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# Anilinotriazoles as potent gamma secretase modulators

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ABSTRACT

The design and synthesis of a novel series of potent gamma secretase modulators is described. Exploration of various spacer groups between the triazole ring and the aromatic appendix in **2** has led to anilinotriazole **28**, which combined high in vitro and in vivo potency with an acceptable drug-like profile.


Dementia affects nearly 36 million people worldwide, a number which is expected to triple by 2050.[1] The most common form of dementia is Alzheimer's disease (AD),[2] a progressive, neurodegenerative illness described in 1906 by the German physician Alois Alzheimer. No treatment exists yet for this condition, the marketed drugs targeting only the amelioration of the symptoms.[3] Characteristic for AD is the deposition of extraneuronal amyloid plaques and intraneuronal neurofibrillary tangles of hyperphosphorylated tau protein in the limbic and cortical regions of the brain, which eventually leads to neurodegeneration. Based on genetic evidence, Hardy and Higgins formulated the amyloid hypothesis, according to which accumulation of amyloid peptides in the brain is the primary driver of the AD.[4,5]

Formation of the amyloid beta peptides in the brain is the result of a sequence of proteolytic cleavages of amyloid precursor protein, APP. This is first cleaved by β-secretase (BACE-1) to form a membrane bound C-terminal fragment (C99), which is further cleaved by gamma secretase (GS) to produce Aβ peptides of lengths varying from 37 to 43 aminoacids. Of these, Aβ42 and Aβ43 are most prone to aggregate and generate the neurotoxic amyloid plaques. The amyloid cascade theory opened the door to anti Aβ therapeutics as strategies for developing anti Alzheimer disease modifying drugs,[6] among which suppression of the Aβ42 production via BACE-1 inhibition and GS inhibition or modulation is intensively pursued.[7] In this regard, GS, the intra-membrane protease complex responsible for the final cleavage step in the amyloid cascade, represents an attractive yet challenging target.[8] Recently, GS inhibitors (GSIs) semagacestat and avagacestat were discontinued in clinical trials due to side effects such as toxicity related to inhibition of other GS substrates and decline in cognition.[8,9] GS modulators (GSMs),[10,11] a class of compounds which are able to reduce the level of longer, neurotoxic Aβ peptides by shifting the APP processing of γ-secretase towards shorter isoforms (such as Aβ37, Aβ38), represent a viable alternative to GS inhibition.[12] Several classes of GSMs are known to date: carboxylic acids derived from non-steroidal anti-inflammatory drugs (NSAIDs), such as tarenflurbil, non-NSAID GSMs such as aryl imidazole-derived GSMs and more recently, triterpene-derived modulators.[7]

Our own search towards aryl-imidazole derived GSMs led to the discovery of the benzimidazole derivative **1** (Fig. 1).[13] It is one of the most potent GSMs to date, suffering however from sub-optimal drug-like properties. More recently, we have described the design and synthesis of bicyclic triazolo-derivatives, such as triazolopiperidine **4** (Scheme 1),[14] as potent in vitro and in vivo GSMs with an improved drug-like profile. These compounds resulted from a conformational restriction of benzyltriazoles **3**, which in turn derived from the introduction of a methylene spacer in **2** between the central triazole ring (C) and the aromatic appendix D, in this case the 4-F-phenyl group. While this work was in progress, an

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0960-894X/

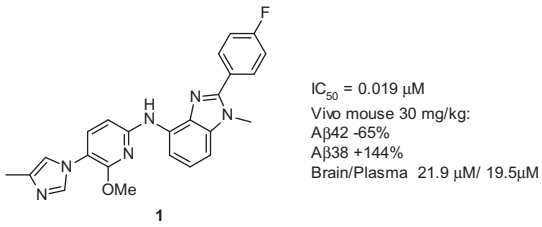



**Figure 1.** Benzimidazole derived GSM.
*Note*: $IC_{50}$ represents the concentration of a compound that is required for reducing the Aβ42 level by 50%. $IC_{50}$ values are a mean of at least 3 determinations. Mouse in vivo at 30 mg/kg p.o. after 4 h ($n = 6$) expressed as a percentage of Aβ42 lowering in brain compared with untreated animals. Plasma and brain samples were analyzed for the tested compound using a qualified research LC–MS/MS method.

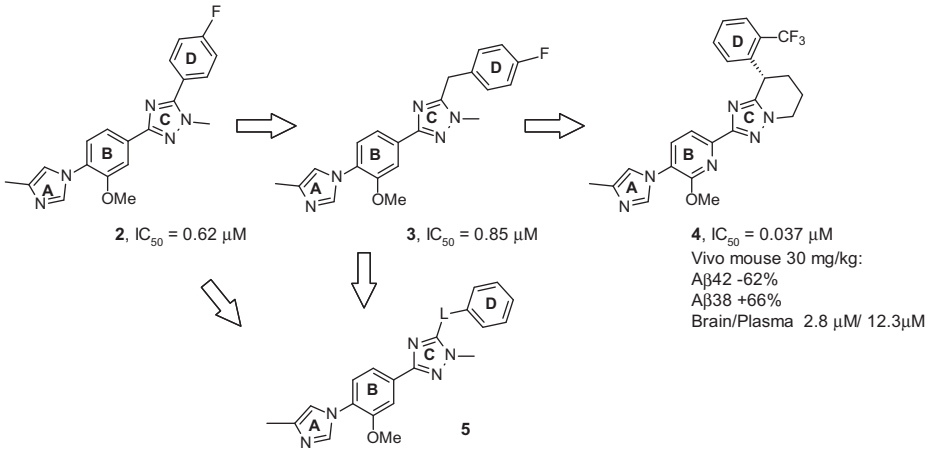
alternative approach to improve the potency of **2**, while maintaining its physicochemical properties (MW < 400, *c*log*P* < 4), was investigated.[15] Considering the influence of torsion between the C and D rings on potency,[14,15] other spacers in **2** that would disrupt the planarity between these rings were also evaluated (Table 1).

Starting from derivative **3**, small substituents were added on the benzylic position with the intent of pointing the D ring towards an active conformation, for instance by hindering free rotation, as in **6** (Table 1). This modification had no effect on potency. Replacement of the linker with a carbonyl function, as in ketone **7** or amide **8**, led to a significant decrease in activity. An extended $sp^2$-carbon linker on the other hand, such as the *E*-alkene in **9**, led to a 10-fold increase in potency. A saturated 2-atom spacer, such as in aniline **10** and benzylamine **11** was probably too flexible and did not manage to bring the $IC_{50}$ under 100 nM. Changing the C linker in **3** to a heteroatom (**12**–**14**) led to the discovery of the highly potent aniline **14**, significantly more active than its phenol- and sulfide-analogs (**14** vs **12** and **13**). This result would indicate that the H-bond donor capability of **14** may contribute to the increase in potency (vide infra). Furthermore, the trifluoromethyl substituent on the 3-position of the aromatic D ring brought a 13-fold increase in potency versus the non-substituted analog **15**. Restriction of the free rotation of **15**, by cyclizing the aniline into the aromatic ring (**16**), did not improve the $IC_{50}$. Swapping the position of the aromatic and the methyl substituents on the triazole ring in **15** (compound **17**, Fig. 2) also had a detrimental effect on potency.

Aniline **14** (MW = 428, *c*log*P* = 4.9) was selected for further evaluation. When **14** was tested orally in mice at 30 mg/kg it showed no significant effect (−17%) on Aβ peptides levels 4 h after dosing, despite high compound brain levels (5.7 μM, B/P = 0.42). This compound was however highly bound to brain proteins, with the fraction of unbound compound in brain ($f_{u,b}$) under the detection limit ($f_{u,b} < 0.05\%$).[16] It can be hypothesized that the free brain concentration of **14** was not high enough for in vivo activity. Nevertheless, in the benzimidazole series of **1** many derivatives with $f_{u,b} < 0.1\%$ demonstrated robust in vivo activity.[13] Further exploration around this hit was aimed at identifying similarly potent analogs that would also demonstrate in vivo efficacy.

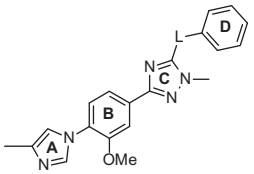
It was found that modifications on the aniline ring (Table 2) resulted in modulation of both in vitro and in vivo activity. Thus, substitution at 3- and 3,5-positions (compounds **18**–**19** in Table 2) with both electron withdrawing and electron donating substituents maintained the in vitro potency of the hit, compound **18** being one of the most potent GSMs in this series. As for **14**, this potency again did not translate in vivo in mice, as Aβ42 levels were not significantly reduced 4 h after administration. Compounds **18** and **19** were also highly bound to brain tissue and thus, despite the high compound brain levels, in both cases the free brain concentration ($C_{u,b}$) was probably not high enough for in vivo activity (estimated as less than 2 nM and 4 nM, respectively).[17] In mouse, the free brain concentration of **18** and **19** was at least 20 fold less than the $IC_{50}$ in vitro as indicated by the coverage ratio ($CR = C_{u,b}/mIC_{50}$, <0.05, see Table 2). Substituents at the 4-position of the aniline ring did not bring an improvement in potency (**20**, not tested in vivo). A substituent present at the 2-position of the aniline (**21**–**28**) turned out to be essential for in vivo potency. Mono 2-substituted anilines, either with electron withdrawing (**21**) or lipophilic electron donating groups (**22**), had moderate in vitro activity, but showed good modulation of Aβ peptides levels in vivo. For **21** and **22**, the free brain concentrations were higher than their respective $IC_{50}$ in vitro (CR = 1.7 and 2.7, respectively). A polar methoxy substituent was weakly active (**23**) and it was not tested in vivo. Additional substitution at the 5-position (compounds **24** and **25**) increased the in vitro potency and maintained the in vivo activity. Compound **24** had high brain and plasma levels and improved B/P ratio (close to 1).

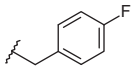
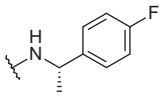
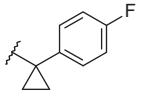
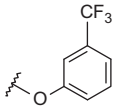
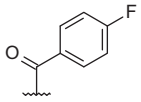
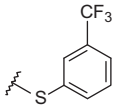
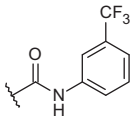
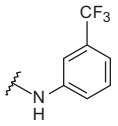
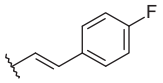
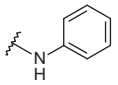
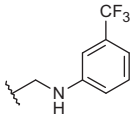
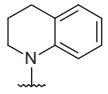
More polar substituents introduced on the 5-position of the aniline ring (e.g., dimethyl amine in **25**) decreased the lipophilicity of the compounds (4.3 for **25** vs 5.1 for **24**), but did not affect their potency. For instance, **25** was highly potent in vitro and demonstrated a surprising 51% lowering of Aβ42 in mice at 30 mg/kg, 4 h from administration, despite a poor brain penetration (B/P = 0.2) and low mouse free brain concentrations ($C_{u,b}$ = 4 nM,



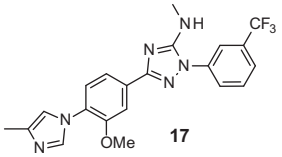

**Scheme 1.**
*Note*: $IC_{50}$ represents the concentration of a compound that is required for reducing the Aβ42 level by 50%. The $IC_{50}$ values are a mean of at least 3 determinations. Mouse in vivo at 30 mg/kg p.o. after 4 h (n = 6) expressed as a percentage of Aβ42 lowering in brain compared with untreated animals. Plasma and brain samples were analyzed for the tested compound using a qualified research LC-MS/MS method.

**Table 1**
Exploration of linkers between C and D rings



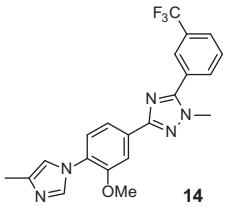

| Compd | L-Ar | $IC_{50}$[a] (μM) | Compd | L-Ar | $IC_{50}$[a] (μM) |
|---|---|---|---|---|---|
| **3** |  | 0.85 | **11** |  | 0.32 |
| **6** |  | 0.71 | **12** |  | 0.27 |
| **7** |  | 6.92 | **13** |  | 0.65 |
| **8** |  | 6.17 | **14** |  | 0.015 |
| **9** |  | 0.085 | **15** |  | 0.20 |
| **10** |  | 0.19 | **16** |  | 0.26 |

[a] $IC_{50}$ represents the concentration of a compound that is required for reducing the Aβ42 level by 50%. The $IC_{50}$ values are a mean of at least 2 determinations.




17

**$hIC_{50}$= 0.28 μM**




14

**$hIC_{50}$ = 0.015 μM**

**$mIC_{50}$ = 0.087 μM**

**Mouse 30 mg/kg PO 4 h**

**Aβ42 -17%**

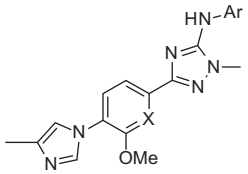
**Mouse Brain total concentration 6 μM (B/P = 0.42)**

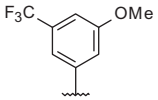
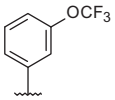
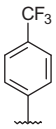
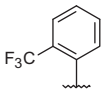
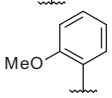
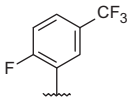
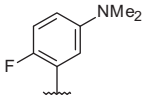
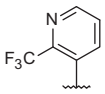
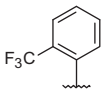
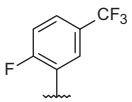
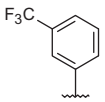
**Mouse Brain free concentration, $C_{u,b}$ < 0.003 μM**

**Figure 2.**
*Note*: $IC_{50}$ represents the concentration of a compound that is required for reducing the Aβ42 level by 50%. The $IC_{50}$ values are a mean of at least 2 determinations. Mouse in vivo at 30 mg/kg p.o. after 4 h ($n$ = 6) expressed as a percentage of Aβ42 lowering in brain compared with untreated animals. Plasma and brain samples were analyzed for the tested compound using a qualified research LC-MS/MS method.

6-fold lower than $mIC_{50}$). Introduction of a heteroatom on the aniline ring was detrimental to potency as exemplified by **26**. Introduction of a N-atom in the anisole ring (compounds **27**–**29**, Table 1) reduced the $c\log P$ by 0.5 while maintaining the in vitro and in vivo potency. This modification led to a reduced brain penetration and a suboptimal B/P ratio for most of the methoxypyridyl derivatives (**27**, **29**). On the other hand, the $f_{u,b}$ of these compounds increased versus their anisole analogs (see **27**–**29** vs **21**, **24** and **14**, respectively). An interesting compound was **27** which, although relatively weak in vitro, was moderately potent in vivo, showing 39% Aβ42 lowering in mice at 30 mg/kg, 4 h after administration. Compounds **28** and **29** had comparable in vitro potencies, both in

**Table 2**
Exploration of the aniline ring




| Compd (clog*P*) | Ar | X | hIC$_{50}$ (μM) Aβ42[a] | mIC$_{50}$ (μM) Aβ42[a] | Brain levels[c] (μM) | Plasma levels[c] (μM) | Inhib Aβ42 vivo[d] (%) | $f_{u,b}$[e] (%) | Free brain levels $C_{u,b}$[f] (μM) | Cover. ratio CR[g] |
|---|---|---|---|---|---|---|---|---|---|---|
| **18** (4.9) |  | CH | 0.005 | 0.050 | 3.4 | 17.1 | 9 | <0.05 | <0.002 | <0.04 |
| **19** (5) |  | CH | 0.025 | 0.078 | 8.6 | 18.9 | 28 | <0.05 | <0.004 | <0.05 |
| **20** (4.9) |  | CH | 0.027 | 0.245 | nd[h] | Nd | nd | nd | nd | nd |
| **21** (4.9) |  | CH | 0.068 | 0.13 | 16 | 19.4 | 40 | 1.4 | 0.22 | 1.7 |
| **22** (5.2) |  | CH | 0.060 | 0.074 | 9.7 | 26.6 | 40 | 2 | 0.20 | 2.7 |
| **23** (3.89) |  | CH | 0.178[b] | 0.257 | nd | Nd | nd | nd | nd | nd |
| **24** (5.1) |  | CH | 0.011 | 0.059 | 11 | 9.85 | 35 | 0.13 | 0.014 | 0.24 |
| **25** (4.33) |  | CH | 0.022[b] | 0.025 | 0.74 | 3.5 | 51 | 0.6 | 0.004 | 0.17 |
| **26** (3.65) |  | CH | 0.603 | nd | nd | Nd | nd | nd | nd | nd |
| **27** (4.5) |  | N | 0.135 | 0.21 | 2.64 | 11.7 | 39 | 4.0 | 0.10 | 0.48 |
| **28** (4.6) |  | N | 0.019 | 0.049 | 14 | 17.8 | 55 | 0.5 | 0.07 | 1.4 |
| **29** (4.5) |  | N | 0.008 | 0.098 | 3.0 | 29 | 14 | 0.3 | 0.009 | 0.09 |

[a] IC$_{50}$ represents the concentration of a compound that is required for reducing the Aβ42 level by 50%. The IC$_{50}$ values are a mean of at least 3 determinations.
[b] Mean of 2 determinations.
[c] Plasma and brain samples were analyzed for the tested compound using a qualified research LC–MS/MS method.
[d] Mouse in vivo at 30 mg/kg p.o. after 4 h ($n$ = 6) expressed as a percentage of Aβ42 lowering in brain compared with untreated animals.
[e] Fraction of unbound compound in rat brain tissue ($f_{u,b}$).
[f] Free brain concentrations, $C_{u,b}$, calculated from $C_{u,b} = f_{u,b} \times$ [brain levels, μM].
[g] Coverage ratio, CR, calculated as CR = $C_{u,b}$/mIC$_{50}$.
[h] nd–not determined.

human and in mouse. Aniline **29** was a less brain penetrant (B/P = 0.1 vs 0.8 for **28**), which combined with a low $f_{u,b}$ (0.3%) resulted in a free brain concentration 10-fold lower than the IC$_{50}$ (Table 2). This might explain the difference in the in vivo activity of the two anilines: while **28** reduced Aβ42 levels significantly 4 h after dosing in mouse at 30 mg/kg, **29** had no effect on the Aβ peptides. Compound **28** was one of the most potent methoxypyridyl derivative both in vitro and in vivo, and maintained a rela-

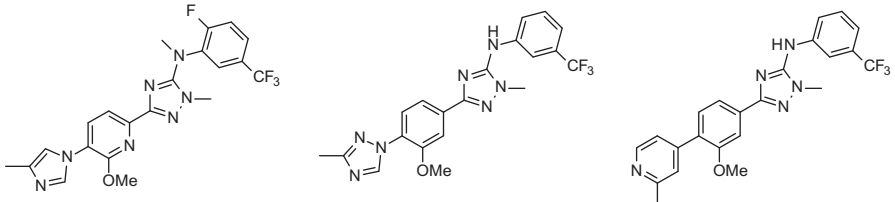
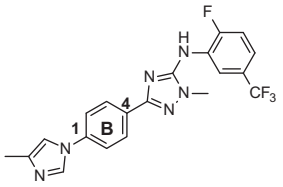
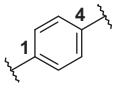
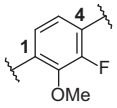
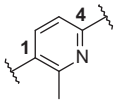
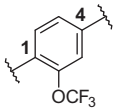
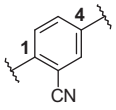
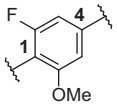



**Figure 3.** Other modifications. Replacements of imidazole ring.
*Note*: $IC_{50}$ represents the concentration of a compound that is required for reducing the Aβ42 level by 50%. The $IC_{50}$ values are a mean of at least 2 determinations.

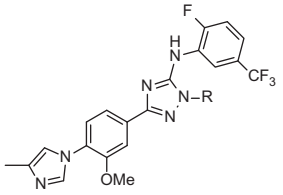
**Table 3**
Variations of the B ring

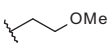


| Compd | B | $IC_{50}$[a] (μM) | Compd | B | $IC_{50}$[a] (μM) |
|---|---|---|---|---|---|
| **33** |  | 0.12 | **36** |  | 0.045 |
| **34** |  | 0.5 | **37** |  | 0.79 |
| **35** |  | 0.036 | **38** |  | 0.058 |

[a] $IC_{50}$ represents the concentration of a compound that is required for reducing the Aβ42 level by 50%. The $IC_{50}$ values are a mean of at least 2 determinations.

**Table 4**
Variations of the alkyl substituents on the triazole core



| Compd | R | $IC_{50}$[a] (μM) | Compd | R | $IC_{50}$[a] (μM) |
|---|---|---|---|---|---|
| **39** | Et | 0.026 | **41** |  | 0.041 |
| **40** |  | 0.052 | **42** |  | 0.076 |

[a] $IC_{50}$ represents the concentration of a compound that is required for reducing the Aβ42 level by 50%. The $IC_{50}$ values are a mean of at least 2 determinations.

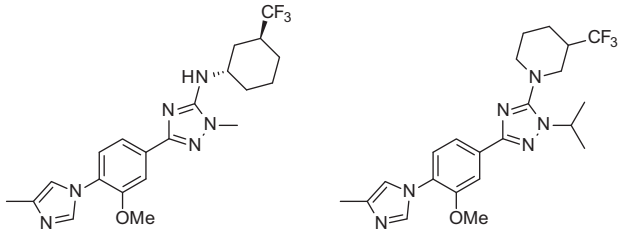



**Figure 4.** Replacement of the aromatic D ring with aliphatic substituents.
*Note*: $IC_{50}$ represents the concentration of a compound that is required for reducing the Aβ42 level by 50%. The $IC_{50}$ values are a mean of at least 2 determinations.

**Table 5**
Compared drug-like profiles of **28** and **1.**

| | **28** | **1** |
|---|---|---|
| *c*log*P* | 4.6 | 5.9 |
| LLE | 3.08 | 1.8 |
| $IC_{50}$ (μM) | 0.019 | 0.019 |
| hLM (%)[a] | 4 | 6 |
| mLM (%)[a] | 0 | 0 |
| Kin. sol. pH 7.4(μM) | 39 | 3 |
| CYP450 $IC_{50}$ (μM) | | |
| 3A4 (T) | 25.4 | 2.9 |
| 3A4 (M) | Activator | 8.7 |
| 2D6 | 16.00 | 3.6 |
| 2C9 | 12.3 | 21.9 |
| 2C19 | 22.1 | 3.5 |
| hHerg $IC_{50}$[b] (μM) | >10 | 8.5 |
| hHerg PX[c] (%) | 52 | 30 |
| $F_{u,h}$ Plasma[d] | 2% | 0.13% |
| $f_{u,b}$[e] | 0.5% | <0.05% |
| Conc mouse plasma 30 mg/kg, μM | 17.8 | 19.5 |
| Conc mouse brain 30 mg/kg, μM | 14 | 22 |
| B/P 4 h | 0.8 | 1.13 |
| Conc mouse brain free 30 mg/kg, μM, 4 h | 0.069 | <0.011 |
| Mouse Aβ42 lowering, 30 mg/kg,[f] 4 h | 55% | 62% |
| Mouse Aβ38 increase, 30 mg/kg,[f] 4 h | 53% | 144% |

[a] % metabolized after 15 min upon incubation with human (hLM) and mouse (mLM) liver microsomes at 1 μM concentration.
[b] Receptor binding on the human Herg ion channel.
[c] % inhibition of IKr current at 3 μM.
[d] Unbound fraction to human plasma proteins.
[e] Unbound fraction to rat brain tissue.
[f] % inhibition of Aβ peptides as determined by Meso Scale.

tively high B/P ratio (0.8). Methylation of the aniline nitrogen of **28** led to a 14-fold loss of potency (**30**, Fig. 3), which underlined the importance of the N–H bond.

Other structural modifications were made, seeking to improve the efficacy in this series of compounds. Replacement of the imidazole ring (see **31**–**32**, Fig. 3, vs **14**) or of the anisole B ring (see **33**–**38**, Table 3, vs **24**) did not improve the in vitro potency. Compound **35**, three times less potent than parent **24**, was one of the most potent compounds resulting from this exercise. Nevertheless when tested at 30 mg/kg in mice, **35** showed no significant lowering of Aβ42 levels 4 h after dosing. Bioanalysis of **35** in mice 4 h post dosing showed that this compound suffered from low exposure both in plasma (0.78 μM) and in brain (0.34 μM), which could be explained by its poor solubility (<1.5 mg/mL in 20%

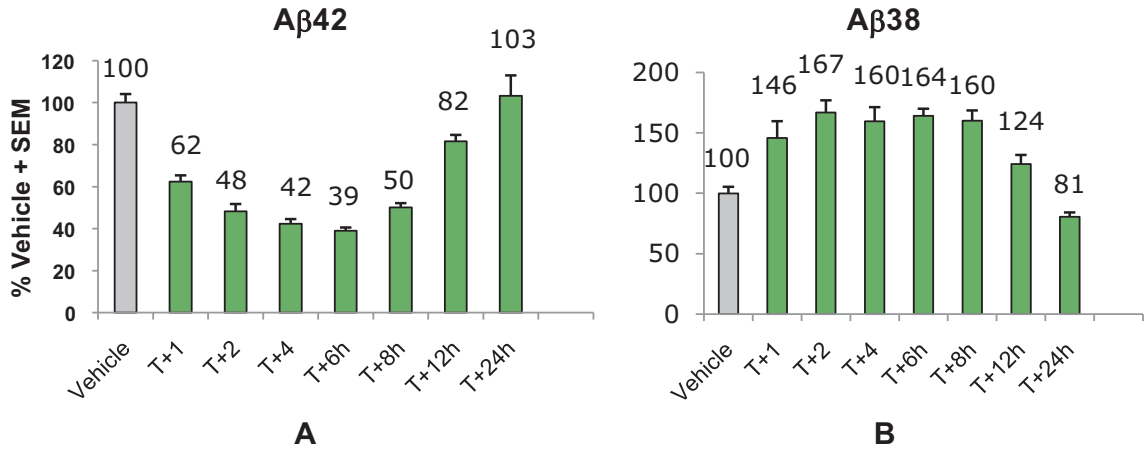



**Figure 5.** Time course of Aβ modulation of **28** in non-transgenic mouse brain after a single oral administration of 30 mg/kg. **A**. Reduction of the Aβ42 levels. **B**. Increase of the Aβ38 levels.

**Table 6**
Modulation of Aβ peptides in CSF dog

| | **28** | **1** |
|---|---|---|
| Conc dog plasma 20 mg/kg, 8 h, μM | 6.7 | 1.7 |
| CSF Aβ42 lowering[a] | 56% | 48% |
| CSF Aβ40 lowering[a] | 54% | 36% |
| CSF Aβ 38 increase[a] | 31% | 84% |

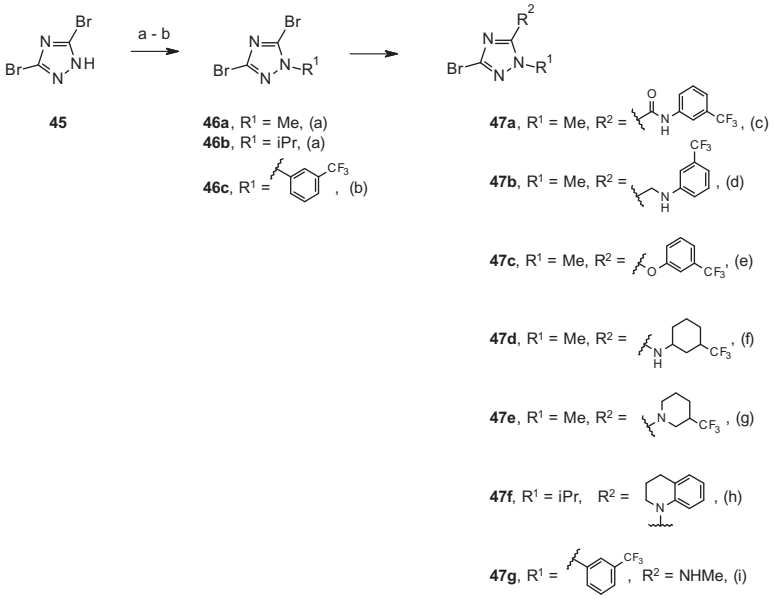
[a] % inhibition of Aβ peptides as determined by alphalisa.

captisol). Replacement of the methyl group on the triazole ring with other aliphatic substituents at best maintained the in vitro potency (**39**–**42**, Table 4), at the expense of the physicochemical properties, such as MW and lipophilicity.

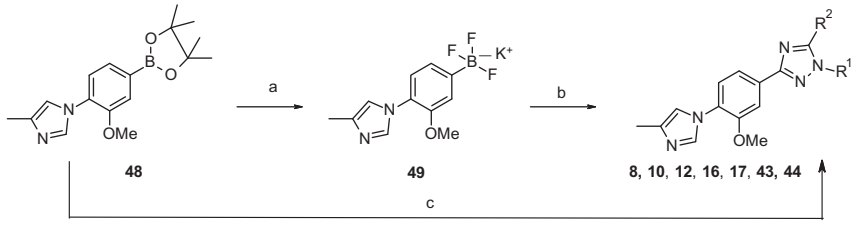
Attempts to reduce aromaticity, as in **43** and **44** (Fig. 4) resulted in a loss of potency.

The most interesting compound in this series remained **28**, with a good balance of the in vitro and in vivo potency. As expected for a GSM, **28** did not affect the total level of Aβ peptides and it did not inhibit Notch processing. Compared with **1**, **28** had improved drug-like properties (reduced aromaticity, lower *c*log*P*, better lipophilic efficiency) which was reflected in the compound's ADMET profile: improved kinetic solubility, weak CyP inhibition potential ($IC_{50}$ >10 μM overall). The moderate inhibition of the potassium membrane current by **28** (52% at 3 μM) did not translate in vivo in anesthetized guinea pigs. When tested orally in mice at 30 mg/kg, **28** showed a significant modulation of the Aβ peptides, inhibiting secretion of Aβ42 by 55% and stimulating production of Aβ38 levels by 53%—again in line with a typical profile of a GSM. While **28** was still highly bound to both plasma and brain proteins, it did show an improvement over benzimidazole **1** (see Table 5).

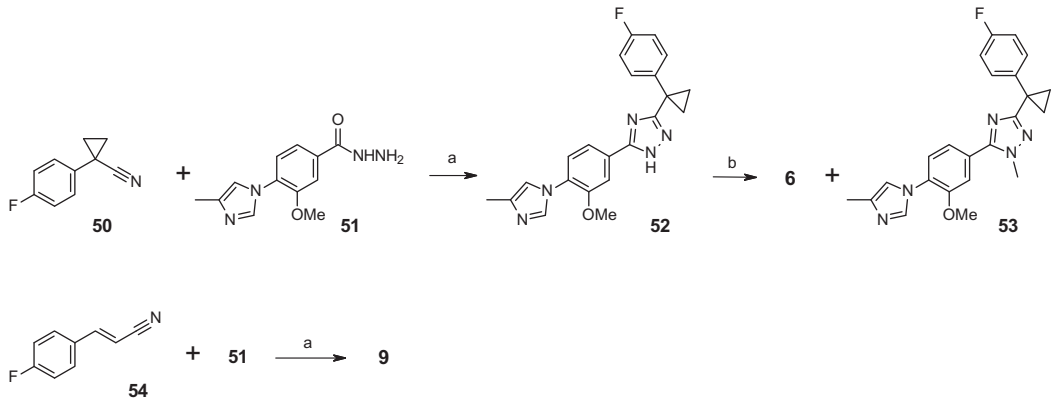
The duration of action of **28** was examined in more detail in a time-course experiment after a single oral dose of 30 mg/kg in non-transgenic mice. Reduction of Aβ42 levels (38%) was observed 1 h after dosing, a maximum inhibition of Aβ42 levels being reached 6 h after treatment. 12 h after dosing, Aβ42 levels remained moderately reduced below vehicle values and returned



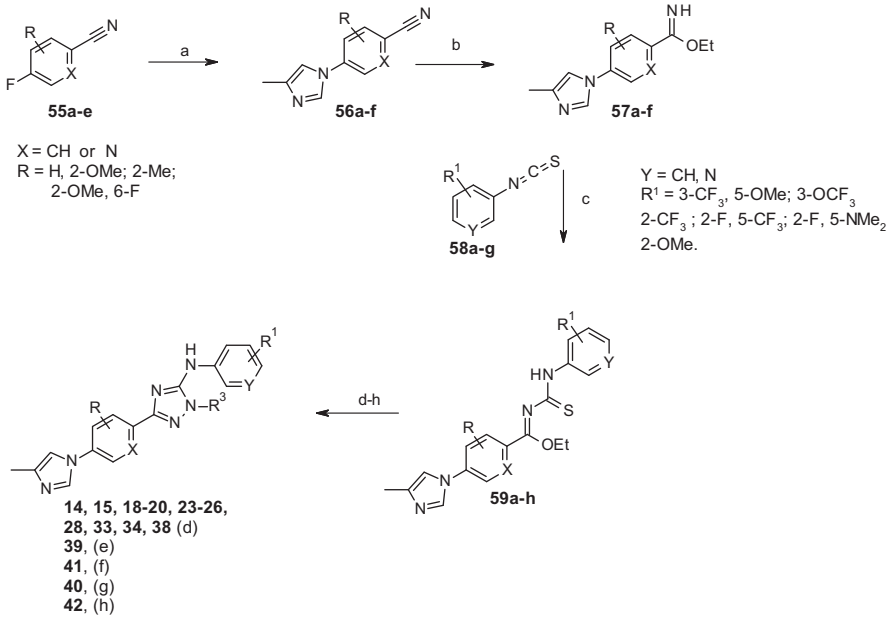

**Scheme 2.** Synthesis of triazolo derivatives from dibromotriazole. Reagents and conditions: (a) $R^1I$, NaH, DMF, 100 °C, 12 h, 93–98%; (b) 3-($CF_3$)-PhB($OH)_2$, $Na_2CO_3$, $Cu(OAc)_2$, Py, tol, 70 °C, 16 h, 68%; (c) i. **46a**, nBuLi, THF, CO (dry ice), −78 °C, 1 h, 82%; ii. BOP-Cl, DIPEA, 3-($CF_3$)-Ph$NH_2$, DCM, 3 h, 80%; (d) i. **46a**, *n*BuLi, DMF, THF, −78 °C to rt, 1 h; ii. 3-($CF_3$)-Ph$NH_2$, NaB$H(OAc)_3$, THF, 0 °C–rt, 16 h, 15%; (e) **46a**, 3-$CF_3$-PhOH, $K_2CO_3$, DMF, MW, 160 °C, 45 min, 88%; (f) **46a**, (RS)(RS)-3-(trifluoromethyl)-cyclohexanamine, $K_2CO_3$, DMF, MW, 160 °C, 90 min, 24%; (g) **46b**, DL-3-(trifluoromethyl)piperidine, $K_2CO_3$, DMF, MW, 160 °C, 45 min, 45%; (h) **46a**, tetrahydroquinoline, LiHMDS, THF, rt, 2 h, 16%; (i) **46c**, $MeNH_2$, $K_2CO_3$, ACN, MW, 140 °C, 20 min, 68%.




**Scheme 3.** Reagents and conditions: (a) $KHF_2$, acetone, rt, 1 h, 80%; (b) **47a**,**b**,**d**,**f**, $Pd(PPh_3)_4$, $K_2CO_3$, ACN, water, MW, 150 °C, 20 min, 15–22%; (c) **47c**,**e**,**g**, $Pd(PPh_3)_4$, $K_2CO_3$, DMF, water, MW, 150 °C, 1 h, 6–38%.




**Scheme 4.** Reagents and conditions: (a) $K_2CO_3$, *n*BuOH, 150 °C, 12 h, 33%; (b) NaH, MeI, DMF, rt, 1 h, 6%.

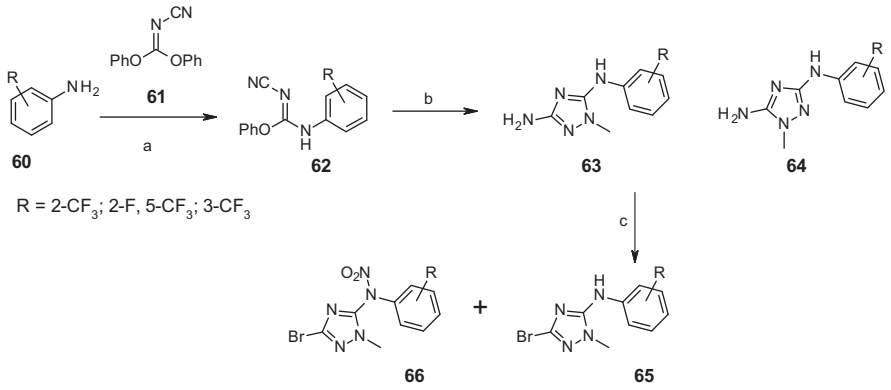



**Scheme 5.** Reagents and conditions: (a) DMSO, 4-Methylimidazole, $K_2CO_3$, 80–120 °C, 1 h, 39–47%; (b) HCl, EtOH, 0 °C to rt, 29–96%; (c) **60**, $CH_3CN$, 50 °C, 32 h, 26–90%; (d) $MeNHNH_2$, MeOH, 50 °C, 1 h, 6–81%; (e) $EtNHNH_2 \times H_2C_2O_4$, DIPEA, MeOH, 50 °C, 1 h, 29%; (f) $CN(CH_2)_2NHNH_2$, MeOH, 50 °C, 1 h, 32%; (g) $MeO(CH_2)_2NHNH_2 \times HCl$, DIPEA, MeOH, 50 °C, 1 h, 35%; (h) *t*-Bu(OH)$NHNH_2$, MeOH, 50 °C, 1 h, 49%.

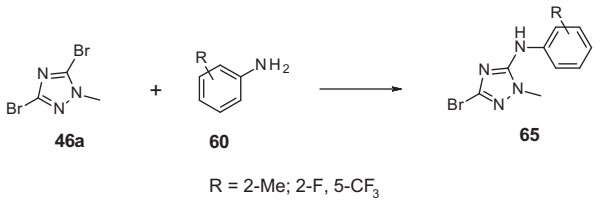
to normal 24 h after administration (Fig. 5A). Similarly, Aβ38 levels in brain were significantly increased from 1 to 12 h post dosing, a 64% increase being reached 6 h after treatment (Fig. 5B). Total levels of Aβ in brain remained unchanged.

When tested in dog at 20 mg/kg, **28** showed a reduction of 56% of Aβ42 in the cerebrospinal fluid (CSF) 8 h post doing, comparable to that of **1** (see Table 6).[19] Upon evaluation of compound **28** in a one week repeated dose study in dog,[18] at 10 and 20 mg/kg/day, no increase in liver enzyme levels (ALT or AST) was observed. Only slight increase in bilirubin was present, which normalized within 24 h after dosing. This represents an improvement versus **1**, which in the same canine model showed increase in both liver enzymes and bilirubin at lower exposure levels.[19]

*Chemistry*. Most analogs from Table 1 were prepared according to the route described in Schemes 2 and 3. N-alkylation or arylation of dibromotriazole **45** provided triazolo-derivatives **46a**–**c** in good to excellent yields. Various substituents were then introduced at the 5-position of **46a**–**c**, as depicted in Scheme 2. The




**Scheme 6.** Reagents and conditions: (a) Method 1: **60**, THF, 100 °C, 12 h or Method 2: **60**, LiHMDS, THF, rt, 3 h, 25–72%; (b) $MeNHNH_2$, iPrOH, 90 °C, 2 h, 59–82%; (c) Method 1: *t*BuONO, $CuBr_2$, acetonitrile, 56–59%; Method 2: $NaNO_2$, CuBr, HBr, acetonitrile, 73%.




**Scheme 7.** Reagents and conditions: LiHMDS (3 equiv), THF, rt, 2 h, 78–81%.

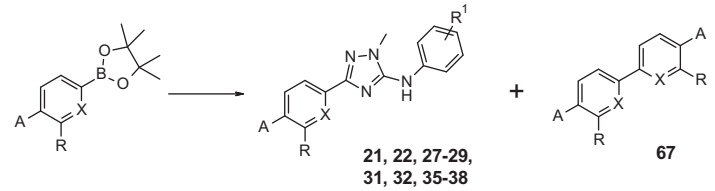
resulting bromotriazoles **47a–g** were then submitted to Suzuki reaction conditions in the presence of either boronate **48** or potassium trifluoroborate salt **49** to yield analogs **8**, **10**, **12**, **16**, **17**, **43**, **44**.[20] Compound **7** was prepared in 68% yield from **3**, via oxidation with manganese oxide.[21]

The synthesis of compounds **6** and **9** is shown in Scheme 4. Commercially available nitriles **50** and **54** were condensed with hydrazide **51**[14] to give **52** and **9**, respectively. Methylation of **52** provided **6** and its regioisomer **53**, in a disappointing 1:6 ratio.

Initially, the anilinotriazoles were prepared by addition of methyl hydrazine to thioureas,[22] as exemplified in Scheme 5. Thus, starting from commercially available 4-F-benzonitriles, nucleophilic aromatic substitution with 4-Me-imidazole provided imidazo-aryl derivatives **56** as the major regioisomers, in a moderate yield. Exposure of **56** to ethanolic HCl provided iminoesters **57**, isolated as HCl salts. When **57** were treated with arylisothiocyanates **58**, thioimidates **59** were obtained. Upon treatment of **59** with hydrazines, anilinotriazoles listed in Scheme 5 were formed in yields varying from poor to excellent.

This route could not provide all desired analogs, and thus formation of triazoles from cyanoimidates using a method developed by Webb and coworkers[23] was investigated (Scheme 6). Addition of anilines to diphenyl cyanocarbonimidate provided cyano isoureas **62** in low to moderate yields. This reaction needed to be performed at higher temperatures and proceeded with longer reaction times than the ones originally reported. The yields were improved when the addition was performed in the presence of a base, LiHMDS in particular. When treated with methyl hydrazine, **62** cyclized to anilinotriazoles **63**, usually as single regioisomers. For the 2-$CF_3$ substituted cyanoisourea **62**, the regioisomer **64** was also formed in a low (22%) yield. When **63** were subjected to Sandmeyer reaction conditions with t-butyl nitrite and Cu(II) bromide,[24] bromotriazoles **65** were formed in low to moderate yields. Side product **66** was difficult to separate from the product mixtures, but its formation was diminished when $NaNO_2$ and HBr were used in the Sandmeyer reaction.

This synthetic pathway proceeded with low overall yields. Formation of the secondary products such as **66** was another drawback. Given the beneficial effect of LiHMDS to the addition reaction of anilines to imidate **61**, it was questioned whether this base would also help in adding anilines **60** directly to bromotriazoles such as **46** (Scheme 7). Previous attempts of introducing anilines on bromotriazole **46a** under classical Buchwald conditions




**48**, A = , X = CH, R = OMe

**48a**, A = , X = N, R = OMe

**48b**, A = , X = CH, R = CN

**48c**, A = , X =CH, R = OMe

**48d**, A = , X =CH, R = OMe

**Scheme 8.** Reagents and conditions: **65**, $Pd(PPh_3)_4$, $Cs_2CO_3$, DME, $H_2O$, 16 h, 90 °C, 19–75%.

failed. Gratifyingly, by addition of 3 equiv of LiHMDS to a mixture of **46a** and anilines **60**, at −78 °C, bromoanilinotriazoles **65** were obtained in high yields and as single regioisomers.

When bromotriazoles **65** were reacted with boronic esters **48** under Suzuki reaction conditions,[25] the desired anilinotriazoles were obtained, in moderate to good yields. A major side product of this reaction was the homocoupled product derived from **48** (**67**, Scheme 8). Formation of **67** was drastically decreased when the boronic ester was added dropwise at reflux to a solution of **65**, base and catalyst. It was also found that using [1,1′-bisdiphenylphosphino)ferrocene]dichloropalladium (II) as catalyst provided better yields than tetrakis(triphenylphosphine)palladium(0).[26]

In conclusion, starting from the weakly potent GSM **3**, exploration of various spacer groups between the triazole ring and the aromatic appendix led to the discovery of a new series of potent gamma secretase modulators, with an improved drug-like profile—compared with the originally reported series around **1**. In this anilinotriazole series, there was a disconnection between the in vitro potency and the in vivo activity of some of the analogs. The presence of an *ortho*-substituent on the aniline ring proved to be crucial for obtaining in vivo activity. This could be the result of the higher free fraction in brain tissue of most of the ortho-substituted anilines in comparison to the *meta*- and or *para*-analogs. In order to better understand the discrepancy between the in vitro and the in vivo activity observed in the anilinotriazole series, the coverage ratio was examined. This parameter represents the ratio between the free brain concentration and the potency, given by $IC_{50}$. Some compounds already showed a significant effect in modulating Aβ peptide levels in brain at a coverage ratio ~0.1. A higher coverage ratio routinely translated into robust reduction in Aβ42 peptide levels. Due to the general low $f_{u,b}$ of all analogs in this series, high exposures of the compounds in the mouse brain were generally required for in vivo activity. Compound **28** was identified, combining good in vitro efficacy with in vivo potency across species. The lower lipophilicity of **28** versus **1** led to an improvement of the overall ADME profile versus **1**. In dog, treatment with **28** resulted in significant changes in Aβ levels and, more importantly, single and repeated dosing did not affect the liver function. Compound **28** was therefore progressed towards further evaluation.

## Acknowledgments

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26. Example: synthesis of **28**. *Step 1. Synthesis of 3-bromo-N-[2-fluoro-5-(trifluoromethyl)phenyl]-1-methyl-1H-1,2,4-triazol-5-amine,* **65**. LiHMDS (1 M in THF, 420 mL, 420 mmol) was added dropwise at 0 °C to a cooled (ice bath) solution of 3-amino-4-fluorobenzotrifluoride (70 mL, 527.8 mmol) and 1*H*-1,2,4-Triazole, 3,5-dibromo-1-methyl-(**46a**, 50 g, 207.6 mmol) in anhydrous THF (500 mL). The reaction mixture was stirred at room temperature (rt) for 20 h. A saturated aqueous $NH_4Cl$ solution was added slowly. The reaction mixture was extracted with dichloromethane (DCM) and the organic layer was washed with brine, dried ($MgSO_4$) and concentrated under reduced pressure. The resulting slurry was triturated in heptane/DIPE and a solid was formed, filtered and dried under vacuum at 60 °C, to provide **65** in 78% yield; mp 159.2 °C. $^1$H NMR (600 MHz, $CDCl_3$) δ ppm 3.77 (s, 3H), 6.37 (d, *J* = 3.9 Hz, 1H), 7.20–7.24 (m, 1H), 7.27–7.29 (m, 1H), 8.34 (dd, *J* = 7.7, 2.2 Hz, 1H). MS (ESI) *m/z* 339 [M+H].$^+$ Anal. ($C_{10}H_7BrF_4N_4$) C, H, N.
*Step 2. Synthesis of N-[2-fluoro-5-(trifluoromethyl)phenyl]-3-[6-methoxy-5-(4-methyl-1H-imidazol-1-yl)-2-pyridinyl]-1-methyl-1H-1,2,4-Triazol-5-amine,* ***28***. A mixture of **65** (47 g, 138.6 mmol) and $Cs_2CO_3$ (104 g, 320 mmol) in a mixture of DME (200 mL) and $H_2O$ (200 mL) was flushed with $N_2$ for 5 min. $PdCl_2$(dppf) (7.88 g, 10.7 mmol) was added and the r.m. was flushed with $N_2$ for an additional 5 min. The mixture was heated at 80 °C and a solution of **48a** (35 g, 107 mmol) in DME (400 mL) was added dropwise over a period of 4 h. After addition, the reaction mixture was stirred at 80 °C for an additional 30 min. The reaction mixture was cooled to rt and the layers were separated. The aqueous layer was extracted with DME (100 mL). The combined organic layers were concentrated to a volume of approximately 250 mL and a precipitate was formed. The precipitated was filtered, washed with DME (50 mL) and dried in vacuo. The solid was dissolved in an aqueous 4 N HCl sol. (600 mL). The resulting solution was washed with DCM (100 mL) and EtOAc (100 mL). The aqueous layer was then cooled with ice and brought to pH 8–9 via the addition of 50% aq NaOH solution. The resulting precipitate was filtered, washed with water and dried in vacuo. The product was recrystallized from iPrOH. The solid was filtered, washed with DIPE and dried to provide **28** in 48% yield; mp 230.1 °C. $^1$H NMR (400 MHz, DMSO-$d_6$) δ ppm 2.17 (s, 3H), 3.89 (s, 3H), 4.02 (s, 3H), 7.28 (s, 1H), 7.36–7.43 (m, 1H), 7.52 (dd, *J* = 11.1, 8.5 Hz, 1H), 7.65 (d, *J* = 7.9 Hz, 1H), 7.89–7.98 (m, 2H), 8.58 (dd, *J* = 7.7, 2.4 Hz, 1H), 9.22 (s, 1H). MS (ESI) *m/z* 447 [M+H].$^+$ Anal. ($C_{20}H_{17}F_4N_7O$) C, H, N.