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Article

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Generation of Leads for γ-Secretase Modulation

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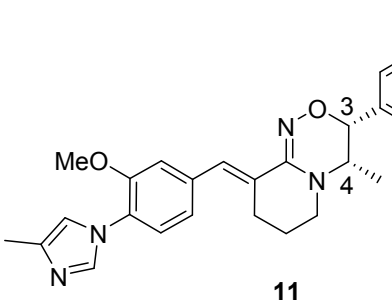
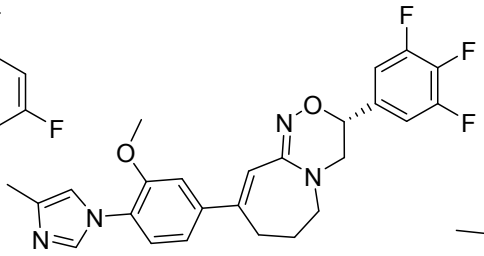
ABSTRACT

Herein we disclose three structurally differentiated γ-secretase modulators (GSMs) based on an oxadiazine scaffold. The analogs from series I potently inhibits the generation of $A\beta_{42}$ in vitro when the substituents at 3 and 4 positions of the oxadiazine moiety adopts an α orientation (cf. **11**). To address the concern around potential reactivity of the exocyclic double bond present in series I towards nucleophilic attack, compounds containing either an endocyclic double bond, such as **20** (series II), or devoid of an olefinic moiety, such as **27** (series III), were designed and validated as novel GSMs. Compound **11** and azepine **20** exhibit robust lowering of CSF $A\beta_{42}$ in rats treated with a 30 mg/kg oral dose.



11
(Series I)



20
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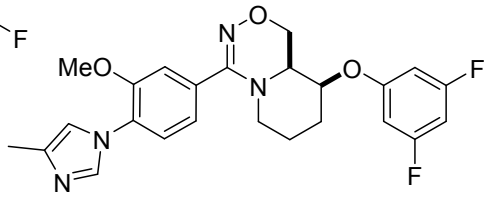


27
(Series III)

Introduction

Despite enormous efforts both in industry and in academia, a disease-modifying therapeutic for Alzheimer's disease (AD) has not yet been approved.[1] Current therapies for AD, which are only palliative, include four acetylcholine esterase inhibitors such as donepezil, rivastigmine, tacrine and galantamine, and one NMDA antagonist, memantine.[2] Thus, the development of agents that will halt or reverse the disease progression still represents an important unmet medical need. Toward this overall goal, testing the amyloid hypothesis by inhibiting BACE and γ-secretase was recognized as a promising strategy.[3,4] Concurrent with the efforts to discover novel BACE1[5] and γ-secretase inhibitors (GSI),[6] designing novel γ-secretase modulators (GSM) to lower the formation of highly amyloidogenic $A\beta_{42}$ with concomitant increase in the formation of $A\beta_{37}$ from APP processing while sparing non-amyloid substrates were undertaken.[7] Our initial efforts resulted in the discovery of two novel chemotypes represented by oxadiazoline **3** and oxadiazine **4** inspired by the classes of compounds represented as **1**[8] and **2**.[9] Representative analogs from these series demonstrated robust lowering of CSF $A\beta_{42}$ after oral dosing.[7h] Subsequently, analog **5**[7d] generated by ring expansion from **4**, and the analog **6**[7i] with substitution at 3 position of oxadiazine were also validated as GSMs.

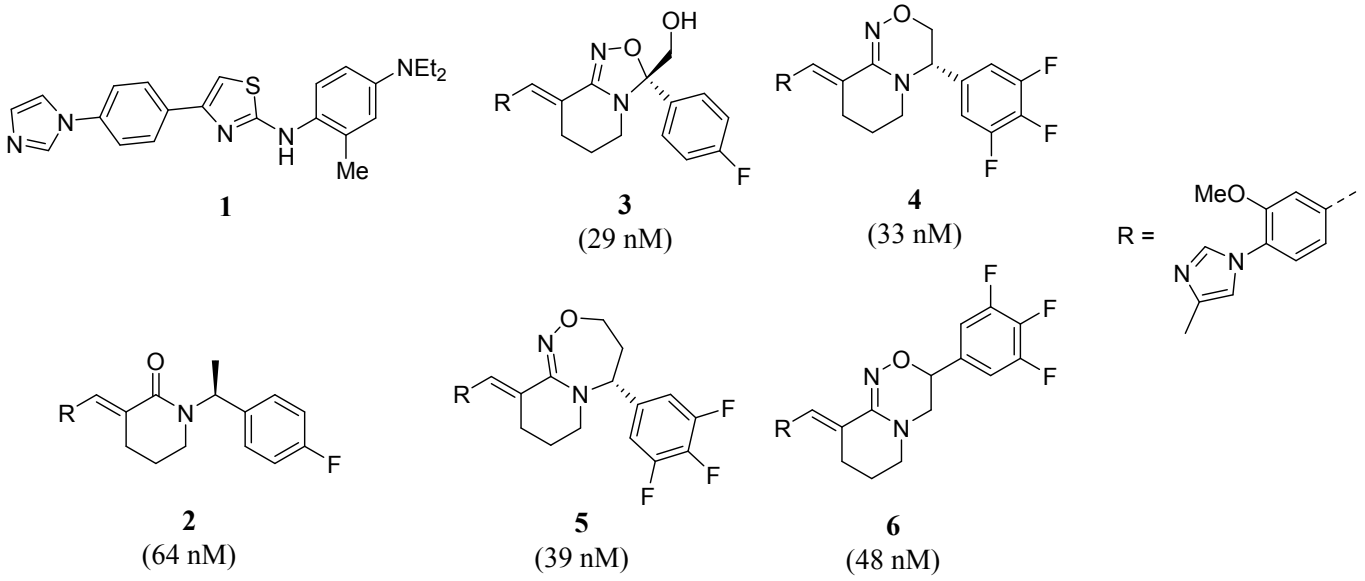


Figure 1. Structures of first non-NSAID γ-secretase modulators **1**, **2** and the discovery of oxadiazoline **3**, oxadiazine **4, 5** and **6**, and in parenthesis $A\beta_{42}$ IC_{50}.

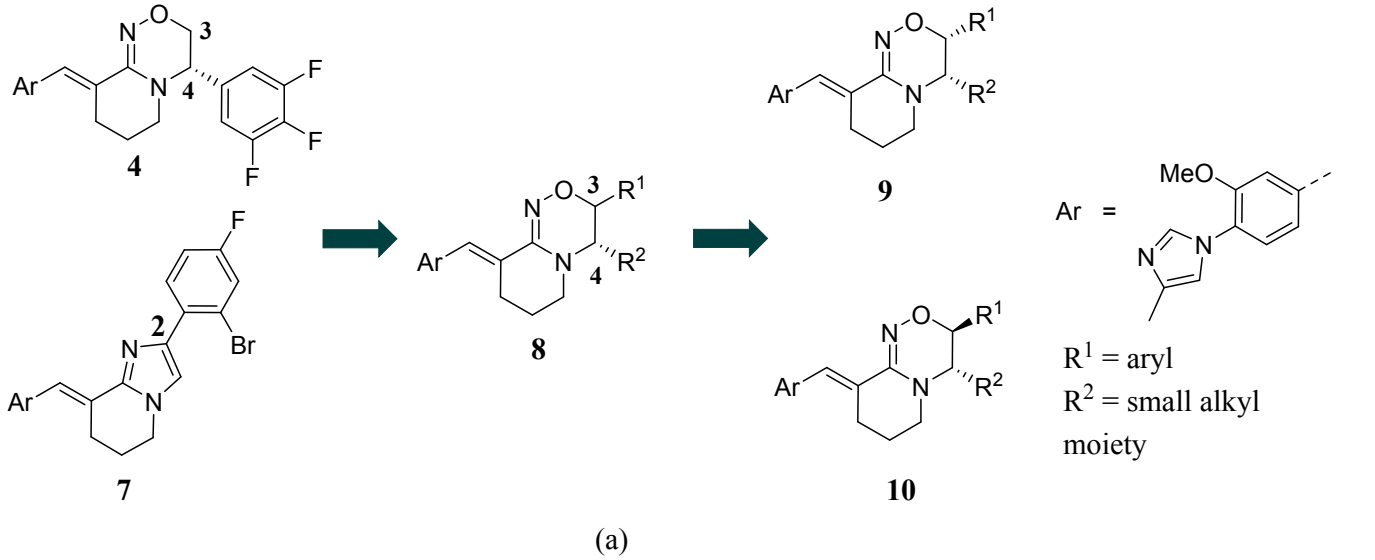
In our further efforts to identify structurally differentiate leads using oxadiazine scaffold, several strategies were undertaken: 1) developing SAR at the C-3 position of oxadiazine moiety of **4** while incorporating smaller groups at the 4 position (C-4) (Figure 2, Strategy I); 2) reconfiguration of the exocyclic double bond into the more hindered β, β-disubstituted endocyclic olefin (Figure 3, Strategy II); and 3) removal of olefinic bond without incorporation of an additional aromatic ring (Figure 5, Strategy III). This article describes the guiding principles for each of these strategies, the synthesis, and evaluation of the resulting molecules as GSMs.

Results and discussion

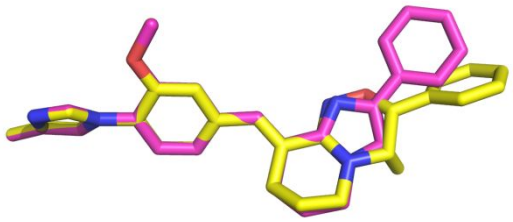
Strategy I: SAR at 3 position (C-3) of oxadiazine moiety of 4 while incorporating smaller groups at the 4 position (C-4).

Design considerations:

Analysis of reported GSMs revealed a novel chemical series exemplified by analog **7** with an IC_{50} of 87 nM for the inhibition of $A\beta_{42}$ in vitro (Figure 2).[10] Structural alignment of compound **7** with **4** suggested that the aryl moiety from the C-2 position of the imidazole **7** projected into a distinct binding pocket previously unexplored using 4-substituated oxadiazine scaffold (cf. **4**). Although direct follow up of this finding by adding the second aryl at the C-3 position of the oxadiazine moiety of **4** was a potential approach, we recognized that this strategy would generate analogs with poor physicochemical properties.



(a)



(b)

Cpd	ΔE (Kcal/mol) **(9ea - 9ae)**	Cpd	ΔE (Kcal/mol) **(10ee - 10aa)**
9	-4.34	**10**	-0.04

(c)

Figure 2. The rationale for designing disubstituted oxadiazines. (a) Structure of **4** and **7**, and conceptualization of 3,4-disubstituted oxadiazine **8**. Compounds **9** and **10** are the diastereomeric pairs differing in orientation of the substitution at their 3 position; (b) Overlay of **9ea** (yellow)and **7** (magenta) with unsubstituted phenyl at its 2 position; (c) Conformational analysis by DFT at the b3lyp/6-31+G(d,p) level of theory. **9ea (**R^1 = equatorial Ph, R^2 = axial Me), **9ae** (R^1 = axial Ph, R^2 = equatorial Me), **10ee** (R^1 = equatorial Ph, R^2 = equatorial Me), and **10aa** (R^1 = axial Ph, R^2 = axial Me).

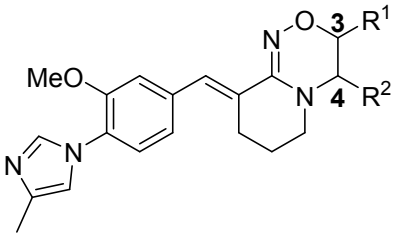
Thus, the computational study was initiated with C-3 aryl and smaller alkyl groups at C-4 (cf. **8**, Figure 2a). Relative to our earlier SAR studies at C-3 with no substitution at C-4, where the $A\beta_{42}$ IC_{50} was independent of the stereochemistry at C-3, the disusbtitution both at C-3 and C-4 would provide the opportunity to probe the effect of conformational restriction. Additionally, we wanted to explore the effect of substitution at C-4 in the context of C-3 only substituted oxadiazine (cf. **6**, Figure 2a) reported earlier.[7i]

Based on the conformational analysis by DFT at the b3lyp/6-31+G(d,p) level of theory we prioritized the synthesis of diastereomer **9** over **10**. Conformer **9ae** (C-3 pseudo axial and C-4 pseudo equatorial orientation) of diastereomer **9** was stabilized by 4.3 kcal/mmol relative to its conformer **9ea** (C-3 pseudo equatorial and C-4 pseudo axial orientation) (Figure 2c). Additionally, conformer **9ae** adopted the desired binding mode with its C-3 aryl projecting into the unexplored binding pocket occupied by the C-2 aryl group of **7**, while positioning the C-4 alkyl substituent in the binding pocket previously explored using C-4-aryl oxadiazine (cf. 3,4,5-trifluorophenyl in **4**) (Figure 2b). By comparison, diastereomer **10** is conformationally more flexible, and the substituents at C-3 and C-4 position of these conformers either did not project into the targeted binding pockets concurrently (**10aa**) or only with significant distortion of the oxadiazine moiety (**10ee**) (Supporting Information, Figure S1).

In vitro Characterization:

Consistent with the conformational analysis, analog **11** with C-3 3,5-difluorophenyl and C-4 methyl, exhibited $A\beta_{42}$ IC_{50} of 52 nM, while the conformationally flexible analog **12** (obtained as the undesired isomer during synthesis), was significantly less potent ($A\beta_{42}$ IC_{50} of 9289 nM). Increasing bulk at C4 (analog **13**), addition of a fluorine into the difluorophenyl at C-3 (analog **14**), and replacement of the methyl with a polar hydroxyl methyl moiety (analog **15**) were tolerated. Compounds **11** and **14** were selected for further profiling based of their potencies, permeabilities, and lack of major off target liabilities (Table 2). Furthermore, the ability of **11** and **14** to lower $A\beta_{42}$ in plasma and CSF was assessed using in vivo studies.

Table 1. GSM Profile of 3,4-Disubstituted Oxadiazine



Cpd	R^1 (orientation)	R^2 (orientation)	IC_{50} (nM) $A\beta_{42}$[a,b]	IC_{50} (μM) $A\beta_{total}$[a]	IC_{50} $A\beta_{total}$/ IC_{50} $A\beta_{42}$
11	3,5-difluorophenyl (α)	Me (α)	52	12	248
12	3,5-difluorophenyl (β)	Me (α)	9289	>20	NA
13	3,5-difluorophenyl (α)	*i*-Pr (α)	113	19	174
14	3,4,5-difluorophenyl (α)	Me (α)	55	>20	>367
15	3,4,5-difluorophenyl (α)	CH_2OH (α)	69	15	221

[a]Protocols for determination of $A\beta_{42}$ IC_{50} (nM) and $A\beta_{total}$ IC_{50} have been previously described.[7h] All the measurements are geometric mean of at least n=2 measurements.

Strategy II - Reconfiguration of the exocyclic double bond into the more hindered β, β disubstituted endocyclic olefin.

Analysis of previous SAR:

Along with the aforementioned efforts to introduce structural diversity, we undertook several strategies[11] to mitigate the concern related to the exocyclic double bond towards nucleophilic attack that was present in our previous GSMs. We generated multiple scaffolds following a cyclization strategy where heterocycles were installed by tethering the distal methylene of the exocyclic double bond and the amide carbonyl as exemplified by compounds **16** and **17** (Figure 3).[11a, b] While successful in masking the double bond with minimal loss of in vitro potency, this strategy introduced an additional aromatic ring into the scaffold. In addition, none of these analogs exhibited statistically significant CSF $A\beta_{42}$ reduction in in vivo at 30 mg/kg oral dose except for **17**, which demonstrated only modest CSF $A\beta_{42}$ lowering at 30 mg/kg with 45% reduction due to poor PK and lack of adequate brain exposure. In another approach,[11c] the exocyclic methylene was replaced by an "NH" (cf. **18**) to create an intramolecular hydrogen bond with the adjacent amide carbonyl to mimic the 5-membered heterocycles present in **16** and **17**.

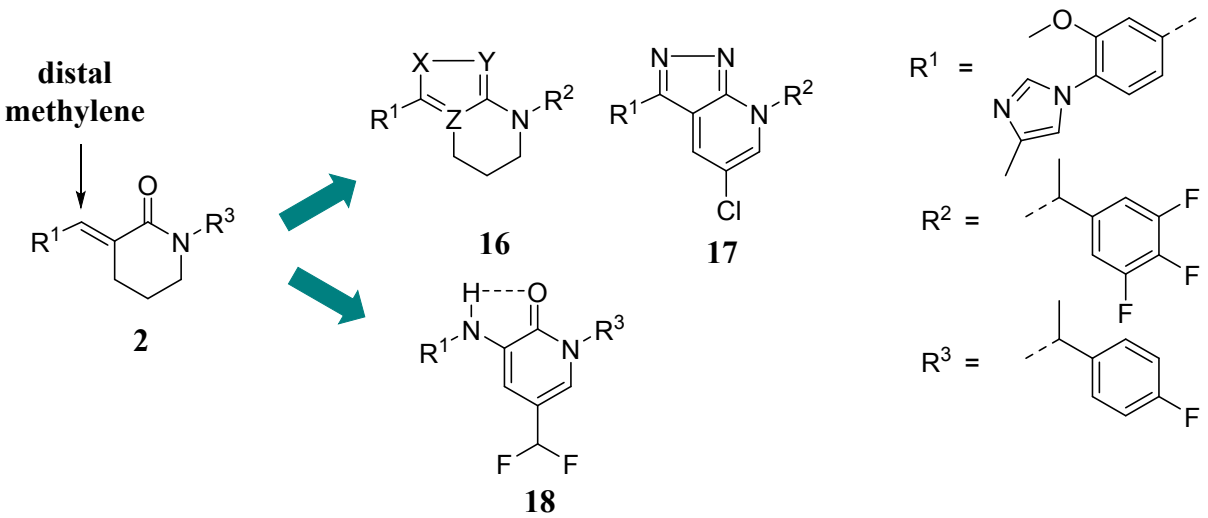


Figure 3: Previous strategies to mask the exocyclic double bond.

While this approach was effective in maintaining the in vitro potency without incorporating an additional aromatic ring, it introduced unsaturation in the piperidinone moiety, and analogs from this series generally lacked robust in vivo efficacy. For example, analog **18** with in vitro potency of 40 nM lowered CSF $A\beta_{42}$ by 40% at 100 mg/kg oral dose in rodents.

Design Considerations:

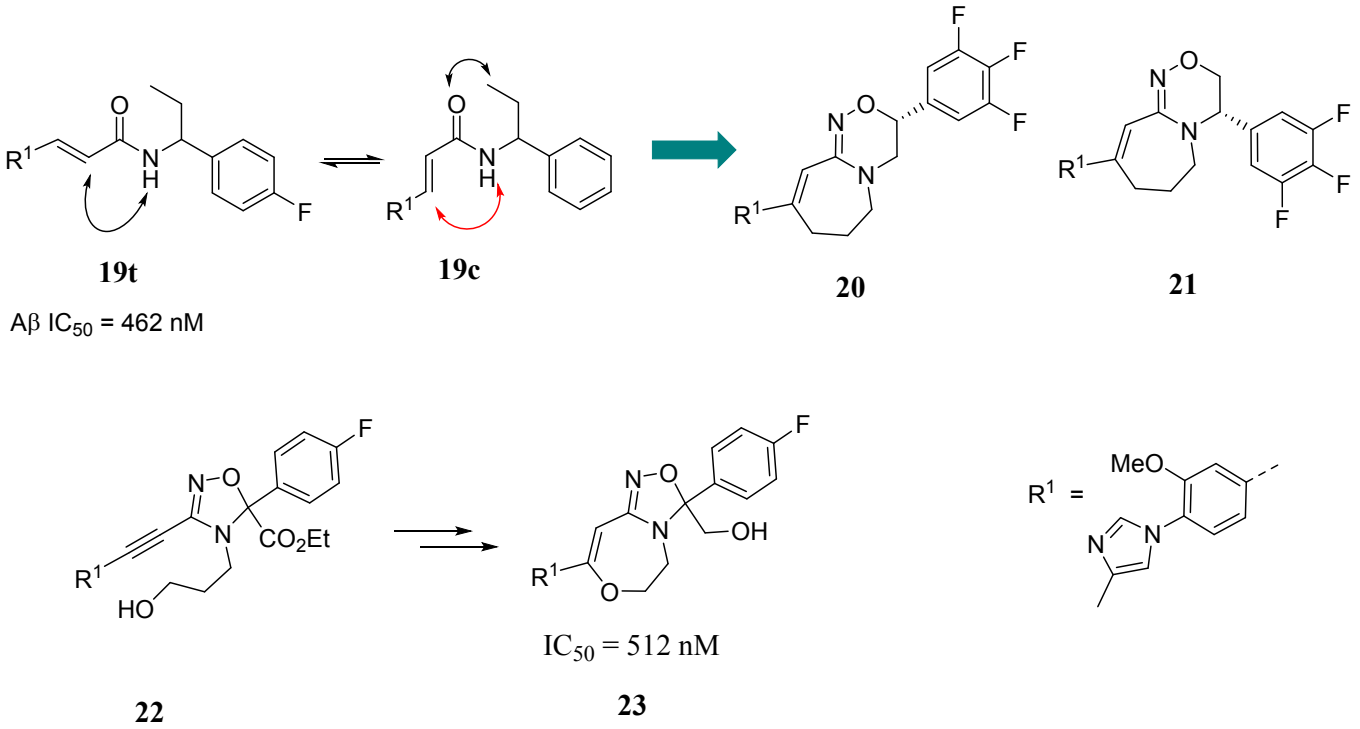


Figure 4: Conformational analysis and building hypothesis for the synthesis of **20** and **21**. The calculated energy difference between **19t** and **19c** is ~ 2.3 kcal/mmol (energy calculations were done at the b3lyp/6-31+g(d,p) level of theory).

Therefore, in subsequent efforts, we focused on designing scaffolds without the introduction of additional aromatic rings or unsaturation while mitigating the concern around the exocyclic double bond. Detailed SAR analysis of close analogs of compound **2** from the literature[12] led to the identification of the acyclic analog **19** with $A\beta_{42}$ IC_{50} of 462 nM (Figure 4). Since it is well known that the conformers with

global minimum energy or even local minimum energy from the potential energy surface often are not the bioactive conformers,[13] we wanted to probe the effect of constraining the cisoid conformer of **19** (cf. **19c)** by linking the amide NH with the methylene distal to amide (bottom red double headed arrow on **19c**) on the in vitro potency. This arrangement forms a sterically encumbered endocyclic β, β-disubstituted double bond with reduced susceptibility for nucleophilic addition.[14] While such strategy could be perceived as high risk due to presence transoid conformation among all the previously reported GSMs, it was inspired by the serendipitous discovery of **23** with an IC_{50} of 512 nM during our SAR studies in the oxadiazoline series (Figure 4). Among several approaches to constrain the cisoid conformation **19c**, azepines **20** and **21** were prepared.

In vitro Characterization:

Azepines **20** and **21** displayed cell $A\beta_{42}$ IC_{50s} of 107 nM and 143 nM respectively while maintaining a good modulator profile as shown by selectivity vs. $A\beta_{total}$ (Table 2). These analogs were highly permeable and exhibited reduced binding to the hERG K^+ channel relative to analog **2** (IC_{50} = 990 nM) in a radiolabeled MK-499 binding[15] assay and possessed no major off target liability (Table 2). These compounds were selected for characterization in a rat pharmacodynamic model to assess their ability to reduce CSF $A\beta_{42}$ in vivo.

Strategy III: Removal of the olefinic bond without incorporation of additional aromaticity

Design considerations and validation as GSM:

Our third strategy to mitigate the concern around the reactivity of the exocyclic double bond began with careful consideration of the structural features of **2** and **4** (Figure 5). This analysis revealed that the lone pair on the oxygen (represented as red) of the amide carbonyl of **2** projected toward the fluorobenzyl moiety was not necessary to maintain potency as it was encapsulated by the formation of oxadiazine ring (**2**→**4**). The importance of the other lone pair (represented as blue) oriented toward the exocyclic double bond was unknown. Generation of analog **24**[11a] with IC_{50} of 116 nM (racemic) eliminating the nitrogen lone pair oriented toward the exocyclic double bond via formation of the 5-membered heterocycles underscored its lack of importance for binding affinity (comparison of IC_{50}s between **24** and **25**). This SAR suggests that the amide carbonyl lone pairs of **2** or the of *N*-1 nitrogen lone pair of triazole **24** may not be playing a significant role on the binding affinity via hydrogen bonding or ionic interactions. Recent reports by Petterssen et al. from Pfizer,[7g] where the exocyclic double bond was replaced by the isosteric amide, further suggested that the amide carbonyl lone pair of **2** was either not important or the distance between the H-bond acceptor and the methoxyaryl-imidazole right-hand-side was flexible.

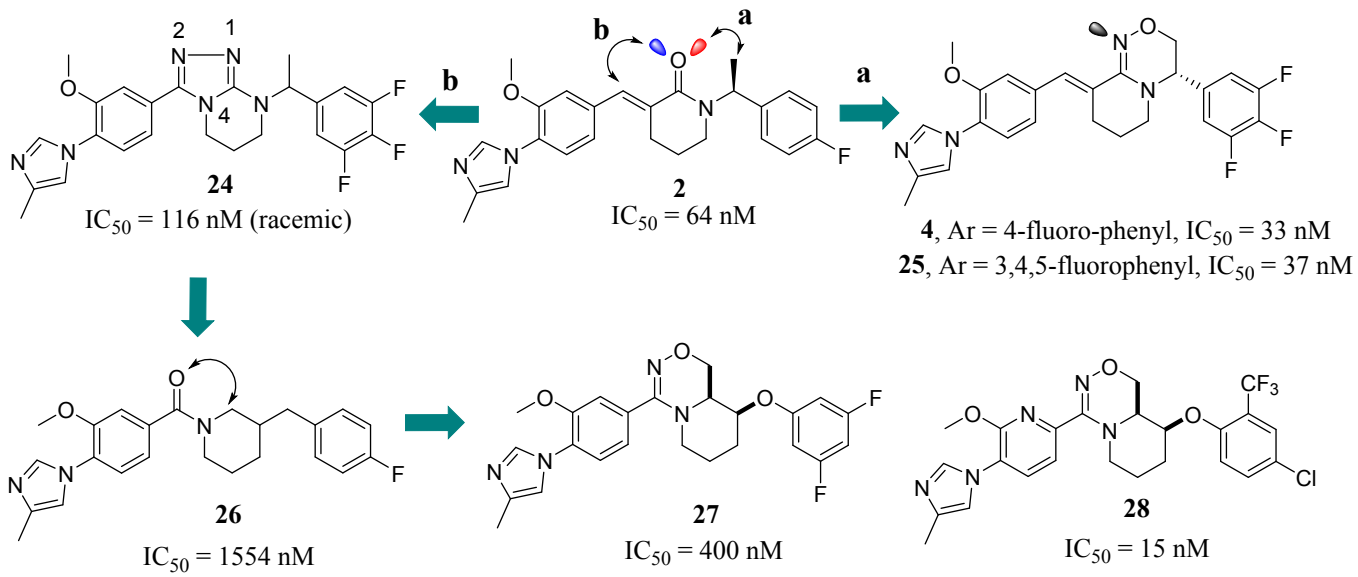


Figure 5. Analysis on the importance of the lone pairs of the amide carbonyl of **2** for potency and building hypothesis for the synthesis **26** and **27** via deconstruction of **24**.

To probe this hypothesis, prototype compound **26** with IC_{50} of 1,554 nM (racemic) was generated by replacing the triazole in **24** with an amide moiety. Importantly, **26** lacked the lone pairs that were initially present at the N1 position in **24**. Furthermore, replacement of the amide of **26** by an oxadiazine along with the introduction of conformational constraint provided analog **27** with an IC_{50} of 400 nM (racemic). The increased sp3 character and reduced aromatic ring count relative to **24** together with the lack of the exocyclic double bond, compound **27** represented a promising novel chemotype for GSM. Further improvement in potency may be necessary before in vivo characterization of this series. Moreover, independently a recent report revealed that enhancement of potency was feasible via SAR studies related to the chemotype exemplified by compound **27**. For example, analog **28** exhibits improved potency and in vivo efficacy.[7a]

Table 2. Collective Profile of 11, 14, 20, and 21

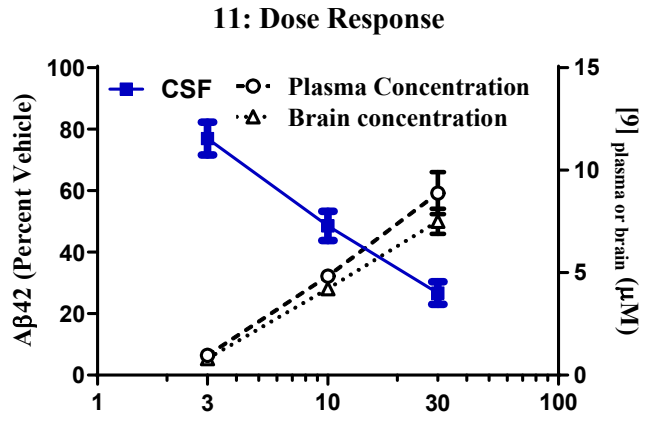
Measured parameters	Compounds			
	11	**14**	**20**	**21**
IC_{50} (nM) $A\beta_{42}$	52	55	107	143
IC_{50} (μM) $A\beta_{total}$	12	>20	14	16
Papp (10^{-6}cm/s)	17	14	22	23
hERG K^+ MK-499 Binding IC_{50} (nM)	2007	1607	3352	2608
PXR (μM)	15	>10	>30	>15
3A4; 2C9; 2D6 (μM)	16; 33; 49	29; 37; 49	34; 18; 48	30; 8.0; 8.0
In vivo rat CSF $A\beta_{42}$ reduction (30 mg/kg)	73	80	52	NS
C_P (μM); C_B (μM)[a]	8.8; 7.5	13.1; 11.1	14.2; 2.1	1.4; 1.3

[a]Measured total concentrations in plasma (C_P), and brain (C_B) of rats at 3h post oral dose of 30 mg/kg. Papp, apparent permeability; hERG K^+, Human ether-a-go-go-related gene (hERG) potassium channels; PXR, Pregnane X receptor.

In vivo characterization:

Concurrent with our efforts to identify novel structural motifs described above, analogs **11** and **14** from the disubstituted oxadiazine series (Table 1) as well as azepines **20** and **21** (Figure 4) were evaluated to measure their ability to lower CSF $A\beta_{42}$ in vivo at a 30 mg/kg oral dose after 3 h drug administration using male CD rats (Table 2). Analogs **11** and **14** exhibited robust CSF $A\beta_{42}$ lowering (73% and 80% respectively) at 3 h post dose. Azepine **20** showed 52% lowering of CSF $A\beta_{42}$ while no significant reduction was observed with azepine **21**. In a dose response study, compound **11** showed a dose dependent effect on $A\beta_{42}$ lowering from 3-30 mg/kg oral dose while no significant reduction was observed with 1 mg/kg dose relative to control levels (Figure 6a). Approximated free drug concentration in the brain at 3 h post dose calculated using plasma free fraction showed that the concentrations of **11** are within 2-3 fold relative to its cellular IC_{50} for 30 and 10 mg/kg oral dose (37 nM and 21 nM for 30

and 10 mg/kg dose respectively). But, the free drug concentration of **11** is much lower at 3 mg/kg dose (4 nM).[16]



(a)

Dose (mg/kg)	CSF $A\beta_{42}$ reduction	C_P (μM)	C_B (μM)
30	73	8.88	7.51
10	52	4.83	4.2
3	24	0.91	0.81

(b)

Figure 6. (a) Dose dependent reduction of $A\beta_{42}$ by compound **11** in male CD rats. The value of CSF $A\beta_{42}$ is the geometric mean of 29 (for 3 mg/kg and 10 mg/kg dosing group) and 10 animals (30 mg/kg dosing group) respectively, and the value of C_P and C_B is the geometric mean of 10 (3 mg/kg dosing group), 15 (10 mg/kg dosing group) and 5 (30 mg/kg dosing group) animals. (b) Measured total concentration of **11** in plasma (C_P), and brain (C_B) of rats at doses ranging from 3 mg/kg to 30 mg/kg. All animal procedures were performed according to the protocol reviewed and approved by the institutional Animal Care and Use Committee of Merck & Co., Inc., Kenilworth, NJ, USA.

As shown in Table 3, compound **11** displayed oral bioavailability of 79% with a C_{max} and total AUC of 1.3 μM and 8 μM·h respectively with 2 mg/kg oral dose. Additionally, it exhibited low in vivo clearance and moderate volume of distribution that resulted in a MRT of 1.7 h.

Table 3. Rat pharmacokinetic parameters of compound 11[a]

$AUC_{(0-\infty)}$ (μM·h)[b]	C_{max} (μM)[b]	F%[b]	PPB (% bound)	Cl (mL/min/kg)[c]	$Vd_{,ss}$ (L/kg)[c]	MRT (h)	Pgp-Efflux (rat)[d]
8	1.3	79	99.5	10	1.1	1.7	2.46

[a]Values are average of two experiments. [b]Data from 2 mg/ kg oral dose [c]Data from 1 mg/ kg iv dose. [d]Rat Pgp Effux was measured in LLC-PK1 cells transfected with rat MDR1A and reported as a ratio of (B to A)/(A to B). AUC, area under the plasma drug concentration-time curve; C_{max}, highest concentration; F%, oral bioavailability; PPB, plasma protein binding; Cl, clearance; Vd, volume of distribution; MRT, mean residence time; Pgp, p-glycoprotein.

Conclusions

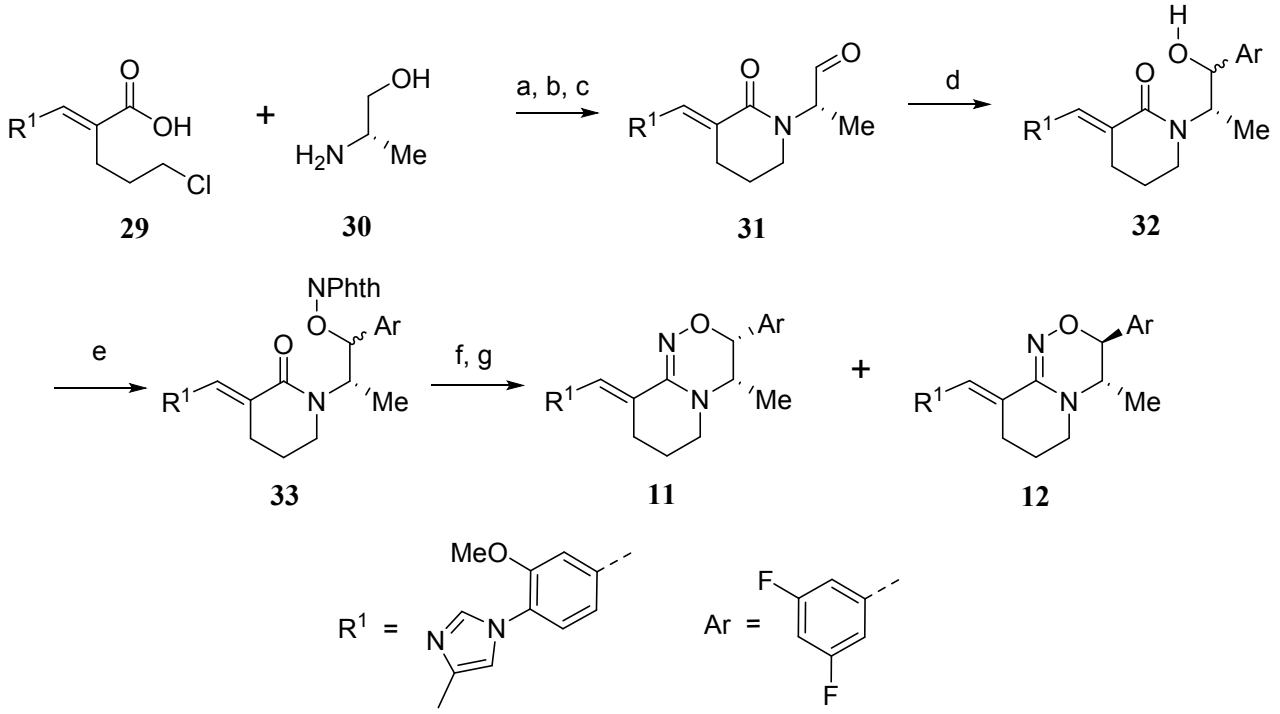
Modulation of γ-secretase to probe the amyloid cascade hypothesis was one of the multipronged approaches that we had undertaken, and our previous work resulted in the identification of two distinct GSM series represented by compound **3** and **4**. To enhance structural diversity and to mitigate concern around the exocyclic double bond present in oxadiazine series, we pursued a number of strategies to identify new leads. Three distinct strategies were inspired by existing chemical matter and executed upon using a "hypothesis driven drug discovery" approach, which is complementary to lead identification via traditional approaches such as uHTS, ALIS and FBLD screens. The conceptualization of the 3,4-disubstituted oxadiazine series was inspired by compound **7** reported in the patent literature. Representative compounds from this series demonstrated robust reduction of CSF $A\beta_{42}$ as exemplified by compounds **11** and **14**. In a further attempt to mask the exocyclic double bonds present in these molecules, analogs **20** and **21** with a β, β- disubstituted endocyclic double bond were designed, synthesized and validated as novel GSMs, and analog **20** exhibited 52% lowering of CSF $A\beta_{42}$ with 30 mg/kg oral dose. Based on the dose responsive study with **11**, we surmised that the free concentration of compounds in the brain is required to be near their IC_{50} for robust lowering of CSF $A\beta_{42}$. Finally,

discovery of **27**, lacking both the exocyclic double bond and the additional aromatic ring led to a novel GSM chemotype. A recent report from the literature confirmed that such molecular motifs embodied the potential for the generation of orally bioavailable molecules that might ultimately translate into novel therapeutics for the treatment of Alzheimer's disease.[7a] Additional investigation of these GSMs in the context of newly developed cryo-electron microscopy structures of human γ-secretase in complex with a Notch fragment and transmembrane APP fragment would provide the opportunity to further optimize these molecules.[17]

Chemistry

Our initial route for the synthesis of **11**, **12** and related analogs commenced with the three-step synthesis of aldehyde **31** from the known intermediate **29** (Scheme 1).[7h] The reaction of **31** with (3,5-difluorophenyl)magnesium bromide led to alcohol **32** as a diastereomeric mixture. Subjection of **32** to three-step sequence, namely Mitsunobu reaction with *N*-hydroxyphthalimide, phthalimide removal and H_3PO_4 mediated dehydrative cyclization resulted in analogs **11** and **12**. A detailed investigation revealed that the addition of (3,5-difluorophenyl)magnesium bromide generated the syn isomer as the major product via chelation control, albeit with low diastereoselectivity (step d). Furthermore, use of the pure diastereomer separated from the mixture of diastereomers represented as **32** in the Mitsunobu reaction with *N*-hydroxyphthalimide resulted in complete retention of stereochemistry at the alcohol bearing center. We surmised that this retention of stereochemistry resulted from double inversion due to the neighboring group participation by the proximal amide moiety.

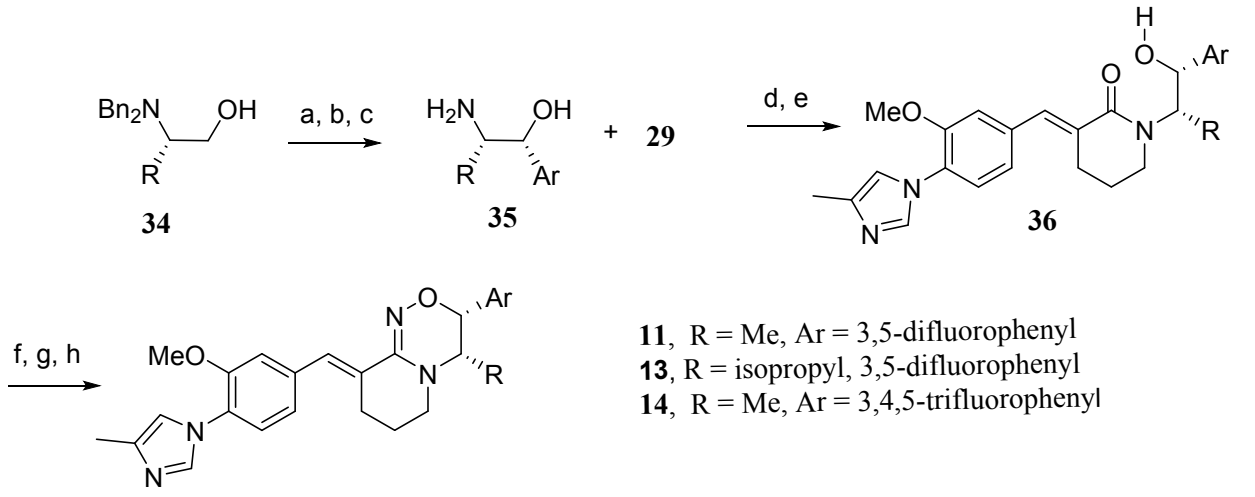
Scheme 1. Initial approach to 3,4- disubstituted oxadiazines 11 and 13-15 based on Mitsunobu reaction.



Reagents and conditions: (a) EDCI, HOBT, DIEA, DMF; (b) NaOMe (25 wt.% in MeOH), THF, 25%; (c) $(COCl)_2$, DMSO, DCM, –78 °C, 100%; (d) 3,5-difluorophenyl magnesium bromide, THF, 48%; (e) *N*-hydroxyphthalimide, n-Bu3P, ADDP, 2MeTHF, 18%; (f) NH_2NH_2, EtOH; (g) H_3PO_4, *n*-BuOH, 100 °C, 29% yield for **11,** and 8% yield for **12**.

Based on these findings, modified synthetic sequences were envisioned to access **11** and close analogs rapidly (Scheme 2). The amino alcohol **35** obtained in three-step from **34** was condensed with acid **29**. Subsequent reaction of the resulting amide with NaOMe led to the cyclized compound **36**. Mitsunobu reaction of **36** with *N*-hydroxyphthalimide followed by hydrazine mediated phthalimide removal and subsequent H_3PO_4 mediated dehydrative cyclization led to the formation of **11** with overall retention in configuration at the alcohol bearing center. Analogs **14** were prepared following this general synthetic sequence.

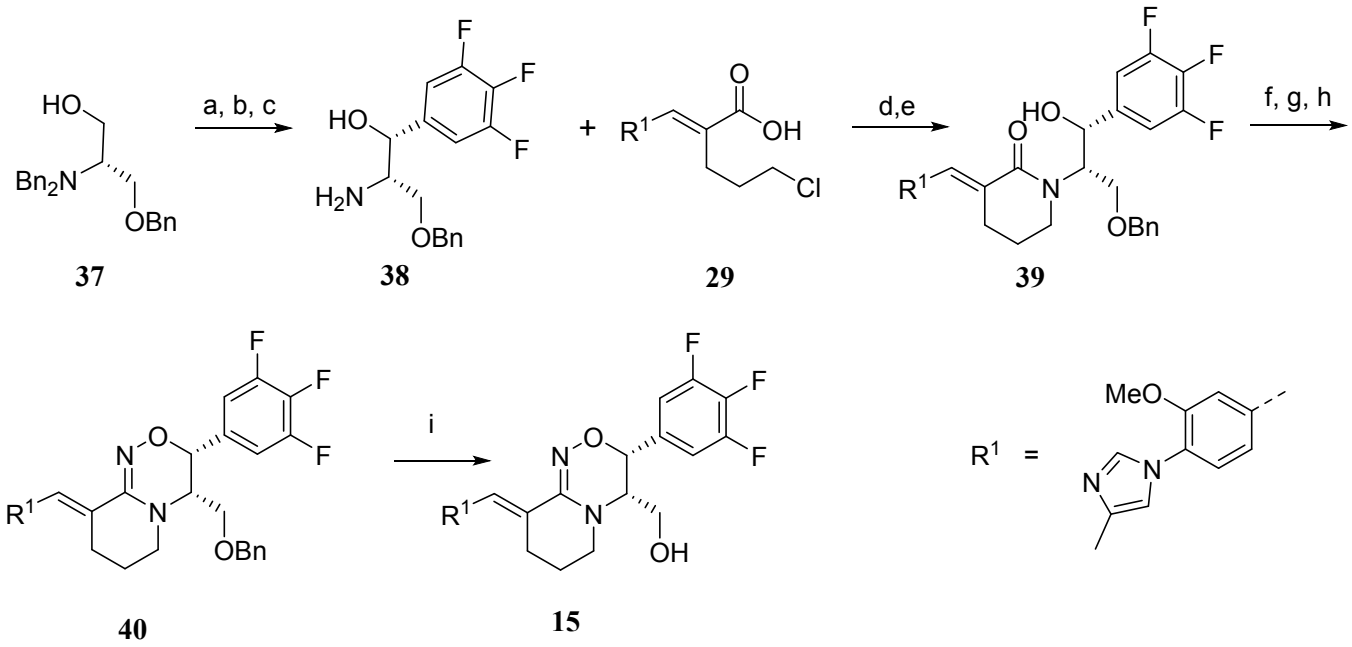
Scheme 2. Stereoselective approach to 3,4-disubstituted oxadiazines based on the observation of retention of configuration Mitsunobu reaction in Scheme 1.



Reagents and conditions: (a) $(COCl)_2$, DMSO, DCM, –78 °C; (b) 3,5-difluorophenyl magnesium bromide, THF, 100%; (c) Pd/C, H_2, MeOH, 84%; (d) EDCI, HOBT, DIEA, DMF; (e) NaOMe (25 wt.% in MeOH), THF, 81%; (f) *N*-hydroxyphthalimide, *n*-Bu_3P, DIAD, THF, 76%; (g) NH_2NH_2, EtOH; (h) H_3PO_4, *n*-BuOH, 100 °C, 51%.

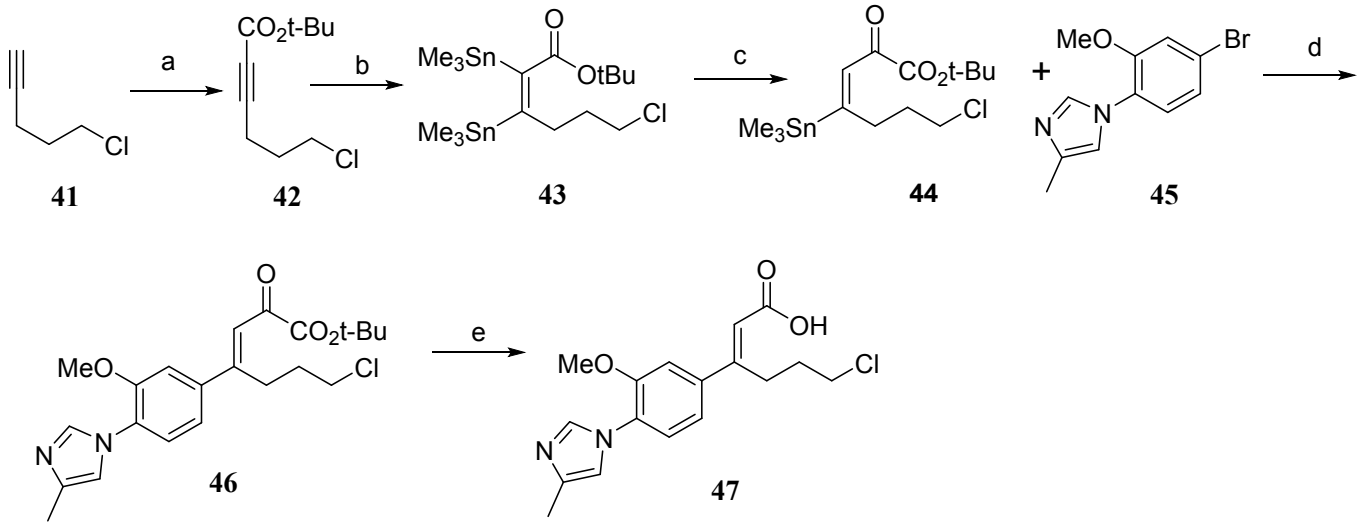
The synthesis of hydroxymethylated analog **15** is described in (Scheme 3). The key intermediate **38** was prepared in three steps from alcohol **37** via Swern oxidation, non-chelation controlled 3,4,5-trifluorophenyl Grignard addition and selective removal of benzyl groups from the amino functionality. Amino alcohol **38** was progressed to compound **40** following the reaction sequence described in Scheme 2. Treatment of **40** with BCl_3 to remove the benzyl group led to **15**.

Scheme 3. Synthesis of analog 15 based on the observation of retention of configuration Mitsunobu reaction.



Reagents and conditions: (a) $(COCl)_2$, DMSO, DCM, –78 °C; (b) 3,5-difluorophenyl magnesium bromide, THF, 94%; (c) Pd/C, H_2, MeOH, 100%; (d) EDCI, HOBT, DIEA, DMF; (e) NaOMe, MeOH, 66%; (f) *N*-hydroxyphthalimide, PPh_3, DIAD, THF, 0 °C; (g) NH_2NH_2, EtOH, 48%; (h) H_3PO_4, *n*-BuOH, 100 °C, 86%; (i) BCl_3, DCM, 0 °C, 23%.

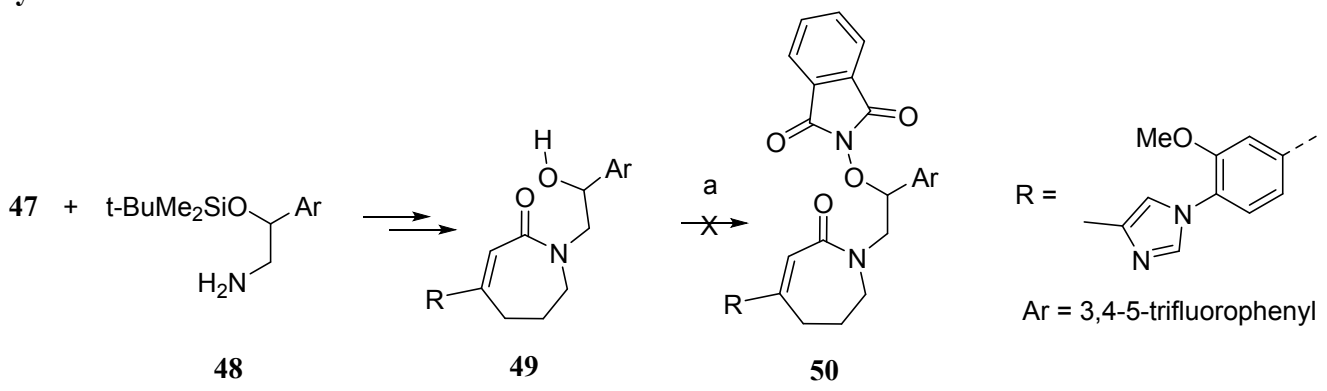
Scheme 4. Synthesis of key intermediate 47 for synthesis of azepine analogs 20 and 21



Reagents and conditions: (a). *n*-BuLi, Et_2O, THF, –30 °C, $(Boc)_2O$, 34%; (b). Me_6Sn_2, $Pd(Ph_3)_4$, 80 °C, THF, 77%; (c). CuCl, DMF, H_2O, rt, 72%; (d). CuCl, $Pd(dppf)Cl_2$, DMF, 100 °C; (e) TFA, DCM, rt, 68%.

The synthesis of acid **47** for the preparation of analogs **20** and **21** was illustrated in (Scheme 4). Alkyne **42** prepared in a single step from **41** was treated with hexamethylditin to provide intermediate **43**. Treatment of **43** with CuCl resulted in selective removal of trimethyltin α to the ester group. Stille coupling between **44** and **45** followed by treatment of the resulting intermediate with TFA provided the key acid **47**, which was used for the synthesis of both core structure **20** and **21**.

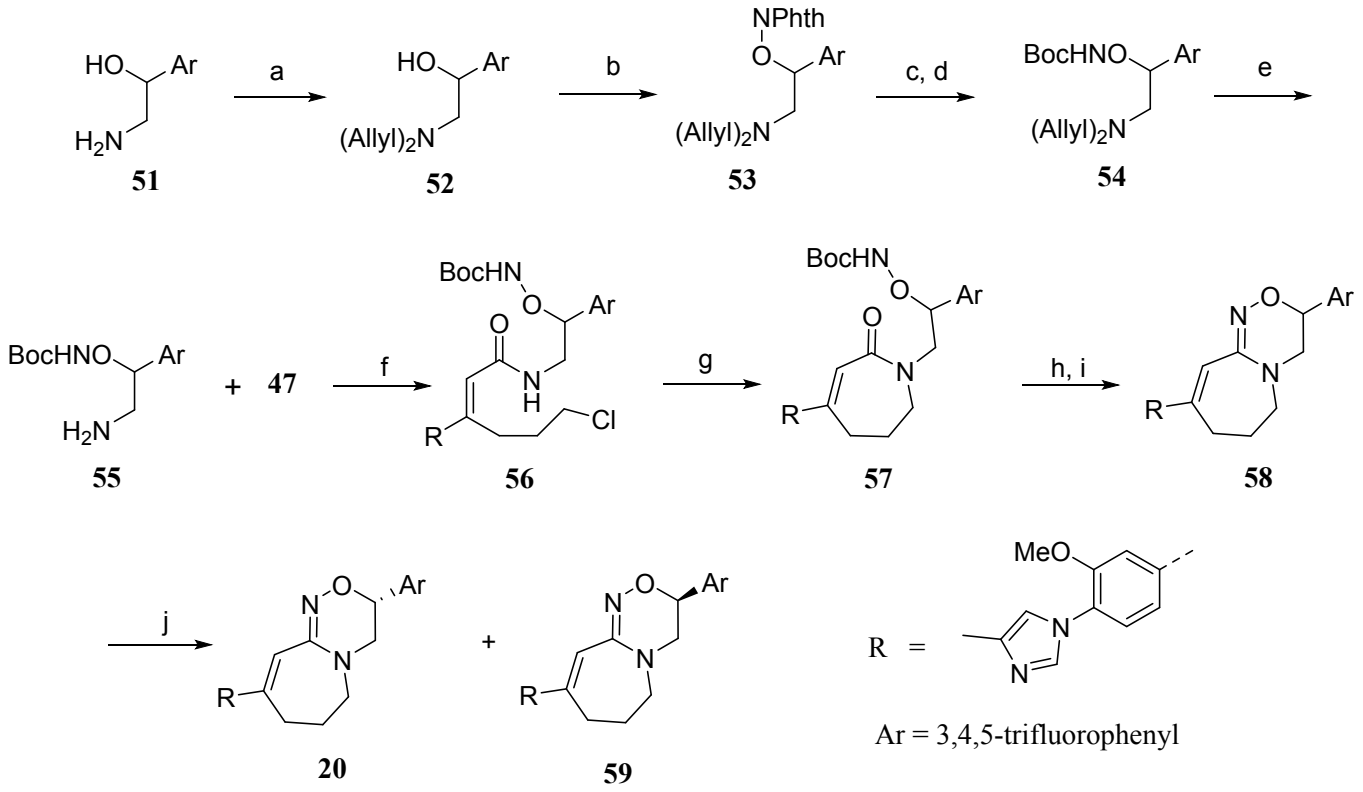
Scheme 5. Failed attempt to transform the hydroxyl functionality to hydroxylamine for the synthesis of 20.



Reagents and conditions: (a) 1. *N*-hydroxyphthalimide, nBu$_3$P, DIAD, THF; 2. *N*-hydroxyphthalimide, nBu$_3$P, ADDP, THF.

With the acid **47** in hand, the initial route for the synthesis of **20** involved the synthetic sequences described in (Scheme 5). And the 7-membered lactam **49** was prepared for the subsequent conversion of its hydroxyl to hydroxyl amine moiety functionality. However, the conversion of **49** to **50** under a variety of conditions was met with failure.

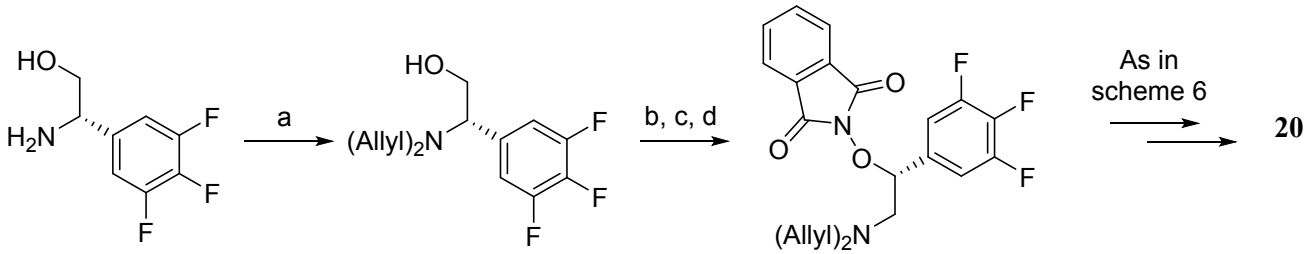
Scheme 6. Synthesis of 20 via development of new synthetic strategy.



Reagents and conditions: (a). Allyl bromide, K_2CO_3, *n*-butyronitrile, 120 °C, 50%; (b) *N*-hydroxyphthalimide, *n*-Bu_3P, DIAD, THF, 0 °C; (c) NH_2NH_2, DCM, EtOH; (d) $(Boc)_2O$, rt, 65%; (e) 1,3-Dimethylbarbituric acid, $Pd(PPh_3)_4$, DCM, rt, 84%; (f) EDCI, HOBt, DIEA, DMF, 0 °C, 85%; (g) KHMDS, THF/DMF, –50 °C, 56%; (h) TFA, DCM, rt; (i) H_3PO_4, 105 °C; (j) Chiralpak AD column, 22% for **20**, 17% for **59**.

The synthetic strategy was revised to preinstall the hydroxylamino moiety into the amino alcohol **51** (c.f. **55**) prior to formation of 7-membered lactam (Scheme 6). The amino moiety of **51** was protected with the bis-allyl group for facile generation of free amine **55** without cleavage of the N-O bond (**54**→**55**). Condensation of amine **55** with acid **47** followed by cyclization using KHMDS led to the 7-membered lactam **57**. Removal of the Boc group followed by treatment of the resulting free hydroxylamine with H_3PO_4 afforded oxadiazine **58**. The two enantiomers of **58** were separated using the AD column with 30% *i*-PrOH in hexanes to afford **20**.

Scheme 7. Unanticipated transformation of 61 to 62 leading to stereocontrolled synthesis of 20.

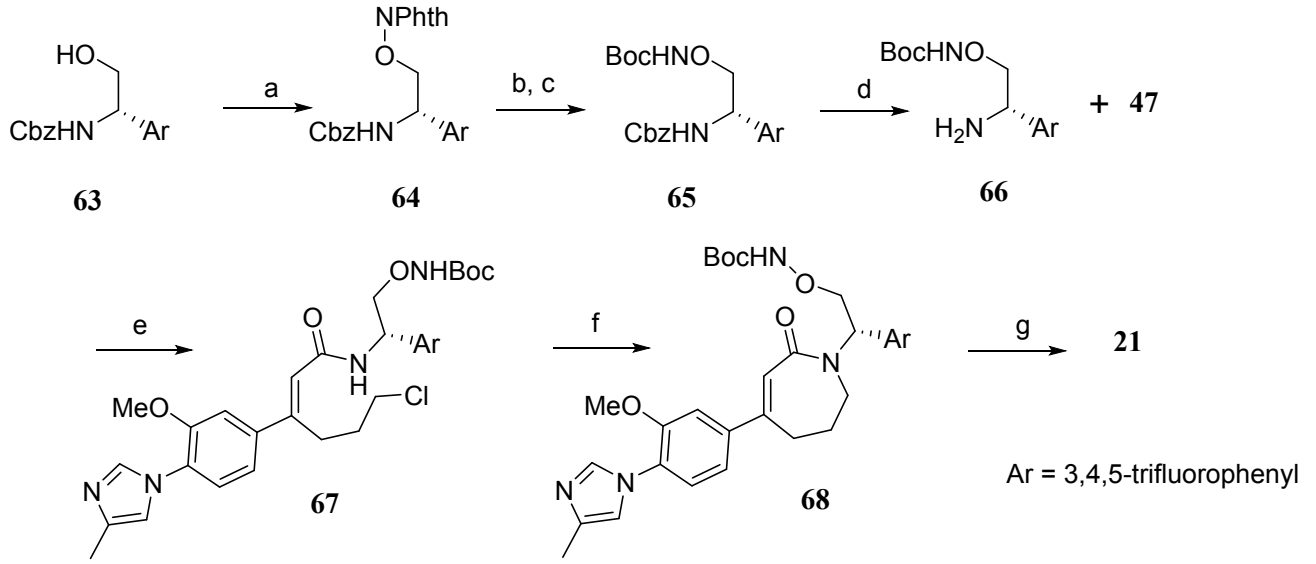


60 **61** **62**

Reagents and conditions: (a). Allyl bromide, K_2CO_3, MeCN, 80 °C, 38%; (b) *N*-hydroxyphthalimide, *n*-Bu_3P, DIAD, THF, 0 °C; (c) NH_2-NH_2, EtOH, DCM, 0 °C; (d) $(BOC)_2O$, 37%.

We recognized that enantiomerically pure amino alcohol **60** may be useful for the synthesis of **21** with the 3,4,5-trifluorophenyl moiety at the 4-position of the oxadiazine moiety (Scheme 8). The route began with the transformation of amino alcohol **60** to the bis-allyl protected amino alcohol **61**. Interestingly, under Mitsunobu reaction conditions, the bis-allyl protected amino alcohol **61** was converted to **62** with apparent migration of the 3,4,5-trifluorophenyl from the nitrogen to the oxygen bearing carbon of the allylated amino alcohol **61**. This transformation occurred presumably via in situ formation of aziridinium ion followed by regio and stereoselective opening of the aziridinium ring by the *N*-hydroxyphthalimide. Although intermediate **62** was not useful for the synthesis of **21**, it could be used for the synthesis of enantiopure **20** following the exact sequences of reactions described in Scheme 6 (details in the supporting information).

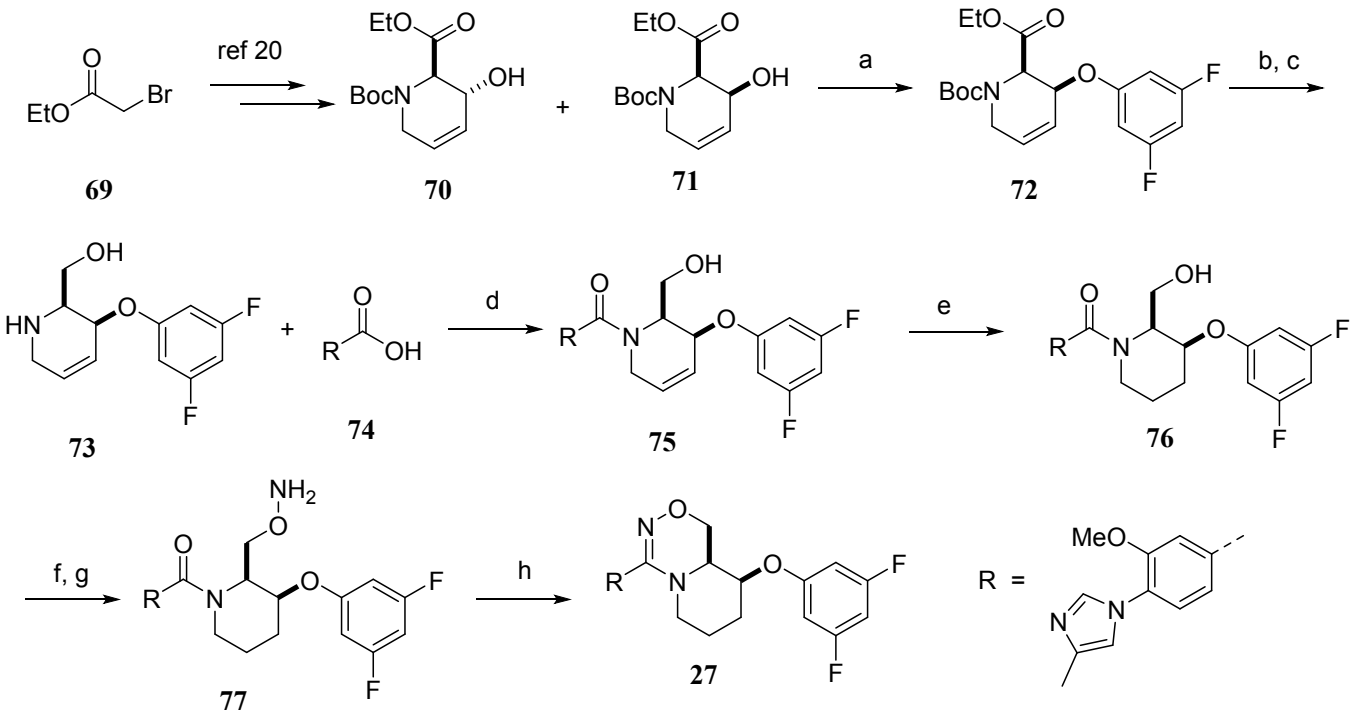
Scheme 8. Stereocontrolled synthesis of 21



Reagents and conditions: (a) *N*-hydroxyphthalimide, Ph_3P, DIAD, THF, –30 °C, 98%; (b) NH_2NH_2, DCM, EtOH, 88%; (c) DCM, $(Boc)_2O$, rt, 80%; (d) Pd/C, H_2, EtOH, rt, 100%; (e) EDCI, HOBt, DIEA, DMF, 0 °C, 65%; (f) KHMDS, DMF, –30 °C, 64%; (g) $POCl_3$, 80 °C, 20%.

For the synthesis of **21**, the amino moiety of amino alcohol **60** was protected with Cbz to attenuate its nucleophilicity, thereby preventing the formation of the aziridinium ion in the following Mitsunobu reaction (Scheme 8). The Cbz protected alcohol **63** underwent Mitsunobu reaction in a desired fashion without rearrangement to generate **64**. Protecting group manipulation followed by removal of Cbz afforded amine **66**, which was progressed to **21** following the similar sequences of reaction as described in Scheme 6.

Scheme 9. Racemic synthesis of 27.



Reagents and conditions: (a) 3,5-Difluorophenol, DEAD, PPh_3, THF, 0 °C; (b) $LiAlH_4$, , THF, 42%; (c) 4N HCl in dioxane, rt; (d) EDCI, HOBt, DIEA, DMF, 50%; (e) Pd/C, H_2, MeOH, 57%; (f) $MeSO_2Cl$, Et_3N, *N*-hydroxyl phthalimide, DCM, 43%; (g) NH_2NH_2, EtOH, DCM, 63%; (h) $POCl_3$, 90 °C, 22%.

Synthesis of **27** began with the ethyl bromoacetate **69**, which was progressed to racemic **70** as the major diastereomer following the literature procedure (Scheme 9).[18] Mitsunobu reaction of **70** with 3,5-difluorophenol resulted in **72** via inversion of stereochemistry at the carbon bearing alcohol. Reduction of ester functionality in **72** to the corresponding alcohol followed by removal of the Boc group provided **73**. Condensation of **73** with acid **74** followed by hydrogenation led to **76**. Subjection of intermediate **76** to Mitsunobu reaction conditions with *N*-hydroxyphthalimide followed by hydrazine mediated phthalimide removal generated **77**, which on treatment with $POCl_3$ resulted in **27**.

EXPERIMENTAL SECTION

General. Measurement of $A\beta_{42}$ IC_{50}, $A\beta_{total}$ IC_{50} and BACE1 K_i and determination of rat CSF $A\beta_{42}$ lowering *in vivo* were carried out following our previously described protocols.[7g]

Unless otherwise mentioned, all reagents and solvents purchased from commercial source were used directly without further purification. Air sensitive reactions were performed under an atmosphere of N_2. Purification of all the final compounds to >95% purity was carried out either by prepacked silica gel cartridge (Analogix, Biotage, or ISCO) or on a reverse phase C18 column. Water and acetonitrile used as mobile phase A and B respectively for the C18 column were supplemented either with 0.05% TFA or 0.1% formic acid. All the NMR data were collected either at 400 or 600 MHz on a Varian or Bruker instrument. Chemical shifts are reported in ppm relative to the residual solvent peak in the indicated solvent, and for ^1H NMR, multiplicities, coupling constants in Hertz, and numbers of protons are indicated parenthetically. Purity and MS information was obtained via LC-electrospray-mass spectroscopy with a C18 column using a gradient of 5% to 95% MeCN in water supplemented with 0.05% TFA or 0.1% formic acid as the mobile phase. The purity of the samples was assessed using a UV detector at 254 nm.

(3R,4S)-3-(3,5-Difluorophenyl)-9-((E)-3-methoxy-4-(4-methyl-1H-imidazol-1-yl)benzylidene)-4-methyl-3,4,6,7,8,9-hexahydropyrido[2,1-c][1,2,4]oxadiazine (11) and (3S,4S)-3-(3,5-difluorophenyl)-9-((E)-3-Methoxy-4-(4-methyl-1H-imidazol-1-yl)benzylidene)-4-methyl-3,4,6,7,8,9-hexahydropyrido[2,1-c][1,2,4]oxadiazine (12)

To a solution of TFA salt of **29** (5 g, 11 mmol), HOBt (2.04 g, 13.3 mmol) and **30** (1 g, 13.3 mmol) in DMF (30 mL) was added EDCI (2.56 g, 13.3 mmol) followed by DIEA (4.28 mL, 24.5 mmol) at 0°C. The resulting mixture was slowly warmed to rt, and stirred for 2 h. Then the reaction mixture was diluted with EtOAc, transferred to a separatory funnel, washed with saturated aqueous solution of

$NaHCO_3$ and then with brine. The organic layer was dried with $MgSO_4$, filtered, concentrated, and the crude product was taken to the next step without further purification. To this crude product in THF (30 mL) was added NaOMe (25 wt. % in MeOH, 3.61 g, 16.71 mmol) at 0 °C. The resulting mixture was slowly warmed to rt, and stirred for 14 h. Then the reaction mixture was diluted with EtOAc, neutralized with 1N HCl and washed with brine. The organic layer was dried with $MgSO_4$, filtered, evaporated, and the crude product was purified by silica gel chromatography using 0–100% EtOAc in hexanes to afford alcohol precursor to aldehyde **31** (1 g, 25 % yield).

To a solution of oxalyl chloride (0.49 mL, 5.63 mmol) in DCM (4 mL) was added a solution of DMSO (0.79 mL, 11.3 mmol) in DCM (2 mL) at −78 °C. The reaction mixture was stirred at –78 °C for 10 min, then to this mixture was added a solution of alcohol precursor to aldehyde **31** (1 g, 2.8 mmol) in DCM (4 mL) and stirred at –78 °C for 30 min. Then was added Et_3N (1.96 mL, 14 mmol), and the resulting solution was slowly warmed to rt over 1 h. The reaction mixture was diluted with EtOAc, transferred to a separatory funnel, washed first with saturated aqueous solution of $NaHCO_3$ and then with brine. The organic layer was dried with $MgSO_4$, filtered, evaporated, and the crude aldehyde **31** was taken directly to the next step without further purification.

To a solution of this crude **31** (0.99 g, 2.8 mmol) in THF (10 mL) was added a solution of (3,5-difluorophenyl)magnesium bromide (2.81 mL, 5.6 mmol, 2M in diethyl ether) at 0 °C, and the resulting mixture was slowly warmed to rt and stirred for 2 h. Then the reaction mixture was diluted with EtOAc, transferred to a separatory funnel, neutralized with 1N HCl and washed with brine. The organic layer was separated, dried with $MgSO_4$, filtered, evaporated and the crude product was purified by silica gel chromatography using 0–10 % methanol in DCM to provide **32** as the mixture of diastereomers (0. 63 g, 48 % yield).

To a solution of **32** (0.17 g, 0.36 mmol) in 2-MeTHF (5 mL) was added *N*-hydroxyphthalimide (0.10 g, 0.73 mmol), ADDP (0.12 g, 0.47 mmol) and *n*-Bu_3P (0.11 mL, 0.47 mmol). The resulting mixture was degassed and heated at 80 °C for 18 h. The reaction mixture was diluted with EtOAc, transferred to a separatory funnel, washed with saturated aqueous solution of $NaHCO_3$ and then with brine. The organic layer was dried with $MgSO_4$, filtered, concentrated, and the crude product was purified by silica gel chromatography using 1% Et_3N in EtOAc to provide corresponding Mitsunobu adduct **33** (0.04 g, 18 % yield).

To a solution of **33** (0.04 g, 0.06 mmol) in EtOH (0.2 mL) was added hydrazine (0.07 mL, 2.23 mmol) at 0 °C, and the resulting solution was slowly warmed to rt and stirred for 30 min. The reaction mixture was diluted with EtOAc, transferred to a separatory funnel, washed with saturated aqueous solution of $NaHCO_3$ and brine, and the crude product was taken to the next step without further purification.

To a solution of this crude product (0.03 g, 0.06 mmol) in butan-1-ol was added H_3PO_4 (0.01 mL, 0.17 mmol) and heated at 100 °C for 12 h. The reaction mixture was concentrated, and the crude reaction mixture was purified by reverse phase HPLC to provide **11** (0.01 g, 29 % yield) and **12** (0.002 g, 7.83 % yield). ^{1}H NMR and *m/z* of compound **11** (400 MHz, CD_3OD) δ 9.19 (d, *J* = 1.6 Hz, 1H), 7.67 – 7.58 (m, 2H), 7.47 (s, 1H), 7.36 (d, *J* = 1.5 Hz, 1H), 7.27 (dd, *J* = 8.2, 1.2 Hz, 1H), 7.10 – 6.98 (m, 3H), 5.34 (d, *J* = 2.2 Hz, 1H), 4.13 (qd, *J* = 6.6, 2.4 Hz, 1H), 3.97 (s, 3H), 3.78 (m, 1H), 3.58 (m, 1H), 3.04 – 2.79 (m, 2H), 2.44 (d, *J* = 1.1 Hz, 3H), 2.21 – 1.95 (m, 2H), 1.15 (d, *J* = 6.6 Hz, 3H). *m/z*: 465. ^{1}H NMR and *m/z* of compound **12** (400 MHz, CD_3OD) δ 9.16 (d, *J* = 1.6 Hz, 1H), 7.63 – 7.51 (m, 2H), 7.35 – 7.26 (m, 2H), 7.22 – 7.12 (m, 3H), 7.04 (tt, *J* = 8.9, 2.2 Hz, 1H), 5.16 (d, *J* = 3.6 Hz, 1H), 4.16 (td, *J* = 6.5, 3.7 Hz, 1H), 3.93 (s, 3H), 3.68 (m, 1H), 3.46 (m 1H), 2.79 (m, 2H), 2.42 (d, *J* = 1.1 Hz, 3H), 2.07 – 1.78 (m, 2H), 1.47 (d, *J* = 6.5 Hz, 3H). *m/z*: 465.

(3R,4S)-3-(3,5-Difluorophenyl)-9-((E)-3-methoxy-4-(4-methyl-1H-imidazol-1-yl)benzylidene)-4-methyl-3,4,6,7,8,9-hexahydropyrido[2,1-c][1,2,4]oxadiazine (11) according to the synthetic route described in Scheme 2.

To a solution of oxalyl chloride (2 mL, 23.5 mmol) in DCM (100 mL) was added a solution of DMSO (2.78 mL, 39.2 mmol) in DCM (10 mL) at –78 °C and stirred for 10 min. Then was added a solution of **34** (5 g, 19.5 mmol) in DCM (10 mL) and stirred at –78 °C for 0.5 h before the addition of Et_3N (10.9 mL, 78 mmol). The reaction mixture was slowly warmed to 0 °C, diluted with EtOAc, transferred to a separatory funnel, washed with saturated aqueous solution of $NaHCO_3$ and then with brine. The organic layer was dried with $MgSO_4$, filtered, concentrated, and the crude aldehyde was taken to the next step without further purification.

To a solution of (3,5-difluorophenyl)magnesium bromide (100 mL, 50 mmol, 0.5M in diethyl ether) was added a solution of the crude aldehyde from the previous step (4.81 g, 19 mmol) in THF (30 mL) at –78 °C, and the reaction mixture was slowly warmed to rt and stirred for 30 min. The reaction mixture was diluted with EtOAc, transferred to a separatory funnel, washed with saturated aqueous solution of $NaHCO_3$ and then with brine. The organic layer was dried with $MgSO_4$, filtered, concentrated, and the crude product was purified by silica gel chromatography using 0–10% EtOAc in hexanes to afford corresponding alcohol (6.98 g, 100% yield).

To a solution of this alcohol (6.98 g, 19 mmol) in MeOH (100 mL) was added 10% Pd/C (2 g, 1.8 mmol), and the resulting solution was degassed and stirred under the atmosphere of H_2 for 12 h. The reaction mixture was filtered, and the filtrate was evaporated to dryness to afford **33** (3 g, 84 % yield) which was taken to the next step without further purification.

To a mixture of **35** (3 g, 16 mmol), TFA salt of **29** (7.19 g, 16 mmol) and HOBt (2.95 g, 19.2 mmol) in DMF (32 mL) was added EDCI (3.69 g, 19.2 mmol) followed by DIEA (6.16 mL, 35.3 mmol) at 0 °C. The resulting mixture was slowly warmed to rt and stirred for 2 h. The reaction mixture was

diluted with EtOAc, transferred to a separatory funnel, washed with saturated aqueous solution of $NaHCO_3$ and then with brine. The organic layer was dried with $MgSO_4$, filtered, concentrated, and the crude product was taken to the next step without further purification.

To a solution of this crude product (6.78 g, 16 mmol) in THF (50 mL) was added sodium methoxide (5.19 g, 24 mmol) at rt and stirred for 12 h. The reaction mixture was diluted water, neutralized with 1N aqueous solution of HCl, transferred to a separatory funnel, extracted with EtOAc, washed with saturated aqueous solution of $NaHCO_3$ and then with brine. The organic layer was dried with $MgSO_4$, filtered, concentrated, and the crude product was purified by silica gel chromatography using 0–100 % methanol/7N NH_3 in DCM to **36** (5 g, 81 % yield). To a solution of **36** (5 g, 10.7 mmol), *N*-hydroxyphthalimide (2.09 g, 12.8 mmol), *n*-Bu_3P (3.17 mL, 12.8 mmol) in THF (60 mL) was added DIAD (2.49 mL, 12.8 mmol) at 0 °C, and the resulting mixture was slowly warmed to rt and stirred for 2 h. The reaction mixture was diluted with EtOAc, transferred to a separatory funnel, washed with saturated aqueous solution of $NaHCO_3$ and then with brine. The organic layer was dried with $MgSO_4$, filtered, concentrated, and the crude product was purified by silica gel chromatography using 0–100% EtOAc in hexanes to afford corresponding Mitsunobu adduct (5 g, 76% yield).

To a solution of this Mitsunobu adduct (5 g, 8.2 mmol) in EtOH (24 mL) was added hydrazine (1.04 mL, 33.3 mmol) at 0 °C and stirred for 30 min. Then the reaction mixture was diluted with EtOAc, transferred to a separatory funnel, washed with saturated aqueous solution of $NaHCO_3$ and then with brine. The organic layer was dried with $MgSO_4$, filtered, concentrated, and the crude hydroxyl amine (3 g, 76%) was taken to the next step for cyclization.

To a solution of this hydroxyl amine (1 g, 2 mmol) in *n*-BuOH (40 mL) was added H_3PO_4 (1 mL, 17.1 mmol), and the resulting mixture was heated at 100 °C for 7 h. Then the reaction mixture was cooled to rt, diluted with EtOAc, washed with saturated aqueous solution of $NaHCO_3$ and then with

brine. The organic layer was dried with $MgSO_4$, filtered, concentrated, and purified by silica gel chromatography using 2% TEA in EtOAc to afford **11** (0.49 g, 51 % yield).

(3R,4S)-3-(3,5-Difluorophenyl)-4-isopropyl-9-((E)-3-methoxy-4-(4-methyl-1H-imidazol-1-yl)benzylidene)-3,4,6,7,8,9-hexahydropyrido[2,1-c][1,2,4]oxadiazine (13)

Compound **13** was prepared following the synthetic sequences described in Scheme 1 for the synthesis of compound **11** using (S)-2-amino-3-methylbutan-1-ol. ^{1}H NMR (400 MHz, CD_3OD) δ 9.19 (d, *J* = 1.6 Hz, 1H), 7.67 – 7.57 (m, 2H), 7.46 – 7.41 (m, 1H), 7.39 (d, *J* = 1.6 Hz, 1H), 7.33 – 7.27 (m, 1H), 7.14 – 7.07 (m, 2H), 7.07 – 6.98 (m, 1H), 5.29 – 5.23 (m, 1H), 4.01 – 3.98 (m, 1H), 3.97 (s, 3H), 3.73 – 3.59 (m, 2H), 3.02 – 2.92 (m, 2H), 2.44 (d, *J* = 1.1 Hz, 3H), 2.20 – 2.01 (m, 2H), 1.96 – 1.83 (m, 1H), 1.03 – 0.86 (m, 6H). *m/z*: 493.

(3R,4S)-9-((E)-3-Methoxy-4-(4-methyl-1H-imidazol-1-yl)benzylidene)-4-methyl-3-(3,4,5-trifluorophenyl)-3,4,6,7,8,9-hexahydropyrido[2,1-c][1,2,4]oxadiazine (14)

Compound **14** was prepared following the synthetic sequences described in scheme 2 for the preparation of compound **11**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.71 (d, *J* = 1.3 Hz, 1H), 7.50 (m, 1H), 7.22 (d, *J* = 7.9 Hz, 1H), 7.07 – 6.88 (m, 5H), 4.85 – 4.75 (m, 1H), 3.83 (s, 3H), 3.53 – 3.44 (m, 1H), 3.40 (m, 1H), 3.14 (m, 1H), 2.81 (m, 1H), 2.74 – 2.60 (m, 1H), 2.29 (d, *J* = 1.0 Hz, 3H), 1.91 (m, 2H), 0.99 (d, *J* = 6.4 Hz, 3H). *m/z*: 483.

((3R,4S)-9-((E)-3-Methoxy-4-(4-methyl-1H-imidazol-1-yl)benzylidene)-3-(3,4,5-trifluorophenyl)-3,4,6,7,8,9-hexahydropyrido[2,1-c][1,2,4]oxadiazin-4-yl)methanol (15)

Compound **15** was prepared following the synthetic sequences described in scheme 3. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.74 (s, 1H), 7.47 (s, 1H), 7.21 (d, *J* = 7.7 Hz, 1H), 7.12 – 7.02 (m, 2H), 6.99 – 6.86 (m, 4H), 4.81 (s, 1H), 3.82 (s, 3H), 3.73 – 3.51 (m, 3H), 3.27 (m, 1H), 2.79 (m, 2H), 2.29 (s, 3H), 1.93 (m, 2H). *m/z*: 499.

(E)-6-Chloro-3-(3-methoxy-4-(4-methyl-1H-imidazol-1-yl)phenyl)hex-2-enoic acid (47)

To a solution of **41** (25 g, 244 mmol) in Et_2O (400 mL) was added *n*-BuLi (117 mL, 293 mmol) at –30 °C and stirred for 30 min. Then was added a solution of $(Boc)_2O$ (67.9 mL, 293 mmol) in Et_2O (50 mL) over 30 min. The reaction mixture was slowly warmed to rt. The resulting mixture was diluted with water, neutralized with 1N aqueous solution of HCl and washed with brine. The organic layer was dried with $MgSO_4$, filtered, concentrated, and the crude product was purified by silica gel chromatography using 5% EtOAc in hexanes to afford **42** (17 g, 34 % yield).

To a solution of **42** (5 g, 24.67 mmol) in THF (30 mL) was added Me_6Sn_2 (6.33 mL, 30.5 mmol) and $Pd(Ph_3P)_4$ (2.85 g, 2.5 mmol). The resulting mixture was degassed and back filled N_2 (3x) and heated at 80 °C for 12 h. Then the reaction mixture was evaporated to dryness, and the crude mixture was purified by silica gel chromatography using 5% EtOAc in hexane to provide **43** (9.57 g, 18 mmol, 77 % yield).

To a solution of **43** (8 g, 15.1 mmol) in a mixture of DMF (60 mL) and water (6 mL) was added cuprous chloride (0.015 g, 0.15 mmol), and stirred at rt under the atmosphere of N_2 for 2 h. Then was added a mixture of NH_4Cl/NH_4OH (20 mL, pH 8), and stirred for additional 1 h while exposing the reaction mixture to the air. The resulting mixture was extracted with Et_2O, dried with $MgSO_4$, filtered, concentrated, and the crude product was purified by silica gel chromatography using 2% EtOAc in hexanes to provide **44** (4 g, 10.9 mmol, 72 % yield).

To solution of **44** (4 g, 10.9 mmol) in DMF (60 mL) was added cuprous chloride (0.323 g, 3.3 mmol), $Pd(dppf)Cl_2$ (0.398 g, 0.54 mmol) and **45** (3.4 g, 12.7 mmol), and the resulting solution was degassed and back filled with N_2 (3x) and heated at 100 °C for 1 h. The reaction mixture was then cooled to rt, diluted with EtOAc, washed with water and then with brine. The organic layer was dried with $MgSO_4$, evaporated and was purified by silica gel chromatography using 0–100% EtOAc in hexanes

to provide **46**, which on treatment with CF_3CO_2H acid in DCM (5 mL, 50% CF_3CO_2H in DCM) afforded **47** (2.5 g, 7.5 mmol, 68 % yield).

(R)-9-(3-Methoxy-4-(4-methyl-1H-imidazol-1-yl)phenyl)-3-(3,4,5-trifluorophenyl)-3,4,7,8-tetrahydro-6H-[1,2,4]oxadiazino[4,3-a]azepine (20)

To a solution of **51** (5 g, 26.2 mmol) in *n*-butyronitrile (130 mL) was added K_2CO_3 (10.85 g, 78 mmol) and allyl bromide (4.7 mL, 54.3 mmol) and heated at 120 °C for 2 h. Then the reaction mixture was filtered, concentrated, and the crude product was purified by silica gel chromatography using 0–10% EtOAc in hexanes to provide **52** (3 g, 50% yield).

To a mixture of **52** (3 g, 11 mmol), *n*-Bu_3P (3.27 mL, 13.3 mmol) and *N*-hydroxyphthalimide (2.16 g, 13.3 mmol) in THF (55 mL) was added DIAD (2.58 mL, 13.3 mmol) at 0 °C. The resulting mixture was slowly warmed to rt and stirred for 2 h. Then the reaction mixture was diluted with EtOAc, washed with saturated aqueous solution of $NaHCO_3$ followed by brine. The organic layer was dried with $MgSO_4$, concentrated, filtered, and the crude product was purified by silica gel chromatography using 15% EtOAc in hexanes to afford corresponding Mitsunobu product, which was dissolved in a mixture of DCM (50 mL) and ethanol (100 mL) and added hydrazine hydrate (1.1 g, 22 mmol) at 0 °C. The reaction mixture was warmed to rt and stirred for 30 min. The resulting mixture was filtered and concentrated to afford corresponding hydroxyl amine. To this hydroxyl amine was added neat $(Boc)_2O$ (2.3 g, 11 mmol) and stirred for 12 h. Then the reaction mixture was concentrated, and the crude product was purified by silica gel chromatography using 0–100% EtOAc in hexanes to afford **54** (2.96 g, 65% yield).

To a solution of **54** (2.6 g, 6.7 mmol) in DCM (33 mL) was added 1,3-dimethylpyrimidine-2,4,6(1H,3H,5H)-trione (6 g, 40 mmol), $Pd(PPh_3)_4$ (0.73 g, 0.67 mmol), and the resulting mixture was degassed and back filled with N_2 (3x) and stirred under the atmosphere of N_2 for 12 h. Then the reaction mixture was washed with saturated aqueous solution of $NaHCO_3$ followed by with brine. The DCM

layer was dried with $MgSO_4$, concentrated, filtered and the crude product was purified by silica gel chromatography using 5% MeOH/7N NH_3 in DCM to afford **55** (1.6 g, 84% yield).

To a solution of ester **46** (2.8 g, 7.2 mmol) in DCM (10 mL) was added CF_3CO_2H (10 mL) at rt and stirred for 2 h. The reaction mixture was evaporated to dryness, and the resulting acid was taken to the next step without further purification. To this acid in DMF (32 mL) was added amine **55** (2 g, 6.5 mmol), HOBt (1.07 g, 7.8 mmol), DIEA (4.5 mL, 26.1 mmol) followed by EDCI (1.5 g, 8.83 mmol) at 0 °C. The resulting mixture was slowly warmed to rt and stirred for 12 h. The reaction mixture was diluted with EtOAc, washed with saturated aqueous solution of $NaHCO_3$ and then with brine. The organic layer was dried with $MgSO_4$, filtered, concentrated, and the crude product was purified by silica gel chromatography using 0–10% MeOH in DCM to afford **56** (3.78 g, 85% yield).

To a solution of **56** (3.78 g, 6.1 mmol) in DMF (35 mL) was added a solution of KHMDS (15 mL, 0.9M in THF, 13.36 mmol) at –50 °C. The resulting mixture was slowly warmed to rt and stirred for 2 h. Then the reaction mixture was diluted with water, carefully neutralized with 1N aqueous solution of HCl, and extracted with EtOAc. The organic layer was dried with $MgSO_4$, filtered, concentrated, and the crude product was purified by silica gel chromatography using 3% MeOH/7N NH_3 in DCM to afford **57** (2 g, 56% yield).

To a solution of this Boc protected intermediate **57** (1.1 g, 1.9 mmol) in DCM (2 mL) was added CF_3CO_2H (2 mL) and stirred at rt for 10 min. The reaction mixture was diluted with EtOAc, carefully neutralized with saturated aqueous solution of $NaHCO_3$. The EtOAc layer was separated, washed with brine, dried with $MgSO_4$, filtered, concentrated and the crude product was purified by silica gel chromatography using 4% MeOH/7N NH_3 in DCM to afford the free amine, which was dissolved in H_3PO_4 (2 mL) and heated at 105 °C for 2 h. The reaction mixture was diluted with EtOAc, carefully neutralized with saturated aqueous solution of $NaHCO_3$. The EtOAc layer was separated, washed with

brine, dried with $MgSO_4$, filtered, concentrated and the crude product was purified by silica gel chromatography using 5% MeOH in DCM to afford the racemic **58**. The enantiomers were separated using chiral HPLC (Chiralpak AD column, 20 μm, 5 cm x 50 cm, 40 mL/min, 70% hexane/isopropanol) to afford desired enantiomer **20** (0.194 g, fast eluting, t_R = 15 min, 22% yield) and enantiomer **59** (0.15 g, slow eluting, t_R = 26.1 min, 17% yield). ^{1}H NMR (400 MHz, CD_3OD) δ 9.17 (d, J = 1.6 Hz, 1H), 7.66 – 7.58 (m, 2H), 7.47 – 7.38 (m, 1H), 7.40 – 7.26 (m, 3H), 6.33 (s, 1H), 5.21 – 5.06 (m, 1H), 4.07 – 3.87 (m, 5H), 3.82 – 3.66 (m, 2H), 3.09 – 2.91 (m, 2H), 2.52 – 2.38 (m, 4H), 2.30 (m, 1H). *m/z*: 469.

(S)-9-(3-Methoxy-4-(4-methyl-1H-imidazol-1-yl)phenyl)-4-(3,4,5-trifluorophenyl)-3,4,7,8-tetrahydro-6H-[1,2,4]oxadiazino[4,3-a]azepine (21)

To a mixture of **63** (5 g, 15.4 mmol), Ph_3P (4.7 g, 17.9 mmol) and *N*-hydroxyphthalimide (3 g, 18.4 mmol) in THF (75 mL) was added DIAD (3.59 mL, 18.5 mmol) at –30 °C, and the resulting mixture was slowly warmed to rt and stirred for 12 h. The reaction mixture was diluted with EtOAc, transferred to a separatory funnel, washed with saturated aqueous solution of $NaHCO_3$ and then with brine. The organic layer was dried with $MgSO_4$, filtered, concentrated, and the crude product was purified by silica gel chromatography using 0–25% EtOAc in hexanes to provide **64** (7.06 g, 98 % yield).

To a solution of **64** (7.06 g, 15 mmol) in the mixture of DCM (100 mL) and ethanol (200 mL) was added hydrazine hydrate (2.25 g, 45 mmol) at 0 °C, and the reaction mixture was warmed to rt and stirred for 30 min. The resulting mixture was filtered, and the filtrate was concentrated to afford crude product, which was purified by silica gel chromatography using 0–60% EtOAc in hexanes to provide the corresponding hydroxyl amine (4.5 g, 88 % yield).

To a solution of this hydroxylamine (4.5 g, 13.2 mmol) in DCM (20 mL) was added $(Boc)_2O$ (6.91 mL, 29.8 mmol) and stirred for 12 h. Then the reaction mixture was concentrated, and the crude

product was purified by silica gel chromatography using 0–30% EtOAc in hexanes to afford **65** (4.54 g, 80% yield).

To a solution of **65** (2 g, 4.5 mmol) in EtOH (20 mL) was added 10 % Pd/C (0.50 g, 0.47 mmol), and the resulting mixture was degassed and back filled with H_2 (3x) and stirred under the atmosphere of H_2 for 1 h. The reaction mixture was filtered, concentrated, and the crude product was purified by silica gel chromatography using 0–100% EtOAc in hexanes to afford **66** (1.5 g, ~100 % yield).

To a mixture of **47** (1.64 g, 4.9 mmol), **66** (1.5 g, 4.9 mmol) and HOBt (0.79 g, 5.2 mmol) in DMF (30 mL) was added DIEA (4 mL, 22.9 mmol) followed by EDCI (1.12 g, 5.9 mmol) at 0 °C. The resulting mixture was slowly warmed to rt and stirred for 12 h. The reaction mixture was diluted with EtOAc, washed with saturated aqueous solution of $NaHCO_3$ and then with brine. The organic layer was dried with $MgSO_4$, filtered, concentrated, and the crude product was purified using 0–5% MeOH in DCM to afford **67** (2 g, 65 % yield).

To a solution of **67** (1 g, 1.6 mmol) in DMF (16 mL) was added a solution of KHMDS (3.50 mL, 3.5 mmol, 1M in THF) at –30 °C. The resulting mixture was slowly warmed to rt and stirred for 2 h. Then the reaction mixture was diluted with water, neutralized with 1N HCl, and extracted with EtOAc. The organic layer was dried with $MgSO_4$, filtered, concentrated to afford **68** (0.60 g, 63.7 % yield), which was taken directly to the next step without further purification.

A mixture of **68** (0.44 g, 0.75 mmol) and $POCl_3$ (8 mL) was heated at 80 °C for 45 min. The resulting mixture was evaporated to dryness, and the crude product was purified by reverse phase HPLC to afford **21** (0.07 g, 20%). ^{1}H NMR (400 MHz, CD_3OD) δ 9.16 (d, J = 1.4 Hz, 1H), 7.61 – 7.54 (m, 2H), 7.40 (s, 1H), 7.31 (dd, J = 8.3, 1.8 Hz, 1H), 7.20 (m, 2H), 6.30 (d, J = 2.5 Hz, 1H), 4.82 (m, 1H), 4.26 – 4.13 (m, 1H), 4.14 – 4.02 (m, 1H), 3.98 (s, 3H), 3.57 – 3.35 (m, 2H), 2.90 (m 2H), 2.43 (d, J = 1.0 Hz, 3H), 2.23 (m, 1H), 2.01 (m, 1H).*m/z*: 469.

Racemic 9-(3,5-difluorophenoxy)-4-(3-methoxy-4-(4-methyl-1H-imidazol-1-yl)phenyl)-1,6,7,8,9,9a-hexahydropyrido[1,2-d][1,2,4]oxadiazine (27)

To a solution of alcohol **70** (1.5 g, 5.5 mmol), 3,5-difluorophenol (0.86 g, 6.6 mmol) and Ph_3P (1.7 g, 6.6 mmol) in THF (10 mL) was added DEAD (1 mL, 6.6 mmol) at 0 °C and stirred for 0.5 h. Then the reaction mixture was slowly warmed to rt and stirred for additional 2 h. The reaction mixture was diluted with EtOAc, washed with saturated aqueous solution of $NaHCO_3$ and then with brine. The organic layer was dried with $MgSO_4$, filtered, concentrated, and the crude product was purified by silica gel chromatography using 10% EtOAc in hexanes to afford crude **72** as a mixture with 3,5-difluorophenol (1.6 g).

To a solution of $LiAlH_4$ (4.17 mL, 4.2 mmol) in THF (10 mL) was added a solution of crude **72** (~1.6 g,~ 4.2 mmol) in THF (3 mL) at 0 °C and stirred for 10 min. Then the ice bath was removed and the mixture was stirred at rt for additional 10 min. Excess of EtOAc (4 mL) was added, and the resulting mixture was stirred for 5 min. Then the reaction mixture was diluted with mixture of EtOAc/water and added solution of aqueous 1N HCl to dissolve the precipitate. The resulting mixture was transferred to separatory funnel, and the organic layer was separated, washed with brine, dried with $MgSO_4$, evaporated and purified using 0 to 20% EtOAc in hexanes to afford **73** in (0.6 g, 42% yield).

To this alcohol **73** (0.2 g, 0.59 mmol) was added to a solution of 4 N HCl in dioxane (2 mL) at 0 °C and stirred for 10 min. Then the ice bath was removed, and the resulting mixture was stirred at rt for 1 h. The reaction mixture was evaporated to dryness, and the resulting HCl salt of the amine was taken to the next step without purification.

To this crude amine salt in DMF (1.6 mL) was added acid **74** (0.129 g, 0.54 mmol), HOBt (0.098 g, 0.64 mmol), DIEA (0.46 mL, 2.7 mmol) followed by EDCI (0.123 g, 0.64 mmol) at rt, and stirred for 18 h. Then the reaction mixture was diluted with EtOAc, washed with saturated aqueous solution of $NaHCO_3$ and then with brine. The organic layer was dried with $MgSO_4$, filtered, concentrated, and the

crude product was purified by silica gel chromatography using 5% MeOH in DCM to afford **75** (0.12 g, 50% yield.

To a solution of **75** (0.122 g, 0.27 mmol) in methanol (2 mL) was added 10% Pd/C (0.028 g, 0.027 mmol) and the reaction mixture was degassed and back filled with H_2 (3x) and stirred under the atmosphere of H_2 for 12 h. Then the reaction mixture was filtered, and the filtrate was evaporated to dryness. The crude product was purified by silica gel chromatography using 0 to 100% EtOAc/Ethanol (3:1v/v) in hexanes as the eluent to give the corresponding hydrogenated alcohol (0.072 g, 57%).

To a mixture of this alcohol (0.072 g, 0.15 mmol) and Et_3N (0.043 mL, 0.31 mmol) in DCM (1 mL) was added $MeSO_2Cl$ (0.024 mL, 0.31 mmol) at –20 °C, and stirred for 30 min. Then to this mixture was added *N*-hydroxyphthalimide (0.049 g, 0.31 mmol) followed by Et_3N (0.043 mL, 0.31 mmol), and the resulting mixture was warmed to rt, and stirred for 1 h. Then the reaction mixture was diluted with EtOAc, washed with saturated aqueous solution of $NaHCO_3$ followed by with brine. The organic layer was dried with $MgSO_4$, concentrated and the crude product was purified by silica gel chromatography using 0 to 100% EtOAc/ethanol (3:1 v/v) in hexanes as the eluent to give the corresponding Mitsunobu adduct (0.04 g, 43% yield).

To this product (0.04 g, 0.07 mmol) in a mixture of EtOH (0.2 mL) and DCM (0.2 mL) was added hydrazine (0.01 mL, 0.33 mmol) at 0 °C. The ice bath was removed and stirred at rt for 0.5 h. The crude reaction mixture was evaporated to dryness and loaded into column and purified by silica gel chromatography using 0 to 100% EtOAc/ethanol (3:1 v/v) in hexanes as the eluent to afford **77** (0.02 g, 64% yield).

A mixture of **77** (0.02 g, 0.04 mmol) and $POCl_3$ (0.5 mL) was heated at 90 °C for 3 h. Then the reaction mixture was evaporated to dryness and the crude product was purified by reverse phase HPLC (C18) using 0 to 90% water/acetonitrile with 0.05% formic acid to afford **27** (0.004 g, 22% yield). ^1H

NMR (599 MHz, $CDCl_3$) δ 7.92 (bs, 1H), 7.33 (d, *J* = 8.0 Hz, 1H), 7.24 (d, J = 1.6 Hz, 1H), 7.19 (dd, *J* = 8.0, 1.6 Hz, 1H), 6.97 (bs, 1H), 6.50 – 6.40 (m, 3H), 4.45 (m, 1H), 4.24 (dd, *J* = 11.6, 5.1 Hz, 1H), 4.12 (dd, *J* = 11.6, 9.3 Hz, 1H), 3.90 (s, 3H), 3.89 – 3.82 (m, 1H), 3.58 (d, *J* = 13.2 Hz, 1H), 2.91 (td, *J* = 13.1, 2.5 Hz, 1H), 2.34 – 2.29 (m, 4H), 1.97 – 1.88 (m, 1H), 1.77 – 1.66 (m, 1H), 1.56 – 1.46 (m, 1H). m/z: 455.

ASSOCIATED CONTENT

Supporting Information. This material is available free of charge via the Internet at http://pubs.acs.org. Protocl for cell based γ-secretase assay; Overlay of **4**, **7**, **10aa** and **10ee**; Synthesis of compound **15**; Chiral synthesis of compound **20**; HPLC traces for compounds **11**-**15**, **20**,**21** and **27**; Molecular Formula Strings (CSV).

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ABBREVIATIONS USED

ALIS, automated ligand identification system; FBLD, fragment based ligand discovery; BACE, beta-site APP-cleaving enzyme; NMDA, N-methyl-D-aspartate; GSM, gamma (γ) secretase modulator; DIEA, diisopropylethylamine; EDCI, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride; DCM, dichloromethane; EtOAc, ethyl acetate; H_3PO_4, phosphoric acid; *n*-Bu_3P,Tri-*n*-butylphosphine; DIAD, Diisopropyl azodicarboxylate; CF_3CO_2H, trifluoroacetic acid; $Pd(Ph_3)_4$, Tetrakis(triphenylphosphine)palladium(0); Ph_3P, Triphenylphosphine; $Pd(dppf)Cl_2$, [1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium(II); ADDP, 1,1'-(azodicarbonyl)dipiperidine; u-HTS, ultra high throughput screen.

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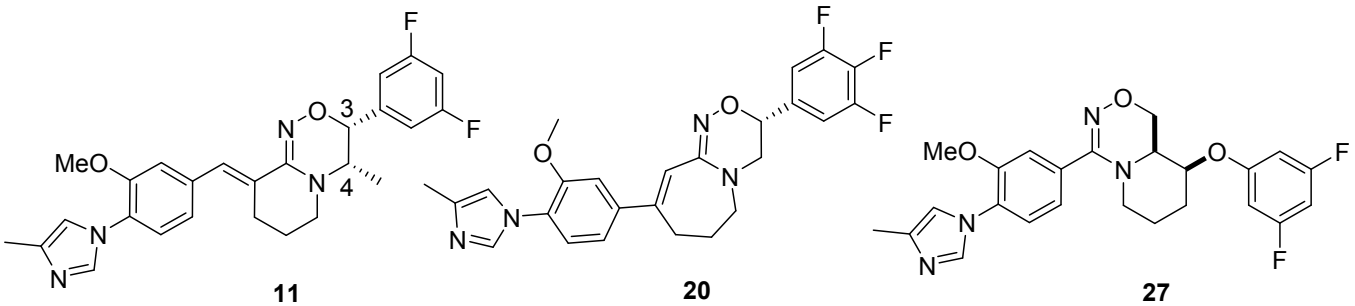

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