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Discovery of (*R*)-4-Cyclopropyl-7,8-difluoro-5-(4-(trifluoromethyl)phenylsulfonyl)-4,5-dihydro-1*H*-pyrazolo[4,3-*c*]quinoline (ELND006) and (*R*)-4-Cyclopropyl-8-fluoro-5-(6-(trifluoromethyl)pyridin-3-ylsulfonyl)-4,5-dihydro-2*H*-pyrazolo[4,3-*c*]quinoline (ELND007): Metabolically Stable γ-Secretase Inhibitors that Selectively Inhibit the Production of Amyloid-β over Notch

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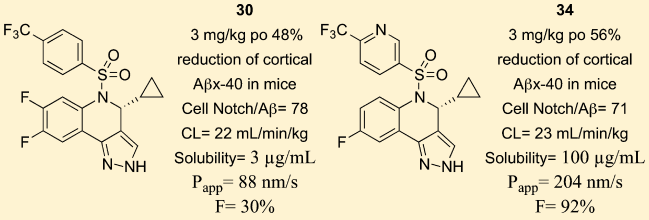
Supporting Information

ABSTRACT: Herein, we describe our strategy to design metabolically stable γ-secretase inhibitors which are selective for inhibition of Aβ generation over Notch. We highlight our synthetic strategy to incorporate diversity and chirality. Compounds **30** (ELND006) and **34** (ELND007) both entered human clinical trials. The in vitro and in vivo characteristics for these two compounds are described. A comparison of inhibition of Aβ generation in vivo between **30**, **34**, Semagacestat **41**, Begacestat **42**, and Avagacestat **43** in mice is made. **30** lowered Aβ in the CSF of healthy human volunteers.



INTRODUCTION

Alzheimer's disease (AD) is an insidious form of senile dementia that causes one to lose their identity. Tens of millions of elderly people worldwide are afflicted with AD.[1] A massive progressive loss of neurons in the hippocampus and cortex results in impaired cognitive and functional ability. Current therapies, *N*-methyl-D-aspartic acid antagonists and acetylcholine esterase inhibitors, to treat those afflicted with AD are merely palliative.[2] With escalating healthcare costs numbering in the hundreds of billions of dollars to treat and care for AD patients, a disease-modifying treatment is urgently needed.

The etiology of AD is not fully understood. However, hints concerning the origins may be found in the pathology. The pathology

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evinces itself with the presence of extraneuronal aggregation of plaques composed of β-amyloid peptides (Aβ)[3] and intracellular neurofibrillary tangles (NFTs),[4] aggregates of aberrantly hyperphosphorylated tau proteins, which are common to numerous neurodegenerative diseases. The amyloid hypothesis[5] states that Aβ initiates a complex pathological cascade including the generation of NFTs, leading to neuronal death.

Aβ-peptides are derived by the sequential proteolytic cleavage of the β-amyloid precursor protein (APP) by two aspartic acid proteases, referred to as β- and γ-secretase, respectively.[6] Inhibitors of either protease offer attractive candidates as potentially disease-modifying therapies for people afflicted with AD.[7] γ-Secretase processes a myriad of substrates[8] in addition to C99, most notably Notch, raising concerns about mechanism-based side-effects (e.g., goblet cell hyperplasia in the gastrointestinal tract)[9] due to a lack of selectivity.

Our objective was to avoid Notch-related toxicity by developing γ-secretase inhibitors with a sufficient therapeutic index. Thus, these inhibitors would be required to selectively inhibit Aβ formation over Notch cleavage. The magnitude of a selectivity ratio that would predict an acceptable in vivo therapeutic index could not be determined a priori, and therefore the strategy for the target product profile was to minimally exceed the selectivity ratios for Semagacestat, **41**, a relatively nonselective γ-secretase inhibitor that had already entered clinical development, by at least a half log interval. In this paper, enzyme and cellular APP:Notch ratios are reported and were used to guide SAR. The selectivity ratios for the benchmark **41** in these assays were 0.41-fold and 15-fold, respectively (Table 4), and therefore the target product profile was to exceed these values by approximately 3-fold. Acute biomarker data, short-term-repeat dose studies in the mouse, and chronic repeat-dose data collected in the Sprague–Dawley rat and cynomolgus monkey retrospectively confirmed this product profile to be adequate for clinical development, i.e., these data provided evidence that brain Aβ could be continuously decreased in the absence of Notch-related toxicity in vivo.

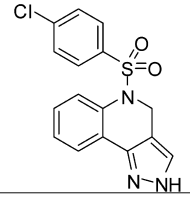
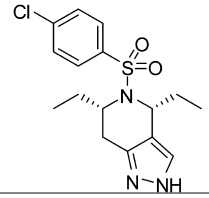
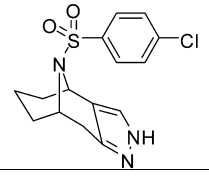
An active site inhibitor would not confer selectivity so we sought an allosteric inhibitor. Existing literature suggested that an allosteric inhibitor of γ-secretase was plausible.[10] An additional requirement was to incorporate favorable pharmacokinetic properties into these inhibitors to avoid peak-to-trough drug levels that could rise above toxic exposures or fall below efficacious concentrations, once-a-day dosing, and sustained levels of exposure to reduce Aβ over a 24 h period.

RESULTS, DISCUSSION, AND CHEMISTRY

Three series of pyrazole/sulfonamide inhibitors **1**–**3** were under development (Table 1). All three series were comparable in terms of their in vitro selectively for inhibiting Aβ production over Notch and low metabolic stability. When dosed in rat PK screens,[11] both compound **2** (67 mL/min/kg) and compound **3** (34 mL/min/kg) resulted in systemic clearances of greater than 60% of hepatic blood flow with oral bioavailabilities of 6% and 1%, respectively. Because of its low oxidative stability, compound **1** was not tested in vivo, however, these results demonstrated a connectivity with low metabolic stability in vitro and high clearance in vivo. Preventing the oxidation of aliphatic carbons can be extremely challenging, however, mitigating the oxidation of aryl rings can be more facile. Thus, the quinoline series **1**[12] ostensibly offered the lowest hurdle to prepare metabolically stable inhibitors. This series had a single aliphatic carbon (the site of oxidation), whereas both the piperidine **2**[13] and bicyclic **3**[14] series bore multiple aliphatic carbons as potential sites for oxidation (see refs 14b and c for early development of the series).

The initial synthesis of the quinolines was unreliable and offered only limited access to functionality (Scheme 1). A reductive amination between the β-keto ester **4** with 4-fluoroaniline provided **5**, which was sulfonylated to provide **6** and hydrolyzed to afford **7**. Formation of the acid chloride followed by Friedel–Crafts cyclization yielded **8**. This cyclization of **7** to **8**

Table 1. SAR for the Three Series

#	Structure	Aβ IC_{50}[a] Notch IC_{50}[a] Notch/Aβ	Cell ED_{50}[a] Notch ED_{50}[a] Notch/Aβ	Ox. Met. % remaining[12] (m, r, d, h)[b] Gluc. Met. % remaining[12] (m, r, d, h)[b]
1		27 nM 250 nM 9.3	100 nM 9,400 nM 94	3, 0, 0, 0 81, 93, 89, 11
2		0.37 nM 4.7 nM 13	0.75 nM 71 nM 95	1, 1, 0, 1 11, 1, 38, 1
3		2.1 nM 29 nM 14	5.2 nM 690 nM 133	15, 5, 2, 50 4, 0, 100, 0

[a]See ref 14c for detailed experimental procedures. [b]m = mouse, r = rat, d =dog, h = human.

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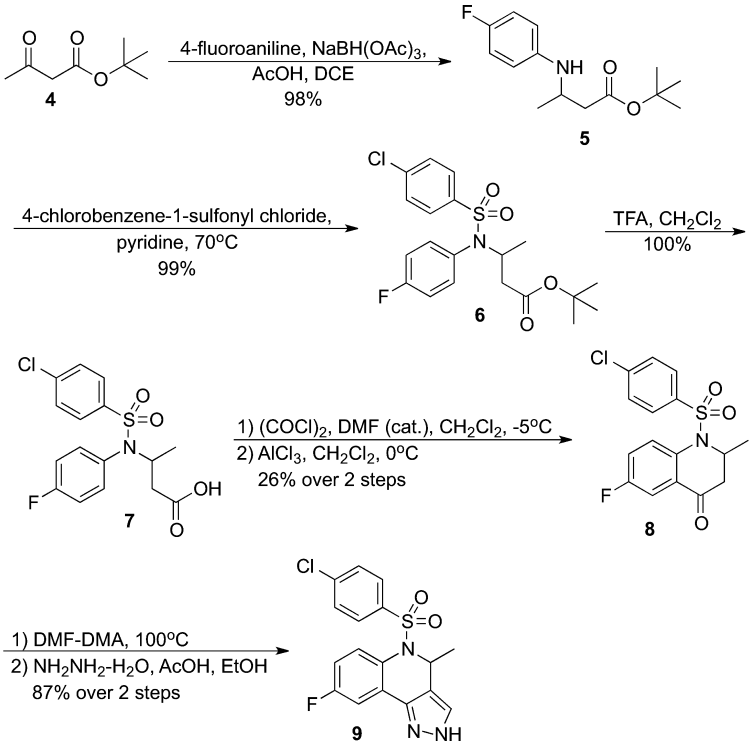
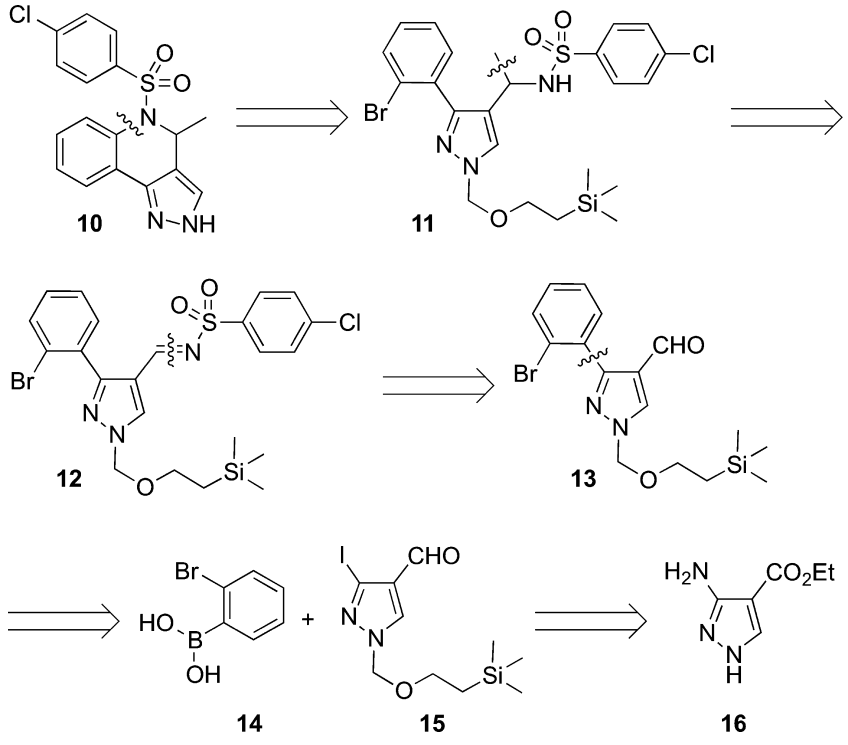
Scheme 1. Initial Synthetic Route



Scheme 2. Retrosynthetic Analysis



was capricious under multiple conditions. Alternatively, **5** could be hydrolyzed and cyclized, but then the sulfonylation was problematic especially in cases where the stereogenic substituent was sterically encumbered by substituents larger than methyl. The cyclization chemistry was not compatible with electron deficient aryl rings (e.g., pyridines), further limiting this approach. Lastly, incorporation of the pyrazole with reliable chemistry furnished **9**. This synthetic route was inefficient. The pyrazole was to be maintained as a constant, and thus should be incorporated early in the synthesis instead of the final step. Areas optimal for SAR were installed in the initial steps of the synthesis rather than the latter stages. Nevertheless, this route did furnish some initial compounds.

An investment into the development of a reliable synthetic route that offered the opportunity to incorporate an eclectic mix

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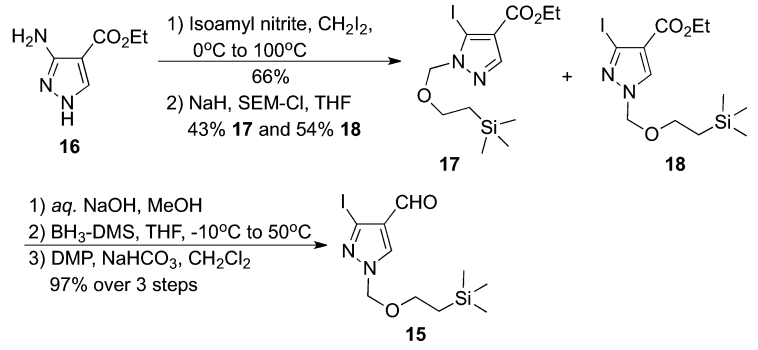
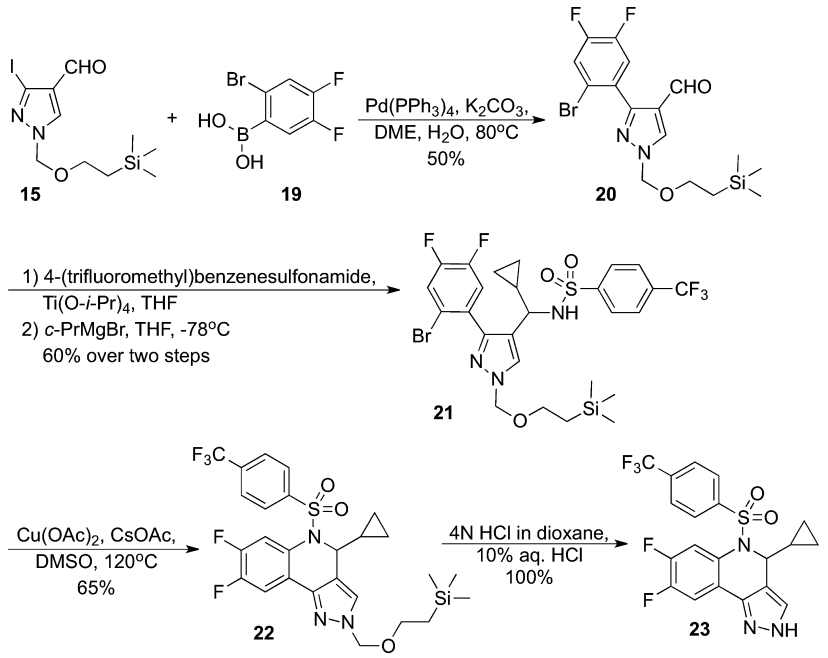
Scheme 3. Synthesis of the Key Intermediate



Scheme 4. Completion of Final Target via the New Synthetic Route



of functionality in the latter stages of the synthesis was necessary for the quinoline series to be successful. The pyrazole was to be maintained as constant (any substitution at the C−H position of the pyrazole was detrimental to the potency), while the other regions of the template were to be varied. A retrosynthetic analysis (Scheme 2) revealed that disconnecting the sulfonamide nitrogen from the aryl ring in **10** would allow for the aliphatic and sulfonamide region to be incorporated separately from the aryl portion. The former areas could be installed via a Grignard addition to a sulfonylimine **12**, which in turn could be derived from the condensation between a sulfonamide and an aldehyde **13**. Biaryl rings can be built efficiently and reliably via transition-metal mediated cross-coupling methodologies. Thus, a protected pyrazole bearing two distinct handles **15** was required as the key intermediate. Ethyl 3-amino-1*H*-pyrazole-4-carboxylate **16** served as the commercially available starting material.

This key intermediate **15** was prepared in multigram quantities in five steps from commercially available starting material **16** (Scheme 3). Conversion of the amino group into an iodide followed by protection of the pyrazole with (2-(chloromethoxy)-ethyl)trimethylsilane afforded **17** and **18** as a mixture of regioisomers[15] that were separable by a laborious chromatographic separation. This material could be advanced, however, as a mixture through all of the subsequent chemistry without incident. Saponification of the ester **18** followed by conversion to the aldehyde yielded **15**.[16] An NOE enhancement between the pyrazole C−H and hemiaminal protons was observed to confirm the regiochemistry of **15** (see Supporting Information).

A Suzuki−Miyaura cross-coupling[17] between **15** and commercially available 2-bromo-4,5-dilfuorobenzeneboronic acid **19** installed the aryl portion (Scheme 4). Condensation of the aldehyde **20** with a sulfonamide followed by Grignard addition to the sulfonylimine afforded the cyclization precursor **21**. A copper-mediated reaction[18] cleanly yielded the cyclized material **22** that was subsequently deprotected to complete the synthesis of **23**. This copper-mediated reaction was extremely robust in that clean starting material was not required, it worked with heterocyclic aryl rings, and was complete within minutes. Hydrodebromination gave rise to the major undesired byproduct, but by placing the reaction mixture into a preheated oil bath this byproduct could largely be suppressed.

Identification of the metabolites was crucial in order to make rational modifications to improve the metabolic stability.[19] Metabolite identification studies with uridine 5′-diphosphate-glucuronic acid (UDP-GA) supplemented rat and human liver microsomses, as well as from bile cannulated rat studies for both

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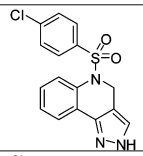
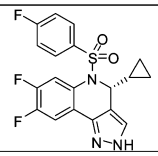
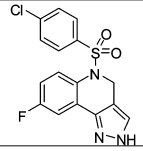
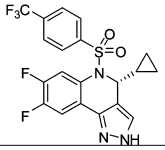
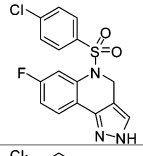
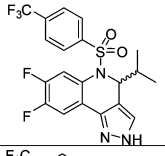
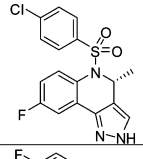
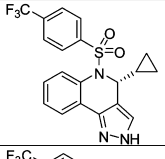
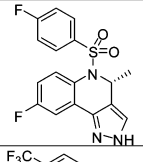
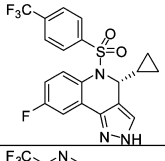
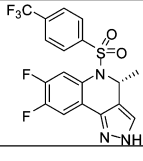
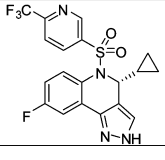
Table 2. Quinoline Series SAR

#	Structure	Aβ IC_{50}[a] Notch IC_{50}[a] Notch/Aβ	Cell ED_{50}[a] Notch ED_{50}[a] Notch/Aβ	Ox. Met. % remaining[12] (m, r, d, h)[b] Gluc. Met. % remaining[12] (m, r, d, h)[b]	#	Structure	Aβ IC_{50}[a] Notch IC_{50}[a] Notch/Aβ	Cell ED_{50}[a] Notch ED_{50}[a] Notch/Aβ	Ox. Met. % remaining[12] (m, r, d, h)[b] Gluc. Met. % remaining[12] (m, r, d, h)[b]
1		27 nM 250 nM 9.3	100 nM 9,400 nM 94	3, 0, 0, 0 81, 93, 89, 11	**29**		0.33 nM 9.6 nM 29	1.3 nM 85 nM 65	52, 51, 47, 24 93, 91, 89, 75
24		22 nM 160 nM 7.3	99 nM 7,400 nM 75	5, 0, 0, 0 80, 91, 86, 69	**30**		0.34 nM 6.6 nM 19	1.1 nM 86 nM 78	72, 66, 75, 80 88, 90, 90, 90
25		38 nM 220 nM 5.8	160 nM 11,000 nM 69	22, 0, 0, 1 77, 99, 83, 3	**31**		1.4 nM 17 nM 12	5.6 nM 410 nM 73	15, 12, 17, 22 95, 93, 87, 76
26		0.62 nM 14 nM 23	2.7 nM 170 nM 63	7, 2, 40, 1 91, 92, 97, 77	**32**		0.18 nM 2.7 nM 15	0.41 nM 26 nM 63	51, 40, 46, 36 98, 87, 99, 99
27		1.3 nM 43 nM 33	7.8 nM 410 nM 54	22, 21, 49, 17 84, 82, 84, 70	**33**		0.14 nM 1.7 nM 12	0.79 nM 53 nM 67	72, 59, 80, 84 97, 91, 88, 82
28		2.1 nM 42 nM 20	13 nM 890 nM 68	20, 36, 54, 28 93, 86, 83, 80	**34**		0.10 nM 1.7 nM 17	0.28 nM 20 nM 71	80, 81, 80, 85 90, 91, 87, 91

[a]See ref 14c for detailed experimental procedures. [b]m = mouse, r = rat, d = dog, h = human.

compounds **1** and **3**, demonstrated that the pyrazole underwent direct *N*-glucuronidation. Glucuronidation is a low affinity, high capacity pathway leading to high clearance in vivo which needed to be addressed. Additionally, cleavage of the glucuronide in the gastrointestinal tract could lead to enterohepatic circulation of the inhibitor, resulting in prolonged plasma concentration−time profile and increased variability between patients. Initial studies indicated that incorporation of a fluoride at C8, as in **24**, attenuated the *N*-glucuronidation. Metabolite identification studies of **24** demonstrated that the methylene of the quinoline ring was susceptible to oxidation (Table 2). A methyl group at C4, as in **26**, was installed to block the metabolism. Virtually no improvement was observed in the in vitro microsomal stability, but fortuitous introduction of the methyl in **26** led to an increase in potency (0.62 vs 22 nM) and an improvement in the biochemical Notch/Aβ selectivity (23 vs 7.3) relative to **24**. Metabolite identification studies of **26** surprisingly revealed that the major site of oxidation was now the aryl ring of the sulfonamide. To prevent this oxidation and retain the potency, electron withdrawing lipophilic functional groups[20] were placed at the *para* position of the sulfonamide. Introduction of a fluoride, as in **27**, in place of the chloride resulted in only a slight improvement in the oxidative stability but resulted in reduced formation of reactive metabolite in trapping studies with glutathione. Virtually no improvement in the oxidative stability was observed with the stronger electron withdrawing trifluoromethyl group as in **28**. The major site of oxidation of **27** appeared relocated onto the quinoline ring system with a minor metabolite was located on the aryl ring of the sulfonamide. We hypothesized that the methyl was the most likely site of oxidation. Cyclopropane has a ring strain of 27 kcal/mol, and incorporation of a single sp^2 center increases the ring strain to 41 kcal/mol.[21b,c] Also the C−H bond strength of cyclopropane (106 kcal/mol) is greater than ethane (101 kcal/mol).[21d,e] Thus, replacing the methyl group with a cyclopropyl ring could prevent hydrogen atom extraction, thereby mitigating the oxidation of the aliphatic region. At this juncture, we began to employ the second synthetic route (Scheme 4) and used commercially available 2-bromo-4,5-difluoroboronic acid **19** in order to expedite the production of targets rather than 2-bromo-4-fluororoboronic acid **36** (Scheme 5) which had to be synthesized. The SAR indicated that the additional C7 fluoride **25** would not be detrimental. The cyclopropyl moiety in **29** enhanced the potency and improved the metabolic stability in rodents but not in humans. At this point, we postulated that the site of metabolic oxidation must have relocated once again, this time back to the aryl ring of the sulfonamide. A trifluoromethyl group **30** was used to prevent the oxidation of aryl ring of the sulfonamide and maintain potency. Furthermore, glutathione adducts were not observed with this substitution. To provide additional support for the hypothesis that the cyclopropyl group prevented the oxidation, the isopropyl derivative **31** was prepared and

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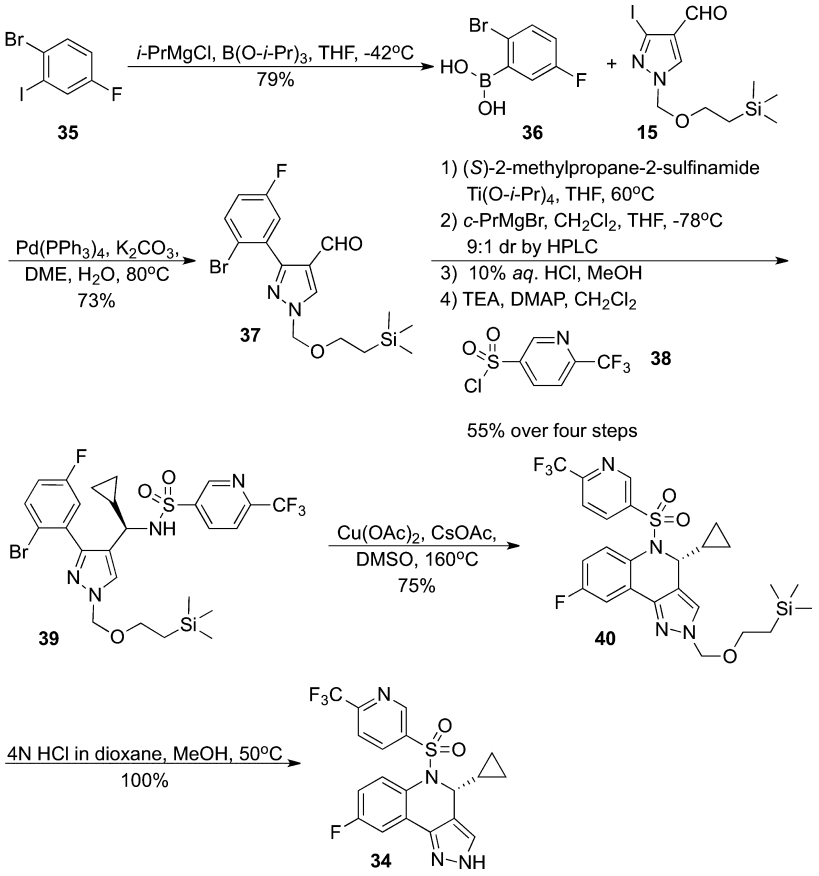
Scheme 5. Asymmetric Synthesis of 34



demonstrated very little stability toward oxidative metabolism. Recall that early in the development of the template, the fluoride at C8 was necessary to mitigate glucuronidation. Removal of the fluorides on the quinoline ring system **32** demonstrated that the C8 fluoride was not necessary to improve the stability against glucuronidation. The cyclopropyl group (or any alkyl group) became the key to abrogate glucuronidation. A single fluoride as in **33** was necessary for stability against oxidation in phase 1 metabolism.

On the basis of these data and the pharmacological data that follows, compound **30** was advanced as far as possible into preclinical development to uncover any hurdles for clinical development for this series because it was the first inhibitor in this genre. Both the in vitro and in vivo profiles for **30**, (*R*)-4-cyclopropyl-7,8-difluoro-5-(4-(trifluoromethyl)phenylsulfonyl)-4,5-dihydro-1*H*-pyrazolo[4,3-*c*]quinoline (ELND006),[22] supported IND, enabling advancement into human clinical trials (ELND numbered compounds are nominated for development). Inhibitor **34**, (*R*)-4-cyclopropyl-8-fluoro-5-(6-(trifluoromethyl)-pyridin-3-ylsulfonyl)-4,5-dihydro-2*H*-pyrazolo[4,3-*c*]quinoline (ELND007),[22] was optimized for both potency and water solubility and eventually advanced into human clinical trials as well. Details regarding the pharmacokinetic/pharmacodynamic (PK/PD) relationship, in vivo Notch selectivity, and other pharmacokinetic properties will be published separately.

The preclinical studies required copious quantities of **30** and **34**. An asymmetric synthesis, which was incorporated into the initial synthetic route, could potentially double the overall yield of the route (Scheme 5) and was implemented immediately upon the discovery of this genre of inhibitors. The commercially available starting material **35** was used to generate the boronic acid **36** that was cross-coupled with **15** to afford **37**. Condensing the (*S*)-(−)-*tert*-butanesulfinamide[23] with the aldehyde **37** afforded the sulfinimine to which cyclopropylmagnesium bromide was added in 9:1 dr as measured by HPLC. Removal of the sulfinyl group afforded the amine, which was sulfonylated with **38** to yield the cyclization precursor **39**. Cyclization followed by deprotection afforded a 9:1 mixture of enantiomers of **34** that were separable via chiral HPLC.

The pharmacological and drug properties for **30** and **34** were suitable for advancing into human clinical trials. The overall chemotype demonstrates favorable physiochemical properties for an orally available central nervous system (CNS) compound with range of lipophilicity ($2 < \log P < 4$), topographical polar surface area (<70 Å^2), hydrogen bond donors (0−1), and moderate molecular weight (≤450 amu).[24] Compound **34** demonstrated a lower octanol−water partition coefficient of 3.65 compared to 3.91 for **30** as measured by the shaker-flask method.[11] This reduced lipophilicity translated to better aqueous solubility for **34** (∼100 μg/mL) compared to **30** (∼3 μg/mL).[11] In addition, compound **34** demonstrated a high rate of passive permeability (204 nm/s) in wild-type Madin−Darby canine kidney (MDCK) cells compared to the moderate rate of permeability for compound **30** (88 nm/s), relative to the high permeability control, propranolol (178 nm/s). Furthermore, both compounds were negligible substrates for permeability-glycoprotein (P-gp) efflux in MDR1 transfected MDCK cells. These properties[25] afforded good to excellent oral bioavailabilities in rats for **34** (92%) and **30** (30%) following administration of an aqueous suspension formulated in 2% methylcellulose and 0.5% Tween 80. Furthermore, their metabolic stabilities conferred a moderately low systemic clearance of 22 mL/min/kg (40% of hepatic blood flow (Q_H)) and 23 mL/min/kg (42% Q_H) for **30** and **34**, respectively, in the rat.

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Table 3. Compound Levels 3 h After Administration

	dose in mice for efficacy studies in Table5[a] brain and plasma concentration			
	1 mg/kg	3 mg/kg	10 mg/kg	30 mg/kg
	30			
[brain] 3 h (μM)	0.12	0.52	2.1	7.2
[brain unbound] 3 h (nM)	1.0	4.5	18	62
[brain] AUC(0−3 h) (μM)	0.64	2.1	7.0	ND[b]
[plasma] 3 h (μM)	0.031	0.19	0.90	3.0
[plasma unbound] 3 h (nM)	0.28	1.7	8.1	27
[plasma] AUC(0−3 h) (μM)	0.19	0.68	0.68	ND[b]
	34			
[brain] 3 h (μM)	0.045	0.22	1.0	2.6
[brain unbound] 3 h (nM)	0.63	3.1	14	36
[brain] AUC(0−3 h) (μM)	0.24	0.92	4.1	11
[plasma] 3 h (μM)	0.014	0.079	1.2	3.5
[plasma unbound] 3 h (nM)	0.87	4.9	74	220
[plasma] AUC(0−3 h) (μM)	0.14	0.57	4.7	12

[a]See ref 14c for detailed experimental procedures. [b]ND = not determined.

Both **30** and **34** also readily partitioned into the CNS (Table 3), which was consistent with their favorable ratio of unbound plasma to unbound brain ratio of 1 and 4, respectively, as determined by equilibrium dialysis for **30** ($f_{u,plasma}$ = 0.0090, $f_{u,brain}$ = 0.0086) and **34** ($f_{u,plasma}$ = 0.062, $f_{u,brain}$ = 0.014).[26] The CNS partitioning was further corroborated by the significant pharmacodynamic effect in the reduction of brain Aβx-40, which plateaued at approximately 60% for all of the compounds in this genre by 3 h following a single oral dose. Both **30** and **34** were superior to **41**,[27] Begacestat **42**,[28] and Avagacestat **43**[29] (Table 4) when comparing the cortical Aβx-40 reduction in mice 3 h after a single oral dose (Table 5). Both **30** and **34** achieved similar Aβ reduction, but at a dose 30-fold lower than other agents (compare **30** and **34** at 1 mg/kg to the other agents at 30 mg/kg) owing to their greater metabolic stability in rodents as well as potency. The effective unbound brain concentration (EC_{u25}) estimated to achieve a 25% reduction of brain Aβx-40 in mice was 18-fold lower for **34** (0.034 nM) than **30** (0.62 nM). Sustained reduction of cortical Aβx-40 above the 25% threshold over a 24 h period was achieved in mice with a single oral 30 mg/kg dose of **30** with a maximal effect of 82% reduction at 6 h post administration (the companion PK/PD study in mice for **34** was not conducted).[19] The ability to reduce central Aβ robustly over a 24 h period with a moderate single daily dose of inhibitor ratified our strategy of focusing on improving the metabolic stability to enhance the exposure. Additionally, the respective in vitro biochemical and cellular selectivity ratios for inhibition of Notch cleavage over Aβ production for **30** (19 and 78) and **34** (17 and 71) were nearly identical and comparable to both **43** (12 and 59) and **42** (6.9 and 45) but superior to **41** (0.41 and 15).

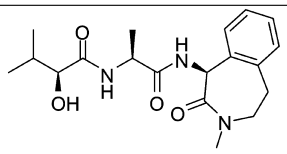
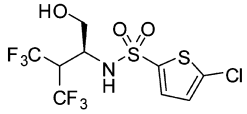
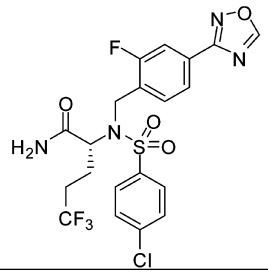
Table 5. Efficacy Comparison 3 h After Administration

	dose (% reduction of cortical Aβx-40 in mice)[a,b]			
inhibitor	1 mg/kg	3 mg/kg	10 mg/kg	30 mg/kg
30	32	48	61	64
34	34	56	61	62
41	5	19	31	47
42	ND[c]	18	20	33
43	7	10	19	35

[a]See ref 14c for detailed experimental procedures. [b]*p < 0.05. [c]Not determined.

The magnitude of a selectivity ratio that would predict an acceptable in vivo therapeutic index could not be determined a priori, and therefore the strategy for the target product profile was to minimally exceed the selectivity ratios for **41**, a relatively nonselective γ-secretase inhibitor that had already entered clinical

Table 4. Competitors Clinical γ-Secretase Inhibitors

#	Structure	Aβ IC_{50}[a] Notch IC_{50}[a] Notch/Aβ	Cell ED_{50}[a] Notch ED_{50}[a] Notch/Aβ	Ox. Met. % remaining[12] (m, r, d, h)[b] Gluc. Met. % remaining[12] (m, r, d, h)[b]
41		7.9 nM 3.2 nM 0.41	15 nM 220 nM 15	100, 87, 100, 92 88, 92, 96, 93
42		1.6 nM 11 nM 6.9	7.8 nM 350 nM 45	89, 54, 78, 78 36, 0, 0, 75
43		0.13 nM 1.5 nM 12	1.2 nM 71 nM 59	1, 2, 79, 75 79, 88, 100, 100

[a]See ref 14c for detailed experimental procedures. [b]m = mouse, r = rat, d = dog, h = human.

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development, by at least a half log interval. It was impractical to assess in vivo selectivity across a large set of compounds to fully model in vitro to in vivo relationships, but for a subset of analogues of interest for potential advancement into preclinical development, acute rodent models were used to explore this translation, i.e., could reductions in CNS Aβ be achieved in the absence of peripheral Notch inhibition in vivo? First, an acute biomarker study was conducted in mice, comparing plasma exposures that decrease brain Aβ with those that decrease the Notch transcription factor *Hairy* and *Enhancer of Split* homologue-1 (HES-1) in thymus (Table 6). Plasma compound

Table 6. Changes in Thymic HES-1 mRNA 3 h Post Administration

	dose (% change of thymic HES-1 mRNA in mice)[a]			
inhibitor	1 mg/kg	3 mg/kg	10 mg/kg	30 mg/kg
30	ND[b]	−17[c]	−14[d]	−25[e]
34	+11[d]	−21[f]	−44[f]	−46[f]

[a]See reference 14c for detailed experimental procedures. [b]Not determined. [c]*p < 0.05. [d]Not statistically significant. [e]*p < 0.001. [f]*p < 0.01.

concentrations were assessed over a range of doses and plasma concentrations associated with a 25% reduction (EC_{25}) in brain Aβ were compared to EC_{25} for thymus HES-1 reduction. To achieve these reductions, **30** afforded a 57-fold difference in plasma levels.[30] Second, 7-day studies in mice dosed twice daily were conducted, examining brain Aβ concentrations biochemically and Notch-responsive tissues histologically (e.g., goblet cell hyperplasia in intestine, atrophy of thymus). Both studies demonstrated a broader therapeutic index relative to the benchmark comparator. Furthermore, longer-term repeat dose studies in the cynomolgus monkey and Sprague−Dawley rat confirmed that acceptable margins of safety were achievable, even after chronic administration. In these repeat dose studies, the cell-based IC_{50} for Notch inhibition was the most informative in predicting plasma concentrations associated with adverse effects. Retrospectively, in vivo studies supported the target product profile for Notch selectivity as being acceptable for clinical development.

In summary, we were able to improve the metabolic stability through rational design based on our understanding of physical properties of functional groups. Fortunately, these perturbations improved the potency and did not adversely affect the Notch selectivity. A single 30 mg oral dose of **30** crystalline material in a gelatin capsule lowered Aβ in the CSF of healthy human volunteers by 31−46% 5 h after administration, however, it was discontinued due to an adverse liver event unrelated to its mechanism of action. The observed liver effects in the clinic were not predicted by repeat-dose toxicity studies up to six months of dosing in the rat and one year of dosing in the cynomolgus monkey. **34** was advanced into human clinical trials but was discontinued due to a concern about the potential for an adverse liver event.

EXPERIMENTAL METHODS

All reagents and solvents were used as purchased from commercial sources. All reactions were run under nitrogen. All of the final compounds were purified on a Varian Prepstar HPLC with a Varian Prostar detector using on a Phenomenex Luna C18 column of either of the following sizes 250 mm × 21.20 mm, 5 μm, or 250 mm × 50.00 mm, 10 μm. Separation of the enantiomers is indicated for each compound (vide infra).

^{1}H NMR data were recorded on a 400 MHz Bruker, 300 MHz Bruker, or 300 MHz Varian Gemini. Chemical shifts are reported in parts per million (ppm) downfield relative to tetramethylsilane (TMS) or to proton resonances resulting from incomplete deuteration of the NMR solvent (*d* scale). The 2D ROSEY NMR was taken on a 700 MHz Bruker.

HMRS data were collected using a LC/MS analysis on a Q-TOFMS. Stock solutions (20 mM) for all standard compounds prepared in DMSO were diluted in water to 5 μM for positive mode mass spectrometric analysis. This typically produced maximum ion abundances on the order of one to five million ions/scan, although for data processing, spectra with ion abundances of 500000 ions/scan were averaged. A tripleTOF 5600 quadrupole time-of-flight hybrid mass spectrometer (AB Sciex, Foster City, CA, USA) equipped with an LC system consisting of a LEAP PAL HTC auto sampler and an Agilent 1290 Infinity LC pump (Agilent, Santa Clara, CA) was used for LC/MS analysis. The mass spectrometer was operated in the positive ionization mode with a spray voltage of 5.5 kV. Ion source gas 1, ion gas source 2, and curtain gas were set at 40, 45, and 25 psi, respectively, and the source temperature was maintained at 500 °C. Samples (5 μL) were introduced to the mass spectrometer via the HPLC system using an Agilent EC-C18 column (2.1 mm × 75 mm, 2.7 μm) under isocratic conditions (50:50 acetonitrile/water with 0.1% formic acid) at a flow rate of 0.8 mL/min. Full scan mass spectra were acquired over the range of 120−900 m/z with an accumulation time of 0.25 s. Total acquisition time was 2 min per sample, and all four time-to-digital converter channels were used for detection. The tripleTOF instrument was calibrated by an integrated calibration delivery system (AB Sciex) using the manufacturer's positive calibration solution, with an injection flow rate of 300 μL/min. These calibrant ions were introduced via an orthogonal atmospheric pressure chemical ionization source in the presence of mobile phase flow. Mass spectral data acquisition and processing were performed using Analyst TF 1.5 and Peak View 1.1.1.2 software.

Mass spectra were recorded on an Agilent series 1100 mass spectrometer connected to an Agilent series 1100 HPLC. Retention times (t_R) are given in minutes. Three methods used for purity analysis: (1) Method 1 = 20% [B], 80% [A] to 70% [B], 30% [A] gradient in 10.0 min, then hold, at 1.5 mL/min, where [A] = 0.1% trifluoroacetic acid in water, [B] = 0.1% trifluoroacetic acid in acetonitrile on a Phenomenex Luna C18 (2) 4.6 mm × 3 cm column, 3 μm packing, 210 nm detection, at 35 °C. (2) Method 2 = 50% [B], 50% [A] to 95% [B], 5% [A] gradient in 10.0 min, then hold, at 1.5 mL/min, where [A] = 0.1% trifluoroacetic acid in water, [B] = 0.1% trifluoroacetic acid in acetonitrile a Phenomenex Luna C18 (2) 4.6 mm × 3 cm column, 3 μm packing, 210 nm detection, at 35 °C. (3) Method 3 = 10% [B], 90% [A] to 40% [B], 60% [A] gradient in 10.0 min, then hold, at 1.5 mL/min, where [A] = 0.1% trifluoroacetic acid in water, [B] = 0.1% trifluoroacetic acid in acetonitrile on a Phenomenex Luna C18 (2) 4.6 mm × 3 cm column, 3 μm packing, 210 nm detection, at 35 °C.

The specific optical rotation values for samples were obtained using a Rudolf Instruments, Model Autopol V polarimeter. The polarimeter was zeroed with 95% ethanol, and a sample was tested at 589 nm. The analysis was performed per USP <781S>.

5-(4-Chlorophenylsulfonyl)-4,5-dihydro-1*H*-pyrazolo[4,3-*c*]-quinoline (1). *Synthesized in a Similar Manner to Scheme 1.* ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.68−7.62 (m, 2H), 7.46−7.41 (m, 3H), 7.32 (d, J = 8.2 Hz, 2H), 7.16 (d, J = 8.8 Hz, 2H), 4.93 (s, 2H). HMRS: calcd for $C_{16}H_{12}ClN_3O_2S$ $(M + H)^+$, 346.04115; found, 346.04057. HPLC purity (method 1): t_R = 4.08, 100%. LCMS m/z 346.0 and 348.0 $(M + H)^+$. HPLC purity (method 2): t_R = 0.87, 100%. LCMS m/z 346.0 and 348.0 $(M + H)^+$. HPLC purity (method 3): t_R = 9.79, 100%. LCMS m/z 346.0 and 348.0 $(M + H)^+$.

(4*R*,6*S*)-5-((4-Chlorophenyl)sulfonyl)-4,6-diethyl-4,5,6,7-tetrahydro-2*H*-pyrazolo[4,3-*c*]pyridine (2).[31] ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.70 (d, J = 8.8 Hz, 2H), 7.41 (s, 1H), 7.35 (d, J = 8.8 Hz, 2H), 4.96 (t, J = 7.2 Hz, 1H), 4.17 (q, J = 7.2 Hz, 1H), 2.52 (d, J = 16.0 Hz, 1H), 2.24 (dd, J = 16.0, 6.4 Hz, 1H), 1.96 (m, 1H), 1.79 (m, 1H), 1.65 (m, 1H), 1.45 (m, 1H), 1.19 (t, J = 7.2 Hz, 3H), 0.98 (t, J = 7.2 Hz, 3H).

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HMRS: calcd for $C_{16}H_{20}ClN_3O_2S$ $(M + H)^+$, 354.10375; found, 354.10350. HPLC purity (method 1): t_R = 5.18, 100%. LCMS m/z 354.1 and 356.1 $(M + H)^+$. HPLC purity (method 2): t_R = 0.85, 100%. LCMS m/z 354.1 and 356.1 $(M + H)^+$. HPLC purity (method 3): t_R = 10.21, 100%. LCMS m/z 354.1 and 356.1 $(M + H)^+$.

(4*R*,8*S*)-10-((4-Chlorophenyl)sulfonyl)-4,5,6,7,8,9-hexahydro-2*H*-4,8-epiminocycloocta[*c*]pyrazole (3).[32] ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.65 (d, J = 8.8 Hz, 2H), 7.48 (s, 1H), 7.36 (d, J = 8.8 Hz, 2H), 5.28 (broad s, 1H), 4.48 (t, J = 5.6 Hz, 1H), 2.96 (dd, J = 17.6, 7.6 Hz, 1H), 2.61 (d, J = 17.6 Hz, 1H), 1.93 (broad m, 2H), 1.61 (broad m, 3H), 1.30 (qt, J = 14.0, 4.0 Hz, 1H). HMRS: calcd for $C_{15}H_{16}ClN_3O_2S$ $(M + H)^+$, 338.07245; found, 338.087212. HPLC purity (method 1): t_R = 2.74, 99%. LCMS m/z 338.0 and 340.1 $(M + H)^+$. HPLC purity (method 2): t_R = 0.49, 100%. LCMS m/z 338.0 and 340.1 $(M + H)^+$. HPLC purity (method 3): t_R = 7.38, 100%. LCMS m/z 338.0 and 340.1 $(M + H)^+$.

***tert*-Butyl 3-((4-Fluorophenyl)amino)butanoate (5).** To a 1,2-dichloroethane solution (146 mL) of 4-fluoroaniline (4.06 g, 36.5 mmol) was added *tert*-butyl acetoacetate **4** (6.05 mL, 36.5 mmol) and glacial acetic acid (3.0 mL, 47.5 mmol). Sodium triacetoxyborohydride (11.6 g, 54.8 mmol) was added in portions, and the reaction was stirred overnight at room temperature. Saturated aqueous sodium bicarbonate (250 mL) was slowly added, and the two-phase solution was well stirred. The separated organic portion was washed with saturated aqueous sodium bicarbonate (1 × 100 mL), water (1 × 100 mL), and brine (1 × 20 mL), dried over anhydrous magnesium sulfate, filtered, and concentrated to yield **5** (9.03 g, 98%) as an oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.88 (t, J = 8.8 Hz, 2H), 6.55 (dd, J = 8.8, 4.4 Hz, 2H), 3.80 (sextet, J = 6.4 Hz, 1H), 2.49 (dd, J = 14.8, 5.6 Hz, 1H), 2.33 (dd, J = 14.8, 6.8 Hz, 1H), 1.44 (s, 9H), 1.25 (d, J = 6.4 Hz, 3H). HPLC (method 1): t_R = 3.28. LCMS m/z 254.1 $(M + H)^+$. HPLC (method 3): t_R = 6.93. LCMS m/z 254.1 $(M + H)^+$.

***tert*-Butyl 3-(4-Chloro-*N*-(4-fluorophenyl)phenylsulfonamido)-butanoate (6).** To a solution of *tert*-butyl 3-((4-fluorophenyl)amino)-butanoate **5** (3.08 g, 12.2 mmol) in pyridine (35 mL) at 0 °C was added 4-chlorobenzenesulfonyl chloride (8.98 g, 42.6 mmol) portionwise. The reaction was warmed to room temperature and then heated at 70 °C for 5.5 h then cooled to room temperature. Dimethylaminopropylamine (40 mL) was added to the reaction mixture, which was then stirred overnight. The reaction mixture was then concentrated and the residue taken up in ethyl acetate (120 mL). The organic phase was washed with cold 1N aqueous hydrogen chloride (6 × 100 mL), water (1 × 100 mL), saturated aqueous sodium bicarbonate (3 × 100 mL), brine (1 × 100 mL), dried over anhydrous magnesium sulfate, filtered, and concentrated to yield **6** (5.2 g, 99%) as an oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.65 (dd, J = 6.8, 1.6 Hz, 2H), 7.43 (dd, J = 6.8, 1.6 Hz, 2H), 7.02 (broad m, 4H), 4.90 (sextet, J = 6.8 Hz, 1H), 2.33 (dd, J = 15.2, 7.6 Hz, 1H), 2.12 (dd, J = 15.2, 7.2 Hz, 1H), 1.46 (s, 9H), 1.13 (d, J = 6.8 Hz, 3H). HPLC (method 1): t_R = 10.14. LCMS m/z 372.0 and 374.0 $(M - C_4H_7)^+$, and 450.1 and 452.1 $(M + Na)^+$. HPLC (method 2): t_R = 4.43. LCMS m/z 372.0 and 374.0 $(M - C_4H_7)^+$, and 450.1 and 452.1 $(M + Na)^+$.

3-(4-Chloro-*N*-(4-fluorophenyl)phenylsulfonamido)butanoic acid (7). To a solution of *tert*-butyl 3-(4-chloro-*N*-(4-fluorophenyl)-phenylsulfonamido)butanoate **6** (5.19 g, 12.13 mmol) in dichloromethane (10 mL) at 0 °C was added trifluoroacetic acid (35 mL). The reaction mixture was allowed to warm to room temperature, stirred overnight, and then concentrated to give **7** (4.51 g, 100%) as a solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.65 (dd, J = 6.8, 1.6 Hz, 2H), 7.43 (dd, J = 6.8, 1.6 Hz, 2H), 7.02 (d, J = 6.4 Hz, 4H), 4.92 (sextet, J = 6.8 Hz, 1H), 2.50 (dd, J = 15.6, 8.0 Hz, 1H), 2.31 (dd, J = 15.6, 6.4 Hz, 1H), 1.20 (d, J = 6.8 Hz, 3H). HPLC (method 1): t_R = 6.16. LCMS m/z 372.0 and 374.0 $(M + H)^+$. HPLC (method 2): t_R = 1.25. LCMS m/z 372.0 and 374.0 $(M + H)^+$.

1-((4-Chlorophenyl)sulfonyl)-6-fluoro-2-methyl-2,3-dihydroquinolin-4(1*H*)-one (8). To an ice chilled solution of 3-(4-chloro-*N*-(4-fluorophenyl)phenylsulfonamido)butanoic acid **7** (3.91 g, 10.52 mmol) in dichloromethane (45 mL) and dimethylformamide (4 drops) was added oxalyl chloride (3.67 mL, 42.10 mmol) dropwise. The resulting solution was stirred at −5 °C for 15 min and then warmed to room temperature and stirred for 3 h. The reaction mixture was then concentrated and the residue taken up in diethyl ether, filtered through a plug of glass wool, and concentrated to give the acid chloride (4.09 g, 99%). A dichloromethane (20 mL) solution of the acid chloride was added dropwise over a 20 min period to a chilled (−5 °C) suspension of aluminum chloride (1.81, 13.6 mmol) in dichloromethane (20 mL). After stirring for 0 °C for 1 h, the reaction was warmed to room temperature and stirred overnight. The reaction mixture was then chilled in an ice bath to which was added 10% aqueous hydrogen chloride (15 mL) dropwise over 15 min. The organic portion was washed with 10% aqueous hydrogen chloride (2 × 50 mL), water (1 × 20 mL), saturated aqueous sodium bicarbonate (3 × 50 mL), brine (1 × 20 mL), dried over anhydrous magnesium sulfate, filtered, and concentrated. The crude product was then dissolved in tetrahydrofuran (40 mL) to which was added saturated aqueous sodium bicarbonate (40 mL). This mixture was stirred for 7 h and then concentrated. The residue was taken up in water and ethyl acetate. The aqueous layer was extracted with ethyl acetate (4 × 30 mL), and the combined ethyl acetate extracts washed with brine (1 × 20 mL), dried over anhydrous magnesium sulfate, filtered, and concentrated to the give the crude product, which was purified by flash chromatography eluting with 8:1 hexanes:ethyl acetate to give **8** (980 mg, 26%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.91 (dd, J = 9.2, 4.8 Hz, 1H), 7.66 (dd, J = 8.4, 3.2 Hz, 1H), 7.57 (dd, J = 6.8, 1.6 Hz, 2H), 7.45 (dd, J = 6.8, 1.6 Hz, 2H), 7.36 (ddd, J = 9.2, 7.6, 3.2 Hz, 1H), 4.91 (quintuplet d, J = 7.2, 2.0 Hz, 1H), 2.40 (dd, J = 18.0, 5.6 Hz, 1H), 2.30 (dd, J = 18.0, 2.0 Hz, 1H), 1.31 (d, J = 7.2 Hz, 3H). HPLC (method 1): t_R = 7.80, LCMS m/z 354.0 and 356.0 $(M + H)^+$. HPLC (method 2): t_R = 2.38, LCMS m/z 354.0 and 356.0 $(M + H)^+$.

5-((4-Chlorophenyl)sulfonyl)-8-fluoro-4-methyl-4,5-dihydro-2*H*-pyrazolo[4,3-*c*]quinoline (9). Dimethylformamide−dimethyl acetal (5.2 mL) solution of 1-((4-chlorophenyl)sulfonyl)-6-fluoro-2-methyl-2,3-dihydroquinolin-4(1*H*)-one **8** (922 mg, 2.61 mmol) was heated at 100 °C for 3 h. HPLC/MS analysis of the reaction mixture determined no starting material remained, so the reaction was cooled to room temperature and poured into ethyl acetate and water (50 mL each). The organic portion was washed with water (3 × 50 mL) and brine (1 × 20 mL), dried over anhydrous magnesium sulfate, filtered, and concentrated to give a reddish foam which was used directly in the next reaction. To an ethanol:glacial acetic acid solution (25:1 v/v, 12 mL) of the intermediate (982 mg, 2.40 mmol) was added hydrazine hydrate (582 mL, 12.0 mmol). The reaction mixture was stirred overnight at room temperature, concentrated, and the residue taken up in ethyl acetate (20 mL). The organic phase was washed with saturated aqueous sodium bicarbonate (3 × 25 mL) and brine (1 × 20 mL), dried over anhydrous magnesium sulfate, filtered, and concentrated. The crude product was purified by flash chromatography eluting with 2:1 hexanes:ethyl acetate to give **9** as a yellow solid. Chiral separation of the enantiomers of **9** provided **26**. See **26** for details concerning chiral separation and characterization.

3-Iodo-1-((2-(trimethylsilyl)ethoxy)methyl)-1*H*-pyrazole-4-carbaldehyde (15). Ethyl 3-iodo-1-((2-(trimethylsilyl)ethoxy)-methyl)-1*H*-pyrazole-4-carboxylate **18** (22.50 g, 56.8 mmol) and 3N aqueous sodium hydroxide (50.0 mL, 150 mmol) in dioxane (200 mL) was stirred for four days. The solution was brought to pH = 3 with 10% aqueous hydrogen chloride and extracted with methylene chloride. The combined organic extracts were dried over anhydrous magnesium sulfate, filtered, and concentrated under reduced pressure to yield 20.91 g (100%) of 3-iodo-1-((2-(trimethylsilyl)ethoxy)methyl)-1*H*-pyrazole-4-carboxylic acid as a light-yellow solid. The material was used in the subsequent reaction without further purification. HPLC (method 1): t_R = 6.22. LCMS m/z 369.0 $(M + H)^+$ and 391.0 $(M + Na)^+$. Neat borane dimethylsulfide ca. 10 M (25 mL, 250 mmol) was slowly added to 3-iodo-1-((2-(trimethylsilyl)ethoxy)methyl)-1*H*-pyrazole-4-carboxylic acid (20.91 g, 56.8 mmol) in tetrahydrofuran (250 mL) at 0 °C. After stirring for 30 min, the solution was placed into a preheated oil bath at 50 °C. After stirring for 18 h, the solution was cooled to 0 °C and ice was added. After stirring for 30 min, saturated aqueous ammonium chloride was added and stirred for an additional 30 min. The solution was extracted with methylene chloride, and the combined organic extracts were dried over anhydrous magnesium sulfate, filtered, and concentrated under reduced pressure to yield 19.50 g (97%) of 3-iodo-1-((2-(trimethylsilyl)ethoxy)methyl)-1*H*-pyrazol-4-yl)methanol as a light-yellow liquid. The material was used

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in the subsequent reaction without further purification. HPLC (method 1): t_R = 5.72. LCMS m/z 355.0 $(M + H)^+$. Dess−Martin periodinane (13.80 g, 32.5 mmol) was added to a heterogeneous mixture of (3-iodo-1-((2-(trimethylsilyl)ethoxy)methyl)-1*H*-pyrazol-4-yl)methanol (7.19 g, 20.3 mmol) and sodium bicarbonate (17.44 g, 208 mmol) in methylene chloride (200 mL). After stirring for 24 h, the heterogeneous mixture was diluted with saturated aqueous sodium sulfite and water and then extracted with diethyl ether. The combined organic extracts were dried over anhydrous magnesium sulfate, filtered, and concentrated. The residue was flash chromatographed on silica using 99:1 49:1, 24:1, 23:2, 22:3, 21:4, and 4:1 hexanes:ethyl acetate as the eluant to yield 7.15 g (100%) of **15** as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.71 (s, 1H), 8.00 (s, 1H), 5.44 (s, 2H), 3.58 (m, 2H), 0.90 (m, 2H), −0.03 (s, 9H). HPLC (method 1): t_R = 7.51. LCMS m/z 294.9 $(M + H)^+$. HPLC (method 2): t_R = 2.23. LCMS m/z 294.9 $(M + H)^+$.

Ethyl 5-Iodo-1-((2-(trimethylsilyl)ethoxy)methyl)-1*H*-pyrazole-4-carboxylate (17) and Ethyl 3-Iodo-1-((2-(trimethylsilyl)-ethoxy)methyl)-1*H*-pyrazole-4-carboxylate (18). Isoamylnitrite (400 mL, 2.98 mol) was added to a heterogeneous mixture of ethyl 3-amino-1*H*-pyrazole-4-carboxylate **16** (47.69 g, 307 mmol) in diiodomethane (800 mL) at −10 °C over a period of 30 min. The heterogeneous mixture was placed into a preheated oil bath at 100 °C for 2 h. The solution was cooled to ambient temperature, diluted with saturated aqueous sodium sulfite, and extracted with ethyl acetate. The combined organic extracts were dried over anhydrous magnesium sulfate, filtered, and concentrated under reduced pressure. The residue was flash chromatographed on silica using 9:1, 4:1, 7:3, 3:2, and 1:1 hexanes:ethyl acetate as the eluant to yield 53.85 g (66%) of ethyl 3-iodo-1*H*-pyrazole-4-carboxylate as a light-yellow solid. Sodium hydride (6.00 g, 150 mmol) as a 60% dispersion in mineral oil was added to a solution of ethyl 3-iodo-1*H*-pyrazole-4-carboxylate (28.21 g, 106 mmol) in tetrahydrofuran (400 mL). After stirring for 1 h, (2-(chloromethoxy)ethyl)trimethylsilane (25.0 mL, 142 mmol) was added. After stirring for 18 h, the heterogeneous mixture was diluted with saturated aqueous sodium bicarbonate and extracted with diethyl ether. The combined organic extracts were dried over anhydrous magnesium sulfate, filtered, and concentrated under reduced pressure. The residue was flash chromatographed with 49:1, 24:1, 23:2, 22:3, 21:4, and 4:1 hexanes:ethyl acetate as the eluant to first 18.00 g (43%) of ethyl 5-iodo-1-((2-(trimethylsilyl)ethoxy)methyl)-1*H*-pyrazole-4-carboxylate **17** as a light-yellow liquid then 22.50 g (54%) of ethyl 3-iodo-1-((2-(trimethylsilyl)-ethoxy)methyl)-1*H*-pyrazole-4-carboxylate **18** as a light-yellow liquid.

Ethyl 5-Iodo-1-((2-(trimethylsilyl)ethoxy)methyl)-1H-pyrazole-4-carboxylate (17). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.02 (s, 1H), 5.58 (s, 2H), 4.33 (q, J = 7.2 Hz, 2H), 3.60 (t, J = 8.1 Hz, 2H), 1.37 (t, J = 7.2 Hz, 3H), 0.91 (t, J = 8.1 Hz, 2H), −0.03 (s, 9H).

Ethyl 3-Iodo-1-((2-(trimethylsilyl)ethoxy)methyl)-1H-pyrazole-4-carboxylate (18). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.97 (s, 1H), 5.40 (s, 2H), 4.31 (q, J = 7.2 Hz, 2H), 3.58 (t, J = 8.4 Hz, 2H), 1.35 (t, J = 7.2 Hz, 3H), 0.90 (t, J = 8.4 Hz, 2H), −0.03 (s, 9H).

3-(2-Bromo-4,5-difluorophenyl)-1-((2-(trimethylsilyl)-ethoxy)methyl)-1*H*-pyrazole-4-carbaldehyde (20). Tetrakis-(triphenylphosphine)palladium(0) (1.29 g, 1.12 mmol), 3-iodo-1-((2-(trimethylsilyl)ethoxy)methyl)-1*H*-pyrazole-4-carbaldehyde **15** (3.53 g, 10.0 mmol), 2-bromo-4,5-difluorophenylboronic acid **19** (10.17 g, 42.9 mmol), and potassium carbonate (15.91 g, 115 mmol) was placed into a flask that was then evacuated and refilled with nitrogen three times. Water (40 mL) and 1,2-dimethoxyethane (40 mL) were added, and the solution placed into a preheated oil bath at 80 °C. After stirring for 18 h, the solution was extracted with diethyl ether. The combined organic extracts were dried over anhydrous magnesium sulfate, filtered, and concentrated. The residue was flash chromatographed on silica using 49:1, 24:1, 23:2, 22:3, 21:4, and 4:1 hexanes:ethyl acetate as the eluant to yield 2.10 g pure (50%) and 1.00 g slightly impure (<24%) of **20** as an orange oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.76 (s, 1H), 8.19 (s, 1H), 7.53 (dd, J = 9.3, 7.2 Hz, 1H), 7.30 (dd, J = 10.2, 8.4 Hz, 1H), 5.50 (s, 2H), 3.65 (m, 2H), 0.94 (m, 2H), −0.03 (s, 9H). HPLC (method 1): t_R = 9.67. LCMS m/z 417.0 and 419.0 $(M + H)^+$. HPLC (method 2): t_R = 3.96. LCMS m/z 417.0 and 419.0 $(M + H)^+$.

***N*-((3-(2-Bromo-4,5-difluorophenyl)-1-((2-(trimethylsilyl)ethoxy)-methyl)-1*H*-pyrazol-4-yl)(cyclopropyl)methyl)-4-(trifluoromethyl)-benzenesulfonamide (21).** Titanium(IV) isopropoxide (3.0 mL, 10.2 mmol) was added to a solution of 3-(2-bromo-4,5-difluorophenyl)-1-((2-(trimethylsilyl)ethoxy)methyl)-1*H*-pyrazole-4-carbaldehyde (slightly impure 1.00 g, < 2.40 mmol) and 4-(trifluoromethyl)-benzenesulfonamide (970 mg, 4.31 mmol) in tetrahydrofuran (10 mL). After stirring for 18 h, the solution was diluted with methylene chloride and then water, and the heterogeneous solution was filtered through a pad of Celite. The filtrate was extracted with methylene chloride, the combined organic extracts were dried over anhydrous magnesium sulfate, filtered, and concentrated. The residue was flash chromatographed on silica using 9:1, 4:1, 7:3, and 3:2 hexanes:ethyl acetate as the eluant to afford a mixture of products containing *N*-((3-(2-bromo-4,5-difluorophenyl)-1-((2-(trimethylsilyl)ethoxy)methyl)-1*H*-pyrazol-4-yl)-methylene)-4-(trifluoromethyl)benzenesulfonamide which was used without further purification. A 1.0 M solution of cyclopropyl magnesium bromide in tetrahydrofuran (20.0 mL, 20.0 mmol) was slowly added along the walls of the flask to a solution of *N*-((3-(2-bromo-4,5-difluorophenyl)-1-((2-(trimethylsilyl)ethoxy)methyl)-1*H*-pyrazol-4-yl)methylene)-4-(trifluoromethyl)benzenesulfonamide in tetrahydrofuran (20 mL) at −78 °C. After stirring for 30 min, the dry ice/acetone bath was removed and saturated aqueous ammonium chloride was added followed by water until the heterogeneous mixture became homogeneous. The biphasic solution was extracted with methylene chloride. The combined organic extracts were dried over anhydrous magnesium sulfate, filtered, and concentrated. The residue was flash chromatographed on silica using 9:1, 4:1, 7:3, and 3:2 hexanes:ethyl acetate as the eluant to yield 1.13 g (>60% yield over two steps) of **21** as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45 (dd, J = 9.2, 7.2 Hz, 1H), 7.11 (dd, J = 10.4, 8.0 Hz, 1H), 5.37 (d, J = 10.4 Hz, 1H), 5.33 (d, J = 10.4 Hz, 1H), 4.88 (d, J = 6.0 Hz, 1H), 3.76 (dd, J = 8.8, 6.0 Hz, 1H), 3.59 (dd, J = 8.8, 7.6 Hz, 2H), 1.01 (m, 1H), 0.95 (t, J = 10.4 Hz, 2H), 0.37 (broad m, 2H), 0.06 (m, 2H), −0.02 (s, 9H). HPLC (method 1): t_R = 12.03. LCMS m/z 666.1 and 668.1 $(M + H)^+$. HPLC (method 2): t_R = 6.04. LCMS m/z 666.1 and 668.1 $(M + H)^+$.

4-Cyclopropyl-7,8-difluoro-5-(4-(trifluoromethyl)-phenylsulfonyl)-2-((2-(trimethylsilyl)ethoxy)methyl)-4,5-dihydro-2*H*-pyrazolo[4,3-*c*]quinoline (22). Cesium acetate (2.71g, 14.1 mmol), cuprous iodide (797 mg, 4.19 mmol), and *N*-((3-(2-bromo-4,5-difluorophenyl)-1-((2-(trimethylsilyl)ethoxy)methyl)-1*H*-pyrazol-4-yl)(cyclopropyl)methyl)-4-(trifluoromethyl)benzenesulfonamide **21** (1.13 g, 1.69 mmol) were placed into a flask that was then evacuated and refilled with nitrogen. Degassed dimethylsulfoxide (1.7 mL) was added, and the heterogeneous mixture was placed into a preheated oil bath at 120 °C. After stirring for 2 h, the solution was cooled to ambient temperature. The solid that formed was washed with methylene chloride and filtered. The filtrate was concentrated and the residue was flash chromatographed on silica using 19:1, 9:1, 17:3, and 4:1 hexanes:ethyl acetate as the eluant to yield 645 mg (65%) of **22** as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.74 (dd, J = 11.2, 7.2 Hz, 1H), 7.52 (dd, J = 10.0, 8.4 Hz, 1H), 7.39 (d, J = 8.4 Hz, 2H), 7.34 (d, J = 8.4 Hz, 2H), 7.15 (s, 1H), 5.23 (s, 2H), 4.92 (d, J = 7.6 Hz, 1H), 3.50 (t, J = 8.0 Hz, 2H), 1.04 (m, 1H), 0.90 (m, 2H), 0.54 (m, 1H), 0.37 (m, 2H), 0.07 (m, 1H), −0.01 (s, 9H). HPLC (method 2): t_R = 7.22. LCMS m/z 586.2 $(M + H)^+$.

4-Cyclopropyl-7,8-difluoro-5-(4-(trifluoromethyl)-phenylsulfonyl)-4,5-dihydro-2*H*-pyrazolo[4,3-*c*]quinoline (23). 4-Cyclopropyl-7,8-difluoro-5-(4-(trifluoromethyl)phenylsulfonyl)-2-((2-(trimethylsilyl)ethoxy)methyl)-4,5-dihydro-2*H*-pyrazolo[4,3-*c*]-quinoline **22** (645 mg, 1.10 mmol) was stirred in 4N hydrogen chloride in dioxane (10 mL) and 10% aqueous hydrogen chloride (2 mL) for 18 h. The solution was concentrated to yield 500 mg (100%) of **23** as a light-yellow solid. Chiral separation of the enantiomers of **23** provided **29**. See **29** for details concerning chiral separation and characterization.

5-(4-Chlorophenylsulfonyl)-8-fluoro-4,5-dihydro-1*H*-pyrazolo[4,3-*c*]quinoline (24). Synthesized in a similar manner to Scheme 1. ^{1}H NMR (300 MHz, CD_3OD) δ 7.77−7.74 (dd, J = 8.9, 5.0 Hz, 1H), 7.40 (s, 1H), 7.36−7.33 (dd, J = 8.8, 3.0 Hz, 1H), 7.20−7.14 (m, 5H), 4.95 (s, 2H). HMRS: calcd for $C_{16}H_{11}ClFN_3O_2S$ $(M + H)^+$, 364.03173; found, 364.03148. HPLC purity (method 1): t_R = 5.61, 96%. LCMS m/z 364.0 and 366.0 $(M + H)^+$. HPLC purity (method 2): t_R = 1.09,

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95%. LCMS m/z 364.0 and 366.0 $(M + H)^+$. HPLC purity (method 3): t_R = 10.79, 97%. LCMS m/z 364.0 and 366.0 $(M + H)^+$.

5-(4-Chlorophenylsulfonyl)-7-fluoro-4,5-dihydro-1*H*-pyrazolo[4,3-*c*]quinoline (25). Synthesized in a similar manner to Scheme 1. ^{1}H NMR (300 MHz, CD_3OD) δ 7.68−7.63 (dd, J = 8.6, 6.2 Hz, 1H), 7.53−7.49 (dd, J = 10.1, 2.6 Hz, 1H), 7.38 (s, 1H), 7.22−7.14 (m, 5H), 4.96 (s, 2H). HMRS: calcd for $C_{16}H_{11}ClFN_3O_2S$ $(M + H)^+$, 364.03173; found, 364.03157. HPLC purity (method 1): t_R = 5.64, 100%. LCMS m/z 364.0 and 366.0 $(M + H)^+$. HPLC purity (method 2): t_R = 1.07, 100%. LCMS m/z 364.0 and 366.0 $(M + H)^+$. HPLC purity (method 3): t_R = 10.86, 100%. LCMS m/z 364.0 and 366.0 $(M + H)^+$.

(*R*)-5-((4-Chlorophenyl)sulfonyl)-8-fluoro-4-methyl-4,5-dihydro-2*H*-pyrazolo[4,3-*c*]quinoline (26). Separation of the enantiomers of **9** was achieved using 10% [A]:90% [B] isocrat, 1.0 mL/min, where [A] = 2-propanol, [B] = hexanes, on a Chiral Technologies OD column (4.6 mm × 250 mm), 10 μm packing, 220 nm detection at ambient temperature. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.78 (dd, J = 6.0, 5.1 Hz, 1H), 7.38 (dd, J = 6.0, 3.3 Hz, 1H), 7.21 (s, 1H), 7.10 (broad m, 5H), 5.60 (q, J = 6.6 Hz, 1H), 1.29 (d, J = 6.6 Hz, 3H). HMRS: calcd for $C_{17}H_{13}ClFN_3O_2S$ $(M + H)^+$, 378.04738; found, 378.04726. HPLC purity (method 1): t_R = 6.21, 97%. LCMS m/z 378.0 and 380.0 $(M + H)^+$. HPLC purity (method 2): t_R = 1.33, 95%. LCMS m/z 378.0 and 380.0 $(M + H)^+$.

(*R*)-8-Fluoro-5-(4-fluorophenylsulfonyl)-4-methyl-4,5-dihydro-1*H*-pyrazolo[4,3-*c*]quinoline (27). Synthesized in a similar manner to Scheme 1. Separation of the enantiomers was achieved using 15% [A]:85% [B] isocrat, 1.0 mL/min, where [A] = ethanol, [B] = hexanes, on a Chiral Technologies OD column (4.6 mm × 250 mm), 10 μm packing, 220 nm detection at ambient temperature. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.84−7.79 (dd, J = 5.1, 3.8 Hz, 1H), 7.38−7.34 (dd, J = 5.5, 2.9 Hz, 1H), 7.23 (s, 1H), 7.22−7.16 (m, 2H), 7.15−7.08 (dt, J = 5.7, 2.9 Hz, 1H), 6.80−6.75 (m, 2H), 5.67−5.60 (q, J = 6.9 Hz, 1H), 1.33−1.30 (d, J = 6.8 Hz, 3H). HMRS: calcd for $C_{17}H_{13}F_2N_3O_2S$ $(M + H)^+$, 362.07693; found, 362.07682. HPLC purity (method 1): t_R = 5.53, 100%. LCMS m/z 362.0 $(M + H)^+$. HPLC purity (method 2): t_R = 1.04, 100%. LCMS m/z 362.1 $(M + H)^+$.

(*R*)-7,8-Difluoro-4-methyl-5-(4-(trifluoromethyl)-phenylsulfonyl)-4,5-dihydro-1*H*-pyrazolo[4,3-*c*]quinoline (28). Synthesized in a similar manner to Scheme 4. Separation of the enantiomers was achieved using 10% [A]:90% [B] isocrat, 1.0 mL/min, where [A] = ethanol, [B] = hexanes, on a Chiral Technologies OD column (4.6 mm × 250 mm), 10 μm packing, 220 nm detection at ambient temperature. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.84 (broad s, 1H), 7.74 (dd, J = 11.1, 7.5 Hz, 1H), 7.41 (m, 3H), 7.32 (d, J = 9.0 Hz, 2H), 7.29 (s, 1H), 5.63 (q, J = 6.9 Hz, 1H), 1.32 (q, J = 6.9 Hz, 3H). HMRS: calcd for $C_{18}H_{12}F_5N_3O_2S$ $(M + H)^+$, 430.06432; found, 430.06420. HPLC purity (method 1): t_R = 7.52, 100%. LCMS m/z 430.0 $(M + H)^+$. HPLC purity (method 2): t_R = 2.08, 100%. LCMS m/z 430.0 $(M + H)^+$.

(*R*)-4-Cyclopropyl-7,8-difluoro-5-(4-fluorophenylsulfonyl)-4,5-dihydro-2*H*-pyrazolo[4,3-*c*]quinoline (29). Synthesized in a similar manner to Scheme 4. Separation of the enantiomers was achieved using 10% [A]:90% [B] isocrat, 1.0 mL/min, where [A] = 2-propanol, [B] = hexanes, on a Chiral Technologies IA column (4.6 mm × 250 mm), 5 μm packing, 220 nm detection at ambient temperature. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.75 (dd, J = 11.4, 7.5 Hz, 1H), 7.48 (dd, J = 9.9, 8. Hz, 1H), 7.20 (m, 4H), 6.79 (m, 2H), 4.96 (d, J = 7.8 Hz, 1H), 1.02 (m, 1H), 0.51 (m, 1H), 0.36 (m, 2H), 0.06 (m, 1H). HMRS: calcd for $C_{19}H_{14}F_3N_3O_2S$ $(M + H)^+$, 406.08316; found, 406.08314. HPLC purity (method 1): t_R = 7.04, 100%. LCMS m/z 406.1 $(M + H)^+$. HPLC purity (method 2): t_R = 1.75, 100%. LCMS m/z 406.1 $(M + H)^+$.

(*R*)-4-Cyclopropyl-7,8-difluoro-5-(4-(trifluoromethyl)-phenylsulfonyl)-4,5-dihydro-1*H*-pyrazolo[4,3-*c*]quinoline (30). Separation of the enantiomers of **23** was achieved using 10% [A]:90% [B] isocrat, 1.0 mL/min, where [A] = 2-propanol, [B] = hexanes, on a Chiral Technologies OD column (4.6 mm × 250 mm), 10 μm packing, 220 nm detection at ambient temperature. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.90 (broad s, 1H), 7.76 (dd, J = 11.2, 7.2 Hz, 1H), 7.50 (t, J = 9.2 Hz, 1H), 7.38 (d, J = 8.4 Hz, 2H), 7.33 (d, J = 8.4 Hz, 2H), 7.18 (s, 1H), 4.96 (d, J = 7.6 Hz, 1H), 1.03 (m, 1H), 0.54 (m, 1H), 0.40 (m, 2H), 0.09 (m, 1H). HMRS: calcd for $C_{20}H_{14}F_5N_3O_2S$ $(M + H)^+$, 456.08051; found, 456.07959. HPLC purity (method 1): t_R = 8.24, 100%. LCMS m/z 456.1 $(M + H)^+$. HPLC purity (method 2): t_R = 2.62, 100%. LCMS m/z 456.1 $(M + H)^+$. $[\alpha]_D^{25.0}$ = −133.40° (c = 5 mg/mL in 95% ethanol).

(*R*)-7,8-Difluoro-4-isopropyl-5-(4-(trifluoromethyl)-phenylsulfonyl)-4,5-dihydro-2*H*-pyrazolo[4,3-*c*]quinoline (31). Synthesized in a similar manner to Scheme 4. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.70 (dd, J = 11.2, 7.2 Hz, 1H), 7.48 (d, J = 10.0, 8.4 Hz, 1H), 7.39 (d, J = 8.8 Hz, 2H), 7.34 (d, J = 8.8 H, 2H), 7.18 (s, 1H), 4.89 (d, J = 9.6 Hz, 1H), 2.69 (s, 1H), 1.51 (m, 1H), 1.09 (d, J = 6.8 Hz, 3H), 0.83 (d, J = 6.4 Hz, 3H). HMRS: calcd for $C_{20}H_{16}F_5N_3O_2S$ $(M + H)^+$, 458.09562; found, 458.09518. HPLC purity (method 1): t_R = 8.69, 100%. LCMS m/z 458.1 $(M + H)^+$. HPLC purity (method 2): t_R = 3.02, 100%. LCMS m/z 458.1 $(M + H)^+$.

(*R*)-4-Cyclopropyl-5-(4-(trifluoromethyl)phenylsulfonyl)-4,5-dihydro-1*H*-pyrazolo[4,3-*c*]quinoline (32). Synthesized in a similar manner to Scheme 5. Separation of the major and minor enantiomers was achieved using 10% [A]:90% [B] isocrat, 1.0 mL/min, where [A] = ethanol, [B] = hexanes, on a Chiral Technologies OD column (4.6 mm × 250 mm), 10 μm packing, 220 nm detection at ambient temperature. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.90 (dd, J = 9.2, 2.0 Hz, 1H), 7.64 (dd, J = 7.0, 1.5 Hz, 1H), 7.48−7.32 (m, 4H), 7.28−7.25 (m, 2H), 7.21 (s, 1H), 4.95 (d, J = 7.8 Hz, 1H), 1.06−1.03 (m, 1H), 0.52−0.34 (m, 3H), 0.15−0.05 (m, 1H). HMRS: calcd for $C_{20}H_{16}F_3N_3O_2S$ $(M + H)^+$, 420.09881; found, 420.09842. HPLC purity (method 1): t_R = 7.12, 100%. LCMS m/z 420.1 $(M + H)^+$. HPLC purity (method 2): t_R = 1.80, 100%. LCMS m/z 420.1 $(M + H)^+$.

(*R*)-4-Cyclopropyl-8-fluoro-5-(4-(trifluoromethyl)-phenylsulfonyl)-4,5-dihydro-1*H*-pyrazolo[4,3-*c*]quinoline (33). Synthesized in a similar manner to Scheme 5. Separation of the major and minor enantiomers was achieved using 10% [A]:90% [B] isocrat, 1.0 mL/min, where [A] = ethanol, [B] = hexanes, on a Chiral Technologies OD column (4.6 mm × 250 mm), 10 μm packing, 220 nm detection at ambient temperature. ^{1}H NMR (300 MHz, CD_3OD) δ 7.85−7.80 (dd, J = 8.9, 5.1 Hz, 1H), 7.50−7.46 (m, 2H) 7.40−7.34 (m, 4H), 7.22−7.16 (td, J = 8.7, 2.9 Hz, 1H), 5.07−5.04 (d, J = 7.5 Hz, 1H), 1.02−0.94 (m, 1H), 0.54−0.47 (m, 1H), 0.41−0.31 (m, 2H), 0.17−0.12 (m, 1H). HMRS: calcd for $C_{20}H_{15}F_4N_3O_2S$ $(M + H)^+$, 438.08939; found, 438.08958. HPLC purity (method 1): t_R = 7.65, 100%. LCMS m/z 438.1 $(M + H)^+$. HPLC purity (method 2): t_R = 2.16, 100%. LCMS m/z 438.1 $(M + H)^+$.

(*R*)-4-Cyclopropyl-8-fluoro-5-(6-(trifluoromethyl)pyridin-3-ylsulfonyl)-4,5-dihydro-2*H*-pyrazolo[4,3-*c*]quinoline (34). Hydrogen chloride (4N) in dioxane (10 mL) was added to a solution of (*R*)-4-cyclopropyl-8-fluoro-5-(6-(trifluoromethyl)pyridin-3-ylsulfonyl)-2-((2-(trimethylsilyl)ethoxy)methyl)-4,5-dihydro-2*H*-pyrazolo[4,3-*c*]-quinoline **40** (1.30 g, 2.29 mmol) in methanol (20 mL), which was placed into a preheated oil bath at 50 °C. After stirring for 1.5 h, the solution was concentrated. The residue was diluted with saturated aqueous sodium bicarbonate and extracted with methylene chloride. The combined organic extracts were dried over anhydrous magnesium sulfate, filtered, and concentrated. The residue was flash chromatographed on silica using 9:1, 4:1, 7:3, and 3:2 hexanes:ethyl acetate as eluant to yield 1.00 g (100%) of **34** as a light-yellow oil. Separation of the major and minor enantiomers was achieved using HPLC 5% [A]:95% [B] isocrat, 55 mL/min, where [A] = 2-propanol, [B] = hexanes, on a Chiral Technologies AD column (5 cm × 50 cm), 20 μm packing, 220 nM detection at ambient temperature. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.95 (braod s, 1H), 8.53 (s, 1H), 7.85 (dd, J = 8.8 and 4.8 Hz, 1H), 7.40 (m, 3H), 7.14 (dt, J = 2.8, 8.4 Hz, 1H), 5.00 (d, J = 7.6 Hz, 1H), 1.01 (m, 1H), 0.54 (m, 1H), 0.39 (m, 2H), 0.09 (m, 1H). HMRS: calcd for $C_{19}H_{14}F_4N_4O_2S$ $(M + H)^+$, 439.08464; found, 439.08427. HPLC purity (method 1): t_R = 6.98, 100%. LCMS m/z 439.1 $(M + H)^+$. HPLC purity (method 2): t_R = 1.74, 100%. LCMS m/z 439.1 $(M + H)^+$. $[\alpha]_D^{25.0}$ = −91.36° (c = 5 mg/mL in 95% ethanol).

2-Bromo-5-fluorophenylboronic acid (36).[33] Isopropylmagnesium chloride (36.5 mmol, 2.0 M in tetrahydrofuran) was slowly added to a −42 °C tetrahydrofuran (2.0 M) solution of 1-bromo-4-fluoro-2-iodobenzene **35** (10 g, 33.2 mmol) under nitrogen and stirred at −42 °C for 2 h. Triisopropylborate (11.4 mL, 49.8 mmol) was added, and the reaction was warmed to room temperature and stirred overnight.

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Then 1N aqueous sodium hydroxide (20 mL) was added and the mixture stirred for 1h. The pH was adjusted to 3 with 3N aqueous hydrogen chloride, and the sample was extracted (2 × 20 mL) with ethyl acetate. The combined organics were dried over anhydrous magnesium sulfate and filtered through Celite, and the solvent was removed to obtain (5.75 g, 79% yield) of **36** as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.68−7.64 (dd, J = 9.1, 3.2 Hz, 1H), 7.54−7.49 (dd, J = 8.8, 4.8 Hz, 1H), 7.08−7.01 (1H, m), 5.76 (2H, s).

3-(2-Bromo-5-fluorophenyl)-1-((2-(trimethylsilyl)ethoxy)-methyl)-1*H*-pyrazole-4-carbaldehyde (37). Tetrakis-(triphenylphosphine)palladium(0) (224 mg, 194 mmol), 3-iodo-1-((2-(trimethylsilyl)ethoxy)methyl)-1*H*-pyrazole-4-carbaldehyde **15** (1.71 g, 10.0 mmol), 2-bromo-5-fluorophenylboronic acid **36** (1.36 g, 6.23 mmol), and potassium carbonate (2.86 g, 20.7 mmol) were placed into a flask that was evacuated and refilled with nitrogen three times. Water (5 mL) and 1,2-dimethoxyethane (5 mL) were added and the solution placed into a preheated oil bath at 80 °C. After stirring for 18 h, the solution was extracted with diethyl ether. The combined organic extracts were dried over anhydrous magnesium sulfate, filtered, and concentrated. The residue was flash chromatographed on silica using 49:1, 24:1, 23:2, 22:3, 21:4, and 4:1 hexanes:ethyl acetate as eluant to yield 590 mg (73%) of **37** as a brown oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.73 (s, 1H), 8.19 (s, 1H), 7.43 (m, 2H), 7.14 (dt, J = 1.8, 8.0 Hz, 1H), 5.52 (s, 2H), 3.67 (t, J = 8.4 Hz, 2H), 0.95 (t, J = 8.4 Hz, 2H), 0.01 (s, 9H). HPLC (method 1): t_R = 9.26. LCMS m/z 399.0 and 401.0 $(M + H)^+$. HPLC (method 2): t_R = 3.58. LCMS m/z 399.0 and 401.0 $(M + H)^+$.

6-(Trifluoromethyl)pyridine-3-sulfonyl chloride (38).[34] Thionyl chloride (31 mL, 0.43 mol) was slowly added to 175 mL of water at 0 °C. During the addition, the temperature was maintained between 0 and 5 °C. After addition, the solution was warmed to 15 °C and 0.47 g (4.8 mmol) of cuprous chloride was added. The solution was diluted with 100 mL of water and cooled back to 0 °C. A solution of 7.21 g (0.10 mol) of sodium nitrite in 100 mL of water was slowly added to a solution of 15.39 g (0.10 mol) of 5-amino-2-(trifluoromethyl)pyridine in concentrated aqueous hydrogen chloride (125 mL) at 0 °C. During addition, the temperature was maintained between 0 and 5 °C. This mixture was then slowly added to the above prepared solution so as to maintain a temperature between 0 and 5 °C. A voluminous precipitate formed. The mixture was stirred for an additional 30 min after addition, and the solid was then collected by filtration. The solid was washed with water and dissolved in chloroform. The solution was dried over anhydrous magnesium sulfate and filtered, and the solvent was removed to afford 18.03 g (77%) of **38** as a tan solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.35 (s, 1H), 8.53 (d, J = 8.4 Hz, 1H), 7.98 (d, J = 8.4 Hz, 1H).

(*R*)-*N*-((3-(2-Bromo-5-fluorophenyl)-1-((2-(trimethylsilyl)-ethoxy)methyl)-1*H*-pyrazol-4-yl)(cyclopropyl)methyl)-6-(trifluoromethyl)pyridine-3-sulfonamide (39). Titanium(IV) isopropoxide (1.1 mL, 3.75 mmol) was added to a solution of 3-(2-bromo-5-fluorophenyl)-1-((2-(trimethylsilyl)ethoxy)methyl)-1*H*-pyrazole-4-carbaldehyde **37** (590 mg, 1.48 mmol) and (*S*)-(−)-*tert*-butanesulfinamide (254 mg, 2.10 mmol) in tetrahydrofuran (10 mL). After stirring for 18 h, the solution was placed into a preheated oil bath at 60 °C. After stirring for 2 h, the solution was cooled to ambient temperature, diluted with methylene chloride and then water, and the heterogeneous solution was filtered through Celite. The filtrate was extracted with methylene chloride, and the combined organic extracts were dried over anhydrous magnesium sulfate, filtered, and concentrated to yield (*S*)-(*E*)-*N*-((3-(2-bromo-5-fluorophenyl)-1-((2-(trimethylsilyl)ethoxy)methyl)-1*H*-pyrazol-4-yl)methylene)-2-methylpropane-2-sulfinamide as an oil which was used without further purification. A 1.0 M solution of cyclopropyl magnesium bromide in tetrahydrofuran (15.0 mL, 15.0 mmol) was slowly added dropwise to a solution of (*S*)-(*E*)-*N*-((3-(2-bromo-5-fluorophenyl)-1-((2-(trimethylsilyl)ethoxy)methyl)-1*H*-pyrazol-4-yl)methylene)-2-methylpropane-2-sulfinamide in methylene chloride (15 mL) at −78 °C. After stirring for 30 min, the dry ice/acetone bath was removed and saturated aqueous sodium bicarbonate was added followed by water until the heterogeneous mixture became homogeneous. The biphasic solution was extracted with methylene chloride, the combined organic extracts were dried over anhydrous magnesium sulfate, filtered, and concentrated to yield (*S*)-*N*-((*R*)-(3-(2-bromo-5-fluorophenyl)-1-((2-(trimethylsilyl)ethoxy)methyl)-1*H*-pyrazol-4-yl)(cyclopropyl)methyl)-2-methylpropane-2-sulfinamide as a brown oil which was used without further purification. HPLC (method 1): t_R = 9.63, LCMS m/z 544.0 and 546.0 $(M + H)^+$ for the major diastereomer and t_R = 10.03, LCMS m/z 544.0 and 546.0 $(M + H)^+$ for the minor diastereomer. Aqueous hydrogen chloride (4N) in dioxane (1 mL) was added to a solution of (*S*)-*N*-((*R*)-(3-(2-bromo-5-fluorophenyl)-1-((2-(trimethylsilyl)ethoxy)methyl)-1*H*-pyrazol-4-yl)-(cyclopropyl)methyl)-2-methylpropane-2-sulfinamide in methanol (20 mL). After stirring for 1 h, the solution was concentrated. The residue was diluted with 1N aqueous sodium hydroxide and extracted with methylene chloride, and the combined organic extracts were dried over anhydrous magnesium sulfate, filtered, and concentrated to yield (*R*)-(3-(2-bromo-5-fluorophenyl)-1-((2-(trimethylsilyl)ethoxy)-methyl)-1*H*-pyrazol-4-yl)(cyclopropyl)methanamine as a brown oil which was used without further purification. HPLC (method 1): t_R = 5.57, LCMS m/z 423.0 and 425.0 $(M - NH_2)^+$, 440.0 and 442.0 $(M + H)^+$. 6-(Trifluoromethyl)pyridine-3-sulfonyl chloride **38** (835 mg, 3.40 mmol) was added to a solution of (*R*)-(3-(2-bromo-5-fluorophenyl)-1-((2-(trimethylsilyl)ethoxy)methyl)-1*H*-pyrazol-4-yl)(cyclopropyl)-methanamine and 4-dimethylaminopyridine (120 mg, 982 mmol) in methylene chloride (5 mL) and triethylamine (1 mL). After stirring for 18 h, the solution was concentrated and the residue was flash chromatographed on silica using 9:1, 4:1, 7:3, and 3:2 hexanes:ethyl acetate as eluant to yield 525 mg (55% over four steps) of **39** as an oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.03 (s, 1H), 8.16 (d, J = 8.0 Hz, 1H), 7.74 (d, J = 8.0 Hz, 1H), 7.53 (s, 1H), 7.37 (dd, J = 8.0, 2.4 Hz, 1H), 7.24 (m, 1H), 7.07 (dt, J = 2.4, 8.0 Hz, 1H), 5.39 (d, J = 10.8 Hz, 1H), 5.35 (d, J = 10.8 Hz, 1H), 5.00 (d, J = 6.4 Hz, 1H), 3.82 (dd, J = 8.8, 6.4 Hz, 1H), 3.61 (t, J = 8.4 Hz, 2H), 1.04 (m, 1H), 0.92 (t, 8.4 Hz, 2H), 0.45 (m, 1H), 0.37 (m, 1H), 0.18 (m, 1H), 0.09 (m, 1H), −0.02 (s, 9H). HPLC (method 1): t_R = 11.01 LCMS m/z 649.1 and 651.1 $(M + H)^+$. HPLC (method 2): t_R = 5.30. LCMS m/z 649.1 and 651.1 $(M + H)^+$.

(*R*)-4-Cyclopropyl-8-fluoro-5-(6-(trifluoromethyl)pyridin-3-ylsulfonyl)-2-((2-(trimethylsilyl)ethoxy)methyl)-4,5-dihydro-2*H*-pyrazolo[4,3-*c*]quinoline (40). Cesium acetate (1.46 g, 7.61 mmol), cuprous iodide (446 mg, 2.34 mmol), and (*R*)-*N*-((3-(2-bromo-5-fluorophenyl)-1-((2-(trimethylsilyl)ethoxy)methyl)-1*H*-pyrazol-4-yl)-(cyclopropyl)methyl)-6-(trifluoromethyl)pyridine-3-sulfonamide **39** (613 mg, 944 mmol) were placed into a flask that was evacuated and refilled with nitrogen. Degassed dimethylsulfoxide (1.0 mL) was added, and the heterogeneous mixture was placed into a preheated oil bath at 160 °C. After stirring for 30 min, the solution was cooled to ambient temperature, the solid that formed was washed with methylene chloride and filtered through Celite. The filtrate was concentrated, and the residue was flash chromatographed on silica using 19:1, 9:1, 17:3, 4:1, and 3:1 hexanes:ethyl acetate as eluant to yield 405 mg (75%) of **40** as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.57 (d, J = 1.6 Hz, 1H), 7.70 (dd, J = 8.8, 6.0 Hz, 1H), 7.62 (dd, J = 9.6, 2.4 Hz, 1H), 7.44 (dd, J = 8.0, 1.6 Hz, 1H), 7.36 (d, J = 8.4 Hz, 1H), 7.23 (s, 1H), 7.11 (dt, J = 2.4, 8.4 Hz, 1H), 5.29 (d, J = 10.8 Hz, 1H), 5.22 (d, J = 10.8 Hz, 1H), 4.99 (d, J = 7.6 Hz, 1H), 3.53 (t, J = 8.4 Hz, 2H), 1.04 (m, 1H), 0.89 (m, 2H), 0.55 (m, 1H), 0.37 (m, 2H), 0.08 (m, 1H), −0.02 (s, 9H). HPLC (method 1): t_R = 12.08, LCMS m/z 569.1 $(M + H)^+$. HPLC (method 2): t_R = 6.18, LCMS m/z 569.1 $(M + H)^+$.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

NMR and HPLC/MS data for all numbered compounds as well as the DMPK experimental protocols are provided. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

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■ ABBREVIATIONS USED

AcOH, acetic acid; AD, Alzheimer's disease; Aβ, β-amyloid peptides; amu, atomic mass units; APP, amyloid precursor protein; AUC, area under the curve; CNS, central nervous system; *c*-PrMgBr, cyclopropylmagnesium bromide; DCE, 1,2-dichloroethane; DMAP, 4-dimethylaminopyridine; DME, 1,2-dimethoxyethane; DMF, dimethylformamide; DMF-DMA, dimethylformamide−dimethylacetal; DMP, Dess−Martin periodinane; DMPK, drug metabolism and pharmacokinetics; DMS, dimethylsulfide; DMSO, dimethylsulfoxide; dr, diastereomeric ratio; EtOH, ethanol; Gluc Met, glucuronidation metabolism; HES-1, hairy and enhancer of split homologue-1; HPLC, High pressure liquid chromatography; IND, investigational new drug; *i*-PrMgCl, isopropylmagnesium chloride; log *P*, octanol−water partition coefficient; MDCK cells, Madin−Darby canine kidney cells; MDR1-MDCK cells, multidrug resistance gene 1 transfected Madin−Darby canine kidney cells; MeOH, methanol; ND, not determined; NFT, neurofibrillary tangle; Ox Met, oxidative metabolism; $Pd(PPh_3)_4$, tetrakis(triphenylphosphine)-palladium(0); P-gp, permeability-glycoprotein; PK/PD, pharmacokinetic/pharmacodynamic; po, oral administration; Q_H, hepatic blood flow; SAR, structure−activity relationship; SEM-Cl, (2-(chloromethoxy)ethyl)trimethylsilane; TEA, triethylamine; TFA, trifluoroacetic acid; THF, tetrahydrofuran; Ti(O-*i*-Pr), titanium(IV) isopropoxide; UDP-GA, uridine 5′-diphosphate−glucuronic acid

■ REFERENCES

(1) Alzheimer's Association. **2012** Alzheimer's disease facts and figures. *Alzheimer's Dementia***2012**, *8*, 131−168.

(2) (a) Querfurth, H. W.; LaFerla, F. M. Alzheimer's Disease. *N. Engl. J. Med.* **2010**, *362*, 329−344. (b) Ballard, C.; Gauthier, S.; Corbett, A.; Brayne, C.; Aarsland, D.; Jones, E. Alzheimer's disease. *Lancet* **2011**, *377*, 1019−1031.

(3) Hardy, J.; Allsop, D. Amyloid deposition as the central event in the aetiology of Alzheimer's disease. *Trends Pharmacol. Sci.* **1991**, *12*, 383−388.

(4) Lee, V. M. Y.; Goedert, M.; Trojanowski, J. Q. Neurodegenerative tauopathies. *Annu. Rev. Neurosci.* **2001**, *24*, 1121−1159.

(5) (a) Hardy, J. A.; Higgins, G. A. Alzheimer's Disease: The Amyloid Cascade Hypothesis. *Science* **1992**, *256*, 184−185. (b) Selkoe, D. J. The molecular pathology of Alzheimer's disease. *Neuron* **1991**, *6*, 487−498.

(6) Sinha, S.; Lieberburg, I. Cellular mechanisms of β-amyloid production and secretion. *Proc. Natl. Acad. Sci. U. S. A.* **1999**, *96*, 11049−11053.

(7) (a) D'Onofrio, G.; Panza, F.; Frisardi, V.; Solfrizzi, V.; Imbimbo, B. P.; Paroni, G.; Cascavilla, L.; Seripa, D.; Pilotto, A. Advances in the identification of γ-secretase inhibitors for the treatment of Alzheimer's disease. *Expert Opin. Drug Discovery* **2012**, *7*, 19−37. (b) De Strooper, B.; Vassar, R.; Golde, T. The secretases: enzymes with therapeutic potential in Alzheimer disease. *Nature Rev. Neurol.* **2010**, *6*, 99−107. (c) Citron, M. Alzheimer's disease: strategies for disease modification. *Nature Rev. Drug Discovery* **2010**, *9*, 387−398. (d) Kreft, A. F.; Martone, R.; Porte, A. Recent Advances in the Identification of γ-Secretase Inhibitors To Clinically Test the Aβ Oligomer Hypothesis of Alzheimer's Disease. *J. Med. Chem.* **2009**, *52*, 6169−6188.

(8) (a) Lleo, A.; Saura, C. A. γ-Secretase Substrates and their Implications for Drug Development in Alzheimer's Disease. *Curr. Top. Med. Chem.* **2011**, *11*, 1513−1527. (b) Haapasalo, A.; Kovacs, D. M. The Many Substrates of Presenilin/γ-Secretase. *J. Alzheimer's Dis.* **2011**, *25*, 3−28. (c) Lleo, A. Activity of γ-Secretase on Substrates Other than APP. *Curr. Top. Med. Chem.* **2008**, *8*, 9−16.

(9) (a) Wong, G. T.; Manfras, D.; Poulet, F. M.; Zhang, Q.; Josien, H.; Bara, T.; Engstrom, L.; Pinzon-Ortiz, M.; Fine, J. S.; Lee, H. J. J.; Zhang, L.; Higgins, G. A.; Parker, E. M. Chronic Treatment with the γ-Secretase Inhibitor LY-411,575 Inhibits β-Amyloid Peptide Production and Alters Lymphopoiesis and Intestinal Cell Differentiation. *J. Biol. Chem.* **2004**, *279*, 12876−12882. (b) Milano, J.; McKay, J.; Dagenais, C.; Foster-Brown, L.; Pognan, F.; Gadient, R.; Jacobs, R. T.; Zacco, A.; Greenberg, B.; Ciaccio, P. J. Modulation of Notch Processing by γ-Secretase Inhibitors Causes Intestinal Goblet Cell Metaplasia and Induction of Genes Known to Specify Gut Secretory Lineage Differentiation. *Toxicol. Sci.* **2004**, *82*, 341−358.

(10) (a) Clarke, E. E.; Churcher, I.; Ellis, S.; Wrigley, J. D. J.; Lewis, H. D.; Harrison, T.; Shearman, M. S.; Beher, D. Intra- or Intercomplex Binding to the γ-Secretase Enzyme. A Model to Differentiate Inhibitor Classes. *J. Biol. Chem.* **2006**, *281*, 31279−31289. (b) Tian, G.; Sobotka-Briner, C. D.; Zysk, J.; Liu, X.; Birr, C.; Sylvester, M. A.; Edwards, P. D.; Scott, C. D.; Greenberg, B. D. Linear Non-competitive Inhibition of Solubilized Human γ-Secretase by Pepstatin A Methylester, L685458, Sulfonamides, and Benzodiazepines. *J. Biol. Chem.* **2002**, *277*, 31499−31505.

(11) See Supporting Information for experimental details.

(12) Truong, A. P.; Aubele, D. L.; Probst, G. D.; Neitzel, M. L.; Semko, C. M.; Bowers, S.; Dressen, D.; Hom, R. K.; Konradi, A. W.; Sham, H. L.; Garofalo, A. W.; Keim, P. S.; Wu, J.; Dappen, M. S.; Wong, K.; Goldbach, E.; Quinn, K. P.; Sauer, J.-M.; Brigham, E. F.; Wallace, W.; Nguyen, L.; Hemphill, S. S.; Bova, M. P.; Bard, F.; Yednock, T. A.; Basi, G. Design, synthesis, and structure−activity relationship of novel orally efficacious pyrazole/sulfonamide based dihydroquinoline γ-secretase inhibitors. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 4920−4923.

(13) (a) Ye, X. M.; Konradi, A. W.; Smith, J.; Xu, Y-z.; Dressen, D.; Garofalo, A. W.; Marugg, J.; Sham, H. L.; Truong, A. P.; Jagodzinski, J.; Pleiss, M.; Zhang, H.; Goldbach, E.; Sauer, J.-M.; Brigham, E.; Bova, M.; Basi, G. S. Discovery of a novel sulfonamide−pyrazolopiperidine series as potent and efficacious γ-secretase inhibitors. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 2195−2199. (b) Ye, X. M.; Konradi, A. W.; Smith, J.; Aubele, D. L.; Garofalo, A. W.; Marugg, J.; Neitzel, M. L.; Semko, C. M.; Sham, H. L.; Sun, M.; Truong, A. P.; Wu, J.; Zhang, H.; Goldbach, E.; Sauer, J.-M.; Brigham, E. F.; Bova, M.; Basi, G. S. Discovery of a novel sulfonamide-pyrazolopiperidine series as potent and efficacious γ-secretase inhibitors (Part II). *Bioorg. Med. Chem. Lett.* **2010**, *20*, 3502−3506.

(14) (a) Aubele, D. L.; Truong, A. P.; Dressen, D. B.; Probst, G. D.; Bowers, S.; Mattson, M. N.; Semko, C. M.; Sun, M.; Garofalo, A. W.; Konradi, A. W.; Sham, H. L.; Zmolek, W.; Wong, K.; Goldbach, E.; Quinn, K. P.; Sauer, J.-M.; Brigham, E. F.; Wallace, W.; Nguyen, L.; Bova, M. P.; Hemphill, S. S.; Basi, G. Design, synthesis and structure−activity relationship of novel [3.3.1] bicyclic sulfonamide-pyrazoles as potent γ-secretase inhibitors. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 5791−5794. (b) Mattson, M. N.; Neitzel, M. L.; Quincy, D. A.; Semko, C. M.; Garofalo, A. W.; Keim, P. S.; Konradi, A. W.; Pleiss, M. A.; Sham, H. L.; Brigham, E. F.; Goldbach, E. G.; Zhang, H.; Sauer, J.-M.; Basi, G. S. Discovery of sulfonamide-pyrazole γ-secretase inhibitors. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 2148−2150. (c) Basi, G. S.; Hemphill, S.; Brigham, E. F.; Liao, A.; Aubele, D. L.; Baker, J.; Barbour, R.; Bova, M.; Chen, X.-H.; Dappen, M. S.; Eichenbaum, T.; Goldbach, E.; Hawkinson, J.; Lawler-Herbold, R.; Hu, K.; Hui, T.; Jagodzinski, J. J.; Keim, P. S.; Kholodenko, D.; Latimer, L. H.; Lee, M.; Marugg, J.; Mattson, M. N.; McCauley, S.; Miller, J. L.; Motter, R.; Mutter, L.; Neitzel, M. L.; Ni, H.; Nguyen, L.; Quinn, K.; Ruslim, L.; Semko, C. M.; Shapiro, P.; Smith, J.; Soriano, F.; Szoke, B.; Tanaka, K.; Tang, P.; Tucker, J. A.; Ye, X. M.; Yu, M.; Wu, J.; Xu, Y-z.; Garofalo, A. W.; Sauer, J. M.; Konradi, A. W.; Ness, D.; Shopp, G.; Pleiss, M. A.; Freedman, S. B.; Schenk, D. Amyloid precursor protein selective gamma-secretase inhibitors for treatment of Alzheimer's disease. *Alzheimer's Res. Ther.* **2010**, *2*, 36−56. (d) Bowers, S.; Probst, G. D.; Truong, A. P.; Hom, R. K.; Konradi, A. W.; Sham, H. L.; Garofalo, A. W.; Wong, K.; Goldbach, E.; Quinn, K. P.; Sauer, J.-M.; Wallace, W.; Nguyen, L.; Hemphill, S. S.; Bova, M. P.; Basi, G. S. N-

Bridged bicyclic sulfonamides as inhibitors of γ-secretase. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 6952−6956.
(15) Gavina, B. D.; Forte, B.; Mantegani, S.; Varasi, M.; Vianello, P. Pyrazolyl-indole derivatives active as kinase inhibitors, process for their preparation and pharmaceutical compositions comprising them. WO2005005414, 2005.
(16) Dess, D. B.; Martin, J. C. Readily accessible 12-I-5 oxidant for the conversion of primary and secondary alcohols to aldehydes and ketones. *J. Org. Chem.* **1983**, *48*, 4155−4156.
(17) Miyaura, N.; Suzuki, A. Palladium-Catalyzed Cross-Coupling Reactions of Organoboron Compounds. *Chem. Rev.* **1995**, *95*, 2457−2483.
(18) Yamada, K.; Kubo, T.; Tokuyama, H.; Fukuyama, T. A Mild Copper-Mediated Intramolecular Amination of Aryl Halides. *Synlett* **2002**, 231−234.
(19) Experimental findings regarding the metabolite identification, PK/PD, and other pharmacokinetic characteristics will be published separately.
(20) (a) Smart, B. E. Fluorine substituent effects (on bioactivity). *J. Fluorine Chem.* **2001**, *109*, 3−11. (b) Hansch, C.; Leo, A.; Taft, R. W. A Survey of Hammett Substituents Constants and Resonance and Parameters. *Chem. Rev.* **1991**, *91*, 165−195. (c) McDaniel, D. H.; Brown, H. C. An Extended Table of Hammett Substituent Constants based on the Ionization of Substituted Benzoic Acids. *J. Org. Chem.* **1958**, *23*, 420−427.
(21) (a) Staas, D. D.; Savage, K. L.; Sherman, V. L.; Shimp, H. L.; Lyle, T. A.; Tran, L. O.; Wiscount, C. M.; McMasters, D. L.; Sanderson, P. E. J.; Williams, P. D.; Lucas, B. J.; Krueger, J. A.; Lewis, S. D.; White, R. B.; Yu, S.; Wong, B. K.; Kochansky, C. J.; Anari, M. R.; Yan, Y.; Vacca, J. P. Discovery of potent, selective 4-fluoroproline-based thrombin inhibitors with improved metabolic stability. *Bioorg. Med. Chem.* **2006**, *14*, 6900−6916. (b) Wiberg, K. B. The Concept of Strain in Organic Chemistry. *Angew. Chem., Int. Ed. Engl.* **1986**, *25*, 312. (c) Wiberg, K. B.; Fenoglio, R. A. Heats of Formation of C_4H_6 Hydrocarbons. *J. Am. Chem. Soc.* **1968**, *90*, 3395−3397. (d) Baghal-Vayjooee, M. H.; Benson, S. W. Kinetics and Thermochemistry of the Reaction Cl + Cyclpropane ↔ HCl + Cyclopropyl. Heat of Formation of the Cyclopropyl Radical. *J. Am. Chem. Soc.* **1979**, *101*, 2838−2840. (e) Blanksby, S. J.; Ellison, G. B. Bond Dissociation Energies of Organic Molecules. *Acc. Chem. Res.* **2003**, *36*, 255−263.
(22) Neitzel, M. L.; Aubele, D. L.; Hom, R.; Konradi, A. W.; Probst, G.; Semko, C. M.; Truong, A. P.; Garofalo, A. W. Fused, tricyclic sulfonamide inhibitors of gamma secretase. WO2007143523, 2007.
(23) Robak, M. T.; Herbage, M. A.; Ellman, J. A. Synthesis and Applications of *tert*-Butanesulfinamide. *Chem. Rev.* **2010**, *110*, 3600−3740.
(24) Hitchcock, S. A.; Pennington, L. D. Structure−Brain Exposure Relationships. *J. Med. Chem.* **2006**, *49*, 7559−7583.
(25) (a) Varma, M. V. S.; Obach, R. S.; Rotter, C.; Miller, H. R.; Chang, G.; Steyn, S. J.; El-Kattan; Troutman, M. D. Physicochemical Space for Optimum Oral Bioavailability Contribution of Human Intestinal Absorption and First-Pass Elimination. *J. Med. Chem.* **2010**, *53*, 1098−1108. (b) Veber, D. F.; Johnson, S. R.; Cheng, H. Y.; Smith, B. R.; Ward, K. W.; Kopple, K. D. Molecular Properties that Influence the Oral Bioavailability of Drug Candidates. *J. Med. Chem.* **2002**, *45*, 2615−2623. (c) Wils, P.; Warnery, A.; Phung-Ba, V.; Legrain, S.; Scherman, D. High Lipophilicity Decreases Drug Transport Across Intestinal Epithelial Cells. *J. Pharmacol. Exp. Ther.* **1994**, *269*, 654−658.
(26) (a) Di, L.; Umland, J. P.; Chang, G.; Huang, Y.; Lin, Z.; Scott, D. O.; Troutman, M. D.; Liston, T. E. Species Independence in Brain Tissue Binding Using Brain Homogenate. *Drug Metab. Dispos.* **2011**, *39*, 1270−1277. (b) Read, K. D.; Braggio, S. Assessing brain free fraction in early drug discovery. *Expert Opin. Drug Metab. Toxicol.* **2010**, *6*, 337−334.
(27) (a) Yi, P.; Hadden, C.; Kulanthaivel, P.; Calvert, N.; Annes, W.; Brown, T.; Barbuch, R. J.; Chaudhary, A.; Ayan-Oshodi, M. A.; Ring, B. J. Disposition and metabolism of semagacestat, a γ-secretase inhibitor, in humans. *Drug Metab. Dispos.* **2010**, *38*, 554−565. (b) Henley, D. B.; May, P. C.; Dean, R. A.; Siemers, E. R. Development of semagacestat (LY450139), a functional γ-secretase inhibitor, for the treatment of Alzheimer's disease. *Expert Opin. Pharmacother.* **2009**, *10*, 1657−1664.
(28) (a) Martone, R. L.; Zhou, H.; Atchison, K.; Comery, T.; Xu, J. Z.; Huang, X.; Gong, X.; Jin, M.; Kreft, A.; Harrison, B.; Mayer, S. C.; Aschmies, S.; Gonzales, C.; Zaleska, M. M.; Riddell, D. R.; Wagner, E.; Lu, P.; Sun, S.-C.; Sonnenberg-Reines, J.; Oganesian, A.; Adkins, K.; Leach, M. W.; Clarke, D. W.; Huryn, D.; Abou-Gharbia, M.; Magolda, R.; Bard, J.; Frick, G.; Raje, S.; Forlow, S. B.; Balliet, C.; Burczynski, M. E.; Reinhart, P. H.; Wan, H. I.; Pangalos, M. N.; Jacobsen, J. S. Begacestat (GSI-953): a novel, selective thiophene sulfonamide inhibitor of amyloid precursor protein γ-secretase for the treatment of Alzheimer's disease. *J. Pharmacol. Exp. Ther.* **2009**, *331*, 598−608. (b) Mayer, S. C.; Kreft, A. F.; Harrison, B.; Abou-Gharbia, M.; Antane, M.; Aschmies, S.; Atchison, K.; Chlenov, M.; Cole, D. C.; Comery, T.; Diamantidis, G.; Ellingboe, J.; Fan, K.; Galante, R.; Gonzales, C.; Ho, D. M.; Hoke, M. E.; Hu, Y.; Huryn, D.; Jain, U.; Jin, M.; Kremer, K.; Kubrak, D.; Lin, M.; Lu, P.; Magolda, R.; Martone, R.; Moore, W.; Oganesian, A.; Pangalos, M. N.; Porte, A.; Reinhart, P.; Resnick, L.; Riddell, D. R.; Sonnenberg-Reines, J.; Stock, J. R.; Sun, S.-C.; Wagner, E.; Wang, T.; Woller, K.; Xu, Z.; Zaleska, M. M.; Zeldis, J.; Zhang, M.; Zhou, H.; Jacobsen, J. S. Discovery of Begacestat, a Notch-1-Sparing γ-Secretase Inhibitor for the Treatment of Alzheimer's Disease. *J. Med. Chem.* **2008**, *51*, 7348−7351.
(29) (a) Tong, G.; Wang, J.-S.; Sverdlov, O.; Huang, S.-P.; Slemmon, R.; Croop, R.; Castaneda, L.; Gu, H.; Wong, O.; Li, H.; Berman, R. M.; Smith, C.; Albright, C. F.; Dockens, R. C. Multicenter, Randomized, Double-Blind, Placebo-Controlled, Single-Ascending Dose Study of the Oral γ-Secretase Inhibitor BMS-708163 (Avagacestat): Tolerability Profile, Pharmacokinetic Parameters, and Pharmacodynamic Markers. *Clin. Ther.* **2012**, *34*, 654−667. (b) Gillman, K. W.; Starrett, J. E.; Parker, M. F.; Xie, K.; Bronson, J. J.; Marcin, L. R.; McElhone, K. E.; Bergstrom, C. P.; Mate, R. A.; Williams, R.; Meredith, J. E.; Burton, C. R.; Barten, D. M.; Toyn, J. H.; Roberts, S. B.; Lentz, K. A.; Houston, J. G.; Zaczek, R.; Albright, C. F.; Decicco, C. P.; Macor, J. E.; Olson, R. E. Discovery and Evaluation of BMS-708163, a Potent, Selective and Orally Bioavailable γ-Secretase Inhibitor. *ACS Med. Chem. Lett.* **2010**, *1*, 120−124.
(30) Ye, X. M.; Konradi, A. W.; Sun, M.; Yuan, S.; Aubele, D. L.; Dappen, M.; Dressen, D.; Garofalo, A. W.; Jagodzinski, J. J.; Latimer, L.; Probst, G. D.; Sham, H. L.; Wone, D.; Xu, Y-z.; Ness, D.; Brigham, E.; Kwong, G. T.; Willits, C.; Tonn, G.; Goldbach, E.; Quinn, K. P.; Zhang, H. H.; Sauer, J.-M.; Bova, M.; Basi, G. S. Discovery of a novel [3.2.1] benzo fused bicyclic sulfonamide-pyrazoles as potent, selective and efficacious γ-secretase inhibitors. *Bioorg. Med. Chem. Lett.* **2013**, *23*, 996−1000.
(31) Garofalo, A. W.; Jagodzinski, J. J.; Konradi, A. W.; Semko, C. M.; Smith, J. L.; Xu, Y-z.; Ye, X. M. 5-(Arylsulfonyl)-pyrazolopiperidines. WO2007064914, 2007.
(32) (a) Konradi, A. W.; Ye, X. M.; Bowers, S.; Garofalo, A. W.; Aubele, D. L.; Dressen, D.; Ng, R.; Probst, G.; Semko, C. M.; Sun, M.; Truong, A. P.; Dappen, M. S. *N*-Sulfonamido polycyclic pyrazolyl compounds. WO2010028213, 2010; (b) Bowers, S.; Garofalo, A. W.; Hom, R. K.; Konradi, A. W.; Mattson, M. N.; Neitzel, M. L.; Semko, C. M.; Truong, A. P.; Wu, J.; Xu, Y.-z. Bridged *N*-cyclic sulfonamido inhibitors of gamma secretase. WO2007022502, 2007.
(33) 2-Bromo-5-fluorophenylboronic acid is now commercially available from Combi-Blocks.
(34) 6-(Trifluoromethyl)pyridine-3-sulfonyl chloride is now commercially available from Matrix Scientific.