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Synthesis and structure–activity relationship of a novel series of heterocyclic sulfonamide γ-secretase inhibitors

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ABSTRACT

γ-Secretase inhibitors have been shown to reduce the production of β-amyloid, a component of the plaques that are found in brains of patients with Alzheimer's disease. A novel series of heterocyclic sulfonamide γ-secretase inhibitors that reduce β-amyloid levels in cells is reported. Several examples of compounds within this series demonstrate a higher propensity to inhibit the processing of amyloid precursor protein compared to Notch, an alternative γ-secretase substrate.

1. Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder, which is the most common cause of dementia in the elderly population. To date, disease-modifying therapeutic agents to treat this disease are not available.[1] AD is characterized pathologically by abnormal deposits of extracellular plaques composed of β-amyloid peptides (Aβ) and intracellular neurofibrillary tangles composed of hyperphosphorylated tau protein in the brain.[2] Aβ peptides are released by sequential cleavage of the amyloid precursor protein (APP) by two membrane associated aspartic proteases. Firstly, β-secretase (BACE1) cleaves APP to form β-C-terminal fragment (βCTF) and then γ-secretase cleaves βCTF to form Aβ peptides, predominantly Aβ40 and Aβ42. Hindrance of Aβ synthesis via inhibition of either of these secretases is an attractive strategy for the treatment of AD.[3,4] Several γ-secretase inhibitors have been tested in preclinical models and reductions in levels of Aβ in plasma, brain and cerebrospinal fluid have been demonstrated. In addition to APP, γ-secretase cleaves a range of transmembrane peptide substrates including Notch. Inhibition of Notch processing is associated with toxicity in the gastrointestinal tract, thymus and spleen.[5] Recently, it has been shown that the GSI clinical candidate BMS-299897, a compound that prevents cleavage of APP 15-fold more effectively than Notch in vitro, lowered Aβ levels in mice without any Notch related side effects.[6]

Herein, we report a series of γ-secretase inhibitors that were derived from a sulfonamide series represented by structure **1** (Fig. 1).[7] This compound was shown to inhibit production of Aβ40 and Aβ42 in CHO cells transfected with human APP with an average EC_{50} value of 29 nM and increased β-CTF levels without effecting APP levels, consistent with the mechanism of a γ-secretase inhibitor. Based on the sulfonamide series, we proposed a new scaffold, whereby the alkyl alcohol moiety of structure **1** is replaced by a heterocycle. Initial lead **2**, with a pyrazole in place of the alkyl alcohol, had weak activity against Aβ production (EC_{50} = 19 μM). From this new starting point, analogs with improved Aβ lowering activity were identified and their mechanism of action, as γ-secretase inhibitors, was demonstrated. Moreover, compounds within this series demonstrated selectivity for APP processing over Notch processing.

2. Chemistry

Several heterocyclic cores were chosen as alkyl alcohol replacements of lead structure **2**, including pyrazole, imidazole, triazole and pyrrole. These derivatives were prepared with the general synthetic sequence described in Scheme 1a. N-Substituted pyrazoles,

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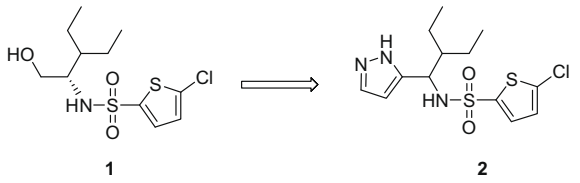


Figure 1. Structures of initial leads **1** and **2**.

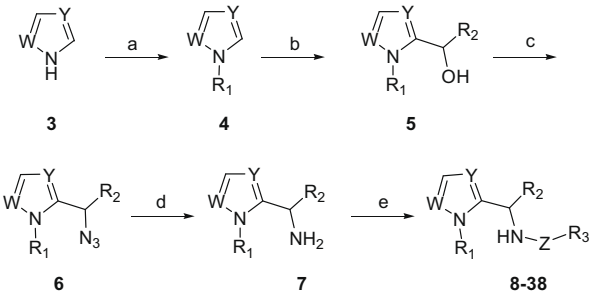
triazoles or imidazoles **4** (W = N, Y = CH; W = N, Y = N; W = CH, Y = N) were prepared by treatment with base (NaH for pyrazoles and imidazoles, DBU for triazoles) and appropriate alkyl halide or sulfonyl chloride. Alternatively, phenyl pyrazoles **4** (R_1 = phenyl), were made by treatment of pyrazole with a phenyl boronic acid, pyridine and cupric acetate.[8] Intermediate **4** was treated with *n*-BuLi[9] or *t*-BuLi[10] and the resulting lithiated heterocycle was reacted with an aldehyde to provide the corresponding alcohol **5**. A pyrrole analog was made from 1-(4-methoxybenzyl)-1*H*-pyrrole-2-carbaldehyde (**39**) by treatment with a Grignard reagent (Scheme 1b). Conversion of pyrazole, pyrrole and imidazole alcohols **5** to azides **6** was performed with a Mitsunobu reaction.[11] For triazole alcohols, **5**, a 2-step sequence, which entailed formation of the mesylate, followed by treatment with sodium azide was used. Standard catalytic hydrogenation of azide **6** provided amines **7**, which were converted to sulfonamides or amides by treatment with base and a suitable sulfonyl chloride or carboxyl chloride to give targets **8–38**.

Treatment of *para*-methoxybenzyl pyrazole **8** with TFA and anisole removed the *para*-methoxybenzyl group to afford **2** (Scheme 2).[9] Demethylation of pyrazole **8** was affected with BBr_3 to afford *p*-hydroxyl benzyl heterocycle **40.** Analogously, *p*-hydroxyl phenyl **41** was made from methoxyl phenyl **28** (see Table 2 for structure).

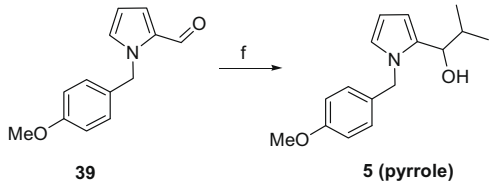
N-Alkylation of sulfonamides **11** and **18** afforded **42** and **43** (Scheme 3).

The synthesis of optically pure pyrazole targets was accomplished with methodology that was developed by Pirc et al. (Scheme 4).[12] *N*-CBz L-Amino acids **44**, which were either purchased or prepared from the corresponding amino acid,[13] were transformed to the corresponding Weinreb amides **45** by conventional conditions. Treatment of **45** with ethynylmagnesium bromide afforded ynones **46** which were subsequently converted to enamino ketones **47** by reaction with diethylamine. Cyclocondensation with phenyl hydrazines gave pyrazoles **48** as single regioisomers. Deprotection by catalytic hydrogenation afforded free amines, which were converted to sulfonamides **49–52**. (See Table 4 for structures.) Hydroxyl phenyl **53** was prepared by demethylation of methoxyl phenyls **51** and **52**, respectively. The corresponding (***R***)-enantiomers were obtained from D-amino acids following the same synthetic route (compounds **54**, **55**). The chiral purity of these compounds were determined to be ⩾95% as determined by chiral HPLC.

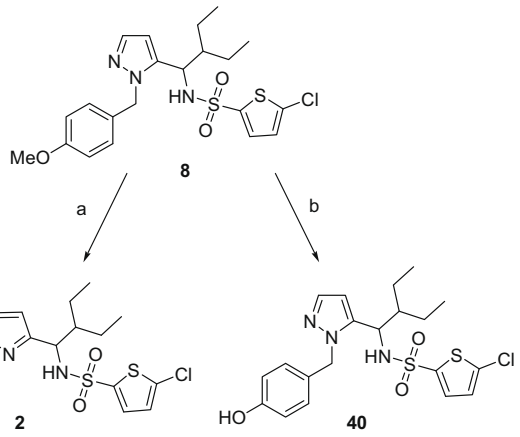
The chiral approach was used for the synthesis of phenyl pyrazoles. For enantiomers **56–59** chiral HPLC was used to separate enantiomers (Table 4).



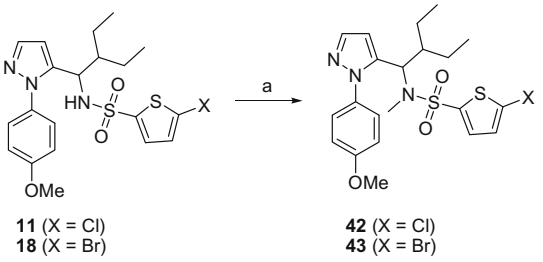
Scheme 1a. Reagents and conditions: (a) R_1X, base (NaH or DBU), THF, rt or $ArB(OH)_2$, $Cu(OAc)_2$, pyridine, rt; (b) (i) *n*-BuLi or *t*-BuLi, THF, −78 °C; (ii) R_2CHO, −78 °C to rt; (c) DPPA, DEAD, PPh_3, THF, 0 °C to rt or (i) MsCl, pyr; (ii) NaN_3, DMF; (d) H_2, Pd/C, MeOH, rt; (e) R_3SO_2Cl or R_3COCl, Et_3N, DMAP, CH_2Cl_2, rt.



Scheme 1b. Reagents and conditions: (f) *i*-PrMgBr, THF, 0 °C to rt.



Scheme 2. Reagent and conditions: (a) TFA, anisole, CH_2Cl_2, reflux; (b) BBr_3, CH_2Cl_2, −78 °C to rt.

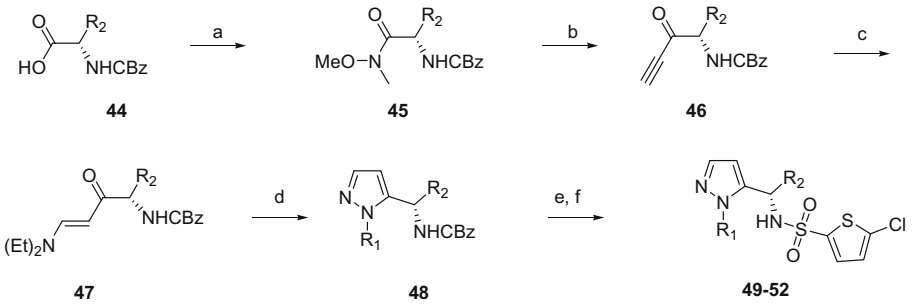


Scheme 3. Reagent and conditions: (a) NaH, MeI, THF, 0 °C to rt.

3. Biology

Inhibition of Aβ production was determined by two methods. The first is a whole cell assay consisting of CHO cells transfected with human APP. Compounds were assayed for inhibition of the secretion of both Aβ40 and Aβ42 by a double sandwich ELISA assay, whereby peptide was captured by monoclonal 6E10 (anchored by goat anti-mouse IgG1) and detected by Aβ40 and Aβ42 carboxy specific rabbit antibody. Secondary detection was by APL-donkey anti-rabbit IgG.[14]

Compounds with an EC_{50} of less than 10 μM were retested with a recently developed electrochemiluminescent immuno assay (MSD, Gaithersburg, MD). This method is an adaptation of the sandwich ELISA assay, which is performed in liquid phase (see Sec-



Scheme 4. Reagents and conditions: (a) EDCI, NMM, MeNHOMe, CH_2Cl_2, rt; (b) ethynylmagnesium bromide, −78 °C to rt, THF; (c) $NH(Et)_2$, CH_2Cl_2, 0 °C to rt; (d) R_1NHNH_2, EtOH, HCl (37% aqueous), 80 °C; (e) H_2, Pd/C, MeOH; (f) 5-chlorothiophene-2-sulfonyl chloride, TEA, CH_2Cl_2, rt. R_1: substituted phenyl; R_2: $(CH_3)_2CH$ or $(CH_3CH_2)_2CH$.

tion 6 for details). In general, EC_{50} values for Aβ40 and Aβ42 were similar, typically with less then twofold difference.

Notch receptor activity was assayed using a constitutively active Notch (mΔ*E* Notch) to drive expression of soluble alkaline phosphatase (SEAP) reporter via transactivation of HES. SEAP levels were measured in conditioned media by fluorescent assay.

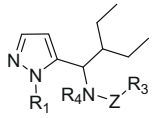
4. Discussion

The structure–activity relationships of the prepared compounds will be discussed based on their Aβ40-lowering effects. Measurements of inhibition of Aβ42 were found to be similar to that of Aβ40 (data not shown). Table 1 shows a set of pyrazole inhibitors. From the start, it was apparent that N-substitution of the pyrazole significantly improves activity compared to initial lead **2** and that a variety of substituents are tolerated including, aliphatic, aromatic, or sulfonyl (**8**–**11**). The preferred substitution on the aliphatic nitrogen was shown to be thiophene sulfonyl. A change to phenyl sulfonyl leads to a decrease of activity as exemplified by **8** versus **12**, **13** and **11** versus **14**–**16**. 5-Chlorothiophene or 5-bromothiophene sulfonamides are more active than 4,5-dichlorothiophene sulfonamide (**11** and **18** vs **19**). Replacement of the sulfonamide linkage with an amide linkage leads to a significant decrease in activity (**12** vs **17**). N-Alkylation of the sulfonamide decreases the activity by approximately 40-fold (**11** vs **42** and **18** vs **43**). Similar trends in the thiophene sulfonamide region of the molecule were also observed in the original alkyl alcohol series represented by lead **1**.[7]

Additional targets were prepared to explore the effects of the R_1 group in combination with variations of the R_2 alkyl (Table 2). In the benzyl pyrazole series, R_2 can be methyl (**20**), straight chain alkyl (**21**), or branched chain alkyl (**8**, **22**), with 3-pentyl derivative **8** possessing the highest activity (Aβ40 EC_{50} = 0.13 μM). When R_1 = phenyl, the 3-pentyl alkyl chain did not provide an advantage over the isopropyl group (**11** vs **23**) as it did with the benzyl derivatives (**8** vs **22**). Replacement of the methoxyl of analog **8** with hydroxyl (compound **40**) does not affect activity. Different R_1 phenyl substituents including 4-Me, 3-Me, 4-F, 4-CF_3, 4-OMe and OH (**24**–**28**, **41**) were tolerated with the 4-Me analog **24** providing a 2.7-fold improvement in activity compared to unsubstituted analog **23**.

Replacement of the pyrazole core with other nitrogen-containing 5-membered heterocycles was investigated (Table 3). Imidazoles **29** and **30** and pyrrole **31** are weaker inhibitors (Aβ40 EC_{50} >13 μM) than their corresponding pyrazole analogs. On the other hand, compounds of the 1,2,4-triazole series (**32**–**38**) demonstrate

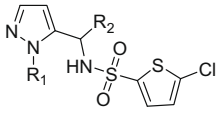
Table 1
Effects of N-substituents on the pyrazole derivatives



Compound	R_1	R_3	R_4	Z	Aβ40 EC_{50}[a] (μM)
2	H	5-Cl-Thiophene	H	SO_2	19.49[b]
8	4-OMe-Benzyl	5-Cl-Thiophene	H	SO_2	0.13
9	Butyl	5-Cl-Thiophene	H	SO_2	1.00
10	4-Me-PhSO_2	5-Cl-Thiophene	H	SO_2	1.68
11	Phenyl	5-Cl-Thiophene	H	SO_2	1.62
12	4-OMe-Benzyl	4-Cl-Benzene	H	SO_2	1.98
13	4-OMe-Benzyl	4-Br-Benzene	H	SO_2	2.27
14	Phenyl	4-Cl-Benzene	H	SO_2	22.97[b]
15	Phenyl	4-CN-Benzene	H	SO_2	85.24[b]
16	Phenyl	3,4-Di-Cl-benzene	H	SO_2	25.51[b]
17	4-OMe-Benzyl	4-Cl-Benzene	H	CO	42.36[b]
18	Phenyl	5-Br-Thiophene	H	SO_2	1.53
19	Phenyl	4,5-Di-Cl-thiophene	H	SO_2	13.36[b]
42	Phenyl	5-Cl-Thiophene	CH_3	SO_2	70.15[b]
43	Phenyl	5-Br-Thiophene	CH_3	SO_2	65.12[b]

[a] Unless otherwise noted, the MSD ELISA assay was used to measure Aβ levels. Values are averages of three experiments, with 10 data points in each experiment.
[b] The double sandwich ELISA was used to measure Aβ levels.

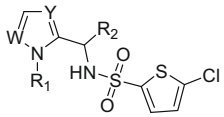
Table 2
Structure–activity relationships of pyrazole derivatives: R_1 and R_2 variation



Compound	R_1	R_2	Aβ40 EC_{50}[a] (μM)
8	4-OMe-Benzyl	$CH(CH_2CH_3)_2$	0.13
11	Phenyl	$CH(CH_2CH_3)_2$	1.67
20	4-OMe-Benzyl	CH_3	1.20
21	4-OMe-Benzyl	$CH_2CH_2CH_3$	0.93
22	4-OMe-Benzyl	$CH(CH_3)_2$	0.41
23	Phenyl	$CH(CH_3)_2$	1.12
24	4-Me-Phenyl	$CH(CH_3)_2$	0.42
25	3-Me-Phenyl	$CH(CH_3)_2$	2.53
26	4-F-Phenyl	$CH(CH_3)_2$	2.76
27	4-CF_3-Phenyl	$CH(CH_2CH_3)_2$	2.19
28	4-OMe-Phenyl	$CH(CH_2CH_3)_2$	1.56
40	4-OH-Benzyl	$CH(CH_2CH_3)_2$	0.14
41	4-OH-Phenyl	$CH(CH_2CH_3)_2$	0.63

[a] The MSD ELISA assay was used to measure Aβ levels. Values are averages of three experiments, with 10 data points in each experiment.

Table 3
Heterocyclic variations

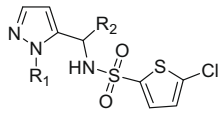


Compound	W	Y	R_1	R_2	Aβ40 EC_{50}[a] (μM)
8	N	CH	4-OMe-Benzyl	$CH(CH_2CH_3)_2$	0.13
29	CH	N	4-OMe-Benzyl	$CH(CH_2CH_3)_2$	50.63[b]
30	CH	N	Benzyl	$CH(CH_2CH_3)_2$	19.4[b]
31	CH	CH	4-OMe-Benzyl	$CH(CH_3)_2$	13.32[b]
32	N	N	4-OMe-Benzyl	$CH(CH_2CH_3)_2$	0.94
33	N	N	Benzyl	$CH(CH_2CH_3)_2$	1.01
34	N	N	4-Me-Benzyl	$CH(CH_2CH_3)_2$	0.88
35	N	N	4-OCF_3-Benzyl	$CH(CH_2CH_3)_2$	0.99
36	N	N	4-F-Benzyl	$CH(CH_2CH_3)_2$	0.48
37	N	N	Benzyl	$CH(CH_3)_2$	3.36
38	N	N	4-Me-Benzyl	$CH(CH_3)_2$	2.89

[a] Unless otherwise noted, the MSD ELISA assay was used to measure Aβ levels. Values are averages of three experiments, with 10 data points in each experiment.
[b] The double sandwich ELISA was used to measure Aβ levels.

activities that are similar to those of the pyrazole series. From these results, it is evident that a nitrogen in the W position gives improved activity compared to carbon–hydrogen in this position. For the triazole series, as in the benzyl pyrazole series, compounds with the $CH(CH_2CH_3)_2$ side chain are more active than those with the $CH(CH_3)_2$ side chain (**34** vs **38** and **33** vs **37**), although the fold improvement is not as large (~7 vs 2–3).

Table 4
Stereochemistry



Compound	Configuration	Rotation	R_1	R_2	Aβ40 EC_{50}[c] (μM)
49	*S*[a]	+	4-Me-Phenyl	$CH(CH_3)_2$	0.32
50	*S*[a]	+	4-F-Phenyl	$CH(CH_3)_2$	1.01
51	*S*[a]	+	4-OMe-Phenyl	$CH(CH_3)_2$	0.55
52	*S*[a]	+	4-OMe-Phenyl	$CH(CH_2CH_3)_2$	0.99
53	*S*[a]	+	4-OH-Phenyl	$CH(CH_3)_2$	0.47
54	*R*[a]	–	4-Me-Phenyl	$CH(CH_3)_2$	>15
55	*R*[a]	–	4-F-Phenyl	$CH(CH_3)_2$	>15[d]
56	*S*[b]	+	4-OMe-Benzyl	$CH(CH_2CH_3)_2$	0.11
57	*R*[b]	–	4-OMe-Benzyl	$CH(CH_2CH_3)_2$	>15
58	*S*[b]	+	4-OH-Benzyl	$CH(CH_2CH_3)_2$	0.092
59	*R*[b]	–	4-OH-Benzyl	$CH(CH_2CH_3)_2$	8.5

[a] Stereoisomers were obtained by chiral synthesis.
[b] Enantiomers were isolated from racemates by chiral HPLC; stereochemical assignment deduced from optical rotation compared with similar analogs of known stereochemical assignment.
[c] Unless otherwise noted, the MSD ELISA assay was used to measure Aβ levels. Values are averages of three experiments, with 10 data points in each experiment.
[d] The double sandwich ELISA was used to measure Aβ levels.

Table 5
Notch selectivity

Compound	Aβ40 EC_{50} (μM)	Notch EC_{50}[a] (μM)	Notch sparing selectivity[b]
49	0.32	12.56	39
58	0.092	1.8	20
56	0.11	2.65	24
51	0.55	6.12	11
52	0.99	3.97	4
53	0.47	3.26	6.9

[a] Values are averages of 2 experiments with 10 data points in each experiment.
[b] Notch sparing selectivity = notch EC_{50}/Aβ40 EC_{50}.

To determine the effect of stereochemistry on the activity, several optically pure analogs were tested (Table 4). It was found that the *S* enantiomers (compounds **49**–**53**, **56**, **58**) are much more active than the *R* enantiomers (e.g., **54**, **55**, **57**, **59**). This is in agreement with the original alkyl alcohol series.[7]

Importantly, compounds within this series are selective for inhibition of APP processing versus Notch processing, as calculated by the Notch sparing selectivity, which is the ratio of Notch EC_{50} to Aβ40 EC_{50} (Table 5). Notably, compound **49** demonstrates 39-fold selectivity and compounds **58** and **56** demonstrate ~20-fold selectivity.

To show that γ-secretase is the mechanism by which this series of compounds inhibits Aβ production, experiments which measure CTF and APP levels were run on representative compounds (data not shown). Increased levels of CTF with no change in APP levels were found, which is indicative of γ-secretase inhibition. Further validation of mechanism was demonstrated with a γ-secretase ligand displacement assay. This assay employs membranes isolated from human neuroblastoma SH-SY5Y cells and a proprietary tritiated γ-secretase inhibitor. Benchmark γ-secretase inhibitors including compound **1**, *N*-[*N*-(3,5-diflurophenacetyl)-L-alanyl]-*S*-phenylglycine-*t*-butylester (DAPT), LY411575[15] and LY450139[16] competitively displace this radiolabeled ligand at concentrations comparable to their EC_{50} values for inhibition of Aβ synthesis. Compound **58** provides an IC_{50} value of 154 nM in this assay, which is similar to its Aβ lowering potency (EC_{50} = 92 nM). This result suggests that compound **58** and its analogs bind to γ-secretase at the same site as other benchmark inhibitors.

5. Conclusions

Starting from lead pyrazole **2**, a series of small molecule heterocyclic γ-secretase inhibitors has been discovered. Expansion of the series, lead to compounds with up to 200-fold improvement in activity compared to the lead. The elements to maintain good activity in this series include N-substitution of the pyrazole, the presence of 5-chlorothiophene sulfonamide, and (*S*)-configuration at the chiral centre. Several potent analogs, including compounds **49**, **56** and **58** show good selectivity for inhibition of APP versus Notch processing. Unfortunately, characteristics such as poor solubility and microsomal instability caused compounds within the series to have poor in vivo efficacy. Further research on the series is required to optimize drug-like characteristics.

6. Experimental

6.1. APP stable cell line

The characteristics of a CHO-cell line stably expressing an APP reporter construct containing the Swedish KM/NL mutation (APP-Rep 751NL) were reported previously.[17] Cells were plated in 96-well plates and allowed to adhere overnight in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% certified fetal

bovine serum. For compound testing, compounds were diluted from stock solutions in dimethylsulfoxide (DMSO) to yield a final concentration of 0.1% DMSO in media. Cells were treated for 24 h at 37 °C with compounds.

6.2. Cellular Aβ assay

Aβ in conditioned media was measured by sandwich $A\beta_{40}$ and $A\beta_{42}$ end-specific ELISA using monoclonal antibody 6E10 (Signet/Covance Labs) for capture, and rabbit C-terminal specific antibodies (Biosource) coupled with an alkaline phosphatase-conjugated anti-Rabbit detection antibody and attophos (alkaline phosphatase substrate) for detection. ELISA plates were read using a Cytofluor fluorescence plate reader to determine the concentration of the fluorescent product of the alkaline phosphatase activity. Standard curves were made using synthetic $A\beta_{40}$ and $A\beta_{42}$ peptides (AnaSpec). Reductions in Aβ levels were measured relative to control cells treated with 0.1% DMSO and expressed as a percentage inhibition. Data from 5 doses in triplicate were fitted to a four-parameter logistical model using LSW software in order to determine EC_{50} values. Cells from the same assay were washed 2× in PBS and a CellTiter 96 Aqueous Non-Radioactive Cell Proliferation Assay (MTS: Promega, Madison, WI) solution was plated onto the cells to assess cell viability. After 1 h, plates were read on a plate reader at 560 nm.

6.3. MDS cellular Aβ assay

Human Aβ40 and Aβ42 in conditioned medium were measured by a double-sandwich Meso Scale Discovery (MSD) based homogenous immunoassay. Briefly, 96-well streptavidin coated MSD plates (MSD, Gaithersburg, MD) were washed three times in Tris buffered saline containing 0.1% Tween 20 (TBST). Twenty microlitres samples or standards were loaded into wells together with 20 μL antibody mastermix [Aβ40: 1/1000 biotinylated 6E10 (Signet), 1/5000 anti-human Aβ40 rabbit polyclonal antibody (Biosource) and 1/2000 anti-rabbit MSD-Tag (MSD) in MSD blocker A; or Aβ42: 1/500 biotinylated 6E10 (Signet), 1/625 anti-human Aβ42 rabbit polyclonal antibody (Biosource) and 1/1250 anti-rabbit MSD-SulfoTag (MSD) in MSD blocker A]. Plates were incubated overnight at 4 °C while shaking. The plates were then washed three times in TBST and 150 μL of MSD read buffer-T was added to each well. Plates were read in a Sector Imager 6000.

6.4. Radiolabeled γ-secretase binding and displacement assay

A tritiated analog of compound **1** ([^{3}H] **1**-analog) was prepared in-house as an 11.00 mCi/mL ethanol stock solution with the specific activity of 26.6 Ci/mol and the radiochemical purity of >99%. γ-Secretase enzyme was prepared in-house as CHAPSO-solubilized microsomes from human neuroblastoma SH-SY5Y cells. Filter plates were purchased from Millipore. Microscint-20 was purchased from Perkin–Elmer. Polyethyleneimine solution (PEI, 50% solution in water) was purchased from Sigma. Multiscreen 96-well FB filter plate was prepared by incubating with 200 μL of 0.6% (w/v) PEI solution at 4 °C overnight. The filter plate was vacuum filtrated and washed twice with 200 μL of water and twice with 200 μL of assay buffer (25 mM MES, pH 6.5, 1 mM EDTA, 0.01% β-mercaptoethanol, 0.01% BSA). Reactions were set up in Costar 96-well clear polypropylene plates (Corning) in the following addition sequence: 160 μL γ-secretase containing microsomes diluted 1:10 in the assay buffer, 20 μL test compound serial dilution in 10% DMSO and 90% assay buffer) and 20 μL 250 nM [^{3}H] GSI-Analog in the assay buffer. Each plate contained 3 test compounds and cold-compound **1**-analog (as positive control) in duplicate. The final concentrations in the reactions were 25 nM [^{3}H] **1**-analog, 0–2 μM or 0–10 μM test compound, 1% DMSO and estimated 92 pM GS. Following 1 h incubation at room temperature with shaking, 180 μL reactions were transferred to the filter plate. The filter plate was then vacuum filtrated, followed by washing 4 times with 200 μL wash buffer (5 mM Tris–HCl, pH 7.4) and vacuum dried for 5 addition min. The filter plate bottom was removed and the plate was blotted dry with paper towel. Microscint-20 (30 μL) was added and ^{3}H radioactivity was counted on a Microbeta (Wallac Model# 1450). Data were analyzed in GaphPad Prism 4.0 using the following equation: $\text{Counts} = a + (b - a) * IC_{50}^{n}/(\text{Conc}^{n} + IC_{50}^{n})$, in which n is the Hill number and IC_{50} is the compound concentration that yields 50% competition.

6.5. Notch functional assay

A constitutively active mouse Notch construct with a deletion of the extracellular domain containing the M1726V mutation as previously described[18] and a reporter construct employing soluble alkaline phosphatase driven by the Hes1 promoter (pSEAP-Basic vector, BD Biosciences) were generated. CHO K1 cells were transiently transfected with both constructs using Polyfect (Qiagen) transfection reagent in Opti-MEM media. Cells were plated in 96 well plates and treated with compounds for 48 h. SEAP levels in the conditioned media were assessed using the Great EscAPe SEAP Chemiluminescence detection kit (Clontech) according to manufacturer's instruction. Briefly, 15 μL of conditioned media were mixed with a dilution buffer and incubated at 65 °C for 45 min. After cooling, assay buffer was added, and the samples were incubated with CSPD substrate. Luminescence was measured in a Wallac 1450 Victor luminescence counter.

6.6. Chemistry—general details

NMR spectra were determined on a 400 MHz Varian Unity Plus. Mass spectra were recorded on a Micromass LCT mass spectrometer. HRMS were recorded in FAB mode on an Agilent mass spectrometer. Elemental analysis was taken by Robertson Microlit Inc. using Perkin–Elmer elemental 2400 CHN analyzer. Optical rotation was recorded with a Jasco P-1020 polarimeter with a 30 mm cell. IR was obtained on an Avatar 360 FT-IR. Melting points were taken on a Mel-Temp 3.0 capillary melting point apparatus and were uncorrected. All reagents were purchased from commercial sources and used without further purification.

6.6.1. General synthesis for pyrazoles 8–28 and imidazoles 29, 30

Step A. To the corresponding N-substituted pyrazole[8,9] or imidazole (**4**) (12.9 mmol) in THF (100 mL) at −78 °C was added a solution of *n*-BuLi (2 M in cyclohexane, 7.1 mL, 14.2 mmol) dropwise. After 75 min, the corresponding aldehyde (10.6 mmol) was added. After 45 min the reaction mixture was quenched with saturated aqueous ammonium chloride (50 mL), extracted with EtOAc (3 × 100 mL), dried (Na_2SO_4) and concentrated. Column chromatography provided alcohol **5**.

Step B. To the appropriate alcohol **5** (4.20 mmol) in THF (40 mL) at 0 °C was added triphenylphosphine (1.65 g, 6.29 mmol), diethyl azodicarboxylate (1.0 mL, 6.3 mmol) and diphenylphosphoryl azide (1.4 mL, 6.3 mmol). The reaction mixture was warmed to room temperature and stirred for 15.5 h. The solvent was removed in vacuo and the resulting residue was purified by column chromatography to give azide **6**.

Step C. Azide **6** (2.39 mmol) in MeOH (24 mL) with 5% Pd/C (75 mg) was hydrogenated under atm pressure for 24 h. The reaction mixture was filtered through a plug of Celite and the filtrate was concentrated to give amine **7** as an oil.

Step D. To amine **7** (1.74 mmol) in CH_2Cl_2 (15 mL) was added triethylamine (0.29 mL, 2.1 mmol) and the appropriate sulfonyl chloride or carbonyl chloride (2.09 mmol). After 1 h to 2 days the reaction mixture was diluted with EtOAc (30 mL), washed with 1 N HCl (30 mL), dried (Na_2SO_4) and concentrated. Column chromatography provided target compounds.

6.6.1.1. 5-Chloro-*N*-{2-ethyl-1-[1-(4-methoxybenzyl)-1*H*-pyrazol-5-yl]butyl}thiophene-2-sulfonamide (8). ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.62 (t, 3H, J = 7.4 Hz), 0.70 (t, 3H, J = 7.3 Hz), 0.93–1.04 (m, 2H), 1.16–1.40 (m, 3H), 3.71 (s, 3H), 4.46 (m, 1H), 5.20 (q, 2H, J = 15.8 Hz), 6.12 (d, 1H, J = 1.8 Hz), 6.84 (d, 1H, J = 4.1 Hz), 6.88 (d, 2H, J = 8.7 Hz), 6.95 (d, 1H, J = 4.0 Hz), 7.05 (d, 2H, J = 8.7 Hz), 7.24 (d, 1H, J = 1.8 Hz), 8.46 (d, 1H, J = 9.5 Hz); MS (−ESI): 466 $(M-H)^-$. Anal. Calcd for $C_{21}H_{26}ClN_3O_3S_2$: C, 53.89; H, 5.60; N, 8.98. Found: C, 53.96; H, 5.72; N, 8.66.

6.6.1.2. *N*-[1-(1-Butyl-1*H*-pyrazol-5-yl)-2-ethylbutyl]-5-chlorothiophene-2-sulfonamide (9). ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.76 (t, 3H, J = 7.2 Hz), 0.82 (t, 3H, J = 7.4 Hz), 0.88 (t, 3H, J = 7.4 Hz), 1.07–1.19 (m, 1H), 1.20–1.33 (m, 4H), 1.34–1.48 (m, 2H), 1.59–1.70 (m, 2H), 3.81–3.94 (m, 2H), 4.44 (m, 1H), 6.09 (d, 1H, J = 1.7 Hz), 7.02 (d, 1H, J = 4.1 Hz), 7.10 (d, 1H, J = 4.0 Hz), 7.19 (d, 1H, J = 1.7 Hz), 8.53 (d, 1H, J = 9.17 Hz); MS (−ESI): 402 $(M-H)^-$. Anal. Calcd for $C_{19}H_{22}ClN_3O_3S_2$: C, 50.54; H, 6.49; N, 10.40. Found: C, 50.58; H, 6.65; N, 10.31.

6.6.1.3. 5-Chloro-*N*-(2-ethyl-1-{1-[(4-methylphenyl)sulfonyl]-1*H*-pyrazol-5-yl}butyl)thiophene-2-sulfonamide (10). ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.68 (t, 3H, J = 7.4 Hz), 0.94 (t, 3H, J = 7.4 Hz), 1.10–1.30 (m, 4H), 1.42–1.57 (m, 1H), 2.41 (s, 3H), 5.28 (dd, 1H, J = 4.2, 10.0 Hz), 6.37 (s, 1H), 6.91 (d, 1H, J = 4.0 Hz), 6.95 (d, 1H, J = 4.0 Hz), 7.51 (d, 2H, J = 8.2 Hz), 7.61 (s, 1H), 7.81 (d, 2H, J = 8.2 Hz), 8.51 (d, 1H, J = 10.2 Hz). MS (−ESI): 500 $(M-H)^-$. Anal. Calcd for $C_{20}H_{24}ClN_3O_4S_3$: C, 47.85; H, 4.82; N, 8.37. Found: C, 48.00; H, 4.75; N, 8.38.

6.6.1.4. 5-Chloro-*N*-[2-ethyl-1-(1-phenyl-1*H*-pyrazol-5-yl)-butyl]thiophene-2-sulfonamide (11). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.60 (d, 1H, J = 9.3 Hz), 7.57 (t, 2H, J = 7.6 Hz), 7.51 (m, 1H), 7.47 (m, 1H), 7.34 (m, 2H), 7.22 (d, 1H, J = 4.0 Hz), 7.12 (d, 1H, J = 4.0 Hz), 6.36 (m, 1H), 4.57 (m, 1H), 1.31 (m, 1H), 1.16 (m, 2H), 1.04 (m, 2H), 0.58 (t, 3H, J = 7.3 Hz), 0.48 (t, 3H, J = 7.3 Hz); MS (+ESI): 424 $(M-H)^-$. Anal. Calcd for $C_{19}H_{22}N_3O_2ClS_2$: C, 53.82; H, 5.23; N, 9.91. Found: C, 54.07; H, 5.31; N, 9.87.

6.6.1.5. 4-Chloro-*N*-{2-ethyl-1-[2-(4-methoxy-benzyl)-2*H*-pyrazol-3-yl]-butyl}-benzenesulfonamide (12). ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.61 (t, 3H, J = 7.2 Hz), 0.65 (t, 3H, J = 7.5 Hz), 0.79–0.99 (m, 2H), 1.17–1.36 (m, 3H), 3.72 (s, 3H), 4.39 (t, 1H, J = 7.6 Hz), 5.02 (q, 2H, J = 15.8 Hz), 6.01 (d, 1H, J = 1.4 Hz), 6.87 (d, 2H, J = 8.8 Hz), 6.99 (d, 2H, J = 8.8 Hz), 7.14 (d, 1H, J = 1.9 Hz), 7.39 (s, 4H), 8.19 (d, 1H, J = 8.6 Hz); MS (+ESI): 462.1 $(M+H)^+$. Anal. Calcd for $C_{23}H_{28}ClN_3O_3S_2$: C, 59.79; H, 6.11; N, 9.10. Found: C, 54.69; H, 5.89; N, 8.95.

6.6.1.6. 4-Bromo-*N*-{2-ethyl-1-[1-(4-methoxybenzyl)-1*H*-pyrazol-5-yl]butyl}-benzene sulfonamide (13). ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.61 (t, 3H, J = 7.3 Hz), 0.65 (t, 3H, J = 7.3 Hz), 0.84–0.99 (m, 2H), 1.17–1.36 (m, 3H), 3.72 (s, 3H), 4.40 (t, 1H, J = 7.5 Hz), 5.05 (q, 2H, J = 15.7 Hz), 6.01 (d, 1H, J = 1.5 Hz), 6.87 (d, 2H, J = 8.6 Hz), 6.99 (d, 2H, J = 8.6 Hz), 7.14 (d, 1H, J = 1.7 Hz), 7.32 (d, 2H, J = 8.4 Hz), 7.53 (d, 2H, J = 8.4 Hz), 8.21 (d, 1H, 8.9 Hz); MS (+ESI): 506 $(M+H)^+$. Anal. Calcd for $C_{23}H_{28}BrN_3O_3S_2C$: 54.55; H, 5.57; N, 8.30. Found: C, 54.73; H, 5.56; N, 8.21.

6.6.1.7. 4-Chloro-*N*-[2-ethyl-1-(2-phenyl-2*H*-pyrazol-3-yl)-butyl]-benzenesulfonamide (14). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.34 (d, 1H, J = 6.86 Hz), 7.63–7.61 (m, 2H), 7.56–7.51 (m, 4H), 7.49–7.44 (d, 1H, J = 7.5 Hz), 7.38 (s, 1H), 7.24 (d, 2H, J = 7.47 Hz), 6.27 (s, 1H), 4.48 (m, 1H), 1.33 (m, 1H), 1.19 (m, 2H), 1.09–1.02 (m, 2H), 0.56 (t, 3H, J = 6.9 Hz), 0.46 (t, 3H, J = 7.01 Hz). MS (+ESI): 418.1 $(M+H)^+$. Anal. Calcd for $C_{21}H_{24}ClN_3O_2S$: C, 60.35; H, 5.79; N, 10.05. Found: C, 60.11; H, 6.00; N, 10.11.

6.6.1.8. 4-Cyano-*N*-[2-ethyl-1-(2-phenyl-2*H*-pyrazol-3-yl)-butyl]-benzenesulfonamide (15). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.51 (d, 1H, J = 9.61 Hz), 7.97 (d, 2H, J = 8.39 Hz), 7.75 (d, 2H, J = 8.08 Hz), 7.57 (d, 2H, J = 7.4 Hz), 7.55 (s, 1H), 7.35 (s, 1H), 7.25 (d, 2H, J = 6.2 Hz), 6.21 (s, 1H), 4.52 (m, 1H), 1.36 (m, 1H), 1.11 (m, 2H), 1.05–1.02 (m, 2H), 0.59 (t, 3H, J = 6.7 Hz) 0.48 (t, 3H, J = 7.05 Hz). MS (+ESI): 409.1 $(M+H)^+$. Anal. Calcd for $C_{22}H_{24}N_4O_2S$: C, 64.68; H, 5.92; N, 13.71. Found: C, 64.36; H, 5.86; N, 13.70.

6.6.1.9. 3,4-Dichloro-*N*-[2-ethyl-1-(2-phenyl-2*H*-pyrazol-3-yl)-butyl]-benzenesulfonamide (16). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.44 (d, 1H, J = 8.23 Hz), 7.75 (d, 1H, J = 8.4 Hz), 7.72 (1H, d, J = 2.2 Hz), 7.58–7.48 (m, 4H), 7.38 (s, 1H), 7.25 (d, 2H, J = 7.6 Hz), 6.27 (s, 1H), 4.48 (m, 1H), 1.39–1.31 (m, 1H), 1.28–1.15 (m, 2H), 1.13–1.07 (m, 2H), 0.61 (t, 3H, J = 6.89 Hz), 0.51 (t, 3H, J = 7.07 Hz). MS (+ESI): 452.1 $(M+H)^+$. Anal. Calcd for $C_{21}H_{23}N_3O_2S$: C, 55.75; H, 5.12; N, 9.29. Found: C, 55.72; H, 5.15; N, 9.08.

6.6.1.10. 4-Chloro-*N*-{2-ethyl-1-[2-(4-methoxy-benzyl)-2*H*-pyrazol-3-yl]-butyl}-benzamide (17). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.69 (1H, d, J = 9.01 Hz), 7.79 (2H, d, J = 8.56 Hz), 7.50 (2H, d, J = 8.55 Hz), 7.41 (1H, s), 7.08 (2H, d, J = 8.56 Hz), 6.83 (2H, d, J = 8.55 Hz), 6.29 (1H, s), 5.40 (2H, q, J = 15.5 Hz), 5.20 (1H, t, J = 9.4 Hz), 3.68 93H, s), 1.87 (1H, m), 1.55 (1H, m), 1.35 (1H, m), 0.99 (1H, m), 0.89 (1H, m), 0.75 (3H, t, J = 7.3 Hz), 0.58 (3H, t, J = 7.2 Hz). MS (+ESI): 426.3 $(M+H)^+$. Calcd for $C_{24}H_{28}ClN_3O_2$.

6.6.1.11. 5-Bromo-*N*-[2-ethyl-1-(1-phenyl-1*H*-pyrazol-5-yl)-butyl]thiophene-2-sulfonamide (18). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.58 (d, 1H, J = 8.7 Hz), 7.59–7.52 (m, 2H), 7.50–7.49 (d, 1H, J = 7.2 Hz), 7.46 (s, 1H), 7.34 (d, 2H, J = 8.2 Hz), 7.21 (d, 1H, J = 5.02 Hz), 7.17 (d, 1H, J = 5.01 Hz), 6.35 (s, 1H), 4.56 (m, 1H), 1.34–1.32 (m, 1H), 1.19–1.11 (m, 2H), 1.10–1.01 (m, 2H), 0.55 (t, 3H, J = 7.05 Hz), 0.49 (t, 3H, J = 6.99 Hz). MS (+ESI): 468.1 $(M+H)^+$. Anal. Calcd for $C_{19}H_{22}BrN_3O_2S_2$: C, 48.72; H, 4.73; N, 8.97. Found: C, 48.72; H, 4.31; N, 8.84.

6.6.1.12. 4,5-Dichloro-*N*-[2-ethyl-1-(1-phenyl-1*H*-pyrazol-5-yl)-butyl]thiophene-2-sulfonamide (19). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.76 (d, 1H, J = 9.4 Hz), 7.60–7.53 (m, 2H), 7.51–7.48 (m, 2H), 7.36 (d, 2H, J = 8.4 Hz), 7.29 (s, 1H), 6.35 (s, 1H), 4.55 (m, 1H), 1.37–1.31 (m, 1H), 1.29–1.24 (m, 2H), 1.19–1.15 (m, 1H), 1.11–1.05 (m, 1H), 0.62 (t, 3H, J = 7.01 Hz), 0.52 (t, 3H, J = 6.93 Hz). MS (+ESI): 458.1 $(M+H)^+$. Anal. Calcd for $C_{19}H_{21}Cl_2N_3O_2S_2$: C, 49.78; H, 4.62; N, 9.17. Found: C, 51.52; H, 4.97; N, 8.56. ^{1}H NMR is included in the Supplementary data as an indication of purity.

6.6.1.13. 5-Chloro-*N*-{1-[1-(4-methoxybenzyl)-1*H*-pyrazol-5-yl]ethyl}thiophene-2-sulfonamide (20). ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.16 (d, 3H, J = 8.4 Hz) 3.71 (s, 3H), 4.59 (m, 1H), 5.23 (m, 2H), 6.15 (d, 1H, J = 1.7 Hz), 6.86 (d, 1H, J = 8.7) 7.05 (d, 2H, J = 8.7 Hz), 7.11 (d, 1H, J = 4.0 Hz), 7.21 (d, 1H, J = 4.1 Hz), 7.29 (d, 1H, J = 1.7 Hz), 8.64 (br s, 1H); MS (−ESI): 410 $(M-H)^-$. Anal. Calcd for $C_{17}H_{18}ClN_3O_3S_2$: C, 49.47; H, 4.40; N, 10.20. Found: C, 49.48; H, 4.38; N, 9.87.

6.6.1.14. 5-Chloro-*N*-{1-[1-(4-methoxybenzyl)-1*H*-pyrazol-5-yl]butyl}thiophene-2-sulfonamide (21). ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.62 (t, 3H, J = 7.4 Hz), 1.00–1.11 (m, 2H), 1.30–1.37 (m, 1H), 1.42–1.49 (m, 1H), 3.71 (s, 3H), 4.40 (br s, 1H), 5.17–5.23 (m, 2H), 6.11 (d, 1H, J = 1.7 Hz), 6.88 (d, 2H, J = 8.7 Hz), 7.04 (d, 2H, J = 8.6 Hz), 7.05 (d, 1H, J = 4.4 Hz), 7.09 (d, 1H, J = 4.1 Hz), 7.27 (d, 1H, J = 1.5 Hz), 8.61 (br s, 1H); MS (+ESI): 440 $(M+H)^+$. Anal. Calcd for $C_{19}H_{22}ClN_3O_3S_2$: C, 51.87; H, 5.04; N, 9.55. Found: C, 52.00; H, 5.10; N, 9.48.

6.6.1.15. 5-Chloro-*N*-{1-[1-(4-methoxybenzyl)-1*H*-pyrazol-5-yl]-2-methylpropyl}thiophene-2-sulfonamide (22). ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.57 (d, 3H, J = 6.7 Hz), 0.78 (d, 3H, J = 6.7 Hz), 1.72–1.78 (m, 1H), 3.71 (s, 3H), 4.27 (m, 1H), 5.12–5.18 (m, 2H), 6.11 (d, 1H, J = 1.7 Hz), 6.87 (d, 2H, J = 8.6 Hz), 6.93 (d, 1H, J = 4.0 Hz), 6.97 (d, 1H, J = 4.0 Hz), 7.11 (d, 2H, J = 8.6 Hz), 7.25 (d, 1H, J = 1.8 Hz), 8.52 (d, 1H, J = 7.9 Hz); MS (+ESI): 440 $(M+H)^+$. Anal. Calcd for $C_{19}H_{22}ClN_3O_3S_2$: C, 51.87; H, 5.04; N, 9.55. Found: C, 51.80; H, 5.05; N, 9.32.

6.6.1.16. 5-Chloro-*N*-[2-methyl-1-(1-phenyl-1*H*-pyrazol-5-yl)propyl]thiophene-2-sulfonamide (23). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.71 (m, 1H), 7.57 (m, 2H), 7.51 (m, 2H), 7.33 (d, 2H, J = 7.1 Hz), 7.14 (d, 1H, J = 4.0 Hz), 7.11 (d, 1H, J = 4.0 Hz), 6.36 (m, 1H), 4.23 (m, 1H), 1.80 (m, 1H), 0.76 (d, 3H, J = 6.6 Hz), 0.63 (d, 3H, J = 6.7 Hz); MS (+ESI): 396 $(M+H)^+$. Anal. Calcd for $C_{17}H_{18}N_3O_2ClS_2$: C, 51.57; H, 4.58; N, 10.61. Found: C, 51.53; H, 4.61; N, 10.55.

6.6.1.17. 5-Chloro-*N*-{2-methyl-1-[1-(4-methylphenyl)-1*H*-pyrazol-5-yl]propyl}thiophene-2-sulfonamide (24). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.69 (d, 1H, J = 8.1 Hz), 7.48 (m, 1H), 7.36 (d, 2H, J = 7.9 Hz), 7.18 (d, 2H, J = 8.2 Hz), 7.13 (d, 1H, J = 4.1 Hz), 7.11 (d, 1H, J = 4.1 Hz), 6.34 (m, 1H), 4.21 (m, 1H), 2.40 (s, 3H), 1.80 (m, 1H), 0.76 (d, 3H, J = 6.7 Hz), 0.63 (d, 3H, J = 6.7 Hz); MS (−ESI): 408 $(M-H)^-$. Anal. Calcd for $C_{18}H_{20}N_3O_2ClS_2$: C, 52.74; H, 4.92; N, 10.25. Found: C, 51.55; H, 5.0; N, 9.19.

6.6.1.18. 5-Chloro-*N*-{2-methyl-1-[1-(3-methylphenyl)-1*H*-pyrazol-5-yl]propyl}thiophene-2-sulfonamide (25). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.73 (br s, 1H), 7.51 (m, 1H), 7.44 (t, 1H, J = 7.8 Hz), 7.31 (d, 1H, J = 7.6 Hz), 7.12 (m, 3H), 7.08 (s, 1H), 6.38 (m, 1H), 4.23 (m, 1H), 2.39 (s, 3H), 1.81 (m, 1H), 0.77 (d, 3H, J = 6.7 Hz), 0.64 (d, 3H, J = 6.8 Hz). MS (−ESI): 408 $(M-H)^-$. Anal. Calcd for $C_{18}H_{20}N_3O_2ClS_2$: C, 52.74; H, 4.92; N, 10.25. Found: C, 52.71; H, 4.94; N, 9.95.

6.6.1.19. 5-Chloro-*N*-{1-[1-(4-fluorophenyl)-1*H*-pyrazol-5-yl]-2-methylpropyl}thiophene-2-sulfonamide (26). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.72 (m, 1H), 7.51 (m, 1H), 7.42 (m, 2H), 7.37 (m, 2H), 7.16 (d, 1H, J = 4.1 Hz), 7.12 (d, 1H, J = 4.1 Hz), 6.35 (m, 1H), 4.16 (m, 1H), 1.80 (m, 1H), 0.77 (d, 3H, J = 6.7 Hz), 0.63 (d, 3H, J = 6.7 Hz); MS (−ESI): 412 $(M-H)^-$. Anal. Calcd for $C_{17}H_{17}N_3O_2ClFS_2$: C, 49.33; H, 4.14; N, 10.15. Found: C, 49.41; H, 3.99; N, 9.97.

6.6.1.20. 5-Chloro-*N*-(2-ethyl-1-{1-[4-(trifluoromethyl)phenyl]-1*H*-pyrazol-5-yl}butyl)thiophene-2-sulfonamide (27). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.62 (d, 1H, J = 8.0 Hz), 7.93 (d, 2H, J = 8.4 Hz), 7.56 (d, 2H, J = 8.2 Hz), 7.51 (m, 1H), 7.19 (d, 1H, J = 4.0 Hz), 7.06 (d, 1H, J = 4.1 Hz), 6.37 (m, 1H), 4.50 (m, 1H), 1.28 (m, 1H), 1.19 (m, 2H), 1.01 (m, 2H), 0.54 (t, 3H, J = 7.3 Hz), 0.48 (t, 3H, J = 7.3 Hz); MS (−ESI): 490 $(M-H)^-$. Anal. Calcd for $C_{20}H_{21}N_3O_2ClF_3S_2$: C, 48.83; H, 4.30; N, 8.54. Found: C, 49.26; H, 4.36; N, 8.28. ^{1}H NMR is included in the Supplementary data as an indication of purity.

6.6.1.21. 5-Chloro-*N*-{2-ethyl-1-[1-(4-methoxyphenyl)-1*H*-pyrazol-5-yl]butyl}thiophene-2-sulfonamide (28). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.57 (d, 1H, J = 9.5 Hz), 7.41 (m, 1H), 7.24 (m, 3H), 7.10 (m, 3H), 6.31 (m, 1H), 4.50 (m, 1H), 3.83 (s, 3H), 1.31 (m, 1H), 1.19 (m, 2H), 1.06 (m, 2H), 0.59 (t, 3H, J = 7.3 Hz), 0.50 (t, 3H, J = 7.3 Hz); MS (ESI+): 454 $(M+H)^+$. Anal. Calcd for $C_{20}H_{24}N_3O_3S_2Cl$: C, 52.91; H, 5.33; N, 9.26. Found: C, 53.08; H, 5.33; N, 9.16.

6.6.1.22. 5-Chloro-*N*-{2-ethyl-1-[1-(4-methoxybenzyl)-1*H*-imidazol-2-yl]butyl}thiophene-2-sulfonamide (29). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.57 (br m, 1H), 7.08 (d, 2H, J = 8.5 Hz), 7.03 (m, 2H), 6.99 (m, 1H), 6.91 (d, 2H, J = 8.9 Hz), 6.77 (m, 1H), 5.02 (m, 2H), 4.40 (d, 1H, J = 8.2 Hz), 3.73 (s, 3H), 1.59 (m, 1H), 1.47 (m, 1H), 1.31 (m, 1H), 0.79 (m, 2H), 0.63 (t, 3H, J = 7.4 Hz), 0.57 (t, 3H, J = 7.4 Hz); MS (ES+): 468 $(M+H)^+$. Anal. Calcd for $C_{21}H_{26}ClN_3O_3S_2$: C, 53.89; H, 5.60; N, 8.98. Found: C, 53.76; H, 5.41; N, 8.87.

6.6.1.23. *N*-[1-(1-Benzyl-1*H*-imidazol-2-yl)-2-ethylbutyl]-5-chlorothiophene-2-sulfonamide (30). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.51 (br s, 1H), 7.29 (m, 2H), 7.26 (m, 1H), 7.08 (m, 2H), 6.99 (m, 3H), 6.75 (s, 1H), 5.05 (m, 2H), 4.33 (d, 1H, J = 8.05 Hz), 1.51 (m, 1H), 1.41 (m, 1H), 1.26 (m, 1H), 0.69 (m, 2H), 0.55 (t, 3H, J = 7.44 Hz), 0.48 (t, 3H, J = 7.38 Hz). MS (ES+): 438 $(M+H)^+$. Anal. Calcd for $C_{20}H_{24}ClN_3O_2S_2$: C, 54.84; H, 5.52; N, 9.59. Found: C, 54.64; H, 5.58; N, 9.51.

6.6.1.24. 5-Chloro-thiophene-2-sulfonic acid [2-ethyl-1-(2*H*-pyrazol-3-yl)-butyl]-amide (2). To a solution of 8 (0.09 g, 0.192 mmol) in anhydrous CH_2Cl_2 (2 mL) was added anisole (0.21 mL, 1.92 mmol) and trifluoroacetic acid (0.074 mL, 0.96 mmol). The mixture was heated to reflux for 18 h. The solvent was removed under reduced pressure. Column chromatography (EtOAc/hexane, 1:1) afforded **2** (0.04 g, 60%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.41 (br s, 1H), 8.29 (br s, 1H), 7.45 (s, 1H), 7.07 (s, 1H), 6.98 (s, 1H), 5.99 (s, 1H), 4.36 (m, 1H), 1.48–1.19 (m, 3H), 1.02–0.91 (m, 2H), 0.70 (t, 3H, J = 7.3 Hz), 0.62 (t, 3H, J = 7.4 Hz); MS (+ESI) 348.0 $(M+H)^+$. Anal. Calcd for $C_{13}H_{18}ClN_3O_2S_2$: C, 44.88; H, 5.22; N, 12.08. Found: C, 45.13; H, 5.10; N, 12.06.

6.6.1.25. 5-Chloro-*N*-{1-[1-(4-methoxybenzyl)-1*H*-pyrrol-2-yl]-2-methylpropyl}thiophene-2-sulfonamide (31). ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.53 (d, 3H, J = 6.6 Hz), 0.80 (d, 3H, J = 6.6 Hz), 1.73–1.77 (m, 1H), 3.73 (s, 3H), 4.11 (t, 1H, J = 7.8 Hz), 5.87 (s, 2H), 5.86–5.89 (m, 1H), 5.91–5.94 (m, 1H), 6.57 (s, 1H), 6.87 (d, 2H, J = 8.7 Hz), 6.92 (d, 1H, J = 4.0 Hz), 7.01 (d, 2H, J = 8.7 Hz), 8.34 (d, 1H, J = 9.0 Hz). MS (+ESI): 439 $(M+H)^+$. Anal. Calcd for $C_{20}H_{23}ClN_2O_3S_2$: C, 54.72; H, 5.28; N, 6.38. Found: C, 55.28; H, 5.41; N, 6.24.

6.6.2. General procedure for triazoles 32–38

Step A. To a stirred solution of 1,2,4-triazole (0.803 g, 11.6 mmol) in THF (100 mL) at 0 °C was added the corresponding benzyl bromide (10.6 mmol), then 1,8-diazabicyclo[5.4.0]undec-7-ene (1.9 mL, 12.7 mmol) and the mixture was slowly warmed to room temperature. The solution was concentrated in vacuo, then purified by column chromatography (SiO_2, MeOH/EtOAc, 5:95).

Step B. To a stirred solution of benzyl triazole **4** (2.82 mmol) in THF (30 mL) at −78 °C was added a solution of *n*-butyllithium (2.5 M in hexanes, 1.1 mL, 2.9 mmol) dropwise. The mixture was stirred at −78 °C for 1.5 h, then the corresponding aldehyde (3.4 mmol) was added. The solution was slowly warmed to room temperature and

stirred for 18 h. It was then quenched with saturated aqueous NH_4Cl and extracted twice with EtOAc. The organic extracts were dried ($MgSO_4$), filtered, concentrated in vacuo and purified by column chromatography (SiO_2, EtOAc/hexane, 1:1) to give alcohol **5**.

Step C. To a stirred solution of alcohol **5** (1.32 mmol) in pyridine (15 mL) at 0 °C was added methanesulfonyl chloride (0.61 mL, 7.9 mmol) and the solution was slowly warmed to room temperature. The mixture was diluted with H_2O, extracted twice with EtOAc and dried ($MgSO_4$), filtered and concentrated in vacuo to give methane sulfonate which was used in the next step without further purification.

Step D. To a stirred solution of the methane sulfonate prepared above (1.42 mmol) in DMF (10 mL) was added sodium azide (0.277 g, 4.26 mmol) and the mixture was heated to reflux for 3 h. After cooling, the reaction mixture was diluted with H_2O and extracted with EtOAc twice. The organic extracts were dried ($MgSO_4$), filtered, concentrated in vacuo and purified by column chromatography (SiO_2, EtOAc/hexane, 1:9) to give azide **6**.

Step E. Azide **6** (2.39 mmol) in MeOH (24 mL) with 5% Pd/C (75 mg) was hydrogenated under atm pressure for 24 h. The reaction mixture was filtered through a plug of Celite and the filtrate was concentrated to give amine **7**. To amine **7** (1.74 mmol) in CH_2Cl_2 (15 mL) was added triethylamine (0.29 mL, 2.1 mmol) and 5-chloro-2-sulfonyl chloride (2.09 mmol). After 1–24 h the reaction mixture was diluted with EtOAc (30 mL), washed with 1 N HCl (30 mL), dried (Na_2SO_4) and concentrated. Column chromatography provided target compounds.

6.6.2.1. 5-Chloro-*N*-{2-ethyl-1-[1-(4-methoxybenzyl)-1*H*-1,2,4-triazol-5-yl]butyl}thiophene-2-sulfonamide (32). ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.58 (t, 3H, J = 7.4), 0.64 (t, 3H, J = 7.5 Hz), 0.71–0.86 (m, 2H), 1.24–1.37 (m, 1H), 1.40–1.47 (m, 1H), 1.62 (br s, 1H), 3.73 (s, 3H), 4.52 (d, 1H, J = 8.1 Hz), 5.26 (s, 2H), 6.91 (d, 2H, J = 8.7 Hz), 7.05 (d, 1H, J = 4.0 Hz), 7.11 (d, 1H, J = 4.1 Hz), 7.18 (d, 2H, J = 8.7 Hz), 7.81 (s, 1H), 8.87 (br s, 1H); MS (+ESI): 469 $(M+H)^+$. Anal. Calcd for $C_{20}H_{25}ClN_4O_3S_2$: C, 51.22; H, 5.37; N, 11.95. Found: C, 51.35; H, 5.38; N, 11.87.

6.6.2.2. *N*-[1-(1-Benzyl-1*H*-1,2,4-triazol-5-yl)-2-ethylbutyl]-5-chlorothiophene-2-sulfonamide (33). ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.55 (t, 3H, J = 7.4 Hz), 0.61 (t, 3H, J = 7.4 Hz), 0.70–0.82 (m, 2H), 1.29–1.37 (m, 1H), 1.40–1.46 (m, 1H), 1.60 (br s, 1H), 4.51 (t, 1H, J = 8.1 Hz), 5.33 (d, 1H, J = 15.9 Hz), 5.37 (d, 1H, J = 15.9 Hz), 7.05 (d, 1H, J = 4.0 Hz), 7.13 (d, 1H, J = 4.1 Hz), 7.22 (d, 2H, J = 8.1 Hz), 7.30–7.38 (m, 3H), 7.84 (s, 1H), 8.87 (d, 1H, J = 7.9 Hz); MS (+ESI): 439 $(M+H)^+$. Anal. Calcd for $C_{19}H_{23}ClN_4O_2S_2$: C, 51.98; H, 5.28; N, 12.76. Found: C, 51.86; H, 5.43; N, 12.68.

6.6.2.3. 5-Chloro-*N*-{2-ethyl-1-[1-(4-methylbenzyl)-1*H*-1,2,4-triazol-5-yl]butyl}thiophene-2-sulfonamide (34). ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.57 (t, 3H, J = 7.3 Hz), 0.63 (t, 3H, J = 7.5 Hz), 0.73–0.87 (m, 2H), 1.29–1.35 (m, 1H), 1.41–1.47 (m, 1H), 1.60 (br s, 1H), 2.28 (s, 3H), 4.52 (t, 1H, J = 8.2 Hz), 5.29 (s, 2H), 7.05 (d, 1H, J = 4.1 Hz), 7.10–7.17 (m, 5H), 7.82 (s, 1H), 8.86 (d, 1H, J = 8.2 Hz); mp 103–104 °C; MS (+ESI): 453 $(M+H)^+$. Anal. Calcd for $C_{20}H_{25}ClN_4O_2S_2$: C, 53.03; H, 5.56; N, 12.37. Found: C, 52.63; H, 5.63; N, 12.21.

6.6.2.4. 5-Chloro-*N*-(2-ethyl-1-{1-[4-(trifluoromethoxy)benzyl]-1*H*-1,2,4-triazol-5-yl}butyl)thiophene-2-sulfonamide (35). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.90 (d, 1H, J = 8.4 Hz), 7.87 (s, 1H), 7.37 (m, 4H), 7.17 (m, 1H), 7.07 (m, 1H), 5.42 (m, 2H), 4.50 (t, 1H, J = 8.4 Hz), 1.60 (m, 1H), 1.42 (m, 1H), 1.32 (m, 1H), 0.77 (m, 1H), 0.71 (m, 1H), 0.62 (t, 3H, J = 7.4 Hz), 0.54 (t, 3H, J = 7.4 Hz); MS (−ESI): 521 $(M-H)^-$. Anal. Calcd for $C_{20}H_{22}N_4O_3ClF_3S_2$: C, 45.93; H, 4.24; N, 10.71. Found: C, 46.35; H, 4.11; N, 10.63.

6.6.2.5. 5-Chloro-*N*-{2-ethyl-1-[1-(4-fluorobenzyl)-1*H*-1,2,4-triazol-5-yl]butyl}thiophene-2-sulfonamide (36). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.83 (br m, 1H), 7.79 (s, 1H), 7.22 (m, 2H), 7.11 (m, 3H), 7.01 (d, 1H, J = 4.0 Hz), 5.30 (s, 2H), 4.47 (d, 1H, J = 8.4 Hz), 1.56 (m, 1H), 1.39 (m, 1H), 1.29 (m, 1H), 0.79 (m, 1H), 0.61 (m, 1H), 0.59 (t, 3H, J = 7.4 Hz), 0.52 (t, 3H, J = 7.3 Hz); MS (+ESI): 457 $(M+H)^+$. Anal. Calcd for $C_{19}H_{22}N_4O_2S_2ClF$: C, 49.94; H, 4.85; N, 12.26. Found: C, 50.24; H, 4.84; N, 12.23.

6.6.2.6. *N*-[1-(1-Benzyl-1*H*-1,2,4-triazol-5-yl)-2-methylpropyl]-5-chlorothiophene-2-sulfonamide (37). ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.46 (d, 3H, J = 6.7 Hz), 0.85 (d, 3H, J = 6.6 Hz), 1.85–2.02 (m, 1H), 4.22–4.26 (m, 1H), 5.38 (d, 2H, J = 7.5 Hz), 7.08 (d, 1H, J = 4.0 Hz), 7.18 (s, 1H), 7.25 (d, 2H, J = 6.9 Hz), 7.31–7.36 (m, 3H), 7.85 (s, 1H), 8.84 (s, 1H); MS (−ESI): 409 $(M-H)^-$. Anal. Calcd for $C_{17}H_{19}ClN_4O_2S_2$: C, 49.69; H, 4.66; N, 13.63. Found: C, 49.85; H, 4.71; N, 13.55.

6.6.2.7. 5-Chloro-*N*-{2-methyl-1-[1-(4-methylbenzyl)-1*H*-1,2,4-triazol-5-yl]propyl}thiophene-2-sulfonamide (38). General procedure I from **5w** to form azide, then general procedure C to form the amine, followed by general procedure D. ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.85 (d, 6H, J = 7.8 Hz), 1.97–2.02 (m, 1H), 2.28 (s, 3H), 4.38 (t, 1H, J = 7.8 Hz), 5.31 (d, 2H, J = 5.7 Hz), 7.08 (d, 1H, J = 4.0 Hz), 7.15–7.17 (m, 5H), 7.83 (s, 1H), 8.88 (d, 1H, J = 7.0 Hz); MS (+ESI): 425 $(M+H)^+$. Anal. Calcd for $C_{18}H_{21}ClN_4O_2S_2$: C, 50.87; H, 4.98; N, 13.18. Found: C, 51.01; H, 4.91; N, 13.15.

6.6.3. General procedure for phenols 40, 41, 53

To the corresponding methoxyphenyl analog (121 μmol) in CH_2Cl_2 (3 mL) at −78 °C was added a solution of boron tribromide (1 M in CH_2Cl_2, 400 μL, 400 μmol) dropwise. The reaction mixture was warmed to room temperature gradually over several hours and allowed to stir overnight. Water (10 mL) was added slowly to the mixture. It was then extracted with EtOAc (3 × 20 mL), dried (Na_2SO_4) and concentrated. Column chromatography (EtOAc/hex) provided the target compounds.

6.6.3.1. 5-Chloro-*N*-{2-ethyl-1-[1-(4-hydroxybenzyl)-1*H*-pyrazol-5-yl]butyl}thiophene-2-sulfonamide (40). Mp = 90–92 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.59 (t, 3H, J = 7.4 Hz), 0.67 (t, 3H, J = 7.3 Hz), 0.87–0.99 (m, 2H), 1.11–1.32 (m, 3H), 4.43 (br s, 1H), 5.04 (q, 2H, J = 15.8 Hz), 6.06 (d, 1H, J = 1.8 Hz), 6.66 (d, 2H, J = 8.5 Hz), 6.80 (d, 1H, J = 4.0 Hz), 6.89 (d, 1H, J = 4.0 Hz), 6.90 (d, 2H, J = 8.5 Hz), 7.18 (d, 1H, J = 1.8 Hz), 8.38 (br s, 1H), 9.34 (s, 1H); MS (−ESI): 452 $(M-H)^-$. Anal. Calcd for $C_{20}H_{24}ClN_3O_3S_2$: C, 52.91; H, 5.33; N, 9.26. Found: C, 52.67; H, 5.41; N, 8.86.

6.6.3.2. 5-Chloro-*N*-{2-ethyl-1-[1-(4-hydroxyphenyl)-1*H*-pyrazol-5-yl]butyl}thiophene-2-sulfonamide (41). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.87 (s, 1H), 8.54 (d, 1H, J = 7.9 Hz), 7.38 (d, 1H, J = 1.7 Hz), 7.21 (d, 1H, J = 4.1 Hz), 7.10 (m, 3H), 6.89 (d, 2H, J = 8.9 Hz), 6.29 (m, 1H), 4.49 (m, 1H), 1.31 (m, 1H), 1.16 (m, 1H), 1.11 (m, 1H), 1.08 (m, 2H), 0.59 (t, 3H, J = 7.2 Hz), 0.50 (t, 3H, J = 7.3 Hz); MS (+ESI): 440 $(M+H)^+$. Anal. Calcd for $C_{19}H_{22}N_3O_3S_2Cl$: C, 51.87; H, 5.04; N, 9.55. Found: C, 51.77; H, 5.04; N, 9.40.

6.6.3.3. 5-Chloro-*N*-{(1S)-1-[1-(4-hydroxyphenyl)-1*H*-pyrazol-5-yl]-2-methylpropyl}thiophene-2-sulfonamide (53). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.80 (br s, 1H), 8.59 (d, 1H, J = 9.2 Hz), 7.37 (d, 1H, J = 1.9 Hz), 7.09 (d, 1H, J = 4.1 Hz), 7.06 (d, 1H,

J = 4.0 Hz), 7.04–7.00 (m, 2H), 6.84 (d, 2H, J = 8.8 Hz), 6.25 (d, 1H, J = 1.9 Hz), 4.10 (m, 1H), 1.79–1.69 (m, 1H), 0.71 (d, 3H, J = 6.75 Hz), 0.58 (d, 3H, J = 6.82 Hz). MS (−ESI): 410.1 $(M-H)^-$. Anal. Calcd for $C_{17}H_{18}ClN_3O_3S_2$: C, 49.57; H, 4.40; N, 10.20. Found: C, 49.35; H, 4.03; N, 9.86. $[\alpha]_D^{25} = +66.0$ (c 1.0, $CHCl_3$).

6.6.3.4. 5-Chloro-*N*-[2-ethyl-1-(1-phenyl-1*H*-pyrazol-5-yl)butyl]-*N*-methylthiophene-2-sulfonamide (42). To a solution of **11** (0.238 g, 0.51 mmol) in anhydrous THF (10 mL) at 0 °C under N_2 was added NaH (0.289 g, 2.03 mmol, 60% in mineral oil). The mixture was stirred for 30 min at 0 °C. Iodomethane (0.049 g, 2.03 mmol) was added to this mixture. After 12 h the mixture was diluted with H_2O (10 mL), then extracted with EtOAc (2 × 30 mL) and washed with brine (30 mL). The organic extract was dried (Na_2SO_4) and concentrated under reduced pressure. Chromatography purification (15% EtOAc/hexane) afforded the title compound (0.128 g, 52%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.61s, 1H), 7.53–7.45 (m, 2H), 7.44–7.42 (m, 1H), 7.29 (d, 2H, J = 5.01 Hz), 7.14 (d, 1H, J = 4.01 Hz), 6.97 (d, 1H, J = 4.2 Hz), 6.56 (s, 1H), 5.04 (d, 1H, J = 11.3 Hz), 2.75 (s, 3H), 2.00 (m, 1H), 1.42–1.38 (m, 1H), 1.31–1.22 (m, 1H), 1.02–0.98 (m, 2H), 0.72 (t, 3H, J = 7.01 Hz), 0.62 (t, 3H, J = 6.99 Hz). MS (+ESI): 438.1 $(M+H)^+$. Anal. Calcd for $C_{20}H_{24}N_3O_2S_2$: C, 54.84; H, 5.52; N, 9.59. Found: C, 55.16; H, 5.25; N, 9.34.

6.6.3.5. 5-Bromo-*N*-[2-ethyl-1-(1-phenyl-1*H*-pyrazol-5-yl)butyl]-*N*-methylthiophene-2-sulfonamide (43). This compound was prepared with a similar procedure to that described for **42**, but used **18** as the starting material. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.65 (s, 1H), 7.56–7.53 (m, 1H), 7.50–7.47 (m, 1H), 7.35 (d, 1H, J = 7.2 Hz), 7.25 (d, 1H, J = 4.2 Hz), 6.96 (d, 1H, J = 4.3 Hz), 6.61 (s, 1H), 5.06 (d, 1H, J = 11.1 Hz), 2.79 (s, 3H), 2.19–2.02 (m, 1H), 1.52–1.42 (m, 1H), 1.32–1.25 (m, 1H), 1.09–1.01 (m, 2H), 0.75 (t, 3H, J = 7.05 Hz), 0.68 (t, 3H, J = 6.97 Hz). MS (+ESI): 482.1 $(M+H)^+$. Anal. Calcd for $C_{20}H_{24}BrN_3O_2S_2$: C, 49.79; H, 5.01; N, 8.71. Found: C, 50.48; H, 4.89; N, 8.46.

6.6.3.6. (*S*)-2-(Benzyloxycarbonylamino)-3-ethylpentanoic acid (44b). (S)-2-Amino-3-ethyl-pentanoic acid[13] (1.32 g, 9.1 mmol) was dissolved in a mixed solvent of 1,4-dioxane/H_2O (1:1, 20/20 mL). A 1 N sodium hydroxide solution (18.2 mL, 18.2 mmol) was added slowly to the stirred solution and benzyl chloroformate (1.54 mL, 11.0 mmol) was added dropwise. The mixture was stirred at room temperature for 36 h, during which time a white precipitate formed. A 2 N hydrochloric acid solution (approximately 50 mL) was added to adjust pH to 1–2. The mixture was extracted with ethyl acetate (3 × 80 mL). The combined organic layers were washed with brine (50 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure to give 2.54 g of an oil, which was used in the next step without further purification. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.22 (1H, br s), 7.36–7.25 (m, 5H), 5.00 (m, 2H), 4.05 (m, 1H), 1.61–1.58 (m, 1H), 1.31–1.18 (m, 4H), 0.82–0.69 (m, 6H). MS (+ESI): 280.2 $(M+H)^+$.

6.6.3.7. (*S*)-Benzyl 3-ethyl-1-(methoxy(methyl)amino)-1-oxopentan-2-ylcarbamate (45b). To a solution of 44b (2.55 g, 9.1 mmol) in anhydrous CH_2Cl_2 (40 mL) was added *N*-methylmorpholine (2.1 mL, 18.3 mmol), 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide) hydrochloride (2.63 g, 13.7 mmol) and *N*,*O*-dimethylhydroxylamine hydrochloride (1.34 g, 13.7 mmol). The mixture was stirred at room temperature for 16 h. Saturated aqueous $NaHCO_3$ (50 mL) was added carefully and the organic layer was separated from aqueous layer and the aqueous layer was extracted with ethyl acetate (3 × 80 mL). The combined organic layer was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was purified by flash chromatography (EtOAc/hexane, 1:1) to afford 45b (1.58 g, 54% yield), which solidified on standing. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.39–7.24 (m, 5H), 6.60 (br s, 1H), 4.99 (m, 2H), 4.58 (m, 1H), 3.70 (s, 3H), 3.07 (s, 3H), 1.59–1.51 (m, 1H), 1.41–1.21 (m, 3H), 1.19–1.06 (m, 1H), 0.80 (t, 3H, J = 7.45 Hz), 0.74 (t, 3H, J = 7.43 Hz). MS (+ESI): 323.2 $(M+H)^+$.

6.6.4. General procedure for the chiral synthesis of 49–52, 54, 55

Step A. To a solution of **45a**[19] or **45b** (3.4 mmol) in anhydrous THF (20 mL) at −78 °C was added a solution of ethynylmagnesium bromide (0.5 M in THF, 27 mL, 14 mmol) dropwise over a period of 10 min. The reaction mixture was warmed to room temperature and stirred for 16 h. The solution was cooled to 0 °C and quenched with saturated aqueous $NaHSO_4$ (10 mL). The solution was stirred for 1 h and then extracted with EtOAc (3 × 60 mL). The combined organic layers were washed with saturated aqueous $NaHCO_3$ (50 mL) and brine (50 mL), dried (Na_2SO_4) and concentrated. Column chromatography (EtOAc/hexane, 3:7) afforded title compound **46**.

Step B. Diethylamine (1.1 mL, 11 mmol) was added dropwise over 5 min to a solution of **46** (10 mmol) in anhydrous CH_2Cl_2 (40 mL) at 0 °C under N_2 atmosphere. The mixture was allowed to warm to 25 °C overnight (16 h). The solution was diluted with H_2O (50 mL). The aqueous and organic layers were separated and the aqueous layer was extracted with CH_2Cl_2 (2 × 100 mL). The combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to obtain a yellow-oil. The crude product was purified by flash chromatography (EtOAc/hexane, 1:1) to afford title compound **47**.

Step C. To a solution of **47** (6.0 mmol) in EtOH (30 mL) was added the corresponding phenyl hydrazine hydrochloride (6.32 mmol) and aqueous HCl (37%, 0.5 mL, 6.3 mmol). The solution was heated to reflux for 6 h. The reaction mixture was cooled to room temperature and the solvent was removed under reduced pressure. The mixture was then diluted with H_2O (50 mL), and extracted with EtOAc (3 × 60 mL). The combined organic layers were washed with saturated aqueous $NaHCO_3$ (50 mL) and brine (50 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was purified by flash chromatography (EtOAc/hexane, 1:1) to give **48**.

Step D. Pyrazole **48** (2 mmol) in MeOH (10 mL) with Pd/C (10%, 0.1 g) was hydrogenated under atm pressure for 16 h. The reaction mixture was filtered through a plug of Celite and the filtrate was concentrated to give amine intermediate which was used in the next step without further purification.

Step E. To a solution of amine (2.0 mmol) in CH_2Cl_2 (20 mL) was added triethylamine (0.83 mL, 6.0 mmol) and 5-chloro-thiophene-2-sulfonyl chloride (0.54 mL, 4.0 mmol). The mixture was stirred at room temperature for 16 h, then diluted with CH_2Cl_2 (50 mL) and washed with aqueous HCl (1 N, 20 mL). The aqueous layer was extracted with CH_2Cl_2 (3 × 50 mL). The combined organic layers were washed with saturated aqueous $NaHCO_3$ (30 mL), and brine (30 mL), then dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was purified by flash chromatography (EtOAc/hexane, 1:1) to give target compound.

6.6.4.1. 5-Chloro-*N*-{(1S)-2-methyl-1-[1-(4-methylphenyl)-1*H*-pyrazol-5-yl]propyl}thiophene-2-sulfonamide (49). ^{1}H NMR (same as **24**). MS (+ESI): 410.2 $(M+H)^+$. Anal. Calcd for $C_{18}H_{20}ClN_3O_2S_2$: C, 52.74; H, 4.92; N, 10.25. Found: C, 52.87; H, 4.82; N, 10.08. $[\alpha]_D^{25} = +67$ (c 1.0, $CHCl_3$). Chiral purity = 99.2% (Chiralcel AD column, 0.45 × 25 cm, 30% EtOH in hexane, racemate used as reference).

6.6.4.2. 5-Chloro-*N*-{(1S)-1-[1-(4-fluorophenyl)-1*H*-pyrazol-5-yl]-2-methylpropyl}thiophene-2-sulfonamide (50). ^{1}H NMR (same as **26**). MS (+ESI): 414.2 $(M+H)^+$. Anal. Calcd for

$C_{17}H_{17}ClFN_3O_2S_2$: C, 49.33; H, 4.14; N, 10.15. Found: C, 49.42; H, 4.14; N, 9.99. $[\alpha]_D^{25} = +21$ (*c* 1.0, $CHCl_3$). Chiral purity = 100% (Chiralcel AD column, 0.45 × 25 cm, 30% EtOH in hexane, racemate used as reference).

6.6.4.3. (5-Chloro-*N*-{(1*R*)-1-[1-(4-methoxyphenyl)-1*H*-pyrazol-5-yl]-2-methylpropyl}thiophene-2-sulfonamide (51). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.64 (d, 1H, *J* = 8.95 Hz), 7.42 (dHz, 1H, *J* = 1.7), 7.17 (d, 2H, *J* = 8.93 Hz), 7.02–7.04 (m, 4H), 6.18 (d, 1H, *J* = 1.9 Hz), 4.14–4.12 (m, 1H), 3.80 (s, 3H), 1.79–1.70 (1H, m), 0.72 (d, 3H, *J* = 6.73 Hz), 0.60 (d, 3H, *J* = 6.84 Hz). MS (−ESI): 424.0 $(M-H)^-$. Anal. Calcd for $C_{18}H_{20}ClN_3O_3S_2$: C, 50.76; H, 4.73; N, 9.86. Found: C, 50.89; H, 4.58; N, 9.86. $[\alpha]_D^{25} = +66$ (*c* 1.0, $CHCl_3$).

6.6.4.4. 5-Chloro-*N*-{(1S)-2-ethyl-1-[1-(4-methoxyphenyl)-1*H*-pyrazol-5-yl]butyl}thiophene-2-sulfonamide (52). ^{1}H NMR (same as **28**); MS (+ESI): 454.1 $(M+H)^+$. Anal. Calcd for $C_{20}H_{24}ClN_3O_3S_2$: C, 52.91; H, 5.33; N, 9.26. Found: C, 53.24; H, 5.33; N, 8.85. $[\alpha]_D^{25} = +63.0$ (*c* 1.0, $CHCl_3$).

6.6.4.5. 5-Chloro-*N*-{(1*R*)-2-methyl-1-[1-(4-methylphenyl)-1*H*-pyrazol-5- yl]propyl}thiophene-2-sulfonamide (54). ^{1}H NMR (same as **24**). MS (+ESI): 410.2 $(M+H)^+$. Anal. Calcd for $C_{18}H_{20}ClN_3O_2S_2$: C, 52.74; H, 4.92; N, 10.25. Found: C, 52.97; H, 4.81; N, 10.16. $[\alpha]_D^{25} = -66$ (*c* 1.0, $CHCl_3$). Chiral purity = 99.2% (Chiralcel AD column, 0.45 × 25 cm, 30% EtOH in hexane, racemate used as reference).

6.6.4.6. 5-Chloro-*N*-{(1R)-1-[1-(4-fluorophenyl)-1*H*-pyrazol-5-yl]-2-methylpropyl}thiophene-2-sulfonamide (55). ^{1}H NMR (same as **26**). MS (+ESI): 414.2 $(M+H)^+$. Anal. Calcd for $C_{17}H_{17}ClFN_3O_2S_2$: C, 49.33; H, 4.14; N, 10.15. Found: C, 49.65; H, 4.24; N, 10.13. $[\alpha]_D^{25} = -17$ (*c* 1.0, $CHCl_3$). Chiral purity = 100% (Chiralcel AD column, 0.45 × 25 cm, 30% EtOH in hexane, racemate used as reference).

The following compounds were isolated by chiral chromatography (56–59).

6.6.4.7. 5-Chloro-*N*-{(1*S*)-2-ethyl-1-[1-(4-methoxybenzyl)-1*H*-pyrazol-5-yl]butyl}thiophene- 2-sulfonamide (56). Racemate **8** was separated into enantiomers by chiral HPLC (Chiralcel AD-H, 2 × 25 cm, 10% MeOH/EtOH—8/2 in hexane/DEA; retention time = 5.03 min); ^{1}H NMR (same as **8**); MS (−ESI): 466 $(M-H)^-$. $[\alpha]_D^{25} = +30.0$ (*c* 1.0, $CHCl_3$). Purity determined by LC–MS at 254 and 215 nm: >99%.

6.6.4.8. 5-Chloro-*N*-{(1R)-2-ethyl-1-[1-(4-methoxybenzyl)-1*H*-pyrazol-5-yl]butyl}thiophene- 2-sulfonamide (57). Racemate **8** was separated into enantiomers by chiral HPLC (Chiralcel AD-H, 2 × 25 cm, 10% MeOH/EtOH—8/2 in hexane/DEA; retention time = 6.18 min); ^{1}H NMR (same as **8**); MS (−ESI): 466 $(M-H)^-$. $[\alpha]_D^{25} = -25.0$ (*c* 1.0, $CHCl_3$). Purity determined by LC–MS at 254 and 215 nm: >99%.

6.6.4.9. 5-Chloro-*N*-{(1*S*)-2-ethyl-1-[1-(4-hydroxybenzyl)-1*H*-pyrazol-5-yl]butyl}thiophene- 2-sulfonamide (58). Racemate **40** was separated into enantiomers by chiral HPLC (Chiralcel AD-H, 2 × 25 cm, 10% MeOH/EtOH—8/2 in hexane/DEA; retention time = 6.51 min); ^{1}H NMR (same as **40**); MS (−ESI): 452 $(M-H)^-$. $[\alpha]_D^{25} = +79.0$ (*c* 1.0, $CHCl_3$). Purity determined by LC–MS at 254 and 215 nm: >99%.

6.6.4.10. 5-Chloro-*N*-{(1*R*)-2-ethyl-1-[1-(4-hydroxybenzyl)-1*H*-pyrazol-5-yl]butyl}thiophene- 2-sulfonamide (59). Racemate **40** was separated into enantiomers by chiral HPLC (Chiralcel AD-H, 2 × 25 cm, 10% MeOH/EtOH—8/2 in hexane/DEA; retention time: 7.59 min); ^{1}H NMR (same as **40**); MS (−ESI): 452 $(M-H)^-$. $[\alpha]_D^{25} = -80.0$ (*c* 1.0, $CHCl_3$). Purity determined by LC–MS at 254 and 215 nm: >99%.

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Supplementary data

Synthetic details and characterization of intermediates **4**–**7** and ^{1}H NMR and HPLC data for compounds **56**–**59** for proof of purity and ^{1}H NMR of compounds **19** and **27** are included. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2009.04.052.

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