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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

# Structure–activity relationship investigation of triazole-based kappa opioid receptor agonists

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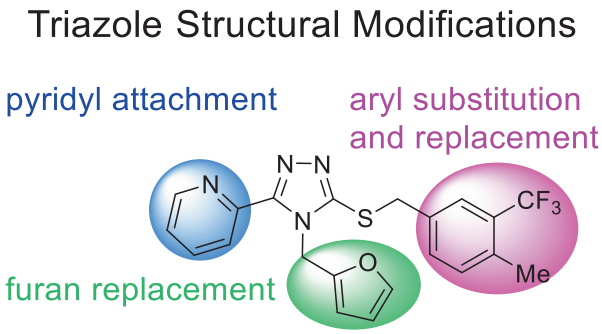
## Abstract

Select triazole-based small molecules possess potent and selective kappa opioid receptor (KOR) agonism. Here, we designed twenty new analogs to investigate the structure–activity relationship effects for all three functional groups attached to the triazole core. We identified specific groups that are critical for KOR potency and further extended the range of moieties explored. These efforts revealed analogs with potency on par with our lead triazole probe molecule, Triazole 1.1, in addition to analogs possessing a spectrum of less potent KOR agonist activity.

## Graphical Abstract




**Keywords** Opioids · Kappa opioid receptor · Triazoles · Antinociceptive agents · Anti-itch agents

## Introduction

Kappa opioid receptor (KOR) agonists have long been of interest for their antinociceptive properties [1]. Additionally, KOR activation opposes the effect of mu opioid receptor (MOR) activation [2]. However, the therapeutic adoption of KOR agonist analgesics has been limited due to the severe dysphoria associated with KOR activation within the CNS [3]. The endogenous peptide dynorphin

With affection to Professor Gary Grunewald in honor of his sustained and profound contributions to science and his commitment to supporting those who practice it.

**Supplementary information** The online version contains supplementary material available at https://doi.org/10.1007/s00044-021-02746-1.

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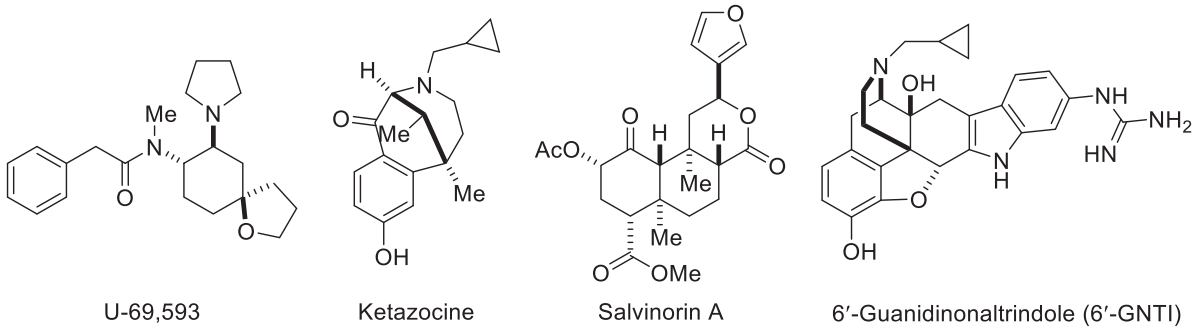



**Fig. 1** Structures of representative KOR small-molecule agonsists

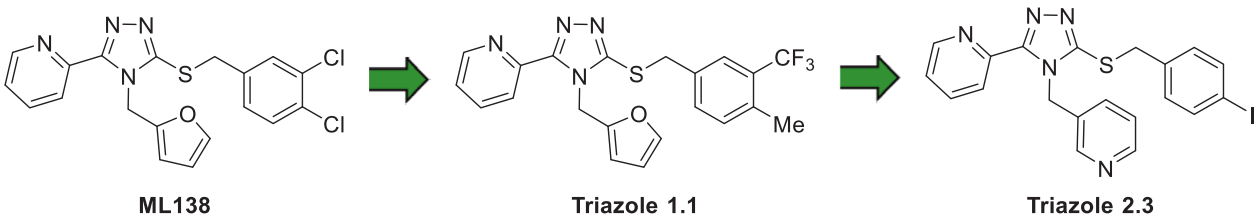
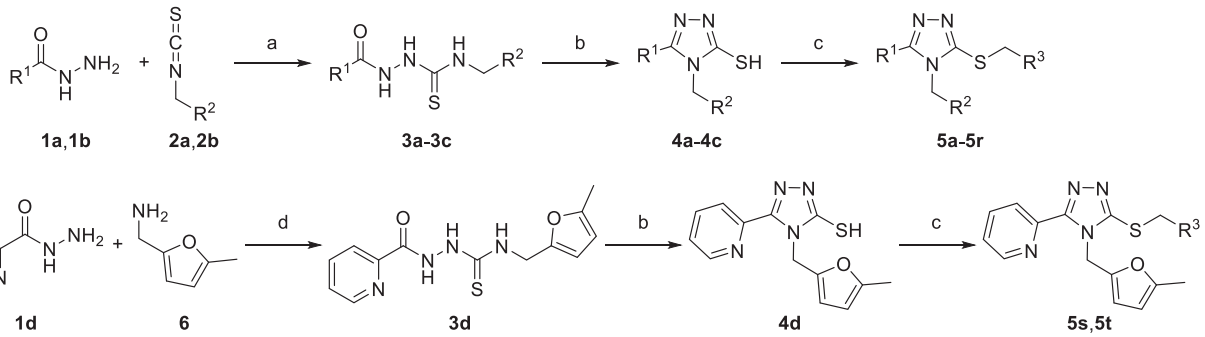


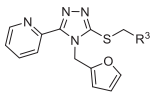

**Fig. 2** Discovery and development of a triazole-based KOR agonist series




**Scheme 1** General synthetic routes to triazole analogs **5**[a]. [a]Reagents and conditions: (**a**) MeCN, rt, 16 h; (**b**) $NaOH_{(aq)}$, reflux, 2 h; $HCl_{(aq)}$; (**c**) $K_2CO_3$, $R^3CH_2Br$, or $R^3CH_2Cl$, acetone, rt, 15 h; (**d**) di-2-pyridyl thiocarbonate, THF, rt, 2 h

selectively activates the KOR and initiates our natural stress response [4]. Numerous small-molecule KOR agonists have been reported [5], notably U-69,593 [6], ketazocine [7], salvinorin A [8], and 6′-guanidinonaltrindole [9] (Fig. 1). We have also reported small-molecule KOR agonist chemotypes, including the discovery and further development of a triazole-based KOR agonist series (Fig. 2). The KOR agonist activity of this triazole scaffold was first reported for the KOR agonist probe molecule ML138 and a limited set of fifteen related analogs, which possessed a wide KOR agonist potency range and high selectivity for the KOR over the MOR and the delta opioid receptor (DOR) [10]. We subsequently optimized the potency and further characterized the signaling properties for several new analogs (e.g., the improved probe molecule Triazole 1.1), revealing a notable trend for biased signaling of the G protein-mediated pathway over β-arrestin recruitment [11]. More recently, we identified additional analogs (e.g., Triazole 2.3) that possessed signaling bias for KOR activation of the ERK 1/2 pathway [12]. The lead compound, Triazole 1.1 has been further characterized in striatal neurons and several mouse models [13]. Triazole 1.1 was found to produce the desired analgesic and anti-pruritic effects associated with KOR agonists, notably without a reduction in dopamine levels typically observed with traditional KOR agonists [14]. Here we report the synthesis and KOR agonist activity of triazole analogs that investigate structural modifications at all three side chains on the triazole core.

**Table 1** Structures and KOR potency of triazole analogs exploring thioether moiety

| Cmpd |  $R^3$ | G protein-mediated KOR activation[a] | | KOR β-arrestin recruitment[b] | |
|---|---|---|---|---|---|
| | | $EC_{50}$ (nM) | $E_{max}$ (%) | $EC_{50}$ (nM) | $E_{max}$ (%) |
| U69,593 | – | 51.3 ± 3.6 | 100 | 6.4 ± 0.8 | 100 |
| Triazole 1.1 | 4-methyl-3-(trifluoromethyl)phenyl | 77.2 ± 17.9 | 90.1 ± 4.7 | 4960 ± 540 | 115.0 ± 3.6 |
| ML138 | 3,4-dichlorophenyl | 15.0 ± 6.0 | 97.5 ± 1.2 | 1380 ± 320 | 100 ± 15 |
| **5a** | 4-chloro-2-(trifluoromethyl)phenyl | 169 ± 42 | 100 ± 3.8 | >10,000 | – |
| **5b** | 4-chlorophenyl | 122.0 ± 23.1 | 90.4 ± 1.6 | >10,000 | – |
| **5c** | 2-methoxyphenyl | not pursued[c] | (26.3)[d] | No data | – |
| **5d** | 3-methoxyphenyl | 1340 ± 330 | 88.6 ± 3.5 | >10,000 | – |
| **5e** | 2,6-difluorophenyl | 2250 ± 270 | 87.7 ± 3.3 | >10,000 | – |
| **5f** | 2-naphthyl | 75.0 ± 20.7 | 96.4 ± 1.0 | 4290 ± 1250 | 63.3 ± 6.2 |
| **5g** | 2-pyridyl | >10,000 | (68 ± 6)[d] | No data | – |
| **5h** | 4-pyridyl | Not pursued[c] | (58)[d] | No data | – |
| **5i** | Cyclohexyl | Not pursued[c] | (35)[d] | No data | – |
| **5j** | Cyclopropyl | Not pursued[c] | (14)[d] | No data | – |
| **5k** | Ethylene | Not pursued[c] | (11)[d] | No data | – |
| **5l** | Ethyne | Not pursued[c] | (6)[d] | No data | – |

[a]Measured by [$^{35}$S]GTPγS membrane binding

[b]Measured using the DiscoverX PathHunter assay platform

[c]Compound was not pursued based on 2 point screens in the [$^{35}$S]GTPγS assay (i.e., compound did not show promising effects at 50 nM (<5% stim) or 10 μM (<80%) testing)

[d]For curves that do not plateau at maximum concentration (10 μM), the potency is estimated as >10,000 nM; the maximum stimulation observed at 10 μM is provided in parentheses for the $E_{max}$

## Results and discussion

The triazole analogs were synthesized using analogous methods to those for our previously reported triazole analogs (Scheme 1) [10–12]. In most cases, the appropriate hydrazide **1** and isothiocyanate **2** were coupled to afford the hydrazinecarbothioamides **3**, which were subsequently cyclized/dehydrated to afford the penultimate triazole intermediates **4**. Nucleophilic addition of **4** to benzyl or alkyl halide components provided the final triazole analogs **5**. When the requisite isothiocyanate component **2** was not commercially available (i.e., for the synthesis of methylfuranyl thione **4d**), the amine component **6** was instead combined with the hydrazide **1a** using the coupling reagent di-2-pyridyl thiocarbonate to directly afford the hydrazinecarbothioamide **3d**, which was cyclized/dehydrated as above to afford **4d**. The synthesized triazole analogs **5** were analyzed by HPLC/MS for purity and evaluated for their ability to activate the KOR using two complementary assays for KOR activation (Table 1). Measurement of [$^{35}$S]GTPγS membrane binding [15] at the KOR provided an indication of G protein activation while the DiscoverX PathHunter assay platform [16] provided assessment of β-arrestin recruitment. To enable direct comparison, the KOR agonist potency for U69,593 and the lead compound from this series, Triazole 1.1, were also determined in the identical assay conditions and U69,593 included as a positive control for each assay set. Compounds were first evaluated in a two-point screen (compound concentrations of 50 nM and 10 μM) using the [$^{35}$S]GTPγS assay. Compounds were only pursued further if they showed promising effects at either 50 nM (>5% stim) or 10 μM (>80%) test concentrations.

Having previously established a critical effect for the aromatic ring substitution on the benzyl thioether moiety [10], we began our investigation by surveying alternative aromatic substitution. Our lead compound, Triazole 1.1, bears a 4-methyl-3-(trifluoromethyl)phenyl group and we had previously explored several other permutations of methyl- or chloro-substitution in combination with a trifluoromethyl group. The 3,4-dichlorophenyl analog ML138 had previously only been screened for KOR agonist activity in β-arrestin recruitment assays (the DiscoverX PathHunter assay [15] and a high-content imaging assay using GFP labeled β-arrestin [17]. Therefore, we evaluated ML138 in the [$^{35}$S]GTPγS assay and found that it was an even more potent KOR agonist in this assay than Triazole 1.1. We expanded the substitution patterns investigated to include the 4-chloro-2-(trifluoromethyl)phenyl-bearing analog **5a**, which was 2.2-fold less potent in the GTPγS assay and inactive in the DiscoverX assay up to 10 μM. Inspired by ML138, we synthesized the simple 4-chlorophenyl analog **5b**, which was

nearly as potent as Triazole 1.1 (within experimental error) and completely inactive in the DiscoverX assay up to 10 μM. Other substitution patterns investigated were markedly less potent with both the 3-methoxyphenyl analog **5d** and the 2,6-difluorophenyl analog **5e** over tenfold less potent in the GTPγS assay and inactive in the DiscoverX assay up to 10 μM, while the 2-methoxyphenyl analog **5c** possessed no significant KOR agonist activity. The 2-naphthyl analog **5f** was on par with the potency for Triazole 1.1 in both assays and appeared to possess reduced efficacy ($E_{max}$) in the DiscoverX assay, compared to Triazole 1.1. Replacement of the substituted phenyl ring for the heterocyclic moieties 2-pyridyl or 4-pyridyl (analogs **5g** and **5h**, respectively) afforded analogs with no significant activity. Similarly inactive analogs were obtained by replacement of the phenyl with cyclohexyl (**5i**), cyclopropyl (**5j**), ethylene (**5k**), or ethyne (**5l**), none of which were pursued beyond the two-point screen. The further exploration of phenyl ring substitution detailed here reinforces the critical effect that aryl substitution has for KOR activation potency and identified two analogs with useful activity profiles similar to Triazole 1.1, namely the 4-chlorophenyl analog **5b** and the 2-naphthyl analog **5f**. Among the analogs surveyed here, replacement of the substituted phenyl ring with anything other than 2-naphthyl was not tolerated and resulted in inactive analogs.

We next investigated a limited set of modifications at the other positions of the triazole core (Table 2). Insertion of a methylene linker between the triazole and 2-pyridyl group was not tolerated and resulted in complete loss of activity (analogs **5m** and **5n**). Replacement of the furan with a cyclohexyl group was similarly detrimental with only the 4-cloro-3-(trifluoromethyl)phenyl analog **5q** possessing even modest KOR agonist potency. Neither the unsubstituted phenyl analog **5o** nor the 4-methyl-3-(trifluoromethyl)phenyl analog **5p**, which shared the phenyl substitution pattern of Triazole 1.1, possessed any significant KOR agonist activity. The more subtle modification of adding a methyl group to the furan at the 5-position was less detrimental to KOR agonist activity, though these analogs were still less potent than the corresponding unsubstituted furan analogs. While the unsubstituted phenyl analog **5r** possessed no significant KOR agonist activity, the 4-bromophenyl analog **5s** possessed modest potency in the GTPγS assay. The 3,4-dichlorophenyl analog **5t** possessed only slightly reduced KOR agonist potency compared to Triazole 1.1, reinforcing our previous observations comparing the activity of methylfuran analogs **5u** and **5v** to Triazole 1.1 [11].

## Conclusion

We have prepared twenty triazole analogs and evaluated their KOR agonist activity in two complementary assays for KOR activation ($^{35}$S-GTPγS binding and β-arrestin recruitment via the DiscoverX PathHunter assay platform). We compared the new analog activity profiles to U69,593 as well as the reported triazole KOR agonists, Triazole 1.1, and ML238. The triazole analogs synthesized here have extended the range of modifications explored and revealed critical functional groups for potent KOR agonism (i.e., 2-pyridyl directly attached to the triazole core and furan or other aromatic moiety tethered to the triazole nitrogen). The 5-methylfuran analogs were 3–8-fold less potent than their unsubstituted furan counterparts, whereas the cyclohexyl analogs were essentially inactive. On the thioether sidechain, we determined that a substituted phenyl ring was required for potency with either 4-chlorophenyl or 2-naphthyl affording potent KOR agonist compounds. Both analogs could serve as suitable stand-in molecules for Triazole 1.1, if needed. Other phenyl substitutions explored afforded substantially less potent analogs. Overall, the synthesis and evaluation of the analogs described here has revealed new SAR trends on aryl substitution to investigate further and several unproductive modifications to avoid in future optimization efforts. The further refinement of this series and continued evaluation of Triazole 1.1 is ongoing and will be reported in due course.

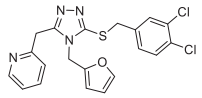
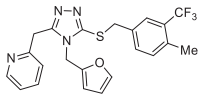
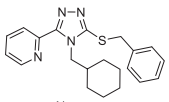
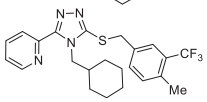
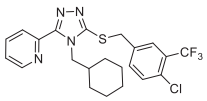
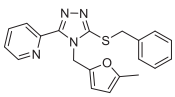
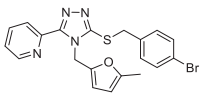
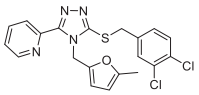
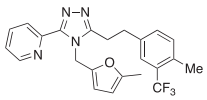
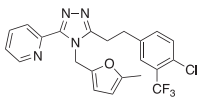
## Experimental section

### Chemistry

#### General synthesis information

All reagents and materials were purchased from commercial vendors and used as received. Ethyl ether, toluene, THF, MeCN and $CH_2Cl_2$ were degassed with nitrogen and passed through two columns of basic alumina on an Innovative Technology solvent purification system. Reactions and chromatography were monitored by thin-layer chromatography on 0.25 mm Analtech GHLF silica gel plates and visualized by UV light (254 nm) or Seebach's stain and heating. Purification was achieved by flash chromatography on a CombiFlash Rf (automated flash chromatography) system. Automated preparative RP HPLC purification was performed using an Agilent 1200 Mass-Directed Fractionation system (Prep Pump G1361 with gradient extension, make-up pump G1311A, pH modification pump G1311A, HTS PAL autosampler, UV-DAD detection G1315D, fraction collector G1364B, and Agilent 6120 quadrapole spectrometer G6120A). The preparative chromatography conditions included a Waters X-Bridge C18 column (19 × 150 mm, 5 μm, with 19 × 10 mm guard column), elution with a water and

**Table 2** Structures and KOR potency of triazole analogs exploring 2-pyridyl or furan moiety replacements

| Cmpd | Structure | G protein-mediated KOR activation[a] | | KOR β-arrestin recruitment[b] | |
|---|---|---|---|---|---|
| | | $EC_{50}$ (nM) | $E_{max}$ (%) | $EC_{50}$ (nM) | $E_{max}$ (%) |
| U69,593 | – | 51.3 ± 3.6 | 100 | 6.4 ± 0.8 | 100 |
| **5m** |  | Not pursued[c] | (62)[d] | – | – |
| **5n** |  | Not pursued[c] | (45)[d] | NC | – |
| **5o** |  | Not pursued[c] | (74)[d] | No data | – |
| **5p** |  | Not pursued[c] | (55)[d] | No data | – |
| **5q** |  | 720 ± 130 | 108.0 ± 5.5 | >10,000 | (53 ± 7)[d] |
| **5r** |  | NC[e] | – | No data | – |
| **5s** |  | 641 ± 49 | 98.4 ± 2.4 | >10,000 | (34 ± 11)[d] |
| **5t** |  | 122 ± 25 | 93.8 ± 1.6 | 6400 ± 1700 | 64.6 ± 3.1 |
| **5u**[f] |  | 250 ± 54 | 91.6 ± 4.8 | 6300 ± 2050 | 68 ± 10 |
| **5v**[f] |  | 101 ± 21 | 92.9 ± 4.0 | 3210 ± 610 | 65 ± 4 |

[a]Measured by [$^{35}$S]GTPγS membrane binding

[b]Measured using the DiscoverX PathHunter assay platform

[c]Compound was not pursued based on 2 point screens in the [$^{35}$S]GTPγS assay (i.e., compound did not show promising effects at 50 nM (<5% stim) or 10 μM (<80%) testing

[d]For curves that do not plateau at maximum concentration (10 μM), the potency is estimated as >10,000 nM; the maximum stimulation observed at 10 μM is provided in parentheses for the $E_{max}$

[e]NC, nonconverging curve due to negligible potency

[f]Data taken from reference [11] and provided for comparison

acetonitrile gradient, which increases 20% in acetonitrile content over 4 min at a flow rate of 20 mL/min (modified to pH 9.8 through addition of $NH_4OH$ by auxiliary pump), and sample dilution in DMSO. The preparative gradient, triggering thresholds, and UV wavelength were selected according to the analytical RP HPLC analysis of each crude sample. The analytical method used an Agilent 1200 RRLC system with UV detection (Agilent 1200 DAD SL) and mass detection (Agilent 6224 TOF). The analytical method conditions included a Waters Aquity BEH C18 column (2.1 × 50 mm, 1.7 μm) and elution with a linear gradient of 5% acetonitrile in pH 9.8 buffered aqueous ammonium formate to 100% acetonitrile at 0.4 mL/min flow rate. Compound purity was measured on the basis of

peak integration (area under the curve) from UV/Vis absorbance (at 214 nm), and compound identity was determined on the basis of mass analysis. $^{1}H$ and $^{13}C$ NMR spectra were recorded on a Bruker AM 400 or Varian 400MR spectrometer (operating at 400 and 100 MHz, respectively) in $CDCl_3$ with 0.03% TMS as an internal standard, unless otherwise specified. Chemical shifts are reported in parts per million (ppm) downfield from TMS. When acquired, $^{13}C$ multiplicities were determined with the aid of an APT pulse sequence, differentiating the signals for methyl and methyne carbons as "d" from methylene and quaternary carbons as "u". The infrared (IR) spectra were acquired as thin films using a universal ATR sampling accessory on a PerkinElmer Spectrum 100 FT-IR spectrometer and the absorption frequencies are reported in $cm^{-1}$. Melting points were determined on a Stanford Research Systems Optimelt automated melting point system interfaced through a PC and are uncorrected.

### *N*-(Furan-2-ylmethyl)-2-(2-(pyridin-2-yl)acetyl)hydrazine-1-carbothioamide (3b)

2-(Pyridin-2-yl)acetohydrazide **1b** (415 mg, 2.75 mmol) and 2-(isothiocyanatomethyl)furan (382 mg, 2.75 mmol) were combined in MeCN (10 mL) and stirred at rt for 16 h. The reaction was filtered and the solid product washed with MeCN (3 × 10 mL) to afford the carbothioamide **3b** as an off-white solid (464 mg, 1.60 mmol, 58% yield), which was used without further purification. $^{1}H$ NMR (400 MHz, DMSO-$d_6$) δ 3.68 (s, 2H), 4.76 (d, $J = 5.6$ Hz, 2H), 6.26 (dd, $J = 0.9$, 3.2 Hz, 1H), 6.37 (dd, $J = 1.9$, 3.2 Hz, 1H), 7.25 (ddd, $J = 1.2$, 4.9, 7.6 Hz, 1H), 7.35 (d, $J = 7.8$ Hz, 1H), 7.56 (dd, $J = 0.9$, 1.9 Hz, 1H), 7.75 (td, $J = 1.9$, 7.7 Hz, 1H), 8.32 (d, $J = 4.5$ Hz, 1H), 8.76 (br s, 1H), 9.53 (s, 1H), and 10.14 (s, 1H); $^{13}C$ NMR (101 MHz, DMSO-$d_6$) δ 41.1, 43.0, 107.7, 110.9, 122.5, 124.5, 137.4, 142.6, 149.2, 152.3, 156.2, 169.0, and 182.1.

### 4-(Furan-2-ylmethyl)-5-(pyridin-2-ylmethyl)-4*H*-1,2,4-triazole-3-thiol (4b)

Carbothioamide **3b** (434 mg, 1.50 mmol) was slurried in aqueous NaOH (2 N, 20 mL) and the mixture heated at reflux for 2 h. After cooling to rt, the now transparent solution was acidified to pH = 7 with concentrated HCl precipitating the thione substrate **4b** as a tan solid (342 mg, 1.26 mmol, 84% yield), which was used without further purification. $^{1}H$ NMR (400 MHz, DMSO-$d_6$) δ 4.29 (s, 2H), 5.24 (s, 2H), 6.35 (d, $J = 14.2$ Hz, 2H), 7.27–7.82 (m, 3H), 8.38–8.61 (m, 1H), and 13.71 (s, 1H); $^{13}C$ NMR (101 MHz, DMSO-$d_6$) δ 34.1, 40.1, 109.6, 111.1, 122.8, 123.9, 137.5, 143.6, 148.5, 149.7, 150.7, 155.4, and 167.5.

### *N*-(Cyclohexylmethyl)-2-picolinoylhydrazine-1-carbothioamide (3c)

Picolinohydrazide **1a** (243 mg, 1.77 mmol) and cyclohexylmethyl isothiocyanate (275 mg, 1.77 mmol) were combined in MeCN (10 mL) and stirred at rt for 16 h. The reaction was filtered and the solid product washed with MeCN (3 × 10 mL) to afford the carbothioamide **3c** as an off-white solid (348 mg, 1.19 mmol, 67% yield), which was used without further purification. $^{1}H$ NMR (400 MHz, DMSO-$d_6$) δ 0.76–0.90 (m, 2H), 1.04–1.20 (m, 3H), 1.52–1.70 (m, 6H), 3.25 (t, $J = 6.2$ Hz, 2H), 7.62 (td, $J = 2.4$, 4.8 Hz, 1H), 7.87–8.12 (m, 3H), 8.65 (d, $J = 4.7$ Hz, 1H), 9.27 (br s, 1H), and 10.50 (br s, 1H); $^{13}C$ NMR (101 MHz, DMSO-$d_6$) δ 25.9, 26.6, 30.7, 37.3, 50.3, 122.9, 127.3, 138.1, 148.88, 148.92, 149.9, and 181.8.

### 4-(Cyclohexylmethyl)-5-(pyridin-2-yl)-4*H*-1,2,4-triazole-3-thiol (4c)

Carbothioamide **3c** (332 mg, 1.14 mmol) was slurried in aqueous NaOH (2 N, 22 mL) and the mixture heated at reflux for 2 h. After cooling to rt, the now transparent solution was acidified to pH = 7 with concentrated HCl precipitating the thione substrate **4c** as a tan solid (308 mg, 1.12 mmol, 99% yield), which was used without further purification. $^{1}H$ NMR (400 MHz, DMSO-$d_6$) δ 0.76–0.93 (m, 2H), 1.11 (t, $J = 9.5$ Hz, 3H), 1.50–1.73 (m, 6H), 3.25 (t, $J = 6.3$ Hz, 2H), 7.48–7.67 (m, 1H), 7.83–8.08 (m, 2H), 8.65 (d, $J = 4.5$ Hz, 1H), 9.25 (br s, 1H), and 10.50 (br s, 1H); $^{13}C$ NMR (101 MHz, DMSO-$d_6$) δ 25.9, 26.6, 30.7, 37.3, 50.3, 122.9, 127.3, 138.1, 148.9, 149.9, 181.79, and 181.81.

### General Procedure A: synthesis of thioether triazole analogs

To a solution of triazole thione (1.0 equiv) in acetone (2 mL/mmol triazole substrate) was added $K_2CO_3$ (3.0 equiv) followed by the appropriate bromide or chloride (1.2–2.0 equiv) and the reaction stirred at rt for 24 h. The solvent was removed under a stream of nitrogen and the residue purified by either flash chromatography (EtOAc/hexanes, eluents) or preparative, reverse-phase, mass-directed fractionation HPLC (MDF HPLC) to afford the thioether product. The thione substrates **4a** and **4d** were prepared as previously described [10, 11].

### 2-(5-((4-Chloro-2-(trifluoromethyl)benzyl)thio)-4-(furan-2-ylmhyl)-4*H*-1,2,4-triazol-3-yl)pyridine (5a)

Furan thione **4a** (51.8 mg, 0.20 mmol) and 4-chloro-2-(trifluoromethyl)benzyl bromide (65.6 mg, 0.24 mmol, 1.2 equiv) were reacted according to General Procedure A and purified by flash chromatography to afford the thioether

product **5a** as a white solid (46.2 mg, 0.102 mmol, 51% yield). $R_f = 0.28$ (50% EtOAc/hexanes); $^1$H NMR (400 MHz, $CDCl_3$) δ 4.66 (d, $J = 1.3$ Hz, 2H), 5.84 (s, 2H), 6.11 (dd, $J = 0.9$, 3.3 Hz, 1H), 6.18 (dd, $J = 1.9$, 3.3 Hz, 1H), 7.20–7.23 (m, 1H), 7.35 (ddd, $J = 1.2$, 4.8, 7.6 Hz, 1H), 7.41 (dd, $J = 2.3$, 8.4 Hz, 1H), 7.63 (d, $J = 2.2$ Hz, 1H), 7.68 (d, $J = 8.3$ Hz, 1H), 7.82 (td, $J = 1.8$, 7.8 Hz, 1H), 8.24–8.30 (m, 1H), 8.65 (ddd, $J = 1.0$, 1.8, 4.8 Hz, 1H); $^{13}$C NMR (101 MHz, $CDCl_3$) δ 33.7, 41.9, 109.1, 110.3, 123.39, 123.43 (q, $J = 275.9$ Hz), 124.2, 126.5 (q, $J = 5.8$ Hz), 129.9 (q, $J = 31.3$ Hz), 132.3, 133.6, 134.0, 134.2, 137.1, 142.7, 147.6, 148.6, 148.7, 152.5, and 152.9; $^{19}$F NMR (376 MHz, $CDCl_3$) δ −59.7; IR (neat) 1589, 1463, 1445, 1411 cm$^{-1}$; HRMS (ESI) *m/z* calcd for $C_{20}H_{15}ClF_3N_4OS$ $[M + H]^+$ 451.0602, found 451.0606; HPLC purity = 94.4%.

### 2-(5-((4-Chlorobenzyl)thio)-4-(furan-2-ylmethyl)-4*H*-1,2,4-triazol-3-yl)pyridine (5b)

Furan thione **4a** (51.8 mg, 0.20 mmol) and 4-chlorobenzyl bromide (49.3 mg, 0.24 mmol, 1.2 equiv) were reacted according to General Procedure A and purified by flash chromatography to afford the thioether product **5b** as a white solid (47.8 mg, 0.125 mmol, 62% yield). $R_f = 0.47$ (EtOAc); $^1$H NMR (400 MHz, $CDCl_3$) δ 4.44 (s, 2H), 5.80 (s, 2H), 6.05–6.13 (m, 1H), 6.16–6.24 (m, 1H), 7.19–7.36 (complex, 6H), 7.80 (td, $J = 1.8$, 7.8 Hz, 1H), 8.25 (d, $J = 8.0$ Hz, 1H), and 8.57–8.68 (m, 1H); $^{13}$C NMR (101 MHz, $CDCl_3$) δ 37.4, 41.9, 109.0, 110.4, 123.4, 124.2, 128.8, 130.5, 133.6, 135.3, 137.0, 142.6, 147.6, 148.6, 148.9, 152.6, 152.6; IR (neat) 1588, 1489, 1462, 1444, and 1421 cm$^{-1}$; HRMS (ESI) *m/z* calcd for $C_{19}H_{16}ClN_4OS$ $[M + H]^+$ 383.0728, found 383.0729; HPLC purity = 97.1%.

### 2-(4-(Furan-2-ylmethyl)-5-((3-methoxybenzyl)thio)-4*H*-1,2,4-triazol-3-yl)pyridine (5c)

Furan thione **4a** (87.4 mg, 0.338 mmol) and 3-methoxybenzyl chloride (132.0 mg, 0.677 mmol, 2 equiv) were reacted according to General Procedure A and purified by flash chromatography to afford the thioether product **5c** as a colorless oil (103.4 mg, 0.273 mmol, 81% yield). $R_f =$ 0.44 (70% EtOAc/hexanes); $^1$H NMR (400 MHz, $CDCl_3$) δ 3.82 (s, 3H), 4.50 (s, 2H), 5.75 (d, $J = 0.8$ Hz, 2H), 6.05 (dd, $J = 0.9$, 3.2 Hz, 1H), 6.15 (dd, $J = 1.9$, 3.3 Hz, 1H), 6.85 (d, $J = 7.8$ Hz, 2H), 7.18–7.34 (m, 4H), 7.77 (td, $J =$ 1.8, 7.8 Hz, 1H), 8.25 (dt, $J = 1.1$, 8.0 Hz, 1H), 8.61 (ddd, $J = 1.0$, 1.8, 4.9 Hz, 1H); $^{13}$C NMR (101 MHz, $CDCl_3$) δ 33.4, 41.7, 55.5, 108.7, 110.3, 110.5, 120.5, 123.3, 124.0, 125.2, 129.3, 130.8, 136.9, 142.5, 147.9, 148.5, 149.3, 152.5, 153.6, and 157.4; IR (neat) 1588, 1493, 1461, and 1443 cm$^{-1}$; HRMS (ESI) *m/z* calcd for $C_{20}H_{19}N_4O_2S$ $[M + H]^+$, 379.1223, found 379.1197; HPLC purity = 97.0%.

### 2-(4-(Furan-2-ylmethyl)-5-((3-methoxybenzyl)thio)-4*H*-1,2,4-triazol-3-yl)pyridine (5d)

Furan thione **4a** (38 mg, 0.15 mmol) 3-methoxybenzyl chloride (27 mg, 0.18 mmol) were reacted according to General Procedure A and purified by MDF HPLC to afford the thioether product **5d** as a colorless oil (48.8 mg, 0.13 mmol, 88% yield). $^1$H NMR (400 MHz, $CDCl_3$) δ 3.76 (s, 3 H), 4.46 (s, 2 H), 5.79 (s, 2 H), 6.11 (d, $J = 3.2$ Hz, 1 H), 6.19 (m, 1 H), 6.81 (dd, $J = 2.0$, 8.4 Hz, 1 H), 6.91 (t, $J = 2.0$ Hz, 1 H), 6.96 (d, $J = 7.6$ Hz, 1 H), 7.20 (d, $J =$ 7.6 Hz, 1 H), 7.23 (d, $J = 1.6$ Hz, 7.32 (ddd, $J = 1.2$, 4.8, 7.6 Hz, 1 H), 7.80 (dt, $J = 1.6$, 8.0 Hz, 1 H), 8.27 (d, $J =$ 8.0 Hz, 1 H), and 8.63 (d, $J = 4.0$ Hz, 1 H); $^{13}$C NMR (101 MHz, $CDCl_3$, APT pulse sequence) δ d 55.2, 108.8, 110.3, 113.6, 114.3, 121.3, 124.0, 129.6, 136.9, 142.5, 148.5; u 38.4, 41.8, 138.0, 147.7, 149.0, 152.5, 152.8, and 159.7; IR (neat) 2940, 1588, 1490, and 1462 cm$^{-1}$; HRMS (ESI) *m/z* calcd for $C_{20}H_{19}N_4O_2S$ ($[M + H]^+$), 379.1223, found 379.1223; HPLC purity = 98.0%.

### 2-(5-((2,6-Difluorobenzyl)thio)-4-(furan-2-ylmethyl)-4*H*-1,2,4-triazol-3-yl)pyridine (5e)

Furan thione **4a** (38 mg, 0.15 mmol) and 2,6-difluorobenzyl chloride (28 mg, 0.18 mmol) were reacted according to General Procedure A and purified by MDF HPLC to afford the thioether product **5e** as a colorless oil (40.9 mg, 0.11 mmol, 73% yield). $^1$H NMR (400 MHz, $CDCl_3$) δ 4.51 (s, 2 H), 5.88 (s, 2 H), 6.13 (d, $J = 3.2$ Hz, 1 H), 6.18 (m, 1 H), 6.89 (t, $J = 7.6$ Hz, 2 H), 7.23 (m, 1 H), 7.25 (m, 1 H), 7.34 (dd, $J = 4.8$, 6.8 Hz, 1 H), 7.81 (dt, $J = 0.8$, 7.6 Hz, 1 H), 8.29 (d, $J = 7.6$ Hz, 1 H), and 8.65 (d, $J = 4.0$ Hz, 1 H); $^{13}$C NMR (101 MHz, $CDCl_3$, APT pulse sequence) δ d 108.9, 109.0, 110.3, 111.3 (d, $J = 25$ Hz), 123.4, 124.1, 129.7 (t, $J = 10$ Hz), 136.9, 142.6, 148.5; u 25.8, 41.9, 112.7 (t, $J = 19$ Hz), 147.7, 149.0, 152.1, 152.7, and 161.3 (d, $J = 250$ Hz); IR (neat) 3056, 1625, 1590, 1470 cm$^{-1}$; HRMS (ESI) *m/z* calcd for $C_{19}H_{15}F_2N_4OS$ $[M + H]^+$ 385.0929, found 385.0931; HPLC purity = 98.7%.

### 2-(4-(furan-2-ylmethyl)-5-((naphthalen-2-ylmethyl)thio)-4H-1,2,4-triazol-3-yl)pyridine (5f)

Furan thione **4a** (41.0 mg, 0.159 mmol) and 2-(bromomethyl) naphthalene (42.1 mg, 0.190 mmol, 1.2 equiv) were reacted according to General Procedure A and purified by flash chromatography to afford the thioether product **5f** as an off-white solid (16.9 mg, 0.042 mmol, 27% yield). $^1$H NMR (400 MHz, $CDCl_3$) δ 4.66 (s, 2H), 5.78 (s, 2H), 6.08 (dd, $J =$ 0.9, 3.3 Hz, 1H), 6.15 (dd, $J = 1.9$, 3.3 Hz, 1H), 7.19 (dd, $J =$ 0.9, 1.9 Hz, 1H), 7.31 (ddd, $J = 1.2$, 4.8, 7.6 Hz, 1H), 7.43–7.47 (m, 2H), 7.52 (dd, $J = 1.8$, 8.5 Hz, 1H), 7.76–7.84

(m, 5H), 8.19–8.35 (m, 1H), and 8.60 (ddd, $J = 1.0$, 1.8, 4.9 Hz, 1H); $^{13}$C NMR (101 MHz, $CDCl_3$, APT pulse sequence) δ d 108.9, 110.3, 123.4, 124.1, 126.1, 126.3, 127.0, 127.7, 127.9, 128.0, 128.6, 137.0, 142.6, and 148.5; u 38.6, 41.9, 132.8, 133.3, 134.0, 147.8, 149.1, 152.6, and 153.0; HRMS (ESI) *m/z* calcd for $C_{23}H_{19}N_4OS$ $[M + H]^+$ 399.1274, found 399.1277; HPLC purity >99.5%.

### 2-(4-(Furan-2-ylmethyl)-5-((pyridin-2-ylmethyl)thio)-4*H*-1,2,4-triazol-3-yl)pyridine (5g)

Furan thione **4a** (51.8 mg, 0.200 mmol) and 2-(chloromethyl)pyridine (39.3 mg, 0.240 mmol, 1.2 equiv) were reacted according to General Procedure A and purified by flash chromatography to afford the thioether product **5g** as a yellow solid (68.0 mg, 0.195 mmol, 97% yield). $R_f = 0.56$ (20% MeOH/EtOAc); $^1$H NMR (400 MHz, $CDCl_3$) δ 4.63 (s, 2H), 5.83 (s, 2H), 6.12 (d, $J = 3.3$ Hz, 1H), 6.16 (dd, $J =$ 1.8, 3.3 Hz, 1H), 7.15 (ddd, $J = 1.2$, 4.9, 7.7 Hz, 1H), 7.24–7.36 (m, 2H), 7.44 (d, $J = 7.8$ Hz, 1H), 7.58 (td, $J =$ 1.8, 7.7 Hz, 1H), 7.77 (td, $J = 1.8$, 7.8 Hz, 1H), 8.23 (d, $J =$ 8.0 Hz, 1H), 8.55 (d, $J = 4.9$ Hz, 1H), and 8.61 (d, $J =$ 4.9 Hz, 1H); $^{13}$C NMR (101 MHz, $CDCl_3$) δ 39.4, 41.9, 109.0, 110.3, 122.5, 123.3, 123.7, 124.1, 136.8, 137.0, 142.6, 147.7, 148.5, 148.9, 149.6, 152.6, 152.9, and 156.4; IR (neat) 1588, 1568, 1462, and 1422 $cm^{-1}$; HRMS (ESI) *m/z* calcd for $C_{18}H_{16}N_5OS$ $[M + H]^+$ 350.1070, found 350.1069; HPLC purity >99.5%.

### 2-(4-(Furan-2-ylmethyl)-5-((pyridin-4-ylmethyl)thio)-4*H*-1,2,4-triazol-3-yl)pyridine (5h)

Furan thione **4a** (51.8 mg, 0.200 mmol) and 4-(chloromethyl)pyridine (39.3 mg, 0.240 mmol, 1.2 equiv) were reacted according to General Procedure A and purified by flash chromatography to afford the thioether product **5h** as a yellow solid (27 mg, 0.08 mmol, 39% yield). $R_f = 0.57$ (20% MeOH/EtOAc); $^1$H NMR (400 MHz, $CDCl_3$) δ 4.45 (s, 2H), 5.82 (s, 2H), 6.12 (d, $J = 3.3$ Hz, 1H), 6.20 (dd, $J = 1.9$, 3.3 Hz, 1H), 7.24 (d, $J = 1.3$ Hz, 1H), 7.31–7.36 (m, 4H), 7.81 (td, $J = 1.8$, 7.8 Hz, 2H), 8.26 (d, $J = 8.0$ Hz, 1H), 8.53 (d, $J = 6.1$ Hz, 2H), and 8.63 (ddd, $J = 1.0$, 1.8, 4.9 Hz, 1H); $^{13}$C NMR (101 MHz, $CDCl_3$) δ 36.4, 41.9, 109.1, 110.4, 123.4, 124.0, 124.2, 137.0, 142.7, 146.0, 147.6, 148.5, 148.8, 150.0, 152.0, and 152.8; IR (neat) 1598, 1589, 1462, and 1414 $cm^{-1}$; HRMS (ESI) *m/z* calcd for $C_{18}H_{16}N_5OS$ $[M + H]^+$ 350.1070, found 350.1067; HPLC purity >99.5%.

### 2-(5-((Cyclohexylmethyl)thio)-4-(furan-2-ylmethyl)-4*H*-1,2,4-triazol-3-yl)pyridine (5i)

Furan thione **4a** (25.8 mg, 0.10 mmol) and (bromomethyl) cyclohexane (21.3 mg, 0.12 mmol, 1.2 equiv) were reacted according to General Procedure A and purified by flash chromatography to afford the thioether product **5i** as a yellow oil (23 mg, 0.065 mmol, 65% yield). $R_f = 0.57$ (EtOAc); $^1$H NMR (400 MHz, $CDCl_3$) δ 0.93–1.06 (m, 2H), 1.10–1.28 (m, 3H), 1.58–1.73 (m, 4H), 1.84–1.93 (m, 2H), 3.18 (d, $J = 6.9$ Hz, 2H), 5.88 (s, 2H), 6.13 (d, $J = 3.3$ Hz, 1H), 6.19 (dd, $J = 1.8$, 3.3 Hz, 1H), 7.23 (d, $J = 2.1$ Hz, 1H), 7.25–7.32 (m, 1H), 7.76 (td, $J = 1.8$, 7.8 Hz, 1H), 8.18–8.26 (m, 1H), and 8.61 (d, $J = 4.7$ Hz, 1H); $^{13}$C NMR (101 MHz, $CDCl_3$) δ 25.9, 26.2, 32.4, 37.6, 40.5, 41.8, 108.9, 110.3, 123.3, 124.0, 136.9, 142.5, 147.9, 148.5, 149.2, 152.4, and 154.0; IR (neat) 1589, 1462, 1446, 1421 $cm^{-1}$; HRMS (ESI) *m/z* calcd for $C_{19}H_{23}N_4OS$ $[M + H]^+$ 355.1587, found 355.1589; HPLC purity = 98.0%.

### 2-(5-((Cyclopropylmethyl)thio)-4-(furan-2-ylmethyl)-4*H*-1,2,4-triazol-3-yl)pyridine (5j)

Furan thione **4a** (25.8 mg, 0.10 mmol) and (bromomethyl) cyclopropane (16.2 mg, 0.12 mmol, 1.2 equiv) were reacted according to General Procedure A and purified by flash chromatography to afford the thioether product **5j** as a white solid (13 mg, 0.042 mmol, 42% yield). $R_f = 0.34$ (EtOAc); $^1$H NMR (400 MHz, $CDCl_3$) δ 0.32 (dt, $J = 4.6$, 6.2 Hz, 2H), 0.53–0.67 (m, 2H), 1.21 (dtd, $J = 2.6$, 7.3, 12.4 Hz, 1H), 3.22 (d, $J =$ 7.3 Hz, 2H), 5.94 (s, 2H), 6.16 (d, $J = 3.3$ Hz, 1H), 6.22 (dd, $J$ = 1.9, 3.3 Hz, 1H), 7.33 (ddd, $J = 1.2$, 4.8, 7.6 Hz, 2H), 7.80 (td, $J = 1.8$, 7.8 Hz, 1H), 8.26 (d, $J = 8.0$ Hz, 1H), and 8.62–8.67 (m, 1H); $^{13}$C NMR (101 MHz, $CDCl_3$) δ 6.0, 10.9, 39.9, 41.9, 108.9, 110.3, 123.4, 124.0, 137.0, 142.6, 147.9, 148.5, 149.2, 152.5, and 153.6; IR (neat) 1589, 1463, 1446, and 1422 $cm^{-1}$; HRMS (ESI) *m/z* calcd for $C_{16}H_{17}N_4OS$ $[M + H]^+$ 313.1118, found 313.1119; HPLC purity >99.5%.

### 2-(5-(Allylthio)-4-(furan-2-ylmethyl)-4*H*-1,2,4-triazol-3-yl) pyridine (5k)

Furan thione **4a** (51.8 mg, 0.20 mmol) and allyl bromide (29.0 mg, 0.24 mmol, 1.2 equiv) were reacted according to General Procedure A and purified by flash chromatography to afford the thioether product **5k** as a yellow oil (58 mg, 0.11 mmol, 73% yield). $R_f = 0.46$ (EtOAc); $^1$H NMR (400 MHz, $CDCl_3$) δ 3.88 (d, $J = 7.2$ Hz, 2H), 5.09–5.14 (m, 2H), 5.25 (dd, $J = 1.4$, 16.9 Hz, 1H), 5.92 (s, 2H), 5.93–6.05 (m, 1H), 6.15 (d, $J = 3.3$ Hz, 1H), 6.20 (dd, $J =$ 1.8, 3.3 Hz, 1H), 7.25 (d, $J = 1.1$ Hz, 1H), 7.32 (ddd, $J =$ 1.2, 4.9, 7.6 Hz, 1H), 7.79 (td, $J = 1.8$, 7.8 Hz, 1H), 8.26 (d, $J = 8.0$ Hz, 1H), and 8.61–8.65 (m, 1H); $^{13}$C NMR (101 MHz, $CDCl_3$) δ 36.6, 41.9, 108.9, 110.4, 119.0, 123.4, 124.1, 132.6, 137.0, 142.6, 147.8, 148.5, 149.1, 152.6, and 152.7; IR (neat) 1588, 1461, 1444, 1421, 1347 $cm^{-1}$; HRMS (ESI) *m/z* calcd for $C_{15}H_{15}N_4OS$ $[M + H]^+$ 299.0961, found 299.0965; HPLC purity = 98.3%.

### 2-(4-(Furan-2-ylmethyl)-5-(prop-2-yn-1-ylthio)-4*H*-1,2,4-triazol-3-yl)pyridine (5l)

Furan thione **4a** (120.6 mg, 0.467 mmol) and propargyl bromide (85 weight % solution in toluene, 138.9 mg, 0.934 mmol, 2.0 equiv) were reacted according to General Procedure A and purified by flash chromatography to afford the thioether product **5l** as a colorless oil (87.3 mg, 0.295 mmol, 63% yield). $R_f = 0.18$ (50% EtOAc/hexanes); $^1$H NMR (400 MHz, $CDCl_3$) δ 2.30 (s, 1H), 4.00 (d, $J = 2.7$ Hz, 2H), 5.98 (s, 2H), 6.15–6.26 (m, 2H), 7.26 (d, $J = 1.0$ Hz, 1H), 7.34 (ddd, $J =$ 1.2, 4.8, 7.6 Hz, 1H), 7.81 (td, $J = 1.8$, 7.8 Hz, 1H), 8.28 (d, $J = 8.0$ Hz, 1H), and 8.66 (dd, $J = 0.9$, 4.9 Hz, 1H); $^{13}$C NMR (101 MHz, $CDCl_3$) δ 22.6, 42.1, 72.7, 78.3, 109.1, 110.4, 123.4, 124.2, 137.0, 142.7, 147.7, 148.6, 148.9, 151.6, and 152.9; IR (neat) 1588, 1462, 1444, and 1422 $cm^{-1}$; HRMS (ESI) *m/z* calcd for $C_{15}H_{13}N_4OS$ $[M + H]^+$ 297.0805, found 297.0801; HPLC purity >99.5%.

### 2-((5-((3,4-Dichlorobenzyl)thio)-4-(furan-2-ylmethyl)-4*H*-1,2,4-triazol-3-yl)methyl)pyridine (5m)

Furan thione **4b** (27 mg, 0.10 mmol) and 3,4-dichlorobenzyl chloride (24 mg, 0.12 mmol, 1.2 equiv) were reacted according to General Procedure A and purified by MDF HPLC to afford the thioether product **5m** as a tan solid (28.8 mg, 0.0668 mmol, 67% yield). $^1$H NMR (400 MHz, $CDCl_3$) δ 4.30 (s, 2H), 4.42 (s, 2H), 5.07 (s, 2H), 6.15 (dd, $J = 0.8$, 3.3 Hz, 1H), 6.25 (dd, $J = 1.8$, 3.3 Hz, 1H), 7.11 (dd, $J = 2.1$, 8.2 Hz, 1H), 7.18 (ddd, $J = 1.2$, 4.9, 7.6 Hz, 1H), 7.22–7.30 (m, 3H), 7.40 (d, $J = 2.1$ Hz, 1H), 7.63 (td, $J = 1.8$, 7.7 Hz, 1H), and 8.46–8.53 (m, 1H); $^{13}$C NMR (101 MHz, $CDCl_3$) δ d 109.3, 110.5, 122.3, 123.3, 128.4, 130.5, 130.9, 137.1, 143.2, and 149.4; u 34.6, 37.3, 40.7, 131.8, 132.6, 137.2, 147.5, 149.9, 154.0, and 155.9; HRMS (ESI) *m/z* calcd for $C_{20}H_{17}Cl_2N_4OS$ $[M + H]^+$ 431.0495, found 431.0498; HPLC purity = 98.9%.

### 2-((4-(Furan-2-ylmethyl)-5-((4-methyl-3-(trifluoromethyl)benzyl)thio)-4*H*-1,2,4-triazol-3-yl)methyl)pyridine (5n)

Furan thione **4b** (27 mg, 0.10 mmol) and 4-methyl-3-(trifluoromethyl)benzyl bromide (30 mg, 0.12 mmol, 1.2 equiv) were reacted according to General Procedure A and purified by MDF HPLC to afford the thioether product **5n** as a tan solid (18.8 mg, 0.0423 mmol, 42% yield). $^1$H NMR (400 MHz, $CDCl_3$) δ 4.38 (s, 2H), 4.43 (s, 2H), 5.07 (s, 2H), 6.15 (dd, $J = 0.9$, 3.3 Hz, 1H), 6.24 (dd, $J = 1.9$, 3.3 Hz, 1H), 7.10–7.20 (m, 2H), 7.26–7.28 (m, 2H), 7.35 (dd, $J = 1.9$, 7.9 Hz, 1H), 7.55 (s, 1H), 7.62 (td, $J = 1.8$, 7.7 Hz, 1H), and 8.49 (dd, $J = 0.9$, 4.9 Hz, 1H); $^{13}$C NMR (101 MHz, $CDCl_3$) δ d 109.3, 110.5, 122.3, 123.3, 128.4, 130.5, 130.9, 137.1, 143.2, and 149.4; u 34.6, 37.7, 40.6, 124.3 (q, $J = 274.8$ Hz), 128.8 (q, $J = 33.0$ Hz), 134.5, 136.1, 147.6, 150.3, 153.9, and 156.0; HRMS (ESI) *m/z* calcd for $C_{22}H_{20}F_3N_4OS$ $[M + H]^+$ 445.1304, found 445.1312; HPLC purity = 98.0%.

### 2-(4-(Cyclohexylmethyl)-5-((4-methyl-3-(trifluoromethyl)benzyl)thio)-4*H*-1,2,4-triazol-3-yl)pyridine (5o)

Furan thione **4c** (27 mg, 0.10 mmol) and benzyl bromide (21 mg, 0.12 mmol, 1.2 equiv) were reacted according to General Procedure A and purified by MDF HPLC to afford the thioether product **5o** as a tan solid (20.2 mg, 0.0554 mmol, 55% yield). $^1$H NMR (400 MHz, $CDCl_3$) δ 0.87 (tt, $J = 6.1$, 12.1 Hz, 2H), 1.07–1.27 (m, 2H), 1.39 (dddd, $J = 3.4$, 6.9, 11.4, 18.1 Hz, 1H), 1.55–1.72 (m, 4H), 3.17 (dd, $J = 5.4$, 6.8 Hz, 2H), 4.07 (s, 2H), 7.21–7.36 (m, 5H), 7.40–7.45 (m, 1H), 7.84 (td, $J = 1.7$, 7.7 Hz, 1H), 8.25 (d, $J = 7.8$ Hz, 1H), and 8.58 (ddd, $J = 0.9$, 1.8, 4.8 Hz, 1H); $^{13}$C NMR (101 MHz, $CDCl_3$) δ d 37.4, 122.3, 125.9, 127.8, 128.8, 128.9, 137.3, and 148.1; u 25.8, 26.4, 30.9, 36.9, 49.8, 136.8, 149.7, 150.3, and 158.6; HRMS (ESI) *m/z* calcd for $C_{21}H_{24}N_4S$ $[M + H]^+$ 365.1794, found 365.1790; HPLC purity = 95.6%.

### 2-(4-(Cyclohexylmethyl)-5-((4-methyl-3-(trifluoromethyl)benzyl)thio)-4H-1,2,4-triazol-3-yl)pyridine (5p)

Furan thione **4c** (27 mg, 0.10 mmol) and 4-methyl-3-(trifluoromethyl)benzyl bromide (30 mg, 0.12 mmol, 1.2 equiv) were reacted according to General Procedure A and purified by MDF HPLC to afford the thioether product **5p** as a tan solid (4.4 mg, 0.00985 mmol, 10% yield). $R_f = 0.25$ (50% EtOAc/hexanes); $^1$H NMR (400 MHz, $CDCl_3$) δ 0.80–0.94 (m, 2H), 0.99–1.12 (m, 3H), 1.45 (d, $J =$ 12.6 Hz, 2H), 1.56–1.66 (m, 2H), 2.44 (d, $J = 1.9$ Hz, 2H), 4.32 (d, $J = 7.3$ Hz, 2H), 4.53 (s, 2H), 7.20 (d, $J = 7.8$ Hz, 1H), 7.31 (ddd, $J = 1.2$, 4.8, 7.6 Hz, 1H), 7.49 (dd, $J = 2.0$, 7.8 Hz, 1H), 7.63 (s, 1H), 7.81 (td, $J = 1.8$, 7.8 Hz, 1H), 8.28 (dt, $J = 1.1$, 8.0 Hz, 1H), and 8.60 (ddd, $J = 0.9$, 1.8, 4.8 Hz, 1H); $^{19}$F NMR (376 MHz, $CDCl_3$) δ -61.81; $^{13}$C NMR (101 MHz, $CDCl_3$) δ 19.0, 25.6, 26.1, 30.3, 36.9, 38.7, 51.2, 123.4, 123.8, 124.3 (q, $J = 275.2$ Hz), 126.4 (q, $J = 5.7$ Hz), 129.1 (q, $J = 35.7$ Hz), 132.2, 132.4, 134.7, 136.1 (q, $J = 1.8$ Hz), 136.9, 148.1, 148.6, 152.6, and 153.3; IR (neat) 1589, 1468, 1446, and 1417 $cm^{-1}$; HRMS (ESI) *m/z* calcd for $C_{23}H_{26}F_3N_4S$ $[M + H]^+$ 447.1825, found 447.1832; HPLC purity = 94.0%.

### 2-(5-((4-Chloro-3-(trifluoromethyl)benzyl)thio)-4-(cyclohexylmethyl)-4*H*-1,2,4-triazol-3-yl)pyridine (5q)

Furan thione **4c** (27 mg, 0.10 mmol) and 4-chloro-3-(trifluoromethyl)benzyl bromide (33 mg, 0.12 mmol, 1.2

equiv) were reacted according to General Procedure A and purified by MDF HPLC to afford the thioether product **5q** as a tan solid (5.8 mg, 0.0124 mmol, 12% yield). $R_f = 0.29$ (50% EtOAc/hexanes); $^1$H NMR (400 MHz, $CDCl_3$) δ 0.82–0.93 (m, 2H), 0.99–1.11 (m, 3H), 1.45 (d, $J =$ 12.7 Hz, 2H), 1.55–1.67 (m, 4H), 4.33 (d, $J = 7.3$ Hz, 2H), 4.54 (s, 2H), 7.32 (ddd, $J = 1.2$, 4.8, 7.6 Hz, 1H), 7.42 (d, $J = 8.3$ Hz, 1H), 7.59 (dd, $J = 2.2$, 8.3 Hz, 1H), 7.74 (d, $J =$ 2.2 Hz, 1H), 7.81 (td, $J = 1.8$, 7.8 Hz, 1H), 8.27 (dt, $J = 1.1$, 8.0 Hz, 1H), and 8.61 (ddd, $J = 0.9$, 1.8, 4.9 Hz, 1H); $^{19}$F NMR (376 MHz, $CDCl_3$) δ −62.73; $^{13}$C NMR (101 MHz, $CDCl_3$) δ 25.5, 26.1, 30.3, 36.2, 38.7, 51.3, 123.4, 123.9, and 122.6 (q, $J = 274.7$ Hz), 128.1 (q, $J = 5.3$ Hz), 128.6 (q, $J = 31.5$ Hz), 131.6, 133.7, 133.7, 136.4, 136.9, 148.0, 148.6, 152.1, and 153.4; IR (neat) 1589, 1468, 1447, 1418 $cm^{-1}$; HRMS (ESI) *m/z* calcd for $C_{22}H_{23}ClF_3N_4S$ $[M + H]^+$ 467.1279, found 467.1278; HPLC purity >99.5%.

### 2-(5-(Benzylthio)-4-((5-methylfuran-2-yl)methyl)-4*H*-1,2,4-triazol-3-yl)pyridine (5r)

Furan thione **4d** (27 mg, 0.10 mmol) and benzyl bromide (21 mg, 0.12 mmol, 1.2 equiv) were reacted according to General Procedure A and purified by MDF HPLC to afford the thioether product **5r** as a tan solid (20.4 mg, 0.0563 mmol, 56% yield). $^1$H NMR (400 MHz, $CDCl_3$) δ 2.13 (s, 3H), 4.49 (s, 2H), 5.72 (s, 2H), 5.75 (dd, $J = 1.1$, 3.1 Hz, 1H), 5.95 (d, $J = 3.1$ Hz, 1H), 7.24–7.35 (m, 4H), 7.37–7.42 (m, 2H), 7.80 (td, $J = 1.8$, 7.8 Hz, 1H), 8.25 (dt, $J = 1.1$, 8.1 Hz, 1H), and 8.64 (ddd, $J = 1.0$, 1.8, 4.9 Hz, 1H); $^{13}$C NMR (101 MHz, $CDCl_3$) δ d 13.5, 106.2, 109.8, 123.4, 124.0, 127.8, 128.7, 129.2, 136.9, 148.5; u 38.4, 41.9, 136.7, 147.2, 147.9, 152.4, 152.7, and 152.9; IR (neat) 1588, 1462, 1444, and 1421 $cm^{-1}$; HRMS (ESI) *m/z* calcd for $C_{20}H_{19}N_4OS$ $[M + H]^+$ 363.1274, found 363.1278; HPLC purity = 98.5%.

### 2-(5-((4-Bromobenzyl)thio)-4-((5-methylfuran-2-yl)methyl)-4*H*-1,2,4-triazol-3-yl)pyridine (5s)

Furan thione **4d** (27 mg, 0.10 mmol) and 4-bromorobenzyl bromide (30 mg, 0.12 mmol, 1.2 equiv) were reacted according to General Procedure A and purified by MDF HPLC to afford the thioether product **5s** as a tan solid (24.2 mg, 0.0548 mmol, 55% yield). $^1$H NMR (400 MHz, $CDCl_3$) δ 2.13 (s, 3H), 4.43 (s, 2H), 5.75 (s, 2H), 5.76 (dd, $J = 1.1$, 3.1 Hz, 1H), 5.95 (d, $J = 3.2$ Hz, 1H), 7.27 (d, $J =$ 1.2 Hz, 1H), 7.29 (d, $J = 2.1$ Hz, 1H), 7.33 (ddd, $J = 1.2$, 4.9, 7.6 Hz, 1H), 7.41 (d, $J = 2.0$ Hz, 1H), 7.42 (d, $J =$ 1.9 Hz, 1H), 7.80 (td, $J = 1.8$, 7.8 Hz, 1H), 8.25 (dt, $J = 1.1$, 8.0 Hz, 1H), and 8.64 (ddd, $J = 1.0$, 1.8, 4.9 Hz, 1H); $^{13}$C NMR (101 MHz, $CDCl_3$) δ d 13.5, 106.3, 109.8, 123.4, 124.1, 130.9, 131.8, 137.0, and 148.6; u 37.4, 42.0, 121.7, 136.0, 147.1, 147.8, 152.4, 152.5, and 152.8; IR (neat) 1588, 1567, 1486, and 1462 $cm^{-1}$; HRMS (ESI) *m/z* calcd for $C_{20}H_{18}BrN_4OS$ $[M + H]^+$ 443.0359, found 443.0363; HPLC purity = 95.8%.

### 2-(5-((3,4-Dichlorobenzyl)thio)-4-((5-methylfuran-2-yl)methyl)-4*H*-1,2,4-triazol-3-yl)pyridine (5t)

Furan thione **4d** (27 mg, 0.10 mmol) and 3,4-dichlorobenzyl chloride (24 mg, 0.12 mmol, 1.2 equiv) were reacted according to General Procedure A and purified by MDF HPLC to afford the thioether product **5t** as a tan solid (32.4 mg, 0.0751 mmol, 75% yield). $^1$H NMR (400 MHz, $CDCl_3$) δ 2.14 (d, $J = 1.0$ Hz, 3H), 4.43 (s, 2H), 5.76–5.78 (m, 1H), 5.77 (s, 2H), 5.97 (d, $J = 3.1$ Hz, 1H), 7.23–7.29 (m, 1H), 7.31–7.35 (m, 2H), 7.51 (d, $J = 2.1$ Hz, 1H), 7.81 (td, $J = 1.8$, 7.8 Hz, 1H), 8.25 (dt, $J = 1.1$, 8.0 Hz, 1H), and 8.64 (ddd, $J = 1.0$, 1.8, 4.9 Hz, 1H); $^{13}$C NMR (101 MHz, $CDCl_3$) δ d 13.5, 106.3, 109.9, 123.4, 124.1, 128.6, 130.5, 131.0, 137.0, and 148.6; u 36.7, 42.1, 131.8, 132.6, 137.3, 147.0, 147.8, 152.2, 152.5, and 152.8; HRMS (ESI) *m/z* calcd for $C_{20}H_{17}Cl_2N_4OS$ $[M + H]^+$ 431.0495, found 431.0498; HPLC purity = 99.1%.

## In vitro assay methods

### Compounds and reagents

Reference compound U69,593 was purchased from Sigma Aldrich and prepared in ethanol as a 10 mM stock solution. Test compounds were prepared as 10 mM stock solutions in DMSO. All compounds were then diluted to working concentrations in vehicle for each assay without exceeding 1% DMSO or 1% EtOH concentrations.

### Cell lines and cell culture

Chinese hamster ovary (CHO) cells expressing recombinant human kappa opioid receptor (CHO-hKOR cell line) were maintained in DMEM/F-12 media (Invitrogen, Carlsbad, CA) supplemented with 10% fetal bovine serum, 1% penicillin/streptomycin, and 500 µg/ml geneticin maintained. The DiscoveRx PathHunter™ U2OS cell line expressing βarrestin2 and hKOR (U2OS-hKOR-βarrestin2-DX) were purchased from Eurofins DiscoverX Products (Fremont, CA) and maintained MEM with 10% fetal bovine serum, 1% penicillin/streptomycin, 500 µg/ml geneticin, and 250 µg/ml hygromycin B. All cells were grown at 37 °C (5% $CO_2$ and 95% relative humidity) [11].

### Signaling assays

$^{35}$S-GTPγS binding assays were performed on membranes prepared from the CHO-hKOR cells as previously

described [11, 18] The βarrestin2 recruitment (DiscoverX PathHunter™) assay was performed according to the manufacturer's protocol with slight modification and following previously published protocols [11].

### Data analysis

GraphPad Prism 6.01 software (GraphPad) was used to generate sigmoidal concentration–response curves using a three-parameter, nonlinear regression analysis. All compounds were run in parallel assays (2–4 replicates per individual experiment). All studies were performed $n \geq 3$ independent experiments in multiple replicates. For some compounds, a two-point screen showed marginal stimulation at 50 nM, and submaximal stimulation at 10 μM; these were not pursued for further characterization. The efficacy and potency values were obtained from the averages of the nonlinear regression analysis performed on each individual curve and are reported as the mean ± S.E.M.

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## Compliance with ethical standards

**Conflict of interest** KJF, LMB, and JA are co-inventors on a patent that includes some of the compounds reported in this paper.

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