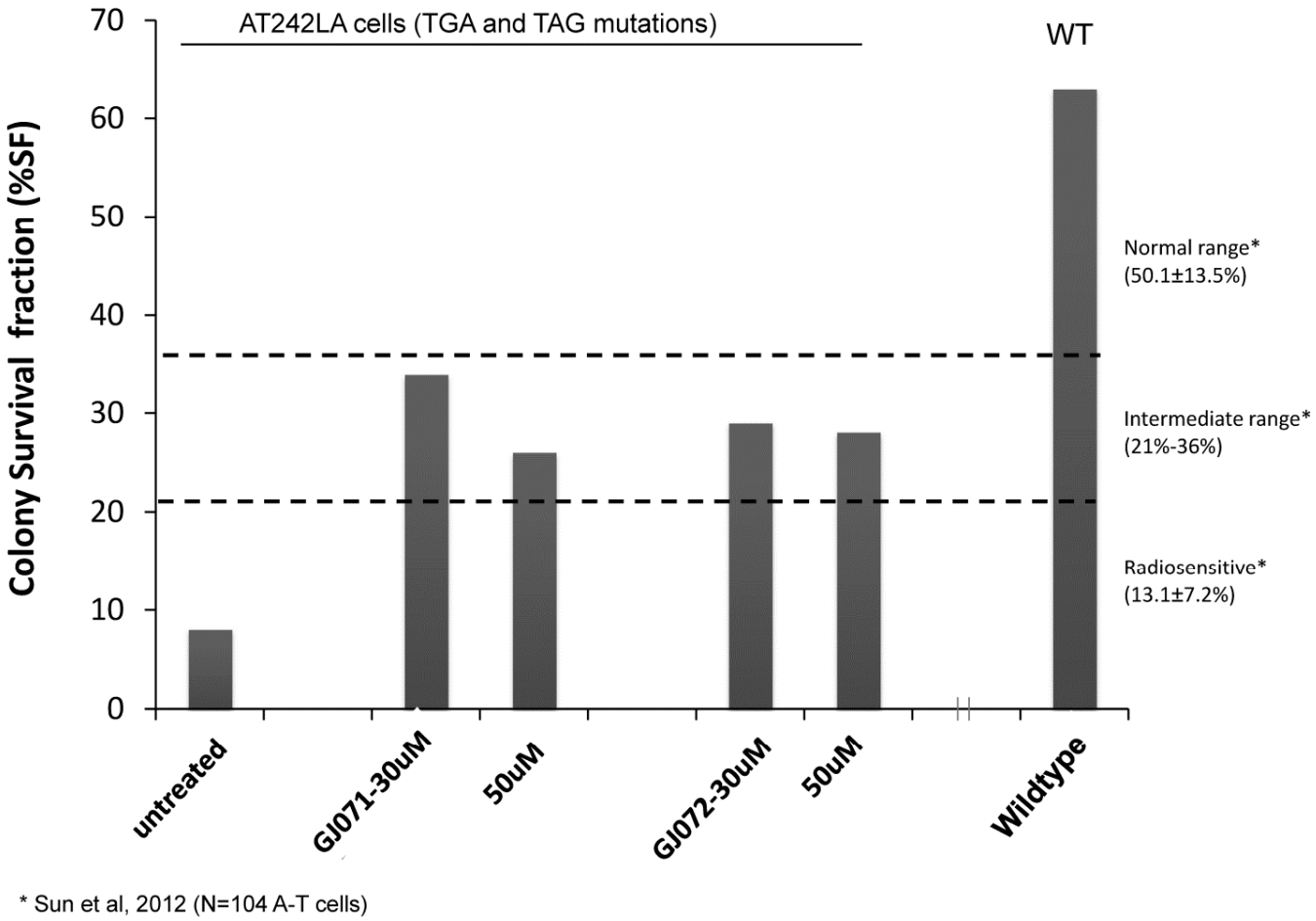


Figure S1. GJ071 and GJ072 abrogated the radiosensitivity of A-T LCLs. AT242LA cells (containing TGA and TAG stop codons) were treated with parent RTC compounds and the CSA was measured. Both RTCs increased cell survival fractions from “radiosensitive” to “intermediate” range (as previously described, Sun et al, 2002).

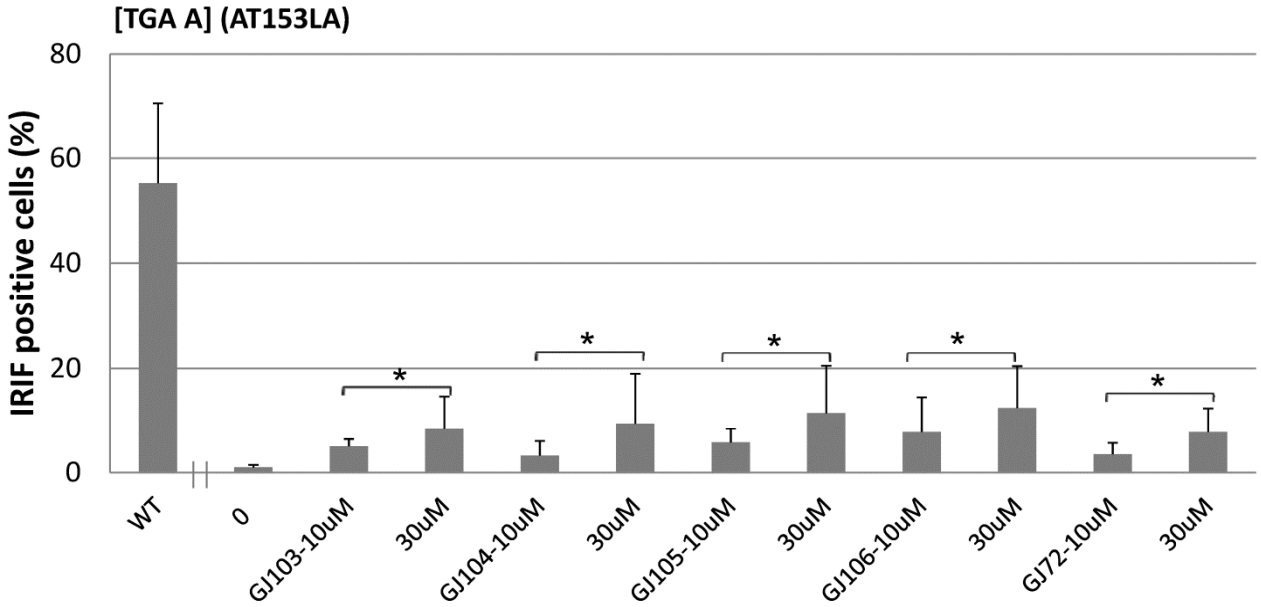


Figure S2. Active GJ072 analogs induced ATMs1981 foci in AT153LA cells with <u>TGA</u> PTC. AT153LA cells were treated with each RTC for 4 days and then followed by IRIF assay. The data shown in the figure were derived from 3 independent experiments. * p≤0.05 compared with untreated A-T cells.

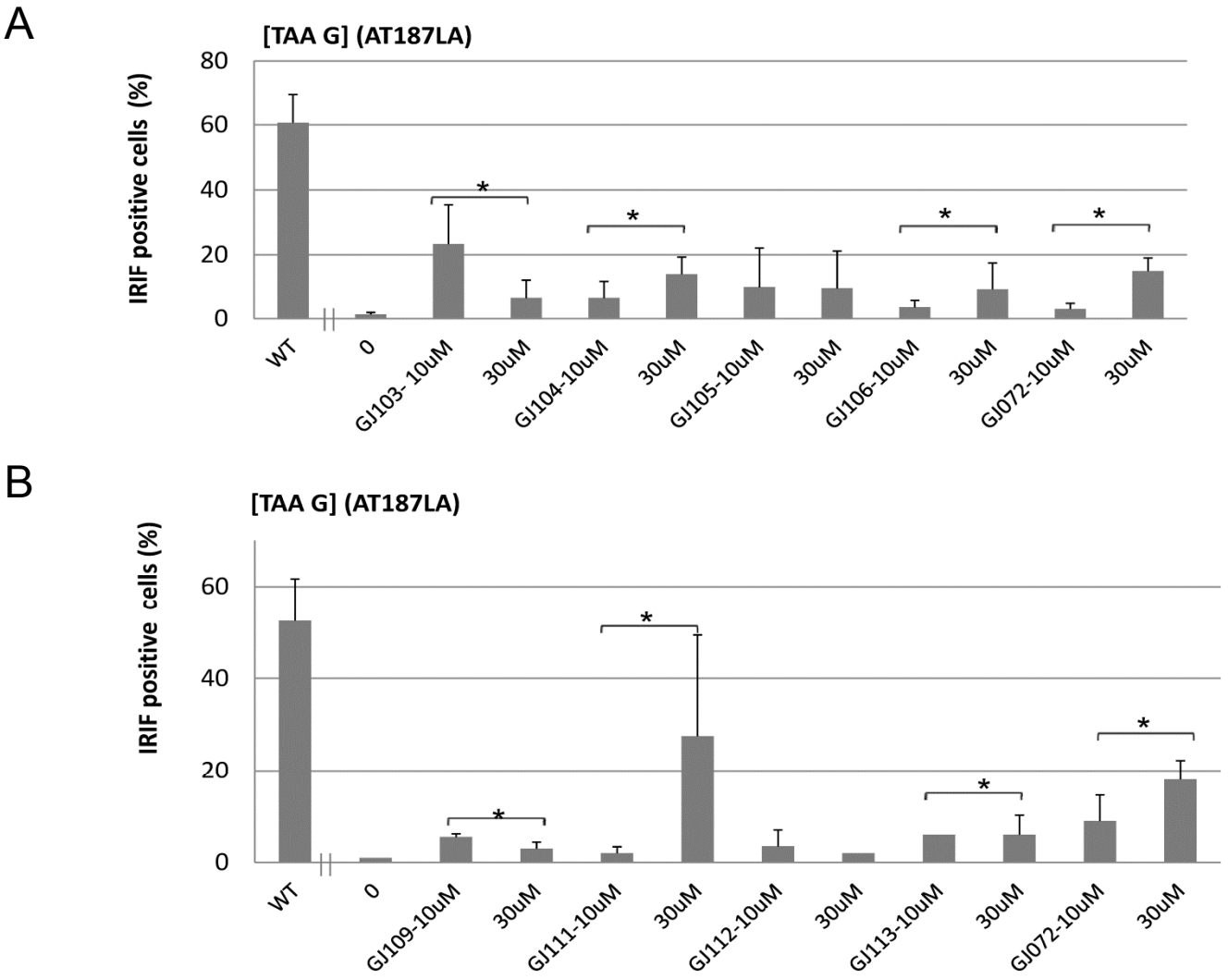


Figure S3. Active GJ072 analogs induced ATMs1981 foci in AT187LA cells with <u>TAA</u> PTC. AT187LA cells were treated with each RTC for 4 days and then followed by IRIF assay. **A)** Activity of GJ103, GJ104, GJ105 and GJ106; **B)** Activity of GJ109, GJ111, GJ112 and GJ113. The data shown in the A and B were derived from 3 independent experiments, respectively. * $p \leq 0.05$ compared with untreated A-T cells.

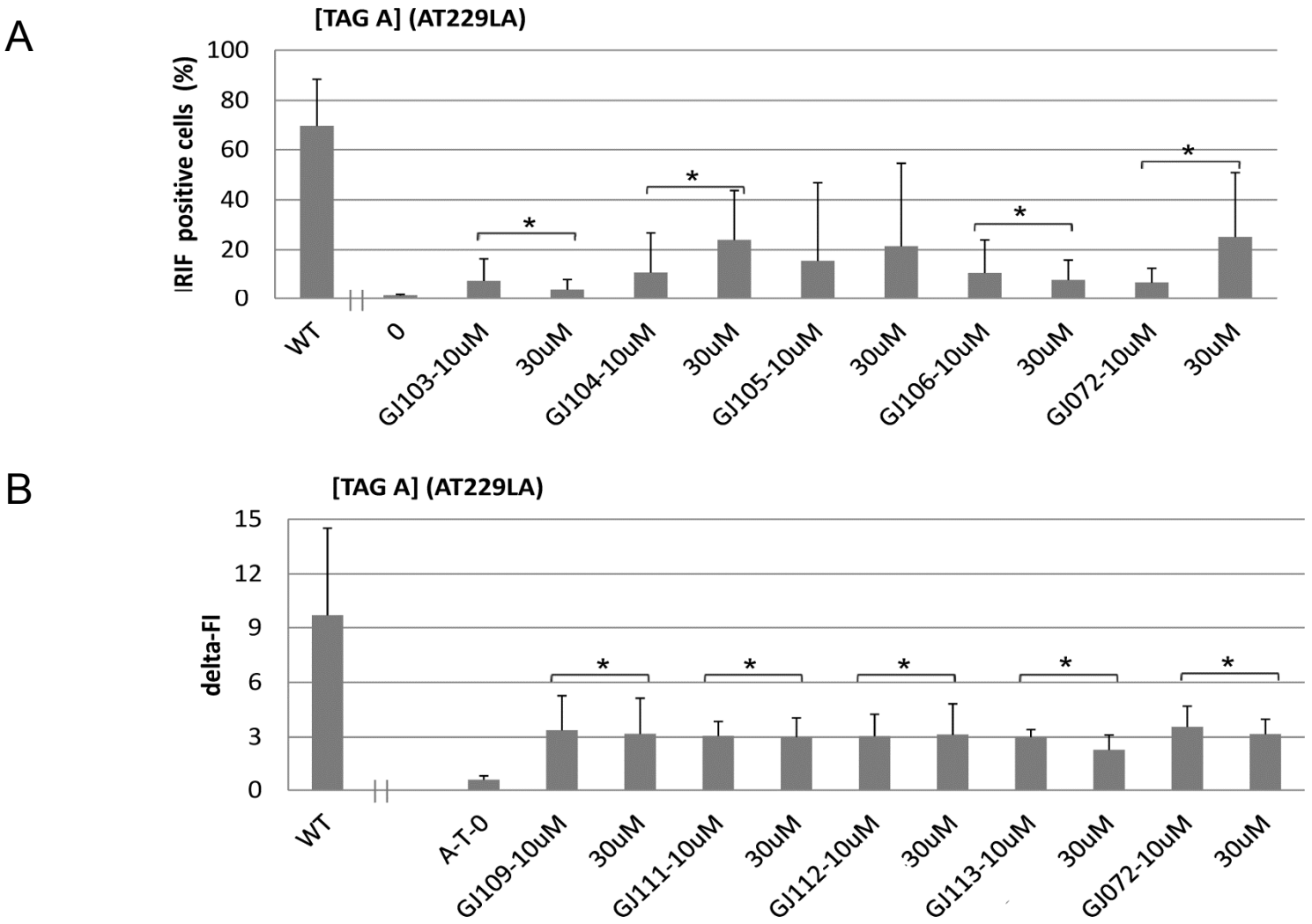


Figure S4. GJ072 analogs induced readthrough of <u>TAG</u> PTC in A-T cells. AT229LA cells with homozygous TAG mutation were treated with RTCs for 4 days and collected for assaying. Restored ATM kinase was measured by ATMpSer1981-IRIF or FCATMpSer1981 assay. **A)** Activity of GJ103, GJ104, GJ105 and GJ106 by ATMs1981-IRIF. The data in the figure are derived from 5 independent experiments; **B**) Activity of GJ109, GJ111, GJ112 and GJ113 by FCATMs1981. These data shown in the figure are derived from 3 independent experiments. * $p \leq 0.05$ as compared with untreated A-T cells.

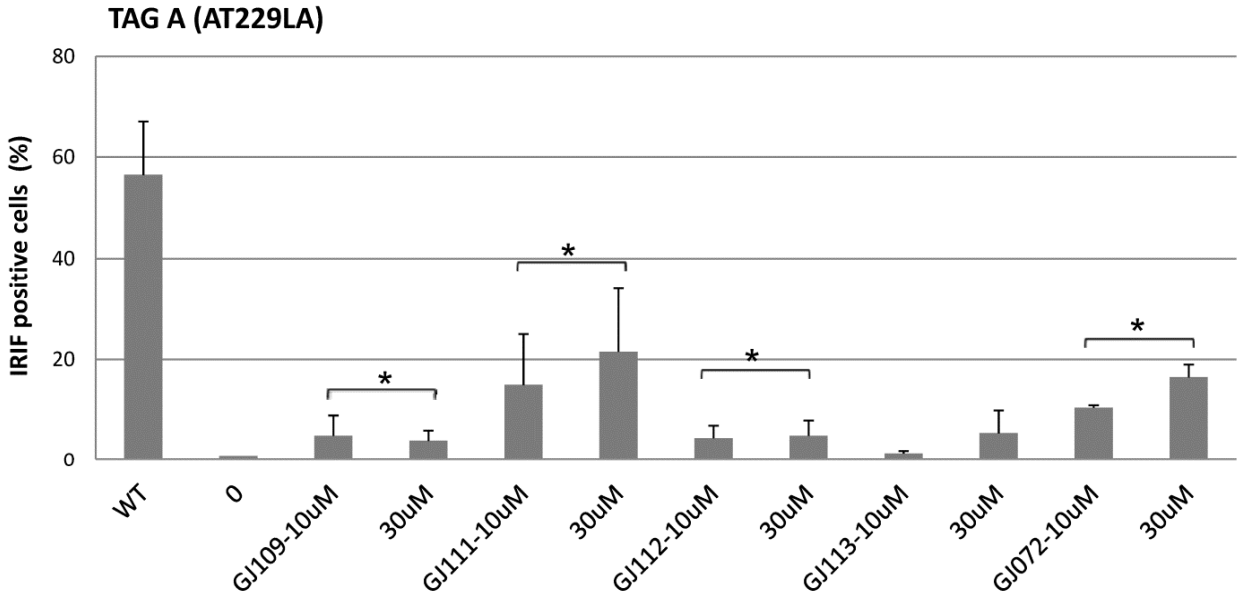
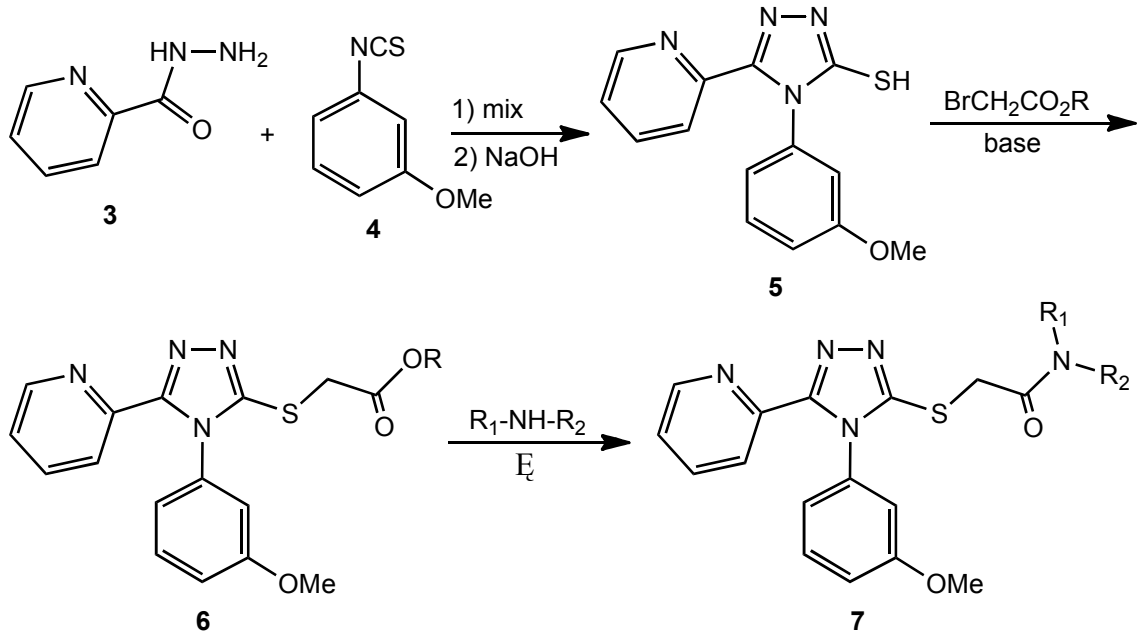


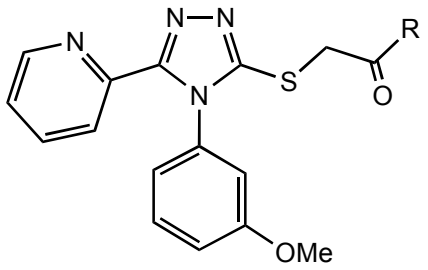
Figure S5. Active GJ072 analogs induced ATMs1981 foci in AT229LA cells with <u>TAG</u> PTC. AT229LA cells were treated with each RTC for 4 days and then followed by IRIF assay. Activity of GJ109, GJ111, GJ112 and GJ113 is shown here. IRIF data on GJ103, GJ104, GJ105 and GJ106 were shown in Suppl. Fig. 4. The data shown in the figure are derived from 3 independent experiments. *: $p \leq 0.05$ compared with untreated A-T cells.

Compound Preparation:

The 1,2,4-triazole-3-thiones were prepared by the condensation of an acyl hydrazide, e.g., the 2-pyridyl compound **3**, with a substituted isothiocyanate, e.g., the 3-methoxyphenyl compound **4**, to give, after treatment of the intermediate with base, the triazolethione **5**. Alkylation of this thione **5** in base, e.g., with chloroacetic acid or an alkyl bromoacetate, afforded the 3-[(carboxymethyl)thio]triazole or the 3-[(carboalkoxymethyl)thio]triazole **6**. Condensation of the acid or esters with mono- and disubstituted amines afforded the amides **7**. In this manner,

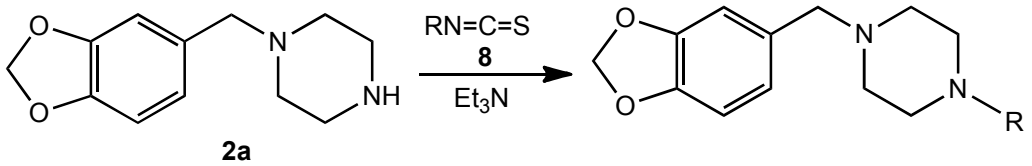


the following compound were made, namely the acid **1a** and its sodium salt **1k**, and the series of amides, **1b** – **1j**. The second series of compounds **2** were made easily from the commercially



1a R = OH
1b R = NHPh
1c R = NH-4-pyr
1d R = NH-4-(OH)C_6H_4
1e R = NH-4-(F)C_6H_4
1f R = NH-2,6-$F_2C_6H_3$
1g R = NH-4-$CF_3C_6H_4$
1h R = NHMe
1i R = NMe_2
1i R = NH-cPr
1k R = O Na

available monosubstituted piperazine **2a** by any of several methods. Thus addition of the isothiocyanate **8**, which was prepared from the corresponding amine, e.g., with thiophosgene, to **2a** in the presence of base afforded the thioureas **2b** – **2f**.



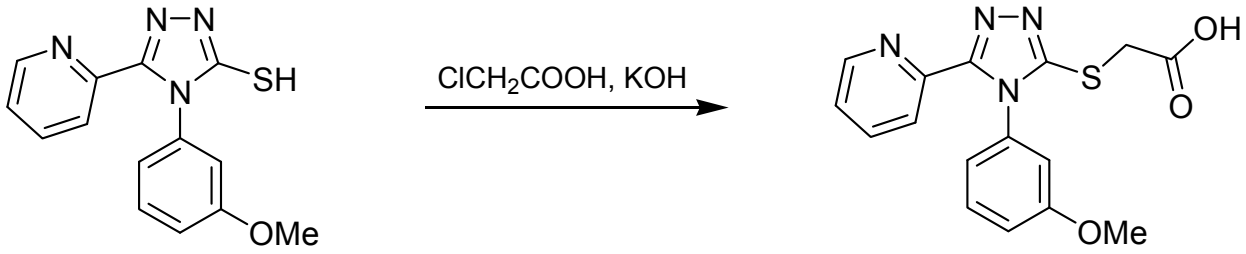
2a

2b R = C=(S)NH-Pr
2c R = C=(S)NH-Bn
2d R = C=(S)NH-allyl
2e R = C=(S)NH-CH_3
2f R = C=(S)NH-C_6H_5

Chemical Experimental Section.

General Procedures. Materials were obtained from commercial suppliers and were used without further purification. Air or moisture sensitive reactions were conducted under argon atmosphere using oven-dried glassware and standard syringe/septa techniques. The reactions were monitored with a silica gel TLC plate under UV light (254 nm) followed by visualization with a *p*-anisaldehyde or ninhydrin staining solution. Column chromatography was performed on silica gel 60. ^{1}H NMR spectra were measured at 400

MHz in $CDCl_3$ unless stated otherwise and data were reported as follows in ppm (□) from the internal standard (TMS, 0.0 ppm): chemical shift (multiplicity, integration, coupling constant in Hz.).



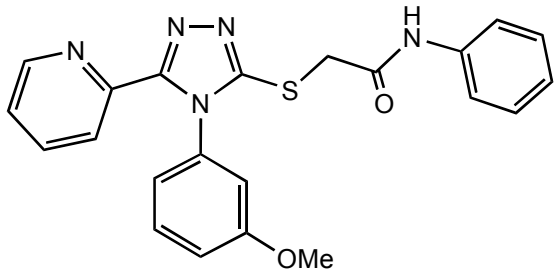
5 6

Preparation of 2-(4-(3-methoxyphenyl)-5-(pyridin-2-yl)-4*H*-1,2,4-triazol-3-ylthio)acetic acid 1a. A mixture of triazolethione **5** (1.75g, 6.2 mmol), 2-chloroacetic acid (584 mg, 5.1 mmol) and aqueous potassium hydroxide solution (2M) (170 mL, 5.1 mmol) was refluxed for 3 h. The hot reaction mixture was filtered and the filtrate acidified with 2M hydrochloric acid. The compound was precipitated out, filtered, washed with water. Recrystallization from an ethyl acetate/hexanes mixture gave **6** in 83% yield (1.76 g, light yellow solid). ^{1}H NMR (500 MHz, $(CD_3)_2SO$): 8.37 (d, J = 4.0 Hz, 1H), 7.99-7.91 (m, 2H), 7.44-7.38 (m, 2H), 7.09 (d, J = 6.5 Hz, 1H), 6.98 (s, 1H), 6.90 (d, J = 7.5 Hz, 1H), 4.10 (s, 2H), 3.73 (s, 3H). ^{13}C NMR (125 MHz, $(CD_3)_2SO$): δ 169.6, 159.9, 153.9, 152.7, 149.4, 146.5, 137.5, 135.7, 130.5, 124.7, 123.9, 119.6, 115.2, 113.5, 55.8, 34.5 ppm.

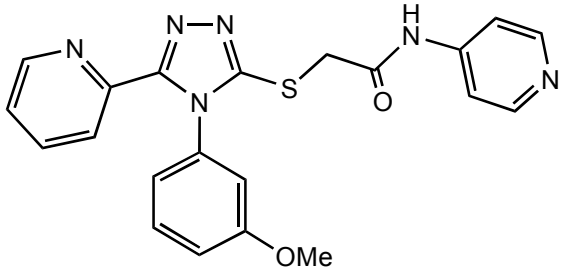
Salt **1k**: To a solution of **1a** (1.0 mmol) in THF, was added 1.0 M aqueous NaOH (1.3 mmol). The reaction mixture was stirred overnight at rt. The organic solvent wsa evaporated and the aqueous solution was stirred at 0°C for 2 h. The precipitate formed was filtered off and the filtrate was concentrated. The yellow solid was washed with cold water and dried using a lyophilizer. The desired salt **1k** was obtained as a yellow solid. ^{1}H NMR (500 MHz, DMSO-d_6): 8.37 (d, J = 4.0 Hz, 1H), 7.99-7.91 (m, 2H), 7.44-7.38 (m, 2H), 7.09 (d, J = 6.5 Hz, 1H), 6.98 (s, 1H), 6.90 (d, J = 7.5 Hz, 1H), 4.10 (s, 2H), 3.73 (s, 3H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 169.6, 159.9, 153.9, 152.7, 149.4, 146.5, 137.5, 135.7, 130,5 124.7, 123.9, 119.6, 115.2, 113.5, 55.8, 34.5 ppm.

General procedure for the synthesis of compounds 1b-1j.

To a solution of triazole **1a** (100 mg, 0.30 mmol) and the appropriate amine, R_1R_2NH, e. g. aniline (34 mg, 0.36 mmol) in dichoromethane (2 mL), *O*-Benzotriazole-*N,N,N',N'*-tetramethyl-uronium-hexafluoro-phosphate (HBTU) (120 mL, 0.36 mmol), and triethylamine (0.05 mL, 0.36 mmoL) were added. The reaction mixture was stirred for 4 h. After removal of solvent under reduced pressure, the residue was dissolved in ethyl acetate, washed with water, aqueous $NaHCO_3$, and brine solution. The organic layer was dried over anhydrous $MgSO_4$, then concentrated *in vacuo*. The crude mixture was purified by column chromatography on silica gel (ethyl acetate:hexane, 1:1) and obtained the desired product (1b to 1i) at 70-90% yield.

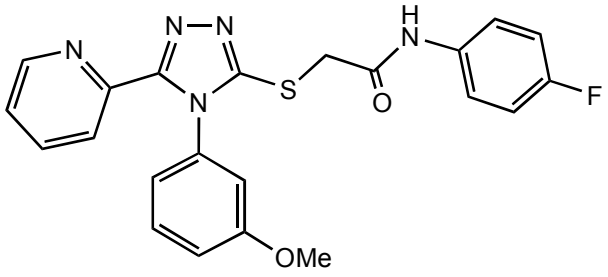


2-(4-(3-Methoxyphenyl)-5-(pyridin-2-yl)-4*H*-1,2,4-triazol-3-ylthio)-N-phenylacetamide 1b: ^{1}H NMR (300 MHz, $CDCl_3$): δ 10.40 (s, 1H), 8.00 (d, *J* = 8.1 Hz, 1H), 7.74 (dt, *J* = 7.8, 1.8 Hz, 1H), 7.63 (d, *J* = 8.7 Hz, 1H), 7.38-7.21 (m, 4H), 7.09-6.99 (m, 2H), 6.83-6.79 (m, 2H), 4.01 (s, 2H), 3.85 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 166.7, 160.3, 154.9, 154.3, 149.3, 146.1, 138.4, 136.8, 135.2, 130.3, 128.9, 124.3, 124.1, 123.5, 119.8, 119.2, 115.7, 112.7 ppm

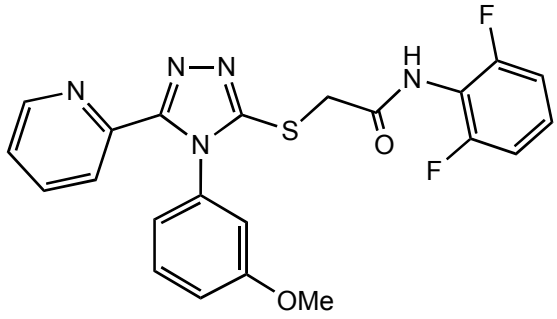


2-(4-(3-Methoxyphenyl)-5-(pyridin-2-yl)-4*H*-1,2,4-triazol-3-ylthio)-N-(pyridin-4-yl)acetam-ide 1c: ^{1}H NMR (500 MHz, $CDCl_3$): δ 10.97 (s, 1H), 8.45 (d, *J* = 6.0 Hz, 1H),

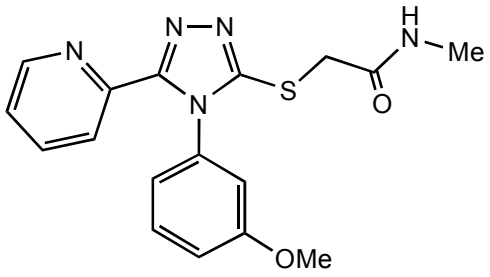
8.36 (d, *J* = 4.5 Hz, 1H), 7.97 (d, *J* = 8.0 Hz, 1H), 7.76 (dt, *J* =1.5, 7.5 Hz , 1H), 7.56-7.54 (m, 2H), 7.35 (t, *J* = 8.5 Hz, 1H), 7.25 (d, *J* = 8.5 Hz, 1H), 7.02 (dd, *J* = 2.0, 8.5 Hz, 1H), 6.82-6.79 (m, 2H), 3.98 (s, 2H), 3.80 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 167.4, 160.2, 154.7, 150.5, 149.2, 145.8, 145.1, 136.7, 134.9, 130.2, 124.3, 123.4, 118.9, 115.5, 113.7, 113.6, 112.6, 55.5, 36.2 ppm.



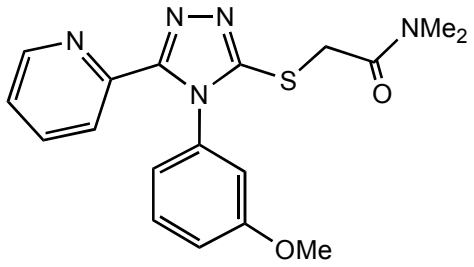
2-(4-(3-Methoxyphenyl)-5-(pyridin-2-yl)-4*H*-1,2,4-triazol-3-ylthio)-N-(4-fluorophenyl)acet-amide 1e: ^{1}H NMR (300 MHz, $CDCl_3$): δ 10.47 (s, 1H), 8.34 (bs,1H), 8.05 (d, *J* = 7.5 Hz, 1H), 7.74 (t, *J* = 7.5 Hz, 1H), 7.35 (t, *J* = 7.5 Hz, 1H), 7.26-7.13 (m, 2H), 7.02 (dd, *J* = 8.0, 0.5 Hz, 1H), 6.91 (t, , *J* = 8.0 Hz, 2H), 6.84-6.81 (m, 2H), 4.08 (s, 2H), 3.79 (s, 3H). ^{19}F (400 MHz, $CDCl_3$): δ-118.9 ppm.



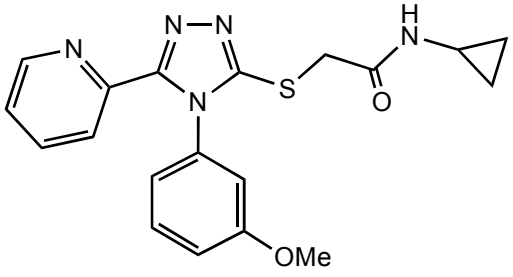
2-(4-(3-Methoxyphenyl)-5-(pyridin-2-yl)-4*H*-1,2,4-triazol-3-ylthio)-N-(2,6-difluorophenyl)-acetamide 1f: ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.94 (s, 1H), 8.34 (bs,1H), 8.05 (d, *J* = 7.5 Hz, 1H), 7.74 (t, *J* = 7.5 Hz, 1H), 7.35 (t, *J* = 7.5 Hz, 1H), 7.26-7.13 (m, 2H), 7.02 (dd, *J* = 8.0, 0.5 Hz, 1H), 6.91 (t, , *J* = 8.0 Hz, 2H), 6.84-6.81 (m, 2H), 4.08 (s, 2H), 3.79 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 167.3, 160.2, 158.8, 158.7, 156.8, 156.7, 154.6, 154.1, 149.1, 146.0, 136.6, 135.2, 130.1, 127.409, 127.3, 127.3, 124.2, 123.4, 119.0, 115.4, 112.7, 111.6, 111.6, 111.5, 111.4, 55.5, 34.9 ppm. ^{19}F NMR (376.5 MHz, $CDCl_3$) δ -117.93 ppm.



2-(4-(3-Methoxyphenyl)-5-(pyridin-2-yl)-4H-1,2,4-triazol-3-ylthio)-N-methylacetamide 1h: ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.34 (d, *J* = 4.2 Hz, 1H), 8.05 (d, *J* = 7.8 Hz, 1H), 7.75 (t, *J* = 7.5 Hz, 1H), 7.35 (t, *J* = 7.5 Hz, 1H), 7.22 (d, *J* = 9.0 Hz, 1H), 7.02 (dd, *J* = 8.7, 2.4 Hz, 1H), 6.83-6.78 (m, 2H), 4.28 (s, 2H), 3.79 (s, 3H), 2.77 (s, 3H).

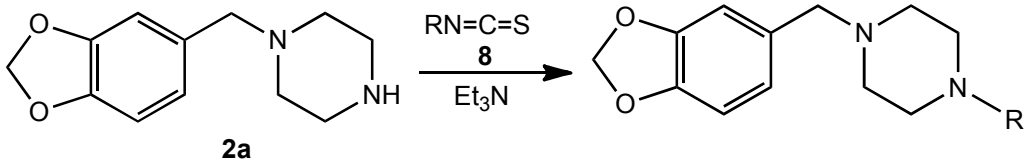


2-(4-(3-Methoxyphenyl)-5-(pyridin-2-yl)-4H-1,2,4-triazol-3-ylthio)-N,N-dimethylacetamide 1i: ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.34 (d, *J* = 4.5 Hz, 1H), 8.05 (d, *J* = 7.8 Hz, 1H), 7.73 (dt, *J* = 7.5, 1.5 Hz, 1H), 7.35 (t, *J* = 8.4 Hz, 1H), 7.22 (dd, *J* = 7.5, 4.8 Hz, 1H), 7.02 (dd, *J* = 8.4, 2.4 Hz, 1H), 6.85-6.63 (m, 2H), 4.38 (s, 2H), 3.78 (s, 3H), 3.16 (s, 3H), 2.99 (s, 3H).



2-(4-(3-Methoxyphenyl)-5-(pyridin-2-yl)-4H-1,2,4-triazol-3-ylthio)-N-cyclopropylacetamide 1j: ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.35 (d, *J* = 4.2 Hz, 1H), 8.00

(s, 1H), 7.98 (d, J = 8.1 Hz, 1H), 7.75 (dt, J = 7.5, 1.5 Hz, 1H), 7.36 (t, J = 8.2 Hz, 1H), 7.22 (dd, J = 7.5, 4.8 Hz, 1H), 7.02 (dd, J = 8.4, 2.4 Hz, 1H), 6.81-6.77 (m, 2H), 3.79 (s, 2H), 3.71 (s, 3H), 2.77-2.71 (m, 1H), 0.78-0.75 (m, 1H), 0.59-0.54 (m. 1H).

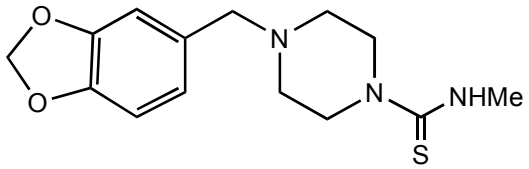


2a

2b R = C=(S)NH-Pr
2c R = C=(S)NH-Bn
2d R = C=(S)NH-allyl
2e R = C=(S)NH-CH_3
2f R = C=(S)NH-C_6H_5

General procedure for the synthesis of compounds 2b-2f.

To a solution of 1-piperonylpiperazine (157 mg, 0.71 mmol) in dichloromethane (5 mL), was added the appropriate isothiocyanate, e.g. allyl isothiocyanate (69 µL, 0.71 mmoL). The reaction mixture was stirred overnight at room temperature. The reaction mixture was poured into water, extracted with dichloromethane and dried with anhydrous sodium sulfate. The residue after removal of solvent was purified by silica gel column chromatography with ethyl acetate:hexane (2:1) and afforded compounds **2b-2f**.



4-((Benzo[*d*][1,3]dioxol-6-yl)methyl)-N-methylpiperazine-1-carbothioamide 2e: ^{1}H NMR (500 MHz, $CDCl_3$): δ 6.83 (s, 1H), 6.75-6.70 (m, 2H), 5.93 (s, 2H), 5.67 (bs, 1H), 3.79 (t, J = 5.0 Hz, 1H), 3.42 (s, 2H), 3.14 (d, J = 10.5 Hz, 3H), 2.45 (t, J = 5.0 Hz, 4H). ^{13}C NMR (125 MHz, $CDCl_3$): δ182.9, 147.6, 146.7, 131.3, 122.1, 109.2, 107.8, 100.8, 62.3, 52.2, 47.3, 32.8 ppm.