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Design, synthesis and biological activities of thiourea containing sorafenib analogs as antitumor agents

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ABSTRACT

A novel series of diaryl thiourea containing sorafenib derivatives **9a**–**t** was designed and synthesized. The structures of all the newly synthesized compounds were determined by ^{1}H NMR, ^{13}C NMR and HRMS. Their antiproliferative activities against HCT116 and MDA-MB-231 cell lines, and their inhibitory activities against the phosphorylation of VEGFR were evaluated and described. Some of the compounds showed significant activities against both cell lines and VEGFR. Compounds **9g**, **9m**, **9o** and **9p** demonstrated competitive antiproliferative activities to sorafenib, the reference standard, while compounds **9d**, **9m**, and **9p** showed significant inhibitory activities against the phosphorylation of VEGFR.

1. Introduction

Due to the diverse side effects of traditional cytotoxic anticancer drugs, it is necessary to exploit novel antitumor drugs with high efficiency and low toxicity. Recent advances and applications on multiple targeted agents may afford effective chemotherapeutics with low toxicity.

Sorafenib, a diaryl urea multiple-targeted antitumor agent, can inhibit several kinases involved in tumor proliferation and angiogenesis including Raf, VEGFR, PDGFR and KIT.[1–4] Due to the advantages of multi-mechanisms, broad-spectrum anticancer potency, and well-tolerated results in combination trials, more and more researchers have focused on the optimization of sorafenib.[5–10]

Inspired by the classic bioisosteric paradigm of thiopental replacing urea in pentobarbital with thiourea, a series of novel sorafenib derivatives were designed and synthesized by substituting thiourea for urea in sorafenib. The antitumor effect of all the newly synthesized compounds on the in vitro growth of two cell lines, namely human colorectal carcinoma (HCT116) and human breast cancer cell (MDA-MB-231) was evaluated. Apparent growth inhibition was observed for most of the compounds, with **9d**, **9g**, **9m**, **9o** and **9p** demonstrating more potent activities against cancer cells as compared to sorafenib, respectively. Furthermore, some structure–activity relationships have also been established.

2. Chemistry

The synthetic routes to the library compounds were illustrated as outlined in Scheme 1.

The substituted diaromatic ethers were prepared in a four-step sequence. The starting material 2-picolinic acid **1** was treated with $SOCl_2$ to generate 4-chloropicolinoyl chloride **2**, and then treated with methanol to produce methyl 4-chloropicolinate **3**. Subsequently, **3** was treated with corresponding amine (methylamine, *n*-butylamine, phenylmethanamine, cyclopropanamine and cyclohexanamine) to form **4a**–**e**. Then, they were treated with 4-aminophenol to generate corresponding diaromatic ethers (**5a**–**e**) with the total yields of 32–54%. Furthermore, substituted anilines(2,4-dichloroaniline, 4-chloroaniline, 3,4-difluoroaniline, 4-fluoroaniline, 3-(trifluoromethyl)aniline, 3,5-bis(trifluoromethyl)aniline, 4-chloro-3-(trifluoromethyl)aniline and 4-methoxyaniline), **6a**–**h**, were treated with CS_2 to generate **7a**–**h**, and then treated with triphosgene (BTC) to produce various isothiocyanates (**8a**–**h**). Finally, these isothiocyanates were reacted with substituted diaromatic ethers (**5a**–**e**) in DCM to afford thiourea (**9a**–**t**) in 19–48% total yields. The final products were purified by column chromatography and their structures were characterized by ^{1}H NMR, ^{13}C NMR and HRMS.

3. Results and discussion

3.1. Cell inhibition

In vitro cell cytotoxicity of the 20 new diaryl thiourea derivatives was initially evaluated against HCT116 and MDA-MB-231

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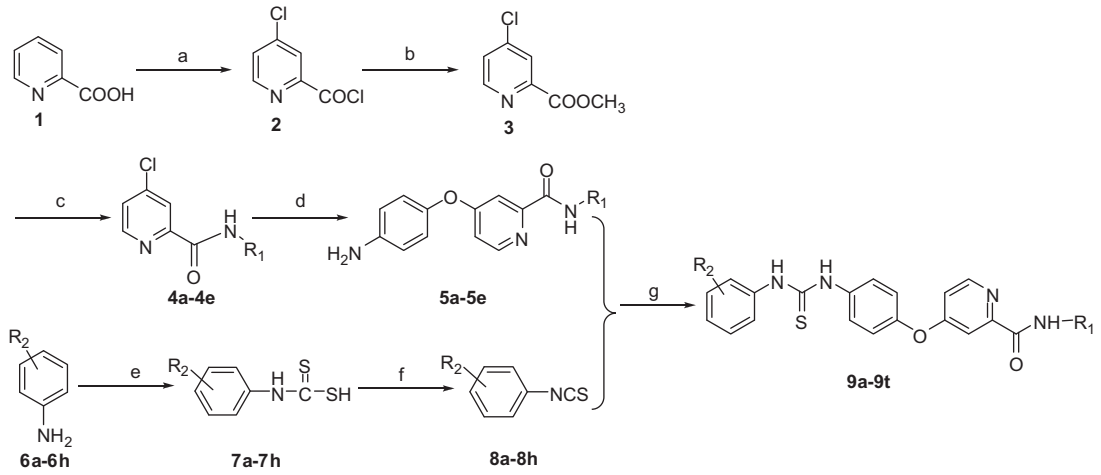


Scheme 1. Reagents and conditions: (a) $SOCl_2$ (b) CH_3OH (c) RNH_2, THF (d) 4-aminophenol, DMF, KOBu-t (e) Dabco, CS_2, toluene (f) BTC, DCM (g) DCM.

cells by MTT assay using sorafenib as a positive control. As shown in Table 1, most compounds (except **9b**, **9e**, **9l** and **9t**) exhibited potent antiproliferative activity against HCT116 cells with IC_{50} = 9.15–53.2 μM. Compounds **9d**, **9g**, **9m**, **9o** and **9p** showed significant inhibitory activities against both cell lines. Among them, two compounds **9o** and **9p** demonstrated competitive antiproliferative activities to sorafenib, the reference standard. The basic structure–activity relationships (SAR) were also studied: (1) The size and shape of the hydrophobic R group: **a**. The longer substitutes (such as *n*-butyl and benzyl groups) of R might cause a decrease in cytotoxicity in general. For example, among the 20 compounds, only **9b**, **9e**, **9l** and **9t** whose R substitutes are *n*-butyl and benzyl groups have no antiproliferative activity to HCT116 and MDA-MB-231 cells. **b**. The size and shape of R group may influence the selectivity on different cancer cells. It is notable that only the compounds having methyl group show the inhibitory activity against MDA-MB-231 cell, and these compounds also have potent activities against HCT-116 cell line (IC_{50} = 16.1–30.6 μM). While the other compounds (except for **9b**, **9e**, **9l** and **9t**) can only inhibit the growth of HCT116 cells. This trait is especially obvious in the compounds containing cyclohexyl ring. The compounds **9j**, **9n** and **9r** containing cyclohexyl group showed good antiproliferative activity to HCT116 (IC_{50} = 9.15–26.15 μM), which are even better than those of compounds containing methyl group. (2) The substitutes on the terminal phenyl ring: **a**. Apart from the terminal R group, the terminal Ar group can also affect the selectivity against cancer cells. The compounds against MDA-MB-231 cells not only contained methyl group of R terminal, but also contained 3,4-difluo-substitue, 3-CF_3, 4-Cl or both of them on the phenyl ring. **b**. The substitutes on the terminal phenyl ring may also influence the activity against HCT116 cells. For example, the compound **9s** containing the hydrophobic group (-OCH_3) on the 4-position of phenyl ring has less cytotoxicity compared with other compounds (**9a**, **9d**, **9g**, **9k**, **9m**, **9o** and **9p**) which containing the same R group with **9s**. The influence of substitutes on phenyl ring still needs further investigation.

3.2. Phosphorylation inhibition of VEGFR

The inhibition against the phosphorylation of VEGFR was tested in cell level using western blotting analysis. Compounds **9d**, **9m**, and **9p** showed inhibition against the phosphorylation of VEGFR. As shown in Figure 1, sorafenib can inhibit the phosphorylation of VEGFR at the concentration of 0.01 μg/mL; **9m** can partly inhibit the phosphorylation of VEGFR at the concentration of 0.1 μg/mL; **9d** and **9p** can inhibit the phosphorylation of VEGFR at the concentration of 1 μg/mL. All these three compounds (**9d**, **9m**, and **9p**) have the methyl substitute on the terminal amide, and their substitutes in phenyl ring are 4-Cl (**9d**), 3-CF_3 (**9m**) or both of them (**9p**). These results indicate that the methyl on the terminal amide, 4-Cl and 3-CF_3 on the phenyl ring contribute more to the phosphorylation inhibition of VEGFR. And these compounds also showed potent cytotoxicity against HCT116 and MDA-MB-231 cells as mentioned above. While other compounds such as **9g** and **9o** which have no phosphorylation inhibition of VEGFR also have significant cytotoxicities, which indicates that the compounds **9g** and **9o** have the other mechanism to inhibit the growth of cancer cells, which need further investigation.

4. Conclusions

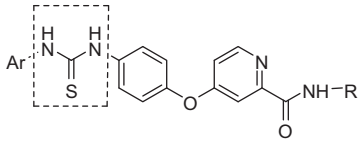
In summary, we report here the synthesis and biological evaluation of a new class of sorafenib derivatives as potential antitumor agents. Most compounds exhibited good antiproliferative activity to HCT116 cells. Compounds **9d**, **9g**, **9m**, **9o** and **9p** which contains methyl group on the terminal amide showed significant inhibitory activities against both HCT116 and MDA-MB-231 cell lines, and two compounds **9o** and **9p** demonstrated competitive antiproliferative activities to sorafenib. Notably, compared with sorafenib, most of the other diaryl thiourea compounds showed excellent selectivity toward HCT-116 over MDA-MB-231 cells. On the other hand, **9d**, **9m**, and **9p** showed inhibition against the phosphorylation of VEGFR. These three compounds also have remarkable antiproliferative activities against both HCT116 and MDA-MB-231 cell lines, while compounds **9g** and **9o** which also have good cytotoxicities have no phosphorylation inhibition of VEGFR, which indicates that the compounds **9g** and **9o** have the other mechanism to inhibit the growth of cancer cells. It is of interest to continue studying cytotoxicity and kinase activities of diaryl thiourea derivatives.

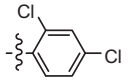
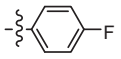
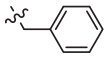
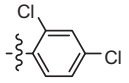
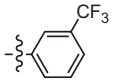
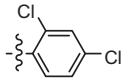
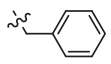
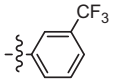
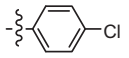
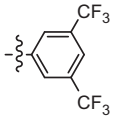
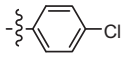
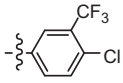
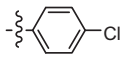
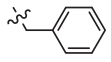
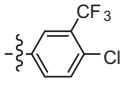
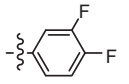
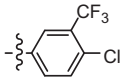
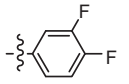
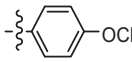
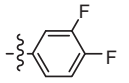
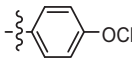
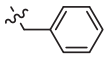
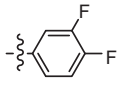
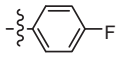
5. Experimental

5.1. Cell inhibition assays

HCT116 and MDA-MB-231 cell lines were plated on 96-well plates at a density of 5000 per well and incubated overnight. The cells were treated with compounds and sorafenib at final concentrations ranging from 0.5 to 200 μM, while control cells were treated with equal volume DMSO. After 48 hr treatment, 0.5% MTT (Amresco, USA) solution was added to each well, and further

Table 1
The structures and IC_{50} values of the target compounds



Compd No.	Substituent		IC_{50} (μM)[a]		Compd No.	Substituent		IC_{50} (μM)[a]	
	Ar	R	HCT116	MDA-MB-231		Ar	R	HCT116	MDA-MB-231
9a			21.7 ± 1.2	>200	**9l**			>200	>200
9b			>200	>200	**9m**			22.6 ± 0.9	64.9 ± 1.8
9c			53.2 ± 1.0	>200	**9n**			19.6 ± 2.1	>200
9d			17.3 ± 1.8	122.5 ± 7.8	**9o**			30.6 ± 0.9	33.4 ± 1.5
9e			>200	>200	**9p**			25.3 ± 1.0	29.7 ± 0.7
9f			24.6 ± 0.8	>200	**9q**			15.9 ± 1.5	>200
9g			16.1 ± 2.5	80.1 ± 4.2	**9r**			9.15 ± 0.7	>200
9h			32.5 ± 1.4	>200	**9s**			43.1 ± 1.9	>200
9i			52.5 ± 2.0	>200	**9t**			>200	>200
9j			26.15 ± 3.1	>200	Sorafenib	—	—	7.8 ± 0.8	36 ± 2.1
9k			31.1 ± 1.1	>200					

[a] Mean value of three experiments and standard deviation are given.

Figure 1. Western blots showing protein expression of VEGFR after treatment with compounds **9d**, **9m**, **9p** and sorafenib at various concentrations (0.01, 0.1 and 1 μg/mL).

incubation for 4 h, then cells centrifuged at 2,500 rpm for 15 min, removed the culture medium, and added 150 μL DMSO to dissolve the formazan. After mixing for 5 min, optical density was detected at 570 nm on a microplate reader (Thermo, USA).

5.2. Phosphorylation inhibition of VEGFR

HUVEC cells were incubated with different concentrations of compounds for 2 h. Cells in each group were harvested, washed twice with ice-cold PBS, and lysed with cell lysis buffer except for the blank group after 5 min of addition of VEGF. Then western blotting was performed to test the VEGFR using anti-phospho-VEGF receptor 2 as the antibody.

5.3. Chemistry: general procedures

All the material we used were purchased from commercial suppliers and used without further purification. Solvents were distilled prior to use and flash chromatography was performed using silica gel (60 Å, 200–300 mesh). All reactions were monitored by thin-layer chromatography on 0.25 mm silica gel plates (60GF-254) and visualized with UV light, or chloride ferric. Melting points were

determined on an electrothermal melting point apparatus and were uncorrected. Proton nuclear magnetic resonance (^{1}H NMR) spectra were determined on a Brucker Avance 600 spectrometer using TMS as an internal standard in DMSO-d_6 solutions. Chemical shifts are reported in delta (δ) units, parts per million (ppm) downfield from trimethylsilane. ESI-MS were determined on an API 4000 spectrometer. High-resolution mass spectral (HRMS) data were conducted by Shandong Analysis and Test Center, and are reported as m/z (relative intensity).

5.3.1. Methyl 4-chloropicolinate (3)

2-Picolinic acid (15 g, 0.122 mol) was slowly added to thionyl chloride (50 mL) at a temperature range of 45–50 °C under stirring. Then the solution was heated to 72 °C, and kept refluxing for about 14 h. After cooled to room temperature, the mixture was diluted with toluene (100 mL), and concentrated to near dryness in vacuo. To the obtained oily residue, methanol (40 mL) was added. The contents were stirred for 2 h at 30 °C. After filtration, the filter cake was added 15% Na_2CO_3 solution to adjust its pH value to neutral, filtrate and washed with cold methanol to obtained light yellow solid 17.5 g (83.7%), mp: 52–54 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.88 (s, 3H); 7.89 (dd, J = 5.4, 2.0 Hz, 1H); 8.10 (d, J = 2.1 Hz, 1H); 8.70 (d, J = 5.4 Hz, 1H).

5.3.2. 4-Chloro-*N*-methylpicolinamide (4a)[11]

The compound **3** (10 g, 58.3 mmol) was dissolved in 70 mL methanol/THF (2/5) mixture at 0 °C, then a solution of methylamine (5 g, 161.3 mmol) in THF (60 mL) was added at a rate that kept the internal temperature below 2 °C. The mixture was stirred at room temperature for 3 h and then was concentrated in vacuo. The crude product was dissolved in 100 mL ethyl acetate and was washed with brine, dried over anhydrous sodium sulfate, and concentrated under reduced pressure to afford 4a (9.4 g, 94.5%) as light yellow crystalline. Mp: 41–43 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.80 (d, J = 4.6 Hz, 3H), 7.78 (dd, J = 5.2, 2.1 Hz, 1H); 8.05 (d, J = 2.1 Hz, 1H), 8.62 (d, J = 5.2 Hz, 1H), 8.85 (br d, J = 4.6 Hz, 1H); ESI-MS m/z 171([M+H]$^+$).

5.3.3. *N*-Butyl-4-chloropicolinamide (4b)

The synthesis is analogous to (**4a**) with *n*-butylamine as the starting material. The product was obtained as a orange solid. Yield = 82.9%. Mp: 42 – 45 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.97 (t, J = 7.3 Hz, 3H), 1.40 (m, 2H), 1.59 (m, 2H), 3.42 (q, J = 6.8 Hz, 2H), 7.39 (dd, J = 5.5, 2.2 Hz, 1H), 7.72 (d, J = 2.2 Hz, 1H), 8.05 (br d, 1H), 8.40 (d, J = 5.5 Hz, 1H).

5.3.4. *N*-Benzyl-4-chloropicolinamide (4c)

The synthesis is analogous to (**4a**) with phenylmethanamine as the starting material. The product was obtained as a light yellow solid. Yield = 70.8%. Mp: 78–82 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.62 (d, J = 6.0 Hz, 2H), 7.09 (dd, J = 5.5, 2.5 Hz, 1H), 7.25 (m, 1H), 7.32 (m, 4H), 7.73 (d, J = 2.5 Hz, 1H), 8.35 (d, J = 5.5 Hz, 1H), 8.38 (t, J = 6.2 Hz, 1H).

5.3.5. 4-Chloro-*N*-cyclopropylpicolinamide (4d)

The synthesis is analogous to (**4a**) with cyclopropanamine as the starting material. The product was obtained as a yellow solid. Yield = 90.1%. Mp: 99–102 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.86 (d, J = 7.0 Hz, 4H), 2.88 (m, 1H), 7.21 (dd, J = 5.5, 2.4 Hz, 1H), 8.04 (d, J = 2.4 Hz, 1H), 8.65 (d, J = 5.6 Hz, 1H), 8.80 (br d, 1H).

5.3.6. 4-Chloro-*N*-cyclohexylpicolinamide (4e)

The synthesis is analogous to (**4a**) with cyclohexanamine as the starting material. The product was obtained as a yellow solid. Yield = 84.6%. Mp: 117–119 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.26–1.48 (m, 6H), 1.85 (m, 4H), 3.84 (m, 1H), 7.64 (dd, J = 5.4, 2.3 Hz, 1H), 8.06 (d, J = 2.3 Hz, 1H), 8.58 (d, J = 5.4 Hz, 1H), 8.79 (br d, 1H).

5.3.7. 4-(4-Aminophenoxy)-*N*-methylpicolinamide (5a)

A solution of 4-aminophenol (10.8 g, 99 mmol) in dry *N*,*N*-dimethylformamide (DMF, 100 mL) was treated with potassium *tert*-butoxide (11.2 g, 100 mmol), and the mixture was stirred at room temperature for 1 h under nitrogen atmosphere, then a solution of (**4a**) (16.0 g, 94 mmol) in DMF (50 mL) and potassium carbonate were added respectively. The mixture was heated to 80–85 °C for 10 h. After the temperature was cooled to room temperature, the mixture was diluted with water (400 mL) and extracted with EtOAc (3 × 200 mL). The extract was washed with brine (2 × 200 mL), dried over anhydrous sodium sulfate, and concentrated under reduced pressure to give light-brown solid (**5a**) (20.3 g, 88.6%); mp: 109–112 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.01 (d, J = 4.8 Hz, 3H), 3.74 (br d, 2H), 6.68, 6.87 (AA′BB′, q, J = 8.7 Hz, 4H), 7.01 (dd, J = 5.0, 2.2 Hz, 1H), 7.63 (d, J = 2.2 Hz, 1H), 8.44 (d, J = 5.0 Hz, 1H), 8.75 (br d, J = 4.6 Hz, 1H).

5.3.8. 4-(4-Aminophenoxy)-*N*-butylpicolinamide (5b)

The synthesis is analogous to (**5a**) with (**4b**) as the starting material. The product was obtained as a brown solid. Yield = 73.5%. Mp: 85–88 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.94 (t, J = 7.3 Hz, 3H), 1.39 (m, 2H), 1.58 (m, 2H), 3.42 (q, J = 6.8 Hz, 2H), 3.72 (br d, 2H), 6.69, 6.87 (AA′BB′, q, J = J = 8.7 Hz, 4H), 6.92 (dd, J = 5.6, 2.4 Hz, 1H), 7.67 (d, J = 2.4 Hz, 1H), 8.03 (br d, 1H), 8.32 (d, J = 5.6 Hz, 1H).

5.3.9. 4-(4-Aminophenoxy)-*N*-benzylpicolinamide (5c)

The synthesis is analogous to (**5a**) with (**4c**) as the starting material. The product was obtained as a yellow solid. Yield = 67.7%. Mp: 71–74 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.72 (br d, 2H), 4.63 (d, J = 6.0 Hz, 2H), 6.70, 6.87 (AA′BB′, q, J = 8.7 Hz, 4H), 6.92 (dd, J = 5.6, 2.6 Hz, 1H), 7.26 (m, 1H), 7.33 (m, 4H), 7.70 (d, J = 2.6 Hz, 1H), 8.32 (d, J = 5.6 Hz, 1H), 8.38 (t, J = 6.2 Hz, 1H). ESI-MS m/z 320.1 [M+H]$^+$.

5.3.10. 4-(4-Aminophenoxy)-*N*-cyclopropylpicolinamide (5d)

The synthesis is analogous to (**5a**) with (**4d**) as the starting material. The product was obtained as a yellow solid. Yield = 72.5%. Mp: 92–95 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.83 (d, J = 7.2 Hz, 4H), 2.81 (m, 1H), 3.73 (br d, 2H), 6.67, 6.86 (AA′BB′, q, J = 8.7 Hz, 4H), 7.17 (dd, J = 5.6, 2.4 Hz, 1H), 7.40 (d, J = 2.4 Hz, 1H), 8.58 (d, J = 5.6 Hz, 1H), 8.68 (br d, 1H). ESI-MS m/z 270.1 [M+H]$^+$.

5.3.11. 4-(4-Aminophenoxy)-*N*-cyclohexylpicolinamide (5e)

The synthesis is analogous to (**5a**) with (**4e**) as the starting material. The product was obtained as a yellow solid. Yield = 64.9%. Mp: 135–138 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.25–1.48 (m, 6H), 1.78 (m, 4H), 3.72 (br d, 2H), 3.80 (m, 1H), 6.77 (q, J = 8.7 Hz, 4H), 7.24 (dd, J = 5.5, 2.4 Hz, 1H), 7.54 (d, J = 2.4 Hz, 1H), 8.54 (d, J = 5.5 Hz, 1H), 8.76 (br d, 1H). ESI-MS m/z 312.1 [M+H]$^+$.

5.3.12. 2,4-Dichloro-1-isothiocyanatobenzene (8a)

To a solution of 2,4-dichloroaniline (2.51 g, 15.5 mmol) and triethylenediamine (2.08 g, 18.6 mmol) in 40 mL toluene, CS_2 (3.5 g, 46.5 mmol) was added slowly. The resulting mixture was stirred at room temperature for 8 h. After filtration, the filter cake was washed with toluene and dried. Obtained **7a** was suspended in 40 mL DCM. Then, a 25 mL DCM solution of BTC (5.0 g, 17.0 mmol) was added slowly under −5–0 °C. The mixture was stirred for 2 h at room temperature, and then was refluxed for 1.5–2 h. After filtration, the filtrate was concentrated in vacuo. The crude product was purified by chromatography (ethyl acetate/petroleum ether = 1:1) on silica gel to give 0.19 g white solid (60%). Mp: 37–42 °C.

5.3.13. 1-Chloro-4-isothiocyanatobenzene (8b)

The synthesis is analogous to (**8a**) with 4-chloroaniline as the starting material. The product was obtained as a light brown solid. Yield = 84.7%; mp: 43–45 °C.

5.3.14. 1,2-Difluoro-4-isothiocyanatobenzene (8c)

The synthesis is analogous to (**8a**) with 3,4-difluoroaniline as the starting material. The product was obtained as a light yellow liquid. Yield = 74.5%; bp: 170 °C.

5.3.15. 1-Fluoro-4-isothiocyanatobenzene (8d)

The synthesis is analogous to (**8a**) with 4-fluoroaniline as the starting material. The product was obtained as a light yellow solid. Yield = 88.5%; mp: 25–28 °C.

5.3.16. 1-Isothiocyanato-3-(trifluoromethyl)benzene (8e)

The synthesis is analogous to (**8a**) with 3-(trifluoromethyl)aniline as the starting material. The product was obtained as a light yellow liquid. Yield = 74.3%; bp: 206–208 °C.

5.3.17. 1-Isothiocyanato-3,5-bis(trifluoromethyl)benzene (8f)

The synthesis is analogous to (**8a**) with 3,5-bis(trifluoromethyl)aniline as the starting material. The product was obtained as a colorless liquid. Yield = 68.0%; bp 63 °C (2.5 mmHg).

5.3.18. 1-Chloro-4-isothiocyanato-2-(trifluoromethyl)benzene (8g)

The synthesis is analogous to (**8a**) with 4-chloro-3-(trifluoromethyl)aniline as the starting material. The product was obtained as a light yellow liquid. Yield = 81.0%; bp 248–251 °C.

5.3.19. 1-Isothiocyanato-4-methoxybenzene (8h)

The synthesis is analogous to (**8a**) with 4-methoxyaniline as the starting material. The product was obtained as a colorless oily liquid. Yield = 78.6%; bp: 280 °C.

5.3.20. 4-(4-(3-(2,4-Dichlorophenyl)thioureido)phenoxy)-*N*-methylpicolinamide (9a)

To a 10 mL DCM solution of compound (**5a**) (1.5 g, 6.16 mmol), a DCM solution of (**8a**) (1.26 g, 6.16 mmol) was added slowly at 0–5 °C under nitrogen atmosphere. The mixture was stirred for 2 h in ice bath, then stirred for another 18 h at room temperature. The mixture was poured into petroleum ether (60 mL) and then filtered. The solids were washed with DCM (2 × 5 mL), and dried under vacuum for 6 h at 50 °C to afford light brown solid in the yield of 69.4%. Mp: 165–167 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 2.79 (d, J = 4.8 Hz, 3H), 7.19 (dd, J = 5.6, 2.5 Hz, 1H), 7.25 (d, J = 8.8 Hz ,2H), 7.43 (d, J = 2.5 Hz, 1H), 7.45 (dd, J = 8.6, 2.3 Hz ,1H), 7.59 (d, J = 8.4 Hz, 1H), 7.61 (d, J = 8.7 Hz, 2H), 7.71 (d, J = 2.3 Hz, 1H), 8.53 (d, J = 5.6 Hz, 1H), 8.81 (br d, J = 4.8 Hz, 1H), 9.59 (s, 1H), 10.13 (s, 1H); ^{13}C NMR (300 MHz, DMSO-d_6) δ 26.95, 109.91, 115.18, 121.97, 126.87, 128.34, 129.89, 131.95, 132.31, 136.64, 137.69, 150.95, 151.39, 153.49, 164.71, 166.56, 181.50; HRMS(AP-ESI) m/z: calcd for $C_{20}H_{17}Cl_2N_4O_2S$ $[M+H]^+$ 447.0444, found 447.04547. HPLC purity = 98.34%.

The other compounds of (**9**) series were synthesized following the general procedure as described above.

5.3.21. *N*-Butyl-4-(4-(3-(2,4-dichlorophenyl)thioureido) phenoxy)picolinamide (9b)

Light yellow solid (yield 67.2%), mp: = 161–164 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 0.88 (t, J = 7.3 Hz, 3H), 1.28 (m, J = 7.4 Hz, 2H), 1.49 (m, J = 7.3 Hz, 2H), 3.26 (q, J = 6.7 Hz, 2H), 7.19 (dd, J = 5.6, 2.5 Hz, 1H), 7.23 (d, J = 8.8 Hz ,2H), 7.42 (d, J = 2.5 Hz, 1H),7.45 (dd, J = 8.6, 2.3 Hz ,1H), 7.59 (d, J = 8.4 Hz, 1H), 7.62 (d, J = 8.6 Hz, 2H), 7.71 (d, J = 2.3 Hz, 1H), 8.53 (d, J = 5.6 Hz, 1H), 8.80 (t, J = 6.0 Hz, 1H), 9.60 (s, 1H), 10.14 (s, 1H); ^{13}C NMR (300 MHz, DMSO-d_6) δ 14.62, 20.47, 32.11, 39.40, 109.98, 115.22, 122.01, 126.88, 128.34, 129.90, 131.96, 132.34, 136.63, 137.68, 150.91, 151.42, 153.41, 164.64, 166.60, 181.51; HRMS(AP-ESI) m/z: calcd for $C_{23}H_{23}Cl_2N_4O_2S$ $[M+H]^+$ 489.0914, found 489.0916. HPLC purity = 99.04%.

5.3.22. *N*-Benzyl-4-(4-(3-(2,4-dichlorophenyl)thioureido) phenoxy)picolinamide (9c)

Light yellow solid (yield 75.0%), mp: = 116–118 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 4.46 (d, J = 6.2 Hz, 2H), 7.20 (dd, J = 5.6, 2.5 Hz, 1H), 7.21 (m, 1H), 7.23 (d, J = 8.8 Hz, 2H), 7.29 (m, 4H), 7.43 (d, J = 2.5 Hz, 1H), 7.45 (dd, J = 7.2, 2.3 Hz ,1H), 7.59 (d, J = 7.2 Hz, 1H), 7.62 (d, J = 8.6 Hz, 2H), 7.71 (d, J = 2.3 Hz, 1H), 8.55 (d, J = 5.6 Hz, 1H), 9.38 (t, J = 6.4 Hz, 1H), 9.59 (s, 1H), 10.13 (s, 1H); ^{13}C NMR (300 MHz, DMSO-d_6) δ 43.39, 110.08, 115.42, 122.04, 126.88, 127.72, 128.30, 128.36, 129.19, 129.90, 131.97, 132.36, 136.64, 137.70, 140.35, 150.88, 151.47, 153.32, 164.30, 166.64, 181.46; HRMS(AP-ESI) m/z: calcd for $C_{26}H_{21}Cl_2N_4O_2S$ $[M+H]^+$ 523.0757, found 523.0758. HPLC purity = 98.7%.

5.3.23. 4-(4-(3-(4-Chlorophenyl)thioureido)phenoxy) -*N*-methylpicolinamide (9d)

Light yellow solid (yield 69.8%), mp: = 146–148 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 2.79 (d, J = 4.8 Hz, 3H), 7.19 (dd, J = 5.6, 2.5 Hz, 1H), 7.24 (d, J = 8.8 Hz ,2H), 7.39 (d, J = 8.6 Hz ,2H), 7.43 (d, J = 2.5 Hz, 1H), 7.52 (d, J = 8.6 Hz, 2H), 7.60 (d, J = 8.8 Hz, 2H), 8.53 (d, J = 5.6 Hz, 1H), 8.81 (br d, J = 4.8 Hz, 1H), 9.98 (s, 2H); ^{13}C NMR (300 MHz, DMSO-d_6) δ 26.96, 109.90, 115.28, 121.96, 126.28, 126.66, 129.31, 137.96, 139.37, 150.77, 151.29, 153.54, 164.11, 166.64, 180.79; HRMS(AP-ESI) m/z: calcd for $C_{20}H_{18}ClN_4O_2S$ $[M+H]^+$ 413.0834, found 413.0840. HPLC purity = 97.8%.

5.3.24. *N*-Butyl-4-(4-(3-(4-chlorophenyl)thioureido)phenoxy)picolinamide (9e)

Light yellow solid (yield 67.6%), mp: = 110–112 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 0.88 (t, J = 7.2 Hz, 3H), 1.28 (m, 2H), 1.49 (m, 2H), 3.26 (q, J = 6.6 Hz, 2H), 7.18–7.22 (m, 3H), 7.38–7.42 (m, 3H), 7.52 (d, J = 8.4 Hz, 2H), 7.59 (d, J = 8.4 Hz, 2H), 8.53 (d, J = 5.4 Hz, 1H), 8.79 (t, J = 6.6 Hz, 1H), 9.97 (s, 2H); ^{13}C NMR (300 MHz, DMSO-d_6) δ 14.60, 20.50, 32.16, 39.44, 109.87, 115.25, 121.93, 126.27, 126.68, 129.28, 137.95, 139.38, 150.72, 151.33, 153.51, 164.06, 166.63, 180.78; HRMS(AP-ESI) m/z: calcd for $C_{23}H_{24}ClN_4O_2S$ $[M+H]^+$ 455.1303, found 455.1298; HPLC purity = 98.3%.

5.3.25. *N*-Benzyl-4-(4-(3-(4-chlorophenyl)thioureido) phenoxy)picolinamide (9f)

Light yellow solid (yield 66.3%), mp: = 164–166 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 4.46 (d, J = 6.2 Hz, 2H), 7.20 (dd, J = 5.6, 2.3 Hz, 1H), 7.21 (m, 1H), 7.23 (d, J = 8.6 Hz, 2H), 7.29 (m, 4H), 7.39 (d, J = 8.6 Hz ,2H), 7.43 (d, J = 2.3 Hz, 1H), 7.52 (d, J = 8.6 Hz, 2H), 7.58 (d, J = 8.6 Hz, 2H), 8.55 (d, J = 5.6 Hz, 1H), 9.39 (t, J = 6.3 Hz, 1H), 9.98 (s, 2H); ^{13}C NMR (300 MHz, DMSO-d_6) δ 43.40, 110.02, 115.43, 121.96, 126.28, 126.69, 127.72, 128.30, 129.19, 129.29, 137.97, 139.37, 140.35, 150.66, 151.46, 153.31, 164.31, 166.67, 180.76; HRMS(AP-ESI) m/z: calcd for $C_{26}H_{22}ClN_4O_2S$ $[M+H]^+$ 489.1147, found 489.1142. HPLC purity = 99.21%.

5.3.26. 4-(4-(3-(3,4-Difluorophenyl)thioureido)phenoxy)-*N*-methylpicolinamide (9g)

Light yellow solid (yield 83.8%), mp: = 144–146 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 2.79 (d, J = 4.8 Hz, 3H), 7.19 (dd, J = 5.6, 2.6 Hz, 1H), 7.20 (d, J = 2.1 Hz, 1H), 7.24 (d, J = 8.8 Hz, 2H), 7.38(d,

J = 8.5 Hz 1H), 7.43 (d, *J* = 2.5 Hz, 1H), 7.58 (d, *J* = 8.8 Hz, 2H), 7.70 (m, 1H), 8.53 (d, *J* = 5.6 Hz, 1H), 8.79 (*J* = 4.8 Hz 1H), 9.99 (s, 1H), 10.01 (s, 1H); ^{13}C NMR (300 MHz, DMSO-d_6) δ 26.96, 109.95, 114.07, 114.30, 115.35, 117.81, 118.04, 121.48 (q, *J* = 12), 121.97, 126.87, 137.38 (dd, *J* = 33, 12), 137.76, 150.85, 151.11, 153.47, 163.06, 165.99, 180.97; HRMS(AP-ESI) *m/z*: calcd for $C_{20}H_{17}F_2N_4O_2S$ $[M+H]^+$ 415.1035, found 415.1032. HPLC purity = 96.2%.

5.3.27. *N*-Butyl-4-(4-(3-(3,4-difluorophenyl)thioureido) phenoxy)picolinamide (9h)

Light yellow solid (yield 68.3%), mp = 161–163 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 0.88 (t, *J* = 7.4 Hz, 3H), 1.28 (m, *J* = 7.5 Hz, 2H), 1.48 (m, *J* = 7.3 Hz, 2H), 3.26 (q, *J* = 6.6 Hz, 2H), 7.19 (dd, *J* = 5.6, 2.5 Hz, 1H), 7.20 (d, *J* = 2.2 Hz, 1H), 7.23 (d, *J* = 8.7 Hz ,2H), 7.39 (d, *J* = 8.5 Hz, 1H), 7.42 (d, *J* = 2.5 Hz, 1H), 7.58 (d, *J* = 8.7 Hz, 2H), 7.70 (m, 1H), 8.53 (d, *J* = 5.6 Hz, 1H), 8.81 (t, *J* = 5.9 Hz, 1H), 9.99 (s, 1H), 10.01 (s, 1H); ^{13}C NMR (300 MHz, DMSO-d_6) δ 14.61, 20.50, 32.17, 39.44, 109.94, 114.06, 114.31, 115.40, 117.80, 118.04, 121.47 (q, *J* = 12), 121.97, 126.88, 137.38 (dd, *J* = 33, 12), 137.73, 150.86, 151.11, 153.47, 163.07, 165.98, 180.98; HRMS(AP-ESI) *m/z*: calcd for $C_{23}H_{23}F_2N_4O_2S$ $[M+H]^+$ 457.1505, found 457.1501. HPLC purity = 99.2%.

5.3.28. *N*-Cyclopropyl-4-(4-(3-(3,4-difluorophenyl)thioureido) phenoxy)picolinamide (9i)

Light yellow solid (yield 77.9%), mp: = 162–163 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 0.67 (d, *J* = 7.1 Hz, 4H), 2.86 (m, 1H), 7.19 (dd, *J* = 5.6, 2.6 Hz, 1H), 7.20 (d, *J* = 2.4 Hz ,1H), 7.23 (d, *J* = 8.8 Hz, 2H), 7.39 (d, *J* = 8.5 Hz 1H), 7.43 (d, *J* = 2.5 Hz, 1H), 7.58 (d, *J* = 8.8 Hz, 2H), 7.70 (m, 1H), 8.51 (d, *J* = 5.6 Hz, 1H), 8.76 (d, *J* = 5.0 Hz, 1H), 9.98 (s, 1H), 10.00 (s, 1H); ^{13}C NMR (300 MHz, DMSO-d_6) δ 6.65, 23.83, 109.96, 114.11, 114.32, 115.41, 117.80, 118.03, 121.48 (q, *J* = 12), 121.98, 126.89, 137.38 (dd, *J* = 33, 12), 137.77, 150.87, 151.08, 153.48, 163.09, 166.01, 180.98; HRMS(AP-ESI) *m/z*: calcd for $C_{22}H_{19}F_2N_4O_2S$ $[M+H]^+$ 441.1192, found 441.1196. HPLC purity = 98.3%.

5.3.29. *N*-Cyclohexyl-4-(4-(3-(3,4-difluorophenyl)thioureido) phenoxy)picolinamide (9j)

Light yellow solid (yield 67.0%), mp: = 162–164 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 1.10–1.16 (m, 1H), 1.27–1.33 (m, 2H), 1.36–1.42 (m, 2H), 1.59 (d, *J* = 12 Hz, 1H), 1.67–1.71 (m, 2H), 1.75–1.77 (m, 2H), 3.70–3.76 (m, 1H), 7.19–7.26 (m, 4H), 7.41 (m, 2H), 7.58 (d, *J* = 9.0 Hz, 2H), 7.68–7.62 (m, 1H), 8.47 (d, *J* = 9.0 Hz, 1H), 8.53 (d, *J* = 5.4 Hz, 1H), 9.98 (s, 1H), 10.00 (s, 1H); ^{13}C NMR (300 MHz, DMSO-d_6) δ 25.67, 26.02, 33.05, 48.92, 109.95, 114.04, 114.31, 115.37, 117.81, 118.04, 121.46 (q, *J* = 12), 121.91, 126.87, 137.37 (dd, *J* = 36, 12), 137.79, 150.89, 151.32, 153.47, 163.07, 166.63, 180.98. HRMS (AP-ESI) *m/z*: calcd for $C_{25}H_{25}F_2N_4O_2S$ $[M+H]^+$ 483.1661, found 483.1655. HPLC purity = 98.9%.

5.3.30. 4-(4-(3-(4-Fluorophenyl)thioureido)phenoxy) -*N*-methylpicolinamide (9k)

Light yellow solid (yield 70.3%), mp: = 172–173 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 2.79 (d, *J* = 4.8 Hz, 3H), 7.19 (dd, *J* = 5.6, 2.5 Hz, 1H), 7.21 (d, *J* = 8.8 Hz, 2H), 7.23 (d, *J* = 8.8 Hz ,2H), 7.43 (d, *J* = 2.5 Hz, 1H), 7.48 (d, *J* = 8.8 Hz, 2H), 7.59 (d, *J* = 8.8 Hz, 2H), 8.53 (d, *J* = 5.6 Hz, 1H), 8.81 (br d, *J* = 4.8 Hz, 1H), 9.86 (s, 1H), 9.89 (s, 1H); ^{13}C NMR (300 MHz, DMSO-d_6) δ 26.96, 109.80, 115.18, 115.89, 116.19, 121.92, 126.71, 127.22 (d, *J* = 33), 136.63 (d, *J* = 12), 138.02, 150.64, 151.38, 153.45, 164.72, 166.61, 181.07; HRMS(AP-ESI) *m/z*: calcd for $C_{20}H_{18}FN_4O_2S$ $[M+H]^+$ 397.1129, found 397.1132. HPLC purity = 97.84%.

5.3.31. *N*-Benzyl-4-(4-(3-(4-fluorophenyl)thioureido) phenoxy)picolinamide (9l)

Light-brown solid (yield 87.4%), mp: = 104–107 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 4.46 (d, *J* = 6.3 Hz, 2H), 7.18 (dd, *J* = 5.6, 2.5 Hz, 1H), 7.19 (m, 1H), 7.21 (d, *J* = 8.8 Hz, 2H), 7.23 (d, *J* = 8.8 Hz ,2H), 7.30 (m, 4H), 7.43 (d, *J* = 2.5 Hz, 1H), 7.48 (d, *J* = 8.8 Hz, 2H), 7.58 (d, *J* = 8.8 Hz, 2H), 8.55 (d, *J* = 5.6 Hz, 1H), 9.38 (t, *J* = 6.4 Hz, 1H), 9.86 (s, 1H), 9.89 (s, 1H); ^{13}C NMR (300 MHz, DMSO-d_6) δ 43.39, 110.10, 115.42, 115.89, 116.19, 121.95, 126.71, 127.18, 127.22 (d, *J* = 33), 128.30, 129.19, 136.63 (d, *J* = 9), 138.05, 140.35, 150.60, 151.45, 153.30, 164.31, 166.69, 181.06; HRMS (AP-ESI) *m/z*: calcd for $C_{26}H_{22}FN_4O_2S$ $[M+H]^+$ 473.1442, found 473.1440. HPLC purity = 98.5%.

5.3.32. *N*-Methyl-4-(4-(3-(3-(trifluoromethyl)phenyl) thioureido)phenoxy)picolinamide (9m)

White solid (yield 69.3%), mp: = 108–110 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 2.79 (d, *J* = 4.8 Hz, 3H), 7.19 (dd, *J* = 5.6, 2.6 Hz, 1H), 7.23 (d, *J* = 8.8 Hz, 2H), 7.43 (d, *J* = 2.5 Hz, 1H), 7.48 (d, *J* = 7.9 Hz, 1H), 7.55 (d, *J* = 7.6 Hz, 1H), 7.58 (d, *J* = 8.8 Hz, 2H), 7.77 (m, 1H), 7.97 (s, 1H), 8.53 (d, *J* = 5.6 Hz, 1H), 8.81 (br d, *J* = 4.8 Hz 1H), 10.12 (br d, 1H), 10.13 (br d, 1H); ^{13}C NMR (300 MHz, DMSO-d_6) δ 26.96, 109.07, 115.42, 120.82, (d, *J* = 15), 121.62 (d, *J* = 15), 122.02, 126.81, 128.33, 129.74, 130.16, 130.48, 137.68, 141.35, 150.86, 151.36, 153.42, 163.02, 166.60, 180.89; HRMS (AP-ESI) *m/z*: calcd for $C_{21}H_{18}F_3N_4O_2S$ $[M+H]^+$ 447.1098, found 447.1103. HPLC purity = 98.04%.

5.3.33. *N*-Cyclohexyl-4-(4-(3-(3-(trifluoromethyl)phenyl) thioureido)phenoxy)picolinamide (9n)

Light yellow solid (yield 68.9%), mp: = 151–154 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 1.11–1.16 (m, 1H) , 1.27–1.33 (m, 2H), 1.36–1.42 (m, 2H), 1.58 (d, *J* = 12 Hz, 1H), 1.67–1.71 (m, 2H), 1.75–1.77 (m, 2H), 3.70–3.75 (m, 1H), 7.23 (dd, *J* = 5.6, 2.5 Hz, 1H), 7.40 (d, *J* = 8.8 Hz, 2H), 7.48 (d, *J* = 7.9 Hz, 1H), 7.54 (d, *J* = 2.5 Hz, 1H), 7.55 (d, *J* = 7.6 Hz, 1H), 7.68 (d, *J* = 8.7 Hz, 2H), 7.77 (m, 1H), 7.97 (s, 1H), 8.54 (d, *J* = 5.6 Hz, 1H), 8.75 (br d, 1H),, 10.12 (br d, 1H), 10.13 (br d, 1H); ^{13}C NMR (300 MHz, DMSO-d_6) δ 25.69, 26.01, 33.05, 48.92, 109.03, 115.39, 120.82, (d, *J* = 15), 121.62 (d, *J* = 15), 122.02, 126.80, 128.34, 129.74, 130.15, 130.48, 137.70, 141.37, 150.89, 151.33, 153.46, 163.06, 166.61, 180.89; HRMS (AP-ESI) *m/z*: calcd for $C_{26}H_{26}F_3N_4O_2S$ $[M+H]^+$ 515.1724, found 515.1728. HPLC purity = 98.9%.

5.3.34. 4-(4-(3-(3,5-Bis(trifluoromethyl)phenyl) thioureido)phenoxy)-*N*-methylpicolinamide (9o)

White solid (yield 76.9%), mp: = 170–172 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 2.79 (d, *J* = 4.8 Hz, 3H), 7.19 (dd, *J* = 5.6, 2.6 Hz, 1H), 7.25 (d, *J* = 8.8 Hz, 2H), 7.43 (d, *J* = 2.5 Hz, 1H), 7.58 (d, *J* = 8.8 Hz, 2H), 7.83 (s, 1H), 8.27 (s, 2H), 8.53 (d, *J* = 5.6 Hz, 1H), 8.81 (br d, *J* = 4.8 Hz 1H), 10.34 (br d, 1H), 10.38 (br d, 1H); ^{13}C NMR (300 MHz, DMSO-d_6) δ 26.95, 109.87, 115.31, 122,19, 124.59, 127.10, 130.76, 131.20, 137.26, 142.78, 151.27, 151.42, 153.48, 164.71, 166.47, 180.96; HRMS (AP-ESI) *m/z*: calcd for $C_{22}H_{17}F_6N_4O_2S$ $[M+H]^+$ 515.0971, found 515.0970. HPLC purity = 99.0%.

5.3.35. 4-(4-(3-(4-Chloro-3-(trifluoromethyl)phenyl) thioureido)phenoxy)-*N*-methylpicolinamide (9p)

Light brown solid (yield 82.3%), mp: = 152–154 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 2.79 (d, *J* = 4.8 Hz, 3H), 7.19 (dd, *J* = 5.6, 2.6 Hz, 1H),7.24 (d, *J* = 8.8 Hz, 2H) 7.43 (d, *J* = 2.5 Hz, 1H), 7.58 (d, *J* = 8.8 Hz, 2H), 7.69 (d, *J* = 8.7 Hz, 1H), 7.82 (dd, *J* = 8.7, 2.1 Hz, 1H), 8.09 (d, *J* = 2.2 Hz, 1H), 8.53 (d, *J* = 5.6 Hz, 1H), 8.79 (q, *J* = 4.8 Hz 1H), 10.16 (br d, 1H), 10.18 (br d, 1H); ^{13}C NMR (300 MHz, DMSO-d_6) δ 26.95, 109.68, 115.43, 122..07, 123.40 (m),

125.49, 126.19, 126.96, 127.36, 129.57, 132.52, 137.55, 140.12, 151.04, 151.31, 153.33, 165.45, 166.49, 180.82; HRMS (AP-ESI) *m/z*: calcd for $C_{21}H_{17}ClF_3N_4O_2S$ $[M+H]^+$ 481.0708, found 481.0714. HPLC purity = 98.6%.

5.3.36. 4-(4-(3-(4-Chloro-3-(trifluoromethyl)phenyl) thioureido)phenoxy)-*N*-cyclopropylpicolinamide (9q)

Off-white solid (yield 77.6%), mp: = 149–152 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 0.64–0.69 (m, 4H), 2.84–2.88 (m, 1H), 7.19 (dd, J = 5.8, 3.0 Hz, 1H), 7.24 (m, 2H), 7.41 (d, J = 3.0, 1H), 7.58 (m, 2H), 7.69 (d, J = 8.4 Hz, 1H), 7.82 (dd, J = 8.4, 2.4 Hz, 1H), 8.09 (d, J = 2.4, 1H), 8.51 (d, J = 5.6 Hz, 1H), 8.76 (d, J = 5.4 Hz, 1H), 10.17 (s, 1H), 10.20 (s, 1H); ^{13}C NMR (300 MHz, DMSO-d_6) δ 6.66, 23.83, 109.75, 115.40, 122.10, 123.40 (m), 125.47, 126.12, 126.91, 127.37, 129.51, 132.51, 137.55, 140.13, 151.00, 151.33,153.32, 165.47, 166.52, 180.84; HRMS (AP-ESI) *m/z*: calcd for $C_{23}H_{19}ClF_3N_4O_2S$ $[M+H]^+$ 507.0864, found 507.0861. HPLC purity = 98.5%.

5.3.37. 4-(4-(3-(4-Chloro-3-(trifluoromethyl)phenyl) thioureido)phenoxy)-*N*-cyclohexylpicolinamide (9r)

White solid (yield 63.8%), mp: = 178–180 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 1.10–1.16 (m, 1H), 1.27–1.33 (m, 2H), 1.36–1.42 (m, 2H), 1.59 (d, J = 12 Hz, 1H), 1.67–1.71 (m, 2H), 1.75–1.77 (m, 2H), 3.70–3.76 (m, 1H), 7.24 (dd, J = 5.6, 2.5 Hz, 1H), 7.40 (d, J = 8.8 Hz, 2H), 7.54 (d, J = 2.5 Hz, 1H), 7.68 (d, J = 8.7 Hz, 2H), 7.70 (d, J = 8.7 Hz, 1H), 7.82 (dd, J = 8.7, 2.1 Hz, 1H), 8.09 (d, J = 2.2 Hz, 1H), 8.54 (d, J = 5.6 Hz, 1H), 8.75 (br d, 1H), 10.17(s, 1H), 10.19(s, 1H); ^{13}C NMR (300 MHz, DMSO-d_6) δ 25.68, 26.02, 33.05, 48.92, 109.76, 115.44, 122.08, 123.40 (m), 125.47, 126.12, 126.93, 127.38, 129.52, 132.54, 137.55, 140.11, 151.00, 151.30,153.34, 165.42, 166.54, 180.87; HRMS(AP-ESI) *m/z*: calcd for $C_{26}H_{25}ClF_3N_4O_2S$ $[M+H]^+$ 549.1334, found 549.1331. HPLC purity = 97.9%.

5.3.38. 4-(4-(3-(4-Methoxyphenyl)thioureido)phenoxy)-*N*-methylpicolinamide (9s)

White solid (yield 69%), mp: = 174–176 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 2.79 (d, J = 4.7 Hz, 3H), 3.75 (s, 3H), 6.92 (d, J = 8.8 Hz, 2H), 7.17 (dd, J = 5.6, 2.5 Hz, 1H), 7.20 (d, J = 8.7 Hz, 2H) 7.33 (d, J = 8.8 Hz, 2H), 7.41 (d, J = 2.4 Hz, 1H), 7.58 (d, J = 8.7 Hz, 2H), 8.52 (d, J = 5.6 Hz, 1H), 8.80 (br d, J = 4.8 Hz ,1H), 9.72 (br d, 2H); ^{13}C NMR (300 MHz, DMSO-d_6) δ 26.96, 56.19, 109.81, 114.98, 115.14, 121.82, 126.64, 127.00, 133.02, 138.24, 150.47, 151.36, 153.43, 157.58, 164.71, 166.66, 180.94; HRMS(AP-ESI) *m/z*: calcd for $C_{21}H_{21}N_4O_3S$ $[M+H]^+$ 409.1329, found 409.1333. HPLC purity = 98.63%.

5.3.39. *N*-Benzyl-4-(4-(3-(4-methoxyphenyl)thioureido) zphenoxy)picolinamide (9t)

White solid (yield 69.4%), mp: = 148–152 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 3.75 (s, 3H), 4.46 (d, J = 6.6 Hz, 2H), 6.91–6.93 (m, 2H), 7.18–7.24 (m, 4H), 7.29–7.36 (m, 6H), 7.43 (d, J = 2.4 Hz, 1H), 7.57–7.59 (m, 2H), 8.54 (d, J = 5.4 Hz, 1H), 9.36 (t, J = 6.6 Hz, 1H), 9.71 (s, 2H); ^{13}C NMR (300 MHz, DMSO-d_6) δ 43.40, 56.19, 110.03, 114.68, 115.38, 121.84, 126.65, 127.01, 127.71, 128.30, 129.19, 133.02, 138.26, 140.35, 150.45, 151.44, 153.30, 157.58, 164.32, 166.72, 180.95; HRMS(AP-ESI) *m/z*: calcd for $C_{27}H_{25}N_4O_3S$ $[M+H]^+$ 485.1642, found 485.1643. HPLC purity = 97.34%.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2012.03.018. These data include MOL files and InChiKeys of the most important compounds described in this article.

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