Synthesis of 5-acetyl-2-arylamino-4-methylthiazole thiosemicarbazones under microwave irradiation and their *in vitro* anticancer activity

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A series of 21 new tri- and tetra-cyclic thiosemicarbazone derivatives were prepared *via* the condensation of morpholine, piperazine or *N*-(4-methoxyphenyl)piperazine with seven methyl hydrazine-carbodithioate derivatives of 5-acetyl-2-arylamino-4-methylthiazoles under microwave irradiation. All compounds were tested for their cytotoxic activity *in vitro* against human gastric, lung and breast cancer cell lines. The results showed that some of the compounds displayed moderate anticancer activity. The most potent compound, a morpholino-substituted analogue, exhibited significant activity against human breast cancer cells.

Keywords: thiazole, thiosemicarbazone, synthesis, anticancer activity, microwave

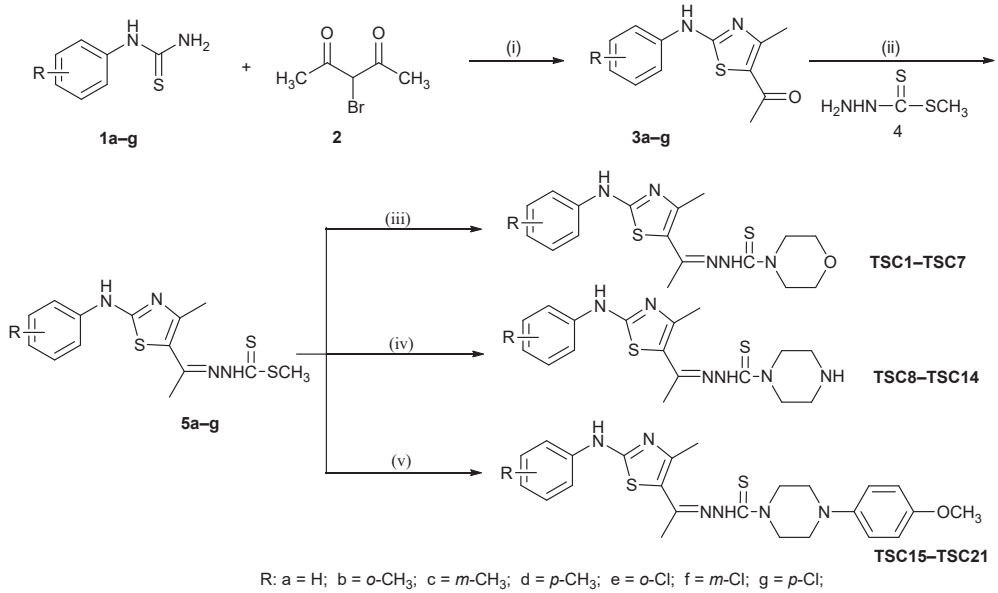
The thiosemicarbazone (TSC) is considered as one of the most important scaffolds and is embedded in many biologically active compounds.[1] In 1956, Brockman *et al*. first showed that 2-formylpyridine TSC possesses antileukemic activity in mice.[2] Since then, various aliphatic, aromatic and heteroaromatic carbaldehyde TSCs were synthesised and many of them exhibited a broad range of biological activities such as antimalarial,[3,4] antiviral,[5] antibacterial,[6] and antitumour activities.[7–11] To date, the most significant progress in the treatment of cancer by this class of compounds has been made with 3-aminopyridine-2-carboxaldehyde TSC (3-AP or triapine) which is currently in phase II clinical trials.[12] TSCs were considered to suppress tumour growth by virtue of their ability to chelate iron (Fe). They are capable of removing Fe from biological systems and inhibit the activity of Fe-requiring proteins, including ribonucleotide reductase (RRa), a key enzyme involved in DNA synthesis and repair.[13] Therefore, there is increasing interest in the structural modification of TSC derivatives with the aim of improving the pharmaceutical profile of existing candidates or of discovering novel derivatives.

Heterocycles are the most common structural units present in marketed drugs and in target molecules in the drug discovery process.[14] Heterocyclic compounds with a variety of shapes and electronic and physicochemical properties provide fertile ground for optimisation of drug candidates. Thiazole TSCs are known to possess a wide range of pharmacological activities including antibacterial, antifungal,[15,16] and anti-inflammatory[17] properties. They are also anti-hypertension,[18] anti-convulsion,[19] anti-Parkinson's disease,[20] anti-Alzheimer's disease,[21] anti-HIV[22] and anticancer[23–27] agents. The work in this paper is an extension of our ongoing efforts towards the synthesis and biological evaluation of new aminothiazole derivatives,[28,29] and is concerned with the synthesis of novel thiosemicarbazone derivatives of 5-acetyl-2-arylamino-4-methylthiazoles and determination of their antiproliferative activity *in vitro* against three human cancer cell lines.

Results and discussion

Synthesis

We designed a series of novel 5-acetyl-2-arylamino-4-methylthiazole thiosemicarbazone derivatives **TSC1–21** and prepared them by the series of reactions shown in Scheme 1. The first step, the preparation of aminothiazoles **3a–g** was adapted from the Hantzsch method described previously by us,[30] and



Reagents and conditions: (i) NBS/C_6H_6,Benzoyl peroxide, reflux, K_2CO_3; (ii) *i*-PrOH, HCl, reflux; (iii) morpholine, *i*-PrOH, MW, 100 °C, 45 mir (iv) piperazine, i-PrOH, MW, 100 °C, 45 min; (v) *N*4-(4-methoxyphenyl)piperazine, *i*-PrOH, MW, 100 °C, 60 min;

Scheme 1

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involved reaction of appropriate thioureas **1a–g** and 3-bromo-acetylacetone **2**. Despite the success of this preparation, the method suffered from an intricate and tedious work-up. Therefore, it was necessary to develop a more convenient preparation. We found that we could prepare 3-bromo-acetylacetone *in situ* by treatment of acetylacetone **2** with *N*-bromosuccinimide and benzoylperoxide and, by this means, we were able in a one-pot reaction to convert a series of *N*-arylthioureas **1a–g** into a series of arylaminothiazoles **3a–g** in good yield. Compared with the previous one, our method is much simpler with more general applicability. Reaction of arylaminothiazoles **3a–g** with methyl hydrazinecarbodithioate **4** (prepared by a literature method[8]) in the presence of concentrated hydrochloric acid gave the thiosemicarbazone derivatives **5a–g**. The catalytic amount of hydrochloric acid that was used is believed to readily drive the reaction to completion thus improving the yield of the reaction. The preparation of thiosemicarbazone derivatives **TSC1–21** was carried out by condensations between compounds **5a–g** and morpholine, piperazine or N^4-substituted piperazine, the latter prepared by the literature method.[31] We first carried out the reaction of compound **5a** with morpholine by conventional heating and found that the reaction was completed in 24 h. When the reaction was repeated under microwave (MW) conditions, the reaction time was reduced to 45 min. We thus adopted the latter method to prepare **TSC1–21**.

The synthesised compounds (**TSC1–21**) were characterised by their ^{1}H NMR, MS, and IR spectra and by elemental analysis or HRMS. The IR spectra of all compounds showed absorption peaks at 1023–1138 cm^{-1} which are attributed to the C=S group, while the bands at 1552–1600 cm^{-1} are due to the C=N group. In addition, the ^{1}H NMR spectra of the compounds showed signals within the range of δ 7.87–10.52 for the –NHCS– group.

Anticancer activity

A preliminary evaluation of compounds **TSC1–21** was performed by MTT assays[32] *in vitro* against three human cancer cell lines including human gastric cancer cell SGC-7901, human lung cancer cell H446 and human breast cancer cell MCF-7. Cisplatin was employed as a positive control in the assay. The data shown in Table 1 are IC_{50} values against tested tumour cells.

As shown in Table 1, most of the tested compounds showed moderate antiproliferative activity against some or all of SGC-7901, H446 and MCF-7. Compound **TSC6** exhibited the highest anticancer activity against MCF-7 cells with an IC_{50} value of 5.49 μM, comparable with that of cisplatin (6.53 μM). Comparing the results of compounds **TSC1–7** bearing the same morpholine ring but different substituents R, it can be seen that the three compounds **TSC5–7** with a chloro group showed better antitumour activity than the three compounds **TSC2–4** with a methyl group against SGC-7901, H446 and MCF-7. Compound **TSC6** also has the best antitumour activity against H446 with an IC_{50} value of 10.33 μM. The results implied that when the R group is an electron-withdrawing group such as a 3-Cl moiety and there is a morpholine ring, this feature enhances the inhibitory activity.

The compounds which contain a piperazine ring **TSC8–14** were only active towards SGC-7901 cells. The activities of compounds **TSC15–21** which have a 4-methoxyphenyl group in the N^4 position in the piperazine ring showed higher activities against all cell lines than compounds unsubstituted at this position. It is clear that a N^4-(4-methoxyphenyl) substituent on the piperazine enhances anticancer activity.

In conclusion, we have designed and synthesised a series of novel compounds **TSC1–TSC21** and evaluated their activities against three human cancer cell lines *in vitro* and some of them showed high or moderate cytotoxic activities. Among them, the most potent compound **TSC6** exhibited significant activity against breast cancer cell line MCF-7. The preliminary structure-activity relationships showed that an electron-withdrawing group R on the benzene ring increased the cytotoxic inhibitory effect and introduction of a 4-methoxyphenyl group into the N^4 position in piperazine ring also enhanced inhibitory activity.

Table 1 Antiproliferative activity of compounds **TSC1–21** (Scheme 1) against human gastric (SGC-7901), lung (H446) and breast cancer (MCF-7) cell lines

Compounds	Substituent R	Cell lines (IC_{50}, μM)[a,b] SGC-7901	H446	MCF-7
TSC1	H	17.54	54.00	13.94
TSC2	2-CH_3	45.91	48.37	20.52
TSC3	3-CH_3	64.06	> 100	57.07
TSC4	4-CH_3	33.29	40.32	53.97
TSC5	2-Cl	25.09	21.13	17.74
TSC6	3-Cl	16.55	10.33	5.49
TSC7	4-Cl	26.47	34.05	11.11
TSC8	H	> 100	> 100	> 100
TSC9	2-CH_3	18.25	> 100	> 100
TSC10	3-CH_3	33.04	> 100	> 100
TSC11	4-CH_3	71.99	> 100	> 100
TSC12	2-Cl	> 100	> 100	> 100
TSC13	3-Cl	31.03	> 100	> 100
TSC14	4-Cl	> 100	> 100	> 100
TSC15	H	65.95	> 100	38.28
TSC16	2-CH_3	18.54	56.13	15.04
TSC17	3-CH_3	30.21	71.19	39.51
TSC18	4-CH_3	21.35	81.79	49.61
TSC19	2-Cl	16.50	> 100	34.65
TSC20	3-Cl	38.49	83.95	45.43
TSC21	4-Cl	11.58	35.03	23.29
Cisplatin	–	1.74	2.23	6.53

[a]IC_{50}, compound concentration required to inhibit tumour cell proliferation by 50%.
[b]Values are the mean of three experiments.

Experimental

Reagents and solvents were purchased from the SinoPharm Chemical Reagent Co., Ltd and were used without further purification. Melting points were taken on a X-4 apparatus and were uncorrected (Shanghai Optical instrument, China). IR spectra (KBr pellets) were obtained on a Bruker TENSOR27 spectrometer. ^{1}H NMR spectra were recorded on a Bruker AVANCE spectrometer operating at 400 MHz in $CDCl_3$ using TMS as the internal standard. Chemical shifts (δ) are given in ppm and coupling constants (*J*) in Hz. High resolution mass spectra (HRMS) were obtained on an Agilent 6210 TOF LC/MS instrument. MS spectra were obtained on an Agilent 1260 Ion Trap LC/MS 500 analysis system. Elemental analyses were performed on a Flash EA1112 Elemental analyser (CE, Thermo Finnigan). Progress of reaction was monitored by thin layer chromatography (TLC). The microwave-assisted reactions were performed in an Initiator EXPmicrowave system (Biotage, Inc.) at the specified temperatures using the standard mode of operation.

Anticancer assays

The anticancer activities of compounds **TSC1–21** were evaluated against human cell lines by the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) method.[32] MTT solution (10.0 μL/well) in RPMI-1640 (Roswell Park Memorial Institute, Sigma, St. Louis, MO, USA) was added after cells were treated with the test compound for 72 h and cells were incubated for a further 3 h at 37 °C. The purple formazan crystals were dissolved in DMSO

150 μL. After 5 min, the plates were read on an automated microplate spectrophotometer (Bio-Tek Instruments, Winooski, VT, USA) at λ = 570 nm. Assays were performed in triplicate in three independent experiments. The concentration required for 50% inhibition of cell viability (IC_{50}) was calculated using the software, Dose-Effect Analysis with Microcomputers. The tumour cell line panel consisted of human gastric (SGC-7901), lung (H446) and breast cancer (MCF-7) cell lines. In all of these experiments, three replicate wells were used to determine each point.

Synthesis

Compounds **1a–g** was prepared according to the reported method.[33, 34]

Aminothiazoles **3a–g***; general procedure*

A mixture of *N*-arylthiourea **1a**–**g** (0.01 mol), acetylacetone **2** (1 g, 0.01 mol), *N*-bromosuccinimide (1.79 g, 0.01 mol) and benzoyl peroxide (0.12 g) in anhydrous benzene (40 mL) was heated to reflux for 6 h (completion indicated by TLC). The solvent was evaporated and the residue was dissolved in water and basified with 5% aqueous K_2CO_3 to pH 9. The resulting precipitate was filtered off and washed thoroughly with ethanol and water. Recrystallisation of the crude product from ethyl acetate afforded the title compound **3a**.

5-Acetyl-4-methyl-2-phenylaminothiazole (**3a**): Pale yellow needles; yield 78%; m.p. 153–154 °C (EtOH) (lit.[29] 154–155 °C); IR (ν_{max} cm^{-1}): 3279 (NH), 3095 (benzene ring), 1617 (C=O); ^{1}H NMR: δ 7.42 (t, *J* = 8.0 Hz, 2H, ArH), 7.34 (d, *J* = 8.0 Hz, 2H, ArH), 7.20 (t, *J* = 7.2 Hz, 1H, ArH), 2.61 (s, 3H, CH_3), 2.47 (s, 3H, CH_3CO); APCI-MS: *m/z* (%): 232 ($[M + H]^+$, 100).

*5-Acetyl-4-methyl-2-(*o-*methylphenylamino)thiazole* (**3b**): White needles; yield 75%; m.p. 156–157 °C (EtOH) (lit.[35] 169–170 °C); IR (ν_{max} cm^{-1}): 3167 (NH), 3060 (benzene ring), 1596 (C=O); ^{1}H NMR: δ 7.50 (d, *J* = 8.0 Hz, 1H, ArH), 7.32–7.27 (m, 2H, ArH), 7.20 (d, *J* = 7.6 Hz, 1H, ArH), 2.52 (s, 3H, CH_3), 2.41 (s, 3H, CH_3CO), 2.33 (s, 3H, $ArCH_3$); APCI-MS: *m/z* (%): 246 ($[M + H]^+$, 100).

*5-Acetyl-4-methyl-2-(*m-*methylphenylamino)thiazole* (**3c**): Grey white needles; yield 92%; m.p. 162–164 °C (EtOH) (lit.[29] 165–166 °C); IR (ν_{max} cm^{-1}): 3220 (NH), 3071 (benzene ring), 1613 (C=O); ^{1}H NMR: δ 7.29 (t, *J* = 7.6 Hz, 1H, ArH), 7.14 (d, *J* = 8.4 Hz, 2H, ArH), 7.00 (d, *J* = 7.2 Hz, 1H, ArH), 2.56 (s, 3H, CH_3), 2.44 (s, 3H, CH_3O), 2.38 (s, 3H, $ArCH_3$); APCI-MS: *m/z* (%): 246 ($[M + H]^+$, 92).

*5-Acetyl-4-methyl-2-(*p-*methylphenylamino)thiazole* (**3d**): White needles; yield 92%; m.p. 190–191 °C (EtOH) (lit.[29] 190–192 °C); IR (ν_{max} cm^{-1}): 3282 (NH), 3084 (benzene ring), 1611 (C=O); ^{1}H NMR: δ 7.23–7.06 (m, 4H, ArH), 2.59 (s, 3H, CH_3), 2.45 (s, 3H, CH_3CO), 2.35 (s, 3H, $ArCH_3$); APCI-MS: *m/z* (%): 246 ($[M + H]^+$, 100).

*5-Acetyl-4-methyl-2-(*o-*chlorophenylamino)thiazole* (**3e**): White needles; yield 79%, m.p. 163–164 °C (EtOH) (lit.[29] 163–165 °C); IR (ν_{max} cm^{-1}): 3236 (NH), 3001 (benzene ring), 1634 (C=O); ^{1}H NMR: δ 7.94 (d, *J* = 8.0 Hz, 1H, ArH), 7.44 (d, *J* = 8.0 Hz, 1H, ArH), 7.34 (t, *J* = 8.0 Hz, 1H, ArH), 7.06 (t, *J* = 7.8 Hz, 1H, ArH), 2.65 (s, 3H, CH_3), 2.46 (s, 3H, CH_3CO); APCI-MS: *m/z* (%): 266 ($[M + H]^+$, 75).

*5-Acetyl-4-methyl-2-(*m-*chlorophenylamino)thiazole* (**3f**): Pale yellow needles; yield 76%, m.p. 192–193 °C (EtOH) (lit.[29] m.p. 191–193 °C); IR (ν_{max} cm^{-1}): 3290 (NH), 3129 (benzene ring), 1615 (C=O); ^{1}H NMR: δ 7.42 (s, 1H, ArH), 7.34 (t, *J* = 8.0 Hz, 1H, ArH), 7.25 (d, *J* = 7.8 Hz, 1H, ArH), 7.15 (d, *J* = 7.5 Hz, 1H, ArH), 2.62 (s, 3H, CH_3), 2.46 (s, 3H, CH_3CO); APCI-MS: *m/z* (%): 266 ($[M + H]^+$, 86); HRMS (APCI) calcd for $C_{12}H_{11}ClN_2OS + H$: 267.0359; found: 267.0359.

*5-Acetyl-4-methyl-2-(*p-*chlorophenylamino)thiazole* (**3g**): Grey white needles; yield 65%; m.p. 193–194 °C (EtOH) (lit.[29] 195–197 °C); IR (ν_{max} cm^{-1}): 3278 (NH), 3085 (benzene ring), 1610 (C=O); ^{1}H NMR: δ 7.38 (d, *J* = 8.8 Hz, 2H, ArH), 7.34 (d, *J* = 8.8 Hz, 2H, ArH), 2.62 (s, 3H, CH_3), 2.46 (s, 3H, CH_3CO); APCI-MS: *m/z* (%): 266 ($[M + H]^+$, 90).

Methyl hydrazinecarbodithioate **4***; general procedure*

Hydrazine hydrate (39.0 g, 0.75 mol) was added to a mixture of distilled water (60 mL), isopropyl alcohol (50 mL) and potassium hydroxide (49.5 g, 0.75 mol) at 20°C. The resulting mixture was cooled to 10 °C with an ice–water bath and then carbon disulfide (57.5 g, 0.75 mol) was added dropwise over the course of 100 min. After the addition was completed, the reaction was allowed to proceed for 2 h. Dimethyl sulfate (94.5 g, 0.75 mol) was then added to the mixture at 15 °C and the mixture was stirred for 1 h. The resulting precipitate was filtered off, washed with water and dried in vacuum. The crude product was recrystallised from methylene dichloride to give 55.0 g of compound (**4**): White platelets; yield 60.1%; m.p. 81–82 °C (lit.[3] 81–83 °C).

Synthesis of compounds **5a–g***; general procedure*

1–2 Drops of hydrochloric acid were added to a solution of 2-phenylamino-4-methyl-5-acetyl-thiazole **3a** (4.64 g, 0.02 mol) and methyl hydrazinecarbodithioate **4** (2.928 g, 0.024 mol) in isopropanol (60 mL). The reaction mixture was heated to reflux for 18 h (monitored by TLC, EtOAc/toluene, 1:2). After concentration of the reaction mixture, the crude solid was filtered off and washed with cold isopropanol. Recrystallisation of the crude product from 95% ethanol afforded the title compound **5a** as a dark-yellow solid.

Methyl 2-{1-[4-methyl-2-(phenylamino)thiazol-5-yl]ethylidene} hydrazinecarbodithioate (**5a**): Yellow powder; yield 81.9%; m.p. 179–180°C (*i*-PrOH); IR (υ_{max} $cm^{-1)}$: 3172 (NH), 2913 (benzene ring), 1531 (C=N), 1049 (C=S); ^{1}H NMR: δ 9.84 (bs, 1H, –NHCS–), 7.41 (t, 2H, *J* = 7.8 Hz, ArH), 7.33 (d, 2H, *J* = 7.6 Hz, ArH), 7.17 (t, 1H, *J* = 7.4 Hz, ArH), 2.65 (s, 3H, $–SCH_3$), 2.58 (s, 3H, thiazole–CH_3), 2.30 (s, 3H, –N=C–CH_3); APCI-MS: *m/z* (%)*:* 337.0 ($[M + H]^+$, 32). Anal. calcd for $C_{14}H_{16}N_4S_3$: C, 49.97; H, 4.79; N, 16.65; found: C, 49.92; H, 4.71; N, 16.61%.

*Methyl 2-{1-[4-methyl-2-(*o-*tolylamino)thiazol-5-yl]ethylidene} hydrazinecarbodithioate* (**5b**): Yellow powder; yield 77.4%; m.p. 181–182 °C (*i*-PrOH); IR (υ_{max} cm^{-1}): 3154 (NH), 2914 (benzene ring), 1566 (C=N), 1048 (C=S); ^{1}H NMR: δ 9.85 (bs, 1H, –NHCS–), 7.53 (d, 1H, *J* = 7.6 Hz, ArH), 7.31–7.27 (m*, 2H, ArH), 7.17 (t, 1H, *J* = 7.6 Hz, ArH), 2.63 (s, 3H, $–SCH_3$), 2.57 (s, 3H, thiazole–CH_3), 2.34 (s, 3H, –N=C–CH_3), 2.27 (s, 3H, $ArCH_3$) (*overlapped with $CHCl_3$); APCI-MS: *m/z* (%): 351.3 ($[M + H]^+$, 100). Anal. calcd for $C_{15}H_{18}N_4S_3$: C, 51.40; H, 5.18; N, 15.98; found: C, 51.48; H, 5.24; N, 16.02%.

*Methyl 2-{1-[4-methyl-2-(*m-*tolylamino)thiazol-5-yl]ethylidene} hydrazinecarbodithioate* (**5c**): Yellow powder; yield 43.7%; m.p. 169–170 °C (*i*-PrOH); IR (υ_{max} cm^{-1}): 3189 (NH), 2916 (benzene ring), 1564 (C=N), 1047 (C=S); ^{1}H NMR: δ 9.91 (bs, 1H, –NHCS–), 7.30–7.28 (m*, 1H, ArH), 7.16–7.09 (m, 2H, ArH), 6.96 (d, 1H, *J* = 6.8 Hz, ArH), 2.65 (s, 3H, $–SCH_3$), 2.55 (s, 3H, thiazole–CH_3), 2.38 (s, 3H, –N=C–CH_3), 2.30 (s, 3H, $ArCH_3$) (*overlapped with $CHCl_3$); APCI-MS: *m/z* (%): 351.1 ($[M + H]^+$, 20). Anal. calcd for $C_{15}H_{18}N_4S_3$: C, 51.40; H, 5.18; N, 15.98; found: C, 51.45; H, 5.21; N, 16.03%.

*Methyl 2-{1-[4-methyl-2-(*p-*tolylamino)thiazol-5-yl]ethylidene} hydrazinecarbodithioate* (**5d**): Yellow powder; yield 63.6%; m.p. 177–178 °C (*i*-PrOH); IR (υ_{max} cm^{-1}): 3114 (NH), 2917 (benzene ring), 1560 (C=N), 1048 (C=S); ^{1}H NMR: δ 9.87 (bs, 1H, –NHCS–), 7.25–7.18 (m, 4H, ArH), 2.64 (s, 3H, $–SCH_3$), 2.54 (s, 3H, thiazole–CH_3), 2.35 (s, 3H, –N=C–CH_3), 2.28 (s, 3H, $ArCH_3$); APCI-MS: *m/z* (%): 351.1 ($[M + H]^+$, 60). Anal. calcd for $C_{15}H_{18}N_4S_3$: C, 51.40; H, 5.18; N, 15.98; found: C, 51.32; H, 5.13; N, 15.93%.

*Methyl 2-{1-[4-methyl-2-(*o-*chlorophenylamino)thiazol-5-yl] ethylidene}hydrazinecarbodithioate* (**5e**): Yellow powder; yield 50.7%; m.p. 173–74 °C (*i*-PrOH); IR (υ_{max} cm^{-1}): 3126 (NH), 2918 (benzene ring), 1543 (C=N), 1050 (C=S); ^{1}H NMR: δ 9.87 (bs, 1H, –NHCS–), 8.02 (d, 1H, *J* = 8.4 Hz, ArH), 7.42 (d, 1H, *J* = 8.4 Hz, ArH), 7.35–7.32 (t, 1H, *J* = 7.2 Hz, ArH), 7.05–7.01 (t, 1H, *J* = 7.8 Hz, ArH), 2.65 (s, 3H, $–SCH_3$), 2.60 (s, 3H, thiazole–CH_3), 2.32 (s, 3H, –N=C–CH_3); APCI-MS: *m/z* (%): 371.3 ($[M + H]^+$, 100). Anal. calcd for $C_{14}H_{15}ClN_4S_3$: C, 45.33; H, 4.08; N, 15.10; found: C, 45.38; H, 4.15; N, 15.16%.

*Methyl 2-{1-[4-methyl-2-(*m-*chlorophenylamino)thiazol-5-yl] ethylidene}hydrazine carbodithioate* (**5f**): Yellow powder; yield 34.3%; m.p. 177–178 °C (*i*-PrOH); IR (υ_{max} cm^{-1}): 3231 (NH), 2914 (benzene ring), 1554 (C=N), 1030 (C=S); ^{1}H NMR: δ 9.84 (bs, 1H, –NHCS–), 7.41 (s, 1H, ArH), 7.35–7.31 (t, 1H, *J* = 8.0 Hz, ArH), 7.23

(m*, 1H, ArH), 7.12 (d, 1H, *J* = 7.2 Hz, ArH), 2.66 (s, 3H, –SCH_3), 2.60 (s, 3H, thiazole–CH_3), 2.32 (s, 3H, –N=C–CH_3) (*overlapped with $CHCl_3$); APCI-MS: *m/z* (%): 371.3 ([M + H]$^+$, 100). Anal. calcd for $C_{14}H_{15}ClN_4S_3$: C, 45.33; H, 4.08; N, 15.10; found: C, 45.41; H, 4.19; N, 15.14%.

*Methyl 2-{1-[4-methyl-2-(*p*-chlorophenylamino)thiazol-5-yl]ethylidene}hydrazine carbodithioate* (**5g**)**:** Yellow powder; yield 69.1%; m.p. 187–189 °C (*i*-PrOH); IR (υ_{max} cm^{-1}): 3175 (NH), 2925 (benzene ring), 1536 (C=N), 1045 (C=S); ^{1}H NMR: δ 9.83 (bs, 1H, –NHCS–), 7.33 (dd, 4H, J_1 = 8.8 Hz, J_2 = 9.2 Hz, ArH), 2.65 (s, 3H, –SCH_3), 2.57 (s, 3H, thiazole–CH_3), 2.31 (s, 3H, –N=C–CH_3); APCI-MS: *m/z* (%): 371.4 ([M + H]$^+$, 100). Anal. calcd for $C_{14}H_{15}ClN_4S_3$: C, 45.33; H, 4.08; N, 15.10; found: C, 45.38; H, 4.14; N, 15.13%.

Synthesis of compounds **TSC1–21***; general procedure*

Conventional heating

Morpholine (0.957 g, 0.011 mol) was added to a stirred mixture of **5a** (3.36 g, 0.01 mol) in isopropanol (60 mL). The mixture was heated to reflux under N_2 for 24 h and TLC showed the reaction was completed. After concentration, the residue was filtered off and washed with cold isopropanol to give a crude product. Recrystallisation from methanol/propan-2-ol afforded the title compound **TSC1** as a light-yellow powder.

Microwave heating

Morpholine (0.55 mmol) was added to a suspension of **5a** (0.5 mmol) and propan-2-ol (5 mL) in a 10 mL glass vial equipped with a small magnetic stirring bar at room temperature under air. After 0.5 h (arrested N_2 bubbles), the reaction vial was sealed with an aluminium/Teflon crimp top and stirred at 100 °C for 45 min under microwave-irradiation. After completion of the reaction, the vial was cooled to 50 °C by air jet cooling before it was opened. The precipitated product was collected by filtration and washed with cold propan-2-ol to give a crude product. Recrystallisation of which from methanol/propan-2-ol afforded the title compound **TSC1** as a light-yellow powder.

N'-{1-[4-Methyl-2-(phenylamino)thiazol-5-yl]ethylidene} morpholine-4-carbothiohydrazide (**TSC1**): Light-yellow powder; yield 69.3%; m.p. 176–178 °C (*i*-PrOH); IR (υ_{max} cm^{-1}): 3425 (NH), 2916 (benzene ring), 1562 (C=N), 1106 (C=S); ^{1}H NMR: δ 7.87 (bs, 1H, NH), 7.41–7.34 (m, 4H, ArH), 7.18 (t, 1H, *J* = 6 Hz, ArH), 4.01 (t, *J* = 4.6 Hz, 4H, 2OCH_2), 3.71 (t, *J* = 4.6 Hz, 4H, 2NCH_2), 2.68 (s, 3H, thiazole–CH_3), 2.66 (s, 3H, –N=C–CH_3); APCI-MS: *m/z* (%)*:* 376.2 ([M + H]$^+$, 60); HRMS (APCI) calcd for $C_{17}H_{21}N_5OS_2$ + H: 376.1266; found: 376.1272.

*N'-{1-[4-Methyl-2-(*o*-tolylamino)thiazol-5-yl]ethylidene} morpholine-4-carbothiohydrazide* (**TSC2**): Light-yellow powder; yield 51.7%; m.p. 164–166 °C (*i*-PrOH); IR (υ_{max} cm^{-1}): 3418 (NH), 2914 (benzene ring), 1561 (C=N), 1113 (C=S); ^{1}H NMR: δ 8.09 (bs, 1H, NH), 7.49 (d, *J* = 7.6 Hz, 1H, ArH), 7.31–7.19 (m*, 3H, ArH), 4.00 (t, *J* = 4.6 Hz, 4H, 2OCH_2), 3.72 (t, *J* = 4.6 Hz, 4H, 2NCH_2), 2.65 (s, 3H, thiazole–CH_3), 2.59 (s, 3H, –N=C–CH_3), 2.33 (s, 3H, $ArCH_3$) (*overlapped with $CHCl_3$); APCI-MS: *m/z* (%)*:* 390.2 ([M + H]$^+$, 100); Anal. calcd for $C_{18}H_{23}N_5OS_2$: C, 55.50; H, 5.95; N, 17.98; found: C, 55.45; H, 5.89; N, 17.93%.

*N'-{1-[4-Methyl-2-(*m*-tolylamino)thiazol-5-yl]ethylidene} morpholine-4-carbothiohydrazide* (**TSC3**): Light-yellow powder; yield 56.6%; m.p. 157 °C (*i*-PrOH); IR (υ_{max} cm^{-1}): 3425 (NH), 2909 (benzene ring), 1554 (C=N), 1115 (C=S); ^{1}H NMR: δ 8.07 (bs, 1H, NH), 7.29–7.25 (m*, 1H, ArH), 7.17–7.10 (m, 2H, ArH), 7.00–6.94 (m, 1H, ArH), 4.02 (t, *J* = 4.6 Hz, 4H, 2OCH_2), 3.69 (t, *J* = 4.4 Hz, 4H, 2NCH_2), 2.68 (s, 3H, thiazole–CH_3), 2.64 (s, 3H, –N=C–CH_3), 2.38 (s, 3H, $ArCH_3$) (*overlapped with $CHCl_3$); APCI-MS: *m/z* (%): 390.2 ([M + H]$^+$, 42). Anal. calcd for $C_{18}H_{23}N_5OS_2$: C, 55.50; H, 5.95; N, 17.98; found: C, 55.43; H, 5.90; N, 17.91%.

*N'-{1-[4-Methyl-2-(*p*-tolylamino)thiazol-5-yl]ethylidene} morpholine-4-carbothiohydrazide* (**TSC4**): Yellow powder; yield 46.9%; m.p. 177–179 °C (*i*-PrOH); IR (υ_{max} cm^{-1}): 3424 (NH), 2917 (benzene ring), 1560 (C=N), 1076 (C=S), 1049; ^{1}H NMR: δ 8.30 (bs, 1H, NH), 7.25–7.18 (m, 4H, ArH), 3.99 (t, *J* = 4.6 Hz, 4H, 2OCH_2), 3.69 (t, *J* = 4.4 Hz, 4H, 2NCH_2), 2.65 (s, 3H, thiazole–CH_3), 2.54 (s, 3H, –N=C–CH_3), 2.36 (s, 3H, $ArCH_3$); APCI-MS: *m/z* (%)*:* 390.2 ([M + H]$^+$, 38); HRMS (APCI) calcd for $C_{18}H_{23}N_5OS_2$ + H: 390.1422; found: 390.1429.

N'-(1-{2-[(2-Chlorophenyl)amino]-4-methylthiazol-5-yl} ethylidene)morpholine-4-carbothiohydrazide (**TSC5**): Yellow powder; yield 37.9%; m.p. 159–161 °C (*i*-PrOH); IR (υ_{max} cm^{-1}): 3424 (NH), 2966 (benzene ring), 1593 (C=N), 1025 (C=S); ^{1}H NMR: δ 8.07 (bs, 1H, NH), 7.95 (d, 1H, *J* = 8.0 Hz, ArH), 7.46 (d, 1H, *J* = 7.6 Hz, ArH), 7.30 (t, 1H, *J* = 8.0 Hz, ArH), 7.09 (t, 1H, *J* = 7.2 Hz, ArH), 4.03 (t, *J* = 4.8 Hz, 4H, 2OCH_2), 3.73 (t, *J* = 4.4 Hz, 4H, 2NCH_2), 2.70 (s, 3H, thiazole–CH_3), 2.68 (s, 3H, –N=C–CH_3); APCI-MS: *m/z* (%): 410.2 ([M + H]$^+$, 45). Anal. calcd for $C_{17}H_{20}ClN_5OS_2$: C, 49.81; H, 4.92; N, 17.08; found: C, 49.76; H, 4.89; N, 17.04%.

N'-(1-{2-[(3-Chlorophenyl)amino]-4-methylthiazol-5-yl} ethylidene)morpholine-4-carbothiohydrazide (**TSC6**): Dark-yellow powder; yield 55.0%; m.p. 178–180 °C (*i*-PrOH); IR (υ_{max} cm^{-1}): 3425 (NH), 2969 (benzene ring), 1597 (C=N), 1026 (C=S); ^{1}H NMR: δ 7.64 (bs, 1H, NH), 7.48 (s, 1H, ArH), 7.30 (t, 1H, *J* = 8.0 Hz, ArH), 7.17 (d, 1H, *J* = 8.0 Hz, ArH), 7.12 (d, 1H, *J* = 7.6 Hz, ArH), 4.04 (t, *J* = 4.8 Hz, 4H, 2OCH_2), 3.75 (t, *J* = 4.8 Hz, 4H, 2NCH_2), 2.71 (s, 3H, thiazole–CH_3), 2.69 (s, 3H, –N=C–CH_3); APCI-MS: *m/z* (%): 410.3 ([M + H]$^+$, 35). Anal. calcd for $C_{17}H_{20}ClN_5OS_2$: C, 49.81; H, 4.92; N, 17.08; found: C, 49.78; H, 4.88; N, 17.01%.

N'-(1-{2-[(4-Chlorophenyl)amino]-4-methylthiazol-5-yl} ethylidene)morpholine-4-carbothiohydrazide (**TSC7**): Yellow powder; yield 50.4%; m.p. 173–174 °C (*i*-PrOH); IR (υ_{max} cm^{-1}): 3422 (NH), 2920 (benzene ring), 1598 (C=N), 1044 (C=S); ^{1}H NMR: δ 7.96 (bs, 1H, NH), 7.34 (dd, J_1 = 8.8 Hz, J_2 = 9.2 Hz, 4H, ArH), 4.03 (t, *J* = 4.6 Hz, 4H, 2OCH_2), 3.73 (t, *J* = 4.6 Hz, 4H, 2NCH_2), 2.69 (s, 3H, thiazole–CH_3), 2.66 (s, 3H, –N=C–CH_3); APCI-MS: *m/z* (%): 410.1 ([M + H]$^+$, 47). Anal. calcd for $C_{17}H_{20}ClN_5OS_2$: C, 49.81; H, 4.92; N, 17.08; found: C, 49.79; H, 4.91; N, 17.03%.

N'-{1-[4-Methyl-2-(phenylamino)thiazol-5-yl]ethylidene} piperazine-1-carbothiohydrazide (**TSC8**): Yellow powder; yield 70.4%; m.p. 216–217 °C (*i*-PrOH); IR (υ_{max} cm^{-1}): 3425 (NH), 2923 (benzene ring), 1562 (C=N), 1074 (C=S); ^{1}H NMR: δ 10.47 (bs, 1H, NH), 7.47–7.39 (m, 4H, ArH), 7.23 (t, 1H, *J* = 6 Hz, ArH), 4.15 (t, *J* = 4.6 Hz, 4H, 2NCH_2), 3.71 (t, *J* = 4.6 Hz, 4H, 2NCH_2), 2.68 (s, 3H, thiazole–CH_3), 2.61 (s, 3H, –N=C–CH_3); APCI-MS: *m/z* (%): 375.0 ([M + H]$^+$, 100); HRMS (APCI) calcd for $C_{17}H_{22}N_6S_2$ + H: 375.1426; found: 375.1431.

*N'-{1-[4-Methyl-2-(*o*-tolylamino)thiazol-5-yl]ethylidene} piperazine-1-carbothiohydrazide* (**TSC9**): Yellow powder; yield 35.9%; m.p. 210–211 °C (*i*-PrOH); IR (υ_{max} cm^{-1}): 3431 (NH), 3174 (benzene ring), 1555 (C=N), 1138 (C=S); ^{1}H NMR: δ 10.05 (bs, 1H, NH), 7.29–7.19 (m, 4H, ArH), 4.16 (t, *J* = 4.8 Hz, 4H, 2NCH_2), 3.90 (s, 1H, NH, pip), 3.75 (t, *J* = 4.8 Hz, 4H, 2NCH_2), 2.68 (s, 3H, thiazole–CH_3), 2.62 (s, 3H, –N=C–CH_3), 2.33 (s, 3H, $ArCH_3$); APCI-MS: *m/z* (%): 389.1 ([M + H]$^+$, 100). Anal. calcd for $C_{18}H_{24}N_6S_2$: C, 55.64; H, 6.23; N, 21.63; found: C, 55.58; H, 6.19; N, 21.61%;

*N'-{1-[4-Methyl-2-(*m*-tolylamino)thiazol-5-yl]ethylidene} piperazine-1-carbothiohydrazide* (**TSC10**): Light-yellow powder; yield 55.9%; m.p. 199–200 °C (*i*-PrOH); IR (υ_{max} cm^{-1}): 3439 (NH), 2923 (benzene ring), 1563 (C=N), 1133 (C=S); ^{1}H NMR: δ 10.11 (bs, 1H, NH), 7.49–7.36 (m, 2H, ArH), 7.30 (t, *J* = 7.6 Hz, 1H, ArH), 7.24–7.19 (m, 1H, ArH), 4.16 (t, *J* = 4.8 Hz, 4H, 2NCH_2), 3.98 (s, 1H, NH, pip), 3.75 (t, *J* = 4.8 Hz, 4H, 2NCH_2), 2.68 (s, 3H, thiazole–CH_3), 2.62 (s, 3H, –N=C–CH_3), 2.33 (s, 3H, $ArCH_3$); APCI-MS: *m/z* (%): 389.1 ([M + H]$^+$, 52). Anal. calcd for $C_{18}H_{24}N_6S_2$: C, 55.64; H, 6.23; N, 21.63; found: C, 55.62; H, 6.21; N, 21.59%.

*N'-{1-[4-Methyl-2-(*p*-tolylamino)thiazol-5-yl]ethylidene} piperazine-1-carbothiohydrazide* (**TSC11**): Yellow powder; yield 56.1%; m.p. 236–237 °C (*i*-PrOH); IR (υ_{max} cm^{-1}): 3440 (NH), 2918 (benzene ring), 1555 (C=N), 1126 (C=S); ^{1}H NMR: δ 10.17 (bs, 1H, NH), 7.48 (d, *J* = 8.4 Hz, 2H, ArH), 7.13 (d, *J* = 8.0 Hz, 2H, ArH), 4.37 (t, *J* = 4.8 Hz, 4H, 2NCH_2), 4.11 (s, 1H, NH, pip), 3.61 (t, *J* = 4.8 Hz,

4H, 2NCH$_2$), 2.65 (s, 3H, thiazole–CH$_3$), 2.61 (s, 3H, –N=C–CH$_3$), 2.32 (s, 3H, ArCH$_3$); APCI-MS: *m/z* (%): 389.3 ([M + H]$^+$, 41); HRMS (APCI) calcd for $C_{18}H_{24}N_6S_2$ + H: 389.1582; found: 389.1585.

N'-(1-{2-[(2-Chlorophenyl)amino]-4-methylthiazol-5-y}) ethylidene)piperazine-1-carbothiohydrazide (**TSC12**): Light-yellow powder; yield 56.7%; m.p. 200–201 °C (*i*-PrOH); IR (υ_{max} cm^{-1}): 3444 (NH), 3167 (benzene ring), 1586 (C=N), 1140 (C=S); ^{1}H NMR: δ 10.24 (bs, 1H, NH), 7.85 (d, 1H, *J* = 8.0 Hz, ArH), 7.58 (d, *J* = 7.6 Hz, 1H, ArH), 7.46 (t, 1H, *J* = 6.4 Hz, ArH), 7.09 (t, 1H, *J* = 7.2 Hz, ArH), 4.37 (t, *J* = 4.8 Hz, 4H, 2NCH$_2$), 4.03 (s, 1H, NH, pip), 3.61 (t, *J* = 4.8 Hz, 4H, 2NCH$_2$), 2.70 (s, 3H, thiazole–CH$_3$), 2.65 (s, 3H, –N=C–CH$_3$); APCI-MS: *m/z* (%): 409.5 ([M + H]$^+$, 58); HRMS (APCI) calcd for $C_{17}H_{21}ClN_6S_2$ + H: 409.1036; found: 409.1046.

N'-(1-{2-[(3-Chlorophenyl)amino]-4-methylthiazol-5-yl} ethylidene)piperazine-1-carbothiohydrazide (**TSC13**): Light-yellow powder; yield 59.3%; m.p. 201–202 °C (*i*-PrOH); IR (υ_{max} cm^{-1}): 3424 (NH), 2923 (benzene ring), 1596 (C=N), 1097 (C=S); ^{1}H NMR: δ 10.24 (bs, 1H, NH), 7.61 (s, 1H, ArH), 7.43–7.32 (m, 3H, ArH), 4.35 (t, *J* = 4.8 Hz, 4H, 2NCH$_2$), 4.07 (s, 1H, NH, pip), 3.62 (t, *J* = 4.8 Hz, 4H, 2NCH$_2$), 2.69 (s, 3H, thiazole–CH$_3$), 2.63 (s, 3H, –N=C–CH$_3$); APCI-MS: *m/z* (%): 409.2 ([M + H]$^+$, 100). Anal. calcd for $C_{17}H_{21}ClN_6S_2$: C, 49.93; H, 5.18; N, 20.55; found: C, 49.91; H, 5.17; N, 20.52%.

N'-(1-{2-[(4-Chlorophenyl)amino]-4-methylthiazol-5-yl} ethylidene)piperazine-1-carbothiohydrazide (**TSC14**): Light-yellow powder; yield 73.1%; m.p. 226–227 °C (*i*-PrOH); IR (υ_{max} cm^{-1}): 3429 (NH), 2922 (benzene ring), 1554 (C=N), 1091 (C=S); ^{1}H NMR: δ 10.52 (bs, 1H, NH), 7.67 (d, *J* = 8.8 Hz, 2H, ArH), 7.38 (d, *J* = 8.8 Hz, 2H, ArH), 4.37 (t, *J* = 4.8 Hz, 4H, 2NCH$_2$), 3.93 (s, 1H, NH, pip), 3.61 (t, *J* = 4.8 Hz, 4H, 2NCH$_2$), 2.70 (s, 3H, thiazole–CH$_3$), 2.65 (s, 3H, –N=C–CH$_3$); APCI-MS: *m/z* (%): 409.1 ([M + H]$^+$, 52). Anal. calcd for $C_{17}H_{21}ClN_6S_2$: C, 49.93; H, 5.18; N, 20.55; found: C, 49.85; H, 5.13; N, 20.53%.

N'-{1-[4-Methyl-2-(phenylamino)thiazol-5-yl]ethylidene}-4-(4-methoxyphenyl)piperazine-1-carbothiohydrazide (**TSC15**): Yellow powder; yield 50.7%; m.p. 170–171 °C (*i*-PrOH); IR (υ_{max} cm^{-1}): 3440 (NH), 2924 (benzene ring), 1600 (C=N), 1080 (C=S); ^{1}H NMR: δ 9.92 (bs, 1H, NH), 7.63–7.62 (m, 4H, ArH), 7.33 (t, 3H, *J* = 6 Hz, ArH), 7.01–6.86 (m, 2H, ArH), 4.36 (t, *J* = 5.2 Hz, 4H, 2NCH$_2$), 3.70 (s, 3H, OCH$_3$), 3.45 (t, *J* = 6.2 Hz, 4H, 2NCH$_2$), 2.49 (s, 3H, thiazole–CH$_3$), 2.38 (s, 3H, –N=C–CH$_3$); APCI-MS: *m/z* (%): 481.3 ([M + H]$^+$, 41); HRMS (APCI) calcd for $C_{24}H_{28}N_6OS_2$ + H: 481.1844; found: 481.1852.

N'-{1-[2-(o-tolylamino)-4-methylthiazol-5-yl]ethylidene}-4-(4-methoxyphenyl)piperazine-1-carbothiohydrazide (**TSC16**): Yellow powder; yield 41.5%; m.p. 166–167 °C (*i*-PrOH); IR (υ_{max} cm^{-1}): 3445 (NH), 2922 (benzene ring), 1595 (C=N), 1044 (C=S); ^{1}H NMR: δ 9.58 (bs, 1H, NH), 7.49 (d, *J* = 8.4 Hz, 2H, ArH), 7.26–7.20 (m*, 4H, ArH), 7.08 (t, *J* = 7.6 Hz, 2H, ArH), 4.36 (t, *J* = 5.0 Hz, 4H, 2NCH$_2$), 3.71 (s, 3H, OCH$_3$), 3.45 (t, *J* = 6.0 Hz, 4H, 2NCH$_2$), 2.68 (s, 3H, thiazole–CH$_3$), 2.62 (s, 3H, –N=C–CH$_3$), 2.34 (s, 3H, ArCH$_3$) (*overlapped with CHCl$_3$); APCI-MS: *m/z* (%): 495.4 ([M + H]$^+$, 34). Anal. calcd for $C_{25}H_{30}N_6OS_2$: C, 60.70; H, 6.11; N, 16.99; found: C, 60.61; H, 6.05; N, 16.97%.

N'-{1-[2-(m-Tolylamino)-4-methylthiazol-5-yl]ethylidene}-4-(4-methoxyphenyl)piperazine-1-carbothiohydrazide (**TSC17**): Yellow powder; yield 55.3%; m.p. 184–185 °C (*i*-PrOH); IR (υ_{max} cm^{-1}): 3444 (NH), 2920 (benzene ring), 1565 (C=N), 1024 (C=S); ^{1}H NMR: δ 9.91 (bs, 1H, NH), 7.47–7.34 (m, 2H, ArH), 7.27 (t, *J* = 7.8 Hz, 1H, ArH), 7.22–7.17 (m, 1H, ArH), 6.94–6.79 (m, 4H, ArH), 4.36 (t, *J* = 4.8 Hz, 4H, 2NCH$_2$), 3.70 (s, 3H, OCH$_3$), 3.47 (t, *J* = 5.2 Hz, 4H, 2NCH$_2$), 2.68 (s, 3H, thiazole–CH$_3$), 2.63 (s, 3H, –N=C–CH$_3$), 2.32 (s, 3H, ArCH$_3$); APCI-MS: *m/z* (%): 495.3 ([M + H]$^+$, 40). Anal. calcd for $C_{25}H_{30}N_6OS_2$: C, 60.70; H, 6.11; N, 16.99; found: C, 60.65; H, 6.09; N, 16.96%.

N'-{1-[2-(p-Tolylamino)-4-methylthiazol-5-yl]ethylidene}-4-(4-methoxyphenyl)piperazine-1-carbothiohydrazide (**TSC18**): Brown-yellow powder; yield 43.5%; m.p. 176–178 °C (*i*-PrOH); IR (υ_{max} cm^{-1}): 3428 (NH), 2919 (benzene ring), 1554 (C=N), 1023 (C=S); ^{1}H NMR: δ 9.86 (bs, 1H, NH), 7.46 (t, *J* = 8.4 Hz, 2H, ArH), 7.19 (d, *J* = 8.4 Hz, 1H, ArH), 7.08 (d, *J* = 8.0 Hz, 1H, ArH), 7.94 (d, *J* = 8.8 Hz, 2H, ArH), 6.85 (d, *J* = 6.8 Hz, 2H, ArH), 4.36 (t, *J* = 4.4 Hz, 4H, 2NCH$_2$), 3.71 (s, 3H, OCH$_3$), 3.47 (t, *J* = 5.2 Hz, 4H, 2NCH$_2$), 2.71 (s, 3H, thiazole–CH$_3$), 2.65 (s, 3H, –N=C–CH$_3$), 2.46 (s, 3H, ArCH$_3$); APCI-MS: *m/z* (%): 495.1 ([M + H]$^+$, 35). Anal. calcd for $C_{25}H_{30}N_6OS_2$: C, 60.70; H, 6.11; N, 16.99; found: C, 60.69; H, 6.09; N, 16.97%.

N'-(1-{2-[(2-Chlorophenyl)amino]-4-methylthiazol-5-yl} ethylidene)-4-(4-methoxyphenyl)piperazine-1-carbothiohydrazide (**TSC19**): Yellow powder; yield 39.2%; m.p. 161–162 °C (*i*-PrOH); IR (υ_{max} cm^{-1}): 3440 (NH), 2920 (benzene ring), 1585 (C=N), 1027 (C=S); ^{1}H NMR: δ 9.91 (bs, 1H, NH), 7.89 (d, 1H, *J* = 8.0 Hz, ArH), 7.59 (d, 1H, *J* = 7.6 Hz, ArH), 7.48 (t, 1H, *J* = 6.4 Hz, ArH), 7.09 (t, 1H, *J* = 7.2 Hz, ArH), 6.97–6.84 (m, 4H, ArH), 4.36 (t, *J* = 4.8 Hz, 4H, 2NCH$_2$), 3.70 (s, 3H, OCH$_3$), 3.45 (t, *J* = 6.0 Hz, 4H, 2NCH$_2$), 2.70 (s, 3H, thiazole–CH$_3$), 2.63 (s, 3H, –N=C–CH$_3$); APCI-MS: *m/z* (%): 515.3 ([M + H]$^+$, 60). Anal. calcd for $C_{24}H_{27}ClN_6OS_2$: C, 55.96; H, 5.28; N, 16.32; found: C, 55.91; H, 5.22; N, 16.31%.

N'-(1-{2-[(3-Chlorophenyl)amino]-4-methylthiazol-5-yl} ethylidene)-4-(4-methoxyphenyl)piperazine-1-carbothiohydrazide (**TSC20**): Yellow powder; yield 41.3%; m.p. 163–165 °C (*i*-PrOH); IR (υ_{max} cm^{-1}): 3441 (NH), 2921 (benzene ring), 1595 (C=N), 1044 (C=S); ^{1}H NMR: δ 9.96 (bs, 1H, NH), 7.86 (t, 1H, *J* = 8.0 Hz, ArH), 7.52–7.30 (m, 2H, ArH), 7.00 (d, 1H, *J* = 8.0 Hz, ArH), 6.94 (d, 2H, *J* = 8.8 Hz, ArH), 6.85 (d, 2H, *J* = 8.4 Hz, ArH), 4.36 (t, *J* = 4.8 Hz, 4H, 2NCH$_2$), 3.70 (s, 3H, OCH$_3$), 3.45 (t, *J* = 6.0 Hz, 4H, 2NCH$_2$), 2.74 (s, 3H, thiazole–CH$_3$), 2.70 (s, 3H, –N=C–CH$_3$); APCI-MS: *m/z* (%): 515.1 ([M + H]$^+$, 86); HRMS (APCI) calcd for $C_{24}H_{27}ClN_6OS_2$ + H: 515.1455; found: 515.1463.

N'-(1-{2-[(4-Chlorophenyl)amino]-4-methylthiazol-5-yl} ethylidene)-4-(4-methoxyphenyl)piperazine-1-carbothiohydrazide (**TSC21**): Yellow powder; yield 36.3%; m.p. 178–180 °C (*i*-PrOH); IR (υ_{max} cm^{-1}): 3440 (NH), 2922 (benzene ring), 1552 (C=N), 1026 (C=S); ^{1}H NMR: δ 9.94 (bs, 1H, NH), 7.66 (dd, J_1 = 8.8 Hz, J_2 = 8.8 Hz, 2H, ArH), 7.43 (d, *J* = 8.8 Hz, 1H, ArH), 7.34 (d, *J* = 8.4 Hz, 1H, ArH), 6.94 (d, *J* = 8.8 Hz, 2H, ArH), 6.85 (d, *J* = 9.2 Hz, 2H, ArH), 4.36 (t, *J* = 4.8 Hz, 4H, 2NCH$_2$), 3.70 (s, 3H, OCH$_3$), 3.73 (t, *J* = 6.0 Hz, 4H, 2NCH$_2$), 2.73 (s, 3H, thiazole–CH$_3$), 2.68 (s, 3H, –N=C–CH$_3$); APCI-MS: *m/z* (%): 515.5 ([M + H]$^+$, 72). Anal. calcd for $C_{24}H_{27}ClN_6OS_2$: C, 55.96; H, 5.28; N, 16.32; found: C, 56.01; H, 5.32; N, 16.35%.

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