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The synthesis of ethacrynic acid thiazole derivatives as glutathione S-transferase pi inhibitors

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ABSTRACT

Glutathione S-transferase pi (GSTpi) is a phase II enzyme which protects cells from death and detoxifies chemotherapeutic agents in cancer cells. Ethacrynic acid (EA) is a weak GSTpi inhibitor. Structure modifications were done to improve the ability of EA to inhibit GSTpi activity. Eighteen EA thiazole derivatives were designed and synthesized. Compounds **9a**, **9b** and **9c** with a replacement of carboxyl group of EA by a heterocyclic thiazole exhibited improvement over EA to inhibit GSTpi activity.

1. Introduction

Glutathione transferases (GSTs) (EC 2.5.1.18) are a family of Phase II detoxification enzymes in plants and animals. GSTs can catalyse the conjugation of glutathione (GSH) to a wide range of endogenous and exogenous electrophilic compounds to generate less toxic and more water soluble products easily to be eliminated. Among these enzymes, GSTpi is an abundant enzyme which also has a role in the regulation of signaling pathway of cell death and proliferation.[1–4] GSTpi has been found to inhibit c-Jun N-terminal kinase (JNK) through direct protein-protein interaction.[5] GSTpi was also found to elicit protection against cell death induced by reactive oxygen species (ROS).[6] GSTpi was found to be able to block tumor necrosis factor receptor-associated factor 2-apoptosis signal-regulating kinase 1 (TRAF2–ASK1) interaction and to suppress TRAF2–ASK1-triggered cell death.[7] Overexpression of GSTpi suppressed mitogen-activated protein kinase/extracellular signal-regulated kinase kinase kinase 1 (MEKK1)-mediated apoptosis in HEK293 cells.[8] In addition, GSTpi is linked to the forward S-glutathionylation reaction of proteins, a critical process responded to cellular stress.[9–11] In view of the multiple functions of GSTpi in protecting cancer cells from death,[11,12] GSTpi inhibitors have potential to be developed as therapeutic agents for cancer treatment.

Ethacrynic acid (EA), a known inhibitor of GSTpi, has been shown to enhance the cytotoxicity of several anticancer agents.[13] EA inhibits the activity of GSTpi by binding directly to the substrate-binding site of the isozyme and by depleting GSH via conjugation of the Michael addition to the thiol group of GSH.[3,14] Previously we have done structural modifications of EA by replacing the carboxyl with esters or heterocyclic oxadiazole. The EA derivatives containing heterocyclic oxadiazole showed better antiproliferative ability than EA but have variant ability of inhibiting GSTpi activity.[15] Recently it has been found that bromo-thiazolide could bind to GSTpi and inhibit GSTpi activity.[16] We designed and synthesized 18 new EA derivatives using a thiazole group to replace carboxyl acid of EA and to compare their abilities of inhibiting GSTpi activity as well as of inhibiting human leukemia HL-60 cell proliferation.

2. Results and discussion

2.1. Chemistry

The synthesis of thiazole derivatives from EA was shown in Scheme 1. The intermediates **2** and **10** were obtained by the methods reported before.[17]

The intermediates **5** were synthesized in two routes. One is started with the appropriate substituted phenols which reacted with chloroacetic acid to generate compounds **2**. With thionyl chloride as a chlorinating agent and solvent, compounds **2** were converted to acyl chlorides and then reacted with aqueous ammonia at 0–5 °C to obtain acetamides **3**. Phosphorus pentasulfide was added to a solution of compounds **3** in tetrahydrofuran at 50 °C in batches to yield ethanethioamides **4**. The intermediates **5** were obtained by Fridel–Crafts reaction of compounds **4**. Most of

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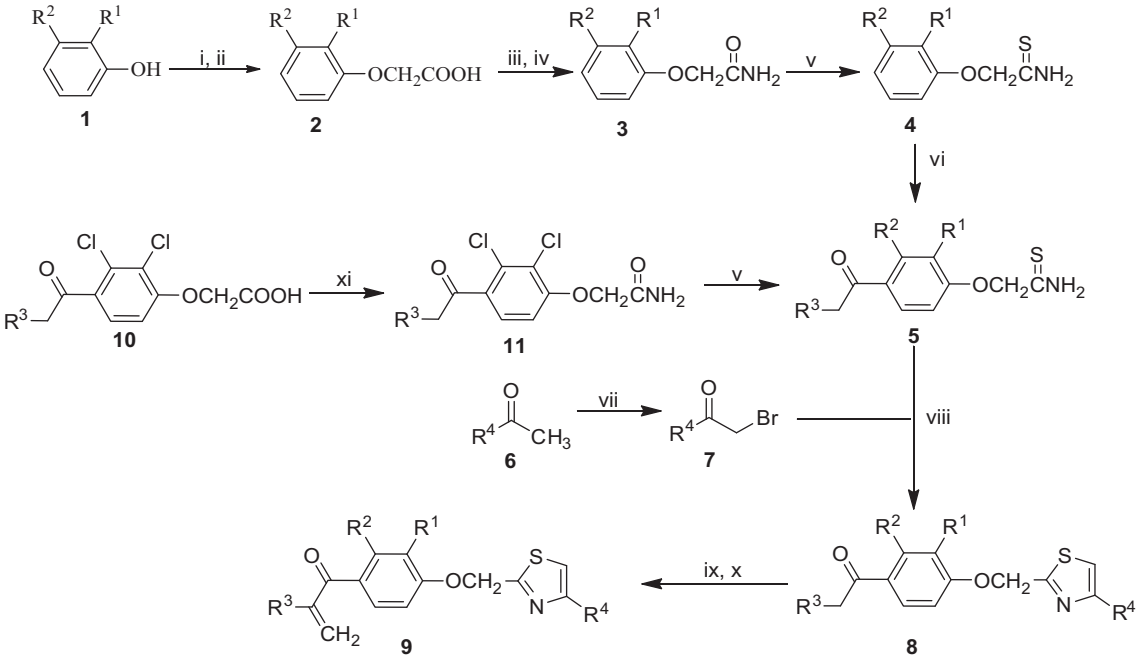


Scheme 1. Reagents and conditions: (i) NaOH, chloroacetic acid, 100 °C; (ii) HCl; (iii) $SOCl_2$, 80 °C; (iv) $NH_3{\cdot}H_2O$, 0–5 °C; (v) P_2S_5, THF, 50 °C; (vi) propionyl chloride or butyryl chloride, $AlCl_3$, CS_2, 50 °C, HCl; (vii) $CuBr_2$, AcOEt, $CHCl_3$, 60 °C; (viii) CH_3OH, 66 °C; (ix) paraformaldehyde, dimethylamine hydrochloride, 100–150 °C; (x) 10% $NaHCO_3$, 85 °C, HCl; (xi) NH_3 (g), DCC, rt.

compounds **5** were synthesized by this method. Another method to obtain **5** was that ammonia gas was passed into the solution of compounds **10** in tetrahydrofuran for 24 h at room temperature in the presence of DCC. Compounds **5** with dichloro-substituted benzene ring were synthesized using this method. The key intermediates **7** were synthesized from the corresponding substituted methyl ketones **6** by refluxing for 5–12 h together with copper bromide in ethyl acetate and chloroform. The thiazoles **8** were obtained by refluxing the mixture of compounds **5** and compounds **7** in anhydrous methanol for 24 h.[18] Then compounds **8** reacted with paraformaldehyde and dimethylamine hydrochloride in the presence of a catalytic amount of acetic acid to give Mannich compounds which were then converted to target compounds **9** under alkaline condition via an elimination reaction.[15]

2.2. The inhibition of EA thiazole derivatives on GSTpi activity and HL-60 cell proliferation

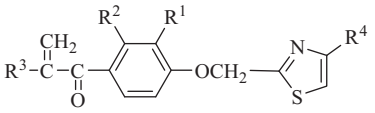
Table 1 showed the structures of EA analogues containing a heterocyclic thiazole and the inhibition of each compound on GSTpi activity as well as on HL-60 cell proliferation. The inhibitory effects of these compounds on GSTpi activity of HL-60 cell lysates were determined in vitro as we reported previously.[17] The cell growth inhibitory effects of these compounds were determined by counting cell number treated with a variety of concentrations of each compounds and the concentration inhibiting half of the cell proliferation (GI_{50}) was calculated.

From the results shown in Table 1, it was found that **9a–9c** exhibited much more potent inhibition than EA on the GSTpi activity. The inhibition rate of GSTpi activity of EA was 65.1% at the concentration of 5 μM, while the inhibition rates of **9a–9c** were 54.1%, 73.1%, 44.0% at the concentration of 1 μM, respectively. All **9a–9c** had a phenyl substituted thiazole, a middle aromatic ring with one or double substituents and a R^3-methyl. Among the three compounds **9b** was the most active in inhibiting GSTpi activity, which has a chlorine-substituted aromatic ring as EA has. Compared to **9a**, compounds **9g** and **9i**, which only have a different substituted benzene ring, had significantly decreased abilities of inhibiting GSTpi activity. The inhibitory effects of compounds **9m–9o**, which have a deleted chlorine at R^1 and a substituted benzene ring, on GSTpi activity were similar to that of EA, but were weaker than that of **9b**. Compared **9m–9o** with **9j–9l**, it was inferred that compounds with methyl group at R^3 position were much more efficient than those with ethyl substitute at R^3 in inhibiting GSTpi activity. The antiproliferative activity of these thiazole derivatives on HL-60 cell proliferation were similar to that of our previously synthesized EA oxadiazole analogues[15] but were much greater than that of the lead compound EA, suggesting that antiproliferative effects of these compounds are independent of their inhibition on GSTpi activity. Some compounds with weaker inhibition on GSTpi activity exhibited improved antiproliferative activity, such as **9j** and **9k**. These data revealed that (1) compounds with R^3-methyl were much more efficient than those with R^3-ethyl in inhibiting GSTpi activity; (2) compounds with larger substituents, such as 4-NO_2 phenyl, 2-naphthyl or 4-CF_3 phenyl on thiazole showed decreased ability to inhibit GSTpi activity compared to those compounds with phenyl at the same position; (3) EA thiazole derivatives had improved antiproliferative ability over EA but that is not correlated with their inhibitory effects on GSTpi activity; (4) EA thiazole derivatives with R^3-methyl and R^4-phenyl had much greater inhibitory effects on the GSTpi activity. Compounds **9a–9c** may represent a new group of GSTpi inhibitors with antiproliferative activity in leukemia cells and are worthy of further study.

3. Experimental

Unless otherwise noted, all materials were obtained from commercial suppliers and used without further purification. All melting points were determined on a Büchi capillary melting point apparatus and were uncorrected. The infrared (IR) spectra were measured on KBr pellets using a Nicolet Nexus 470FT-IR spectrometer and were expressed in cm^{-1}. The proton nuclear magnetic resonance (^1H NMR) spectra were recorded with a Bruker Avance DRX600 instrument with tetramethyl-silane (TMS) as the internal

Table 1
The structures of targeting compounds and their inhibitory effects on GSTpi activities



Code	R^1	R^2	R^3	R^4	GSTpi activity inhibition (%, 5 μM)[a]	GSTpi activity inhibition (%, 1 μM)[b]	GI_{50} (μM) for HL-60[c]
EA	Cl	Cl	CH_2CH_3	—	65.1	24.2	44.2
9a	CH_3	CH_3	CH_3	Phenyl	100	54.1	3.4
9b	H	Cl	CH_3	Phenyl	100	73.1	6.4
9c	H	CH_3	CH_3	Phenyl	100	44.0	4.7
9d	CH_3	CH_3	CH_2CH_3	4-NO_2 Phenyl	4.4	—	3.6
9e	CH_3	CH_3	CH_2CH_3	2-Naphthyl	0	—	2.0
9f	CH_3	CH_3	CH_2CH_3	4-CF_3 Phenyl	0	—	1.5
9g	CH_3	CH_3	CH_3	4-NO_2 Phenyl	17.2	—	5.1
9h	CH_3	CH_3	CH_3	2-Naphthyl	11.3	—	2.3
9i	CH_3	CH_3	CH_3	4-CF_3 Phenyl	3.6	—	3.1
9j	H	Cl	CH_2CH_3	4-NO_2 Phenyl	10.2	—	0.8
9k	H	Cl	CH_2CH_3	2-Naphthyl	9.5	—	0.9
9l	H	Cl	CH_2CH_3	4-CF_3 Phenyl	19.7	—	2.0
9m	H	Cl	CH_3	4-NO_2 Phenyl	86.7	—	5.9
9n	H	Cl	CH_3	2-Naphthyl	55.2	—	2.9
9o	H	Cl	CH_3	4-CF_3 Phenyl	72.3	—	1.4
9p	Cl	Cl	CH_2CH_3	4-NO_2 Phenyl	8.1	—	1.0
9q	Cl	Cl	CH_2CH_3	2-Naphthyl	4.3	—	3.6
9r	Cl	Cl	CH_2CH_3	4-CF_3 Phenyl	22.2	—	1.6

[a] The inhibition rates of GSTpi were calculated by the value of control group minus the value of treated group divided by the value of control group and multiplying by 100 at the concentration of 5 μM. Each group had triplicate samples.
[b] The inhibition rates of GSTpi were tested at the concentration of 1 μM. Each group had triplicate samples.
[c] GI_{50} is the concentration that inhibits 50% of proliferation compared to untreated cells.

standard. The chemical shifts (δ) were reported in parts per million (ppm) and were relative to the central peak of the solvent, which was DMSO-d_6 or $CDCl_3$. Coupling constants (J) were given in Hz. Data were reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, br = broad, m = multiplet), coupling constants, and number of protons. Mass spectra (MS) were measured with an API 4000 and the high resolution mass spectra data were obtained using an Accela UPLC-LTQ Orbitrap Mass Spectrometer. Column chromatography was carried out in the solvents indicated with silica gel. Petroleum ether used for column chromatography had a boiling range of 60–90 °C.

3.1. Synthesis of the compound 3 series

The intermediates **2** were synthesized via methods reported previously.[17] Compounds **2** (20 mmol) and thionyl chloride (15 mL, 200 mmol) were added to a 50 mL round-bottomed flask, the mixture was refluxed for 4 h, and then thionyl chloride was evaporated at reduced pressure. Anhydrous toluene (10 mL) was added to the acyl chloride, and the yielded solution was added dropwise to aqueous ammonia (10 mL) in a 100 mL three-necked flask equipped with a drying tube at 0–5 °C. The mixture was stirred at that temperature for another 1.5 h. After filtration the solvent was removed, the remaining residue was solved in hot acetone, the insoluble was discarded. Crude product was obtained by evaporating the solvent and purified by recrystallization from ethanol–water to obtain compounds **3**.

Compound **3a**: 2-(2,3-dimethylphenoxy)acetamide, white crystalline solid, yield 74.6%. ^{1}H NMR (DMSO-d_6) δ (ppm): 7.34 (s, 1H), 7.35 (s, 1H), 7.01 (t, J = 8.40 Hz, 1H), 6.78 (d, J = 7.80 Hz, 1H), 6.67 (d, J = 8.40 Hz, 1H), 4.39 (s, 2H,), 2.21 (s, 3H), 2.13 (s, 3H). MS (calcd/found) $[M+H]^+$: 180.09/180.9.

Compound **3b**: 2-(3-chlorophenoxy)acetamide, white crystalline solid, yield 45.7%. ^{1}H NMR (DMSO-d_6) δ (ppm): 7.56 (s, 1H), 7.41 (s, 1H), 7.32 (t, J = 7.20 Hz, 1H), 7.01–7.03 (m, 2H), 6.92–6.94 (m, 1H), 4.46 (s, 2H). MS (calcd/found) $[M+H]^+$: 185.61/185.3.

Compound **3c**: 2-(3-methylphenoxy)acetamide, white crystalline solid, yield 56.9%, ^{1}H NMR (DMSO-d_6) δ (ppm): 7.48 (s, 1H), 7.38 (s, 1H), 7.16 (t, J = 8.40 Hz, 1H), 6.77–6.78(m, 2H), 6.73 (d, J = 9.00 Hz, 1H), 4.38 (s, 2H), 2.27 (s, 3H). MS (calcd/found) $[M+H]^+$: 165.19/166.4.

3.2. Synthesis of the compound 11 series

Compounds **10** were synthesized via methods reported previously.[17] Tetrahydrofuran (40 mL) was added to the mixture of compounds **10** (20 mmol) and DCC (6.18 g, 30 mmol). After mixing well, amount of ammonia gas was passed into the mixture for 24 h at room temperature. After filtration the filter cake was washed with tetrahydrofuran and the filtrate was collected. The crude product was obtained with the solvent evaporating and purified by column chromatography. The eluent was ethyl acetate/petroleum ether (1:2).

Compound **11**: 2-(4-butyryl-2,3-dichlorophenoxy)acetamide, white powder, yield 69.9%, mp 95–96 °C. ^{1}H NMR (DMSO-d_6) δ: 7.65 (d, J = 9.00 Hz, 1H), 7.47 (s, 1H), 7.45 (s, 1H), 7.07 (d, J = 9.00 Hz, 1H), 4.73 (s, 2H), 2.91 (t, J = 7.20 Hz, 2H), 1.56–1.60 (m, 2H), 0.90 (t, J = 7.20 Hz, 3H). MS (calcd/found) $[M]^+$: 290.14/290.2.

3.3. Synthesis of the compound 4 series

Compounds **3** (20 mmol) were dissolved in tetrahydrofuran (5 mL) in a 50 mL flask, then the solution was heated up to 50 °C slowly, phosphorus pentasulfide (40 mmol) was added in batches and stirred for 1.5 h at the same temperature. After cooling to room temperature, the reaction solution was poured to a beaker with 100 mL mixture of ice-cold water standing for another 2 h. And then it was extracted with ethyl acetate for 3 times, the organic

phase was evaporated to obtain the crude product. Compounds **4** were yielded by dissolving the crude product in hot ethyl acetate, filtering while hot, and the solvent evaporating at reduced pressure.

Compound **4a**: 2-(2,3-dimethylphenoxy)ethanethioamide, white crystalline solid, yield 94.3%, mp 109–111 °C. ^{1}H NMR (DMSO-d_6) δ (ppm): 10.00 (s, 1H), 9.17 (s, 1H), 7.01 (t, J = 7.80 Hz, 1H), 6.80 (d, J = 7.80 Hz, 1H), 6.64 (d, J = 8.40 Hz, 1H), 4.71 (s, 2H), 2.22 (s, 3H), 2.15 (s, 3H). MS (calcd/found) [M+H]$^+$: 196.07/196.3.

Compound **4b**: 2-(3-chlorophenoxy)ethanethioamide, white crystalline solid, yield 75.5%, mp 119–122 °C. ^{1}H NMR (DMSO-d_6) δ (ppm): 10.04 (s, 1H), 9.40 (s, 1H), 7.33 (t, J = 8.40 Hz, 1H), 7.03–7.06 (m, 2H), 7.01 (dd, J_1 = 2.40 Hz, J_2 = 7.80 Hz, 1H), 4.79 (s, 2H). MS (calcd/found) [M+H]$^+$: 202.00/202.2.

Compound **4c**: 2-(m-tolyloxy)ethanethioamide, white crystalline solid, yield 95.6%. ^{1}H NMR (DMSO-d_6) δ (ppm): 9.97 (s, 1H), 9.30 (s, 1H), 7.14 (t, J = 7.80 Hz, 1H), 6.75–6.77 (m, 2H), 6.72 (dd, J_1 = 7.80 Hz, J_2 = 2.40 Hz, 1H), 4.69 (s, 2H), 2.41 (s, 3H). MS (calcd/found) [M+H]$^+$: 182.06/182.3.

3.4. Synthesis of the compound 5 series

Method A: carbon disulfide (30 mL) was added to compounds **4** in a three-necked flask and anhydrous aluminum chloride (4.0 g, 30 mmol) was added in batches at room temperature. A constant pressure dropping funnel was used to add propionyl chloride or butyryl chloride (15 mmol) dropwise to the mixture to yield viscous material after stirring for 0.5 h, then it was heated to 50 °C slowly and refluxed for 4 h, cooled to room temperature and the supernatant fluid was decanted. Amount of ice-cold water was added to the flask at 0 °C to yield yellow viscous solid which was extracted with ethyl acetate. The solvent of organic layer was evaporating at reduced pressure to yield the crude product which was purified by flash chromatography with ethyl acetate /petroleum ether (1:3) to give **5**.

Compound **5a**: 2-[2,3-dimethyl-4-(1-oxo-propyl)phenoxy]ethanethioamide, yellowish solid, yield 51.8%, mp 139–141 °C. ^{1}H NMR (DMSO-d_6) δ: 10.02 (s, 1H), 9.21 (s, 1H), 7.53 (d, J = 8.40 Hz, 1H), 6.71 (d, J = 8.40 Hz, 1H), 4.81 (s, 2H), 2.86 (q, J = 7.80 Hz, 2H), 2.26 (s, 3H), 2.19 (s, 3H), 1.03 (t, J = 7.80 Hz, 3H). MS (calcd/found) [M+H]$^+$: 252.10/252.4.

Compound **5b**: 2-[3-chloro-4-(1-oxo-propyl)phenoxy]ethanethioamide, white solid, yield 36.6%, mp 116–119 °C. ^{1}H NMR (DMSO-d_6) δ: 10.05 (s, 1H), 9.43 (s, 1H), 7.73 (d, J = 9.00 Hz, 1H), 7.13 (d, J = 1.80 Hz, 1H), 7.02 (dd, J_1 = 8.40 Hz, J_2 = 1.80 Hz, 1H) , 4.87 (s, 2H), 2.92 (q, J = 7.20 Hz, 2H), 1.05 (t, J = 7.20 Hz, 3H). MS (calcd/found) [M+H]$^+$: 257.74/258.2.

Compound **5c**: 2-[3-methyl-4-(1-oxo-propyl)phenoxy]ethanethioamide, yellow solid, yield 51.5%, ^{1}H NMR (DMSO-d_6) δ: 10.04 (s, 1H), 9.40 (s, 1H), 7.84 (d, J = 8.40 Hz, 1H), 6.89 (s, 1H), 6.87 (d, J = 7.20 Hz , 1H), 4.82 (s, 2H), 2.91 (q, J = 7.20 Hz, 2H), 2.42 (s, 3H), 1.04 (t, J = 7.20 Hz, 3H). MS (calcd/found) [M+H]$^+$: 238.08/238.2.

Compound **5d**: 2-[2,3-dimethyl-4-(1-oxo-butyl)phenoxy] ethanethioamide, yellowish solid, yield 73.5%, mp 147–149 °C. ^{1}H NMR (DMSO-d_6) δ: 10.02 (s, 1H), 9.21 (s, 1H), 7.52 (d, J = 8.40 Hz, 1H), 6.72 (d, J = 8.40 Hz, 1H), 4.81 (s, 2H), 2.82 (t, J = 7.20 Hz, 2H), 2.26 (s, 3H), 2.19 (s, 3H), 1.55–1.59 (m, 2H), 0.89 (t, J = 7.20 Hz, 3H). MS (calcd/found) [M+H]$^+$: 265.37/266.4.

Compound **5e**: 2-[3-chloro-4-(1-oxo-butyl)phenoxy]ethanethioamide, white solid, yield 54.6%, mp 115–117 °C. ^{1}H NMR (DMSO-d_6) δ: 10.05 (s, 1H), 9.43 (s, 1H), 7.71 (d, J = 8.40 Hz, 1H), 7.13 (d, J = 3.00 Hz, 1H), 7.02 (dd, J_1 = 9.00 Hz, J_2 = 2.4 Hz, 1H), 4.87 (s, 2H), 2.89 (t, J = 7.20 Hz, 2H), 1.57–1.61 (m, 2H), 0.90 (t, J = 7.20 Hz, 3H). MS (calcd/found) [M+H]$^+$: 271.76/272.4.

Method B: compounds **11** and tetrahydrofuran (5 mL) were added to a 50 mL flask, and the next steps were same with the synthesis of the compound **4** series mentioned above.

Compound **5f**: 2-[2, 3-dichloro-4-(1-oxo-butyl) phenoxy] ethanethioamide, white solid, yield 85.6%, mp 135–137 °C. ^{1}H NMR (DMSO-d_6) δ: 10.08 (s, 1H), 9.21 (s, 1H), 7.65 (d, J = 9.00 Hz, 1H), 7.03 (d, J = 9.00 Hz, 1H), 5.01 (s, 2H), 2.88 (t, J = 7.20 Hz, 2H), 1.55 (m, 2H), 0.91 (t, J = 1.20 Hz, 3H). MS (calcd/found) [M+H]$^+$: 306.21/306.4.

3.5. Synthesis of the compound 7 series

To a solution of substituted methyl ketones (**6**, 20 mmol) in ethyl acetate (15 mL) and chloroform (15 mL), copper bromide (8.92 g, 40 mmol) was added in batches and it was slowly heated to 60 °C for 5–12 h, filtered while hot, the filtrate was washed with 10% hydrochloric acid (30 mL × 3) and water (30 mL × 3), dried with anhydrous sodium sulfate. After filtration the solvent was evaporated at reduced pressure and the compounds **7** were recrystallized from ethanol.

3.6. Synthesis of the compound 8 series

An equimolar mixture of the compounds **5** (10 mmol) and compounds **7** (10 mmol) in anhydrous methanol was refluxed for 24 h. The mass was cooled to room temperature, the solid obtained was filtered and dried.[18]

Compound **8a**: 2-[2,3-dimethyl-4-(1-oxo-propyl)phenoxymethyl]-4-phenylthiazole, yellowish solid, yield 53.4%, mp 81–83 °C. ^{1}H NMR (DMSO-d_6) δ: 8.17 (s, 1H), 7.98 (d, J = 7.80 Hz, 2H), 7.57 (d, J = 8.40 Hz, 1H), 7.46 (t, J = 7.80 Hz, 2H), 7.36 (t, J = 7.80 Hz ,1H), 7.06 (d, J = 8.40 Hz, 1H), 5.57 (s, 2H), 2.88 (q, J = 7.20 Hz, 2H), 2.28 (s, 3H), 2.22(s, 3H), 1.04 (t, J = 7.20 Hz, 3H). HRMS (ESI) m/z for $C_{21}H_{22}NO_2S$ [M+H]$^+$: calculated 352.1366 found 352.1391.

Compound **8b**: 2-[3-chloro-4-(1-oxo-propyl)phenoxymethyl]-4-phenylthiazole, yellow solid, yield 48.1%. ^{1}H NMR (DMSO-d_6) δ: 8.19 (s, 1H), 7.97 (d, J = 7.20 Hz ,2H), 7.74 (d, J = 9.00 Hz, 1H), 7.46 (t, J = 7.80 Hz, 2H), 7.36 (t, J = 7.80 Hz ,1H), 7.32 (d, J = 2.40 Hz ,1H), 7.17 (dd, J = 8.40 Hz, J = 2.40 Hz, 1H), 5.62 (s, 2H), 2.93 (q, J = 7.20 Hz, 2H), 1.05 (t, J = 7.20 Hz, 3H). MS (calcd/found) [M+H]$^+$: 358.06/358.2.

Compound **8c**: 2-[3-methyl-4-(1-oxo-propyl)phenoxymethyl]-4-phenylthiazole, yellow solid, yield 27.4%. ^{1}H NMR (DMSO-d_6) δ: 8.17 (s, 1H), 7.97 (d, J = 7.80 Hz ,2H), 7.86 (d, J = 9.00 Hz, 1H), 7.46 (t, J = 7.80 Hz, 2H), 7.36 (t, J = 7.20 Hz ,1H), 7.02 (d, J = 7.20 Hz, 2H), 5.58 (s, 2H), 2.92 (q, J = 7.20 Hz, 2H), 2.44 (s, 3H), 1.04 (t, J = 7.20 Hz, 3H). MS (calcd/found) [M+H]$^+$: 338.11/338.5.

Compound **8d**: 2-[2,3-dimethyl-4-(1-oxo-butyl)phenoxymethyl]-4-(4-nitrophenyl)thiazole, yellowish solid, yield 67.6%, mp 155–156 °C. ^{1}H NMR (DMSO-d_6) δ: 8.54 (s, 1H), 8.33 (d, J = 9.60 Hz, 2H), 8.25 (d, J = 9.00 Hz, 2H), 7.57 (d, J = 9.00 Hz, 1H), 7.07 (d, J = 9.00 Hz, 1H), 5.60 (s, 2H), 2.84 (t, J = 7.20 Hz, 2H), 2.28 (s, 3H), 2.23 (s, 3H), 1.56–1.60 (m, 2H), 0.90 (t, J = 7.20 Hz, 3H). MS (calcd/found) [M+H]$^+$: 410.39/411.4.

Compound **8e**: 2-[2,3-dimethyl-4-(1-oxo-butyl)phenoxymethyl]-4-(naphthalen-2-yl)thiazole, yellowish solid, yield 68.9%, mp 119–122 °C. ^{1}H NMR (DMSO-d_6) δ: 8.55 (s, 1H), 8.32 (s, 1H), 8.12 (dd, J_1 = 8.40 Hz, J_2 = 1.20 Hz, 1H), 7.99–8.02 (m, 2H), 7.94 (d, J = 7.80 Hz, 1H), 7.52–7.59 (m, 3H), 7.09 (d, J = 8.40 Hz, 1H), 5.61 (s, 2H), 2.85 (t, J = 7.20 Hz, 2H), 2.29 (s, 3H), 2.24 (s, 3H), 1.57–1.61 (m, 2H), 0.90 (t, J = 7.20 Hz, 3H). MS (calcd/found) [M+H]$^+$: 415.55/416.5.

Compound **8f**: 2-[2,3-dimethyl-4-(1-oxo-butyl)phenoxymethyl]-4-(4-trifluoromethylphenyl)thiazole, white solid, yield

68.5%, mp 114–117 °C. ^{1}H NMR (DMSO-d_6) δ: 8.42 (s, 1H), 8.20 (d, J = 7.80 Hz, 2H), 7.83 (d, J = 7.80 Hz, 2H), 7.56 (d, J = 8.40 Hz, 1H), 7.067 (d, J = 8.40 Hz, 1H), 5.60 (s, 2H), 2.84 (t, J = 7.2 Hz, 2H), 2.28 (s, 3H), 2.23 (s, 3H), 1.56–1.60 (m, 2H), 0.90 (t, J = 7.20 Hz, 3H). MS (calcd/found) $[M+H]^+$: 433.49/434.4.

Compound **8g**: 2-[2,3-dimethyl-4-(1-oxo-propyl)phenoxymethyl]-4-(4-nitrophenyl) thiazole, white powder, yield 65.6%, mp 144–147 °C. ^{1}H NMR (DMSO-d_6) δ: 8.54 (s, 1H), 8.33 (d, J = 9.60 Hz, 2H), 8.26 (d, J = 9.00 Hz, 2H), 7.57 (d, J = 9.00 Hz, 1H), 7.06 (d, J = 9.00 Hz, 1H), 5.60 (s, 2H), 2.88 (q, J = 7.20 Hz, 2H), 2.28 (s, 3H), 2.23 (s, 3H), 1.04 (t, J = 7.20 Hz, 3H). MS (calcd/found) $[M+H]^+$:396.46/397.4.

Compound **8h**: 2-[2,3-dimethyl-4-(1-oxo-propyl)phenoxymethyl]-4-(naphthalen-2-yl)thiazole, yellowish powder, yield 56.0%, mp 107–109 °C. ^{1}H NMR (DMSO-d_6) δ: 8.55 (s, 1H), 8.32 (s, 1H), 8.12 (dd, J_1 = 9.00 Hz, J_1 = 1.80 Hz, 1H), 7.99–8.02 (m, 2H), 7.94 (d, J = 7.80 Hz, 1H), 7.58 (d, J = 8.40 Hz, 1H), 7.55 (m, 2H), 7.09 (d, J = 9.00 Hz, 1H), 5.61 (s, 2H), 2.88 (q, J = 7.20 Hz, 2H), 2.28 (s, 3H), 2.24 (s, 3H), 1.05 (t, J = 7.20 Hz, 3H). MS (calcd/found) $[M+H]^+$:401.52/402.4.

Compound **8i**: 2-[2,3-dimethyl-4-(1-oxo-propyl)phenoxymethyl]-4-(4-trifluoromethylphenyl)thiazole, yellow solid, yield 58.5%, mp 107–110 °C. ^{1}H NMR (DMSO-d_6) δ: 8.41 (s, 1H), 8.20 (d, J = 8.40 Hz, 2H), 7.83 (d, J = 8.40 Hz, 2H), 7.57 (d, J = 8.40 Hz, 1H), 7.06 (d, J = 9.00 Hz, 1H), 5.59 (s, 2H), 2.88 (q, J = 7.2 Hz, 2H), 2.28 (s, 3H), 2.23 (s, 3H), 1.04 (t, J = 7.20 Hz, 3H). MS (calcd/found) $[M+H]^+$: 419.46/420.4.

Compound **8j**: 2-[3-chloro-4-(1-oxo-butyl)phenoxymethyl]-4-(4-nitrophenyl) thiazole, yellow solid, yield 68.2%, mp 149–151 °C. ^{1}H NMR (DMSO-d_6) δ: 8.56 (s, 1H), 8.33 (d, J = 9.00 Hz, 2H), 8.25 (d, J = 9.00 Hz, 2H), 7.73 (d, J = 9.00 Hz, 1H), 7.32 (d, J = 2.40 Hz, 1H), 7.17 (dd, J_1 = 8.40 Hz, J_2 = 2.40 Hz, 1H), 5.65 (s, 2H), 2.89 (t, J = 7.20 Hz, 2H), 1.57–1.61 (m, 2H), 0.90 (t, J = 7.80 Hz, 3H). MS (calcd/found) $[M+H]^+$:416.88/417.4.

Compound **8k**: 2-[3-chloro-4-(1-oxo-butyl)phenoxymethyl]-4-(naphthalen-2-yl)thiazole, white solid, yield 69.5%, mp 88–90 °C. ^{1}H NMR (DMSO-d_6) δ: 8.55 (s, 1H), 8.34 (s, 1H), 8.12 (dd, J_1 = 9.00 Hz, J_2 = 1.80 Hz, 1H), 7.99–8.01 (m, 2H), 7.94 (d, J = 7.20 Hz, 1H), 7.74 (d, J = 8.40 Hz, 1H), 7.52–7.57 (m, 2H), 7.35 (d, J = 3.00 Hz, 1H), 7.19 (dd, J_1 = 8.40 Hz, J_2 = 2.40 Hz , 1H), 5.67 (s, 2H), 2.90 (t, J = 7.20 Hz, 2H), 1.56–1.63 (m, 2H), 0.91 (t, J = 7.20 Hz, 3H). MS (calcd/found) $[M+H]^+$: 421.94/422.3.

Compound **8l**: 2-[3-chloro-4-(1-oxo-butyl)phenoxymethyl]-4-(4-trifluoromethylphenyl)thiazole, white solid, yield 67.6%, mp 86–88 °C. ^{1}H NMR (DMSO-d_6) δ: 8.43 (s, 1H), 8.20 (d, J = 8.40 Hz, 2H), 7.83 (d, J = 8.40 Hz, 2H), 7.73 (d, J = 8.40 Hz, 1H), 7.32 (d, J = 2.40 Hz, 1H), 7.17 (dd, J_1 = 8.40 Hz, J_2 = 2.40 Hz, 1H), 5.64 (s, 2H), 2.90 (t, J = 7.20 Hz, 2H), 1.57–1.61 (m, 2H), 0.90 (t, J = 7.20 Hz, 3H). MS (calcd/found) $[M+H]^+$: 439.88/440.4.

Compound **8m**: 2-[3-chloro-4-(1-oxo-propyl)phenoxymethyl]-4-(4-nitrophenyl)thiazole, yellow powder, yield 69.6%, mp 148–150 °C. ^{1}H NMR (DMSO-d_6) δ: 8.56 (s, 1H), 8.33 (d, J = 8.40 Hz, 2H), 8.25 (d, J = 9.00 Hz, 2H), 7.75 (d, J = 8.40 Hz, 1H), 7.32 (d, J = 2.40 Hz, 1H), 7.17 (dd, J_1 = 9.00 Hz, J_2 = 2.40 Hz, 1H), 5.66 (s, 2H), 2.93 (q, J = 7.20 Hz, 2H), 1.05 (t, J = 7.20 Hz, 3H). MS (calcd/found) $[M+H]^+$:402.85/403.4.

Compound **8n**: 2-[3-chloro-4-(1-oxo-propyl)phenoxymethyl]-4-(naphthalen-2-yl)thiazole, light red powder, yield 50.0%, mp 109–111 °C. ^{1}H NMR (DMSO-d_6) δ: 8.55 (s, 1H), 8.34 (s, 1H), 8.11 (dd, J_1 = 8.40 Hz, J_2 = 1.80 Hz, 1H), 7.99–8.01 (m, 2H), 7.94 (d, J = 7.20 Hz, 1H), 7.76 (d, J = 9.00 Hz, 1H), 7.55 (m, 2H), 7.35 (d, J = 2.40 Hz, 1H), 7.19 (dd, J_1 = 8.40 Hz, J_2 = 2.40 Hz, 1H), 5.67 (s, 2H), 2.93 (q, J = 7.20 Hz, 2H), 1.06 (t, J = 7.20 Hz, 3H). MS (calcd/found) $[M+H]^+$:407.91/408.4.

Compound **8o**: 2-[3-chloro-4-(1-oxo-propyl)phenoxymethyl]-4-(4-trifluoromethylphenyl)thiazole, white solid, yield 58.5%, mp 88–90 °C. ^{1}H NMR (DMSO-d_6) δ: 8.43 (s, 1H), 8.20 (d, J = 7.80 Hz, 2H), 7.83 (d, J = 7.80 Hz, 2H), 7.75 (d, J = 8.40 Hz, 1H), 7.32 (d, J = 2.40 Hz, 1H), 7.17 (dd, J_1 = 8.40 Hz, J_1 = 2.40 Hz, 1H), 5.65 (s, 2H), 2.93 (q, J = 7.20 Hz, 2H), 1.06 (t, J = 7.20 Hz, 3H). MS (calcd/found) $[M+H]^+$: 425.85/426.2.

Compound **8p**: 2-[2,3-dichloro-4-(1-oxo-butyl)phenoxymethyl]-4-(4-nitrophenyl)thiazole, yellow solid, yield 62.7%, mp 174–176 °C. ^{1}H NMR (DMSO-d_6) δ: 8.57 (s, 1H), 8.33 (d, J = 8.40 Hz, 2H), 8.25 (d, J = 9.00 Hz, 2H), 7.72 (d, J = 9.00 Hz, 1H), 7.45 (d, J = 8.40 Hz, 1H), 5.76 (s, 2H), 2.92 (t, J = 3.60 Hz, 2H), 1.60 (m, 2H), 0.91 (t, J = 7.20 Hz, 3H). MS (calcd/found) $[M+H]^+$: 451.32/451.2.

Compound **8q**: 2-[2,3-dichloro-4-(1-oxo-butyl)phenoxymethyl]-4-(naphthalen-2-yl)thiazole, white powder, yield 58.3%, mp 135–137 °C. ^{1}H NMR (DMSO-d_6) δ: 8.45 (s, 1H), 7.97 (dd, J_1 = 7.20 Hz, J_2 = 1.20 Hz, 1H), 7.90–7.93(m, 2H), 7.86 (d, J = 6.60 Hz, 1H), 7.67 (s, 1H), 7.65 (s, 1H), 7.48–7.53 (m, 2H), 7.23 (s, 1H), 5.56 (s, 2H), 2.92 (t, J = 7.20 Hz, 2H), 1.70–1.76 (m, 2H), 0.98 (t, J = 7.20 Hz, 3H). MS (calcd/found) $[M]^+$: 456.38/456.0729.

Compound **8r**: 2-[2,3-dichloro-4-(1-oxo-butyl)phenoxymethyl]-4-(4-trifluoromethylphenyl)thiazole, white solid, yield 58.5%, mp 88–90 °C. ^{1}H NMR (DMSO-d_6) δ: 8.01 (d, J = 7.80 Hz, 2H), 7.69 (d, J = 8.40 Hz, 2H), 7.65 (s, 1H), 7.38 (d, J = 8.40 Hz, 1H), 7.04 (d, J = 8.40 Hz, 1H), 5.53 (s, 2H), 2.91 (t, J = 7.20 Hz, 2H), 1.72 (m, 2H), 0.98 (t, J = 7.20 Hz, 3H). MS (calcd/found) $[M]^+$: 474.32/474.0307.

3.7. Synthesis of the compound 9 series

In a 50 mL round-bottomed flask a mixture of compounds **8** (9.6 mmol), paraformaldehyde (0.38 g, 11.5 mmol), dimethylamine hydrochloride (0.91 g, 10.4 mmol) and acetic acid (0.05 mL) was heated to 100 °C for about 1.5 h, during which period suction was applied. When the mixture was cooled to room temperature, the obtained Mannich compounds were dissolved in 10% sodium bicarbonate and modulated to slight alkalinity. The resulting solution was heated to 85 °C for 40 min and extracted with ethyl acetate, the solvent of organic layer was evaporated at reduced pressure to yield oil. The target compounds **9** were obtained by column chromatography, the eluent was acetone/petroleum ether (1:20).

Compound **9a**: 2-[2,3-dimethyl-4-(2-methylene-1-oxo-propyl) phenoxymethyl]-4-phenylthiazole, white powder, yield 39.5%, mp 69–70 °C. ^{1}H NMR ($CDCl_3$-d_3) δ: 7.90 (d, J = 7.80 Hz, 2H), 7.51 (s, 1H), 7.44 (t, J = 7.80 Hz, 2H), 7.35 (t, J = 7.80 Hz, 1H), 7.09 (d, J = 7.80 Hz ,1H), 6.81 (d, J = 7.80 Hz, 1H), 5.92 (s, 1H), 5.57 (s, 1H), 5.45 (s, 2H), 2.29 (s, 3H), 2.21 (s, 3H), 2.04 (s, 3H). IR (KBr, cm^{-1}) $\nu_{=CH}$: 3113.78, 3068.66, 3025.33; ν_{CH}: 2976.26, 2922.56; $\nu_{C=O}$: 1653.48; $\nu_{C=C}$: 1624.97, 1589.43, 1516.50; δ_{CH}: 1444.25, 1357.02, 1324.59; ν_{C-O}: 1266.54, 1230.89; $\gamma_{=CH}$: 1090.84, 947.82, 803.22, 741.60. HRMS (ESI) m/z for $C_{22}H_{22}NO_2S$ $[M+H]^+$: calculated 364.1366 found 364.1381.

Compound **9b**: 2-[3-chloro-4-(2-methylene-1-oxo-propyl) phenoxymethyl]-4-phenylthiazole, yield 75.0%, mp 138–139 °C. ^{1}H NMR (DMSO-d_6) δ: 8.20 (s, 1H), 7.97 (d, J = 7.80 Hz, 2H), 7.46 (t, J = 7.80 Hz, 2H), 7.39 (d, J = 8.40 Hz, 1H), 7.36 (t, J = 7.20 Hz ,1H), 7.34 (dd, $J_{1\,=\,7.80}$ Hz, J_2 = 2.40 Hz, 1H), 7.15–7.18 (m, 1H), 6.12 (s, 1H), 5.61 (s, 1H), 5.52 (s, 2H), 1.95 (s, 3H). IR (KBr, cm^{-1}) $\nu_{=CH}$: 3099.94, 3070.24; ν_{CH}: 2976.32, 2922.48, 2880.86; $\nu_{C=O}$: 1650.91; $\nu_{C=C}$: 1598.16, 1559.57, 1507.16, 1495.80; δ_{CH}: 1470.24, 1456.71, 1445.02, 1369.65, 1335.97, 1298.32; ν_{C-O}:1243.95, 1230.95; $\gamma_{=CH}$: 1061.94, 917.04, 841.17, 758.00. HRMS (ESI) m/z for $C_{20}H_{17}ClNO_2S$ $[M+H]^+$: calculated 370.0663 found 370.0676.

Compound **9c**: 2-[3-methyl-4-(2-methylene-1-oxo-propyl) phenoxymethyl]-4-phenylthiazole, white powder, yield 31.3%, mp 129–131 °C. ^{1}H NMR ($CDCl_3$-d_3) δ: 7.91 (d, J = 7.80 Hz, 2H),

7.52 (s, 1H), 7.44 (t, J = 7.80 Hz, 2H), 7.36 (t, J = 7.20 Hz, 1H), 7.29 (d, J = 8.40 Hz ,1H), 6.91 (s, 1H), 6.85 (dd, J_1 = 8.40 Hz, J_2 = 2.40 Hz, 1H), 5.91 (s, 1H), 5.55 (s, 1H), 5.47 (s, 2H), 2.34 (s, 3H), 2.04 (s, 3H). IR (KBr, cm^{-1}) $\nu_{=CH}$: 3095.89, 3059.71; ν_{CH}: 2983.90, 2956.19, 2921.15; $\nu_{C=O}$: 1633.70; $\nu_{C=C}$: 1599.79, 1508.37; δ_{CH}: 1445.40, 1367.85, 1336.32, 1309.41; ν_{C-O}:1248.35, 1232.96; $\gamma_{=CH}$: 1011.83, 957.11, 847.16, 762.30. HRMS (ESI) m/z for $C_{21}H_{20}NO_2S$ $[M+H]^+$: calculated 350.1209 found 350.1223.

Compound **9d**: 2-[2,3-dimethyl-4-(2-methylene-1-oxo-butyl) phenoxymethyl]-4-(4-nitrophenyl)thiazole, white solid, yield 62.3%, mp 145–152 °C. ^{1}H NMR (DMSO-d_6) δ: 8.54 (s, 1H), 8.33 (d, J = 9.00 Hz, 2H), 8.25 (d, J = 9.00 Hz, 2H), 7.10 (d, J = 8.40 Hz, 1H), 7.05 (d, J = 8.40 Hz, 1H), 5.95 (s, 1H), 5.58 (s, 2H), 5.49 (s, 1H), 2.38 (q, J = 7.20 Hz, 2H), 2.23 (s, 3H), 2.14 (s, 3H), 1.07 (t, J = 7.20 Hz, 3H). IR (KBr, cm^{-1}): $\nu_{=CH}$: 3121.10; ν_{CH}: 2963.22, 2921.64, 2873.38; $\nu_{C=O}$: 1641.89; $\nu_{C=C}$: 1599.36, 1578.59, 1522.28, 1509.61, 1482.03; ν_{NO2}: 1341.16; ν_{C-O}: 1276.62; $\gamma_{=CH}$: 1099.88, 857.00, 847.12, 744.80. HRMS (ESI) m/z for $C_{23}H_{23}N_2O_4S$ $[M+H]^+$: calculated 423.1373 found 423.1371.

Compound **9e**: 2-[2,3-dimethyl-4-(2-methylene-1-oxo-butyl) phenoxymethyl]-4-(naphthalen-2-yl)thiazole, white solid, yield 72.6%, mp 134–136 °C. ^{1}H NMR (DMSO-d_6) δ: 8.55 (s, 1H), 8.33(s, 1H), 8.12 (dd, J_1 = 8.40 Hz, J_2 = 1.20 Hz, 1H), 7.99–8.02 (m, 2H), 7.94 (d, J = 7.20 Hz, 1H), 7.52–7.56 (m, 2H), 7.10 (q, J = 8.40 Hz, 2H), 5.95 (s, 1H) 5.59 (s, 2H), 5.50 (s, 1H), 2.38 (q, J = 7.20 Hz, 2H), 2.25 (s, 3H), 2.15 (s, 3H),1.07 (t, J = 7.20 Hz, 3H). IR (KBr, cm^{-1}): $\nu_{=CH}$: 3103.45, 3058.86; ν_{CH}: 2965.60, 2919.85, 2872.20; $\nu_{C=O}$: 1651.76; $\nu_{C=C}$: 1590.94, 1519.59, 1483.20, 1445.57; δ_{CH}: 1353.74; ν_{C-O}: 1268.86; $\gamma_{=CH}$: 1087.32, 943.79, 802.25, 760.07. HRMS (ESI) m/z for $C_{27}H_{26}NO_2S$ $[M+H]^+$: calculated 428.1679 found 428.1677.

Compound **9f**: 2-[2,3-dimethyl-4-(2-methylene-1-oxo-butyl) phenoxymethyl]-4-(4-trifluoromethylphenyl) thiazole, white solid, yield 78.5%, mp 86–87 °C. ^{1}H NMR (DMSO-d_6) δ: 8.42 (s, 1H), 8.20 (d, J = 7.80 Hz, 2H), 7.83 (d, J = 7.80 Hz, 2H), 7.10 (d, J = 8.40 Hz, 1H), 7.05 (d, J = 8.40 Hz, 1H), 5.95 (s, 1H), 5.57 (s, 2H), 5.49 (s, 1H), 2.38 (q, J = 7.20 Hz, 2H), 2.23 (s, 3H), 2.14 (s, 3H), 1.07 (t, J = 7.20 Hz, 3H). IR (KBr, cm^{-1}): $\nu_{=CH}$: 3114.66; ν_{CH}: 2966.08, 2918.55, 2874.16; $\nu_{C=O}$: 1649.55; $\nu_{C=C}$: 1617.86, 1590.25, 1508.20; δ_{CH}: 1329.14; ν_{C-O}: 1271.02; $\gamma_{=CH}$: 1069.70, 854.25, 765.93. HRMS (ESI) m/z for $C_{24}H_{23}F_3NO_2S$ $[M+H]^+$: calculated 446.1396 found 446.1393.

Compound **9g**: 2-[2,3-dimethyl-4-(2-methylene-1-oxo-propyl) phenoxymethyl]-4-(4-nitrophenyl)thiazole, white powder, yield 75.6%, mp 158–160 °C. ^{1}H NMR (DMSO-d_6) δ: 8.54 (s, 1H), 8.33 (d, J = 9.00 Hz, 2H), 8.25 (d, J = 9.00 Hz, 2H), 7.11 (d, J = 8.40 Hz, 1H), 7.05 (d, J = 8.40 Hz, 1H), 6.04 (s, 1H), 5.58 (s, 2H), 5.48 (s, 1H), 2.23 (s, 3H), 2.13 (s, 3H), 1.96 (s, 3H). IR (KBr, cm^{-1}): $\nu_{=CH}$: 3094.47; ν_{CH}: 2922.45, 2848.60; $\nu_{C=O}$: 1653.48; $\nu_{C=C}$: 1597.22, 1579.44, 1507.98, 1482.34; ν_{NO2}: 1336.32; ν_{C-O}: 1267.42; $\gamma_{=CH}$: 1088.68, 862.79, 844.75, 795.59, 753.79. HRMS (ESI) m/z for $C_{22}H_{21}N_2O_4S$ $[M+H]^+$: calculated 409.1217 found 409.1214.

Compound **9h**: 2-[2,3-dimethyl-4-(2-methylene-1-oxo-propyl) phenoxymethyl]-4-(naphthalen-2-yl)thiazole, white powder, yield 66.0%, mp 116–118 °C. ^{1}H NMR (DMSO-d_6) δ: 8.55 (s, 1H), 8.32(s, 1H), 8.12 (dd, J_1 = 7.2 Hz, J_2 = 1.8 Hz, 1H), 7.99–8.02 (m, 2H), 7.94 (d, J = 7.20 Hz, 1H), 7.52–7.56(m, 2H), 7.12 (d, J = 8.40 Hz, 1H), 7.07 (d, J = 8.40 Hz, 1H), 6.04 (s, 1H), 5.59 (s, 2H), 5.49 (s, 1H), 2.25 (s, 3H), 2.14 (s, 3H), 1.96 (s, 3H). IR (KBr, cm^{-1}): $\nu_{=CH}$: 3111.49, 3051.20; ν_{CH}: 2972.96, 2919.32, 2842.02; $\nu_{C=O}$: 1655.22; $\nu_{C=C}$: 1593.12, 1584.01, 1520.90, 1484.39, 1446.79; δ_{CH}: 1354.34; ν_{C-O}: 1268.09; $\gamma_{=CH}$: 1085.37, 943.38, 858.61, 808.86, 769.40, 761.59. HRMS (ESI) m/z for $C_{26}H_{24}NO_2S$ $[M+H]^+$: calculated 414.1522 found 414.1520.

Compound **9i**: 2-[2,3-dimethyl-4-(2-methylene-1-oxo-propyl) phenoxymethyl]-4-(4-trifluoromethylphenyl)thiazole, white solid, yield 68.5%, mp 104–106 °C. ^{1}H NMR (DMSO-d_6) δ: 8.42 (s, 1H), 8.20 (d, J = 8.40 Hz, 2H), 7.83 (d, J = 8.40 Hz, 2H), 7.11 (d, J = 8.40 Hz, 1H), 7.06 (d, J = 9.00 Hz, 1H), 6.04 (s, 1H), 5.57 (s, 2H), 5.48 (s, 1H), 2.23 (s, 3H), 2.13 (s, 3H), 1.96 (s, 3H). IR (KBr, cm^{-1}): $\nu_{=CH}$: 3116.46, 3088.72; ν_{CH}: 2919.31, 2868.88; $\nu_{C=O}$: 1683.15; $\nu_{C=C}$: 1617.62, 1591.85, 1579.96, 1508.90, 1449.19; δ_{CH}: 1329.73; ν_{C-O}: 1272.48; $\gamma_{=CH}$: 1069.72, 854.17, 766.14. HRMS (ESI) m/z for $C_{23}H_{21}F_3NO_2S$ $[M+H]^+$: calculated 432.1240 found 432.1236.

Compound **9j**: 2-[3-chloro-4-(2-methylene-1-oxo-butyl) phenoxymethyl]-4-(4-nitrophenyl)thiazole, white crystalline solid, yield 76.9%, mp 111–112 °C. ^{1}H NMR (DMSO-d_6) δ: 8.56 (s, 1H), 8.33 (d, J = 9.00 Hz, 2H), 8.25 (d, J = 9.00 Hz, 2H), 7.38 (d, J = 8.40 Hz, 1H), 7.34 (d, J = 9.00 Hz, 1H), 7.17 (dd, J_1 = 8.40 Hz, J_2 = 2.40 Hz, 1H), 6.04 (s, 1H), 5.65 (s, 2H), 5.54 (s, 1H), 2.37 (q, J = 7.2 Hz, 2H), 0.90 (t, J = 7.2 Hz, 3H). IR (KBr, cm^{-1}): $\nu_{=CH}$: 3097.71; ν_{CH}: 2969.51, 2935.62, 2875.13, 2851.09; $\nu_{C=O}$: 1653.84; $\nu_{C=C}$: 1599.22, 1566.07, 1522.36, 1514.60, 1492.26; ν_{NO2}: 1344.80; ν_{C-O}: 1294.67; $\gamma_{=CH}$: 1066.83, 981.27, 903.61, 863.04, 844.37, 749.09. HRMS (ESI) m/z for $C_{21}H_{18}ClN_2O_4S$ $[M+H]^+$: calculated 429.0670 found 429.0668.

Compound **9k**: 2-[3-chloro-4-(2-methylene-1-oxo-butyl) phenoxymethyl]-4-(naphthalen-2-yl)thiazole, white crystalline solid, yield 50.0%, mp 101–102 °C. ^{1}H NMR (DMSO-d_6) δ: 8.55 (s, 1H), 8.35(s, 1H), 8.12 (dd, J_1 = 8.40 Hz, J_2 = 1.20 Hz, 1H), 7.99–8.01(m, 2H), 7.94 (d, J = 7.20 Hz, 1H),7.52–7.56 (m, 2H), 7.41 (d, J = 9.00 Hz, 1H), 7.37 (d, J = 2.40 Hz, 1H), 7.18 (dd, J = 9.00 Hz, J = 2.40 Hz, 1H), 6.04(s, 1H) 5.65 (s, 2H), 5.55 (s, 1H), 2.37(q, J = 7.2 Hz, 2H), 1.07 (s, 3H). IR (KBr, cm^{-1}): $\nu_{=CH}$: 3108.01, 3055.33; ν_{CH}: 2971.25, 2935.43, 2873.26; $\nu_{C=O}$: 1664.80; $\nu_{C=C}$: 1599.45, 1559.55, 1506.87, 1490.74, 1453.91; δ_{CH}: 1356.55; ν_{C-O}: 1298.58, 1236.76; $\gamma_{=CH}$: 1060.17, 1043.76, 979.66, 905.48, 861.42, 831.20, 773.77, 751.80. HRMS (ESI) m/z for $C_{25}H_{21}ClNO_2S$ $[M+H]^+$: calculated 434.0976 found 434.0975.

Compound **9l**: 2-[3-chloro-4-(2-methylene-1-oxo-butyl) phenoxymethyl]-4-(4-trifluoromethylphenyl)thiazole, white solid, yield 58.5%, mp 104–106 °C. ^{1}H NMR (DMSO-d_6) δ: 8.44 (s, 1H), 8.20 (d, J = 7.80 Hz, 2H), 7.83 (d, J = 7.80 Hz, 2H), 7.73 (d, J =8.40 Hz, 1H), 7.34 (d, J = 2.40 Hz, 1H), 7.33 (dd, J_1 = 8.40 Hz, J_2 = 2.4 Hz, 1H), 6.04 (s, 1H), 5.64 (s, 2H), 5.54 (s, 1H), 2.37 (q, J = 7.20 Hz, 2H), 1.07 (t, J = 7.20 Hz, 3H). IR (KBr, cm^{-1}): $\nu_{=CH}$: 3115.44; ν_{CH}: 2968.58, 2935.49, 2879.43; $\nu_{C=O}$: 1661.06; $\nu_{C=C}$: 1599.30, 1565.92, 1511.10, 1492.97, 1456.72; δ_{CH}: 1324.07; ν_{C-O}: 1242.93; $\gamma_{=CH}$: 1070.64, 943.38, 980.57, 909.75, 852.56, 843.13, 760.57, 733.83. HRMS (ESI) m/z for $C_{22}H_{18}ClF_3NO_2S$ $[M+H]^+$: calculated 452.0693 found 452.0691.

Compound **9m**: 2-[3-chloro-4-(2-methylene-1-oxo-propyl) phenoxymethyl-4-(4-nitrophenyl)thiazole, light red crystalline solid, yield 76.9%, mp 88–91 °C. ^{1}H NMR (DMSO-d_6) δ: 8.56 (s, 1H), 8.33 (d, J = 9.60 Hz, 2H), 8.25 (d, J = 9.00 Hz, 2H), 7.40 (d, J = 8.40 Hz, 1H), 7.34 (d, J = 2.40 Hz, 1H), 7.17 (dd, J_1 = 8.40 Hz, J_1 = 2.40 Hz, 1H), 6.12 (s, 1H), 5.64 (s, 2H), 5.53 (s, 1H), 1.95 (s, 3H). IR (KBr, cm^{-1}): $\nu_{=CH}$: 3106.73; ν_{CH}: 2924.34, 2850.22; $\nu_{C=O}$: 1665.75; $\nu_{C=C}$: 1598.82, 1521.78, 1493.18, 1449.99; ν_{NO2}: 1346.38; ν_{C-O}: 1294.13; $\gamma_{=CH}$: 1061.96, 918.06, 861.24, 845.77, 742.48. HRMS (ESI) m/z for $C_{20}H_{16}ClN_2O_4S$ $[M+H]^+$: calculated 415.0514 found 415.0511.

Compound **9n**: 2-[3-chloro-4-(2-methylene-1-oxo-propyl) phenoxymethyl]-4-(naphthalen-2-yl)thiazole, light red crystalline solid, yield 50.0%, mp 105–107 °C. ^{1}H NMR (DMSO-d_6) δ: 8.55 (s, 1H), 8.34 (s, 1H), 8.12 (dd, J_1 = 8.40 Hz , J_2 = 1.20 Hz, 1H), 7.99–8.01(m, 2H), 7.94 (d, J = 7.80 Hz, 1H), 7.52–7.56 (m, 2H), 7.41 (d, J = 8.40 Hz, 1H), 7.37 (d, J = 2.40 Hz, 1H), 7.18 (dd, J_1 = 9.00 Hz, J_2 = 2.40 Hz, 1H), 6.12 (s, 1H) 5.67 (s, 2H), 5.54 (s, 1H), 1.96 (s, 3H). IR (KBr, cm^{-1}): $\nu_{=CH}$: 3114.67, 3088.31, 3055.47; ν_{CH}: 2982.74, 2925.09, 2865.53; $\nu_{C=O}$: 1664.62; $\nu_{C=C}$: 1596.16,

1565.31, 1499.09; δ_{CH}: 1344.43; ν_{C-O}: 1295.92; $\gamma_{=CH}$: 1058.32, 940.78, 862.87, 832.42, 753.65. HRMS (ESI) *m*/*z* for $C_{24}H_{19}ClNO_2S$ $[M+H]^+$: calculated 420.0820 found 420.0817.

Compound **9o**: 2-[3-chloro-4-(2-methylene-1-oxo-propyl) phenoxymethyl]-4-(4-trifluoromethylphenyl) thiazole, yellowish solid, yield 58.5%, mp 87–90 °C. ^{1}H NMR (DMSO-d_6) δ: 8.44 (s, 1H), 8.20 (d, J = 7.80 Hz, 2H), 7.83 (d, J = 7.80 Hz, 2H), 7.40 (d, J = 8.40 Hz, 1H), 7.34 (d, J = 2.40 Hz, 1H), 7.17 (dd, J_1 = 8.40 Hz, J_2 = 2.40 Hz, 1H), 6.12 (s, 1H) 5.63 (s, 2H), 5.53 (s, 1H), 1.95 (s, 3H). IR (KBr, cm^{-1}): $\nu_{=CH}$: 3112.45; ν_{CH}: 2931.62, 2882.16; $\nu_{C=O}$: 1663.30; $\nu_{C=C}$: 1614.86, 1599.73, 1565.84, 1492.42; δ_{CH}: 1323.72; ν_{C-O}: 1292.58; $\gamma_{=CH}$: 1070.57, 917.65, 852.17, 841.66, 759.65. HRMS (ESI) *m*/*z* for $C_{21}H_{16}ClF_3NO_2S$ $[M+H]^+$: calculated 438.0537 found 438.0535.

Compound **9p**: 2-[2,3-dichloro-4-(2-methylene-1-oxo-butyl) phenoxymethyl]-4-(4-nitrophenyl)thiazole, yellowish solid, yield 66.7%, mp 179–182 °C. ^{1}H NMR (DMSO-d_6) δ: 8.31 (d, J = 9.00 Hz, 2H), 8.08 (d, J = 9.00 Hz, 2H), 7.76 (s, 1H), 7.18 (d, J = 8.40 Hz, 1H), 7.04 (d, J = 8.40 Hz, 1H), 5.95 (s, 1H) 5.60 (s, 1H), 5.54 (s, 2H), 2.47 (q, J = 7.80 Hz, 2H), 1.15 (t, J = 7.20 Hz, 3H). IR (KBr, cm^{-1}): $\nu_{=CH}$: 3108.52, 3081.01; ν_{CH}: 2966.14, 2933.30; $\nu_{C=O}$: 1663.67; $\nu_{C=C}$: 1599.08, 1585.97, 1523.99, 1466.83; ν_{NO2}: 1342.94; ν_{C-O}: 1294.13; $\gamma_{=CH}$: 1065.81, 860.37, 846.13, 741.40. HRMS (ESI) *m*/*z* for $C_{21}H_{17}Cl_2N_2O_4S$ $[M+H]^+$: calculated 463.0281 found 463.0277.

Compound **9q**: 2-[2,3-dichloro-4-(2-methylene-1-oxo-butyl) phenoxymethyl]-4-(naphthalen-2-yl)thiazole, white powder, yield 58.3%, mp 135–137 °C. ^{1}H NMR (DMSO-d_6) δ: 8.55 (s, 1H), 8.36 (s, 1H), 8.12 (dd, J_1 = 9.00 Hz, J_2 = 1.20 Hz, 1H), 7.99–8.01 (m, 2H), 7.93 (d, J = 1.80 Hz, 1H), 7.52–7.57 (m, 2H), 7.47 (d, J = 7.80 Hz, 1H), 7.65 (d, J = 7.80 Hz, 1H), 6.09 (s, 1H), 5.76 (s, 2H), 5.60 (s, 1H), 2.38 (q, J = 7.20 Hz, 2H), 1.09 (t, J = 7.20 Hz, 3H). IR (KBr, cm^{-1}): $\nu_{=CH}$: 3102.95, 3058.74; ν_{CH}: 2968.67, 2936.43; $\nu_{C=O}$: 1658.25; $\nu_{C=C}$: 1586.82, 1517.54, 1472.29; δ_{CH}: 1384.69, 1357.99; ν_{C-O}: 1292.40, 1265.56; $\gamma_{=CH}$: 1068.18, 1050.99, 1001.76, 948.00, 811.25, 773.01. HRMS (ESI) *m*/*z* for $C_{25}H_{20}Cl_2NO_2S$ $[M+H]^+$: calculated 468.0586 found 468.0583.

Compound **9r**: 2-[2,3-dichloro-4-(2-methylene-1-oxo-butyl) phenoxymethyl]-4-(4-trifluoromethylphenyl)thiazole, white powder, yield 58.5%, mp 88–90 °C. ^{1}H NMR (DMSO-d_6) δ: 8.01 (d, J = 7.80 Hz, 2H), 7.69 (d, J = 8.40 Hz, 2H), 7.67 (s, 1H), 7.17 (d, J = 9.00 Hz, 1H), 7.04 (d, J = 8.40 Hz, 1H), 5.95 (s, 1H) 5.60 (s, 1H), 5.54 (s, 2H), 2.47 (q, J = 7.20 Hz, 2H), 1.15 (t, J = 7.20 Hz, 3H). IR (KBr, cm^{-1}): $\nu_{=CH}$: 3115.37; ν_{CH}: 2971.43, 2933.25, 2876.80; $\nu_{C=O}$: 1660.78; $\nu_{C=C}$: 1616.31, 1584.58, 1512.46, 1469.03; δ_{CH}: 1328.46, 1300.83; ν_{C-O}: 1267.77; $\gamma_{=CH}$: 1069.99, 1047.60, 855.22, 801.43, 766.11. HRMS (ESI) *m*/*z* for $C_{22}H_{17}Cl_2F_3NO_2S$ $[M+H]^+$: calculated 486.0304 found 486.0301.

3.8. GSTpi activity inhibition assay

HL-60 cells were cultured in RPMI-1640 medium supplemented with 100 U/mL penicillin, 100 μg/mL streptomycin, 1 mmol/L L-glutamine, and 10% heat-inactivated fetal bovine serum in a humidified atmosphere of 95% air and 5% CO_2 at 37 °C. Cell lysates were prepared as we reported before and the GSTpi activity was measured spectrophotometrically at 25 °C using 1-cholro-2,4-dinitrobenzene (CDNB) and GSH as substrates.[17] The CDNB–GSH complex has a strong absorptivity at 340 nm and the extinction coefficient of 9.6 mM^{-1}cm^{-1} was used to calculate GSTpi activity. GSTpi activity was defined as nanomoles of product per minute per milligram of protein. The GSTpi activity inhibition rate was calculated as $(V_c-V_t)/V_c \times 100\%$. V_c refers to GSTpi activity of control group; V_t refers to GSTpi activity of treated group.

3.9. Inhibition of HL-60 cell proliferation

Cell proliferation inhibition was determined as we reported previously.[19] HL-60 cells were seeded in 24-well plates at a density of 4×10^4/mL and incubated with various concentrations of the tested compounds for 72 h. The cell number including trypan blue staining positive and negative cells in each group was counted. The growth inhibition rate was calculated as $(1-N_t/N_c) \times 100\%$. N_t refers to the cell number of the treated group, N_c refers to the cell number of the control group. The concentration inhibiting half of the cell proliferation (GI_{50}) was calculated.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2012.02.011.

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