Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry 13 (2005) 4056–4062
Bioorganic & Medicinal Chemistry

Synthesis and structure–activity relationship of ethacrynic acid analogues on glutathione-s-transferase P1-1 activity inhibition

Guisen Zhao,[a] Tao Yu,[a] Rui Wang,[b] Xiaobing Wang[a] and Yongkui Jing[c,*]

[a]*College of Pharmacy, Shandong University, Ji'nan, Shandong Province, 25001Z, PR China*
[b]*Shenyang Pharmaceutical University, Shenyang, 110016, PR China*
[c]*Mount Sinai School of Medicine, Box 1178, One Gustave L. Levy Place, New York, NY 10029, USA*

Received 14 March 2005; revised 28 March 2005; accepted 29 March 2005
Available online 28 April 2005

Abstract—Ethacrynic acid (EA) is a glutathione-s-transferase π (GSTP1-1) inhibitor. Fifteen of EA analogues were designed and synthesized and their inhibition on GSTP1-1 activity was tested in lysate of human leukemia HL-60 cells. These compounds were synthesized using substituted phenol as precursors through reacting with 2-chlorocarboxylic acid and acylation. Structure–activity analysis indicates that replacements of chlorides of EA by methyl, bromide, and fluoride at 3′ position remain the GSTP1-1 inhibitory effect. The compounds without any substitute at 3′ position lose the activity on GSTP1-1 inhibition. These data suggest that the substitution of 3′ position of EA is necessary for inhibiting GSTP1-1 activity.

1. Introduction

Glutathione-s-transferases (GSTs), a family of phase II detoxification enzymes, catalyze the conjugation of glutathione with broad substrates including chemotherapeutic agents. Among these isoenzymes, increased expression of GSTπ (GSTP1) was found to be correlated with the resistance of some chemotherapeutic agents in human tumor cells including colon, stomach, pancreas, uterine cervix, breast, lung cancers, melanoma, and lymphoma.[1–10] Recently, it has been found that GSTπ protein (GSTP1-1) functions as an apoptosis inhibitor by directly inhibiting c-jun-N-terminal kinase (JNK)[11] and catabolizing hydrogen peroxide (H_2O_2).[12] Thus there is a potential to develop GSTP1-1 inhibitors into apoptosis enhancers or agents of overcoming chemotherapy resistance. So far the known GSTπ inhibitors can be grouped into two classes: α,β-unsaturated carbonyl derivatives and their glutathione (GSH) conjugates. Many α,β-unsaturated carbonyl derivatives such as acrolein, cinnamaldehyde, citral, crotonaldehyde, curcumin, ethacrynic acid (EA), and trans-2-hexenal have been found to inhibit GSTP1-1 activity.[13] EA (Fig. 1) has been found to enhance the cytotoxicity of chemotherapeutic agents such as thiotepa, that is correlated with GSTP1-1 inhibition.[14] In addition to being an GSTP1-1 inhibitor, EA also has been found to interact directly with GSH to form an EA-GSH conjugate that is also a GSTP1-1 inhibitor.[15] Fifteen of new EA analogues were designed and synthesized, and their inhibition on GSTP1-1 activity of human HL-60 cell lysate was tested. A structure–activity relationship on GSTπ inhibition has been concluded.

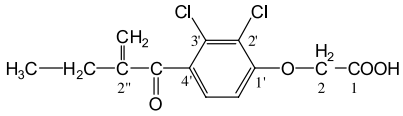


Figure 1. The chemical structure of ethacrynic acid (EA) and the listed potential positions to be modified.

2. Results and discussion

2.1. Chemistry

The synthesis of target compounds VI were accomplished by steps shown in Figure 2. Compounds II were obtained by reacting substituted phenol (I) with 2-chlorocarboxylic acid in the presence of sodium hydroxide.

Keywords: Ehtacrynic acid; Structure–activity relationship; Glutathione-s-transferase.
* Corresponding author. Tel.: +1 212 241 6775; fax: +1 212 996 5787; e-mail: yongkui.jing@mssm.edu

0968-0896/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2005.03.046

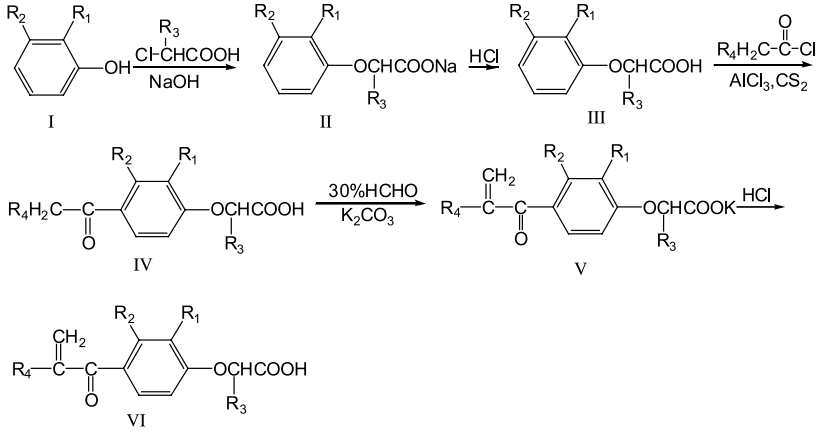


Figure 2. The synthetic pathways of EA derivatives.

Table 1. The substituted groups of EA derivatives (compounds VI)

Compound VI	R_1	R_2	R_3	R_4
VI-1	CH_3	H	CH_3	CH_3
VI-2	CH_3	H	H	CH_3
VI-3	CH_3	H	CH_3	CH_2CH_3
VI-4	CH_3	CH_3	CH_3	CH_2CH_3
VI-5	CH_3	CH_3	H	CH_3
VI-6	CH_3	CH_3	CH_3	CH_3
VI-7	H	Br	H	CH_3
VI-8	Cl	H	H	CH_2CH_3
VI-9	Cl	H	H	CH_3
VI-10	Cl	H	CH_3	CH_3
VI-11	Cl	H	CH_3	CH_2CH_3
VI-12	F	H	H	CH_3
VI-13	F	H	CH_3	CH_2CH_3
VI-14	F	H	CH_3	CH_3
VI-15	F	H	H	CH_2CH_3

Table 2. Dose-dependent inhibition of EA on GSTP1-1 activity of HL-60 cell lysate

Treatment	Concentration (μmol/L)	GSTP1-1 activity (IU/L)	GSTP1-1 activity Inhibition rate (%)
Control	0	74.17 ± 7.23	—
EA	5	31.42 ± 2.27***	57.64
EA	10	17.19 ± 1.10***	76.82
EA	15	12.18 ± 0.89***	83.58
EA	20	8.87 ± 0.90***	88.04
EA	40	2.39 ± 0.77***	96.78

Note: GSTP1-1 activity was assayed in 30 min reaction buffer containing the indicated concentration of EA, 1 mmol/L GSH, 1 mmol/L CDNB, and 100 μg HL-60 cell lysate. A unit of enzyme activity was defined as catalyzing the formation of 1 μmol of product per milligram protein per minute. The data shown are mean plus SD of triplicate samples.

*** $P < 0.001$.

Acidification of compounds II with hydrochloric acid yielded compounds III. The Friedel–Crafts acylation reaction of compound III with acyl chloride was performed in the presence of powdered aluminum chloride in carbon disulfide to generate compounds IV.[16] Aldol condensation of compounds IV with 30% methanol solution was performed in the presence of potassium carbonate to generate compounds V, acidification of V yielded target compounds VI. Total 15 of VI derivatives with different substitutes of R1, R2, R3, and R4 (methyl, ethyl, chloride, bromide, and fluoride as listed in Table 1) at indicated positions were synthesized.

2.2. The inhibitory effect of EA derivatives on GSTP1-1 activity in vitro

By using EA as a model drug, the inhibitory effect on GSTP1-1 activity of HL-60 lysate has been tested in a dose- and time-dependent pattern in vitro. As shown in Table 2, 30 min incubation of EA with HL-60 cell lysate resulted in a dose-dependent inhibition. EA (40 μmol/L) inhibited 97% of GSTP1-1 activity (Table 2). Time-dependent studies indicated that EA inhibited GSTP1-1 activity after incubation as short as 5 min, but the maximal inhibition occurred at 30 min. To compare the inhibitory effect of the synthesized EA derivatives with EA, 30 min incubation and 40 μmol/L concentration had been selected as screening reaction condition, and the results were shown in Table 3. At the concentration of 40 μmol/L, compounds VI-4, VI-5, VI-6, VI-7, and EA inhibited GSTP1-1 activity with a 81.4%, 89.5%, 94.6%, 89.9%, and 93.5% of inhibition rate, respectively. Compound VI-1 had a decreased effect with a 40.9% of inhibition rate. All other compounds did not have significant inhibitory effect on GSTP1-1 activity (<20% of inhibition rate).

Based on the results shown in Table 3, it suggests that (1) compounds with substitutes at both 2′ and 3′ of the aromatic ring are potent GSTP1-1 inhibitors; (2) compounds with one substitute at 3′ are effective as that of compounds with two substitutes at 2′ and 3′ positions; (3) compounds with methyl at 2 and 2″ do not change the activity of compounds with substitute at 3′ position; (4) compounds without any substitute at 3′ position do not have evident inhibitory effect on GSTP1-1 activity. These data suggest that the substitution on position 3′ of EA is necessary for the GSTP1-1 inhibition.

In summary, 15 of EA analogues with substitution of methyl, bromide, and fluoride at different positions are synthesized and their GSTP1-1 inhibitory effect is com-

Table 3. The GSTP1-1 activity inhibition by compounds VI

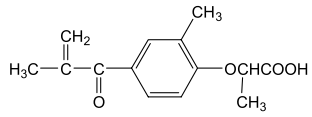
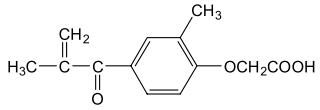
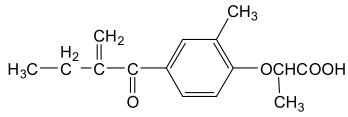
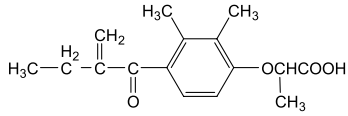
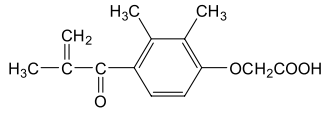
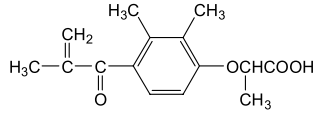
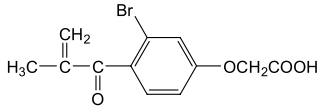
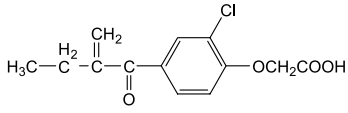
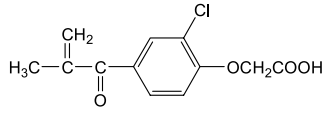
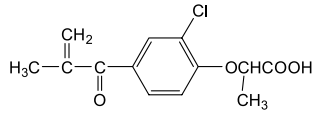
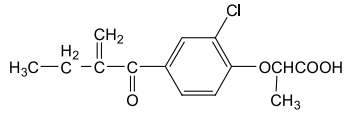
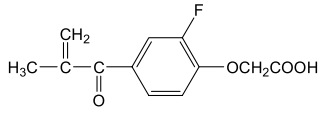
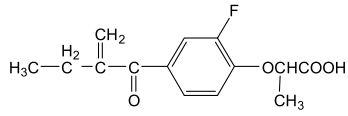
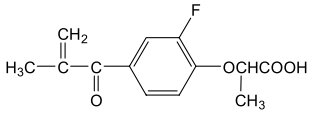
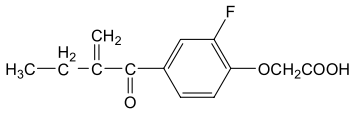
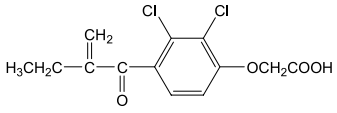
Code number of the compounds	Structure	GSTP1-1 activity Inhibition rate (%)
VI-1		40.9
VI-2		2.2
VI-3		0
VI-4		81.4
VI-5		89.5
VI-6		94.6
VI-7		89.9
VI-8		26.4
VI-9		18.8
VI-10		19.7
VI-11		10.2
VI-12		0
VI-13		14.2

Table 3 (*continued*)

Code number of the compounds	Structure	GSTP1-1 activity Inhibition rate (%)
VI-14		18.6
VI-15		10.7
EA		93.5

The data shown are inhibition rates of GSTP1-1 activity calculated by the value of control group minus the value of treated group divided by the value of control group and multiply 100%. Each group has triplicate samples.

pared using HL-60 cell lysate as GSTπ provider. The synthetic pathway is novel and simple. The replacement of chlorides in EA by methyl remains GSTP1-1 inhibitory effect, but the compounds without substitute group at position 3′ lose GSTP1-1 inhibition ability. Thus in addition of the known α,β-unsaturated carbonyl group of EA, the substitution on 3′ position is also important for inhibiting GSTP1-1 activity. These new compounds would have advantage over EA to overcome chemotherapeutic agent resistance and/or with decreased side effect that worth to be studied further.

3. Experimental

Melting points were determined in Büchi capillary melting point apparatus. Infrared (IR) spectra were measured on KBr pellets using Nicolet Nexus 470FT-IR instrument. Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded by a Bruker Avance DRX600 spectrometer with DMSO-d_6 as solvent and TMS as the internal standard. Mass spectra (MS) were measured on an API 4000 instrument. Thin-layer chromatography (TLC) was done on precoated slide with silica gel GF254 (layer thickness, 0.2 mm).

3.1. General methods for preparing compounds III

Substituted phenol I (100 mmol) was added to a solution of sodium hydroxide (15 g, 375 mmol) in 30 mL of water, then 2-chlorocarboxylic acid (170 mmol) was added slowly at 40 °C. The mixture was heated to 85 °C to reflux with stirring for 2 h. Two hundred milliliters of water was added after the mixture was cooled down. The solution was filtered and acidified to pH 1–2 with concentrated hydrochloric acid. The brown oil fraction was extracted twice with ether (100 mL). The ether fraction was further extracted twice with 5% sodium bicarbonate solution (75 mL). The sodium

bicarbonate solution was acidified to pH 1–2 with concentrated hydrochloric acid and yielded a solid product. The solid product was collected with filtering and dried to obtain compounds III.

III-1: 2-(2-methylphenoxy)propionic acid, yellow powder, yield 32.2%, mp 86–89 °C, TLC R_f = 0.49 (hexane/EtOAc, 2:1, v/v). ^{1}H NMR (DMSO-d_6) δ: 12.9 (s, 1H), 7.13 (d, J = 7.3 Hz, 1H), 7.10 (t, J = 7.7 Hz, 1H), 6.83 (t, J = 7.3 Hz, 1H), 6.75 (d, J = 8.2 Hz, 1H), 4.78 (q, J = 6.7 Hz, 1H), 2.18 (s, 3H), 1.51 (d, J = 6.8 Hz, 3H).

III-2: (2-methylphenoxy)acetic acid, white powder, yield 48.2%, mp 148–151 °C, TLC R_f = 0.51 (hexane/EtOAc, 2:1, v/v). ^{1}H NMR (DMSO-d_6) δ: 12.9 (s, 1H), 7.13 (t, J = 7.3 Hz, 1H), 7.10 (d, J = 7.7 Hz, 1H), 6.85 (t, J = 7.3 Hz, 1H), 6.79 (d, J = 8.2 Hz, 1 H), 4.68 (s, 2H), 2.19 (s, 3H).

III-3: 2-(2,3-dimethylphenoxy)propionic acid, yellow powder, yield 51.5%, mp 114–117 °C, TLC R_f = 0.37 (petroleum ether/acetone, 2:1, v/v). ^{1}H NMR (DMSO-d_6) δ: 12.87 (s, 1H), 6.98 (t, J = 7.9 Hz, 1H), 6.75 (d, J = 7.5 Hz, 1H), 6.61 (d, J = 8.2 Hz, 1H), 4.73 (q, J = 6.7 Hz, 1H), 2.21 (s, 3H), 2.10 (s, 3H), 1.50 (d, J = 6.7 Hz, 3H).

III-4: (2,3-dimethylphenoxy)acetic acid, yellow powder, yield 66.7%, mp 95–97 °C, TLC R_f = 0.72 (petroleum ether/acetone, 2:1, v/v). ^{1}H NMR (DMSO-d_6) δ: 12.9 (s, 1H), 6.99 (t, J = 7.9 Hz, 1H), 6.76 (d, J = 7.5 Hz, 1H), 6.65 (d, J = 8.2 Hz, 1H), 4.64 (s, 2H), 2.21 (s, 3H), 2.11 (s, 3H).

III-5: (3-bromophenoxy)acetic acid, white powder, yield 38.2%, mp 116–118 °C, TLC R_f = 0.56 (petroleum ether/acetone, 1:1, v/v). ^{1}H NMR (DMSO-d_6) δ: 13.1 (s, 1H), 7.30 (t, J = 8.1 Hz, 1H), 7.19 (m, 2H), 6.99 (m, 1H), 4.78 (s, 2H).

III-6: (2-chlorophenoxy)acetic acid, white powder, yield 60.1%, mp 143–146 °C, TLC R_f = 0.47 (petroleum ether/acetone, 3:2, v/v). ^{1}H NMR (DMSO-d_6) δ: 13.1 (s, 1H), 7.42 (d, J = 7.8 Hz, 1H), 7.27 (t, J = 8.0 Hz, 1H), 7.02 (d, J = 8.3 Hz, 1H), 6.96 (t, J = 7.6 Hz, 1H), 4.79 (s, 2H).

III-7: 2-(2-chlorophenoxy)propionic acid, white powder, yield 64.0%, mp 108–111 °C, TLC R_f = 0.52 (petroleum ether/acetone, 2:1, v/v). ^{1}H NMR (DMSO-d_6) δ: 13.1 (s, 1H), 7.42 (d, J = 8.2 Hz, 1H), 7.26 (t, J = 7.2 Hz, 1H), 6.95 (m, 2H), 4.91 (q, J = 6.8 Hz, 1H), 1.53 (d, J = 6.8 Hz, 3H).

III-8: (2-fluorophenoxy)acetic acid, white powder, yield 61.8%, mp 135–138 °C, TLC R_f = 0.53 (petroleum ether/acetone, 2:1, v/v). ^{1}H NMR (DMSO-d_6) δ: 13.07 (s, 1H), 7.21 (m, 1H), 7.07 (m, 2H), 6.94 (m, 1H), 4.76 (s, 2H).

III-9: 2-(2-fluorophenoxy)propionic acid, white powder, yield 86.4%, mp 89–91 °C, TLC R_f = 0.56 (petroleum ether/acetone, 2:1, v/v). ^{1}H NMR (DMSO-d_6) δ: 13.06 (s, 1H), 7.20 (m, 1H), 7.09 (t, J = 7.9 Hz, 1H), 7.00 (t, J = 8.4 Hz, 1H), 6.95 (m, 1H), 4.89 (q, J = 6.8 Hz, 1H), 1.52 (d, J = 6.8 Hz, 3H).

3.2. General methods for preparing compounds IV

The compound III (24 mmol) was added to carbon disulfide (70 mL) with stirring at room temperature. Powdered aluminum chloride (10 g, 75 mmol) was added into batches and then acyl chloride (30 mmol) was added slowly. The mixture was heated up to 70 °C to reflux for 4 h and then the carbon disulfide was decanted after cooling to the room temperature. The residue was added to a mixture of ice (100 g) and concentrated hydrochloric acid (3 mL) and yielded oil fraction. The oil fraction was extracted twice with ether (100 mL), and then the ether fraction was extracted twice with 5% sodium bicarbonate solution (50 mL). The sodium bicarbonate extract was acidified with concentrated hydrochloric acid to pH 1–2. The solid particles were collected by filtering and dried to obtained compounds IV.

IV-1: 2-[2-methyl-4-(1-oxopropyl)phenoxy]propionic acid, yellow powder, yield 72.7%, mp 129–131 °C, TLC R_f = 0.33 (hexane/EtOAc, 2:1, v/v). ^{1}H NMR (DMSO-d_6) δ: 13.05 (s, 1H), 7.84 (m, 2H), 6.92 (m, 1H), 5.01 (q, J = 6.7 Hz, 1H), 3.00 (q, J = 7.2 Hz, 2H), 2.29 (s, 3H), 1.61 (d, J = 6.7 Hz, 3H), 1.12 (t, J = 7.2 Hz, 3H).

IV-2: [2-methyl-4-(1-oxopropyl)phenoxy]acetic acid, white powder, yield 83.4%, mp 114–118 °C, TLC R_f = 0.48 (petroleum ether/EtOAc, 2:1, v/v). ^{1}H NMR (DMSO-d_6) δ: 13.06 (s, 1H), 7.84 (m, 2H), 6.92 (m, 1H), 4.80 (s, 2H), 2.95 (q, J = 7.2 Hz, 2H), 2.24 (s, 3H), 1.06 (t, J = 7.2 Hz, 3H).

IV-3: 2-[2-methyl-4-(1-oxobutyl)phenoxy]propionic acid, yellow powder, yield 82.4%, mp 88–90 °C, TLC R_f = 0.42 (petroleum ether/EtOAc, 2:1, v/v). ^{1}H NMR (DMSO-d_6) δ: 13.07 (s, 1H), 7.78 (m, 2H), 6.85 (m, 1H), 4.95 (q, J = 6.7 Hz, 1H), 2.90 (t, J = 7.2 Hz, 2H), 2.23 (s, 3H), 1.60 (m, 2H), 1.54 (d, J = 6.8 Hz, 3H), 0.91 (t, J = 7.4 Hz, 3H).

IV-4: 2-[2,3-dimethyl-4-(1-oxobutyl)phenoxy]propionic acid, yellow powder, yield 79.7%, mp 113–115 °C, TLC R_f = 0.34 (petroleum ether/acetone, 4:1, v/v). ^{1}H NMR (DMSO-d_6) δ: 13.00 (s, 1H), 7.48 (d, J = 8.6 Hz, 1H), 6.69 (d, J = 8.6 Hz, 1H), 4.88 (q, J = 6.7 Hz, 1H), 2.81 (t, J = 7.2 Hz, 2H), 2.25 (s, 3H), 2.14 (s, 3H), 1.57 (m, 2H), 1.53 (d, J = 6.7 Hz, 3H), 0.89 (t, J = 7.4 Hz, 3H).

IV-5: [2,3-dimethyl-4-(1-oxopropyl)phenoxy]acetic acid, yellow powder, yield 88.0%, mp 103–106 °C, TLC R_f = 0.58 (petroleum ether/EtOAc, 2:1, v/v). ^{1}H NMR (DMSO-d_6) δ: 13.00 (s, 1H), 7.50 (d, J = 8.6 Hz, 1H), 6.75 (d, J = 8.7 Hz, 1H), 4.74 (s, 2H), 2.85 (q, J = 7.2 Hz, 2H), 2.26 (s, 3H), 2.15 (s, 3H), 1.04 (t, J = 7.2 Hz, 3H).

IV-6: 2-[2,3-dimethyl-4-(1-oxopropyl)phenoxy]propionic acid, yellow powder, yield 86.7%, mp 135–138 °C, TLC R_f = 0.42 (petroleum ether/acetone, 2:1, v/v). ^{1}H NMR (DMSO-d_6) δ: 13.00 (s, 1H), 7.49 (d, J = 8.6 Hz, 1H),

6.69 (d, J = 8.6 Hz, 1H), 4.88 (q, J = 6.7 Hz, 1H), 2.84 (q, J = 7.2 Hz, 2H), 2.25 (s, 3H), 2.14 (s, 3H), 1.53 (d, J = 6.8 Hz, 3H), 1.03 (t, J = 7.2 Hz, 3H).

IV-7: [3-bromo-4-(1-oxopropyl)phenoxy]acetic acid, yellow powder, yield 69.0%, mp 96–98 °C, TLC R_f = 0.51 (petroleum ether/acetone, 1:1, v/v). ^{1}H NMR (DMSO-d_6) δ: 13.12 (s, 1H), 7.69 (d, J = 8.6 Hz, 1H), 7.29 (d, J = 2.4 Hz, 1H), 7.08 (dd, J = 2.4, 8.6 Hz, 1H), 4.85 (s, 2H), 2.95 (q, J = 7.3 Hz, 2H), 1.12 (t, J = 7.2 Hz, 3H).

IV-8: [2-chloro-4-(1-oxobutyl)phenoxy]acetic acid, white powder, yield 87.7%, mp 115–118 °C, TLC R_f = 0.67 (petroleum ether/acetone, 1:1, v/v). ^{1}H NMR (DMSO-d_6) δ: 13.10 (s, 1H), 7.97 (d, J = 2.1 Hz, 1H), 7.90 (dd, J = 2.1, 8.7 Hz, 1H), 7.13 (d, J = 8.7 Hz, 1H), 4.90 (s, 2H), 2.94 (t, J = 7.1 Hz, 2H), 1.61 (m, 2H), 0.91 (t, J = 7.4 Hz, 3H).

IV-9: [2-chloro-4-(1-oxopropyl)phenoxy]acetic acid, yellow powder, yield 59.8%, mp 117–119 °C, TLC R_f = 0.59 (hexane/EtOAc, 2:3, v/v). ^{1}H NMR (DMSO-d_6) δ: 13.10 (s, 1H), 7.98 (s, 1H), 7.89 (d, J = 8.7 Hz, 1H), 7.14 (d, J = 8.7 Hz, 1H), 4.92 (s, 2H), 2.99 (q, J = 7.1 Hz, 2H), 1.06 (t, J = 7.1 Hz, 3H).

IV-10: 2-[2-chloro-4-(1-oxopropyl)phenoxy]propionic acid, yellow powder, yield 70.3%, mp 75–78 °C, TLC R_f = 0.44 (petroleum ether/acetone, 2:1, v/v). ^{1}H NMR (DMSO-d_6) δ: 13.19 (s, 1H), 7.97 (d, J = 2.1 Hz, 1H), 7.90 (dd, J = 2.1, 8.7 Hz, 1H), 7.07 (d, J = 8.7 Hz, 1H), 5.09 (q, J = 6.7 Hz, 1H), 2.98 (q, J = 7.2 Hz, 2H), 1.57 (d, J = 6.8 Hz, 3H), 1.06 (t, J = 7.2 Hz, 3H).

IV-11: 2-[2-chloro-4-(1-oxobutyl)phenoxy]propionic acid, yellow oil, yield 80.5%, TLC R_f = 0.66 (petroleum ether/acetone, 2:1, v/v). ^{1}H NMR (DMSO-d_6) δ: 13.23 (s, 1H), 8.04 (s, 1H), 7.95 (d, J = 8.7 Hz, 1H), 7.12 (d, J = 8.7 Hz, 1H), 5.16 (q, J = 6.8 Hz, 1H), 3.00 (t, J = 7.2 Hz, 2H), 1.66 (m, 2H), 1.63 (d, J = 6.8 Hz, 3H), 0.96 (t, J = 7.4 Hz, 3H).

IV-12: [2-fluoro-4-(1-oxopropyl)phenoxy]acetic acid, yellow powder, yield 88.8%, mp 104–106 °C, TLC R_f = 0.45 (petroleum ether/acetone, 2:1, v/v). ^{1}H NMR (DMSO-d_6) δ: 13.20 (s, 1H), 7.76 (m, 2H), 7.19 (m, 1H), 4.89 (s, 2H), 2.98 (q, J = 7.2 Hz, 2H), 1.06 (t, J = 7.2 Hz, 3H).

IV-13: 2-[2-fluoro-4-(1-oxobutyl)phenoxy]propionic acid, yellow oil, yield 72.5%, TLC R_f = 0.47 (petroleum ether/acetone, 2:1, v/v). ^{1}H NMR (DMSO-d_6) δ: 13.04 (s, 1H), 7.78 (m, 2H), 6.85 (m, 1H), 4.95 (q, J = 6.7 Hz, 1H), 2.90 (t, J = 7.2 Hz, 2H), 1.60 (m, 2H), 1.55 (d, J = 6.8 Hz, 3H), 0.91 (t, J = 7.4 Hz, 3H).

IV-14: 2-[2-fluoro-4-(1-oxopropyl)phenoxy]propionic acid, yellow oil, yield 88.2%, TLC R_f = 0.47 (petroleum ether/acetone, 2:1, v/v). ^{1}H NMR (DMSO-d_6) δ: 13.26 (s, 1H), 7.82 (m, 2H), 7.16 (m, 1H), 5.15 (q, J = 6.8 Hz, 1H), 3.42 (q, J = 7.2 Hz, 2H), 1.61 (d, J = 6.8 Hz, 3H), 1.12 (t, J = 7.2 Hz, 3H).

IV-15: [2-fluoro-4-(1-oxobutyl)phenoxy]acetic acid, yellow powder, yield 39.3%, mp 102–105 °C, TLC R_f = 0.58 (petroleum ether/acetone, 2:1, v/v). ^{1}H NMR (DMSO-d_6) δ: 13.40 (s, 1H), 7.82 (m, 2H), 7.23 (m, 1H), 4.92 (s, 2H), 2.99 (t, J = 7.1 Hz, 2H), 1.67 (m, 2H), 0.97 (t, J = 7.3 Hz, 3H).

3.3. General methods for preparing target compounds VI

Compounds IV (17 mmol) and 30% formaldehyde solution (1.7 mL, 17 mmol) were added to ethanol (34 mL) following by addition of potassium carbonate (2.3 g, 17 mmol) in a mixed solution of water (17 mL) with ethanol (10 mL). The solution was added in ethanol (24 mL) while stirring simultaneously. The mixture was refluxed for 3 h and decanted into hydrochloric acid solution (a mixture of 5 mL concentrated hydrochloric acid and 340 mL water) after cooling down to room temperature. The yielded yellow oil was extracted twice with ether (150 mL) and the ether fraction was evaporated to obtain a yellow product, which was then purified through a silica gel column using petroleum ether/chloroform/methanol (10:10:1, v/v) as eluent.

VI-1: 2-[2-methyl-4-(2-methylene-1-oxopropyl)phenoxy]-propionic acid, brown oil, yield 28.6%, TLC R_f = 0.54 (petroleum ether/acetone, 2:1, v/v). ^{1}H NMR (DMSO-d_6) δ: 13.06 (s, 1H), 7.80 (d, J = 8.6 Hz, 1H), 7.78 (s, 1H), 6.86 (d, J = 8.5 Hz, 1H), 5.87 (s, 1H), 5.47 (s, 1H), 4.96 (q, J = 6.6 Hz, 1H), 2.23 (s, 3H), 1.56 (d, J = 6.7 Hz, 3H), 1.06 (s, 3H); MS: m/z = 249.5 (M^++1); IR (KBr) ν_{max}: 3435, 3060, 2979, 2938, 2911, 1731, 1711, 1669, 1599, 1498 cm^{-1}.

VI-2: [2-methyl-4(2-methylene-1-oxopropyl)phenoxy]-acetic acid, white powder, yield 39.0%, mp 112–114 °C, TLC R_f = 0.17 (petroleum ether/acetone, 2:1, v/v). ^{1}H NMR (DMSO-d_6) δ: 13.07 (s, 1H), 7.82 (d, J = 8.6 Hz, 1H), 7.80 (s, 1H), 6.94 (d, J = 8.5 Hz, 1H), 4.85 (s, 2H), 3.69 (s, 2H), 2.29 (s, 3H), 1.06 (s, 3H); MS: m/z = 234.8 (M^+); IR (KBr) ν_{max}: 3398, 3059, 2972, 2939, 1743, 1658, 1599, 1502 cm^{-1}.

VI-3: 2-[2-methyl-4-(2-methylene-1-oxobutyl)phenoxy]-propionic acid, yellow oil, yield 21.8%, TLC R_f = 0.26 (petroleum ether/chloroform/methanol, 5:5:1). ^{1}H NMR (DMSO-d_6) δ: 7.72 (d, J = 8.3 Hz, 1H), 7.68 (d, J = 8.6 Hz, 1H), 6.98 (s, 1H), 4.46 (s, 2H), 3.64 (q, J = 6.8 Hz, 1H), 3.54 (q, J = 7.2 Hz, 2H), 1.77 (s, 3H), 1.45 (d, J = 6.6 Hz, 3H), 0.78 (t, J = 7.4 Hz, 3H); MS: m/z = 263.3 (M^++1); IR (KBr) ν_{max}: 3373, 2966, 2937, 2877, 1669, 1614, 1513 cm^{-1}.

VI-4: 2-[2,3-dimethyl-4-(2-methylene-1-oxobutyl)phenoxy]propionic acid, yellow powder, yield 83.7%, mp 115–118 °C, TLC R_f = 0.57 (petroleum ether/acetone, 2:1, v/v). ^{1}H NMR (DMSO-d_6) δ: 12.86 (s, 1H), 7.04 (d, J = 8.5 Hz, 1H), 6.73 (d, J = 8.4 Hz, 1H), 5.91 (s, 1H), 5.47 (s, 1H), 4.86 (q, J = 6.8 Hz, 1H), 2.39 (q, J = 7.4 Hz, 2H), 2.16 (s, 3H), 2.12 (s, 3H), 1.55 (d, J = 6.7 Hz, 3H), 1.07 (t, J = 7.4 Hz, 3H); MS: m/z = 277.4 (M^++1); IR (KBr) ν_{max}: 2962, 2938, 2878, 1726, 1708, 1650, 1591, 1578, 1482 cm^{-1}.

VI-5: [2,3-dimethyl-4-(2-methylene-1-oxopropyl)phenoxy]acetic acid, yellow powder, yield 51.6%, mp 112–114 °C, TLC $R_f = 0.42$ (petroleum ether/acetone, 2:1, v/v). ^{1}H NMR (DMSO-d_6) δ: 12.88 (s, 1H), 7.06 (d, $J = 8.5$ Hz, 1H), 6.78 (d, $J = 8.5$ Hz, 1H), 5.99 (s, 1H), 5.48 (s, 1H), 4.74 (s, 2H), 2.17 (s, 3H), 2.11 (s, 3H), 1.95 (s, 3H); MS: $m/z = 249.4$ (M^++1); IR (KBr) ν_{max}: 3090, 2972, 2930, 1742, 1641, 1589, 1576 cm^{-1}.

VI-6: 2-[2,3-dimethyl-4-(2-methylene-1-oxopropyl) phenoxy]propionic acid, yellow powder, yield 82.0%, mp 125–128 °C, TLC $R_f = 0.53$ (petroleum ether/acetone, 2:1, v/v). ^{1}H NMR (DMSO-d_6) δ: 12.84 (s, 1H), 7.04 (d, $J = 8.5$ Hz, 1H), 6.71 (d, $J = 8.5$ Hz, 1H), 5.99 (s, 1H), 5.46 (s, 1H), 4.86 (q, $J = 6.7$ Hz, 1H), 2.16 (s, 3H), 2.11 (s, 3H), 1.95 (s, 3H), 1.54 (d, $J = 6.7$ Hz, 3H); MS: $m/z = 263.3$ (M^++1); IR (KBr) ν_{max}: 3402, 2976, 2926, 1727, 1645, 1627, 1590, 1578 cm^{-1}.

VI-7: [3-bromo-4-(2-methylene-1-oxopropyl)phenoxy]-acetic acid, white powder, yield 40.1%, mp 124–126 °C, TLC $R_f = 0.44$ (petroleum ether/acetone, 1:1, v/v). ^{1}H NMR (DMSO-d_6) δ: 12.96 (s, 1H), 7.32 (d, $J = 8.5$ Hz, 1H), 7.24 (d, $J = 2.3$ Hz, 1H), 7.03 (dd, $J = 8.5$, 2.3 Hz, 1H), 6.09 (s, 1H), 5.50 (s, 1H), 4.80 (s, 2H), 1.96 (s, 3H); MS: $m/z = 299.4$ (M^+); IR (KBr) ν_{max}: 3430, 3086, 3060, 2984, 2920, 1737, 1657, 1626, 1593, 1562 cm^{-1}.

VI-8: [2-chloro-4-(2-methylene-1-oxobutyl)phenoxy]acetic acid, yellow oil, yield 22.7%, TLC $R_f = 0.24$ (petroleum ether/chloroform/methanol, 5:5:1, v/v). ^{1}H NMR (DMSO-d_6) δ: 7.94 (d, $J = 6.7$ Hz, 1H), 7.88 (d, $J = 6.6$ Hz, 1H), 6.97 (s, 1H), 4.62 (s, 2H), 3.62 (q, $J = 7.3$ Hz, 2H), 3.51 (s, 2H), 0.79 (t, $J = 7.4$ Hz, 3H); MS: $m/z = 268.3$ (M^+); IR (KBr) ν_{max}: 3365, 2964, 2934, 2876, 1671, 1623, 1593, 1566, 1498 cm^{-1}.

VI-9: [2-chloro-4-(2-methylene-1-oxopropyl)phenoxy]-acetic acid, yellow oil, yield 31.7%, TLC $R_f = 0.26$ (petroleum ether/chloroform/methanol, 5:5:1, v/v). ^{1}H NMR (DMSO-d_6) δ: 7.92 (d, $J = 6.6$ Hz, 1H), 7.86 (d, $J = 6.8$ Hz, 1H), 6.98 (s, 1H), 4.43 (s, 2H), 3.66 (s, 2H), 1.04 (s, 3H); MS: $m/z = 255.1$ (M^+); IR (KBr) ν_{max}: 3441, 2972, 2935, 2878, 1672, 1614, 1594, 1566, 1499 cm^{-1}.

VI-10: 2-[2-chloro-4-(2-methylene-1-oxopropyl)phenoxy]-propionic acid, yellow oil, yield 33.4%, TLC $R_f = 0.32$ (petroleum ether/chloroform/methanol, 5:5:1, v/v). ^{1}H NMR (DMSO-d_6) δ: 7.90 (s, 1H), 7.84 (d, $J = 8.5$ Hz, 1H), 6.97 (d, $J = 8.7$ Hz, 1H), 4.67 (s, 1H), 4.50 (s, 1H), 3.64 (q, $J = 6.9$ Hz, 1H), 1.50 (s, 3H), 1.04 (d, $J = 6.7$ Hz, 3H); MS: $m/z = 268.5$ (M^+); IR (KBr) ν_{max}: 3441, 3071, 2977, 2939, 2877, 1734, 1676, 1660, 1592, 1498 cm^{-1}.

VI-11: 2-[2-chloro-4-(2-methylene-1-oxobutyl)phenoxy]-propionic acid, yellow oil, yield 34.5%, TLC $R_f = 0.36$ (petroleum ether/chloroform/methanol, 5:5:1, v/v). ^{1}H NMR (DMSO-d_6) δ: 7.94 (s, 1H), 7.88 (d, $J = 8.4$ Hz, 1H), 6.99 (d, $J = 8.3$ Hz, 1H), 4.76 (s, 2H), 3.51 (q, $J = 6.2$ Hz, 1H), 1.90 (s, 2H), 1.51 (d, $J = 6.1$ Hz, 3H), 0.79 (t, $J = 7.5$ Hz, 3H); MS: $m/z = 283.2$ (M^++1); IR (KBr) ν_{max}: 3362, 2965, 2936, 2876, 1672, 1592, 1565, 1497 cm^{-1}.

VI-12: [2-fluoro-4-(2-methylene-1-oxopropyl)phenoxy]-acetic acid, yellow powder, yield 31.4%, mp 113–115 °C, TLC $R_f = 0.28$ (petroleum ether/acetone, 1:1, v/v). ^{1}H NMR (DMSO-d_6) δ: 13.20 (s, 1H), 7.81 (d, $J = 8.8$ Hz, 1H), 7.78 (d, $J = 8.6$ Hz, 1H), 7.20 (s, 1H), 4.91 (s, 2H), 3.69 (s, 2H), 1.06 (s, 3H); MS: $m/z = 238.6$ (M^+); IR (KBr) ν_{max}: 3456, 3067, 2986, 2921, 1740, 1674, 1613, 1584, 1518 cm^{-1}.

VI-13: 2-[2-fluoro-4-(2-methylene-1-oxobutyl)phenoxy]-propionic acid, yellow oil, yield 28.4%, TLC $R_f = 0.42$ (petroleum ether/chloroform/methanol, 5:5:1, v/v). ^{1}H NMR (DMSO-d_6) δ: 7.55 (d, $J = 7.8$ Hz, 1H), 7.48 (d, $J = 7.6$ Hz, 1H), 6.71 (s, 1H), 4.01 (s, 2H), 2.83 (q, $J = 6.5$ Hz, 1H), 1.61 (q, $J = 7.3$ Hz, 2H), 1.44 (d, $J = 6.7$ Hz, 3H), 0.92 (t, $J = 7.4$ Hz, 3H); MS: $m/z = 266.3$ (M^+); IR (KBr) ν_{max}: 3374, 2964, 2935, 2876, 1713, 1666, 1599, 1501 cm^{-1}.

VI-14: 2-[2-fluoro-4-(2-methylene-1-oxopropyl)phenoxy]-propionic acid, yellow oil, yield 38.1%, TLC $R_f = 0.26$ (petroleum ether/chloroform/methanol, 5:5:1, v/v). ^{1}H NMR (DMSO-d_6) δ: 7.74 (d, $J = 8.2$ Hz, 1H), 7.70 (d, $J = 8.3$ Hz, 1H), 7.00 (s, 1H), 3.63 (s, 2H), 3.43 (q, $J = 4.9$ Hz, 1H), 1.47 (s, 3H), 1.02 (d, $J = 5.1$ Hz, 3H); MS: $m/z = 253.2$ (M^++1); IR (KBr) ν_{max}: 3346, 3075, 2978, 2938, 2878, 1674, 1610, 1582, 1514 cm^{-1}.

VI-15: [2-fluoro-4-(2-methylene-1-oxobutyl)phenoxy]-acetic acid, gray powder, yield 51.9%, mp 115–117 °C, TLC $R_f = 0.46$ (petroleum ether/acetone, 2:1, v/v). ^{1}H NMR (DMSO-d_6) δ: 7.75 (d, $J = 8.3$ Hz, 1H), 7.73 (d, $J = 8.6$ Hz, 1H), 7.13 (s, 1H), 4.75 (s, 2H), 2.92 (s, 2H), 1.63 (q, $J = 7.3$ Hz, 2H), 0.93 (t, $J = 7.4$ Hz, 3H); MS: $m/z = 253.5$ (M^++1); IR (KBr) ν_{max}: 3424, 3080, 2964, 2936, 2876, 1747, 1679, 1650, 1614, 1583, 1519 cm^{-1}.

3.4. Cell culture and GSTP1-1 activity 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. HL-60 cells in logarithmic growth (3×10^6) were washed twice with PBS, resuspended in 300 μL of 100 mmol/L potassium phosphate buffer, pH 6.8, sonicated for 10 s at 4 °C, and centrifuged at 14000 rpm for 30 min at 4 °C. The supernatant was used for GSTP1-1 activity assay. GSTP1-1 activity was determined spectrophotometrically at 25 °C, with CDNB and GSH as substrates according to the reported method.[17] The linear increase in absorption at 340 nm, caused by conjugation of GSH (1 mmol/L) with CDNB (1 mmol/L) in HL-60 cell lysate with or without presence of compounds VI-1–VI-15 (40 μmol/L), was measured. An extinction coefficient of 9.6 mM^{-1} cm^{-1} was used to calculate GSTP1-1 activity expressed as micromoles

per minute per milligram of protein. Activity inhibition rate was calculated as $(V_c - V_t)/V_c \times 100\%$. V_c represents GSTP1-1 activity of control group; V_t represents GSTP1-1 activity of treated group.

Acknowledgments

This work was partly supported by Joint Research Fund for Overseas Chinese Young Scholars of National Natural Science Foundation of China (30328036) and Natural Science Foundation of China (20472045).

References and notes

1. Coles, B.; Ketterer, B. *Crit. Rev. Biochem. Mol. Biol.* **1990**, *25*, 47.
2. Tsuchida, S.; Sato, K. *Crit. Rev. Biochem. Mol. Biol.* **1992**, *27*, 337.
3. Wang, A. L.; Tew, K. D. *Cancer Treat Rep.* **1985**, *69*, 677.
4. Yang, W. Z.; Begleiter, A.; Johnston, J. B.; Israels, L. G.; Mowat, M. R. *Mol. Pharmacol.* **1992**, *41*, 625.
5. Buller, A. L.; Clapper, M. L.; Tew, K. D. *Mol. Pharmacol.* **1987**, *31*, 575.
6. Batist, G.; Tulpule, A.; Sinha, B. K.; Katki, A. G.; Myers, C. E.; Cowan, K. H. *J. Biol. Chem.* **1986**, *261*, 15544.
7. Taylor, C. W.; Brattain, M. G.; Yeoman, L. C. *Cancer Res.* **1985**, *45*, 4422.
8. Gupta, V.; Singh, S. V.; Ahmad, H.; Medh, R. D.; Awasthi, Y. C. *Biochem. Pharmacol.* **1989**, *38*, 1993.
9. Teicher, B. A.; Holden, S. A.; Kelley, M. J.; Shea, T. C.; Cucchi, C. A.; Rosowsky, A.; Henner, W. D.; Frei, E., III. *Cancer Res.* **1987**, *47*, 388.
10. Armstrong, D. K.; Gordon, G. B.; Hilton, J.; Streeper, R. T.; Colvin, O. M.; Davidson, N. E. *Cancer Res.* **1992**, *52*, 1416.
11. Wang, T.; Arifoglu, P.; Ronai, Z.; Tew, K. D. *J. Biol. Chem.* **2001**, *276*, 20999–21003.
12. Zhou, L.; Jing, Y.; Styblo, M.; Chen, Z.; Wxaman, S. *Blood* **2005**, *105*, 1198–1203.
13. Iersel, M. L.; Ploemen, J. P.; Struik, I.; van Amersfoort, C.; Keyzer, A. E.; Schefferlie, J. G.; van Bladeren, P. J. *Chem. Biol. Interact.* **1996**, *102*, 117–132.
14. O'Dwyer, P. J.; LaCreta, F.; Nash, S.; Tinsley, P. W.; Schilder, R.; Clapper, M. L.; Tew, K. D.; Panting, L.; Litwin, S.; Comis, R. L. *Cancer Res.* **1991**, *51*, 6059–6065.
15. Oakley, A. J.; Rossjohn, J.; Lo Bello, M.; Caccuri, A. M.; Federici, G.; Parker, M. W. *Biochemistry* **1997**, *36*, 576–585.
16. Abraham, D. J.; Mehanna, A. S.; Williams, F. S.; Cragoe, E. J., Jr.; Woltersdorf, O. W., Jr. *J. Med. Chem.* **1989**, *32*, 2460–2467.
17. Habig, W. H.; Pabst, M. J.; Jakoby, W. B. *J. Biol. Chem.* **1974**, *249*, 7130–7139.