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The synthesis of α,β-unsaturated carbonyl derivatives with the ability to inhibit both glutathione *S*-transferase P1-1 activity and the proliferation of leukemia cells

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Abstract—Ethacrynic acid (EA), an α,β-unsaturated carbonyl compound, is a glutathione *S*-transferase P1-1 (GSTP1-1) inhibitor. Twenty-one novel EA derivatives have been synthesized. The effects of these compounds on GSTP1-1 activity and on the proliferation of human leukemia HL-60 cells have been determined. Compounds with a halogen substitution at the 3′-position of the aromatic ring have greater inhibitory effects on GSTP1-1 activity than those of compounds with a methyl substitution there. Compounds with substitutions at both the 2′- and 3′-positions of the aromatic ring have more antiproliferative ability than those with one substitution at 3′-position. Esterification of the carboxyl group appears to increase the antiproliferative ability.

1. Introduction

Glutathione *S*-transferase P1-1 (GSTP1-1, GSTπ), a member of phase II detoxification enzymes, which catalyzes the conjugation of glutathione with a broad range of substrates, including chemotherapeutic agents,[1] has been reported to be overexpressed in a variety of cancer cells and to be involved in multidrug resistance (MDR) and the protection against cell death.[2–12] GSTP1-1 inhibitors have the potential to overcome MDR and/or to induce cell death in cancer cells with overexpressed GSTP1-1. Ethacrynic acid has been found to be a GSTP1-1 inhibitor and, at higher concentrations, has antiproliferative effects in tumor cells.[12,13] We previously synthesized 15 EA derivatives and found that a substitution at the 3′-position of EA is required for the inhibition of GSTP1-1 activity.[14] We have now designed and synthesized 21 novel EA derivatives. The inhibitory effects of these compounds on GSTP1-1 activity were determined in human leukemia HL-60 cell lysates and the antiproliferative effects were determined in HL-60 cells. The structure–activity relationships between GSTP1-1 activity inhibition and cell growth inhibition were analyzed.

2. Results and discussion

2.1. Chemistry

The synthesis of target compounds **VI** and **VII** was accomplished by the steps shown in Figure 1. The target compounds, as typified by the general structures of the **VI** series and the intermediates **III** and **IV**, were prepared by using methods we reported previously.[14] **VI** series compounds were esterified with methanol, with *para*-toluenesulfonic acid as the catalyst to generate compounds of the **VII** series. Twenty-one novel EA derivatives of the **VI** and **VII** series with substitutions termed R1, R2, R3, and R4 were synthesized (Table 1).

2.2. The inhibitory effects of EA derivatives on GSTP1-1 activity and on HL-60 cell proliferation

The inhibitory effects of these compounds on GSTP1-1 activity in HL-60 cell lysates were determined in vitro

Keywords: Glutathione *S*-transferase; α,β-unsaturated carbonyl compounds; Structure–activity relationship.
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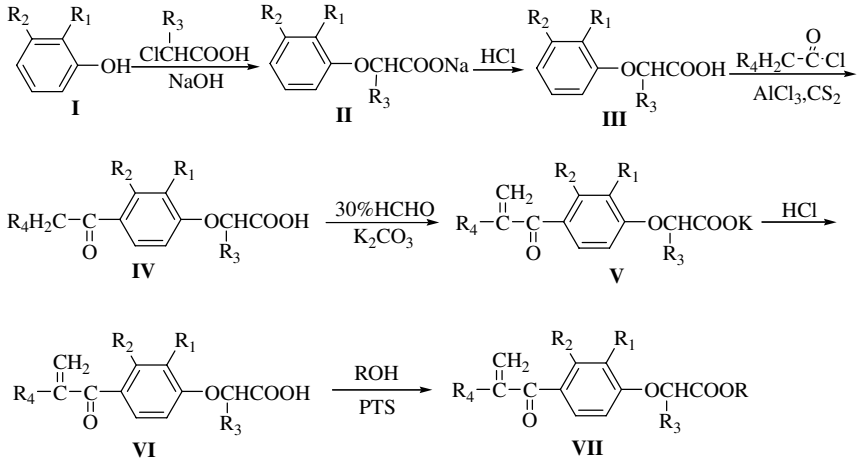
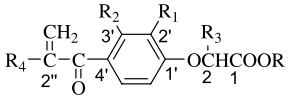


Figure 1. The synthetic pathways of the **VI** and **VII** series.

Table 1. Substituted groups of compounds of the **VI** and **VII** series

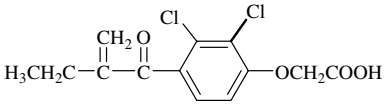
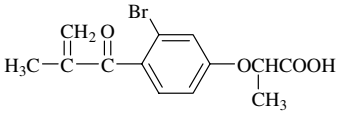
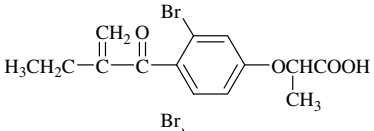
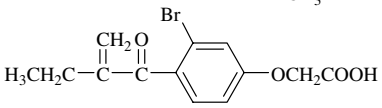
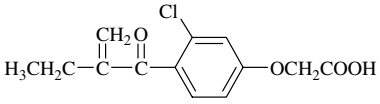
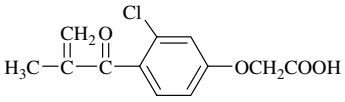
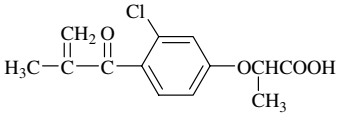
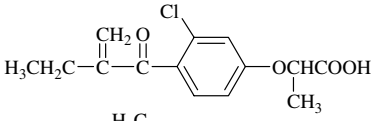
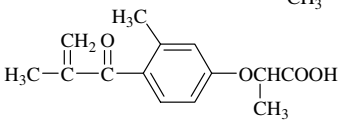
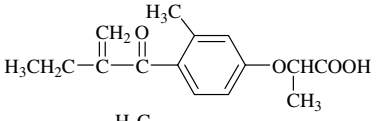
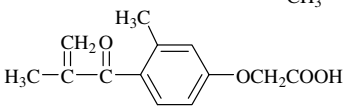
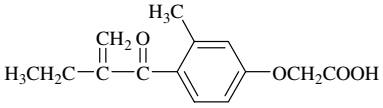
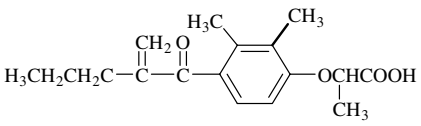
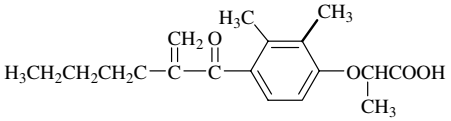
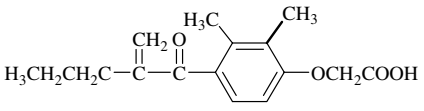


Compound	R_1	R_2	R_3	R_4	R
VI-16	H	Br	CH_3	CH_3	H
VI-17	H	Br	CH_3	CH_2CH_3	H
VI-18	H	Br	H	CH_2CH_3	H
VI-19	H	Cl	H	CH_2CH_3	H
VI-20	H	Cl	H	CH_3	H
VI-21	H	Cl	CH_3	CH_3	H
VI-22	H	Cl	CH_3	CH_2CH_3	H
VI-23	H	CH_3	CH_3	CH_3	H
VI-24	H	CH_3	CH_3	CH_2CH_3	H
VI-25	H	CH_3	H	CH_3	H
VI-26	H	CH_3	H	CH_2CH_3	H
VI-27	CH_3	CH_3	CH_3	$CH_2CH_2CH_3$	H
VI-28	CH_3	CH_3	CH_3	$CH_2CH_2CH_2CH_3$	H
VI-29	CH_3	CH_3	H	$CH_2CH_2CH_3$	H
VI-30	CH_3	CH_3	H	$CH_2CH_2CH_2CH_3$	H
VII-1	CH_3	CH_3	CH_3	$CH_2CH_2CH_3$	CH_3
VII-2	CH_3	CH_3	CH_3	$CH_2CH_2CH_2CH_3$	CH_3
VII-3	CH_3	CH_3	H	$CH_2CH_2CH_3$	CH_3
VII-4	CH_3	CH_3	H	$CH_2CH_2CH_2CH_3$	CH_3
VII-5	CH_3	CH_3	CH_3	CH_2CH_3	CH_3
VII-6	CH_3	CH_3	CH_3	CH_3	CH_3

as we reported previously.[14] To compare the inhibitory effects on GSTP1-1 activity of these derivatives with that of EA, 40 μmol/L of each compound was incubated with cell lysate for 30 min at 37 °C. At this concentration, all compounds had some inhibitory effects (Table 2). Compounds of the **VI-16–22** with a chloro or bromo at 3′-position, like EA, were potent GSTP1-1 activity inhibitors. Replacement of the chloride or bromide by a methyl group decreased inhibitory ability (**VI-23–30**). The inhibitory ability was further decreased when the carboxyl was esterified with methanol (**VII** series). The length of the substitutions at 2″ did not influence the inhibitory effects (compare **VI-27** with **VI-28** or **VII-1** with **VII-2**).

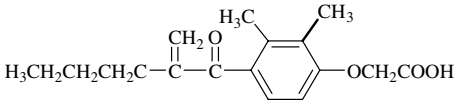
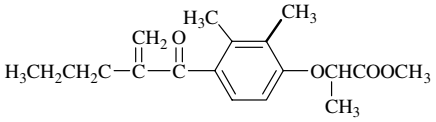
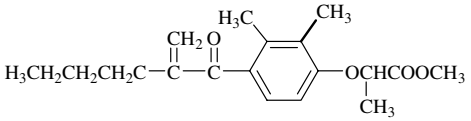
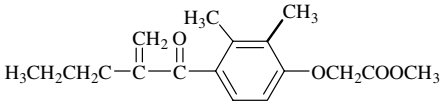
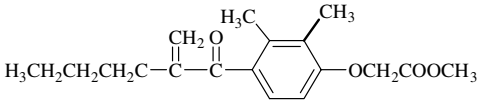
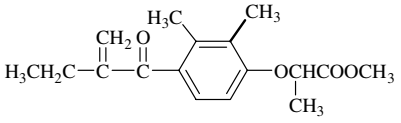
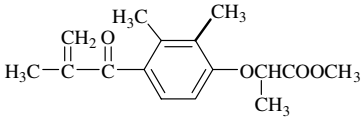
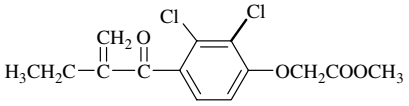
The antiproliferative ability of these compounds was determined by counting total cells. Compounds with a single substitution at the 3′-position had less antiproliferative activity than those with two at the 2′- and 3′-positions (Table 2). Compounds with methyl substitutions at the 3′- and 2′-positions (**VI-27–30**) had greater antiproliferative effects than EA. Esterification of the carboxyl group (**VII**) with methanol significantly improved the antiproliferative ability. The GI_{50} of the EA methyl ester is 3.9 μM which is approximately 10-fold lower than that of EA (44 μM). The length of the substitutions at 2″ did not influence the antiproliferative effects (compare **VI-27** with **VI-28** and **VII-1** with **VII-2**). However, some compounds (**VII-1–4**) with relatively low

Table 2. The inhibitory effects of EA derivatives on GSTP1-1 activity and on HL-60 cell growth

Code	Structure	Inhibition of GSTP1-1 activity[a] (%)	GI_{50}[b] (μM)
EA		94.4	44.2
VI-16		92.9	>60
VI-17		92.4	>60
VI-18		93.8	>60
VI-19		91.7	>60
VI-20		95.1	>60
VI-21		94.7	>60
VI-22		94.8	>60
VI-23		84.4	>60
VI-24		62.9	>60
VI-25		86.9	>60
VI-26		46.1	>60
VI-27		54.8	11.2
VI-28		51.2	12.1
VI-29		62.5	19.3

(*continued on next page*)

Table 2 (*continued*)

Code	Structure	Inhibition of GSTP1-1 activity[a] (%)	GI_{50}[b] (μM)
VI-30		61.1	14.4
VII-1		17.8	5.6
VII-2		20.0	5.2
VII-3		36.1	8.6
VII-4		34.8	8.6
VII-5		74.9	4.6
VII-6		91.3	6.0
EA′		96.7	3.9

[a] The inhibition rates of GSTP1-1 activity are calculated by the value of control group minus the value of treated group divided by the value of control group and multiplying by 100. Data shown in each group are the means of triplicate samples.

[b] GI_{50} is the concentration that inhibits 50% of proliferation compared to untreated cells. The cells were treated with each derivative for 72 h and then the cell number was determined using a haemocytometer.

GSTP1-1 inhibition ability have more potent antiproliferative ability than compounds with potent GSTP1-1 inhibition ability (**VI-16–26**). These data suggest that these compounds may act to inhibit cell proliferation through a GSTP1-1 inhibition-independent pathway.

In summary, these data indicate that (1) compounds with one substitution at the 3′-position of the aromatic ring, as we previously reported,[14] are more potent GSTP1-1 inhibitors than those without substitutions; (2) compounds with one halogen substitution at the 3′-position of the aromatic ring have greater GSTP1-1 activity inhibition than those of compounds with one methyl substitution at the same position; (3) compounds with two substitutions (at 2′- and 3′-positions) have greater antiproliferative effects than those compounds with a single substitution at the 3′-position; (4) the length of the substitution at the 2″-position does not influence the effect of those compounds on inhibition of GSTP1-1 activity and cell proliferation; (5) esterification of the carboxyl group increases the antiproliferative effect; (6) some of these compounds may inhibit cell proliferation independent of GSTP1-1 inhibition.

3. Experimental

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

3.1. Synthesis of the compound III, IV and VI series

The intermediates **III** and **IV** and the target compounds were synthesized as we previously described.[14] The parameters of the target compounds **VI** were as follows.

VI-16: 2-[3-bromo-4-(2-methylene-1-oxopropyl)phenoxy]-propionic acid, white power, yield 66.7%, mp 101.1–104.0 °C, TLC R_f = 0.45 (acetone/petroleum ether, 1:3, v/v). ^{1}H NMR (DMSO-d_6) δ: 7.30 (d, J = 8.50 Hz, 1H), 7.18 (d, J = 2.44 Hz, 1H), 6.93 (dd, J_1 = 2.45 Hz, J_2 = 8.52 Hz, 1H), 6.10 (s, 1H), 5.48 (s, 1H), 4.98 (q, J = 6.81 Hz, 1H), 1.94 (s, 3H), 1.50 (d, J = 6.80 Hz, 3H). IR (KBr, cm^{-1}): υ_{CH}: 2982.9, 2928.8; $\upsilon_{C=O}$: 1729.0, 1713.0; $\upsilon_{C=C}$: 1659.9, 1595.0; υ_{C-O}: 1222.1; $\gamma_{=CH_2}$: 1050.4, 954.1.

VI-17: 2-[3-bromo-4-(2-methylene-1-oxobutyl)phenoxy]-propionic acid, yellow oil, yield 60.6%, TLC R_f = 0.42 (acetone/petroleum ether, 1:3, v/v). ^{1}H NMR (DMSO-d_6) δ: 7.26 (d, J = 8.51 Hz, 1H), 7.16 (d, J = 2.47 Hz, 1H), 6.95 (dd, J_1 = 2.35 Hz, J_2 = 8.60 Hz, 1H), 5.98 (s, 1H), 5.51 (s, 1H), 4.94 (q, J = 6.82 Hz, 1 H), 2.33 (q, J = 7.45 Hz, 2H), 1.51 (d, J = 7.03 Hz, 3H), 1.04 (t, J = 7.58 Hz, 3H). IR (KBr, cm^{-1}): $\upsilon_{=CH}$: 3093.4; υ_{CH}: 2969.5, 2937.4; $\upsilon_{C=O}$: 1729.5; $\upsilon_{C=C}$: 1595.5; υ_{C-O}: 1224.6; $\gamma_{=CH_2}$: 1045.4, 954.7.

VI-18: [3-bromo-4-(2-methylene-1-oxobutyl)phenoxy]-acetic acid, white power, yield 69.8%, mp 104.2–106.0 °C, TLC R_f = 0.45 (acetone/petroleum ether, 1:3, v/v). ^{1}H NMR (DMSO-d_6) δ: 7.28 (d, J = 8.56 Hz, 1H), 7.24 (d, J = 2.41 Hz, 1H), 7.00 (dd, J_1 = 2.41 Hz, J_2 = 8.51 Hz, 1H), 6.03 (s, 1H), 5.49 (s, 1H), 4.81 (s, 2H), 2.34 (q, J = 7.44 Hz, 2H), 1.06 (t, J = 7.43 Hz, 3H). IR (KBr, cm^{-1}): $\upsilon_{=CH}$: 3061.2; υ_{CH}: 2973.1, 2925.1; $\upsilon_{C=O}$: 1738.2, 1709.7; $\upsilon_{C=C}$: 1657.3, 1593.0; υ_{C-O}: 1226.4; $\gamma_{=CH_2}$: 1081.8, 883.2.

VI-19: [3-chloro-4-(2-methylene-1-oxobutyl)phenoxy]-acetic acid, white power, yield 78.7%, mp 102.5–103.5 °C, TLC R_f = 0.43 (acetone/petroleum ether, 1:3, v/v). ^{1}H NMR (DMSO-d_6) δ: 7.32 (d, J = 8.51 Hz, 1H), 7.10 (d, J = 2.44 Hz, 1H), 6.97 (dd, J_1 = 2.47 Hz, J_2 = 8.54 Hz, 1H), 6.02 (s, 1H), 5.52 (s, 1H), 4.80 (s, 2H), 2.34 (q, J = 7.5 Hz, 2H), 1.05 (t, J = 7.45 Hz, 3H). IR (KBr, cm^{-1}): $\upsilon_{=CH}$: 3065.2; υ_{CH}: 2971.9, 2925.9, 2876.7; $\upsilon_{C=O}$: 1737.3, 1712.0; $\upsilon_{C=C}$: 1657.3, 1597.2; υ_{C-O}: 1228.1; $\gamma_{=CH_2}$: 1085.2, 885.3.

VI-20: [3-chloro-4-(2-methylene-1-oxopropyl)phenoxy]-acetic acid, white power, yield 56.1%, mp 107.4–109.5 °C, TLC R_f = 0.45 (acetone/petroleum ether, 1:3, v/v). ^{1}H NMR (DMSO-d_6) δ: 7.33 (d, J = 8.53 Hz, 1H), 7.10 (d, J = 2.44 Hz, 1H), 6.97 (dd, J_1 = 2.45 Hz, J_2 = 8.50 Hz, 1H), 6.10 (s, 1H), 5.51 (s, 1H), 4.80 (s, 2H), 1.94 (s, 3H). IR (KBr, cm^{-1}): $\upsilon_{=CH}$: 3063.7; υ_{CH}: 2983.2, 2920.1; $\upsilon_{C=O}$: 1736.4, 1711.6; $\upsilon_{C=C}$: 1654.6, 1596.4; υ_{C-O}: 1230.4; $\gamma_{=CH_2}$: 1085.5, 886.3.

VI-21: 2-[3-chloro-4-(2-methylene-1-oxopropyl)phenoxy]-propionic acid, white power, yield 37.3%, mp 110.7–112.8 °C, TLC R_f = 0.51 (acetone/petroleum ether, 2:3, v/v). ^{1}H NMR (DMSO-d_6) δ: 7.33 (d, J = 8.52 Hz, 1H), 7.04 (d, J = 2.33 Hz, 1H), 6.92 (dd, J_1 = 2.38 Hz, J_2 = 8.56 Hz, 1H), 6.10 (s, 1H), 5.50 (s, 1H), 4.99 (q, J = 6.79 Hz, 1H), 1.94 (s, 3H), 1.51 (d, J = 6.77 Hz, 3H). IR (KBr, cm^{-1}): υ_{CH}: 2985.3, 2960.3, 2927.9; $\upsilon_{C=O}$: 1726.5, 1712.5; $\upsilon_{C=C}$: 1660.9, 1599.6; υ_{C-O}: 1224.2; $\gamma_{=CH_2}$: 1059.1, 867.8.

VI-22: 2-[3-chloro-4-(2-methylene-1-oxobutyl)phenoxy]-propionic acid, yellow oil, yield 40.9%, TLC R_f = 0.41 (acetone/petroleum ether, 1:3, v/v). ^{1}H NMR (DMSO-d_6) δ: 7.29 (d, J = 8.59 Hz, 1H), 7.01 (d, J = 2.52 Hz, 1H), 6.91 (dd, J_1 = 2.44 Hz, J_2 = 8.61 Hz, 1H), 5.97 (s, 1H), 5.51 (s, 1H), 4.95 (q, J = 6.87 Hz, 1H), 2.33 (q, J = 7.66 Hz, 2H), 1.52 (d, J = 7.13 Hz, 3H), 1.03 (t, J = 7.84 Hz, 3H). IR (KBr, cm^{-1}): $\upsilon_{=CH}$: 3089.3; υ_{CH}: 2975.7, 2936.3, 2875.1; $\upsilon_{C=O}$: 1714.1; $\upsilon_{C=C}$: 1660.4, 1600.8; υ_{C-O}: 1222.2; $\gamma_{=CH_2}$: 1097.4, 862.0.

VI-23: 2-[3-methyl-4-(2-methylene-1-oxopropyl)phenoxy]-propionic acid, yellow oil, yield 47.0%, TLC R_f = 0.26 (chloroform/glacial acetic acid, 80:1, v/v). ^{1}H NMR (DMSO-d_6) δ: 7.24 (d, J = 8.52 Hz, 1H), 6.79 (d, J = 2.65 Hz, 1H), 6.68 (dd, J_1 = 2.65 Hz, J_2 = 8.54 Hz, 1H), 5.95 (s, 1H), 5.44 (s, 1H), 4.87 (q, J = 6.89 Hz, 1H), 2.22(s, 3H), 1.94 (s, 3H), 1.50 (d, J = 7.00 Hz, 3H). IR (KBr, cm^{-1}): $\upsilon_{=CH}$: 3093.4; υ_{CH}: 2987.7, 2927.4; $\upsilon_{C=O}$: 1728.4; $\upsilon_{C=C}$: 1654.7, 1604.2; υ_{C-O}: 1244.5; $\gamma_{=CH_2}$: 1129.2, 802.4.

VI-24: 2-[3-methyl-4-(2-methylene-1-oxobutyl)phenoxy]-propionic acid, yellow oil, yield 57.3%, TLC R_f = 0.40 (acetone/petroleum ether, 1:3, v/v). ^{1}H NMR (DMSO-d_6) δ: 7.76 (d, J = 8.50 Hz, 1H), 6.76 (m, 2H), 5.93 (s, 1H), 5.43 (s, 1H), 3.33 (q, J = 7.49 Hz, 1H), 2.41 (s, 3H), 1.56 (q, J = 7.12 Hz, 2H), 1.51 (d, J = 7.98 Hz, 3H), 0.86 (t, J = 7.34Hz, 3H). IR (KBr, cm^{-1}): υ_{CH}: 2965.4, 2935.4, 2875.6; $\upsilon_{C=O}$: 1735.0; $\upsilon_{C=C}$: 1676.1, 1603.3; υ_{C-O}: 1246.2; $\gamma_{=CH_2}$: 1135.0.

VI-25: [3-methyl-4-(2-methylene-1-oxopropyl)phenoxy]-acetic acid, white power, yield 27.3%, mp 112.0–114.1 °C, TLC R_f = 0.22 (acetone/petroleum ether, 1:3, v/v). ^{1}H NMR (DMSO-d_6) δ: 7.25 (d, J = 8.47 Hz, 1H), 6.79 (d, J = 2.36 Hz, 1H), 6.68 (dd, J_1 = 2.54 Hz, J_2 = 8.48 Hz, 1H), 5.99 (s, 1H), 5.44 (s, 1H), 4.72 (s, 2H), 2.23 (s, 3H), 1.94 (s, 3H). IR (KBr, cm^{-1}): $\upsilon_{=CH}$: 3078.7, 3051.8; υ_{CH}: 2953.6, 2917.8, 2797.6; $\upsilon_{C=O}$: 1733.1; $\upsilon_{C=C}$: 1649.1, 1602.4; υ_{C-O}: 1242.4; $\gamma_{=CH_2}$: 1121.3, 794.5.

VI-26: [3-methyl-4-(2-methylene-1-oxobutyl)phenoxy]-acetic acid, white power, yield 33.3%, mp 110.1–113.1 °C, TLC R_f = 0.25 (acetone/petroleum ether, 1:3, v/v). ^{1}H NMR (DMSO-d_6) δ: 7.24 (d, J = 8.49 Hz, 1H), 6.83 (d, J = 2.48 Hz, 1H), 6.76 (dd, J_1 = 2.62 Hz, J_2 = 8.49 Hz, 1H), 5.86 (s, 1H), 5.42 (s, 1H), 4.72 (s, 2H), 2.22 (s, 3H), 1.55 (q, J = 7.31 Hz, 2H), 1.02 (t, J = 7.40 Hz, 3H). IR (KBr, cm^{-1}): $\upsilon_{=CH}$: 3054.1; υ_{CH}: 2962.6, 2924.6, 2875.0; $\upsilon_{C=O}$: 1732.6; $\upsilon_{C=C}$: 1654.0, 1603.2; υ_{C-O}: 1244.3; $\gamma_{=CH_2}$: 1125.3, 756.5.

VI-27: 2-[2,3-dimethyl-4-(2-methylene-1-oxopentyl)phenoxy]propionic acid, yellow power, yield 86.2%, mp 83.6–85.2 °C, TLC $R_f = 0.75$ (acetone/petroleum ether, 2:3, v/v). ^{1}H NMR (DMSO-d_6) δ: 13.0 (s, 1H), 7.48 (d, $J = 8.66$ Hz, 1H), 7.01 (d, $J = 8.48$ Hz, 1H), 5.93 (s, 1H), 5.48 (s, 1H), 4.84 (q, $J = 6.80$ Hz, 1H), 2.31 (t, $J = 7.43$ Hz, 2H), 2.14 (s, 3H), 2.11 (s, 3H), 1.53 (d, $J = 6.74$ Hz, 3H), 1.45 (m, 2H), 0.90 (t, $J = 7.33$ Hz, 3H). IR (KBr, cm^{-1}): υ_{CH}: 2959.6, 2933.0, 2873.1; $\upsilon_{C=O}$: 1726.5, 1708.8; $\upsilon_{C=C}$: 1653.4, 1591.7, 1577.1; υ_{C-O}: 1263.7; $\gamma_{=CH_2}$: 1105.0, 797.0.

VI-28: 2-[2,3-dimethyl-4-(2-methylene-1-oxohexyl)phenoxy]propionic acid, yellow power, yield 88.2%, mp 75.3–76.7 °C, TLC $R_f = 0.51$ (EtOAc/petroleum ether, 1:3, v/v). ^{1}H NMR (DMSO-d_6) δ: 13.0 (s, 1H), 7.49 (d, $J = 8.65$ Hz, 1H), 7.01 (d, $J = 8.48$ Hz, 1H), 5.93 (s, 1H), 5.48 (s, 1H), 4.88 (q, $J = 6.74$ Hz, 1H), 2.81 (t, $J = 7.27$ Hz, 2H), 2.14 (s, 3H), 2.10 (s, 3H), 1.53 (d, $J = 6.81$ Hz, 3H), 1.44 (m, 4H), 0.85 (t, $J = 7.39$ Hz, 3H). IR (KBr, cm^{-1}): υ_{CH}: 2959.6, 2932.8, 2873.0; $\upsilon_{C=O}$: 1726.5, 1708.8; $\upsilon_{C=C}$: 1653.3, 1591.7, 1577.0; υ_{C-O}: 1263.7; $\gamma_{=CH_2}$: 1104.8, 797.0.

VI-29: [2,3-dimethyl-4-(2-methylene-1-oxopentyl)phenoxy]-acetic acid, yellow power, yield 74.3%, mp 75.1–78.3 °C, TLC $R_f = 0.35$ (EtOAc/petroleum ether, 1:3, v/v). ^{1}H NMR (DMSO-d_6) δ: 13.0 (s, 1H), 7.49 (d, $J = 8.65$ Hz, 1H), 7.02 (d, $J = 8.48$ Hz, 1H), 5.93 (s, 1H), 5.48 (s, 1H), 4.72 (s, 2H), 2.32 (t, $J = 7.42$ Hz, 2H), 2.15 (s, 3H), 2.11 (s, 3H), 1.44 (m, 2H), 0.91 (t, $J = 7.33$ Hz, 3H). IR (KBr, cm^{-1}): υ_{CH}: 2959.7, 2931.5, 2874.8; $\upsilon_{C=O}$: 1747.6, 1714.0; $\upsilon_{C=C}$: 1645.5, 1590.2, 1579.5; υ_{C-O}: 1257.1; $\gamma_{=CH_2}$: 1123.3, 799.1.

VI-30: [2,3-dimethyl-4-(2-methylene-1-oxohexyl)phenoxy]-acetic acid, yellow power, yield 72.3%, mp 79.8–80.9 °C, TLC $R_f = 0.36$ (EtOAc/petroleum ether, 1:3, v/v). ^{1}H NMR (DMSO-d_6) δ: 13.0 (s, 1H), 7.49 (d, $J = 8.63$ Hz, 1H), 7.02 (d, $J = 8.49$ Hz, 1H), 5.93 (s, 1H), 5.47 (s, 1H), 4.72 (s, 2H), 2.34 (t, $J = 7.46$ Hz, 2H), 2.14 (s, 3H), 2.10 (s, 3H), 1.41 (m, 2H), 1.32 (m, 2H), 0.89 (t, $J = 7.30$ Hz, 3H). IR (KBr, cm^{-1}): $\upsilon_{=CH}$: 3056.0; υ_{CH}: 2954.7, 2932.6, 2874.4; $\upsilon_{C=O}$: 1743.1, 1716.5; $\upsilon_{C=C}$: 1673.2, 1644.4, 1591.3, 1580.2; υ_{C-O}: 1252.2; $\gamma_{=CH_2}$: 1124.5, 804.1.

3.2. Synthesis of the target compounds of the VII series

A compound **VI** (2 mmol) and *para*-toluenesulfonic acid (0.58 mmol) were dissolved in methanol (10 mL). The mixture was heated up to 45 °C for 40 min and diluted by ether (15 mL) after the mixture was cooled down to room temperature. Then the mixture was washed with 5% sodium bicarbonate solution (10 mL, 2×). The ether layer was collected and dried with $MgSO_4$ overnight. After filtering, the ether was evaporated under reduced pressure to obtain a yellow product **VII**.

VII-1: methyl 2-[2,3-dimethyl-4-(2-methylene-1-oxopentyl)phenoxy]propionate, yellow oil, yield 41.1%, TLC $R_f = 0.56$ (acetone/petroleum ether, 1:3, v/v). ^{1}H NMR (DMSO-d_6) δ: 7.47 (d, $J = 8.66$ Hz, 1H), 7.00 (d, $J = 8.50$ Hz, 1H), 5.92 (s, 1H), 5.46 (s, 1H), 5.02 (q, $J = 6.72$ Hz, 1H), 3.67 (s, 3H), 2.34 (t, $J = 7.42$ Hz, 2H), 2.15 (s, 3H), 2.10 (s, 3H), 1.54 (d, $J = 6.84$ Hz, 3H), 1.32 (m, 2H), 0.88 (t, $J = 7.32$ Hz, 3H). IR (KBr, cm^{-1}): $\upsilon_{=CH}$: 3087.0; υ_{CH}: 2988.4, 2955.4, 2930.8, 2871.9, 2860.9; $\upsilon_{C=O}$: 1760.1, 1741.2; $\upsilon_{C=C}$: 1656.6, 1591.6, 1578.5; υ_{C-O}: 1262.0; $\gamma_{=CH_2}$: 1105.9, 799.3.

VII-2: methyl 2-[2,3-dimethyl-4-(2-methylene-1-oxohexyl) phenoxy]propionate, yellow oil, yield 61.0%, TLC $R_f = 0.55$ (acetone/petroleum ether, 1:3, v/v). ^{1}H NMR (DMSO-d_6) δ: 7.46 (d, $J = 8.65$ Hz, 1H), 7.00 (d, $J = 8.48$ Hz, 1H), 5.92 (s, 1H), 5.47 (s, 1H), 4.98 (q, $J = 6.76$ Hz, 1H), 3.67 (s, 3H), 2.32 (t, $J = 7.58$ Hz, 2H), 2.15 (s, 3H), 2.11 (s, 3H), 1.55 (d, $J = 7.95$ Hz, 3H), 1.45 (m, 2H), 1.28 (m, 2H), 0.85 (t, $J = 7.39$ Hz, 3H). IR (KBr, cm^{-1}): $\upsilon_{=CH}$: 3087.0; υ_{CH}: 2956.8, 2932.6, 2872.4; $\upsilon_{C=O}$: 1759.8, 1741.0; $\upsilon_{C=C}$: 1656.4, 1591.6, 1578.8; υ_{C-O}: 1261.6; $\gamma_{=CH_2}$: 1105.2, 799.2.

VII-3: methyl [2,3-dimethyl-4-(2-methylene-1-oxopentyl)phenoxy]acetate, yellow oil, yield 51.7%, TLC $R_f = 0.55$ (acetone/petroleum ether, 1:3, v/v). ^{1}H NMR (DMSO-d_6) δ: 7.48 (d, $J = 8.67$ Hz, 1H), 7.02 (d, $J = 8.50$ Hz, 1H), 5.92 (s, 1H), 5.49 (s, 1H), 4.84 (s, 2H), 3.70 (s, 3H), 2.32 (t, $J = 7.42$ Hz, 2H), 2.16 (s, 3H), 2.12 (s, 3H), 1.45 (m, 2H), 0.91 (t, $J = 7.30$ Hz, 3H). IR (KBr, cm^{-1}): $\upsilon_{=CH}$: 3088.0; υ_{CH}: 2957.6, 2931.9, 2872.4; $\upsilon_{C=O}$: 1764.1, 1743.3; $\upsilon_{C=C}$: 1656.1, 1591.8, 1579.5; υ_{C-O}: 1211.3; $\gamma_{=CH_2}$: 1124.2, 798.5.

VII-4: methyl [2,3-dimethyl-4-(2-methylene-1-oxohexyl)-phenoxy]acetate, yellow oil, yield 32.9%, TLC $R_f = 0.57$ (acetone/petroleum ether, 1:3, v/v). ^{1}H NMR (DMSO-d_6) δ: 7.48 (d, $J = 8.67$ Hz, 1H), 7.01 (d, $J = 8.50$ Hz, 1H), 5.92 (s, 1H), 5.47 (s, 1H), 4.84 (s, 2H), 3.70 (s, 3H), 2.34 (t, $J = 7.39$ Hz, 2H), 2.16 (s, 3H), 2.12 (s, 3H), 1.40 (m, 2H), 1.31 (m, 2H), 0.88 (t, $J = 7.28$ Hz, 3H). IR (KBr, cm^{-1}): $\upsilon_{=CH}$: 3087.6; υ_{CH}: 2955.5, 2930.1, 2860.4; $\upsilon_{C=O}$: 1764.3, 1743.4; $\upsilon_{C=C}$: 1656.3, 1591.6, 1579.1; υ_{C-O}: 1209.9; $\gamma_{=CH_2}$: 1124.0, 798.7.

VII-5: methyl 2-[2,3-dimethyl-4-(2-methylene-1-oxobutyl)phenoxy]propionate, yellow oil, yield 42.0%, TLC $R_f = 0.55$ (acetone/petroleum ether, 1:3, v/v). ^{1}H NMR (DMSO-d_6) δ: 7.01 (d, $J = 8.48$ Hz, 1H), 6.69 (d, $J = 8.47$ Hz, 1H), 5.92 (s, 1H), 5.46 (s, 1H), 4.98 (q, $J = 6.74$ Hz, 1H), 3.67 (s, 3H), 2.34 (q, $J = 7.34$ Hz, 2H), 2.15 (s, 3H), 2.11 (s, 3H), 1.54 (d, $J = 6.82$ Hz, 3H), 1.04 (t, $J = 7.42$ Hz, 3H). IR (KBr, cm^{-1}): $\upsilon_{=CH}$: 3087.5; υ_{CH}: 2963.3, 2935.9, 2875.4; $\upsilon_{C=O}$: 1759.7, 1740.9; $\upsilon_{C=C}$: 1656.0, 1591.5, 1579.8; υ_{C-O}: 1261.2; $\gamma_{=CH_2}$: 1136.2, 799.3.

VII-6: methyl 2-[2,3-dimethyl-4-(2-methylene-1-oxopropyl)phenoxy]propionate, yellow oil, yield 41.0%, TLC $R_f = 0.52$ (acetone/petroleum ether, 1:3, v/v). ^{1}H NMR (DMSO-d_6) δ: 7.02 (d, $J = 8.48$ Hz, 1H), 6.68 (d, $J = 8.56$ Hz, 1H), 6.01 (s, 1H), 5.45 (s, 1H), 5.00 (q, $J = 6.77$ Hz, 1H), 3.67 (s, 3H), 2.15 (s, 3H), 2.09 (s, 3H), 1.94 (s, 3H), 1.54 (d, $J = 6.77$ Hz, 3H). IR (KBr, cm^{-1}): $\upsilon_{=CH}$: 3089.2; υ_{CH}: 2987.0, 2953.6; $\upsilon_{C=O}$:

1759.0, 1740.1; $\upsilon_{C=C}$: 1657.2, 1591.7, 1580.2; υ_{C-O}: 1261.4; $\gamma_{=CH_2}$: 1135.9, 799.3.

3.3. Biological activity methods

3.3.1. Cells. HL-60 cells were cultured in RPMI-1640 medium supplemented with 100 U/mL pencillin, 100 μg/mL streptomycin, 1 mmol/L L-glutamine, and 10% heat-inactivated fetal bovine serum.

3.3.2. GSTP1-1 activity. GSTP1-1 activity was measured using CDNB and GSH as substrates as we described previously.[14] Cells (3×10^6) in logarithmic growth were collected, 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, centrifuged at 14,000 rpm for 30 min at 4 °C, and the supernatant fluid was used for enzyme activity assays. The protein content in the cell lysates was determined by the Bradford Protein Assay, with bovine serum albumin as a standard. The assay mixture contained 2.55 mL of 0.1 mol/L sodium phosphate–1 mmol/L EDTA (pH 6.5), 150 μL of 20 mmol/L GSH, 150 μL of 20 mmol/L CDNB, and 150 μL of clear cell lysate. An assay mixture without the cell lysate was used as a control. The absorbance at 340 nm was continuously recorded for 2 min. The nonenzymatic reaction was corrected by blanking the spectrophotometer with the control cuvette before reading a sample cuvette. The CDNB-GSH product has a strong molar absorptivity at 340 nm (=9.6 mM^{-1} cm^{-1}). GSTP1-1 activity was defined as nanomoles of product per min per milligram of protein.

3.3.3. Cell proliferation inhibition. Cells were seeded at a density of 1×10^5 cells/mL and incubated with various concentrations of the tested compounds. Total cell number including trypan blue staining positive and negative cells in each group was counted. The antiproliferative ability was calculated and expressed as the ratio of the cell number in the treated group to that of the untreated group. The concentration (GI_{50}) which inhibited half of the cell proliferation was calculated.

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