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Synthesis and anticancer activity of 2-alkylaminomethyl-5-diaryl-methylenecyclopentanone hydrochlorides and related compounds

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Abstract—Eleven new diaryl-methylenecyclopentanone Mannich hydrochlorides and related compounds were synthesized with different modification on Mannich base and α,β-unsaturated bonds. The glutathione-binding ability, glutathione-s-transferase π (GSTπ) inhibition and antitumor effect of these compounds were compared. Compounds containing both Mannich base and α-unsaturated bond have GSH binding ability, GSTπ inhibitory activity and antitumor effect. Compounds without Mannich base or having a α-saturated bond lose GSH binding ability and the antitumor effect. Converting of Mannich base from dimethylaminomethyl group to morpholino, pyrrolidino, or piperidino-methyl groups do not evidently change the antitumor effect. However replacement of phenyl group with methylphenyl group on β-chain significantly increases cytotoxic effect in breast cancer cells but not in immortalized mammary epithelial cells. Our data suggest that diaryl-methylenecyclopentanones represent a new category of compounds which might inhibit tumor growth through binding to glutathione or thiol proteins.

1. Introduction

Diterpenes and sesquiterpenes containing α,β-unsaturated carbonyl structures have been found to have a strong antineoplastic activity.[1,2] Similarly to these natural compounds, it has been found that many synthetic compounds containing α,β-unsaturated carbonyl structures have remained the antitumor effect.[3–5] These studies suggest that α,β-unsaturated carbonyl structures would be a critical substitute to mediate the observed antitumor effect among these compounds. Moreover, it has been found that compounds containing Mannich base, which is a masked bioactive group, could produce the methylene substances upon 1,2-elimination and have an enhanced antitumor effect.[6–8] The mechanism inhibiting tumor cell growth of these compounds has been thought to be mediated by binding of α,β-unsaturated carbonyl structures to free cysteine sulfhydryl groups including glutathione (GSH) to form thiol-addition product.[8] GSH plays an important role in cells as a substrate of antioxidant enzymes to prevent cell death and as a substrate of glutathione-s-transferases (GSTs) which involve in the drug resistance.[9,10] Among the GST family it has been found that GSTπ was increased in chemotherapy-resistance cancer cells.[11] Recently it has been found that GSTπ functions as an antioxidant enzyme to block apoptosis-induced by some chemotherapeutic agents through diminishing free radical oxygen and directly inhibition of c-Jun N-terminal kinase (JNK).[12,13] Thus GSTπ could be used as a potential target for the cancer treatment.[14] The potential GSH binding ability of these compounds suggests that they may inhibit GSTπ activity directly or indirectly, and would be potent anticancer agents in tumor cells even with higher GSTπ activity.

In our current study, we have introduced two phenyl groups into β-carbon of previously reported cyclopentanone[8] to obtain new compounds (**7a**–**h**). To determine the importance of α, or β-unsaturated bonds on the antitumor activity, a selective reduction of the β-unsaturated bond of **7a** and **7e** was made into the saturated ketone analogues **8a** and **8b,** and further reduction of **8a** generated the α-, β-saturated analogue **9**. To test the importance of α, or β-unsaturated bond or Mannich base,

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the GSH binding ability, GSTπ inhibition ability and antitumor activity, were compared among these compounds (**5**–**9** listed in Fig. 1) with modification of α, or β-unsaturated bond or Mannich base.

2. Results and discussion

2.1. Chemistry

The compounds **7**–**9** were prepared according to the procedure indicated in Figure 1. The starting compound, ethyl 1,4-dioxaspiro[4,4]nonane-6-carboxylate (**4**), was synthesized according to previously described methods.[15] 2-Diarylmethylenecyclopentanones (**5a**, **b**) were synthesized by the reaction of the appropriate aryl Grignard reagents with **4** following by hydrolysis and dehydration using dilute aqueous–ethanolic mineral acid using a general method reported previously.[16] Compounds **7a**, **b** were synthesized from the corresponding 2-diarylmethylenecyclopentanones, secondary amines and paraformaldehyde and the products were isolated as hydrochlorides. The ketonic groups in the compounds **5a**, **b** were reduced into 2-arylmethylcyclopentanones (**6a**, **b**), respectively, by catalytic hydrogenation with hydrogen over Pd–C at atmospheric pressure based on a reported procedure.[17] The reactions of **6a**, **b** with *N*,*N*-dimethylmethyleneammonium chloride led to the formation of **8a**, **b** with Mannich bases, respectively. Compound **8a** was reduced by $NaBH_4$ in methanol to yield the corresponding alcohol analogue **9**. All the synthesized Mannich base hydrochlorides and compound **9** were chemically characterized by melting point (mp), infrared (IR) and nuclear magnetic resonance (^1H NMR) spectra as well as mass spectra (MS).

The reagents used were as follows, namely: (i) ethylene glycol; (ii) aryl Grignard reagent; (iii) dilute aqueous–ethanolic hydrochloric acid; (iv) hydrogen over palladium on carbon powder; (v) concentrated hydrochloric acid, paraformaldehyde and secondary amine; (vi) *N*,*N*-dimethylmethyleneammonium chloride; (vii) sodium borohydride.

2.2. Interaction with GSH

The interaction of these cyclopentanone Mannich base hydrochlorides with GSH were measured by reported method based on the interaction of DTNB [5,5′-dithio-bis(2-nitrobenzoic acid)] with GSH.[18]

Basically, when DTNB [5,5′-dithio-bis(2-nitrobenzoic acid)] is added to a solution containing GSH, a reaction listed in Figure 2 will occur and the yellow color product 5-thio-2-nitrobenzoic acid (TNB) of DTNB and GSH addition will be formed and can quantitatively be used to measure at 412 nm using a spectrometer.[19] The DTNB, GSH, compounds **5**–**9** and their mixture with GSH do not have absorption at 412 nm at concentrations below 10^{-3} mol/L.

2-Dimethylaminomethyl-5-(*E*)-pentylidenecyclopentanone hydrochloride (WB_{852}) has been found to directly bind with GSH measured using MS previously[8] and was used as a positive control in the current method. We use the decrease of 412 nm absorption to evaluate the competition of WB_{852} or compounds **5**–**9** with DTNB to bind GSH. WB_{852} reacts completely with an equimolar amount of GSH in 60 min and prevents TNB formation. Based on the decrease of TNB formation (the intensity of 412 nm absorbance), the addition

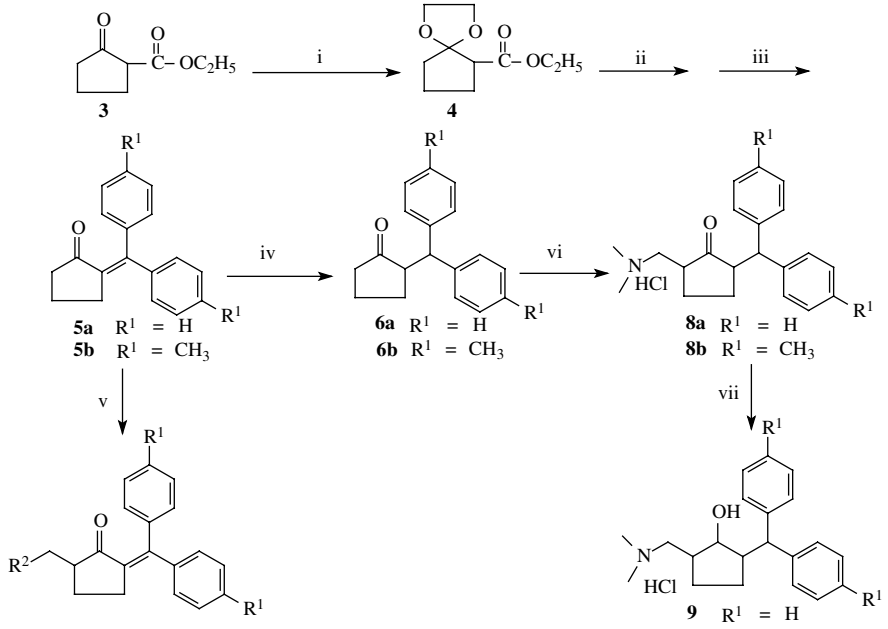

Figure 1. The synthetic pathways of compounds **5**–**9**.

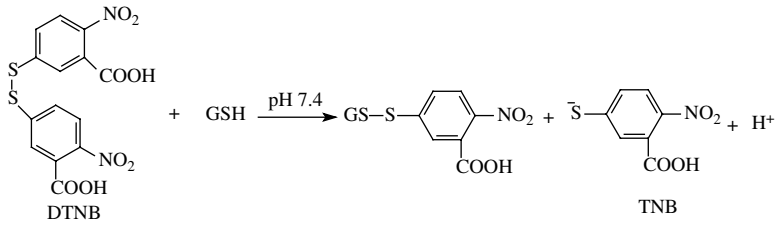

Figure 2. The interaction pathway of DTNB with GSH.

Table 1. The influence of WB_{852} and compounds **5–9** on TNB formation due to DTNB and GSH reaction

Compound	Concentrations (μmol/L)					
	0	10	20	40	50	160
OD 412 nm[a]						
WB_{852}	0.062	0.060	0.059	0.041	0.026	0
5a	0.063	0.063	0.062	0.061	0.062	0.063
7a	0.064	0.057	0.045	0.031	0.026	0.007
7b	0.063	0.052	0.047	0.038	0.030	0.015
7c	0.062	0.055	0.046	0.046	0.036	0.015
7d	0.061	0.054	0.050	0.045	0.040	0.012
7e	0.064	0.064	0.059	0.038	0.032	0.026
7f	0.060	0.057	0.053	0.051	0.047	0.025
7g	0.063	0.060	0.052	0.048	0.042	0.033
7h	0.061	0.058	0.055	0.053	0.052	0.032
8a	0.060	0.054	0.046	0.035	0.031	0.015
8b	0.063	0.060	0.054	0.045	0.038	0.032
9	0.063	0.063	0.062	0.063	0.061	0.045

[a] The OD at 412 nm was assayed in buffer containing 50 μmol/L GSH and indicated concentrations of compounds for 30 min following addition of 500 μmol/L DTNB.

of compounds **5–9** with GSH is estimated and listed in Table 1.

All of the compounds with Mannich base hydrochlorides decrease the absorbance at 412 nm in a concentration-dependent manner. Compounds **5a**, **b** without 2-alkylaminomethyl group adjacent to the α-CH_2 on the cyclopentanone ring, do not change the absorbance at 412 nm. Compound **7a** and **7b** decrease the absorbance at 412 nm to 70.3% and 74.6% of that of control solution at 20 μmol/L, respectively, while WB_{852} only lowers the GSH level to 95.4% of that of control solution at 20 μmol/L (Table 1). Comparing with WB_{852}, compound **7a** at 50 μmol/L has a stronger activity to decrease the absorbance at 412 nm. For compounds containing the same alkylidene moiety, the dimethylaminomethyl analogs are more active than structures containing morpholino, pyrrolidino, or piperidino-methyl groups. Comparing with compound **8a**, compound **9** has a decreased ability of blocking TNB formation. The fact that compounds **5a** and **9** do not/weakly decrease the absorbance at 412 nm indicates that both 2-alkylaminomethyl structure (Mannich base) and cyclopentanone ring are critical functional groups to mediate GSH-binding ability. Since compound **8** has similar ability to decrease the absorbance at 412 nm with that of compounds **7** indicating that β-unsaturated bond is not necessary for the GSH binding.

2.3. Inhibition of GSTπ activity

To test whether these compounds inhibit GSTπ activity, the cell lysate of HL-60 cells, which has been shown to contain higher activity of GSTπ,[20] as a GSTπ source used to measure the inhibitory effect of these compounds on its activity. The GSTπ activity was measured based on the classic method.[21] By using WB_{852} as a represent compound of these synthesized cyclopentanones, we calculated the IC_{50} of its inhibition on GSTπ activity and found that was around 30 μM. Thus, for the purpose to compare the structure–activity relationship of these compounds on GSTπ inhibition, we focused on the 30 μM concentration of each compounds. As shown in Table 2, compounds **7** and **8** had stronger inhibitory effect on GSTπ activity than WB_{852}. However, compound **5a** did not have inhibitory effect on GSTπ activity and compound **9** had a decreased inhibitory effect comparing with WB_{852}. Since the pattern of structure–activity relationship of these compounds on GSTπ activity inhibition is similar to that of their interaction with GSH (Table 1), it seems that the inhibitory effect of these compounds on GSTπ activity results from their interaction with GSH, the substrate of GSTπ, and inhibit GSTπ activity indirectly. In supporting this conclusion, GSTπ activity in cell lysate isolated from HL-60 cells after

Table 2. The inhibitory effect of compounds **7–9** on GSTπ activity in vitro

Compounds	GSTπ activity (nmol/min/mg protein)	Inhibition (%)
Control	88.4 ± 6.0	0
WB_{852}	35.3 ± 3.0[a]	55.8
5a	88.5 ± 6.4	0
7a	3.0 ± 2.1[a]	96.2
7b	2.8 ± 1.1[a]	96.8
7c	6.3 ± 2.1[a]	92.9
7d	9.2 ± 2.6[a]	89.6
7e	6.0 ± 1.3[a]	92.5
7f	2.1 ± 1.7[a]	97.6
7g	14.7 ± 3.7[a]	83.4
7h	19.8 ± 1.3[a]	77.6
8a	1.5 ± 1.3[a]	98.1
8b	1.1 ± 0.6[a]	98.7
9	52.5 ± 4.3[b]	25.6

GSTπ activity was assayed in 2 h reaction buffer containing 30 μmol/L tested compounds, 1 mmol/L GSH, 1 mmol/L CDNB and 100 μg HL-60 cell lysate. The unit of GSTπ activity was defined as the amount of enzyme which catalyze the formation of 1 μmol of product per mg protein per minute. The data show mean ± SD of triple samples.

[a] $P < 0.005$.

[b] $P < 0.05$ in *t*-test.

treatment with any of these compounds was not inhibited. However the intracellular GSH levels were decreased in HL-60 cells after treatment by compounds showing interaction with GSH in vitro (data not shown).

2.4. Antitumor effect

The in vitro antitumor activity of these compounds was conducted by using MTT assay in two human breast cancer cell lines MCF-7 and MCF-7/Adr cells and one immortalized human mammary epithelial cell line 184B5 and by using direct cell number counting in human leukemia HL-60 cells (Table 3). All of the compounds had antitumor effect except compounds **5a** and **5b**. Compound **9** had a decreased antitumor effect comparing with compound **8a**. Since the pattern of cancer cell growth inhibition (Table 3) is also similar to that of GSH binding (Table 1), it suggests that the cytotoxic effect of these compounds is mediated with direct binding to GSH or other thiol proteins that are critical for cell growth. The stronger activity of compounds **7e**–**h** comparing with that of compounds **7a**–**d**, suggest that methyl group in the phenyl of β-chain will either increase the cell membrane penetration ability or stability in solution or medium. Correlated with the antitumor activity, compounds **7e**–**h** have strong GSH decreasing ability than compounds **7a**–**c** (data not shown). Since the IC_{50s} of compounds **7e**–**h** in MCF-7 cells (without expression of GSTπ) is much lower than the IC_{50s} in MCF-7/Adr cells and 184B5 cells (both expressing GSTπ)[22] suggesting that the growth inhibitory effect of these compounds might not result from GSTπ inhibition. Interestingly it seems that GSTπ might play a negative role in cell growth inhibition by these compounds. It is possible that GSTπ catalyzes these compounds to interact with GSH and the GSH-conjugated compounds will have a decreased cell growth inhibitory effect. This assumption needs to be testified in future by synthesizing GSH combined cyclopentanone. The stronger selectivity of compounds **7h** to breast cancer MCF-7 cells comparing to mammary epithelial 184B5 cells, suggesting it could be developed into a novel drug which will selectively target tumor cells, at least in tumor cells without GSTπ expression, without severe toxicity to normal cells at controlled doses.

Table 3. The IC_{50s} of compounds **5**–**9** on cell growth inhibition in vitro

Compounds	MCF-7[a]	MCF-7/Adr[a]	184B5[a]	HL-60[b]
IC_{50} (μmol/L)				
5a	>50	>50	>50	>50
5b	>50	>50	>50	>50
7a	2.5	5.8	2.0	13.3
7b	8.0	15.5	3.5	13.0
7c	7.7	14.0	4.2	9.6
7d	4.4	8.0	1.5	12.7
7e	1.7	3.1	3.0	3.4
7f	2.5	3.8	3.5	0.4
7g	2.2	3.5	3.0	0.9
7h	2.2	3.5	5.5	1.3
8a	6.4	6.7	N	3.2
8b	6.6	7.2	N	4.0
9	>20	>20	N	>20

[a] The cells were treated for 4 days.
[b] The cells were treated for 1 day; N, not tested.

In summary, both Mannich base hydrochlorides and cyclopentanone ring are important for GSH binding, GSTπ inhibition and antitumor effect of our synthesized compounds. The GSTπ inhibitory effect of these compounds result from their direct interaction with GSH, the substrate of GSTπ. The significant changes in antitumor activity are apparent in altering phenyl groups to methylphenyl groups by comparing compounds **7a**–**d** or **7e**–**h**. Our data support the previous postulation of that the activity of these compounds may result from direct binding to GSH and other thiol proteins.[8]

3. Experimental

GSH, DTNB, 1-chloro-2,4-dinitrobenzene(CDNB) and 3-[4,5-dimethylthiazol-2yl]-2,5-di-phenyltetrazolium bromide (MTT) were obtained from J&K-ACROS Chemical Co. Bovine Serum Albumin (BSA) was purchased from Sigma (St. Louis, MO) and prepared in pure water. IR Spectra were recorded on a Bruker IR-27G spectrometer. The ^1H NMR were measured on a Bruker ARX-300 spectrometer with an internal standard of tetramethylsilane. Mass spectra were recorded on a Shimadzu GCMS QP-1000 mass spectrometer.

4. General procedure for preparation of 2-diarylmethylenecyclopentanones (5a, b)

Grignard reagents were prepared by addition of aryl bromide (0.26 mol) in dry ether (75 mL) to magnesium (0.27 mol) in ether (25 mL). The mixture was refluxing for 20 min and then a solution of ethyl 2,2-ethylenedioxycyclopentanecarboxylate (0.11 mol) in ether (50 mL) was added dropwisely with vigorous stirring. The mixture was boiled and stirred for 60 min, cooled and decomposed with aqueous ammonium chloride (156 mL, 37% w/v). The precipitate was removed by filtration, the ether layer was separated and the aqueous layer extracted with ether. The combined ethereal solutions were concentrated and methanol (62 mL), water (43 mL) and concentrated hydrochloric acid (3.1 mL) were added in. The mixture was refluxed with stirring for 5 h. After cooling down, yellow precipitates were collected and was recrystallized from ethanol to obtain yellow needle products.

Compound **5a**: 2-diphenylmethylenecyclopentanone, yield 39%, mp 115–116 °C.

Compound **5b**: 2-[bis-(4-methylphenyl)methylene]cyclopentanone, yield 32%, mp 142–143 °C.

4.1. General procedure for synthesis of 2-alkylaminomethyl-5-diarylmethylenecyclopentanone hydrochlorides (7a–h)

Compounds **7a**–**h** were synthesized according to classical Mannich reaction.[23] A mixture of 2-diarylmethylene-

cyclopentanone (0.005 mol), secondary amine hydrochloride (0.005 mol) and paraformaldehyde (0.013 mol) in the presence of concentrated hydrochloric acid (0.15 mL) was refluxed in absolute ethanol (15 mL) for 4 h. The resulting solution was concentrated in vacuum. For compounds **7a** and **7e**, the residues were treated with dry acetone yielding crystalline material and the products were recrystallized from acetone–ethanol. However, for compounds **7b–d**, **7f–h**, the following purification method was employed. The residue was dissolved in water, the precipitate was removed and the filtrate solution was adjust to alkaline with anhydrous sodium carbonate and was extracted with ethyl ether. The ethereal solution was dried over anhydrous sodium sulfate. After filtration the solution was treated dropwisely with dry ethereal hydrochloric acid to give a white precipitate and was then recrystallized from acetone–ethanol.

4.1.1. 2-Dimethylaminomethyl-5-diphenylmethylenecyclopentanone hydrochloride (7a). Mp: 155–156 °C. Yield: 12%. EI-MS (*m*/*z*): 305 (M^+, 3.77), 259 (12.28), 58 (100.00), 44 (7.64), 36 (7.30); ^{1}H NMR ($CDCl_3$) δ (ppm): 1.68–1.74 (m, 1H), 2.78–3.33 (m, 11H), 3.35–3.39 (m, 1H), 7.07–7.35 (m, 10H, *H*-Ph), 12.56 (s, 1H, HCl); IR (KBr) σ/cm^{-1}: 2965 ($\upsilon_{=CH}$), 2455 (υ_{CH}, CH_3), 1704 ($\upsilon_{C=O}$), 1590, 1469, 1442 ($\upsilon_{C=C}$), 1180 (δ_{CH}), 948, 758 ($\gamma_{C=C}$).

4.1.2. 2-Morpholinomethyl-5-diphenylmethylenecyclopentanone hydrochloride (7b). Mp: 172–173 °C. Yield: 35%. EI-MS (*m*/*z*): 347 (M^+, 9.92), 259 (46.42), 100 (100.00), 44 (40.19), 36 (19.09); ^{1}H NMR ($CDCl_3$) δ (ppm): 1.66–1.77 (m, 1H), 2.75–2.83 (m, 2H), 2.92–3.03 (m, 4H), 3.20–3.47 (m, 4H), 3.93–3.97 (m, 2H), 4.20–4.37 (m, 2H), 7.08–7.36 (m, 10H, *H*-Ph), 13.02 (s, 1H, HCl); IR (KBr) σ/cm^{-1}: 2930 ($\upsilon_{=CH}$), 1701 ($\upsilon_{C=O}$), 1586, 1568, 1441, 1410 ($\upsilon_{C=C}$), 1080 (δ_{CH}), 872 ($\gamma_{C=C}$).

4.1.3. 2-Pipyridylmethyl-5-diphenylmethylenecyclopentanone hydrochloride (7c). Mp: 168–170 °C. Yield: 15%. EI-MS (*m*/*z*): 345 (M^+, 9.20), 259 (100.00), 98 (98.23), 36 (30.77); ^{1}H NMR ($CDCl_3$) δ (ppm): 1.36–1.44 (m, 1H), 1.65–1.71 (m, 1H), 1.79–1.85 (m, 2H), 2.20-2.37 (m, 2H), 2.60–2.96 (m, 7H), 3.16–3.28 (m, 2H), 3.47–3.55 (m, 2H), 7.08–7.36 (m, 10H, *H*-Ph), 12.10 (s, 1H, HCl); IR (KBr) σ/cm^{-1}: 2942 ($\upsilon_{=CH}$), 1705 ($\upsilon_{C=O}$), 1587, 1569, 1431 ($\upsilon_{C=C}$), 1165 (δ_{CH}), 937 ($\gamma_{C=C}$).

4.1.4. 2-Pyrrolidylmethyl-5-diphenylmethylenecyclopentanone hydrochloride (7d). Mp: 164–165 °C. Yield: 28%. EI-MS (*m*/*z*): 331 (M^+, 11.94), 259 (87.90), 84 (100.00), 36 (39.27); ^{1}H NMR ($CDCl_3$) δ (ppm): 1.68–1.73 (m, 1H), 2.05–2.08 (m, 2H), 2.19–2.26 (m, 2H), 2.76–3.12 (m, 7H), 3.37–3.43 (m, 1H), 3.71–3.83 (m, 2H), 7.08–7.36 (m, 10H, *H*-Ph), 12.48 (s, 1H, HCl); IR (KBr) σ/cm^{-1}: 2921 ($\upsilon_{=CH}$), 1706 ($\upsilon_{C=O}$), 1588, 1569, 1491, 1443 ($\upsilon_{C=C}$), 1175 (δ_{CH}), 770 ($\gamma_{C=C}$).

4.1.5. 2-Dimethylaminomethyl-5-[bis-(4-methylphenyl)-methylene]cyclopentanone hydrochloride (7e). Mp: 144–146 °C. Yield: 32%. EI-MS (*m*/*z*): 333 (M^+, 12.32), 287 (16.80), 273 (31.69), 58 (100.00); ^{1}H NMR ($CDCl_3$) δ (ppm): 1.64–1.71 (m, 1H), 2.38 (d, 6H, J = 3.0 Hz), 2.79–3.33 (m, 11H), 3.37–3.39 (m, 1H), 6.96–7.16 (m, 8H, *H*-Ph), 12.57 (s, 1H, HCl); IR (KBr) σ/cm^{-1}: 2507 (υ_{CH}, CH_3), 1704 ($\upsilon_{C=O}$), 1641, 1586, 1561, 1508, 1466 ($\upsilon_{C=C}$), 1181 (δ_{CH}), 915 ($\gamma_{C=C}$).

4.1.6. 2-Morpholinomethyl-5-[bis-(4-methylphenyl)methylene]cyclopentanone hydrochloride (7f). Mp: 168–170 °C. Yield: 40%. EI-MS (*m*/*z*): 375 (M^+, 13.10), 287 (9.03), 273 (18.87), 100 (100.00), 44 (1.32); ^{1}H NMR ($CDCl_3$) δ (ppm): 1.66–1.77 (m, 1H), 2.38 (d, 6H, J = 3.0 Hz), 2.74–2.81 (m, 2H), 2.93–2.97 (m, 4H), 3.16–3.47 (m, 4H), 3.93–3.96 (m, 2H), 4.21–4.39 (m, 2H), 6.96–7.16 (m, 8H, *H*-Ph), 13.09 (s, 1H, HCl); IR (KBr) σ/cm^{-1}: 2424 (υ_{CH}, CH_3), 1702 ($\upsilon_{C=O}$), 1591, 1508, 1446 ($\upsilon_{C=C}$), 1176 (δ_{CH}), 819 ($\gamma_{C=C}$).

4.1.7. 2-Pipyridylmethyl-5-[bis-(4-methylphenyl)methylene]cyclopentanone hydrochloride (7g). Mp: 161–163 °C. Yield: 36%. EI-MS (*m*/*z*): 373 (M^+, 15.57), 287 (20.40), 273 (37.10), 98 (100.00), 44 (1.50); ^{1}H NMR ($CDCl_3$) δ (ppm): 1.36–1.44 (m, 1H), 1.65–1.71 (m, 1H), 1.79–1.85 (m, 2H), 2.18–2.33 (m, 2H), 2.38 (d, 6H, J = 3.0 Hz), 2.61–2.97 (m, 7H), 3.12 (m, 1H), 3.28 (m, 1H), 3.47–3.56 (m, 2H), 6.96–7.16 (m, 8H, *H*-Ph), 12.11 (s, 1H, HCl); IR (KBr) σ/cm^{-1}: 2502 (υ_{CH}, CH_3), 1707 ($\upsilon_{C=O}$), 1596, 1507, 1449 ($\upsilon_{C=C}$), 1159 (δ_{CH}), 917, 820 ($\gamma_{C=C}$).

4.1.8. 2-Pyrrolidylmethyl-5-[bis-(4-methylphenyl)methylene]cyclopentanone hydrochloride (7h). Mp: 165–167 °C. Yield: 42%. EI–MS (*m*/*z*): 359 (M^+, 12.56), 287 (19.02), 273 (37.93), 84 (100.00), 44 (1.52); ^{1}H NMR ($CDCl_3$) δ (ppm): 1.66–1.77 (m, 1H), 2.05–2.09 (m, 2H), 2.18–2.25 (m, 2H), 2.38 (d, 6H, J = 3.0 Hz), 2.75–3.10 (m, 7H), 3.40 (m, 1H), 3.71–3.81 (m, 2H), 6.96–7.16 (m, 8H, *H*-Ph), 12.47 (s, 1H, HCl); IR (KBr) σ/cm^{-1}: 2579 (υ_{CH}, CH_3), 1706 ($\upsilon_{C=O}$), 1592, 1508, 1448 ($\upsilon_{C=C}$), 1169 (δ_{CH}), 820 ($\gamma_{C=C}$).

4.2. General procedure for synthesis of 2-alkylaminomethyl-5-diarylmethylcyclopentanone hydrochlorides (8a, b)

The Mannich bases **8a**, **b** were prepared as follows. *N*,*N*-dimethylmethylene-ammonium chloride (0.01 mol), synthesized based on a literature procedure,[24] was added to a solution of 2-diarylmethyl cyclopentanone (0.0025 mol) in acetonitrile (50 mL) at 80–90 °C and the mixture was heated under reflux. The reaction was monitored by TLC using a solvent system of chloroform–methanol (8:1). After 16 h, the mixture was filtered and the solid product was crystallized from chloroform–acetonitrile to give **8a** or **8b**.

4.2.1. 2-Benzhydryl-5-((dimethylamino)methyl)cyclopentanone hydrochloride (8a). Mp: 175–176 °C. Yield: 52%. EI-MS (*m*/*z*): 307 (M^+, 0.46), 262 (0.29), 58 (100.00), 44 (1.72), 36 (2.28); ^{1}H NMR (DMSO-d_6) δ (ppm): 1.41 (m, 1H), 1.60 (m, 1H), 1.99 (m, 1H), 2.27 (m, 1H), 2.64 (m, 1H), 2.72 (s, 6H), 3.05 (m, 1H), 3.33 (m, 2H), 4.25 (d, 1H, J = 8.4 Hz), 7.13–7.35 (m, 10H), 10.15 (s, 1H, HCl); IR (KBr) σ/cm^{-1}: 2481 (υ_{CH},

CH_3), 1739 ($\upsilon_{C=O}$), 1494, 1479, 1450, 1421 ($\upsilon_{C=C}$), 1157 (δ_{CH}), 746 ($\gamma_{C=C}$).

4.2.2. 2-((Dimethylamino)methyl)-5-(di-*p*-tolylmethyl)-cyclopentanone hydrochloride (8b). Mp: 172–173 °C. Yield: 49%. EI-MS (*m*/*z*): 335 (M^+, 1.23), 290 (0.60), 58 (100.00), 44 (2.31); ^{1}H NMR (DMSO-d_6) δ (ppm): 1.42 (m, 1H), 1.57 (m, 1H), 2.03 (m, 1H), 2.21(s, 6H), 2.24 (m, 1H), 2.58 (m, 1H), 2.70 (s, 6H), 3.05 (m, 1H), 3.23 (m, 2H), 4.20 (d, 1H, J = 8.4 Hz), 7.04–7.19 (m, 8H), 10.05 (s, 1H, HCl); IR (KBr) σ/cm^{-1}: 2922 ($\upsilon_{=CH}$), 2467 (υ_{CH}, CH_3), 1734 ($\upsilon_{C=O}$), 1511, 1468, 1412 ($\upsilon_{C=C}$), 1162 (δ_{CH}), 767 ($\gamma_{C=C}$).

4.2.3. Synthesis of 2-benzhydryl-5-((dimethylamino)-methyl)cyclopentanol (9). 2-Benzhydryl-5-((dimethylamino)methyl)cyclopentanone hydrochloride (**8a**, 0.005 mol) was dissolved in methanol (30 mL) and stirred. $NaBH_4$ (1.0 g, 0.026 mol) was added portionwisely over a period of 0.5 h and stirred at room temperature for another 6 h. Solvent was removed under reduced pressure, 10 ml of water was then added and the precipitates were filtered and recrystallized from acetone–ether to yield **9** as a white powder (76%), mp: 139–141 °C. Yield: 71%. EI-MS (*m*/*z*): 309 (M^+, 1.10), 281 (0.18), 167 (5.56), 142 (21.50), 58 (100.00), 44 (2.51); ^{1}H NMR (DMSO-d_6) δ (ppm): 1.13 (m, 2H), 1.36 (m, 2H), 1.73 (m, 1H), 2.01 (m, 2H), 2.11 (s, 6H), 2.65 (m, 1H), 3.54 (m, 1H), 4.22 (d, 2H, J = 3.8 Hz), 4.53 (m, 1H), 7.10 (m, 2H), 7.25 (m, 4H), 7.36 (m, 4H), 10.18 (s, 1H, HCl); IR (KBr) σ/cm^{-1}: 2956 ($\upsilon_{=CH}$), 2776 (υ_{CH}, CH_3), 1567, 1493, 1449 ($\upsilon_{C=C}$), 1170 (δ_{CH}), 758 ($\gamma_{C=C}$).

4.3. GSH-binding assay

GSH-binding reaction was carried out in sodium phosphate buffer (PBS, 0.1 mol/L, pH 7.4, containing 0.1 mmol/L EDTA) based on a reported method.[18] Briefly, 50 μmol/L GSH in PBS was incubated with cyclopentanone derivatives (0, 10, 20, 40, 50 and 160 μmol/L) for 30 min at 37 °C, the unreacted GSH in the solution was determined by adding an aliquot of 0.3 mL to the sample 1.0 cm quartz cuvette containing 3.0 mL 500 μmol/L DTNB solution in the same buffer and 0.3 mL same PBS to the reference 1.0 cm quartz cuvette containing the same DTNB solution. The unreacted GSH reacts with DTNB to form TNB. The absorbance of TNB was measured with a UV-9100 spectrophotometer at 412 nm. The concentrations of the unreacted GSH can be calculated according to the absorbance of TNB (ε = 13700 M^{-1} cm^{-1} at 412 nm). The equation of sample concentration calculation was done as follows:

$$C_1 = [(\text{Total volume}/\text{sample volume}) \times (\text{Abs}_{412})]/13700$$

4.4. Cell culture

HL-60 cells (obtained from ATCC) 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 in a humidified atmosphere of 95% air and 5% CO_2 at 37 °C. MCF-7 and MCF-7/Adr cells (obtained from Dr. T.-C. Chou)[25] were cultured in D-MEM medium supplemented with 100 U/mL pencillin, 100 μg/mL streptomycin, 1 mmol/L L-glutamine and 5% heat-inactivated fetal bovine serum in a humidified atmosphere of 95% air and 5% CO_2 at 37 °C. 184B5 immortalized mammary epithelial cells (obtained from ATCC) were cultured in MEGM (mammary epithelial growth medium) supplemented with 1 ng/mL cholera toxin in a humidified atmosphere of 95% air and 5% CO_2 at 37 °C.

4.5. Assay of GSTπ activity

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, centrifuged at 14000 rpm for 30 min at 4 °C and the supernatant lysate was used for enzyme assay. GSTπ activity was determined spectrophotometrically at 25 °C, using CDNB and GSH as substrates according to the reported method.[21] The linear increase in absorption at 340 nm, caused by conjugation of GSH (1 mM) with CDNB (1 mM) in HL-60 cell lysate with or without presence of compounds **5**–**9** (30 μM), was measured. A complete assay mixture without enzyme was used as a control. The nonenzymatic reaction was corrected by blanking the spectrophotometer with the control cuvette before each reading of the sample cuvette. An extinction coefficient of 9.6 mM^{-1} cm^{-1} was used to calculate GSTπ activity expressed as nanomoles per minute per milligram of protein.

4.5.1. Assay of cell growth inhibition. HL-60 cells were seeded at 2×10^5 cells/mL and cultured in the above noted medium with or without the indicated concentrations of test compounds for 24 h. Cell numbers in treated with or without compounds were determined by hemocytometer and growth inhibition was calculated to untreated cells (%).[20] The growth inhibition of these compounds on MCF-7, MCF-7/Adr and 184B5 cells were measured by MTT assay.[26] Briefly, cells (2×10^3) were plated in each well of a 96-well plate and were allowed to adhere and spread for 24 h. The various concentrations of compounds **5**–**9** were then added and cultured for 4 days at 37 °C. 50 μL of 2 mg/mL MTT solution was added per well and the cultures were continued for an additional 4 h. The medium was removed by aspiration. The cells were dissolved in 200 μL DMSO and absorbance at 570 nm was measured in the 96-well plate. Growth inhibition was determined as compared to untreated cells (%).

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References and notes

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