Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry 14 (2006) 6539–6547
Bioorganic & Medicinal Chemistry

The synthesis of L-carvone and limonene derivatives with increased antiproliferative effect and activation of ERK pathway in prostate cancer cells

Jiaojiao Chen,[a] Min Lu,[b] Yongkui Jing[b,*] and Jinhua Dong[a,*]

[a]*School of Pharmaceutical Engineering, Shenyang Pharmaceutical University, Shenyang 110016, PR China*
[b]*Department of Medicine, Mount Sinai School of Medicine, New York, NY 10029, USA*

Received 16 May 2006; revised 5 June 2006; accepted 6 June 2006
Available online 27 June 2006

Abstract—Thirty-one novel derivatives of carvone, carveol, and limonene were designed and synthesized using L-carvone as a starting material via chlorination, nucleophilic substitution, and reduction. The structures of these derivatives were characterized by MS and ^{1}H NMR. The antiproliferative effect was evaluated in human prostate cancer LNCaP cells. L-Carvone, L-carveol, and L-limonene were weak cell growth inhibitors and introduction of 4-(2-methoxyphenyl)piperazine to carvone, carveol or limonene significantly increased their antiproliferative effect. The antiproliferative effect was correlated with ERK activation and p21^{waf1} induction.

1. Introduction

Natural isoprenoids have been found to have effects of inhibiting tumor cell growth in vivo and in vitro.[1–3] Among these isoprenoids, monoterpene D-limonene and its in vivo metabolite, perillyl alcohol (Fig. 1), have been observed with preventive and therapeutic effects against a variety of tumors in animal models.[4–6] Although the mechanism of D-limonene and perillyl alcohol of inhibiting tumor cell growth is unclear, it has been proposed that inhibition of farnesyl transferase might be one of their action targets.[7–9] Both agents have been put into Phase I and phase II clinical trials and the preliminary results indicated that both agents were well tolerated in cancer patients.[10–13] Based on the in vitro results, higher concentrations of D-limonene are needed to reach a therapeutic effect. The low polarity of limonene structure might limit its ability to transverse cellular membranes and resulted in the less activity.[9] Structural modifications with increased polarity of limonene might lower its effective concentrations. Some hydroxy-containing derivatives of limonene have been synthesized and their inhibitory effects on tumor cell growth have been compared. It was found that those derivatives with improved polarity without breaking unsaturated double bond of limonene have increased antiproliferative effect.[9] Based on this observation, we designed and synthesized a new group of limonene analogues containing polar groups in the structure.

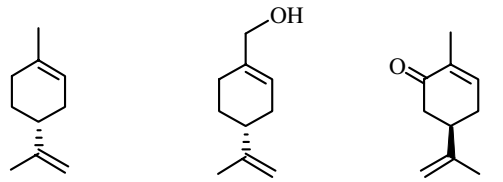


Figure 1. Structures of D-limonene, D-perillyl alcohol, and L-carvone.

Since direct structural modification from limonene might result in multiple by-products, L-carvone was chosen as the starting reagent and derivatives of carvone, carveol, and limonene were generated. The structural modification of L-carvone was focused on the 10-carbon and the carbonyl moiety. Lipophilic benzoates and hydrophilic amines were linked to the terpenoid moiety in order to enhance polarity and to alter hydrophilicity. In this study, thirty-one novel derivatives of limonene, carvone, and carveol were synthesized. Among these

Keywords: L-Limonene; L-Carvone; Synthesis; Antiproliferative effect; ERK; Prostate cancer.
* Corresponding authors. Tel.: +862423986402; fax: +862423904249 (J.D.); e-mail addresses: yongkui.jing@mssm.edu; dongjh66@hotmail.com

0968-0896/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2006.06.013

derivatives, 21 compounds contain carvone skeleton, five compounds contain carveol skeleton, and five compounds contain limonene skeleton. The cell growth inhibitory effect of these compounds was measured in prostate cancer cells in vitro and the potential mechanism was explored.

2. Results and discussion

2.1. Chemistry

The synthetic route of these derivatives starting with L-carvone is listed in Figure 2. The intermediate compound, 5-(1-chloromethyl)vinyl-2-methylcyclohex-2-enone (**I**), was prepared by chlorination of L-carvone with *tert*-butyl hypochlorite in *n*-hexane at room temperature.[14] Compound **I** reacted with a substituted potassium benzoate in presence of potassium carbonate in *N*,*N*-dimethylformamide and a corresponding ester derivative (**II**$_1$–**II**$_7$) was obtained. Reaction of compound **I** with *N*-alkylpiperazines or *N*-arylpiperazines in presence of potassium carbonate in boiling ethanol afforded the derivatives containing a piperazine moiety (**III**$_1$–**III**$_8$). Compounds **IV**$_1$–**IV**$_6$ were prepared by reacting compound **I** with an aliphatic amine or heterocyclic amine. Sodium borohydride reduction of ketones **III**$_1$, **III**$_2$, or **III**$_5$–**III**$_7$ in methanol at room temperature yielded **V**$_1$–**V**$_5$, respectively. Compounds **VI**$_1$–**VI**$_5$ were prepared from related ketone derivatives using 85% hydrazine hydrate and potassium hydroxide based on the method of Wolff–Kishner reduction.[15] Since the conditions of both reduction methods could not change the configuration of L-carvone, all the derivatives of carvone, carveol, and limonene were in L-configuration.

2.2. Antiproliferative effect

The antiproliferative effects of these compounds were measured in human prostate LNCaP cancer cells using MTT assay. The IG$_{50}$ (the concentration which inhibits 50% of the cell growth) was calculated. As shown in Table 1, the IG$_{50}$s of L-carvone, L-carveol, and L-limonene were more than 100 μM (the highest concentration used in this experiment). The addition of a group of 4-methylbenzoic acid (**II**$_2$), 4-methoxybenzoic acid (**II**$_6$) or 4-aminobenzoic acid (**II**$_7$) significantly increased the antiproliferative activity of L-carvone. Addition of substituted groups of *N*-alkylpiperazine or *N*-benzylpiperazine in carvone (**III**$_1$–**III**$_5$) did not increase the antiproliferative effect of L-carvone evidently. However, introduction of *N*-(4-methoxyphenyl)piperazine (**III**$_6$), *N*-(2-methoxyphenyl)piperazine (**III**$_7$), or *N*-(2-chlorophenyl)piperazine (**III**$_8$) significantly increased the antiproliferative effect of L-carvone. Based on these data, it seems that the compounds with increased activity have increased the polarity of L-carvone. The addition of a substituted amine into L-carvone did not increase the antiproliferative effect (**IV**) except for **IV**$_4$ which had a 2-thiopheneethylamine substitute. Since some *N*-arylpiperazine-substituted carvone derivatives (**III**) have an increased antiproliferative effect, we have synthesized related carveol and limonene derivatives by reduction of **III**. As shown in Table 1, although *N*-methylpiperazine or *N*-ethylpiperazine-substituted carvone (**III**$_1$ and **III**$_2$) and carveol (**V**$_1$ and **V**$_2$) did not increase the antiproliferative effect of L-carvone

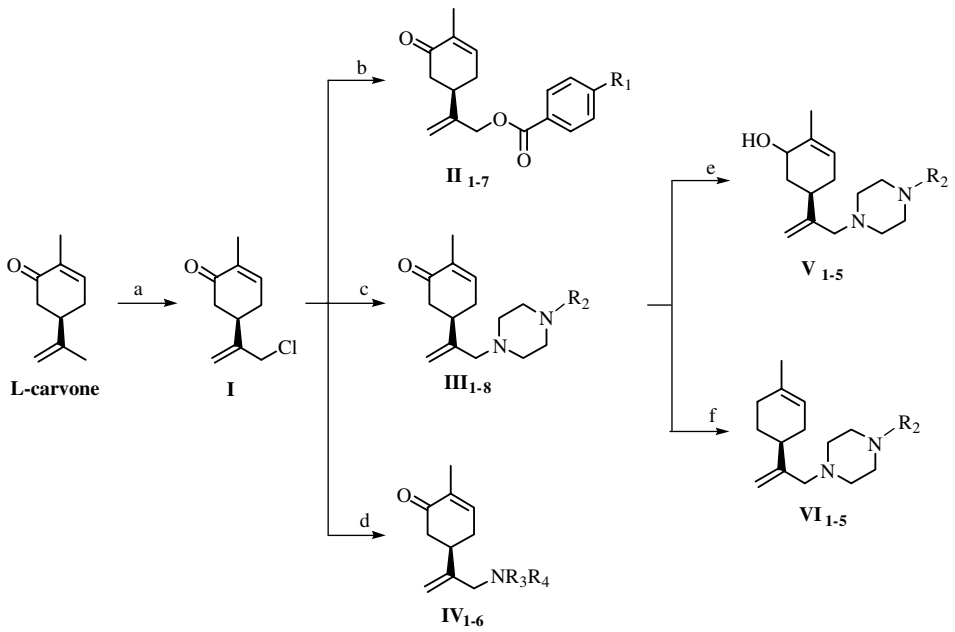


Figure 2. The synthetic route of L-carvone, L-carveol, and L-limonene derivatives. Reagents: (a) *tert*-butyl hypochlorite; (b) a substituted potassium benzoate (**II**$_1$–**II**$_7$: R$_1$ = H, CH$_3$, F, Cl, Br, OCH$_3$, or NH$_2$, respectively) and K$_2$CO$_3$; (c) a *N*-alkylpiperazines or *N*-arylpiperazine (**III**$_1$–**III**$_8$: R$_2$ = CH$_3$, C$_2$H$_5$, isopropyl, isobutyl, benzyl, 4-methoxyphenyl, 2-methoxyphenyl, or 2-chlorophenyl, respectively) and K$_2$CO$_3$; (d) an aliphatic amine or heterocyclic amine (**IV**$_1$–**IV**$_6$: NR$_3$R$_4$ = pyrrolidinyl, piperidinyl, cyclohexylamino, 2-thiopheneethylamino, dimethylamino, or 1-adamantanamino, respectively) and K$_2$CO$_3$; (e) NaBH$_4$; (f) NH$_2$NH$_2$·H$_2$O (85%) and KOH.

Table 1. The structures and IG_{50} values of carvone, carveol, and limonene derivatives in LNCaP cells

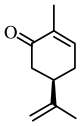
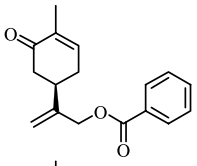
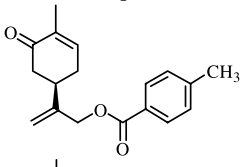
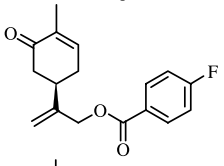
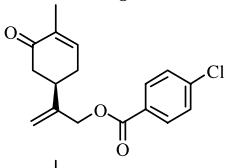
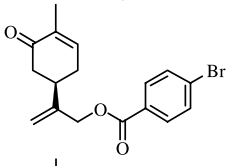
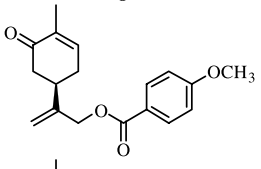
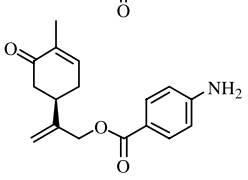
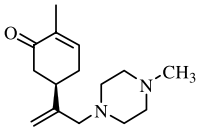
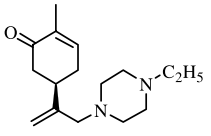
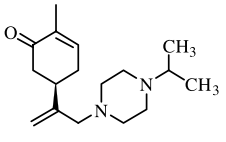
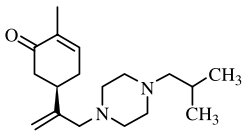
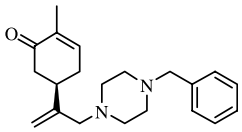
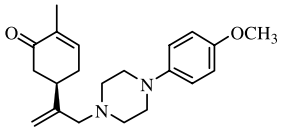
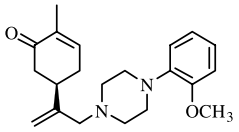
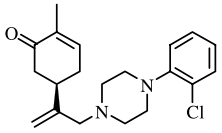
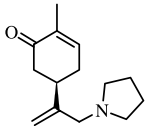
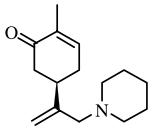
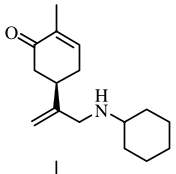
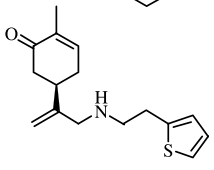
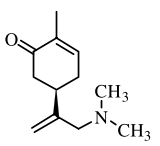
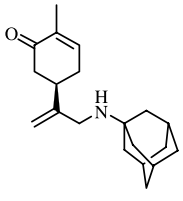
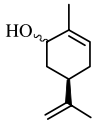
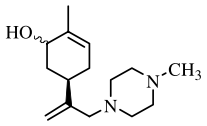
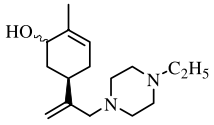
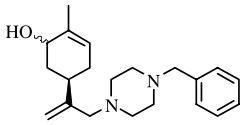
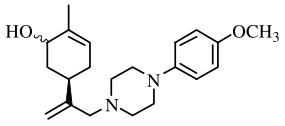
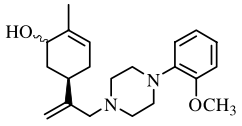
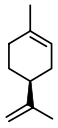
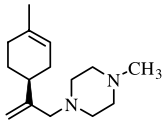
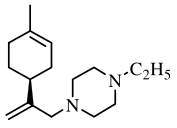
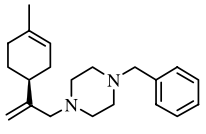
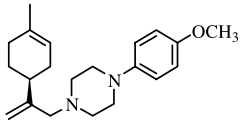
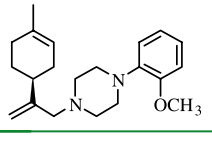
Compound	Structure	IG_{50} (μM)
L-Carvone		>100
II$_1$		>100
II$_2$		57
II$_3$		>100
II$_4$		92
II$_5$		80
II$_6$		21
II$_7$		45
III$_1$		>100
III$_2$		>100
III$_3$		>100

Table 1 (*continued*)

Compound	Structure	IG_{50} (μM)
III$_4$		>100
III$_5$		>100
III$_6$		45
III$_7$		37
III$_8$		19
IV$_1$		>100
IV$_2$		>100
IV$_3$		>100
IV$_4$		24
IV$_5$		75
IV$_6$		83

(*continued on next page*)

Table 1 (*continued*)

Compound	Structure	IG_{50} (μM)
L-Carveol		>100
$\mathbf{V}_1$		>100
$\mathbf{V}_2$		>100
$\mathbf{V}_3$		64
$\mathbf{V}_4$		85
$\mathbf{V}_5$		38
L-Limonene		>100
$\mathbf{VI}_1$		50
$\mathbf{VI}_2$		38
$\mathbf{VI}_3$		24
$\mathbf{VI}_4$		28
$\mathbf{VI}_5$		20

evidently, the addition of *N*-methylpiperazine or *N*-ethylpiperazine into limonene ($\mathbf{VI}_1$ and $\mathbf{VI}_2$) significantly increased the antiproliferative effect of limonene. Based on compounds **III**, it was found that introduction of 4-arylpiperazine moiety significantly increased the antiproliferative effect of carvone than addition of other groups. By focusing on compounds $\mathbf{III}_6$, $\mathbf{III}_7$, $\mathbf{V}_4$, $\mathbf{V}_5$, $\mathbf{VI}_4$, and $\mathbf{VI}_5$, it seems that derivatives of limonene are more potent than the derivatives of carvone and carveol with same substitute.

2.3. The effects of some potent compounds on farnesyl transferase and MAP kinases

It has been suggested that limonene-like monoterpenes are inhibitors of farnesyl transferase and their tumor growth inhibitory effect might result from the inhibition of farnesyl transferase.[8,16] To investigate this possibility, the farnesyl transferase inhibition was investigated by measuring the HDJ2 unfarnesyl form using Western blot analyses as reported.[17] Compounds $\mathbf{II}_7$, $\mathbf{III}_6$, $\mathbf{IV}_4$, $\mathbf{V}_4$, $\mathbf{VI}_4$, L-limonene, and L-carvone were selected for this purpose. LNCaP cells were treated with these compounds at a concentration of 50 μM for 1–3 days. Surprisingly, no one of these compounds at this concentration increased the unfarnesyl form of HDJ2 (data not shown). Since compounds $\mathbf{II}_7$, $\mathbf{III}_6$, $\mathbf{IV}_4$, $\mathbf{V}_4$, and $\mathbf{VI}_4$ significantly inhibited LNCaP cell growth at this concentration (Table 1), it suggests that the cell growth inhibitory effects of these compounds might not be through inhibition of farnesyl transferase. Since these compounds showed cytostatic but not cytotoxic effect, we suspect that the cell growth survival pathways might be regulated by these compounds. It has been found that ERK and p38 MAP kinases are regulators of cell growth and some natural compounds inhibited cell growth through activation or inhibition of these kinases.[18–20] The protein and phosphorylated forms of both p38 and ERK were investigated in LNCaP cells. As shown in Figure 3, phosphorylated ERK was significantly increased in LNCaP cells after treatment with compounds $\mathbf{II}_7$, $\mathbf{III}_6$, $\mathbf{IV}_4$, $\mathbf{V}_4$, and $\mathbf{VI}_4$, but not by L-limonene or L-carvone. The increased levels of phosphorylated ERK sustained for three days. Unlike phosphorylated ERK, phosphorylated p38 was increased transiently. Since compounds $\mathbf{II}_7$, $\mathbf{III}_6$, $\mathbf{IV}_4$, $\mathbf{V}_4$, and $\mathbf{VI}_4$, but not by L-limonene or L-carvone, significantly inhibited growth of LNCaP cells, these data suggest that activated ERK would participate in antiproliferative effect of these compounds. Recently, it has been found that activated ERK leads to an increase of cell cycle regulatory protein $p21^{waf1}$,[21,22] the protein level of $p21^{waf1}$ was compared. As shown in Figure 3, $p21^{waf1}$ protein level was increased in LNCaP cells after treatment with $\mathbf{II}_7$, $\mathbf{III}_6$, $\mathbf{IV}_4$, $\mathbf{V}_4$, and $\mathbf{VI}_4$, but not by L-limonene or L-carvone. The $p21^{waf1}$ induction was correlated with the increased levels of phosphorylated ERK. This result indicates that these derivatives might inhibit cell growth through activation of ERK which leads to upregulation of $p21^{waf1}$ protein and cell cycle arrest. The mechanism of these compounds which activate ERK is worthy of further study.

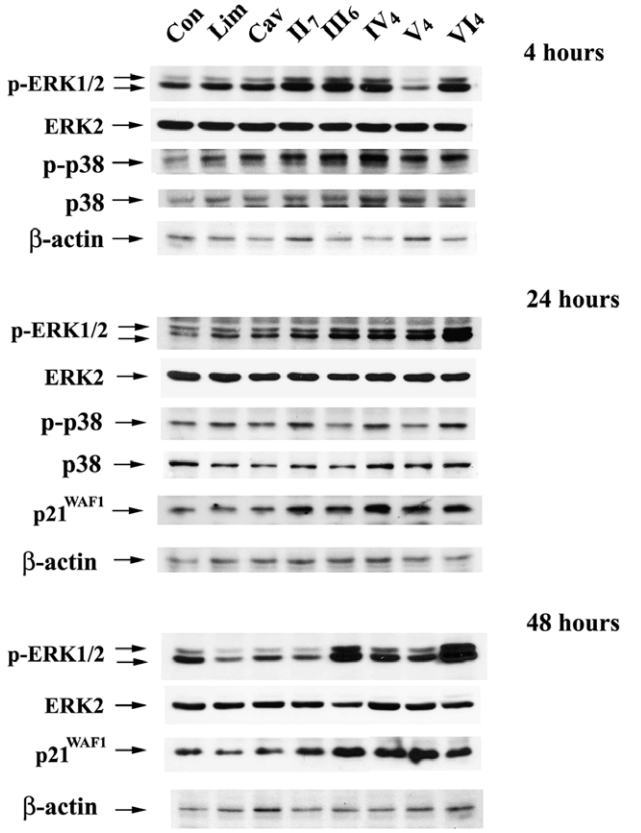


Figure 3. The effect of some synthetic derivatives on the phosphorylation of ERK and p38 as well as $p21^{waf1}$ protein levels. LNCaP cells were treated with indicated compounds at 50 μM for indicated times. Whole cellular protein lysates were prepared with RIPA buffer and Western blot analysis was used to analyze each protein level using indicated antibodies. Lim, L-limonene; Cav, L-carvone.

3. Experimental

3.1. Chemistry

Melting points were determined on a Yanaco micro melting point apparatus and are uncorrected. Mass spectra were determined on an Agilent-5973A spectrometer. ^{1}H NMR spectra were recorded on Varian mercury-300 NMR instrument using tetramethylsilane (TMS) as the internal standard (chemical shifts in δ, ppm) in deuteriochloroform. All the reagents and solvents used in the experiments were obtained from commercial sources and used without further purification.

3.1.1. 5-(1-Chloromethyl)vinyl-2-methylcyclohex-2-enone (I). *tert*-Butyl hypochlorite (13.3 g, 0.11 mol) was added dropwise into a solution of L-carvone (15.0 g, 0.1 mol) in *n*-hexane (300 mL) at 0 °C. The mixture was stirred for 3 h at room temperature, washed with aqueous sodium sulfite and brine, dried over anhydrous sodium sulfate, and filtered. The filtrate was concentrated in vacuo to give brown oil, which was purified on silica gel column with petroleum ether as eluent to give the title compound (10.7 g, 76.0% yield) as yellow oil. ^{1}H NMR 1.76 (3H, s, $-CH_3$), 2.33–2.40 (2H, m, $-CH_2-$), 2.49–2.66 (2H, m, $-CH_2-$), 2.94 (1H, m, >CH–), 4.06 (2H, s, $-CH_2Cl$), 5.03 (1H, s, $=CH_2$), 5.23 (1H, s, $=CH_2$), 6.73 (1H, m, =CH–).

3.1.2. General procedure for the preparation of compounds II_1–II_7

3.1.2.1. Benzoic acid 2-(4-methyl-5-oxocyclohex-3-enyl)allyl ester (II_1). Compound **I** (1.5 g, 8.1 mmol), potassium carbonate (1.2 g, 17.8 mmol), and potassium benzoate (1.4 g, 8.1 mmol) were dissolved in 15 mL dry *N*,*N*-dimethylformamide and heated to 80–85 °C for 6–8 h. Then water (100 mL) was added and the reaction mixture was extracted with methylene chloride (5× 30 mL). The combined organic extracts were washed with brine, dried over anhydrous sodium sulfate, and filtered. The filtrate was concentrated in vacuo. The residue was purified on a silica gel column with petroleum ether–ethylacetate as eluent to give the target product $\mathbf{II}_1$ (0.48 g, yield 22.0%), mp 65–67 °C. ^{1}H NMR 1.79 (3H, s, $-CH_3$), 2.31–2.51 (2H, m, $-CH_2-$), 2.59–2.70 (2H, m, $-CH_2-$), 2.90 (1H, m, >CH–), 4.81 (1H, d, $-CH_2-O-$), 4.87 (1H, d, $-CH_2-O-$), 5.11 (1H, s, $=CH_2$), 5.29 (1H, s, $=CH_2$), 6.70 (1H, m, =CH–), 7.44 (2H, dd, Ar-H), 7.56 (1H, t, Ar-H), 8.04 (2H, d, Ar-H). MS (EI) *m*/*z*: 270 (M^+, 11), 105 (100).

3.1.2.2. 4-Methylbenzoic acid 2-(4-methyl-5-oxocyclohex-3-enyl)allyl ester (II_2). Compound $\mathbf{II}_2$ was obtained in 24.5% yield, mp 63–64 °C. ^{1}H NMR 1.79 (3H, s, $-CH_3$), 2.30–2.50 (5H, m, $-CH_2-$, $-CH_3$), 2.58–2.65 (2H, m, $-CH_2-$), 2.88 (1H, m, >CH–), 4.77 (1H, d, $-CH_2-O-$), 4.88 (1H, d, $-CH_2-O-$), 5.10 (1H, s, $=CH_2$), 5.27 (1H, s, $=CH_2$), 6.75 (1H, m, =CH–), 7.25 (2H, d, Ar-H), 7.93 (2H, d, Ar-H). MS (EI) *m*/*z*: 284 (M^+, 20), 119 (100).

3.1.2.3. 4-Fluorobenzoic acid 2-(4-methyl-5-oxocyclohex-3-enyl)allyl ester (II_3). Compound $\mathbf{II}_3$ was obtained in 29.1% yield, mp 73–75 °C. ^{1}H NMR 1.79 (3H, s, $-CH_3$), 2.31–2.50 (2H, m, $-CH_2-$), 2.58–2.69 (2H, m, $-CH_2-$), 2.88 (1H, m, >CH–), 4.82 (1H, d, $-CH_2-O-$), 4.84 (1H, d, $-CH_2-O-$), 5.12 (1H, s, $=CH_2$), 5.27 (1H, s, $=CH_2$), 6.76 (1H, m, =CH–), 7.13 (2H, t, Ar-H), 8.04 (1H, d, Ar-H), 8.07 (1H, d, Ar-H). MS (EI) *m*/*z*: 288 (M^+, 6), 123 (100).

3.1.2.4. 4-Chlorobenzoic acid 2-(4-methyl-5-oxocyclohex-3-enyl)allyl ester (II_4). Compound $\mathbf{II}_4$ was obtained in 18.6% yield, mp 56–58 °C. ^{1}H NMR 1.79 (3H, s, $-CH_3$), 2.30–2.50 (2H, m, $-CH_2-$), 2.57–2.69 (2H, m, $-CH_2-$), 2.87 (1H, m, >CH–), 4.83 (1H, d, $-CH_2-O-$), 4.84 (1H, d, $-CH_2-O-$), 5.12 (1H, s, $=CH_2$), 5.27 (1H, s, $=CH_2$), 6.75 (1H, m, =CH–), 7.44 (2H, d, Ar-H), 7.97 (2H, d, Ar-H). MS (EI) *m*/*z*: 304 (M^+, 6), 148 (100).

3.1.2.5. 4-Bromobenzoic acid 2-(4-methyl-5-oxocyclohex-3-enyl)allyl ester (II_5). Compound $\mathbf{II}_5$ was obtained in 23.4% yield, mp 56–58 °C. ^{1}H NMR 1.79 (3H, s, $-CH_3$), 2.30–2.50 (2H, m, $-CH_2-$), 2.57–2.69 (2H, m, $-CH_2-$), 2.83 (1H, m, >CH–), 4.80 (1H, d, $-CH_2-O-$), 4.86 (1H, d, $-CH_2-O-$), 5.11 (1H, s, $=CH_2$), 5.27 (1H, s, $=CH_2$), 6.75 (1H, m, =CH–), 7.60 (2H, d, Ar-H), 7.90 (2H, d, Ar-H). MS (EI) *m*/*z*: 349 (M^+, 3), 148 (100).

3.1.2.6. 4-Methoxybenzoic acid 2-(4-methyl-5-oxocyclohex-3-enyl)allyl ester (II_6). Compound II_6 was obtained in 22.7% yield, oily material. ^{1}H NMR 1.79 (3H, s, $-CH_3$), 2.30–2.50 (2H, m, $-CH_2-$), 2.58–2.67 (2H, m, $-CH_2-$), 2.89 (1H, m, >CH–), 3.87 (3H, s, $-OCH_3$), 4.80 (1H, d, $-CH_2-O-$), 4.84 (1H, d, $-CH_2-O-$), 5.09 (1H, s, $=CH_2$), 5.27 (1H, s, $=CH_2$), 6.76 (1H, m, =CH–), 6.92 (2H, d, Ar-H), 7.99 (2H, d, Ar-H). MS (EI) *m/z*: 300 (M^+, 20), 135 (100).

3.1.2.7. 4-Aminobenzoic acid 2-(4-methyl-5-oxocyclohex-3-enyl)allyl ester (II_7). Compound II_7 was obtained in 24.3% yield, mp 64–65 °C. ^{1}H NMR 1.79 (3H, s, $-CH_3$), 2.30–2.50 (4H, m, $-CH_2-$, $-NH_2$), 2.56–2.73 (2H, m, $-CH_2-$), 2.88 (1H, m, >CH–), 4.81 (1H, d, $-CH_2-O-$), 4.85 (1H, d, $-CH_2-O-$), 5.10 (1H, s, $=CH_2$), 5.27 (1H, s, $=CH_2$), 6.75 (1H, m, =CH–), 7.25 (2H, d, Ar-H), 7.94 (2H, d, Ar-H). MS (EI) *m/z*: 284 (M^+-1, 14), 119 (100).

3.1.3. General procedure for the preparation of compounds III_1–III_8

3.1.3.1. 2-Methyl-5-{[1-(4-methylpiperazin-1-yl)methyl]vinyl}cyclohex-2-enone (III_1). Compound **I** (1.5 g, 8.1 mmo1), potassium carbonate (1.2 g, 8.9 mmol), and *N*-methylpiperazine (0.8 g, 8.1 mmol) were dissolved in ethanol (15 mL) and refluxed for 6–10 h. Then the solvent was removed by evaporation. The residue was dissolved in 30 mL of methylene chloride, washed with 3 N hydrochloric acid (3× 30 mL). The combined aqueous solution was neutralized with aqueous sodium carbonate to pH 9.0–10.0, extracted with methylene chloride (5× 30 mL). The combined organic extracts were washed with brine, dried over anhydrous sodium sulfate, and filtered. The filtrate was concentrated in vacuo. The residue was purified on a silica gel column with petroleum ether–ethylacetate as eluent to afford the target product III_1 (1.79 g, yield 67.3%). ^{1}H NMR (DMSO-d_6), 1.75 (3H, s, $-CH_3$), 2.30–2.55 (15H, m, $-CH_2-$, $-N-CH_2-$, $-N-CH_3$), 2.88 (1H, m, >CH–), 2.90 (2H, m, $-CH_2-N-$), 4.88 (1H, s, $=CH_2$), 4.98 (1H, s, $=CH_2$), 6.75 (1H, m, =CH–). MS (EI) *m/z*: 248 (M^+, 86), 58 (100).

3.1.3.2. 5-[1-(4-Ethylpiperazin-1-yl)methyl]vinyl-2-methylcyclohex-2-enone (III_2). Compound III_2 was obtained in 61.5% yield. ^{1}H NMR (DMSO-d_6), 1.51 (3H, t, $-CH_2-CH_3$), 1.79 (3H, s, $-CH_3$), 2.23–2.45 (2H, m, $-CH_2-$), 2.68–2.74 (2H, m, $-CH_2-$), 3.22 (2H, q, $-CH_2-CH_3$), 3.31 (1H, m, >CH–), 3.58 (4H, m, $-N-CH_2-$), 3.76 (2H, m, $-CH_2-N-$), 3.95–4.20 (4H, m, $-N-CH_2-$), 5.47 (1H, s, $=CH_2$), 5.61 (1H, s, $=CH_2$), 6.75 (1H, m, =CH–). MS (EI) *m/z*: 262 (M^+, 100).

3.1.3.3. 5-[1-(4-Isopropylpiperazin-1-yl)methyl]vinyl-2-methylcyclohex-2-enone (III_3). Compound III_3 was obtained in 70.4% yield. ^{1}H NMR 1.50 (6H, d, $-CH(CH_3)_2$), 1.79 (3H, s, $-CH_3$), 2.30 (2H, m, $-CH_2-$), 2.71 (2H, m, $-CH_2-$), 3.25 (1H, m, >CH–), 3.40–4.40 (11H, m, $-N-CH_2-$, $-CH(CH_3)_2$), 5.46 (1H, s, $=CH_2$), 5.63 (1H, s, $=CH_2$), 6.76 (1H, m, =CH–). MS (EI) *m/z*: 276 (M^+, 100).

3.1.3.4. 5-[1-(4-Isobutylpiperazin-1-yl)methyl]vinyl-2-methylcyclohex-2-enone (III_4). Compound III_4 was obtained in 75.4% yield. ^{1}H NMR (DMSO-d_6), 0.89 (6H, d, $-CH(CH_3)_2$), 1.64 (3H, s, $-CH_3$), 1.88 (1H, m, $-CH(CH_3)_2$), 2.21–2.43 (6H, m, $-CH_2-$, $-N-CH_2-$), 2.49 (1H, m, >CH–), 2.57 (4H, m, $-N-CH_2-$), 2.72 (4H, m, $-N-CH_2-$), 2.90 (2H, s, $-N-CH_2-$), 4.84 (1H, s, $=CH_2$), 4.91 (1H, s, $=CH_2$), 6.64 (1H, m, =CH–). MS (EI) *m/z*: 290 (M^+, 38), 247 (100).

3.1.3.5. 5-[1-(4-Benzylpiperazin-1-yl)methyl]vinyl-2-methylcyclohex-2-enone (III_5). Compound III_5 was obtained in 77.3% yield. ^{1}H NMR (DMSO-d_6), 1.76 (3H, s, $-CH_3$), 2.36 (2H, m, $-CH_2-$), 2.70 (2H, m, $-CH_2-$), 3.33 (1H, m, >CH–), 3.45–3.63 (4H, m, $-N-CH_2-$), 3.76 (2H, s, $-CH_2Ph$), 4.03 (4H, m, $-N-CH_2-$), 4.27 (2H, s, $-N-CH_2-$), 5.44 (1H, s, $=CH_2$), 5.58 (1H, s, $=CH_2$), 6.74 (1H, m, =CH–), 7.47 (3H, m, Ar-H), 7.67 (2H, m, Ar-H). MS (EI) *m/z*: 324 (M^+, 22), 91 (100).

3.1.3.6. 5-{1-[4-(4-Methoxyphenyl)piperazin-1-yl]-methyl}vinyl-2-methylcyclohex-2-enone (III_6). Compound III_6 was obtained in 65.9% yield. ^{1}H NMR 1.79 (3H, s, $-CH_3$), 2.37 (2H, m, $-CH_2-$), 2.77 (2H, m, $-CH_2-$), 3.36 (1H, m, >CH–), 3.79 (9H, m, $-N-CH_2-$, $-OCH_3$), 4.27 (2H, m, $-N-CH_2-$), 4.89 (2H, m, $-N-CH_2-$), 5.50 (1H, s, $=CH_2$), 5.67 (1H, s, $=CH_2$), 6.76 (1H, m, =CH–), 7.00 (2H, d, Ar-H), 7.86 (2H, d, Ar-H). MS (EI) *m/z*: 340 (M^+, 100).

3.1.3.7. 5-{1-[4-(2-Methoxyphenyl)piperazin-1-yl]-methyl}vinyl-2-methylcyclohex-2-enone (III_7). Compound III_7 was obtained in 70.5% yield. ^{1}H NMR 1.79 (3H, s, $-CH_3$), 2.35–2.51 (2H, m, $-CH_2-$), 2.56–2.66 (6H, m, $-CH_2-$, $-N-CH_2-$), 2.89 (1H, m, >CH–), 3.00 (2H, m, $-N-CH_2-$), 3.05 (4H, m, $-N-CH_2-$), 3.84 (3H, s, $-OCH_3$), 4.93 (1H, s, $=CH_2$), 5.05 (1H, s, $=CH_2$), 6.75 (1H, m, =CH–), 6.83–7.00 (4H, m, Ar-H). MS (EI) *m/z*: 340 (M^+, 84), 150 (100).

3.1.3.8. 5-{1-[4-(2-Chlorophenyl)piperazin-1-yl]methyl}vinyl-2-methylcyclohex-2-enone (III_8). Compound III_8 was obtained in 66.1% yield. ^{1}H NMR 1.79 (3H, s, $-CH_3$), 2.20–2.42 (2H, m, $-CH_2-$), 2.66–2.86 (2H, m, $-CH_2-$), 3.40 (1H, m, >CH–), 3.44–3.81 (8H, m, $-N-CH_2-$), 4.40 (2H, m, $-N-CH_2-$), 5.48 (1H, s, $=CH_2$), 5.63 (1H, s, $=CH_2$), 6.77 (1H, m, =CH–), 7.24 (1H, m, Ar-H), 7.35 (2H, m, Ar-H), 7.47 (1H, m, Ar-H). MS (EI) *m/z*: 340 (M^+, 84), 150 (100).

3.1.4. General procedure for the preparation of compounds IV_1–IV_6. The title compounds were synthesized using the method described for the synthesis of compound III_1, substituting an aliphatic amine or a heterocyclic amine for *N*-methylpiperazine.

3.1.4.1. 2-Methyl-5-[1-(pyrrolidin-1-ylmethyl)vinyl]-cyclohex-2-enone (IV_1). Compound IV_1 was obtained in 69.3% yield. ^{1}H NMR 1.80 (3H, s, $-CH_3$), 2.08 (2H, m, $-CH_2-$), 2.29 (3H, m, $-CH_2-$), 2.42 (1H, m, $-CH_2-$), 2.67 (1H, m, $-CH_2-$), 2.81 (3H, m, $-CH_2-$, $-N-CH_2-$), 3.30 (1H, m, >CH–), 3.57–3.80 (4H, m, $-N-CH_2-$), 5.35 (1H, s, $=CH_2$), 5.56 (1H, s, $=CH_2$), 6.77 (1H, m, =CH–). MS (EI) *m/z*: 218 (M^+, 25), 84 (100).

3.1.4.2. 2-Methyl-5-[1-(piperidin-1-ylmethyl)vinyl]-cyclohex-2-enone (IV_2). Compound IV_2 was obtained in 74.8% yield. ^{1}H NMR 1.43 (1H, m, –CH_2–), 1.78 (3H, s, –CH_3), 1.86 (3H, m, –CH_2–), 2.18–2.27 (1H, m, –CH_2–), 2.35–2.69 (6H, m, –CH_2–, –N–CH_2–), 2.84 (1H, m, –N–CH_2–), 3.34 (1H, m, >CH–), 3.43 (2H, m, –N–CH_2–), 3.56 (1H, m, –N–CH_2–), 3.70 (1H, m, –N–CH_2–), 5.38 (1H, s, =CH_2), 5.54 (1H, s, =CH_2), 6.76 (1H, m, =CH–). MS (EI) *m*/*z*: 232 (M^+, 43), 98 (100).

3.1.4.3. 5-(1-Cyclohexylaminomethyl)vinyl-2-methyl-cyclohex-2-enone (IV_3). Compound IV_3 was obtained in 69.2% yield. ^{1}H NMR 1.24 (3H, m, –CH_2–), 1.65 (3H, m, –CH_2–), 1.79 (3H, s, –CH_3), 1.86 (2H, m, –CH_2–), 2.18–2.27 (3H, m, –CH_2–), 2.39 (1H, m, –CH_2–), 2.65 (1H, m, –CH_2–), 2.72 (1H, m, –CH_2–), 2.92 (1H, m, >CH–), 3.07 (1H, m, >CH–), 3.55 (1H, d, –N–CH_2–), 3.64 (1H, d, –N–CH_2–), 5.28 (1H, s, =CH_2), 5.52 (1H, s, =CH_2), 6.75 (1H, m, =CH–). MS (EI) *m*/*z*: 246 (M^+, 32), 56 (100).

3.1.4.4. 2-Methyl-5-{1-[(2-thiophen-2-ylethylamino)-methyl]vinyl}cyclohex-2-enone (IV_4). Compound IV_4 was obtained in 66.3% yield. ^{1}H NMR 1.77 (3H, s, –CH_3), 2.30 (2H, m, –CH_2–), 2.64 (2H, m, –CH_2–), 2.97 (1H, m, >CH–), 3.20 (2H, m, –N–CH_2–), 3.52 (2H, m, –N–CH_2–), 3.62 (2H, m, Ar-CH_2–), 5.30 (1H, s, =CH_2), 5.50 (1H, s, =CH_2), 6.71 (1H, m, =CH–), 6.94 (2H, s, Ar-H), 7.18 (1H, s, Ar-H). MS (EI) *m*/*z*: 275 (M^+, 1), 178 (100).

3.1.4.5. 5-(1-Dimethylaminomethyl)vinyl-2-methylcyclohex-2-enone (IV_5). Compound IV_5 was obtained in 65.9% yield. ^{1}H NMR 1.78 (3H, s, –CH_3), 2.15 (6H, s, –N(CH_3)$_2$), 2.27–2.43 (2H, m, –CH_2–), 2.48–2.65 (2H, m, –CH_2–), 2.80–2.91 (3H, m, –N–CH_2–, >CH–), 4.90 (1H, s, =CH_2), 5.01 (1H, s, =CH_2), 6.76 (1H, m, =CH–). MS (EI) *m*/*z*: 192 (M^+, 33), 58 (100).

3.1.4.6. 5-[1-(Adamantan-1-ylamino)methyl]vinyl-2-methylcyclohex-2-enone (IV_6). Compound IV_6 was obtained in 64.8% yield. ^{1}H NMR 1.60–1.62 (3H, m, –CH_2–), 1.69 (2H, m, –CH_2–), 1.78 (7H, m, –CH_2–), 1.79 (3H, s, –CH_3), 2.08 (3H, m, >CH–), 2.34 (1H, m, –CH_2–), 2.47 (1H, m, –CH_2–), 2.52 (1H, m, –CH_2–), 2.64 (1H, m, –CH_2–), 2.88 (1H, m, >CH–), 3.25 (2H, m, –N–CH_2–), 4.91 (1H, s, =CH_2), 5.10 (1H, s, =CH_2), 6.75 (1H, m, =CH–). MS (EI) *m*/*z*: 299 (M^+, 25), 135 (100).

3.1.5. General procedure for the preparation of compounds V_1–V_5

3.1.5.1. 2-Methyl-5-{[1-(4-methylpiperazin-1-yl)methyl]vinyl}cyclohex-2-enol (V_1). Compound III_1 (2.0 g, 8.0 mmol) was dissolved in 15 mL methanol, then sodium borohydride (20 mmol) was added in three portions within 1 h. The mixture was stirred at room temperature for 2 h, then dissolved in 30 mL brine and extracted with methylene chloride (3× 30 mL). The combined organic extracts were washed with brine, dried over anhydrous sodium sulfate, and filtered. The filtrate was concentrated in vacuo. The residue was purified on a silica gel column with petroleum ether–ethylacetate–methanol as eluent to afford the target product V_1 (0.42 g, yield 16.4%). ^{1}H NMR 1.57 (1H, m, –CH_2–), 1.77 (3H, s, –CH_3), 1.96 (2H, m, –CH_2–), 2.14 (2H, m, –CH_2–), 2.21 (1H, m, >CH–), 2.29 (3H, s, –N–CH_3), 2.42–2.44 (8H, m, –N–CH_2–), 2.91–2.95 (2H, d, –N–CH_2–), 4.17 (1H, m, >C*H*OH), 4.93 (1H, s, =CH_2), 4.95 (1H, s, =CH_2), 5.51 (1H, m, =CH–). MS (EI) *m*/*z*: 251 (M^+, 100).

3.1.5.2. 5-{[1-(4-Ethylpiperazin-1-yl)methyl]vinyl}-2-methylcyclohex-2-enol (V_2). Compound V_2 was obtained in 19.2% yield. ^{1}H NMR 1.09 (3H, m, –CH_3), 1.57 (1H, m, –CH_2–), 1.76 (3H, s, –CH_3), 1.88 (1H, m, –CH_2–), 2.10 (1H, m, –CH_2–), 2.44 (1H, m, –CH_2–), 2.65 (1H, m, –CH_2–), 2.87 (1H, m, >CH–), 2.35–2.44 (10H, m, –N–CH_2–), 2.90–2.92 (2H, d, –N–CH_2–), 4.16 (1H, m, >C*H*OH), 4.92 (1H, s, =CH_2), 4.96 (1H, s, =CH_2), 5.50 (1H, m, =CH–). MS (EI) *m*/*z*: 265 (M^+, 80), 72 (100).

3.1.5.3. 5-{[1-(4-Benzylpiperazin-1-yl)methyl]vinyl}-2-methylcyclohex-2-enol (V_3). Compound V_3 was obtained in 12.8% yield, mp 179–180 °C. ^{1}H NMR (DMSO-d_6), 1.56 (1H, m, –CH_2–), 1.76 (3H, s, –CH_3), 1.95 (1H, m, –CH_2–), 2.15 (2H, m, –CH_2–), 2.44 (10H, br, –CH_2–, –N–CH_2–, >CH_2–), 2.90–2.94 (2H, d, –N–CH_2–), 3.50 (2H, s, –CH_2Ph), 4.15 (1H, m, >C*H*OH), 4.86 (1H, s, =CH_2), 4.92 (1H, s, =CH_2), 5.49 (1H, m, =CH–), 7.30 (3H, m, Ar-H), 7.31 (2H, m, Ar-H). MS (EI) *m*/*z*: 326 (M^+, 19), 91 (100).

3.1.5.4. 5-{1-[4-(4-Methoxyphenyl)piperazin-1-yl]-methyl}vinyl-2-methylcyclohex-2-enol (V_4). Compound V_4 was obtained in 15.8% yield. ^{1}H NMR 1.59 (1H, m, –CH_2–), 1.77 (3H, s, –CH_3), 1.95 (2H, m, –CH_2–), 2.20 (2H, m, –CH_2–), 2.45 (1H, m, >CH_2–), 2.55 (4H, m, –N–CH_2–), 2.95 (2H, m, –N–CH_2–), 3.25 (4H, m, –N–CH_2–), 3.75 (3H, s, –OCH_3), 4.19 (1H, m, >C*H*OH), 4.95 (1H, s, =CH_2), 5.02 (1H, s, =CH_2), 5.50 (1H, m, =CH–), 6.82 (2H, d, Ar-H), 6.90 (2H, d, Ar-H). MS (EI) *m*/*z*: 342 (M^+, 38), 150 (100).

3.1.5.5. 5-{1-[4-(2-Methoxyphenyl)piperazin-1-yl]-methyl }vinyl-2-methylcyclohex-2-enol (V_5). Compound V_5 was obtained in 17.3% yield. ^{1}H NMR 1.62 (1H, m, –CH_2–), 1.77 (3H, s, –CH_3), 2.02 (2H, m, –CH_2–), 2.19 (2H, m, –CH_2–), 2.46 (1H, m, >CH_2–), 2.59 (4H, br, –N–CH_2–), 2.99 (2H, m, –N–CH_2–), 3.07 (4H, br, –N–CH_2–), 3.85 (3H, s, –OCH_3), 4.18 (1H, m, >C*H*OH), 4.93 (1H, s, =CH_2), 4.99 (1H, s, =CH_2), 5.51 (1H, m, =CH–), 6.84–6.99 (4H, m, Ar-H). MS (EI) *m*/*z*: 342 (M^+, 3), 150 (100).

3.1.6. General procedure for the preparation of compounds VI_1–VI_5

3.1.6.1. 1-Methyl-4-[2-(4-methylcyclohex-3-enyl)allyl]piperazine (VI_1). A solution of compound III_1 (2.0 g, 8.0 mmol), 80% hydrazine hydrate (3.9 mL, 40 mmol) in 15 mL ethylene glycol was heated at 120 °C for 2 h. The mixture was cooled to 70 °C and treated with potassium hydroxide (3.7 g, 56 mmol), then heated at 180–185 °C for 4–6 h. Then, the mixture was cooled to the room temperature, diluted with brine (100 mL), and extracted with methylene chloride

(3× 30 mL). The combined organic extracts were washed with brine, dried over anhydrous sodium sulfate, and filtered. The filtrate was concentrated in vacuo. The residue was purified on a silica gel column with petroleum ether–acetate as eluent to afford the target product **VI**$_1$, (12.5% yield). ^{1}H NMR 1.51 (2H, m, –CH$_2$–), 1.65 (3H, s, –CH$_3$), 1.79 (1H, m, –CH$_2$–), 1.90 (1H, m, –CH$_2$–), 2.12 (1H, m, –CH$_2$–), 2.15 (1H, m, –CH$_2$–), 2.21 (1H, m, >CH–), 2.27 (3H, s, –N–CH$_3$), 2.46 (8H, m, –N–CH$_2$–), 2.91–2.93 (2H, d, –N–CH$_2$–), 4.87 (1H, s, =CH$_2$), 4.94 (1H, s, =CH$_2$), 5.39 (1H, m, =CH–). MS (EI) *m*/*z*: 234 (M$^+$, 28), 113 (100).

3.1.6.2. 1-Ethyl-4-[2-(4-methylcyclohex-3-enyl)allyl]piperazine (VI$_2$). Compound **VI**$_2$ was obtained in 10.8% yield. ^{1}H NMR 1.09 (3H, m, –CH$_3$), 1.51 (2H, m, –CH$_2$–), 1.65 (3H, s, –CH$_3$), 1.80 (1H, m, –CH$_2$–), 1.93 (1H, m, –CH$_2$–), 2.04 (1H, m, –CH$_2$–), 2.13 (1H, m, –CH$_2$–), 2.21 (1H, m, >CH–), 2.43 (10H, m, –N–CH$_2$–), 2.91–2.94 (2H, d, –N–CH$_2$–), 4.87 (1H, s, =CH$_2$), 4.94 (1H, s, =CH$_2$), 5.40 (1H, m, =CH–). MS (EI) *m*/*z*: 248 (M$^+$, 100).

3.1.6.3. 1-Benzyl-4-[2-(4-methylcyclohex-3-enyl)allyl]piperazine (VI$_3$). Compound **VI**$_3$ was obtained in 14.9% yield. ^{1}H NMR 1.49 (2H, m, –CH$_2$–), 1.65 (3H, s, –CH$_3$), 1.77–2.21 (5H, m, –CH$_2$–, >CH–), 2.44 (8H, br, –N–CH$_2$–), 2.91 (2H, m, –N–CH$_2$–), 3.51 (2H, s, –CH$_2$Ph), 4.85 (1H, s, =CH$_2$), 4.93 (1H, s, =CH$_2$), 5.39 (1H, m, =CH–), 7.30 (3H, m, Ar-H), 7.31 (2H, m, Ar-H). MS (EI) *m*/*z*: 310 (M$^+$, 40), 91 (100).

3.1.6.4. 1-(4-Methoxyphenyl)-4-[2-(4-methylcyclohex-3-enyl)allyl]piperazine (VI$_4$). Compound **VI**$_4$ was obtained in 11.7% yield. ^{1}H NMR (DMSO-d_6), 1.58 (2H, m, –CH$_2$–), 1.70 (3H, s, –CH$_3$), 1.86–2.25 (5H, m, –CH$_2$–, >CH–), 2.59 (4H, br, –N–CH$_2$–), 3.03 (2H, m, –N–CH$_2$–), 3.12 (4H, br, –N–CH$_2$–), 3.80 (3H, s, –OCH$_3$), 4.95 (1H, s, =CH$_2$), 5.03 (1H, s, =CH$_2$), 5.45 (1H, m, =CH–), 6.85 (2H, d, Ar-H), 6.94 (2H, d, Ar-H). MS (EI) *m*/*z*: 326 (M$^+$, 60), 205 (100).

3.1.6.5. 1-(2-Methoxyphenyl)-4-[2-(4-methylcyclohex-3-enyl)allyl]piperazine (VI$_5$). Compound **VI**$_5$ was obtained in 14.6% yield. ^{1}H NMR 1.53 (2H, m, –CH$_2$–), 1.66 (3H, s, –CH$_3$), 1.84 (2H, m, –CH$_2$–), 1.91 (2H, m, –CH$_2$–), 2.22 (1H, m, >CH–), 2.60 (4H, br, –N–CH$_2$–), 3.00 (2H, m, –N–CH$_2$–), 3.08 (4H, br, –N–CH$_2$–), 3.85 (3H, s, –OCH$_3$), 4.90 (1H, s, =CH$_2$), 4.99 (1H, s, =CH$_2$), 5.41 (1H, m, =CH–), 6.84–7.01 (4H, m, Ar-H). MS (EI) *m*/*z*: 326 (M$^+$, 56), 150 (100).

3.2. Biological activity

3.2.1. Cell line and culture. LNCaP cells were obtained from the American Type Culture Collection, Rockville, MD, and were cultured in RPMI-1640 medium supplemented with 100 μg/mL penicillin, 100 μg/mL streptomycin, 1 mM L-glutamine, and 10% heat-inactivated fetal bovine serum.

3.2.2. MTT assay. 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 the compounds to be tested were then added and cultured for 4 days at 37 °C. Fifty microliters of 2 mg/mL 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution was added per well and the cells were continued to be cultured 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 reader. Growth inhibition was determined as compared to untreated cells and calculated as % of untreated cells.[23]

3.2.3. Western blot analysis. Protein extracts (50 μg) prepared with RIPA lysis buffer (50 mM Tris-HCl, 150 mM NaCl, 0.1% SDS, 1% NP-40, 0.5% sodium deoxycholate, 1 mM PMSF, 100 μM leupeptin, and 2 μg/mL aprotinin, pH 8.0) were separated on an 8 or 12% SDS–polyacrylamide gel, and then transferred to nitrocellulose membranes. The membranes were stained with 0.2% Ponceau S red to assure equal protein loading and transfer. After blocking with 5% non-fat milk, the membranes were incubated with antibodies to phospho-ERK1/2, ERK, HDJ-2, phospho-p38, p38, and p21waf1 (Signal Transduction Lab). Immunocomplexes were visualized by chemiluminescence (ECL kit, Amersham).

Acknowledgment

This work was partly supported by National Natural Science Foundation of China (30328030).

References and notes

1. Crowell, P. L.; Gould, M. N. *Crit. Rev. Oncog.* **1994**, *5*, 1–22.
2. Elson, C. E. *J. Nutr.* **1995**, *125*, 1666S–1672S.
3. Elson, C. E.; Peffley, D. M.; Hentosh, P.; Mo, H. *Proc. Soc. Exp. Biol. Med.* **1999**, *221*, 294–311.
4. Haag, J. D.; Lindstrom, M. J.; Gould, M. N. *Cancer Res.* **1992**, *52*, 4021–4026.
5. Mills, J. J.; Chari, R. S.; Boyer, I. J.; Gould, M. N.; Jirtle, R. l. *Cancer Res.* **1995**, *55*, 979–983.
6. Haag, J. D.; Gould, M. N. *Cancer Chemother. Pharmacol.* **1994**, *34*, 477–483.
7. Hardcastle, I. R.; Rowlands, M. G.; Barber, A. M.; Grimshaw, R. M.; Mohan, M. K., et al. *Biochem. Pharmacol.* **1999**, *57*, 801–809.
8. Gelb, M. H.; Tamanoi, F.; Yokoyama, K.; Ghomashchi, F.; Esson, K., et al. *Cancer Lett.* **1995**, *91*, 169–175.
9. Crowell, P. L.; Ren, Z.; Lin, S.; Vedejs, E.; Gould, M. N. *Biochem. Pharmacol.* **1994**, *47*, 1405–1415.
10. Vigushin, D. M.; Poon, G. K.; Boddy, A.; English, J.; Halbert, G. W.; Pagonis, C.; Jarman, M.; Coombes, R. C. *Cancer Chemother. Pharmacol.* **1998**, *42*, 111–117.
11. Hudes, G. R.; Szarka, C. E.; Adams, A.; Ranganathan, S.; McCauley, R. A., et al. *Clin. Cancer Res.* **2000**, *6*, 3071–3080.
12. Liu, G.; Oettel, K.; Bailey, H.; Van Ummersen, L.; Tutsch, K.; Staab, M. J.; Horvath, D.; Alberti, D.; Arzoomanian, R.; Rezazadeh, H.; McGovern, J.; Robinson, E.; DeMets, D.; Wilding, G. *Invest. New Drugs* **2003**, *21*, 367–372.

13. Meadows, S. M.; Mulkerin, D.; Berlin, J.; Bailey, H.; Kolesar, J., et al. *Int. J. Gastrointest. Cancer* **2002**, *32*, 125–128.
14. Nakamura, E.; Aoki, S.; Sekiya, K.; Oshino, H.; Kuwajima, I. *J. Am. Chem. Soc.* **1987**, *109*, 8056–8066.
15. Huang, M. *J. Am. Chem. Soc.* **1946**, *68*, 2487–2488.
16. Kawata, S.; Nagase, T.; Yamasaki, E.; Ishiguro, H.; Matsuzawa, Y. *Br. J. Cancer* **1994**, *69*, 1015–1020.
17. Sun, S. Y.; Zhou, Z.; Wang, R.; Fu, H.; Khuri, F. R. *Cancer Biol. Ther.* **2004**, *3*, 1092–1098, discussion 1099–1101.
18. Hsu, Y. L.; Kuo, P. L.; Lin, L. T.; Lin, C. C. *J. Pharmacol. Exp. Ther.* **2005**, *313*, 333–344.
19. Park, S.; Park, C. H.; Hahm, E. R.; Kim, K.; Kimler, B. F., et al. *Cell. Signalling* **2005**, *17*, 111–119.
20. Nguyen, T. T.; Tran, E.; Nguyen, T. H.; Do, P. T.; Huynh, T. H., et al. *Carcinogenesis* **2004**, *25*, 647–659.
21. Shen, G.; Xu, C.; Chen, C.; Hebbar, V.; Kong, A. N. *Cancer Chemother. Pharmacol.* **2006**, *57*, 317–327.
22. Park, K. S.; Ahn, Y.; Kim, J. A.; Yun, M. S.; Seong, B. L., et al. *Br. J. Pharmacol.* **2002**, *137*, 597–607.
23. Lu, M.; Xia, L.; Luo, D.; Waxman, S.; Jing, Y. *Oncogene* **2004**, *23*, 3945–3952.