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The Role of Substituents of *ar*-Turmerone for its Anticancer Activity

Won-Geun Oh, Kyong-Up Baik, Sang-Hun Jung* and Byung-Zun Ahn
College of Pharmacy, Chung-Nam National University, Yousung Gu, Dae Jeon, 305-764, Korea.
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Abstract □ For the evaluation of the role of substituents of *ar*-turmerone for its anticancer activity, ar-turmerone (**1a**) and its analogs like 2-methyl-6-(4'-methyphenyl)-2-octen-4-one (**1b**), 2-methyl-6-phenyl-2-hepten-4-one (**1c**), 2-methyl-6-phenyl-2-octen-4-one (**1d**), and 2-methyl-6-(*trans*-4'-methylcyclohexyl)-2-hepten-4-one (**1e**) were prepared and their cytotoxic activities against L_{1210} cell were determined. Omission of methyl group at *para*-position dose not variate the cytotoxicity of ar-turmerone. Elongation of alkyl group at 6-position decreases ED_{50} value. Saturation of aromatic ring of ar-turmerone markedly decreases the cytotoxicity. Therefore the smaller size of alkyl group at 6-position and aromatic ring of ar-turmerone should be essential for exhibiting its anticancer activity.

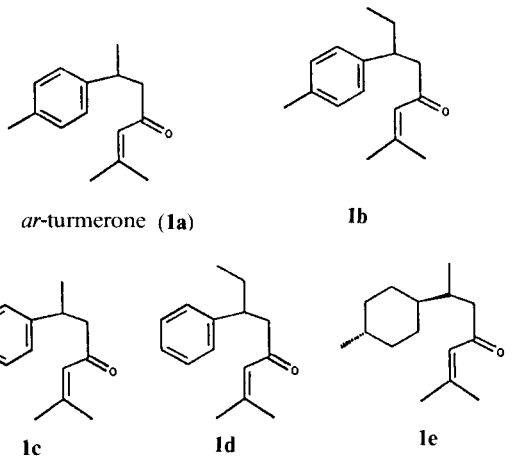
Keywords □ *ar*-turmerone, substituent effect, anticancer activity, cytotoxicity.

The cytotoxicity of the various ingredients from *Curcuma* species against cancer cell lines *in vitro*[1] was initially notified in 1980. Itokawa and his coworkers[2] isolated ar-turmerone[3] from *Curcuma xanthorrhiza* as one of the antineoplastic components with the guidance of bio-assay against Sarcoma 180 ascite in mice in 1985. Ahn and his collegues[4] screened the antitumor activity of the various fractions from forty herbal drugs. As a result, they found the antitumor compound, ar-turmerone, from *Curcuma domestica* in 1986. Although the anticancer activity of ar-turmerone is moderate (ED_{50} 40 μg/ml against L_{1210} cell *in vitro* and T/C(%)=160 against Sarcoma 180 in mice), its side effect expected to be weak due to the traditional use of the root of *Curcuma domestica* in the oriental medicine[4b]. Furthermore its remarkable synergistic effect for the activities of aurapten and other naturally occuring antitumor components[4] is very much attractive. Therfore, to obtain the more potent analogs, modification of ar-turmerone structure has been attempted. Our initial focus was given to the recognition of the role of methyl group at para position of phenyl ring and at 6 position and π-electron or flatness of phenyl ring of ar-turmerone on its anticancer effect. Accordingly compounds **1a-1e** were prepared and tested their cytotoxicity against murine leukemia L_{1210} cell in *in vitro*.

EXPERIMENTAL

General

Melting points were measured using Electrothermal melting point apparatus and uncorrected. All commercial chemicals were used as obtained and all solvents were purified by the standard procedures prior to use[5]. Thin-layer chromatography was performed on E. Merck silica gel GF-254 precoated plates and the identification was done with UV light and colorization with spray 10% phosphomolydic acid followd by heating. Flash Column chromatography was carried out on silica gel (Merck, 230-400 mesh). IR spectra were measured on Perkin-Elmer 780 IR spectrometer and corrected against peak at 1601 cm^{-1} of polystyrene. NMR spectra were determined on Bruker AC 80 (80 MHz), Varian-Gemini 200 (200 MHz), and Bruker AM-300 (300 MHz). Chemical shift are reported in δ ppm



ar-turmerone (**1a**) **1b**

1c **1d** **1e**

relative to tetramethylsilane. Mass spectra were obtained on JMX-DX 303 (Jeol) under standard condition.

General procedure[6c,7] for the preparation of compounds 3

One equivalent of aldehyde **2** and diethyl malonate were dissolved in benzene to be 10% solution. To this solution 0.2 equivalent of acetic acid and 0.03 equivalent of piperidine were added. The resulting mixture was then refluxed through Dean-Stark trap for 18 hours under nitrogen atmosphere. The reaction mixture was cooled to room temperature. After addition of the same volume of benzene, the reaction mixture was washed with 10% aqueous NaCl solution three times, dehydrated with anhydrous $MgSO_4$, and concentrated in vacuo. The purification of crude products were performed by recrystallization or flash column chromatography.

Diethyl (4'-methylbezylidene) malonate, 3a

Recrystallized from ethanol; Rf 0.50 (20% acetone-hexane); white solid; m.p. 49-50℃; yield 85%; IR (KBr) 3030, 2985, 2940, 1720 cm^{-1}; NMR ($CDCl_3$, 80 MHz) δ 1.28 (t, J=7.2 Hz, 3H), 1.30 (t, J=7.2 Hz, 3H), 2.35 (s, 3H), 4.28 (q, J=7.2 Hz, 2H), 4.33 (q, J=7.2 Hz, 2H), 7.14 (d, J=7.4 Hz, 2H), 7.14 (d, J=7.4 Hz, 2H), 7.37 (d, J=7.4 Hz, 2H), 7.70 (s, 1H).

Diethyl benzylidenemalonate, 3c

Rf 0.47 (20% acetone-hexane); colorless oil; yield 76%; IR (neat, NaCl) 3030, 3940, 1720 cm^{-1}; NMR ($CDCl_3$, 80 MHz) δ 1.28 (t, J=7.2 Hz, 3H), 1.30 (t, J=7.2 Hz, 3H), 4.28 (q, J=7.2 Hz, 2H), 4.33 (q, J=7.2 Hz, 2H), 7.14 (s, 5H), 7.70 (s, 1H).

Diethyl trans-4'-methylcyclohexylmethylidenemalonate, 3e

Rf 0.54 (20% Ethyl acetate-hexane); colorless oil; yield 75%; IR (neat, NaCl) 2925, 1725 cm^{-1}; NMR ($CDCl_3$, 80 MHz) δ 0.85-1.80 (m, 18H, containing δ 1.29 (t, J=7.2 Hz), 1.32 (t, J=7.2 Hz), and 1.75 (d, J=6.5 Hz)), 2.31 (m, 1H), 4.22 (q, J=7.2 Hz, 2H), 4.30 (q, J=7.2 Hz, 2H), 6.78 (d, J=10.4 Hz); MS, m/z (rel. intensity) 268 (9), 222 (8), 176 (100), 148 (9), 120 (14).

General procedure[7] for the preparation of compounds 4

In three neck flask equipped with a pressure equalizing funnel, thermometer, and reflux condenser, magnesium metal (1.5 equivalent) and anhydrous ether was placed under nitrogen atmosphere. To this mixture the ethereal solution of methyl iodide (or ethyl iodide, 1.6 equivalent) was added dropwise with stirring. The resulting mixture was refluxed for about one hour until magnesium metal was nearly dissolved. This solution was then cooled to −5-0℃ and CuCl (0.02 equivalent) was added carefully under nitrogen flow. The ethereal solution of compounds **3** (1 equivalent) was added dropwise in the rate to keep the reaction temperature at −5-0℃ and the resulting mixture was then stirred for additional 0.5 hour at the room temperature. The reaction mixture was carefully added to the mixture of ice and 10% aqueous sulfuric acid. Ethereal layer was separated and aqueous phase was extracted with ether three times. All combined ether fraction was washed with 10% aqueous $NaHSO_3$ and water successively, dried over anhydrous magnesium sulfate, and concentrated in vacuo. The crude products **4** was purified with flash column chromatography.

Ethyl 2-ethoxycarbonyl-3-(4'-methylphenyl)butanoate, 4a

Rf 0.60 (20% Ethyl acetate-hexane); colorless oil; yield 90%; IR (neat, NaCl) 3040, 2880, 1750, 1730 cm^{-1}; NMR ($CDCl_3$, 80 MHz) δ 0.97 (t, J=7.2 Hz, 3H), 1.13 (t, J=7.2 Hz, 3H), 1.29 (d, J=6.3 Hz, 3H), 2.31 (s, 3H), 3.47 (m, 2H), 3.92 (q, J=7.2 Hz, 2H), 4.22 (q, J=7.2 Hz, 2H), 7.13 (s, 4H).

Ethyl 2-ethoxycarbonyl-3-(4'-methylphenyl)pentanoate, 4b

Rf 0.63 (20% Ethyl acetate-hexane); colorless oil; yield 75%; IR (neat, NaCl) 3040, 2880, 1750, 1730 cm^{-1}; NMR ($CDCl_3$, 80 MHz) δ 0.92 (t, J=7.2 Hz,

3H), 0.95 (m, 2H), 1.13 (t, J=7.2 Hz, 3H), 1.29 (t, J=7.2 Hz, 3H), 2.31 (s, 3H), 3.48 (m, 2H), 3.92 (t, J=7.2 Hz, 2H), 4.21 (t, J=7.2 Hz, 2H), 7.13 (s, 4H).

Ethyl 2-ethoxycarbonyl-3-phenylbutanoate, 4c

Rf 0.62 (20% Ethyl acetate-hexane); colorless oil; yield 80%; IR (neat, NaCl) 3040, 2880, 1750, 1730 cm^{-1}; NMR ($CDCl_3$, 80 MHz) δ 0.92 (t, J=7.2 Hz, 3H), 1.13 (t, J=7.2 Hz, 3H), 1.29 (t, J=6.3 Hz, 3H), 3.45-3.60 (m, 2H), 3.92 (q, J=7.2 Hz, 3H), 4.21 (q, J=7.2 Hz, 2H), 7.13 (s, 5H).

Ethyl 2-ethoxycarbonyl-3-phenylpentanoate, 4d

Rf 0.65 (20% Ethyl acetate-hexane); colorless oil; yield 72%; IR (neat, NaCl) 3040, 2880, 1750, 1730 cm^{-1}; NMR ($CDCl_3$, 80 MHz) δ 0.92 (t, J=6.8 Hz, 3H), 0.97 (t, J=7.2 Hz, 3H), 1.13 (t, J=7.2 Hz, 3H), 1.29 (m, J=6.8 Hz, 2H), 3.45-3.60 (m, 2H), 3.92 (q, J=7.2 Hz, 2H), 4.21 (q, J=7.2 Hz, 2H), 7.13 (m, 5H).

Ethyl 2-ethoxycarbonyl-3-(trans-4'-methylcyclohexyl)butanoate, 4e

Rf 0.60 (20% Ethyl acetate-hexane); colorless oil; yield 90%; IR (neat, NaCl) 2925, 1725 cm^{-1}; NMR ($CDCl_3$, 200 MHz) δ 0.85-1.80 (m, 22H, containing peaks at δ 0.84 (d, J=6.5 Hz), 0.88 (d, J=6.5 Hz), and 1.27 (t, J=7.2 Hz)), 2.19 (m, 1H), 3.41 (d, J= 8.8 Hz, 1H), 4.20 (t, J=7.2 Hz, 4H), ; MS, m/z (rel, intensity) 284 (100), 187 (50), 160 (53), 132 (12), 123 (15).

General procedure[8] for the preparation of compounds 5

The reaction mixture of compounds **4** and two equivalent of Lithium chloride and water in dimethysulfoxide (180 times of water used) was heated at 160-170 ℃ for 18 hours with stirring. After the mixture was cooled to room temperature, two times of water as that of dimethysulfoxide was added and the resulting mixture was extracted with hexane 4 times. All combined hexane layer was washed with water three times, dried over anhydrous magnesium sulfate, concentrated in vacuo. The products were purified with flash column chromatography and (or) vacuum distillation with Kugelrohr.

Ethyl 3-(4'-methylphenyl)butanoate, 5a

Rf 0.65 (30% Ethyl acetate-hexane); distilled at 60-70℃ under 0.05 torr; colorless oil; yield 85%; IR (neat, NaCl) 3040, 2990, 2940, 1730 cm^{-1}; NMR ($CDCl_3$, 80 MHz) δ 1.21 (t, J=7.2 Hz, 3H), 1.30 (d, J=6.9 Hz, 3H), 2.34 (s, 3H), 2.58 (m, 2H), 3.30 (m, 1H), 4.10 (q, J=7.2 Hz, 2H), 7.14 (s, 4H).

Ethyl 3-(4'-methylphenyl)pentanoate, 5b

Rf 0.68 (30% Ethyl acetate-hexane); distilled at 60-70℃ under 0.05 torr; colorless oli; yield 76%; IR (neat, NaCl) 3040, 2990, 2940, 1730 cm^{-1}; NMR ($CDCl_3$, 80 MHz) δ 0.95 (t, J=6.8 Hz, 3H) 1.12 (t J=7.2 Hz, 3H), 1.31 (m, 2H), 2.31 (s, 3H), 2.51 (m, 2H), 3.35 (m, 1H), 4.18 (q, J=7.2 Hz, 2H), 7.06 (m, 4H).

Ethyl 3-phenylbutanoate, 5c

Rf 0.60 (20% Ethyl acetate-hexane); colorless oil; yield 72%; IR (neat, NaCl) 3040, 2990, 2940, 1730 cm^{-1}; NMR ($CDCl_3$, 80 MHz) δ 1.12 (t, J=7.2 Hz, 3H), 1.31 (d, J=6.9 Hz, 3H), 2.46 (m, 2H), 3.56 (m, 1H), 4.18 (q, J=7.2 Hz, 2H), 7.17 (s, 5H).

Ethyl 3-phenylpentanoate, 5d

Rf 0.63 (20% Ethyl acetate-hexane); distilled at 65-70℃ under 0.05 torr; colorless oil; yield 75%; IR (neat, NaCl) 3040, 2990, 2940, 1730 cm^{-1}; NMR ($CDCl_3$, 80 MHz) δ 0.95 (t, J=6.7 Hz, 3H), 1.13 (t, J=7.2 Hz, 3H), 1.30 (m, 2H), 2.48 (m, 2H), 3.56 (m, 1H), 4.17 (q, J=7.2 Hz, 2H), 7.17 (m, 5H).

Ethyl 3-(trans-4'-methylcyclohexyl)butanoate, 5e

Rf 0.60 (20% Ethyl acetate-hexane); colorless oil; yield 90%; IR (neat, NaCl) 2920, 1730 cm^{-1}; NMR ($CDCl_3$, 200 MHz) δ 0.85-1.80 (m, 19H, containing peaks at δ 0.85 (d, J=6.5 Hz), 0.89 (d, J=5.8 Hz), 1.25 (t, J=7.2 Hz)), 1.86 (m, 1H), 2.20 (m, 2H), 4.12 (q, J=7.2 Hz, 2H); MS, m/z (rel. intensity) 212 (29), 148 (22), 123 (47), 115 (100), 88 (78).

General procedure[6e] for the preparation of compounds 6

Two equivalent of diisopropylamine was dissolved in tetrahydrofuran under nitrogen atmosphere and the solution was cooled to 0-5℃. Two equivalent of n-butyl lithium was injected to the solution. The resulting solution was stirred for 0.5 hour and then cooled to −78℃. One equivalent of compounds **5** in tetrahydrofuran was added dropwise for 0.5 hour and stirred for additional 0.5 hour. To this resulting solution, solution of 3,3-dimethylacryloyl chloride (1.2 equivalent) in tetrahydrofuran was added in one

rated in vacuo. The products **6** were purified with flash column chromatography. Every product was consisted of two diastereomers in a ratio of 1 : 1 approximately indicated by their NMR spectra obtained.

5-Ethoxycarbonyl-6-(4'-methylphenyl)-2-methyl-2-hepten-4-one, 6a

Rf 0.45 (20% Ethyl acetate-hexane); pale yellowish oil; yield 90%; IR (neat, NaCl) 3040, 2980, 2940, 1735, 1680 cm^{-1}; NMR ($CDCl_3$, 80 MHz), Two sets of peaks were tentatively assigned as follows; δ 1.24 (t, J=7.2 Hz, 3H), 1.26 (d, J=6.6 Hz, 3H), 1.91 (d, J=1.0 Hz, 3H), 2.17 (d, J=1.0 Hz, 3H), 2.27 (s, 3H), 3.55 (m, 1H), 3.72 (m, 1H), 4.22 (q, J=7.2 Hz, 2H), 6.31 (m, 1H), 7.10 (m, 4H) and δ 0.92 (t, J=7.2 Hz, 3H), 1.15 (d, J=6.6 Hz, 3H), 1.77 (d, J=1.0 Hz, 3H), 1.85 (d, J=1.0 Hz, 3H), 2.25 (s, 3H), 3.53 (m, 1H), 3.68 (m, 1H), 3.85 (q, J=7.2 Hz, 2H), 6.05 (m, 1H), 7.05 (m, 4H).

5-Ethoxycarbonyl-6-(4'-methylphenyl)-2-methyl-2-octen-4-one, 6b

Rf 0.48 (20% Ethyl acetate-hexane); pale yellowish oil; yield 84%; IR (neat, NaCl) 3040, 2980, 2940, 1735, 1680 cm^{-1}; NMR ($CDCl_3$, 80 MHz), two sets of peaks were tentatively assigned as follows; δ 0.72 (t, J=7.2 Hz, 3H), 1.25 (t, J=7.2 Hz, 3H), 1.40-1.80 (m, 2H), 1.80 (d, J=1.0 Hz, 3H), 2.21 (d, J=1.0 Hz, 3H), 2.30 (s, 3H), 3.25 (m, 1H), 3.80 (m, 1H), 4.20 (q, J=7.2 Hz, 2H), 6.32 (m, 1H), 7.10 (s, 4H) and δ 0.70 (t, J=7.2 Hz, 3H), 0.95 (t, J=7.2 Hz, 3H), 1.40-1.80 (m, 5H, containing 1.75 (d, J=1.0 Hz)), 1.95 (d, J=1.0 Hz, 3H), 2.25 (s, 3H), 3.25 (m, 1H), 3.80 (m, 1H), 3.95 (t, J=7.2 Hz, 3H), 6.02 (m, 1H), 7.01 (s, 4H).

5-Ethoxycarbonyl-6-phenyl-2-methyl-2-hepten-4-one, 6c

Rf 0.42 (17% Ethyl acetate-hexane); pale yellowish oil; yield 86%; IR (neat, NaCl) 3040, 2980, 2940, 1735, 1680 cm^{-1}; NMR ($CDCl_3$, 80 MHz), two sets of peaks were tentatively assigned as follows: δ 1.25 (t, J=7.2 Hz, 3H), 1.30 (d, J=7.1 Hz, 3H), 1.93 (d, J=1.0 Hz, 3H), 2.22 (d, J=1.0 Hz, 3H), 3.60-3.80 (m, 2H), 4.21 (q, J=7.2 Hz, 2H), 6.36 (m, 1H), 7.25 (s, 5H) and δ 0.92 (t, J=7.2 Hz, 3H), 1.25 (d, J=7.1 Hz, 3H), 1.71 (d, J=1.0 Hz, 3H), 1.85 (d, J=1.0 Hz, 3H), 3.50-3.75 (m, 2H), 4.05 (q, J=7.2 Hz, 2H), 5.95 (m, 1H), 7.17 (s, 5H).

portion and the resulting reaction mixture was stirred for 0.5 hour at −78℃. After removal of dry ice-acetone bath, the mixture was stirred for another one hour and then poured to 0.1 N aqueous hydrochloric acid. This mixture was extracted with dichloromethane three times and all combined dichloromethane phase was washed with water three times, dried over anhydrous magnesium sulfate, concent-

5-Ethoxycarbonyl-6-phenyl-2-methyl-2-octen-4-one, 6d

Rf 0.43 (17% Ethyl acetate-hexane); pale yellowish oil; yield 83%; IR (neat, NaCl) 3040, 2980, 2940, 1735, 1680 cm^{-1}; NMR ($CDCl_3$, 80 MHz), two sets of peaks were tentatively assigned as follows; δ 0.82 (t, J=7.1 Hz, 3H), 1.22 (t, J=7.2 Hz, 3H), 1.40-1.80 (m, 2H), 1.90 (d, J=1.0 Hz, 3H), 2.21 (d, J=1.0 Hz, 3H), 3.50-3.80 (m, 2H), 4.23 (q, J=7.2 Hz, 2H), 6.30 (m, 1H), 7.07 (s, 5H) and δ 0.75 (t, J=7.1 Hz, 3H), 0.95 (t, J=7.2 Hz, 3H), 1.40-1.80 (m, 2H), 1.73 (d, J=1.0 Hz, 3H), 1.79 (d, J=1.0 Hz, 3H), 3.45-3.75 (m, 2H), 4.03 (q, J=7.2 Hz, 2H), 6.01 (m, 1H), 7.04 (s, 5H).

5-Ethoxycarbonyl-6-(trans-4'-methylcyclohexyl)-2-methyl-2-hepten-4-one, 6e

Rf 0.45 (20% Ethyl acetate-hexane); colorless oil; yield 73%; IR (neat, NaCl) 2920, 1730, 1680 cm^{-1}; NMR ($CDCl_3$, 300 MHz), two sets of peaks were tentatively assigned as follows: δ 0.80-2.26 (m, 26H, containing peaks at δ 0.83 (d, J=6.5 Hz), 0.86 (d, J=5.8 Hz), 1.21 (t, J=7.2 Hz), 1.87 (s)), 3.46 (d, J=8.8 Hz, 1H), 4.17 (q, J=7.2 Hz, 2H), 6.23 (m, 1H) and δ 0.73 (d, J=6.5 Hz, 3H), 0.80-2.26 (m, 23H, containing peaks at δ 0.84 (d, J=5.8 Hz), 1.21 (t, J=7.2 Hz), 1.87 (s), 2.15 (s)), 3.37 (d, J=8.8 Hz, 1H), 4.09 (q, J=7.2 Hz, 2H), 6.18 (m, 1H); MS, m/z (rel. intensity) 294 (7), 249 (9), 197 (10), 170 (65), 155 (25), 15 (15), 83 (100).

General procedure[b] for the preparation of compounds 1

Employing the same procedure for the preparation of compounds **5**, compounds **1** were synthesized. The purification of compounds **1** was performed with flash column chromatography.

2-Methyl-6-(4'-methylphenyl)-2-hepten-4-one, 1a

Rf 0.70 (25% Ethyl acetate-hexane); colorless oil; yield 76%; IR (neat, NaCl) 3035, 1695 cm^{-1}; NMR ($CDCl_3$, 80 MHz) δ 1.20 (d, J=6.4 Hz, 3H), 1.86

(d, J=1.0 Hz, 3H), 2.11 (d, J=1.0 Hz, 3H), 2.11 (d, J=1.0 Hz, 3H), 2.30 (s, 3H), 2.62 (d, J=7.5 Hz, 2H), 3.21 (m, 1H), 6.01 (m, 1H), 7.06 (s, 4H).

2-Methyl-6-(4'-methylphenyl)-2-octen-4-one, 1b

Rf 0.72 (25% Ethyl acetate-haxane); clorless oil; yield 72%; IR (neat, NaCl) 3040, 1695 cm^{-1}; NMR ($CDCl_3$, 80 MHz) δ 0.78(t, J=7.1 Hz, 3H), 1.60 (m, 2H), 1.75 (d, J=1.0 Hz, 3H), 2.03 (d, J=1.0 Hz, 3H), 2.22 (s, 3H), 2.65 (d, J=7.3 Hz, 2H), 3.05 (m, 1H), 6.02 (m, 1H), 7.12 (s, 4H).

2-Methyl-6-phenyl-2-hepten-4-one, 1c

Rf 0.69 (17% Ethyl acetate-hexane); colorless oil; yield 73%; IR (neat, NaCl) 3035, 1695 cm^{-1}; NMR ($CDCl_3$, 80 MHz) δ 1.21 (d, J=6.4 Hz, 3H), 1.86 (d, J=1.0 Hz, 3H), 2.11 (d, J=1.0 Hz, 3H), 2.62 (d, J=7.5 Hz, 3H), 2.62 (d, J=7.5 Hz, 2H), 3.10 (m, 1H), 6.10 (m, 1H), 7.06 (s, 5H).

2-Methyl-6-phenyl-2-octen-4-one, 1d

Rf 0.70 (17% Ethyl acetate-hexane); colorless oil; yield 67%; IR (neat, NaCl) 3035, 1695 cm $^{-1}$; NMR ($CDCl_3$, 80 MHz) δ 0.85 (t, J=7.2 Hz, 3H), 1.60 (m, 2H), 1.86 (d, J=1.0 Hz, 3H), 2.11 (d, J=1.0 Hz, 3H), 2.65 (d, J=7.5 Hz, 2H), 3.12 (m, 1H), 6.08 (m, 1H), 7.06 (s, 5H).

2-Methyl-6-(trans-4'-methylcyclohexyl)-2-hepten-4-one, 1e

Rf 0.65 (20% Ethyl acetate-hexane); colorless oil; yield 83%; IR (neat, NaCl) 2920, 1685 cm $^{-1}$; NMR ($CDCl_3$, 300 MHz) δ 0.85-1.40 (m, 14H, containing two doublets at δ 0.81 (d, J=6.5 Hz), and 0.84 (d, J=6.0 Hz)), 1.85-1.98 (m, 4H, containing singlet at δ 1.85), 2.05-2.15 (m, 4H, containing singlet at δ 2.11), 2.45 (dd, J=15.0, 4.8 Hz, 1H), 6.04 (m, 1H); MS, m/z (rel. intensity) 222(15) 208(10), 168(20), 149(23), 125(57), 98(98), 83(100).

***Cytotoxicity against L_{1210} cell in vitro*[9]**

Test compounds were initially dissolved in dimethysulfoxide at concentration of 10 mg/m*l* and diluted 10 times with Fisher's medium supplemented with 10% horse serum. In capped culture tubes, these samples were taken 60, 30, 15 μ*l*, respectively and diluted with 3 m*l* of Fisher's medium containing L_{1210} cell 5×10^4 cell/m*l*. After 48 hours incubation at 37℃ in CO_2 incubator containing 5% CO_2 and 95% air, cell numers were determined on hacmacytometer. From the cell number counted, ED_{50} values were calculated with known method. Each compound was tested three times and mean ED_{50} values were obtained.

Table I. Cytotoxicity of *ar*-turmerone derivatives[a].

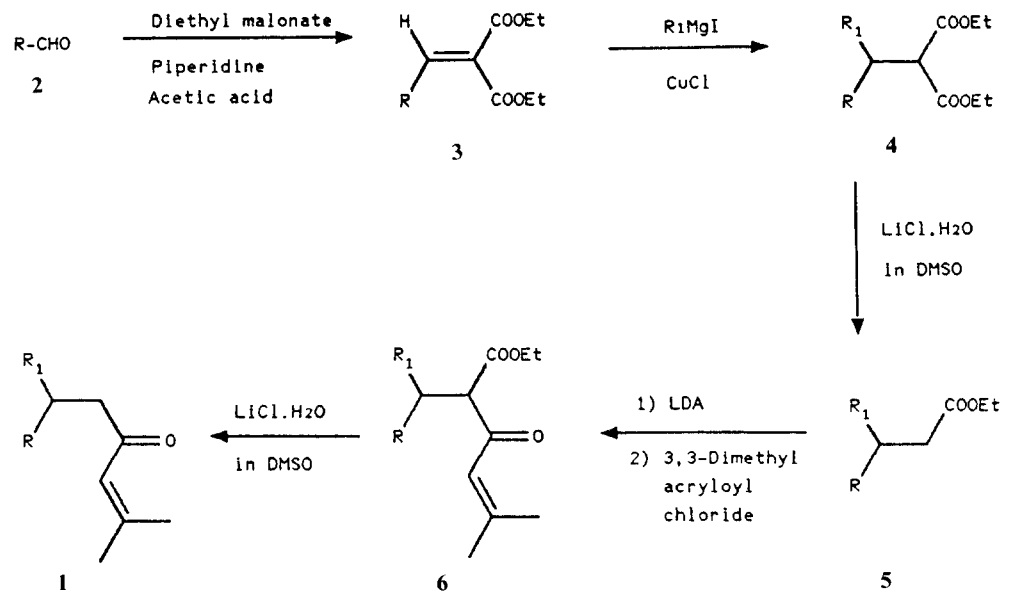
Comp. No.	Substituents R	R_1	ED_{50} (μg/m*l*)
1a	p-MePh	Me	40.0
1b	p-MePh	Et	65.5
1c	Ph	Me	45.4
1d	Ph	Et	53.3
1e	*trans*-4-MeCy[b]	Me	>100
6a	p-MePh	Me	11.3
6b	p-MePh	Et	16.8
6c	Ph	Me	12.1
6e	*trans*-4-MeCy[b]	Me	>100
MeCCNU			4.5

[a]Cytotoxicity was measured against L_{1210} cell *in vitro*. [b]*trans*-4-MeCy means *trans*-4'-methylcyclohexyl.

RESULTS AND DISCUSSION

Synthesis

The various synthetic pathways[6] for the preparation of ar-turmerone have been developped. Among them, we modified Rousseau's procedure[6c] shown in scheme 1 and prepared compounds **1a-1d** in good yields. Diethyl alkylidenemalonates **3** were synthesized by the typical Knoevenagel condensation[7]. The mixture of Diethyl malonate (1 equivalent), aldehyde **2** (1 equivalent), acetic acid (0.2 equivalent), and piperidine (0.03 equivalent) in benzene was refluxed through Dean-Stark trap to remove the water for 18 hours. Standard work-up followed by recrystallization and chromatographic separation gave pure products **3** in very good yield. Intermediates **4** were prepared by the addition[7] of ethereal solution of compounds **3** to organocuprates obtained from methyl magnesium iodide or ethyl magnesium iodide with 10% cuprous chloride at -5-0℃. In the following step, decarboxylation[8] of compounds **4** in the presence of two equivalents of lithium chloride and water in dimethysulfoxide by heating at 160-170℃ for 15 hours gave the products **5**. Conversion of compounds **5** to **6** was performed by the treatment of compounds **5** with two equivalent of Lithium diisopropylamide at −78℃



* R and R1 are found in Table I.

Scheme 1. Synthetic pathway for the preparation of ar-turmerone analog.

in tetrahydrofuran followed by addition of tetrahydrofuran solution of 3,3-dimethylacryloyl chloride (1.2 equivalent)[9c]. From these reactions, diastereomeric mixtures **6** were obtained. The analysis of their NMR spectra indicated the equal ratio of mixtures. However, the mixtures are inseparable due to the fast equilibrium between two diastereomers because of the existence of two carbonyl functional groups at the same carbon. The following decarboxylation[7] of these diastereomers **6** using the same procedure for the preparation of **5** gave racemic final products **1** in good yield.

Structure-activity relationship

For the measurement of anticancer activity of compounds **1** and **6**, their cytotoxicity was tested against L_{1210} cell *in vitro* and their ED_{50} values were calculated with known procedure[9]. As shown in table 1, their ED_{50} values are more than 10 μg/ml, which are weaker than that (2-7 μg/ml) of MeCCNU (N-(2-chloroethyl)-N′-(*trans*-4′-methyl cyclohexyl)-N-nitrosourea).

Comparing ED_{50} values of **1a** and **1c**, **1b** and **1d**, **6a** and **6d**, *para* methyl substituent on phenyl ring is not an important factor for its cytotoxic action. Although data are limited to methyl and ethyl group at 6 position, examination of ED_{50} values of **1a** and **1b**, **1c** and **1d** reflects that increasing the size of alkyl group at 6 position results in decreasing the activity. Therefore elongation of aliphatic chain of ar-turmerone should not be considered for the enhancement of the biological activity of this analog. Saturation of phenyl ring makes a dramatic loss of the biological activity as indicated with ED_{50} value of compound **1e** (>100 μg/ml). This result certainly assures the importance of π-electron or flatness of phenyl part of ar-turmerone for its antineoplastic activity. Introduction of ethoxycarbonyl moiety at 5 position shown in compounds **6a-6d** markedly increases the cytotoxicity. This might indicate that the importance of polarity in the area of α, β-unsaturated ketone of ar-turmerone.

From this study, presence of aromatic ring in ar-turmerone is essential for the antitumor effect. Increment of polarity of derivatives may also contributes for the enhancement of antitumor effect of this analog. Therefore, alteration of π-electron density of phenyl ring and the polarity at α, β-unsaturated ketone area of ar-turmerone might provide the important clues for finding the more potent analogs.

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