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Recognition of Pharmacophore of ar-Turmerone for its Anticancer Activity

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For the evaluation of the role of α,β-unsaturated ketone portion of ar-turmerone for its activity, the structural variation of this structural unit was performed to omit its alkylating property. Thus compounds **2**, **3**, and **4** were prepared and their cytotoxicities were determined against three different leukemia cell lines (HL-60, K-562, and L_{1210}) *in vitro*. The biological inactivity against three different cell lines of these analogues implies that the α,β-unsaturated ketone of ar-turmerone is the essential pharmacophore for its activity.

Key words: Pharmacophore, ar-Turmerone, Cytotoxicity, Anticancer activity

INTRODUCTION

The various antineoplastic components have been isolated from curcuma species (Mattes *et al.*, 1980). A sesquiterpene, ar-turmerone (**1**, Rupe and Wiederkehr, 1924), is one of the unique substances which exhibit moderate antineoplastic activity (ED_{50} 40 μg/ml against L_{1210} cell in vitro and T/C (%)=160 against sarcoma 180 in mice)(Itokawa *et al.*, 1985; Lee *et al.*, 1986). Considersing the traditional use of Curcuma domestica in the oriental medicine (Lee *et al.*, 1986), the side effect of the analogues of ar-turmerone expected to be weak. Therefore the structural modification of ar-turmerone has been attractive for finding the novel antineoplastic agents. Our first investigation addressed on the recognition of the role of substituents at para-position and 6-position and phenyl ring of ar-turmerone on its anticancer activity (Oh *et al.*, 1992). As a result, aromatic ring portion of this compound was the important factor for its activity. Our second question on its structure was about the role of α,β-unsaturated ketone unit of ar-turmerone for its activity. This question prompted us to prepare compounds **2-4** (shown in Scheme 1) for evaluating the role of α,β-unsaturated carbonyl moiety in ar-turmerone for its activity. In this paper we described the preparation of compounds **2-4** and their cytotoxicities against human leukemia HL-60 and K-562 cell and murine leukemia L_{1210} cell *in vitro*.

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MATERIALS AND METHODS

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 (Perrin and Armarego, 1988). 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% phosphomolybdic 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 Jeol JNM-EX 90 (89.45 MHz) and Varian-Gemini 200 (200 MHz) spectrometer. Chemical shift are reported in δ ppm relative to tetramethylsilane. Mass spectra were obtained on JMX-DX 303(Jeol) under standard condition.

Synthesis of 4-Hydroxy-5-(hydroxymethyl)-2-methyl-6-(4'-methylphenyl)-2-heptene (2)

Toluene solution of diisobutylaluminum hydride (1 M, 15 ml, 15 mmole) was cooled to −78℃. The solution of 5-ethoxycarbonyl-2-methyl-6-(4'-methyl-phenyl)-2-hepten-4-one (1.44 g, 5 mmole) in 20 ml of tetrahydrofuran was added dropwise in 0.5 hour and then stirred for additional 6 hours at room temperature. The reaction mixture was carefully quenched with 30 ml of methanol containing **5** equivalents of water and

then extracted with dichloromethane three times (Kraus and Frazier, 1980). The dichloromethane layers were combined, dehydrated with anhydrous magnesium sulfate, and evaporated in vacuo. Flash column chromatographic separation of the crude product gave 290 mg of pure cpmpound **2** (22% yield).

Rf 0.20 (SiO_2, 10% ethyl acetate-hexane); white solid; m.p 63.5-65.0℃; IR(KBr): 3300(br), 2925, 1670 cm^{-1}; NMR(200 MHz, $CDCl_3$) 1.20(d, J=7.0Hz, 3H), 1.36(d, J=1.3Hz, 3H), 1.73(d,J=1.3Hz, 3H), 2.31(s, 3H), 2.50 (m, 1H), 2.91(m, 1H), 3.92(m, 2H), 4.30(m, 1H), 5.42(m, 1H), 7.26(m, 4H); MS m/z (rel. intensity) 248(M^+,4), 175(27), 146(88), 131(100), 119(69), 95(18).

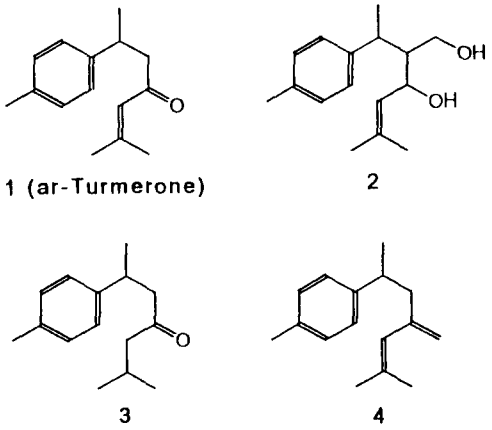
Synthesis of 2-Methyl-6-(4'-methylphenyl)heptan-4-one (3)

Compound **1** (216 mg, 1 mmole) was dissolved in 3 ml of methanol-dichloromethane (1:1). To the reaction mixture was added 10% Pd-C (200 mg) and then bubbled with hydrogen gas for 12 hours at room temperature. After filtration, the filtrate was evaporated *in vacuo* and the pure product **3** was isolated by flash column chromatography (SiO_2, 10% ethylacetate-hexane) in 70% yield (152 mg) (Breitner *et al.*, 1959).

Rf 0.33 (SiO_2, 10% ethyl acetate-hexane); colorless liquid; IR(neat): 2950, 1705, 1510 cm^{-1}; NMR(200 MHz, $CDCl_3$) 0.81(d, J=6.4Hz, 3H), 0.83(d, J=6.4Hz, 3H), 1.22(d, J=6.9Hz, 3H), 2.00-2.20(m, 3H), 2.30(s, 3H), 2.62(m, 2H), 3.25(m, 1H), 7.08(s, 4H); MS m/z (rel. intensity) 218(M_+, 23), 203(35), 161(22), 119(100), 105(11), 85(11).

Synthesis of 2-Methyl-6-(4'-methylphenyl)- 4-methylidene-2-heptene (4)

To the solution of methyltriphenylphosphonium bromide (2.36 g, 6.4 mmole) in 15 ml of tetrahydrofuran was added dropwise 3.8 ml (6.07 mmole) of n-butyl lithium (1.6 M) in hexane at 0℃ for 10 minutes.



1 (ar-Turmerone) 2 3 4

Scheme 1

Table I. Cytotoxicity of compounds **1-4**[a]

Compound	ED_{50} (μg/ml)[b]		
No.	HL-60[c]	K-562[c]	L_{1210}[d]
1	18	20	50
2	30	61	65
3	>100	>100	>100
4	>100	>100	>100

[a]Cytotoxicity of these compounds were measured with known methods (Thayer *et al.*, 1971). [b]Mean ED50 values were obtained from the tests repeated three times. [c]RPMI medium enriched with fetal bovine serums in 10% were used. [d]Fisher's medium supplemented with horse serum in 10% was used.

The resulting mixture was stirred for additional 30 minutes and the solution of 1 (1.25 g, 5.78 mmole) in 15 ml of tetrahydrofuran was added. The reaction mixture was then stirred for 16 hours at room temperature. After addition of 20 ml of methanol, the resulting solution was concentrated in vacuo, triturated with ether, and the ethereal layer was concentrated in vacuo to give the crude product. The flash column chromatography of the crude mixture gave the pure compound 4 (725 mg) in 65% yield (Greenwald *et al.*, 1963).

Rf 0.80 (SiO_2, 10% ethyl acetate-hexane); colorless liquid; IR(neat) 2925, 1620 cm^{-1}; NMR(89.45 MHz, $CDCl_3$) 1.18(d, J=6.9Hz, 3H), 1.73(d, J=1.1Hz, 3H), 1.78(d, J=1.1Hz, 3H), 2.10-2.40(m, 5H, containing tall singlet at 2.30), 2.76(m, 1H), 4.72(m, 1H), 4.87(m, 1H), 5.55(m, 1H), 7.06(s, 4H); MS m/z (rel. intensity) 214 (M^+,13), 199(6), 171(8), 157(13), 119(100), 91(7).

RESULTS AND DISCUSSION

Compound **2-4** are the analogues which are altered at the α,β-unsaturated ketonic portion of ar-turmerone. Structural characteristics of compounds designed, are sterically similar to that of ar-turmerone. However, these are not able to undergo a putative biological alkylation of ar-turmerone through Michael addition reaction since these materials do not possess α,α-unsaturated ketone. This structural variation should be enable for us to determine whether the α,β-unsaturated ketone unit of ar-turmerone is the essential pharmacophore.

Compound **2** possesses 4-hydroxy group instead of 4-keto group in ar-turmerone. Although compound **2** exhibits the moderate cytotoxicities against all three different cell lines as shown in table I, these are very much weak compared to those (ED_{50} 5.0, 8.2, and 11.3 μg/ml against HL-60, K-562, and L_{1210} cell, respectively) of its synthetic precursor, 5-ethoxycarbonyl-6-(4'-methylphenyl)-2-methyl-2-hepten-4-one. This reduced cytotoxicity might reflect the lack of alkylating reactivity

of compound **2** due to the reduction of ketone of α,β-unsaturated ketone of the precursor. Compound **3** is the saturated analogue at the double bond of ar-turmerone (**1**). This saturation results in the complete loss of cytotoxicity against all three different cell lines. This should be considered as the result of structural variation of α,β-unsaturated ketone to saturated moiety of ar-turmerone. Compound 4 contains methylidene group at 4-position as the isoelectronic group to the ketone of α,β-unsaturated ketone. However, electronic nature of diene structure of the compound 4 can not alkylate the nucleophile. The lack of the biological activity of this compound shown in table I also reflects the complete loss of alkylating property.

From the structural alteration of α,β-unsaturated ketone unit of ar-turmerone to the derivatives incapable of alkylation and the examination of cytotoxicity of these analogues, the α,β-unsaturated ketone of ar-turmerone is identified as the essential pharmacophore for its antitumor effect. These results also support that the biological activity of ar-turmerone should occur from the alkylation of its α,β-unsaturated ketone function. Therefore, the antitumor effect of this series could be increased as the increment of alkylating reactivity of α,β-unsaturated ketone potion of ar-turmerone.

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