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Structure Activity Relationship of ar-Turmerone Analogues

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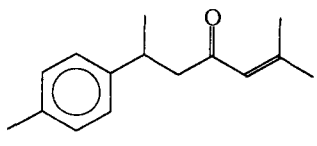
For the analysis of structure activity relationship of ar-turmerone analogues, the compounds containing the various substituents on the phenyl ring and 1(or 2)-naphthyl group in the place of phenyl of ar-turmerone were prepared and tested their cytotoxicity against HL-60, K-562, and L1210 leukemia cells *in vitro*. The substituents at para position are methoxy, phenoxy, methyl, trifluoromethyl, fluoro, and chloro. At *meta* position methoxy, methyl, trifluoromethyl, or chloro groups and at *ortho* position methoxy or chloro group were introduced. Against HL-60 and K-562 cells, ED_{50} values of the analogues are ranged from 0.8 to 30.0 μg/ml. Against L1210 cell, these are located more than 20.0 μg/ml. However, 5-carboethoxy-2-methyl-6-(1-naphthyl)-2-octen-4-one (**5n**) possesses ED50 valuses 0.8, 2.1, 6.5 μg/ml against HL-60, K-562, L1210 cells, respectively. The electronic nature of the subsituents on phenyl ring of ar-turmerone dose not affect the biological activity. Therefore the flat structure of aromatic portion of ar-turmerone analogues is the more important factor for their activity rather than its electronic nature. The potentiation of the cytotoxicity with the enlargement of aromatic ring region also supports the importance of the plane structure of this area. The restriction of the single bond rotation between C-6 and aromatic ring through the introduction of substituents at the *ortho* position of phenyl ring and the increment of size of alkyl group at C-6 position enhances the activity. Therefore the effective conformation should be the one having the orthogonal arrangement between the aromatic ring and the side chain.

Key words: Ar-turmerone, Cytotoxicity, Anticancer activity

INTRODUCTION

Ar-turmerone (Rupe *et al.*, 1924, Honwad and Rao, 1964) was isolated as a antitumor component from Curcuma species(Itokawa *et al.*, 1985; Lee *et al.*, 1986). Its unique synergistic effect for the various antineoplastic agents was also notified (Ahn *et al.*, 1989). These biologically interesting characteristics combined with the traditional use of *Curcuma domestica* in the oriental folk medicine (Ahn and Lee, 1989) was attractive to consider this compound as a model structure for the structural modification. Therefore the identification of the essential structural units of ar-turmerone for its activity was initially attempted (Oh *et al.*, 1992). As the results, the absolute necessity of the presence of phenyl ring portion and α, β-unsaturated ketone function of ar-turmerone for its activity was recognized (Oh *et al.*, 1992; Baik *et al.*, 1993). The subsequent structural feature for the potentiation of its antitumor activity to be identified were the electronic and structural nature of phenyl ring and the conformation between aromatic portion and the side chain. Accordingly the various electron donating or withdrawing substituents were introduced at *ortho*, *meta*, or *para* position of phenyl ring of ar-turmerone and naphthyl analogues were also prepared as shown in Fig. 1. The cytotoxicity of these analogues were measured against the murine leukemia L1210 cell and human leukemia HL-60 and k-562 *in vitro*.



ar-Turmerone

MATERIALS AND METHODS

Chemistry

The preparation of ar-turmerone analogues were performed in five steps from the substituted aromatic aldehydes. The detail procedure was previously descri-

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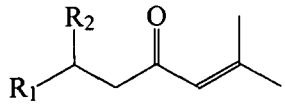


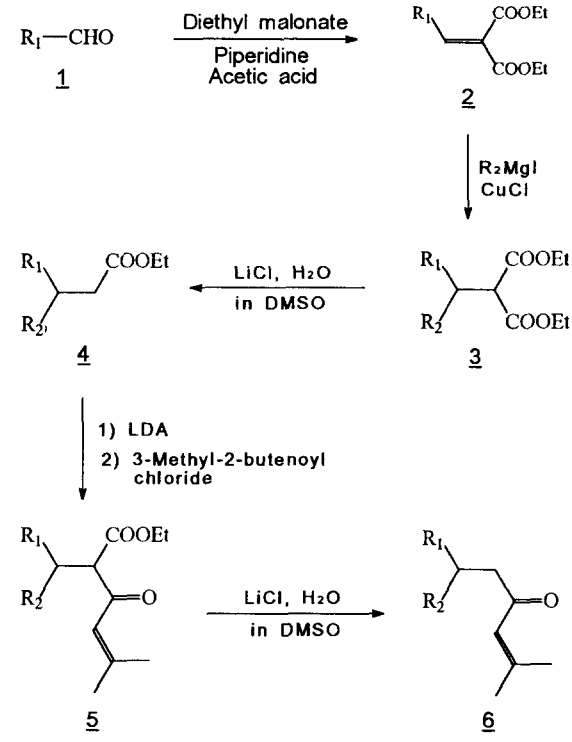
R_1: substituted phenyl or naphthyl substituents on phenyl

Position	e/d group	e/w group
para	OMe, OPh, Me	CF_3, F, Cl
meta	OMe, Me	CF_3, Cl
ortho	OMe	Cl

R_2: Me or Et

Fig. 1. Target structure

bed (Oh *et al.*, 1992). The aromatic aldehyde **1** was refluxed with one equivalent of diethyl malonate in the presence of the catalytic amount of acetic acid and piperidine in benzene through Dean-Stark trap for 18 hours(Rousseau and Blanco, 1985). Arylidenemalonates **2** purified by vacuum distillation or flash column chromatography was then treated with methylmagnesium iodide in the presence of 10% catalytic amount of cuprous chloride in ether at 5-10℃ to give the adducts **3** (Eliel *et al.*, 1988). Decarboxylation of compounds **3** was done by heating with two equivalent of lithium chloride and water in dimethylsulfoxide at 160-170℃ for 15 hours (Kpracho *et al.*, 1978). Decarboxylated products **4** were then acylated by the sequential treatment with two equivalent of lithium diisopropylamide at −78℃ in tetrahydrofuran and 3-methyl-2-butenoyl chloride (1.2 equivalent) to give the inseparable diastereomeric mixtures **5** (Rousseau and Blanco, 1985). Due to the rapid equilibrium between



Scheme 1. Synthetic pathway for the preparation of ar-turmerone analogues
*R_1 and R_2 are found in Table I.

the diastereomers, compounds **5** were inseparable. Conversion of **5** to **6** were accomplished with the same procedure for the preparation of 4. The physical data for compounds **5** and **6** are presented in Table

Table I. Physical data of compounds **5**.

No.	Compound **5**	Substituents R_1	R_2	molecular formula	overall yield (%)	distillation temperature[a]
1	a	p-OMePh	Me	$C_{18}H_{24}O_4$	57.0	112-124
2	b	p-OPhPh	Me	$C_{23}H_{26}O_4$	32.4	—[b]
3	c	P-CF_3Ph	Me	$C_{18}H_{21}O_3F_3$	55.3	—[b]
4	d	p-FPh	Me	$C_{17}H_{21}O_3F$	64.7	100-110
5	e	p-ClPh	Me	$C_{17}H_{21}O_3Cl$	43.5	—[b]
6	f	m-OMePh	Me	$C_{18}H_{24}O_4$	36.2	—[b]
7	g	m-ClPh	Me	$C_{17}H_{21}O_3Cl$	65.3	85-100
8	h	m-CF_3Ph	Me	$C_{18}H_{21}O_3F_3$	55.3	100-110
9	i	m-MePh	Me	$C_{18}H_{24}O_3$	74.0	110-120
10	j	o-OMePh	Me	$C_{18}H_{24}O_4$	39.3	—[b]
11	k	o-OMePh	Et	$C_{19}H_{26}O_4$	23.3	—[b]
12	l	o-ClPh	Me	$C_{17}H_{21}O_3Cl$	63.8	110-124
13	m	1-naphthyl	Me	$C_{21}H_{24}O_3$	35.6	—[b]
14	n	1-naphthyl	Et	$C_{22}H_{26}O_3$	46.3	—[b]
15	o	2-naphthyl	Me	$C_{21}H_{24}O_3$	45.0	—[b]
16	p	2-naphthyl	Et	$C_{22}H_{26}O_3$	50.5	—[b]

[a]Distillation were performed with Kugelrohr under reduced pressure (0.5 torr).
[b]Compounds were purified by flash column chromatography.

Table II. Spectral data of compounds **5**.

No.	Compound[a] **5**	Spectral data[b] ^{1}H-NMR (in $CDCl_3$, δ), IR (ν_{max} cm^{-1}), Mass (m/e, rel. int.)
1	a	NMR: A: 7.15(m, 2H), 6.80(m, 2H), 6.32(m, 1H), 4.21(q, J=7.1 Hz, 2H), 3.78(s, 3H), 3.67(m, 1H), 3.54(m, 1H), 2.22(d, J=1.1 Hz, 3H), 1.96(d, J=1.1 Hz, 3H), 1.28(t, J=7.1 Hz, 3H), 1.26(d, J=6.8 Hz, 3H) B: 7.14(m, 2H), 6.75(m, 2H), 6.00(m, 1H), 3.88(q, J=7.1 Hz, 2H), 3.78(s, 3H), 3.65(m, 1H), 3.53(m, 1H), 1.89(d, J=1.1 Hz, 3H), 1.78(d, J=1.1 Hz, 3H), 1.16(d, J=6.8 Hz, 3H), 0.95(t, J=7.1 Hz, 3H) IR: 2960, 1735, 1615 Mass: 304(M^+, 4), 250(9), 231(4), 221(7), 135(100), 100(26), 83(53)
2	b	NMR: A: 7.45-6.85(m, 9H), 6.32(m, 1H), 4.22(q, J=7.1 Hz, 2H), 3.70-3.45(m, 2H), 1.99(d, J=1.1 Hz, 3H), 1.96(d, J=1.1 Hz, 3H), 1.30(d, J=6.8 Hz, 3H), 1.27(t, J=7.1 Hz, 3H) B: 7.45-6.80(m, 9H), 6.02(m, 1H), 3.91(q, J=7.1 Hz, 2H), 3.68-3.40(m, 2H), 1.90(d, J=1.1 Hz, 3H), 1.78(d, J=1.1 Hz, 3H)1.19(d, J=6.8 Hz, 3H), 0.97(t, J=7.1 Hz, 3H) IR: 2970, 1730, 1620, 1510 Mass: 366(M^+, 9), 283(8), 237(100), 197(91), 109(3), 83(95)
3	c	NMR: A: 7.60-7.30(m, 4H), 6.32(m, 1H), 4.20(q, J=7.2 Hz, 2H), 3.80-3.70(m, 2H), 2.22(d, J=1.0 Hz, 3H), 1.97(d, J=1.0 Hz, 3H), 1.35-1.20(m, 6H) B: 7.60-7.30(m, 4H), 5, 99(m, 1H), 3.88(q, J=7.2 Hz, 2H), 3.75-3.60(m, 2H), 1.89(d, J=1.0 Hz, 3H), 1.79(d, J=1.0 Hz, 3H), 1.22(d, J=7.0 Hz, 3H), 0.93(t, J=7.2 Hz, 3H) IR: 2975, 1730, 1618, 1322 Mass: 342(M^+, 2), 259(4), 173(2), 83(100)
4	d	NMR: A: 7.20(m, 2H), 6.98(m, 2H), 6.30(m, 1H), 4.20(q, J=7.1 Hz, 2H), 3.73-3.55(m, 2H), 2.20(d, J=1.1 Hz, 3H), 1.94(d, J=1.1 Hz, 3H), 1.26(d, J=6.8 Hz, 3H), 1.25(t, J=7.1 Hz, 3H) B: 7.20(m, 2H), 6.96(m, 2H), 5.99(m, 1H), 3.87(q, J=7.1 Hz, 2H), 3.70-3.50(m, 2H), 1.88(d, J=1.1 Hz, 3H), 1.78(d, J=1.1 Hz, 3H), 1.18(d, J=6.8 Hz, 3H), 0.95(t, J=7.1 Hz, 3H) IR: 2970, 1730, 1683 Mass: 292(M^+, 11), 246(7), 209(68), 123(39), 83(100)
5	e	NMR: A: 7.32(m, 4H), 6.30(m, 1H), 4.20(q, J=7.1 Hz, 2H), 3.73-3.53(m, 2H), 2.19(d, J=1.1 Hz, 3H), 1.95(d, J=1.1 Hz, 3H), 1.28(t, J=7.1 Hz, 3H), 1.26(d, J=6.8 Hz, 3H) B: 7.30(m, 4H), 6.03(m, 1H), 3.89(q, J=7.1 Hz, 2H), 3.70-3.50(m, 2H), 1.90(d, J=1.1 Hz, 3H), 1.80(d, J=1.1 Hz, 3H), 1.18(d, J=6.8 Hz, 3H), 0.98(t, J=7.1 Hz, 3H) IR: 2970, 1730, 1683 Mass: 308(M^+, 3), 266(2), 225(20), 141(15), 139(47), 83(100)
6	f	NMR: A: 7.20(m, 2H), 6.78(m, 2H), 6.32(m, 1H), 4.20(q, J=7.1 Hz, 2H), 3.79(s, 3H), 3.70(m, 1H), 3.56(m, 1H), 2.20(d, J=1.1 Hz, 3H), 1.94(d, J=1.1 Hz, 3H), 1.28(d, J=6.8 Hz, 3H), 1.27(t, J=7.1 Hz, 3H) B: 7.18(m, 2H), 6.76(m, 2H), 6.00(m, 1H), 3.89(q, J=7.1 Hz, 2H), 3.77(s, 3H), 3.68(m, 1H), 3.54(m, 1H), 1.94(d, J=1.1 Hz, 3H), 1.89(d, J=1.1 Hz, 3H), 1.27(d, J=6.8 Hz, 3H), 0.96(t, J=7.1 Hz, 3H) IR: 2970, 1730, 1630 Mass: 304(M^+, 4), 175(7), 135(20), 83(100)
7	g	NMR: A: 7.20(m, 4H), 6.30(m, 1H), 4.18(q, J=7.1 Hz, 2H), 3.85-3.50(m, 2H), 2.20(d, J=1.1 Hz, 3H), 1.95(d, J=1.1 Hz, 3H), 1.28(d, J=6.8 Hz, 3H), 1.25(t, J=7.1 Hz, 3H) B: 7.19(m, 4H), 6.01(m, 1H), 3.80(q, J=7.1 Hz, 2H), 3.70-3.20(m, 2H), 3.90(d, J=1.1 Hz, 3H), 1.79(d, J=1.1 Hz, 3H), 1.21(d, (d, J=6.8 Hz, 3H), 0.97(t, J=7.1 Hz, 3H) IR: 2970, 1730, 1683, 1450 Mass: 308(M^+, 4), 225(15), 152(13), 139(12), 83(100)
8	h	NMR: A: 7.48(m, 4H), 6.30(m, 1H), 4.22(q, J=7.1 Hz, 2H), 3.80-3.60(m, 2H), 2.22(d, J=1.1 Hz, 3H), 1.96(d, J=1.1 Hz, 3H), 1.33(d, 1.33(d, J=6.8 Hz, 3H), 1.31(t, J=7.1 Hz, 3H) B: 7.46(m, 4H), 6.02(m, 1H), 3.88(q, J=7.1 Hz, 2H), 3.75-3.60(m, 2H), 1.85(d, J=1.1 Hz, 3H), 1.78(d, J=1.1 Hz, 3H), 1.25(d, (d, J=6.8 Hz, 3H), 0.93(t, J=7.1 Hz, 3H) IR: 2975, 1735, 1620 Mass: 342(M^+, 4), 159(11), 173(8), 83(100)
9	i	NMR: A: 7.20-6.90(m, 4H), 6.33(m, 1H), 4.20(q, J=7.1 Hz, 2H), 3.68(m, 1H), 3.54(m, 1H), 2.31(s, 3H), 2.20(d, J=1.1 Hz, 3H), 1.95(d, J=1.1 Hz, 3H), 1.28(d, J=6.8 Hz, 3H), 1.27(t, J=7.1 Hz, 3H) B: 7.20-6.90(m, 4H), 6.01(m, 1H), 3.87(q, J=7.1 Hz, 2H), 3.67(m, 1H), 3.50(m, 1H), 2.29(s, 3H), 1.87(d, J=1.1 Hz, 3H), 1.77(d, J=1.1 Hz, 3H), 1.20(d, J=6.8 Hz, 3H), 0.94(t, J=7.1 Hz, 3H) IR: 2975, 1730, 1680, 1620 Mass: 288(M^+, 9), 270(8), 205(28), 159(37), 119(22), 83(100)

Table II. continued

No.	Compound[a] **5**	Spectral data[b] ^{1}H-NMR (in $CDCl_3$, δ), IR (ν_{max} cm^{-1}), Mass (m/e, rel. int.)
10	j	NMR: A: 7.18(m, 2H), 6.85(m, 2H), 6.30(m, 1H), 4.18(q, J=7.1 Hz, 2H)4.00-3.85(m, 2H), 3.84(s, 3H), 2.21(d, J=1.1 Hz, 3H), 1.95(d, J=1.1 Hz, 3H), 1.32(d, J=6.8 Hz, 3H), 1.27(t, J=7.1 Hz, 3H) B: 7.16(m, 2H), 6.84(m, 2H),1 6.08(m, 1H), 3.98(q, J=7.1 Hz, 2H), 4.00-3.85(m, 2H), 3.83(s, 3H), 1.85(d, J=1.1 Hz, 3H), 1.79(d, J=1.1 Hz, 3H), 1.19(d, J=6.8 Hz, 3H), 0.93(t, J=7.1 Hz, 3H) IR: 2960, 1730, 1680 Mass: 304(M^+, 7), 205(13), 166(11), 165(100), 83(13)
11	k	NMR: A: 7.25-6.80(m, 4H), 6.35(m, 1H), 4.18(q, J=7.1.Hz, 2H), 3.83(s, 3H), 4.00-3.85(m, 2H), 2.19(d, J=1.1 Hz, 3H), 1.93(d, J=1.1 Hz, 3H), 1.75(m, 2H), 1.25(t, J=7.1 Hz, 3H), 0.70(t, J=7.3 Hz, 3H) B: 7.25-6.80(m, 4H), 6.08(m, 1H), 4.05(q, J=7.1 Hz, 3H), 4.00-3.83(m, 2H), 1.76(d, J=1.1 Hz, 3H), 1.74(d, J=1.1Hz, 3H), 1.71(m, 2H), 0.88(t, J=7.1 Hz, 3H), 0.67(t, J=7.3 Hz, 3H) IR: 2960, 1725, 1680, 1615 Mass: 318(M^+, 2), 189(9), 161(2), 149(21), 121(13), 83(100)
12	l	NMR: A: 7.28(m, 4H), 6.34(m, 1H), 4.24(q, J=7.1 Hz, 2H), 4.05-3.80(m, 2H), 2.24(d, J=1.1 H1z, 3H), 1.98(d, J=1.1 Hz, 3H), 1.31(d, J=6.8 Hz, 3H), 1.28(t, J=7.1 Hz, 3H) B: 7.25(m, 4H), 6.18(m, 1H), 3.95(q, J=7.1 Hz, 2H), 4.00-3.80(m, 2H), 1.88(d, J=1.1 Hz, 3H), 1.83(d, J=1.1 Hz, 3H), 1.21(d, J=6.8 Hz, 3H), 0.99(t, J=7.1 Hz, 3H) IR: 2975, 1730, 1685 Mass: 308(M^+, 3), 273(35), 139(16), 83(100)
13	m	NMR: A: 8.31-7.32(m, 7H), 6.36(m, 1H), 4.52(m, 1H), 4.23(q, J=7.1 Hz, 2H), 4.05(m, 1H), 2.23(d, J=1.1 Hz, 3H), 1.96(d, J=1.1 Hz, 3H), 1.40(d, J=7.0 Hz, 3H), 1.27(t, J=7.1 Hz, 3H) B: 8.30-7.30(m, 7H), 6.02(m, 1H), 4.52(m, 1H), 4.05(m, 1H), 3.72(q, J=7.1 Hz, 2H), 1.62(d, J=1.1 Hz, 3H), 1.31(d, J=1.1 Hz, 3H), 1.28(d, J=7.0 Hz, 3H), 0.68(t, J=7.1 Hz, 3H) IR: 2975, 1740, 1680, 1620, 1440 Mass: 324(M^+, 5), 306(14), 241(6), 195(27), 155(31), 86(27), 83(100)
14	n	NMR: A: 8.40-7.35(m, 7H), 6.37(m, 1H), 4.44(m, 1H), 4.25(q, J=7.1 Hz, 2H), 4.00(m, 1H), 2.25(d, J=1.2 Hz, 3H), 1.97(d, J=1.2 Hz, 3H), 1.85(m, 2H), 1.30(t, J=7.1 Hz, 3H), 0.70(t, J=7.2 Hz, 3H) B: 8.40-7.35(m, 7H), 5.97(m, 1H), 4.40(m, 1H), 4.00(m, 1H), 3.61(q, J=7.1 Hz, 2H), 1.52(d, J=1.2 Hz, 3H), 1.43(d, J=1.2 Hz, 3H), 1.28(t, J=7.1 Hz, 3H), 0.56(t, J=7.2 Hz, 3H) IR: 2975, 1730, 1680, 1615, 1440 Mass: 338(M^+, 4), 320(6), 209(5), 169(23), 153(20), 141(12), 83(100)
15	o	NMR: A: 7.73-7.45(m, 7H), 6.37(m, 1H), 4.23(q, J=7.1 Hz, 2H), 3.85-3.70(m, 2H), 2.22(d, J=1.1 Hz, 3H), 1.96(d, J=1.1Hz, 3H), 1.38(d, J=6.8 Hz, 3H), 1.28(t, J=7.1 Hz, 3H) B: 7.70-7.40(m, 7H), 6.01(m, 1H), 3.82(q, J=7.1 Hz,2 2H), 3.83-3.65(m, 2H), 1.78(d, J=1.1 Hz, 3H), 1.69(d, J=1.1Hz, 3H), 1.26(d, J=6.8 Hz, 3H), 0.87(t, J=7.1 Hz, 3H) IR: 2975, 1730, 1680 MASS : 324(M^+, 45), 306(32), 241(35), 135(100), 155(76), 83(100)
16	p	NMR: A: 7.85-7.30(m, 7H), 6.37(m, 1H), 4.25(q, J=7.1 Hz, 3H), 3.60-3.40(m, 2H), 2.23(d, J=1.1 Hz, 3H), 1.96(d, J=1.1 Hz, 3H), 1.70(m, 2H), 1.29(t, J=7.1 Hz, 3H), 0.73(t, J=7.3Hz, 3H) B: 7.85-7.30(m, 7H), 6.00(m, 1H), 3.78(q, J=7.1 Hz, 3H), 3.60-3.40(m, 2H), 1.70(d, J=1.1 Hz, 3H), 1.66(d, J=1.1 Hz, 3H), 0.78(t, J=7.1 Hz, 3H), 0.71(t, J=7.3 Hz, 3H) IR: 2955, 1730, 1680, 1620 Mass: 338(M^+, 3), 320(16), 265(5), 255(4), 209(29), 170(19), 141(7), 84(100)

[a]A and B are inseparable diastereomers.

[b]NMR spectra were determined on Jeol FT90 (89.45M Hz), Varian-Gemini (200M Hz), FT-NMR spectrometers, chemical shifts are reported in ppm relative to tetramethylsilane, IR spectra were measured on IR Report-100(Jasco) IR spectrometer and corrected against peak at 1601 cm^{-1} of polystyrene, MASS spectra were obtained on JMX-DX303(Jeol) under standard condition.

I and III, respectively. The spectroscopic data of these compounds are shown in Table II and IV, respectively. The spectroscopic data of intermediates **2**, **3**, and **4** obtained are satisfactory. The essential feature of IR and NMR spectral data are the same as those for the corresponding derivatives described previously (Oh *et al.*, 1992) except for the aromatic region.

Table III. Physical data of compounds **6**.

No.	Compound **5**	Substituents R_1	R_2	molecular formula	overall yield (%)	distillation temperature[a]
1	a	p-OMePh	Me	$C_{15}H_{20}O_2$	33.8	—[b]
2	b	p-OPhPh	Me	$C_{20}H_{22}O_2$	23.3	—[b]
3	c	P-CF3Ph	Me	$C_{15}H_{17}OF_3$	53.6	—[bc]
4	d	p-FPh	Me	$C_{14}H_{17}OF$	56.1	85-95
5	e	p-ClPh	Me	$C_{14}H_{17}OCl$	31.8	—[b]
6	f	m-OMePh	Me	$C_{15}H_{20}O_2$	34.1	—[b]
7	g	m-ClPh	Me	$C_{14}H_{17}OCl$	60.5	84-95
8	h	m-CF3Ph	Me	$C_{15}H_{17}OF_3$	53.6	—[b]
9	i	m-MePh	Me	$C_{15}H_{20}O$	72.9	82-87
10	j	o-OMePh	Me	$C_{15}H_{20}O_2$	33.4	—[b]
11	k	o-OMePh	Et	$C_{16}H_{22}O_2$	21.5	—[b]
12	l	o-ClPh	Me	$C_{14}H_{17}OCl$	60.6	—[b]
13	m	1-naphthyl	Me	$C_{18}H_{20}O$	29.2	—[b]
14	n	1-naphthyl	Et	$C_{19}H_{22}O$	42.1	—[b]
15	o	2-naphthyl	Me	$C_{18}H_{20}O$	35.1	—[b]
16	p	2-naphthyl	Et	$C_{19}H_{22}O$	25.3	—[b]

[a]Distillation were performed with Kugelrohr under reduced pressure (0.5 torr).
[b]Compounds were purified by flash column chromatography.

Bioassay

Cytotoxicity of compounds **5** and **6** were measured against leukemia L1210, HL-60, and K-562 cells *in vitro* using the known method (Thayer *et al.*, 1971). Fisher's medium supplented with horse serum in 10% was used for the proliferation of L1210 cell. RPMI medium enriched with fetal bovine serum in 5% was used for the proliferation of HL-60 and K-562 cells. The results are shown in Table V and VI as mean ED50 values (μg/ml) from three independent experiments.

RESULT AND DISCUSSION

The cytotoxic activity of the compounds **5** and **6** are varied with the tumor cell lines tested (Table V and VI). These analogues exhibit relatively strong activity against HL-60 and K-562 cell lines compared to against L1210 cell. Against HL-60 and K-562 cells, ED 50 values of the analogues are ranged from 0.8 to 30.0 μg/ml. Against L1210 cell, these are located more than 20.0 μg/ml with the exception of ED_{50} value (6.5 μg/ml) for compound **5n**. Compound **5n** possesses the most potent activity against all three different cell lines. This level of biological activity of **5n** indicates the necessity of the further biological test of this compound for the development and the high potential of this series to find the potent analogues.

For the investigation of the electronic and structural nature of phenyl ring and the conformation between the aromatic portion and the side chain, the various substituents were introduced at the *ortho, meta,* or *para* position of phenyl ring of ar-turmerone analogues (Fig. 1). The cytotoxicity of compounds (**5a**, **5b**, **5q**) containing the electron releasing group at para position and compounds (**5c**, **5d**, **5e**) possessing the electron attracting group are nearly same level against HL-60 and K-562 cells (Table V). This trends are also evident for the *ortho* or *meta* substituted analogues. Although compound **5a**, **5b**, **5q** are little more potent than the compounds **5c**, **5d**, **5e** against L1210, the trend observed for the cytotoxicity against HL-60 and K-562 cells remains same as in the ortho or meta substituted analogues. These little variation in the biological activity of derivatives **5** with the electron donating and withdrawing groups also maintains in the series of analogues **6** as shown in Table VI. Therefore the π-electron density on the aromatic ring may not be an important factor for the exhibition of the activity of ar-turmerone analogue. The flat nature in this region of ar-turmerones should be the more essential structural feature for its activity. Introduction of the substituents on the various position of phenyl ring variates the biological activity of the analogues substantially. The *ortho* substituted compounds **5j-l** are more potent than the corresponding meta or para substituted isomers against HL-60 and L1210 cells. This characteristics are also found in the analogues **6**. This enhanced acivity of the *ortho* derivatives may be resulted from the more effective conformation of these analogues compared to those of the *meta* or *para* substituted isomers. The substituents at the *ortho* position restrict the single bond rotation between C-6 and phenyl ring. Therefore the side chain of ar-turmerone analogues should be orthogonally arranged to the plane of aromatic ring. The more potent activity of the ortho isomers indicates that this conformational restriction should enhance the activity

Table IV. Spectral data of compounds **6**.

No.	Compound **5**	Spectral data[a] ^{1}H-NMR (in $CDCl_3$, δ), IR (ν_{max} cm^{-1}), Mass (m/e, rel. int.)
1	a	NMR: 7.25-6.89(m, 4H), 6.05(m, 1H), 3.8(s, 3H), 3.2(m, 1H), 2.60 (m, 2H), 2.13(d, J=1.1 Hz, 3H), 1.88(d, J=1.1 Hz, 3H), 1.25(d, J=7.0 Hz, 3H) IR: 2960, 1895, 1610 Mass: 232(M^+, 30), 148(38), 135(100), 83(89)
2	b	NMR: 7.40-6.80(m, 8H), 6.02(m, 1H), 3.70(m, 1H), 2.65(m, 2H), 2.12(d, J=1.1 Hz, 3H), 1.88(d, J=1.1 Hz, 3H), 1.22(d, J=6.9 Hz, 3H) IR: 2975, 1682, 1504 Mass: 294(M^+, 15), 197(100), 183(4), 83(59)
3	c	NMR: 7.60-7.43(m, 4H), 6.01(m, 1H), 3.39(m, 1H), 2.70(dd, J=6.6, 6.1 Hz, 2H), 2.10(d, J=1.1 Hz, 3H), 1.86(d, J=1.1 Hz, 3H), 1.27(d, J=7.0 Hz, 3H) IR: 2975, 1680, 1618, 1322 Mass: 270(M^+, 7), 255(4), 173(4), 98(5), 83(100)
4	d	NMR: 7.30-6.90(m, 4H), 6.05(m, 1H), 3.35(m, 1H), 2.69(m, 2H), 2.15(d, J=1.1 Hz, 3H), 1.99(d, J=1.1 Hz, 3H), 1.27(d, J=6.9 Hz, 3H) IR: 2960, 1730, 1510
5	e	NMR: 7.20(m, 4H), 6.01(m, 1H), 3.32(m, 1H), 2.63(m, 2H), 2.10(s, 3H), 1.84(s, 3H), 1.24(d, J=6.9 Hz, 3H) IR: 2860, 1730, 1620 Mass: 236(M^+, 7), 221(6), 141(10), 139(31), 83(100)
6	f	NMR: 7.20(m, 2H), 6.77(m, 2H), 6.03(m, 1H), 3.78(s, 3H), 3.30(m, 1H) 3.30(m, 1H), 2.67(m, 2H), 2.11(d, J=1.1 Hz, 3H), 1.86(d, J=1.1 Hz, 3H), 1.23(d, J=6.9 Hz, 3H) IR: 2956, 2830, 1682 Mass: 232(M^+, 29), 149(100), 135(42), 86(26)
7	g	NMR: 7.21(m, 4H), 6.02(m, 1H), 3.63(m, 1H), 2.67(m, 2H), 2.11(d, J=1.1 Hz, 3H), 1.86(d, J=1.1 Hz, 3H), 1.24(d, J=6.9 Hz, 3H) IR: 2930, 1730, 1685 Mass: 236(M^+, 6), 152(25), 139(24), 83(100)
8	h	NMR: 7.45(m, 4H), 6.05(m, 1H), 3.45(m, 1H), 2.75(m, 2H), 2.13(d, J= 1.1 Hz, 3H), 1.87(d, J=1.1 Hz, 3H), 1.31(d, J=6.9 Hz, 3H) IR: 2970, 1735, 1685 Mass: 270(M^+, 11), 255(6), 215(3), 83(100)
9	i	NMR: 7.20-6.85(m, 4H), 5.97(m, 1H), 3.25(m, 1H), 2.70-2.45(m, 2H), 2.04(d, J=1.1 Hz, 3H), 1.79(d, J=1.1 Hz, 3H), 1.18(d, J=6.9 Hz, 3H) IR: 2970, 1730, 1680 Mass: 216(M^+, 13), 132(27), 119(49), 83(100)
10	j	NMR: 7.19(m, 2H), 6.87(m, 2H), 6.09(m, 1H), 3.84(s, 3H), 3.70(m, 1H)2.62(m, 2H), 2.12(d, J=1.1 Hz, 3H), 1.87(d, J=1.1 Hz, 3H), 1.21(d, J=6.6 Hz, 3H) IR: 2960, 1680 Mass: 232(M^+, 100), 217(55), 135(100), 88(100)
11	k	NMR: 7.20-6.75(m, 4H), 6.04(m, 1H), 3.81(s, 3H), 3.51(m, 1H), 2.75-2.65(m, 2H), 2.07 (d, J=1.1 Hz, 3H), 1.65(m, 2H), 0.77(t, J=7.2 Hz, 3H) IR: 2955, 1682, 1620, 1490 Mass: 246(M^+, 29), 217(29), 149(57), 121(28), 91(30), 83(100)
12	l	NMR: 7.35-7.00(m, 4H), 6.02(m, 1H), 3.75(m, 1H), 2.80-2.40(m, 2H), 2.05(d, J=1.1 Hz, 3H), 1.80(d, J=1.1 Hz, 3H), 1.19(d, J=6.9 Hz, 3H) IR: 3060, 2960, 1680, 1618, 1440 Mass: 236(M^+, 5), 152(15), 139(53), 83(100)
13	m	NMR: 8.20-7.35(m, 7H), 6.08(m, 1H), 4.22(m, 1H), 2.82(m, 2H), 2.14(d, J=1.2 Hz, 3H), 1.86(d, J=1.2 Hz, 3H), 1.41(d, J=7.0Hz, 3H) IR: 2975, 1680, 1615, 1440 Mass: 252(M^+, 24), 237(5), 181(3), 169(7), 155(63), 141(5), 83(63)
14	n	NMR: 8.30-7.35(m, 7H), 6.03(m, 1H), 4.11(m, 1H), 2.83(d, J=6.9 Hz, 2H), 2.05(d, J=1.1 Hz, 3H), 1.83(m, 2H), 1.81(d, J=1.1 Hz, 3H), 0.82(t, J=7.3 Hz, 3H) IR: 2955, 1680, 1620, 1440 Mass: 266(M^+, 3), 237(2), 168(9), 141(3), 84(34), 49(100)

Table IV. continued

No.	Compound **5**	Spectral data[a] ^{1}H-NMR (in $CDCl_3$, δ), IR (ν_{max} cm^{-1}), Mass (m/e, rel. int.)
15	o	NMR: 7.84-7.25(m, 7H), 6.03(m, 1H), 3.58-3.40(m, 1H), 2.90-2.60(m, 2H), 2.08(d, J=1.1 Hz, 3H), 1.82(d, J=1.1 Hz, 3H), 1.34(d, J=6.9 Hz, 3H) IR: 2960, 1680, 1570 Mass: 252(M^+, 22), 237(3), 169(23), 156(52), 83(100)
16	p	NMR: 8.30-7.35(m, 7H), 6.03(m, 1H), 4.11(m, 1H), 2.83(d, J=6.9 Hz, 2H), 2.05(d, J=1.1 Hz, 3H), 1.83(m, 2H), 1.81(d, J=1.1 Hz, 3H), 0.82(t, J=7.3 Hz, 3H) IR: 2955, 1680, 1620, 1440 Mass: 266(M^+, 3), 237(2), 168(9), 141(3), 84(34), 49(100)

[a]Spectra were obtained as described footnote b under Table 2.

Table V. Cytotoxicity of compounds **5**.

No.	Compound **5**	Substituents R_1	R_2	ED_{50} (μg/ml) HL-60	K-562	L1210
1	a	p-OMePh	Me	10.3	17.2	43.0
2	b	p-OPhPh	Me	4.7	6.9	24.2
3	c	p-CF_3Ph	Me	9.3	9.3	>100
4	d	p-FPh	Me	14.3	7.1	>100
5	e	p-ClPh	Me	8.0	7.3	>100
6	f	m-OMePh	Me	10.8	11.8	40.0
7	g	m-ClPh	Me	11.0	15.0	>100
8	h	m-CF3Ph	Me	11.6	7.4	61.5
9	i	m-MePh	Me	12.2	7.7	67.0
10	j	o-OMePh	Me	8.8	7.2	50.0
11	k	o-OMePh	Et	3.7	9.7	29.7
12	l	o-ClPh	Me	6.0	8.5	45.5
13	m	1-naphthyl	Me	7.8	3.5	10.0
14	n	1-naphthyl	Et	0.8	2.1	6.5
15	o	2-naphthyl	Me	9.6	4.2	33.6
16	p	2-naphthyl	Et	6.4	6.6	50.0
17	q[a]	p-MePh	Me	8.8	9.7	30.0
18		5-Fluorouracil		0.05	4.2	0.1

[a]Compound **5q** was prepared as described previously (Oh *et al.*, 1992).

Table VI. Cytotoxicity of compounds **6**.

No.	Compound **5**	Substituents R_1	R_2	ED_{50} (μg/ml) HL-60	K-562	L1210
1	a	p-OMePh	Me	18.2	20.0	>100
2	b	p-OPhPh	Me	20.4	28.0	>100
3	c	P-CF3Ph	Me	18.6	>100	>100
4	d	p-FPh	Me	18.0	20.8	>100
5	e	p-ClPh	Me	9.4	11.5	>100
6	f	m-OMePh	Me	15.7	28.0	>100
7	g	m-ClPh	Me	17.5	30.0	>100
8	h	m-CF3Ph	Me	11.4	11.5	>100
9	i	m-MePh	Me	11.3	15.3	92.0
10	j	o-OMePh	Me	9.5	13.7	>100
11	k	o-OmePh	Et	6.0	12.0	25.5
12	l	o-ClPh	Me	11.0	17.5	58.7
13	m	1-naphthyl	Me	0.8	9.6	>100
14	n	1-naphthyl	Et	3.1	8.3	50.0
15	o	2-naphthyl	Me	8.1	1.5	>100
16	p	2-naphthyl	Et	7.2	11.9	69.0
17	q[a]	p-MePh	Me	18.0	20.1	50.4
18		5-Fluorouracil		0.05	4.2	0.1

[a]Compound **6q** (synthesized ar-turmerone) was prepared as described previously (Oh *et al.*, 1992).

of the analogues. Such a conformation should be more effective as the size of substituents R1 of ar-turmerone is increased from methyl to ethyl group like **5k** and **6k**. Accordingly the cytotoxicity of **5k** and **6k** is enhanced compared to those of the corresponding methoxy substituted analogues, **5a**, **5f**, **5j**, and **6a**, **6f**, **6j**. The essential feature of this effective conformation for the enhancement of activity in the series becomes more obvious considering the cytotoxicity of naphthyl analogues. Compounds with the side chain connected to one position of naphthalene such as **5n**, **6m**, **6n** are much more potent than the corresponding isomers with the side chain at 2-position like **5o**, **5p**, **6o**, **6p**. Analogues containing 1-naphthyl should have the same effect on the conformation as the *ortho* substituted phenyl derivatives due to the second aromatic ring fusion. The potentiation of cytotoxicity of these naphthalene analogues indicates that the size of the flat area of these analogues may be also related to the variation of antineoplastic activity.

In conclusion, the flat structure of aromatic portion of ar-turmerone analogues is the important factor rather than its electronic nature. This may be an implication for the possibility of intercalation of aromatic portion to bind with the biomacromolecules (Silverman, 1992). This might be supported by the enhancement of cytotoxicity as the enlargement of the aromatic ring size of ar-turmerone analogues. This binding property should increase the effectiveness of the putative bioalkylation (Lien and Li, 1985) of this series to exert the more potent biological effect. The other essential feature for the enhancement of the cytotoxicity of these analogues is the conformational factor between the aromatic area and the side chain. The restriction of the single bond rotation between C-6 and aromatic ring through the introduction of substituents at the *ortho* position of phenyl ring and the increment of size of alkyl group at C-6 position enhance the activity. Therefore the effective conformation should be the one having the orthogonal arrangement between the aromatic ring and the side chain.

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