Synthesis and Biological Evaluation of 7-Hydroxy-3,4-diphenyl-1,2-dihydroisoquinolines as New 4-Hydroxytamoxifen Analogues

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A phenolic 3,4-diphenyl-1,2-dihydroisoquinoline derivative (4a) as a new 4-hydroxytamoxifen analogue and a related compound (4c) were synthesized from 3,4-diphenyl-1,2,3,4-tetrahydroisoquinolin-4-ols (5a, c), which were prepared by intramolecular Barbier reaction of *N*-(2-iodobenzyl)phenacylamines. Anti-proliferative activities of 4a,c and 5a,c, as well as 4b and 5b prepared previously, against human mammary carcinoma MCF-7 cell line and human nasopharyngeal carcinoma KB cell line were evaluated. The 3,4-diphenyl-1,2-dihydroisoquinoline derivatives (4a, c) and isoquinolin-4-ols (5a, b) were active against MCF-7 cells and were nearly equipotent to the corresponding nonphenolic compound (1a). The mechanism of the anti-proliferative activity of 4a—c against MCF-7 cells is discussed.

Key words 1,2-dihydroisoquinoline; tamoxifen analogue; anti-proliferative activity; MCF-7 cell

Tamoxifen is an effective antiestrogenic drug for the treatment of hormone-dependent advanced breast cancer.[1,2]

In the previous paper,[3] we reported the synthesis and evaluation of the anti-proliferative activity against MCF-7 cells of 3,4-diphenyl-1,2-dihydroisoquinolines (**1a—c**) as new tamoxifen analogues, in addition to 3,4-diphenyl-1,2,3,4-tetrahydroisoquinolin-4-ols (**2a—c**) and 3,4-diphenyl-1,2,3,4-tetrahydroisoquinolines (**3a—c**) as analogues of ethamoxytriphetol[4] and centchroman.[5] Since hydroxylated metabolites, such as 4-hydroxytamoxifen, have much greater potency *in vitro* than the parent drug,[6–9] we expected that 7-hydroxy-3,4-diphenyl-1,2,-dihydroisoquinoline (**4a**) would exhibit more potent anti-proliferative activity than **1a**. Thus, we synthesized the monophenolic compound (**4a**) and a diphenolic compound (**4c**) as new 4-hydroxytamoxifen analogues. Further, we evaluated the anti-proliferative activity against MCF-7 cells and human nasopharyngeal carcinoma KB cells of these compounds, the related compounds (**5a—d**) and the isomeric compounds (**4b** and **5b**) reported previously.[3]

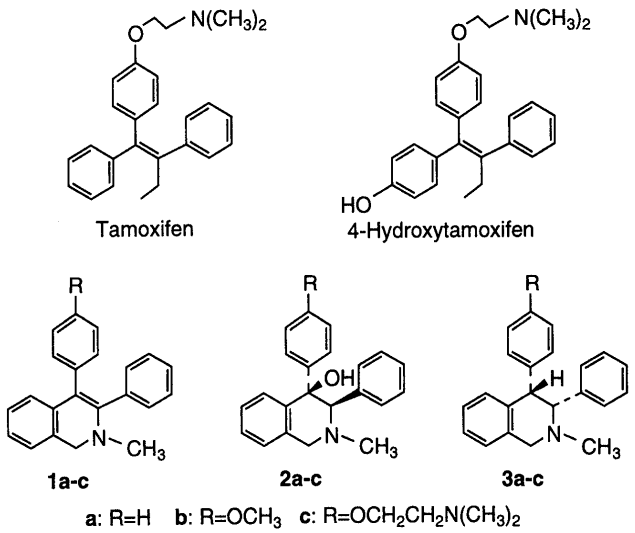

Chart 1

Chemistry

The monophenolic 7-hydroxy-1,2-dihydroisoquinoline derivative (**4a**), a 4-hydroxytamoxifen analogue, was synthesized as shown in Chart 3. 7-(*tert*-Butyldimethylsilyloxy)-1,2-dihydro-3,4-diphenylisoquinolin-4-ol (**5d**) was prepared as a key intermediate by intramolecular Barbier reaction with *n*-BuLi (developed by us[10,11]) of the *O*-silylated phenacylamine (**6**) in 66% yield. The phenacylamine (**6**) was synthesized by condensation of α-bromobenzyl phenyl ketone (**13**) and 5-(*tert*-butyldimethylsilyloxy)-2-iodo-*N*-methylbenzylamine (**12**), which was derived from 3-hydroxybenzaldehyde (**7**) in five steps (Chart 3). The isoquinolin-4-ol (**5d**) thus obtained was deprotected with tetrabutylammonium fluoride (TBAF) to give a phenolic isoquinolin-4-ol (**5a**). Finally, dehydration of **5a** with CF_3SO_3H afforded the monophenolic 3,4-diphenylisoquinoline (**4a**) in a low yield. Thus, the direct dehydration and deprotection of **5d** with CF_3SO_3H gave **4a** in 24% yield. The synthesis of the isomeric compounds (**4b** and **5b**) of **4a** and **5a** was reported previously.[3]

The diphenolic 1,2-dihydro-3,4-diphenylisoquinoline (**4c**) was prepared in a similar manner to the synthesis of

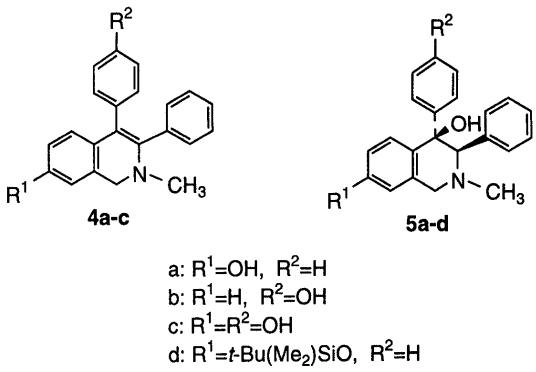

Chart 2

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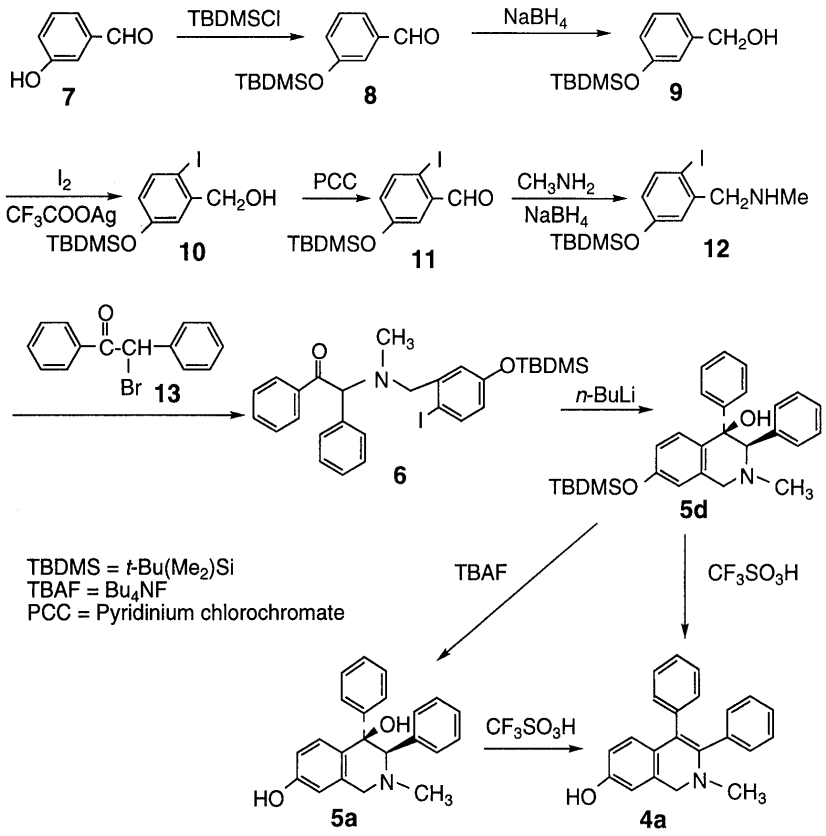

Chart 3

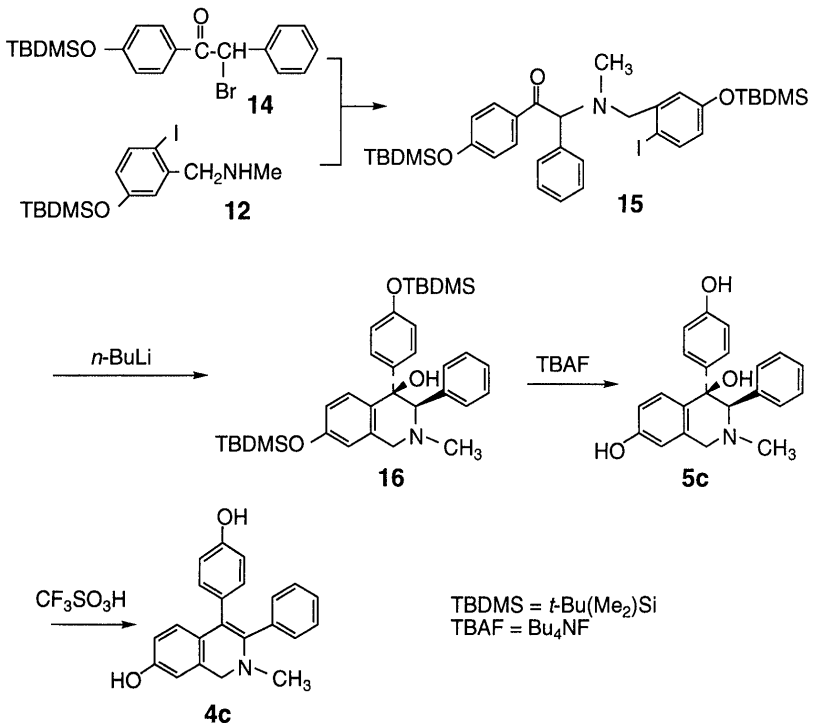

Chart 4

4a, as shown in Chart 4. The key intermediate (**16**), possessing two protected hydroxy groups, was synthesized by intramolecular Barbier reaction of the phenacylamine (**15**) with *n*-BuLi, which was prepared from a bromobenzyl phenyl ketone (**14**) and the benzylamine (**12**) in good yield. The silyl group of **16** was deprotected with TBAF to give the isoquinolin-4-ol (**5c**), and then dehydration of **5c** with CF_3SO_3H afforded the diphenolic 1,2-dihydro-3,4-diphenylisoquinoline (**4c**).

Results and Discussion

The phenolic 1,2-dihydro-3,4-diphenylisoquinolines (**4a—c**) and 3,4-diphenyl-1,2,3,4-tetrahydroisoquinolin-4-ols (**5a—d**) prepared in this study or the previous study[3)]

Table 1. Anti-proliferative Activity of Phenolic 1,2-Dihydroisoquinolines (**4a—c**) and Related Compounds (**5a—d**) against Two Cell Lines

Compound	IC_{50} value (μg/ml)	
	KB cells	MCF-7 cells
1a[a]	3.5	0.94
2a[a]	9.5	>10
3a[a]	>10	>10
4a	>10	1.7
4b	1.2	1.3
4c	>10	5.4
5a	>10	1.9
5b	>10	2.4
5c	>10	>10
5d	>10	>10
Tamoxifen	6.4	0.24
4-Hydroxytamoxifen	1.5	0.040

a) Reference 7.

were tested for anti-proliferative activities against human mammary carcinoma MCF-7 cells and human nasopharyngeal carcinoma KB cells according to the method reported by Saotome *et al.*[12] The results are summarized in Table 1. Of the compounds tested, the 1,2-dihydroisoquinolines (**4a—c**) and the isoquinolin-4-ols (**5a, b**), but not **5c, d**, were active against MCF-7 cells, although these compounds were less active than tamoxifen or 4-hydroxytamoxifen. The 1,2-dihydroisoquinolines (**4a, b**) were nearly equipotent to the isoquinolin-4-ols (**5a, b**). The isoquinolin-4-ol (**2a**)[3] without a phenol group and the isoquinolin-4-ol (**5d**) with a protected phenolic group showed no activity. On the other hand, the phenolic isoquinolin-4-ol (**5a**) showed activity. Furthermore, the 7-hydroxy-1,2-dihydroisoquinoline analogue (**4a**) was equipotent to the isomeric 4-(4-hydroxyphenyl)-1,2-dihydroisoquinoline (**4b**). These findings suggest that the olefinic structure and the location of the phenol group in the phenolic compounds (**4a—c** and **5a—c**) studied in this paper may not be critical for anti-proliferative activity against MCF-7 cells. The diphenolic compounds (**4c** and **5c**) were less active than the monophenolic analogues (**4a, b** and **5a, b**). Only the monophenolic compound (**4b**) inhibited the growth of nonestrogenic KB cells, as 4-hydroxytamoxifen did.

Since 4-hydroxytamoxifen has a higher affinity for ER-receptors than tamoxifen,[6,7] it was expected that the 7-hydroxy-1,2-dihydroisoquinoline (**4a**) corresponding to 4-hydroxytamoxifen would be more active against MCF-7 cells than the prototype compound (**1a**). However, **4a** was found to be nearly equipotent to **1a** [IC_{50} (μg/ml) 0.94].[3] These facts suggest that the 1,2-dihydroisoquinolines (**4a—c**) may have no affinity for the estrogen receptor. In order to test this, we carried out binding tests of **4a—c**, as well as **1a**, to the estrogen receptor, using rat uterine cytosol according to the method reported by Ogawa *et al.*[13] and confirmed that **4a—c** show no affinity for the estrogen receptor.

A number of studies have suggested that there are other macromolecules to which tamoxifen and related compounds bind, in addition to the estrogen receptor,[14] and some of them probably contribute to the anti-proliferative activity.[15,16] In particular, the growth-inhibitory action of triphenylethylene antiestrogens on human breast cancer cell lines has been suggested to correlate with inhibition of the calcium-dependent regulatory protein calmodulin,[17,18] and possibly with other components of the second messenger pathway, such as protein kinase C.[19,20] From the results of these studies and the finding of a lack of affinity of **1a** and **4a—c** for the estrogen receptor in the present study, we consider that the anti-proliferative activities of **1a** and **4a—c** against MCF-7 cell line may depend on some mechanism(s) other than anti-estrogenic effect.

Experimental

Chemistry All melting points are given as uncorrected values. IR spectra were taken with a Perkin–Elmer 1720 infrared Fourier transform spectrometer. High-resolution mass spectra (MS) were recorded on a JEOL JMS-D 300 spectrometer. ^{1}H-NMR spectra were recorded on a JEOL JNM-FX 200 spectrometer with tetramethylsilane as a standard.

3-*tert*-Butyldimethylsilyloxybenzaldehyde (8) A solution of 3-hydroxybenzaldehyde (**7**) (6.459 g, 52.89 mmol), *tert*-butyldimethylsilyl chloride (11.959 g, 79.34 mmol), and imidazole (7.944 g, 132.23 mmol) in dry CH_2Cl_2 (200 ml) was stirred at room temperature for 2 h. The precipitates formed were filtered off, and the filtrate was evaporated *in vacuo* to give an oil (14.890 g). The crude product was purified by flash chromatography on SiO_2 with *n*-hexane–CH_2Cl_2 (1 : 1) to afford **8** as a pale yellow oil (11.936 g, 96%). ^{1}H-NMR ($CDCl_3$) δ: 9.92 (1H, s), 7.49 (1H, d, J=7.6 Hz), 7.40 (1H, t, J=7.6 Hz), 7.33 (1H, br s), 7.11 (1H, ddd, J=7.8, 1.5, 1.5 Hz), 1.00 (9H, d, J=0.7 Hz), 0.23 (6H, d, J=0.7 Hz). IR (film): 2931, 2859, 1704, 1388 cm^{-1}. MS Calcd for $C_{13}H_{20}O_2Si$ (M^+): 236.1233. Found: 236.1251.

3-*tert*-Butyldimethylsilyloxybenzyl Alcohol (9) $NaBH_4$ (3.08 g, 73.17 mmol) was added to a solution of **8** (11.531 g, 48.78 mmol) in MeOH (50 ml) under ice-cooling. The mixture was stirred at 0 °C for 10 min and evaporated *in vacuo*. H_2O (100 ml) was added to the residue, and the mixture was extracted with ether (100 ml × 3). The extract was washed with brine (100 ml), dried over $MgSO_4$, and evaporated *in vacuo* to give a colorless oil (11.284 g). This was purified by flash chromatography on SiO_2 with CH_2Cl_2–acetone (1 : 1) to give **9** as a colorless oil (11.379 g, 98%). ^{1}H-NMR ($CDCl_3$) δ: 7.20 (1H, t, J=7.8 Hz), 6.29 (1H, d, J=7.8 Hz), 6.83 (1H, s), 6.74 (1H, dd, J=8.0, 2.8 Hz), 4.60 (2H, d, J=5.9 Hz), 2.01 (1H, br s), 0.98 (9H, d, J=1.0 Hz), 0.19 (6H, d, J=1.0 Hz). IR (film): 3331, 2931, 2859, 1278 cm^{-1}. MS Calcd for $C_{13}H_{22}O_2Si$ (M^+): 238.1389. Found: 238.1380.

5-*tert*-Butyldimethylsilyloxy-2-iodobenzyl Alcohol (10) A solution of iodine (10.872 g, 42.84 mmol) in CH_2Cl_2 (250 ml) was added to a mixture of **9** (10.213 g, 42.84 mmol) and CF_3COOAg (9.462 g, 42.84 mmol) in CH_2Cl_2 (50 ml) for 10 min. The mixture was stirred at room temperature for 5 min and filtered. The filtrate was washed with a saturated solution of $Na_2S_2O_4$ in H_2O and a saturated solution of K_2CO_3 in H_2O, successively. The CH_2Cl_2 solution was dried over $MgSO_4$ and evaporated *in vacuo* to give an oil (14.458 g). This was purified by flash chromatography on SiO_2 with *n*-hexane–CH_2Cl_2 (1 : 1) to afford **10** as a pale yellow oil (12.122 g, 78%). ^{1}H-NMR ($CDCl_3$) δ: 7.61 (1H, d, J=8.6 Hz), 6.98 (1H, d, J=2.9 Hz), 6.52 (1H, dd, J=8.3, 2.9 Hz), 4.58 (2H, d, J=6.1 Hz), 2.15 (1H, t, J=6.4 Hz), 0.97 (9H, s), 0.19 (6H, s). IR (film): 3348, 2930, 2858, 1289 cm^{-1}. MS Calcd for $C_{13}H_{21}IO_2Si$ (M^+): 364.0356. Found: 364.0336.

5-*tert*-Butyldimethylsilyloxy-2-iodobenzaldehyde (11) Pyridinium chlorochromate (8.620 g, 39.99 mmol) was added to a solution of **10** (9.712 g, 26.66 mmol) in CH_2Cl_2 (150 ml) under ice-cooling. The mixture was stirred at room temperature for 2.5 h. The reaction mixture was passed through the column packed with Florisil and eluted with ether (100 ml). The eluate was evaporated *in vacuo* to give **11** as a yellow oil (9.241 g, 96%). ^{1}H-NMR ($CDCl_3$) δ: 9.97 (1H, s), 7.76 (1H, d, J=7.8 Hz), 7.35 (1H, d, J=2.5 Hz), 6.82 (1H, dd, J=8.6, 2.9 Hz), 0.97 (9H, s), 0.20 (6H, s). IR (film): 2930, 2858, 1697, 1358 cm^{-1}. MS Calcd for $C_{13}H_{19}IO_2Si$ (M^+): 362.0201. Found: 362.0176.

5-*tert*-Butyldimethylsilyloxy-2-iodo-*N*-methylbenzylamine (12) CH_3-NH_2 (0.25 ml of 40% solution in MeOH, 3.24 mmol) was added to a solution of **11** (0.588 g, 1.62 mmol) in MeOH (5 ml). The mixture was

refluxed for 1 h, then 2 N HCl–MeOH (1.62 ml, 3.24 mmol) and $NaBH_4$ (0.102 g, 2.44 mmol) were added. The reaction mixture was stirred at room temperature for 2 h and then evaporated *in vacuo*. A saturated solution (30 ml) of K_2CO_3 in H_2O was added to the residue, and the mixture was extracted with ether. The extract was dried over $MgSO_4$ and evaporated to give a brown oil (0.482 g). This was purified by flash chromatography on SiO_2 with acetone–CH_2Cl_2 (1 : 5) to afford **12** as a pale yellow oil (0.258 g, 42%). ^{1}H-NMR ($CDCl_3$) δ: 7.63 (1H, dd, J=8.6, 1.0 Hz), 6.90 (1H, d, J=2.7 Hz), 6.50 (1H, dd, J=8.3, 2.9 Hz), 3.72 (2H, s), 2.50 (1H, br s), 2.54 (3H, s), 0.97 (9H, d, J=1.2 Hz), 0.19 (6H, d, J=1.2 Hz). IR (film): 2931, 2792, 1288, 1171 cm^{-1}. MS Calcd for $C_{14}H_{24}INOSi$ (M^+): 377.0673. Found: 377.0689.

***N*-(5-*tert*-Butyldimethylsilyloxy-2-iodobenzyl)-*N*-methyl-α-phenylphenacylamine (6)** A solution of **12** (3.423 g, 9.07 mmol) and propylene oxide (1.58 g, 27.2 mmol) in dioxane (20 ml) was added to a solution of α-phenylphenacyl bromide (**13**)[21] (2.496 g, 9.07 mmol) in dioxane (20 ml). The mixture was refluxed for 2 h, and then evaporated. The crude product was purified by flash chromatography on SiO_2 with *n*-hexane–ethyl acetate (10 : 1) to give **6** as a pale yellow oil (4.392 g, 85%). ^{1}H-NMR ($CDCl_3$) δ: 7.97 (2H, d, J=8.1 Hz), 7.60 (1H, dd, J=8.6, 0.7 Hz), 7.53—7.26 (8H, m), 7.11 (1H, d, J=2.9 Hz), 6.49 (1H, dd, J=8.3, 2.7 Hz), 5.41 (1H, s), 3.76 (1H, d, J=14.4 Hz), 3.62 (1H, d, J=14.4 Hz), 2.33 (3H, s), 0.98 (9H, s), 0.19 (6H, s). IR (film): 3061, 2931,2859, 1690, 1287, 1162 cm^{-1}. MS Calcd for $C_{28}H_{33}INO_2Si$ (M−1): 570.1327. Found: 570.1356.

7-(*tert*-Butyldimethylsilyloxy)-2-methyl-3,4-diphenyl-1,2,3,4-tetrahydroisoquinolin-4-ol (5d) *n*-BuLi (7.7 ml of 1.6 M solution in *n*-hexane, 12.29 mmol) was added to a solution of **6** (4.392 g, 7.68 mmol) in dry THF (20 ml) under N_2 at −78 °C. The mixture was stirred at −78 °C for 10 min. H_2O (100 ml) was added and the mixture was extracted with ether (100 ml × 3). The extract was dried over $MgSO_4$ and evaporated *in vacuo* to give a pale brown amorphous solid (4.862 g). This was purified by flash chromatography on SiO_2 with CH_2Cl_2–acetone (30 : 1) to afford **5d** as colorless cubes (2.248 g, 66%); mp 181 °C (from *n*-hexane). ^{1}H-NMR ($CDCl_3$) δ: 7.15—7.01 (10H, m), 6.78 (1H, d, J=9.3 Hz), 6.62—6.59 (2H, m), 4.07 (1H, d, J=15.6 Hz), 3.71 (1H, d, J=15.6 Hz), 3.62 (1H, s), 3.28 (1H, br s), 2.21 (3H, s), 0.98 (9H, s), 0.20 (6H, s). IR (KBr): 3029, 2931, 2858, 1256, 1162 cm^{-1}. MS Calcd for $C_{28}H_{35}NO_2Si$ (M^+): 445.2436. Found: 445.2424. *Anal.* Calcd for $C_{28}H_{35}NO_2Si$: C, 75.46; H, 7.92; N, 3.14. Found: C, 75.17; H, 8.05; N, 3.08.

7-Hydroxy-2-methyl-3,4-diphenyl-1,2,3,4-tetrahydroisoquinolin-4-ol (5a) TBAF (0.67 ml of 1.0 M solution in THF, 0.67 mmol) was added to a solution of **5d** (150 mg, 0.34 mmol) in THF (3 ml) under ice-cooling and the mixture was stirred for 30 min. H_2O (20 ml) was added and the mixture was extracted with CH_2Cl_2 (30 ml × 3). The extract was dried over $MgSO_4$ and evaporated *in vacuo* to give white crystals (131 mg). The crude product was subjected to flash chromatography on SiO_2 with CH_2Cl_2–acetone (3 : 2) to afford **5a** as colorless cubes (100 mg, 90%), mp 217—218 °C (from *n*-hexane–MeOH). ^{1}H-NMR (acetone-d_6) δ: 8.15 (1H, s), 7.13—7.04 (10H, m), 6.99—6.58 (3H, m), 4.24 (1H, s), 3.98 (1H, d, J=15.6 Hz), 3.65 (1H, d, J=15.6 Hz), 3.65 (1H, s), 2.12 (3H, s). IR (KBr): 3320, 1494, 1446 cm^{-1}. MS Calcd for $C_{22}H_{21}NO_2$ (M^+): 331.1572. Found: 331.1553. *Anal.* Calcd for $C_{22}H_{21}NO_2 \cdot 1/8H_2O$: C, 79.19; H, 6.42; N, 4.20. Found: C, 79.04; H, 6.43; N, 4.01.

7-Hydroxy-2-methyl-3,4-diphenyl-1,2-dihydroisoquinoline (4a) From **5a**: CF_3SO_3H (0.3 ml, 3.5 mmol) was added to a solution of **5a** (233 mg, 0.70 mmol) in dry benzene (5 ml) under N_2 and the mixture was refluxed for 30 min. H_2O (30 ml) was added, and the whole was made basic with NH_4OH and extracted with CH_2Cl_2. The extract was dried over $MgSO_4$ and evaporated *in vacuo* to give brown crystals (0.198 g). The crude product was purified by flash chromatography on SiO_2 with CH_2Cl_2–acetone (20 : 1) to afford **4a** as an amorphous solid (27 mg, 13%). ^{1}H-NMR ($CDCl_3$) δ: 7.28—6.96 (11H, m), 6.69 (1H, s), 6.55 (1H, d, J=8.3 Hz), 4.25 (2H, s), 2.47 (3H, s). IR (KBr): 3054, 2800, 1062 cm^{-1}. MS Calcd for $C_{22}H_{19}NO$ (M^+): 313.1466. Found: 313.1448. This free base was crystallized as the hydrochloride from 2 N HCl–MeOH as yellow crystals, mp 253—255 °C (dec.). *Anal.* Calcd for $C_{22}H_{19}NO \cdot HCl \cdot 1/2H_2O$: C, 73.63; H, 5.90; N, 3.90. Found: C, 73.68; H, 5.66; N, 3.78.

From **5d**: CF_3SO_3H (0.38 ml, 4.24 mmol) was added to a solution of **5d** (378 mg, 0.85 mmol) in dry benzene (10 ml) under N_2 and the mixture was refluxed for 30 min. The reaction mixture was treated in the same way as above to give **4a** as an amorphous solid (65 mg, 24%). This compound **4a** was identical with **4a** obtained from **5a** as above, based on a comparison of their ^{1}H-NMR spectra.

***N*-(5-*tert*-Butyldimethylsilyloxy-2-iodobenzyl)-*N*-methyl-α-phenyl-4-(*tert*-butyldimethylsilyloxy)phenacylamine (15)** The phenacylamine (**15**) was prepared in the same way as **6**. Reaction of **12** (1.823 g, 4.83 mmol) in dioxane (10 ml) with α-phenylphenacyl bromide (**14**)[3] (1.959 g, 4.83 mmol) in dioxane (10 ml) in the presence of propylene oxide (0.84 g, 14.5 mmol) gave **15** as a pale yellow oil (3.266 g, 96%). ^{1}H-NMR ($CDCl_3$) δ: 7.91 (2H, d, J=8.8 Hz), 7.59 (1H, d, J=8.6 Hz), 7.67—7.42 (2H, m), 7.34—7.25 (3H, m), 7.12 (1H, d, J=2.9 Hz), 6.79 (2H, d, J=8.8 Hz), 6.48 (1H, dd, J=8.5, 2.9 Hz), 5.35 (1H, s), 3.75 (1H, d, J=14.7 Hz), 3.60 (1H, d, J=14.7 Hz), 2.31 (3H, s), 0.98 (9H, d, J=0.7 Hz), 0.96 (9H, d, J=0.7 Hz), 0.20 (6H, s), 0.19 (6H, s). IR (film): 3061, 2934, 2859, 1682, 1256, 1162, 912 cm^{-1}. MS Calcd for $C_{34}H_{48}INO_3Si_2$ (M^+): 701.2219. Found: 701.2264.

7-(*tert*-Butyldimethylsilyloxy)-4-(4-*tert*-butyldimethylsilyl-oxyphenyl)-2-methyl-3-phenyl-1,2,3,4-tetrahydroisoquinolin-4-ol (16) The isoquinolin-4-ol (**16**) was prepared in the same way as **5d**. Reaction of **15** (0.600 g, 0.85 mmol) with *n*-BuLi (0.85 ml of 1.6 M solution in *n*-hexane, 1.36 mmol) in dry tetrahydrofluran (THF) (5 ml) under N_2 at −78 °C gave a brown oil (0.509 g). This was purified by flash chromatography on SiO_2 with *n*-hexane–ethyl acetate (5 : 1) to afford **16** as a pale yellow amorphous solid (0.300 g, 73%). ^{1}H-NMR ($CDCl_3$) δ: 7.11—7.03 (3H, m), 6.97 (2H, d, J=6.4 Hz), 6.87—6.79 (3H, m), 6.62—6.54 (4H, m), 4.05 (1H, d, J=15.6 Hz), 3.67 (1H, d, J=15.6 Hz), 3.53 (1H, s), 3.21 (1H, br s), 2.20 (3H, s), 0.98 (9H, d, J=1.0 Hz), 0.94 (9H, d, J=0.7 Hz), 0.19 (6H, d, J=1.0 Hz), 0.13 (6H, d, J=0.7 Hz). IR (KBr): 3340, 3029, 2857, 1251, 1165, 915 cm^{-1}. MS Calcd for $C_{28}H_{49}NO_3Si_2$ (M^+): 575.3250. Found: 575.3231.

7-Hydroxy-4-(4-hydroxyphenyl)-2-methyl-3-phenyl-1,2,3,4-tetrahydroisoquinolin-4-ol (5c) The isoquinolin-4-ol (**5c**) was prepared in the same way as **5a**. Reaction of **16** (493 mg, 0.86 mmol) in THF (10 ml) with TBAF (2.6 ml of 1.0 M sol. in THF, 2.6 mmol) gave **5c** as a pale brown amorphous solid (284 mg, 95%). ^{1}H-NMR (MeOH-d_4) δ: 7.14—7.03 (5H, m), 6.79—6.74 (3H, m), 6.62—6.57 (2H, m), 6.50 (2H, dt, J=8.0, 2.2 Hz), 4.06 (1H, d, J=15.4 Hz), 3.65 (1H, d, J=15.4 Hz), 3.54 (1H, s), 2.14 (3H, s). IR (KBr): 3384, 1514, 1455, 1170 cm^{-1}. MS Calcd for $C_{22}H_{21}NO_3$ (M^+): 347.1521. Found: 347.1504.

7-Hydroxy-4-(4-hydroxyphenyl)-2-methyl-3-phenyl-1,2-dihydroisoquinoline (4c) The 1,2-dihydroisoquinoline (**4c**) was prepared in the same way as **4a**. Reaction of **5c** (57 mg, 0.16 mmol) with CF_3SO_3H (0.14 ml, 1.63 mmol) in dry benzene (3 ml) gave a brown oil (89 mg). The crude product was purified by column chromatography on SiO_2 with CH_2Cl_2–acetone (1 : 1) to afford **4a** as a pale brown oil (39 mg, 73%). ^{1}H-NMR (MeOH-d_4) δ: 7.47—6.91 (5H, m), 6.81—6.70 (3H, m), 6.60—6.45 (4H, m), 4.20 (2H, s), 2.43 (3H, s). IR (KBr) (HCl salt): 3057, 1612, 1519, 1445, 1410, 1238 cm^{-1}. MS Calcd for $C_{22}H_{18}NO_2$ (M−1): 328.1337. Found: 328.1308.

Growth-Inhibitory Activity Assay Anti-proliferative activities of the compounds prepared in this study against MCF-7 and KB cells were evaluated by the method reported by Saotome *et al.*[12] These cell lines were grown in RPMI 1640 medium, supplemented with 10% FBS, Kanamycin (100 mg/ml) and L-glutamine (2 mM). The cells were maintained in exponential-phase cultures at 37 °C in 5% CO_2 with 100% relative humidity and harvested using Ca^{2+}/Mg^{2+}-free phosphate-buffered saline from the culture bottles. Two thousand cells/well of MCF-7 cells or 1500 cells/well of KB cells were seeded into 96-well plates (Becton Dickinson, Lincoln Park, NJ) and incubated overnight for reattachment. Compounds were dissolved in dimethyl sulfoxide (Sigma, St. Louis, MO), diluted with medium and applied at the appropriate concentrations. The final concentration of dimethyl sulfoxide (0.1%) had no effect on the cell growth. Five days after drug exposure to MCF-7 cells or three days after drug exposure to KB cells, the plates were fixed with glutaraldehyde and stained with crystal violet. Subsequently, 100 ml of water–ethanol (50 : 50, v/v) was added to each well and the optical density at 540 nm was read on a microplate reader (Bio-Tec Instrument, Winooski, VT) to estimate the cell-growth. The IC_{50} value is the concentration of a test compound, necessary to cause 50% inhibition of the cell growth.

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