Synthesis of 1,1,2-triphenylethylenes and their antiproliferative effect on human cancer cell lines

Lifang Zheng, Qingyi Wei, Bo Zhou, Li Yang and Zhong-Li Liu

Tamoxifen analogs (1–3) and 1,1,2-triphenylethylenes (4–7) have been synthesized by the McMurry coupling reaction. Their antiproliferative effects on MCF-7 human breast-cancer cells, HO-8910 human ovarian-carcinoma cells, and (HL)-60 human promyelocytic-leukemia cells were studied by use of the colorimetric MTT assay or sulphorhodamine B assay. Compounds 2 and 3 exhibited significantly higher antiproliferative activity on all the three cell lines, and compound 6 exhibited a remarkably higher antiproliferative activity on HO-8910 and human peripheral blood HL-60 cell lines, than did tamoxifen. Structure–activity relationship analysis demonstrates that the methoxyl group on the 2-phenyl ring contributes critically to the activity. *Anti-Cancer Drugs* **18:1039–1044 © 2007 Lippincott Williams & Wilkins.**

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Introduction

Tamoxifen ((*Z*)-1-[4-(2-dimethylaminoethoxy)phenyl]-1,2-diphenyl-1-butene, TAM) is a well known selective estrogen-receptor modulator (SERM), which has been extensively used in the treatment of hormone-sensitive breast cancers and has become the first-line endocrine therapy for all stages of breast cancer in premenopausal and postmenopausal women [1–3]. TAM has also been reported to be effective in inducing apoptosis of the human ovarian cell line [4] and in regulating the cell cycle of human pancreatic cancers [5]. By contrast, it has been shown that TAM causes an increased incidence of endometrial cancer in women [3,6], and hepatomacellular tumors in rats [7]. Generally, *Z*-tamoxifen derivatives are considered to be antiestrogenic, whereas 1,1,2-triphenylethylenes without the basic side chain are estrogenic [1]. It is also reported, however, that the antiestrogenic/estrogenic activities are species-specific and that some 1,1-bis(4-hydroxyphenyl)-2-phenylethenes and 1,1,2-tris(4-hydroxyphenyl)ethenes without the basic chain are also antiestrogenic [8,9]. Extensive effort has, therefore, been continuously devoted to the synthesis of new TAM analogs, aiming to find effective SERMs without the disadvantages of TAM [8–14]. We report here the synthesis of TAM analogs (**1** and **3**) and 1,1,2-triphenylethylenes (**4–7**) (Fig. 1) by the McMurry coupling reaction, and their in-vitro antiproliferative effect on MCF-7 human breast-cancer cells, HO-8910 human ovarian-carcinoma cells and (HL)-60 human promyelocytic-leukemia cells. Compounds **2** and **3** exhibited significant higher antiproliferative activity on all three cell lines, and compound **6** exhibited remarkably higher antiproliferative activity on the HO-8910 and HL-60 cell lines, than did the parent TAM. Structure–activity relationship (SAR) analysis demonstrates that the methoxyl group on the phenyl ring contributes critically to the activity. The *N*,*N*-dimethylaminoethoxyl basic side chain and the C-2 alkyl chain length also play a role.

Materials and methods

Chemistry

^{1}H- and ^{13}C-nuclear magnetic resonance (NMR) spectra were recorded on a Bruker AM-400 NMR or a Bruker DRX-300 NMR spectrometer (Fällanden, Switzerland). Infrared (IR) spectra were taken on a Nicolet 170SX IR spectrometer (Fällanden, Switzerland). High-resolution mass spectra (HR-MS) were determined on a Bruker APEX II Fourier transform icon cyclotron resonance mass spectrometer (FTICR-MS) (Billerica, Massachussetts, USA) in electrospray ionization (ESI) mode. Electron impact ionization mass spectrometry (EI-MS) were recorded with an HP 5988 A mass spectrometer. Melting points (m.p.) were determined on a Yanagimoto melting-point apparatus and uncorrected. High-performance liquid chromatography (HPLC) analysis was carried out with a Hewlett Packard 1100 system and a diode-array detector. Best separations were achieved by using a Whatman Partisil Octadecylsilyl (ODS) reversed-phase column (10 × 250 mm) and gradient elution with ethanenitrile methyl cyanide (MeCN)–water containing 50 mmol/l of triethylamine and acetic acid (pH 7.4). Compounds **1–3** and **4–7** were ultraviolet (UV) detected at 280 and 254 nm, respectively.

Experimental procedure for the synthesis of 4,4′-dihydroxybenzophenone (8) [15]

Into a flask was charged 10 g of 4-$HOC_6H_4CO_2H$, 6.4 g of PhOH, 21 g of $ZnCl_2$ and 107 g of H_3PO_4, which was produced by mixing 20 parts polyphosphoric acid and

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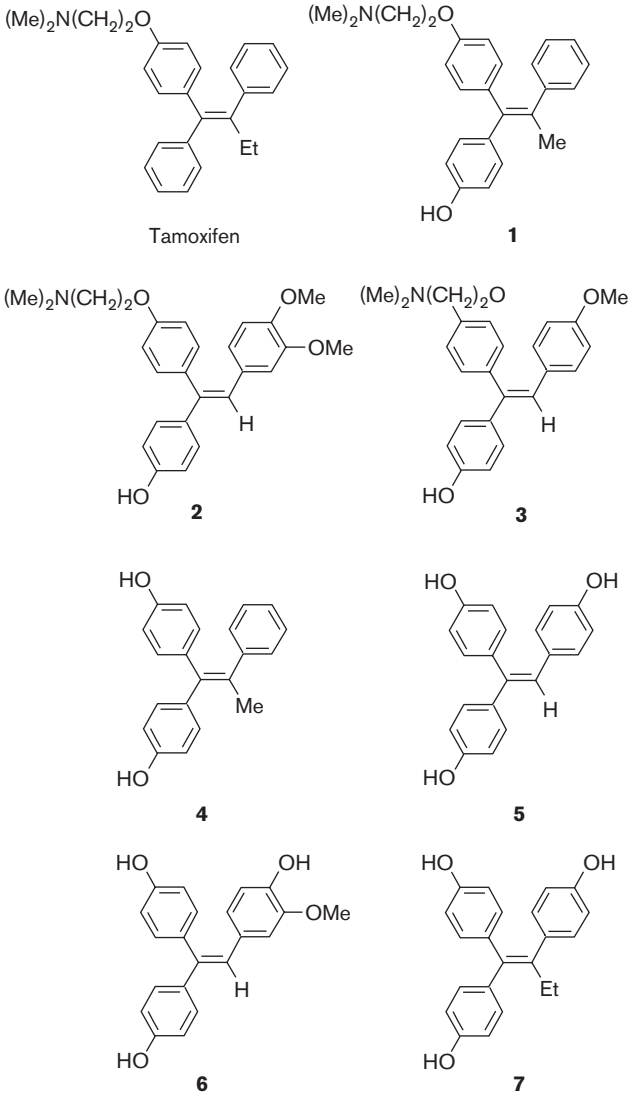
Fig. 1



Molecular structures.

13 parts 85% H_3PO_4. The slurry was heated with stirring to 40°C and 12 g PCl_3 was added in 1.5 h. The mixture was heated slowly to 60°C and held for 16 h, then poured into 200 ml cold H_2O. The precipitate was filtered and washed with cold H_2O, 2.5% $NaHCO_3$ and H_2O, respectively, and dried. The results were as follows. Yield: 13.5 g (91.5%) of a reddish purple color; m.p.: 210°C; ^{1}H-NMR (300 MHz, acetone-d_6): δ6.97 (d, $J = 8.6$ Hz, 4H), 7.70 (d, $J = 8.6$ Hz, 4H) and 9.15 (s, 2H); EI-MS (*m/z*, %): 214 (M^+, 31), 197 (10), 121 (100), 93 (1), 77 (11), and 65 (23).

Experimental procedure for the synthesis of [4-(2-dimethylaminoethoxy)phenyl]-4′-hydroxyphenyl ketone (9) [16]

4,4′-Dihydroxy-benzophenone (**8**, 5.00 *g*) and NaH (2.0 *g*) in tetrahydrofuran (THF; 20 ml) was refluxed for 60 min, then added to freshly distilled dimethylformamide (50 ml), followed by 2-(dimethylamino)ethyl chloride hydrochloride (3.80 g) in solid form. The mixture was further refluxed for 60 min, then cooled to room temperature and filtrated. The mother liquor was extracted with EtOAc and the organic layer was washed with brine, dried over $MgSO_4$, and concentrated *in vacuo*. The product was separated by column chromatography, giving the following results. Yield: 3.15 g (48%) of a white solid; m.p.: 150–152°C; ^{1}H-NMR (300 MHz, CD_3OD): δ2.38 (s, 6H), 2.84 (t, $J = 5.4$ Hz, 2H), 3.31 (t, $J = 5.4$ Hz, 2H), 6.87 (d, $J = 8.8$ Hz, 2H), 7.06 (d, $J = 9.0$ Hz, 2H), 7.67 (d, $J = 8.8$ Hz, 2H) and 7.74 (d, $J = 9.0$ Hz, 2H). EI-MS (*m/z*, %): 285 (M^+, 2), 121 (7), 93 (1) and 58 (100).

General procedure for preparation of compounds 1–7 [14]

$TiCl_4$ (39 mmol) was added dropwise to a stirred suspension of zinc powder (78 mmol) in dry THF (70 ml) under Ar, at −10°C. When the addition was complete, the mixture was warmed to room temperature and then refluxed for 2 h. To the cooled suspension of the titanium reagent was added a solution of [4-(2-dimethyl-aminoethoxy)-phenyl]-4′-hydroxyl-phenyl ketone (**9**) or 4,4′-dihydroxybenzophenone (**8**) (10 mmol) and the appropriate aldehyde or ketone (20 mmol) in dry THF (50 ml) at 0°C, and the mixture was refluxed for 3 h. After being cooled to room temperature, the reaction mixture was quenched with 10% aqueous potassium carbonate (200 ml) and extracted with ethyl acetate (EtOAc; 3 × 200 ml). The organic layer was washed with brine, dried over $MgSO_4$ and concentrated *in vacuo*. The products were separated by column chromatography. Their structures were fully identified with ^{1}H-NMR, ^{13}C-NMR and high resolution electronspray ionization mass spectra (HR-ESI-MS); the data of compounds **4**, **5** and **7** are consistent with those reported in the literature [8,9].

(*Z*)-1-[4-(2-dimethylaminoethoxy)phenyl]-1-(4-hydroxyphenyl)-2-phenylprop-1-ene (1)

Yield: 46% of colorless powder; m.p.: 185°C; HPLC: $t_R = 23.9$ min; ^{1}H-NMR (300 MHz, CD_3OD): δ1.80 (s, 3H), 2.10 (s, 6H), 2.53(t, $J = 6.0$ Hz, 2H), 3.86 (t, $J = 6.0$ Hz, 2H), 6.13 (d, $J = 8.4$ Hz, 2H), 6.38 (d, $J = 8.4$ Hz, 2H), 6.65 (d, $J = 8.7$ Hz, 2H) and 6.77–6.81 (m, 7H); ^{13}C-NMR [75 MHz, dimethylsulfoxide (DMSO)-d_6]: δ23.8, 46.2, 58.4, 66.3, 114.6, 115.0, 126.6, 128.5, 129.7, 131.5, 132.2, 133.8, 134.4, 136.3, 138.9, 144.8, 156.0 and 157.8; IR (KBr, cm^{-1}): 3402, 3041, 2951, 1604 and 1508; HR-ESI-MS (*m/z*): calculated value was for $C_{25}H_{28}NO_2$ (M + H) 374.2115, found 374.2117.

(*Z*,*E*)-1-[4-(2-dimethylaminoethoxy)phenyl]-1-(4-hydroxyphenyl)-2-(3,4-dimethoxyphenyl)ethene (2)

Yield: 39% of pink powder with the *Z*/*E* ratio of 1 : 1. HPLC: $t_R = 17.3$ and 21.0 min respectively; ^{1}H-NMR (300 MHz, $CDCl_3$): δ2.47 (s, 6H), 2.84 (t, $J = 5.4$ Hz,

2H), 3.49 and 3.51 (2 × s, 3H), 3.82 (s, 3H), 4.04 and 4.10 (2 × t, $J = 5.4$ Hz, 2H), 6.46 and 6.52 (2 × s, 1H), 6.60 (d, $J = 8.4$ Hz, 1H), 6.67–6.81 (m, 6H), 7.02–7.08 (m, 2H) and 7.17 (t, $J = 8.7$ Hz, 2H); ^{13}C-NMR (75 MHz, $CDCl_3$): δ 45.1, 45.2, 55.1, 55.7, 57.9, 64.5, 64.8, 110.5, 111.9, 119.8, 114.5, 115.5, 116.1, 122.5, 122.7, 125.2, 128.3, 128,7, 130.9, 131.6, 133.4, 135.3, 136.6, 140.3, 147.5, 147.9, 156.5, 157.7 and 157.9; IR (KBr, cm^{-1}): 3451, 2953, 2833, 1606 and 1511; HR-ESI MS (*m/z*): calculated for $C_{26}H_{30}NO_4$ (M + H) 420.2169, found 420.2175.

(*Z*,*E*)-1-[4-(2-dimethylaminoethoxy)phenyl]-1-(4-hydroxyphenyl)-2-(4-methoxyphenyl)ethene (3)

Yield: 45% of pale yellow powder with the *Z*/*E* ratio of 1 : 1; HPLC: $t_R = 17.3$ and 21.0 min respectively. ^{1}H-NMR (300 MHz; $CDCl_3$): δ2.48 and 2.52 (2 × s, 6H), 2.91 and 2.93 (2 × t, $J = 5.2$ Hz, 2H), 3.76 (s, 3H), 4.09 and 4.13 (2 × t, $J = 5.2$ Hz, 2H), 6.61–6.80 (m, 7H), 6.91–7.05 (m, 4H) and 7.11–7.18 (m, 2H); ^{13}C-NMR (75 MHz, $CDCl_3$): δ45.2, 55.4, 58.0, 64.6, 113.6, 114.1, 114.6, 115.7, 116.3, 122.8, 125.7, 128.8, 129.0, 130.8, 131.8, 133.7, 135.8, 136.9, 137.3, 138.5, 140.2, 142.6, 156.6, 157.9 and 158.1; IR (KBr, cm^{-1}): 3740, 2953, 2833, 1605 and 1510; HR-ESI-MS (*m/z*): calculated for $C_{25}H_{28}NO_3$ (M + H) 390.2060, found 390.2064.

1,1-Bis(4-hydroxyphenyl)-2-phenylprop-1-ene (4)

Yield: 78% of colorless powder, m.p.: 132°C; HPLC: $t_R = 9.7$ min; ^{1}H-NMR (300 MHz, acetone-d_6): δ2.10 (s, 3H), 6.51 (d, $J = 8.7$ Hz, 2H), 6.72 (d, $J = 8.7$ Hz, 2H), 6.85 (d, $J = 8.7$ Hz, 2H), 6.75 (d, $J = 8.7$ Hz, 2H), 7.09–7.16 (m, 5H), 8.11 (s, 1H) and 8.34 (s, 1H); ^{13}C-NMR (75 MHz, acetone-d_6): δ23.1, 114.5, 115.1, 126.1, 128.1, 129.6, 131.4, 132.2, 133.7, 135.2, 135.4, 139.3, 145.1, 155.7 and 156.4; IR (KBr, cm^{-1}): 3359, 3024, 2936, 2855, 1607 and 1509; HR-ESI-MS (*m/z*): calculated for $C_{21}H_{17}O_2$ (M – H) 301.1234, found 301.1240.

1,1,2-Tris(4-hydroxyphenyl)ethene (5)

Yield: 40% of colorless powder, m.p.: 169°C; HPLC: $t_R = 5.5$ min; ^{1}H-NMR (300 MHz, acetone-d_6): δ6.61 (d, $J = 8.7$ Hz, 2H), 6.75 (s, 1H), 6.77 (d, $J = 8.7$ Hz, 2H), 6.85 (d, $J = 8.7$ Hz, 2H), 6.91 (d, $J = 8.7$ Hz, 2H), 6.99 (d, $J = 8.7$ Hz, 2H), 7.15 (d, $J = 8.7$ Hz, 2H), 8.31 (s, 1H), 8.38 (s, 1H) and 8.43 (s, 1H); ^{13}C-NMR (75 MHz, acetone-d_6): δ114.9, 115.1, 115.7, 125.2, 128.7, 129.8, 130.8, 131.6, 132.3, 135.8, 139.8, 156.2, 156.9 and 157.1; IR (KBr, cm^{-1}): 3348, 3027, 1607 and 1511; HR-ESI-MS (*m/z*): calculated for $C_{20}H_{15}O_3$ (M – H) 303.1027, found 303.1028.

1,1-Bis(4-hydroxyphenyl)-2-(4-hydroxy-3-methoxyphenyl)ethene (6)

Yield: 46% of dark pink powder; mp: 96°C. HPLC: $t_R = 5.4$ min; ^{1}H-NMR (400 MHz, acetone-d_6): δ3.48 (s, 3H), 6.52 (s, 1H), 6.64 (s, 2H), 6.77 (s, 1H), 6.78 (d, $J = 8.8$ Hz, 2H), 6.88 (d, $J = 8.2$ Hz, 2H), 7.01 (d, $J = 8.2$ Hz, 2H) and 7.17 (d, $J = 8.8$ Hz, 2H); ^{13}C-NMR (100 MHz, acetone-d_6): δ54.9, 112.4, 115.1, 115.8, 123.4, 125.4, 128.5, 130.2, 131.6, 132.3, 135.4, 139.9, 145.5, 146.8, 159.6 and 157.1; IR (KBr, cm^{-1}): 3358, 2925, 1606 and 1512; HR-ESI-MS (*m/z*): calculated for $C_{21}H_{17}O_4$ (M – H) 333.1132, found 333.1138.

1,1,2-Tris(4-hydroxyphenyl)but-1-ene (7)

Yield: 51% of pale yellow powder, m.p.: 95°C; HPLC: $t_R = 6.4$ min; ^{1}H-NMR (300 MHz, acetone-d_6): δ0.91 (t, $J = 7.5$ Hz, 3H), 2.46 (q, $J = 7.5$ Hz, 2H), 6.51 (d, $J = 8.4$ Hz, 2H), 6.66 (d, $J = 8.4$ Hz, 2H), 6.72 (d, $J = 8.4$ Hz, 2H), 6.83 (d, $J = 8.4$ Hz, 2H), 6.97 (d, $J = 8.4$ Hz, 2H), 7.05 (d, $J = 8.4$ Hz, 2H), 8.09 (s, 1H), 8.17 (s, 1H) and 8.32 (s, 1H); ^{13}C-NMR (75 MHz, acetone-d_6): δ13.4, 28.8, 114.4, 114.9, 115.0, 130.7, 130.9, 132.1, 133.9, 135.4, 135.7, 138.0, 140.1, 155.4, 155.7 and 156.2; IR (KBr, cm^{-1}): 3354, 3030, 2971, 2874, 1607 and 1510; HR-ESI-MS (*m/z*): calculated for $C_{22}H_{24}NO_3$ ($M + NH_4$) 303.1027, found 303.1028.

Biology

MTT, sulforhodamine B (SRB) and sodium dodecyl sulfate (SDS) were from Sigma (St Louis, Missouri, USA) and used as received. Roswell Park Memorial Institute RPMI-1640 medium was from GIBCO (Grand Island, New York, USA). All other chemicals were of the highest quality available.

Cell culture

The human cancer-cell lines were originally obtained from Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences. The cells were grown in RPMI-1640 medium containing 10% (v/v) thermally inactivated fetal bovine serum, penicillin (100 KU/1), streptomycin (100 mg/l) and 2 mmol/l of L-glutamine. For a typical screening experiment, cells were inoculated into 96-well microtiter plates in 100 µl at plating densities ranging from 5 to 10×10^4 cells/ml, depending on the doubling time of individual cell lines. After cell inoculation, the microtiter plates were incubated at 37°C in a humidified 5% CO_2 incubator.

Sample preparation

Each compound was dissolved in DMSO, and diluted to the appropriate experimental concentrations in the tissue medium and protected from light. The final concentration of DMSO in the tissue media was less than 0.1%, which did not interfere with the cell growth.

MTT assay

The cell viability of HL-60 was assessed by the MTT colorimetric assay, which is based on the reduction of MTT by the mitochondrial succinate dehydrogenase of intact cells to a purple formazan product [17]. Briefly,

100-μl aliquots of the exponentially growing HL-60 cells containing 5×10^4 cells/ml were added to each well of a 96-well flat-microtiter plate and incubated with various amounts of the test compound. Three replicate wells were used in each point in the experiments. After a 48-h incubation at 37°C, MTT solution (5 mg/ml in phosphate-buffered saline) was added and incubated for another 4 h at 37°C in a 5% CO_2 incubator. The resulting MTT–formazan product was dissolved by the same volume of lysis buffer (10% SDS–0.1 mol/l HCl) and the incubation was continued overnight at 37°C. The amount of formazan was determined by measuring the absorbance at 570 nm using a Bio-Rad 550 enzyme-linked immunosorbent assay (ELISA) microplate reader.

Sulforhodamine B assay

Growth inhibition was determined using the SRB assay, which estimates cell number indirectly by measuring total basic amino acids [18]. The adherent HO-8910 and MCF-7 cells were incubated in 96-well microtiter plates for 24 h. Following the addition of the test compound, the plates were incubated for an additional 72 h. Then the culture medium was discarded and the cells were fixed *in situ* by the gentle addition of 100 μl of cold 10% (w/v) Trichloroacetic acid (TCA) and incubated for 60 min at 4°C. The supernatant was discarded, and the plates were washed five times with tap water and air-dried. SRB solution (100 μl) at 0.4% (w/v) in 1% acetic acid was added and plates were incubated for 20 min at room temperature. After staining, unbound dye was removed by washing five times with 1% acetic acid and the plates were air-dried. Bound stain was subsequently solubilized with 10 mmol/l of unbuffered Tris and the absorbance was read at 515 nm on a Bio-Rad 550 ELISA microplate reader.

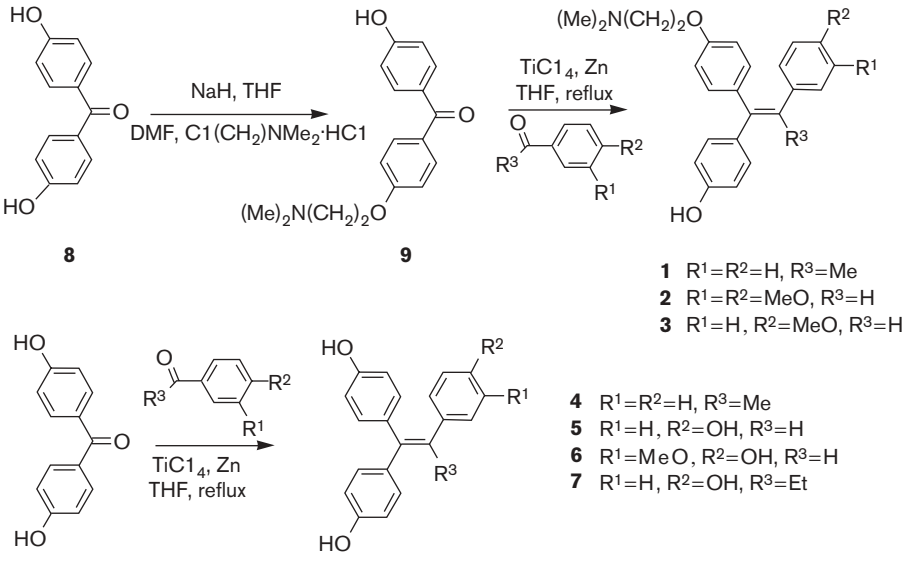
Results and discussion

Chemistry

The McMurry coupling procedure has proved very effective in the synthesis of ethylenic compounds including TAM [14,19]. This reaction was successfully used for the synthesis of compounds **1–7**. Compounds, **4**, **5** and **7**, had been synthesized previously by the use of the Grignard reaction [8,9]. As shown in Fig. 2, 4,4′-dihydroxybenzophenone (**8**) was functionalized by treatment with 2-(dimethylamino)-ethylchloride hydrochloride in dimethylformamide and by the use of NaH as the base, which gave essentially only the monosubstituted [4-(2-dimethylaminoethoxy)-phenyl]- 4′-hydroxyphenyl ketone (**9**) [16]. The key step was then to couple the appropriate aldehyde or ketone with **9** in the presence of $TiCl_4$/Zn under an argon atmosphere to give compounds **1–3**. In the case of compounds **4–7**, 4,4′-dihydroxybenzophenone (**8**) was directly coupled with the aldehyde or ketone. To avoid self coupling of **8**, a large excess of the aldehyde or ketone had to be used. It was noted that the workup of the McMurry product was easier if a large quantity of water was used in quenching the reaction mixture [12].

It has been reported previously that McMurry cross-coupling of carbonyl compounds for synthesis of TAM analogs produces generally a mixture of *Z*-isomers and *E*-isomers and the isomeric ratio depends on the substituents [12–14,16]. The *Z*-isomers and *E*-isomers

Fig. 2



Synthesis of compounds **1–7**.

can be easily assigned based on ^{1}H-NMR analysis of the relative positions of the aryl proton signals arising from the A_2B_2 *para*-system of the 4-substituted phenyl ring and/or the triplet OCH_2 signal arising from the basic side chain [12,14,16]. These proton signals resonate at a lower frequency in the *Z*-isomer, with respect to the corresponding *E*-isomer, because this aromatic ring locates between the two other aromatic rings and subjects through the space-shielding effect [10,20]. In the current case, compound **1,** which was obtained as a single isomer with the chemical shift of the triplet OCH_2 signal, appears at δ3.86 in CD_3OD. Comparison with the chemical shifts of the same OCH_2 signal for *Z*-hydroxytamoxifen and *E*-4-hydroxytamoxifen (δ3.91 and 4.07, respectively, in CD_3OD) [14] in compound **1** was assigned as the *Z*-isomer. Compound **2** was found to be a mixture of the *Z*-isomer and *E*-isomers, which was hardly separated by chromatography. The *Z*-isomeric and *E*-isomeric ratio was determined to be circa 1:1 based on the peak integrations of the triplet OCH_2 signals (δ4.04 and 4.10, respectively) and those of the singlet methoxyl signals (δ3.49 and 3.51, respectively). Similarly, compound **3** was assigned as a mixture of 1 : 1 *Z*-isomer and *E*-isomer based on the peak integration of the triplet OCH_2 signals (δ4.09 and 4.13 respectively) and the singlet $N(CH_3)_2$ signals (δ2.48 and 2.52 respectively). The ^{13}C-NMR spectra of **2** and **3** also gave two sets of carbon signals, but some of them were overlapped.

Antiproliferative effect on human cancer-cell lines

It is well known that in many estrogen-responsive systems, the *Z*-isomer and *E*-isomer of substituted triphenylethylenes possess opposite biological activities [1,21,22]. For instance, *Z*-TAM is an antiestrogen in rats, whereas the *E*-isomer is an estrogen [22–24]. By contrast, it is also known that TAM and its derivatives undergo facile *Z*-/*E*-isomerization in solutions, in cell cultures, and *in vivo* [21,25,26]. TAM derivatives could, therefore, also be assayed as a mixture of *Z*-isomer and *E*-isomer for biological studies [12,27]. In the current work TAM and the compounds **1–7** were assayed *in vitro* for their antiproliferative effect on HL-60 human promyelocytic leukemia cells, MCF-7 human breast cancer cells and HO-8910 human ovarian carcinoma cells. In case of suspension of HL-60 cells, the cell viability was assayed by the standard MTT tetrazolium spectrophotometric method [17], whereas in the case of adherent MCF-7 and HO-8910 cells the SRB method was used to avoid any direct contact of MTT with the drugs [18].

It was found that compounds **1–7** inhibited the cell proliferation of the three human cancer cell lines. The antiproliferative activities of these compounds are reported as the 50% inhibitory concentration (IC_{50}) of the treated cells with respect to the untreated control cells, the IC_{50} of TAM is also listed for comparison (Table 1). The IC_{50} value of TAM in MCF-7 cells is in good agreement with that reported previously [12].

Table 1 Antiproliferative activity of 1,1,2-triphenylethylenes in human cancer-cell lines[a]

Compounds	HL-60 cells	HO-8910 cells	MCF-7 cells
TAM	13.5 ± 0.8	29.1 ± 1.5	14.3 ± 1.2
1	15.2 ± 1.3	28.2 ± 1.6	14.9 ± 1.5
2	8.1 ± 0.5	20.8 ± 1.8	10.7 ± 0.9
3	9.2 ± 0.6	19.1 ± 1.5	8.2 ± 0.6
4	85.1 ± 2.5	50.2 ± 1.8	51.3 ± 2.2
5	64.7 ± 2.0	54.2 ± 1.6	75.4 ± 1.4
6	5.5 ± 0.2	4.6 ± 0.3	39.5 ± 1.2
7	79.3 ± 2.2	51.3 ± 1.1	40.2 ± 0.9

[a]Activities are expressed as median inhibition concentration (IC_{50}) in μmol/l. Cells were incubated with the test compounds for 48 h using the MTT assay in the case of HL-60 cells, and for 72 h using the SRB assay in the case of HO-8910 and MCF-7 cells. Data are the average of three determinations. SRB, sulforhodamine B.

It is seen from Table 1 that compounds **1–3** with the basic side chain are generally more active than compounds **4–7** without the basic side chain, except compound **6** for HL-60 and HO-8910 cells. Compound **1** exhibits similar activity as TAM for all the three cell lines, probably due to the fact that **1** is very similar to 4-hydroxytamoxifen and that the latter is the active metabolite of tamoxifen [22]. Compounds **2** and **3**, especially **3**, show significantly higher activity on all the three cell lines other than TAM and **1**. The IC_{50} values of **3** are 9.2, 19.1 and 8.2 μmol/l for HL-60, HO-8910 and MCF-7 cell lines, respectively, in comparison with those of TAM (13.5, 29.1 and 14.3 μmol/l, respectively). It demonstrates that introducing a methoxyl group on the 2-phenyl ring can significantly enhance the activity. This is also true for those compounds without the basic side chain. In other words, although compounds **4–7** are generally less active, compound **6**, which bears a 3-methoxyl group on the 2-phenyl ring, is remarkably active against the HL-60 (IC_{50} = 5.5 μmol/l) and HO-8910 (IC_{50} = 4.6 μmol/l) cell lines. A similar effect of the methoxyl group was described recently regarding 3,4,5,4′-tetramethoxystilbene, a methoxylated analogue of resveratrol, which shows higher inhibitory effect on the growth of cancer cell lines than the parent resveratrol, but with almost no effect on the growth of normal cells [28]. It is also seen that the C-2 alkyl also exhibits appreciable effect on the activity against the MCF-7 breast cancer cell line. Compound **7** with a C-2 ethyl group is more active than compound **5** without C-2 alkyl substitution (IC_{50} 40.2 and 75.4 μmol/l respectively). A similar effect of the C-2 alkyl chain length has been reported previously [8].

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