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Synthesis and antiproliferative activity of (*Z* + *E*)-1-[4-(2-(cyclopentadienyltricarbonylmanganese)-2-oxo-ethoxy)phenyl]-1,2-di(*p*-hydroxyphenyl)-but-1-ene against breast cancer cells†

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This paper describes the synthesis of (*Z* + *E*)-1-[4-(2-(cyclopentadienyltricarbonylmanganese)-2-oxo-ethoxy)phenyl]-1,2-di(*p*-hydroxyphenyl)-but-1-ene. Two synthetic pathways were explored. The best pathway consisted of the alkylation of 1,2-bis-[4-(*tert*-butyl-dimethylsilyloxy)phenyl]-1-(4-hydroxyphenyl)but-1-ene with $BrCH_2COOEt$. The ester obtained was transformed into the Weinreb amide by reaction with HN(OMe)Me–HCl. The reaction of lithium manganese tricarbonylcyclopentadienide with the Weinreb amide produced 1-[4-(2-(cyclopentadienyltricarbonylmanganese)-2-oxo-ethoxy)phenyl]-1,2-di(*p*-*tert*-butyldimethylsiloxyphenyl)-but-1-ene. The deprotection of phenolic functions of the latter compound led to the formation of the final compound. The *Z* and *E* isomers could be separated but the isomerization of these isomers from one to another is an easy process. The *Z* + *E* compound 2 was tested against the hormone-dependent MCF-7 and hormone-independent MDA-MB-231 breast cancer cell lines. The IC_{50} values of compound 2 were 4.80 ± 2.00 μM and 4.79 ± 0.70 μM for MCF-7 cells and MDA-MB-231 cells, respectively, which was three times better than the ferrocenyl analogue. Copyright © 2012 John Wiley & Sons, Ltd.

Keywords: manganese; cymantrene; tamoxifen; breast cancer

Introduction

Bioorganometallic chemistry, which is meant to use organometallic complexes in real problems of biological interest, is a discipline that lies at the interface between organometallic chemistry and biology. This area has been extensively developed in the last two decades.[1–7] Recently, an important field of application in this topic was described under the term 'medicinal organometallic chemistry'.[8] One of the most prominent examples of the use of organometallic compounds is hydroxyferrocifen (FcOHTAM), a ferrocenyl analogue of tamoxifen (Fig. 1).[9–12]

Tamoxifen is a drug which is extensively used for the treatment of breast cancer.[13–16] It acts as an anti-oestrogen and is only active against hormone-dependent cancer cell lines. Tamoxifen is completely inactive against hormone-independent cancer cell lines. The use of tamoxifen for a long period leads to the development of drug resistance phenomena. In order to find alternative drugs with wider spectra of applications, the organometallic approach has emerged as one of the most innovative strategies. Hydroxyferrocifen was shown to be very active against both hormone-dependent MCF-7 and hormone-independent MDA-MB-231 breast cancer cell lines.[12] This activity was further improved by replacing the ferrocenyl group with a ferrocenophane motif.[17,18]

In order to preserve the triaryl butene core of tamoxifen, the ferrocenyl compound **1** was prepared (Fig. 2).[19,20] With an IC_{50} value of 11.3 μMfor MCF-7 hormone-dependent breast cancer cells, **1** is less active than FcOHTAM and 1,1-bis-(*p*-hydroxyphenyl)-2-ferrocenyl-but-1-ene (IC_{50} = 0.64 ± 03 μM).[11,12] However, **1** still exhibits good affinity for oestrogen receptor alpha (relative binding affinity = 14%).

Pursuing research in this field, we thought it would be interesting to study the cymantrenyl compound **2**, an analogue of **1**, for the following reasons: firstly, **2** may exhibit antiproliferative effects that differ from those of **1**; secondly, compound **2** may be used as a precursor for other cyclopentadienyl metal complexes (for example, via a reaction of decomplexation–recomplexation, it may be possible to transform manganese compounds into rhenium or titanium complexes);[21,22] and thirdly, **2** may be used as a model for the technetium analogue. Technetium compounds play an important role as imaging agents.[23–25] For this purpose, we now describe the synthesis of **2** and its antiproliferative effects on hormone-dependent MCF-7 and hormone-independent MDA-MB-231 breast cancer cell lines.

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† *This paper is dedicated to Professor Stefan Toma on the occasion of his 75th birthday.*

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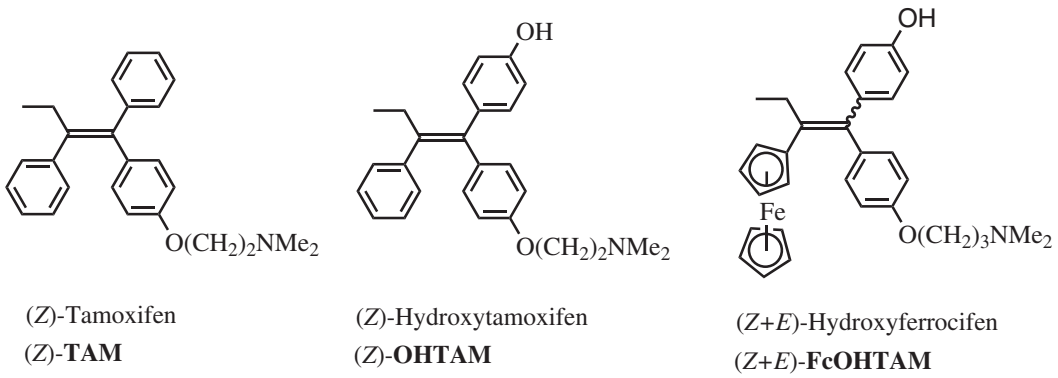


(*Z*)-Tamoxifen
(*Z*)-**TAM**

(*Z*)-Hydroxytamoxifen
(*Z*)-**OHTAM**

(*Z*+*E*)-Hydroxyferrocifen
(*Z*+*E*)-**FcOHTAM**

Figure 1. Molecules of tamoxifen, hydroxytamoxifen and hydroxyferrocifen.

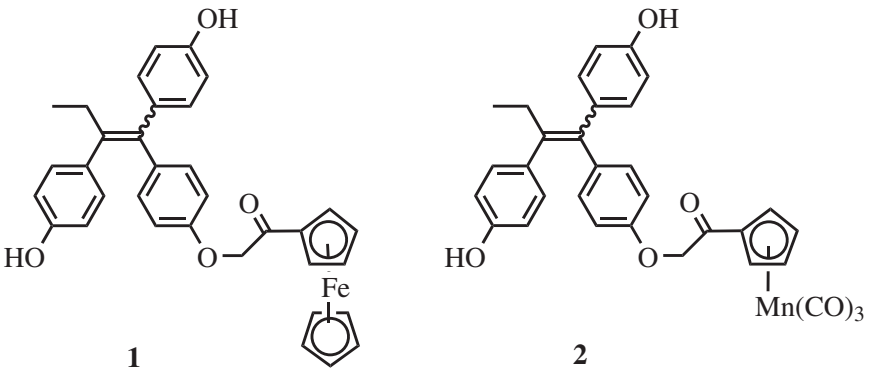


Figure 2. Ferrocenyl and cymantrenyl derivatives of triaryl butane.

Results and Discussion

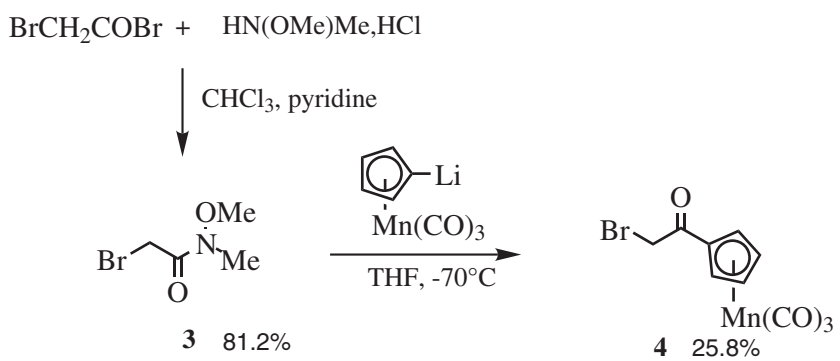
Synthesis

Two different pathways were used for the synthesis of compound **2**.

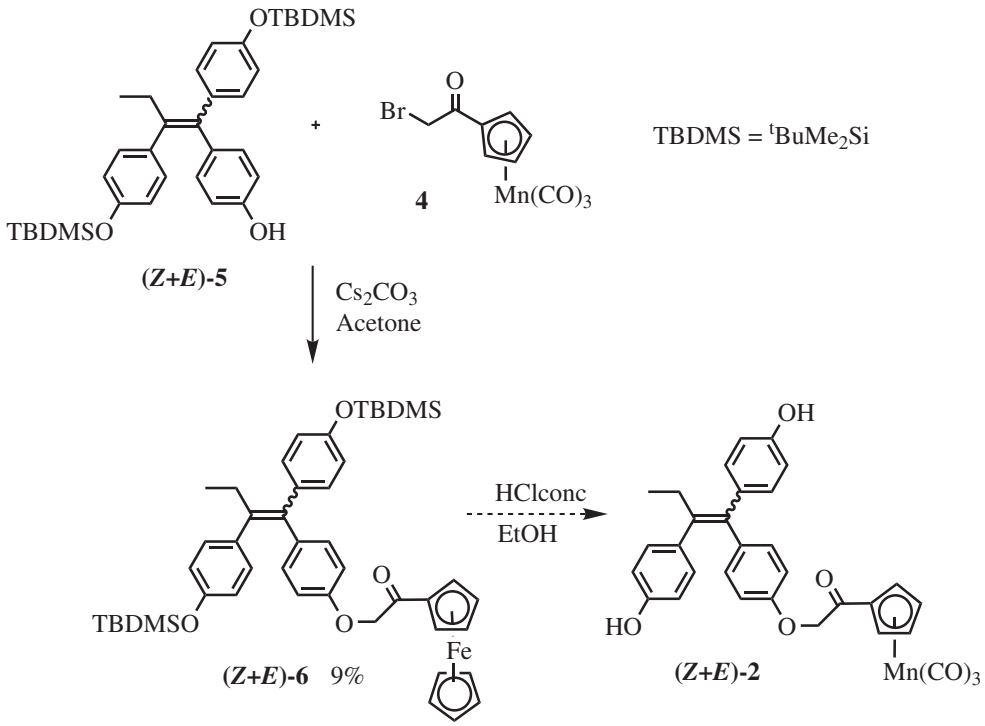
The first synthetic route consisted of the preparation of bromoacetyl-cyclopentadienyltricarbonylmanganese followed by Williamson alkylation of a phenolic derivative of triarylbutene with this halide. Schemes 1 and 2 show the synthetic pathway adopted.

In the first step, the Weinreb amide **3** was prepared by reacting the bromoacetyl bromide with *N,O*-dimethylhydroxylammonium according to the procedures outlined in the literature.[26] Compound **3** was obtained in high yield (81.2%). The reaction of compound **3** with lithium manganese tricarbonylcyclopentadienide in THF at −70 °C for 1 h resulted in only a moderate yield of compound **4** (25.8%). Along with compound **4**, 36% yield of manganese tricarbonylcyclopentadiene was recovered after the reaction.

Following the alkylation procedure of triarylbutene **5** by chloroacetylferrocene,[20] compound **5** was alkylated with bromide **4** in acetone using caesium carbonate (Cs_2CO_3) as a base. Compound **6** was obtained as a mixture of *Z* and *E* isomers in 9% yield. This low yield is due to an incomplete reaction and also to the sensitivity of the *t*-butyldimethylsilyl protecting group to a basic medium leading to the formation of a mixture of several compounds. The use of other bases, such as K_2CO_3 or NaOH, did not improve the reaction yield.



Scheme 1. Synthesis of bromoacetyl-cyclopentadienyltricarbonylmanganese.



Scheme 2. Synthesis of compound (Z + *E*)-**2**.

In order to improve the access of **6**, a second synthetic approach was explored (Scheme 3). This new reaction pathway differed from the first synthetic approach by first performing the alkylation of compound **5** with ethyl bromoacetate.

The reaction of ethyl bromoacetate with **5** in the presence of Cs_2CO_3 provided ester **7** as a mixture of two isomers (*Z* and *E*) that could be separated by silica gel column chromatography using ethyl ether–petroleum ether (1:20) as the eluent. *E*-**7** and *Z*-**7** were obtained in yields of 22.4% and 27%, respectively. The overall yield of the reaction was 49.4%. The structure of *Z*-**7** was identified by correlation spectroscopy (COSY), heteronuclear multiple bond correlation (HMBC), heteronuclear multiple quantum coherence (HMQC) and nuclear Overhauser effect spectroscopy (NOESY) NMR. This identification allowed the structural attribution of all isomers in this study. The reaction of each isomer of **7** with *N*, *O*-dimethylhydroxylamine chloride in the presence of $AlMe_3$ in dichloromethane at −15 °C gave amides *Z*-**8** and *E*-**8** in very good yields (72% and 71%, respectively).

The reaction of lithium manganese tricarbonylcyclopentadienide with Z-**8** at −70 °C furnished a *Z*-**6** yield of 22% (Scheme 4).

The deprotection of phenolic groups was easily achieved by heating the Z-**6** solution in ethanol in the presence of HCl at 80 °C. *E*-**2** was first obtained at a yield of 48%. The *E* configuration was attributed to this isomer because it was prepared from *Z*-**7**. We observed that *Z*-**7** was not stable in solution with respect to the isomerization reaction. In acetone solution at room temperature, *Z*-**7** progressively transformed to *E*-**7** and, after 4 days, the proportion of *Z*:*E* isomers was 42:58 (determined by NMR). Therefore, in biological media, **7** should be considered a mixture of the *Z*/*E* isomers even when using a pure isomer.

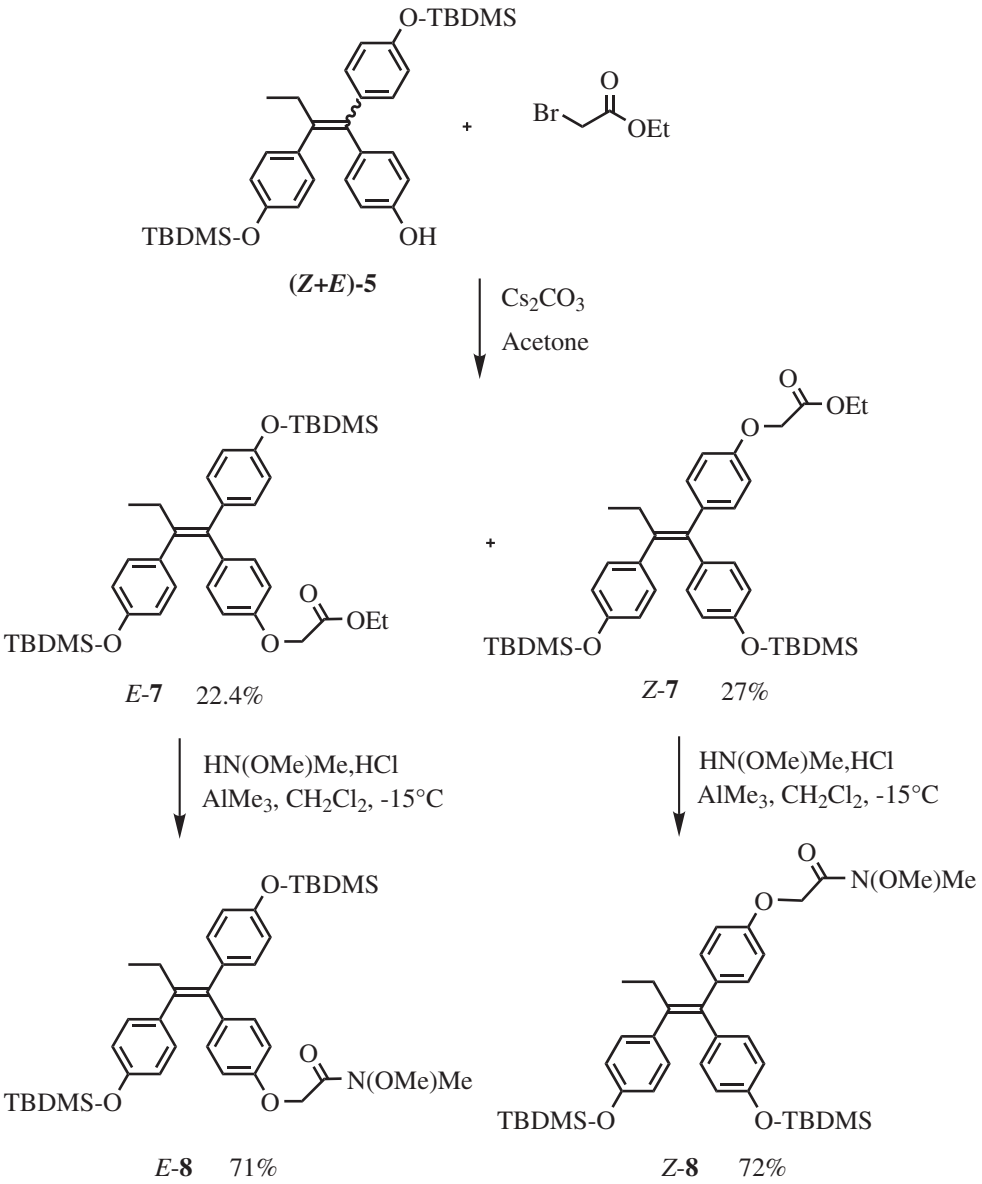
Antiproliferative Effects

The effect of compound **2** on the proliferation of cancer cells was tested on hormone-dependent MCF-7 breast cancer cells and hormone-independent MDA-MB-231 breast cancer cells. Table 1 shows the effects at 1 and 10 μM, as well as the IC_{50} values.

At 10 μM, compound **2** was found to be very active against both cancer cell lines. The IC_{50} values of 4.80 ± 2.00 μM and 4.79 ± 0.70 μM were measured for MCF-7 cells and MDA-MB-231 cells, respectively. Thus compound **2** shows almost the same antiproliferative effects for hormone-dependent and hormone-independent breast cancer cell lines. This result is interesting, as it has been shown before that cymantrene and cyrhetrene compounds are not very active against these cancer cells. Thus 1,1′-bis(4-hydroxyphenyl)-2-(tricarbonylmanganese cyclopentadienyl)-but-1-ene and 1,1′-bis (4-hydroxyphenyl)-2-(tricarbonylrhenium cyclopentadienyl)-but-1-ene did not show an antiproliferative effect on MDA-MB-231 cells at 1 μM, and they even exhibited a slightly proliferative effect on MCF-7 cells.[27] In addition, the attachment of the cymantrenyl group to so-called cell-penetrating peptides has been shown to enhance their cytotoxic activity against MCF-7 cells. However, the IC_{50} values of these compounds were in the range of 15–60 μM and thus higher than that of **2**.[28,29] We show here that, by selecting a judicious structure, cymantrenyl compounds can also have decent antiproliferative effects, as **2** performed even better than its ferrocenyl analogue, compound **1** (IC_{50} = 11.3 μM).[19]

Conclusion

In this study, (*Z* + *E*)-1-[4-(2-(cyclopentadienyltricarbonylmanganese)-2-oxo-ethoxy)phenyl]-1,2-di(*p*-*hydr*oxyphenyl)-but-1-ene, compound **2**, was prepared. Compound **2** is characterized by the presence of a triaryl butene core, like tamoxifen. It is possible to separate the *Z* isomer from the *E* isomer, but the isomerization of these compounds is an easy process. The compounds were found to have antiproliferative effect against the hormone-dependent MCF-7 and hormone-independent MDA-MB-231 breast cancer cell lines. We have shown



Scheme 3. Synthesis of compounds *Z* and *E*-**8**.

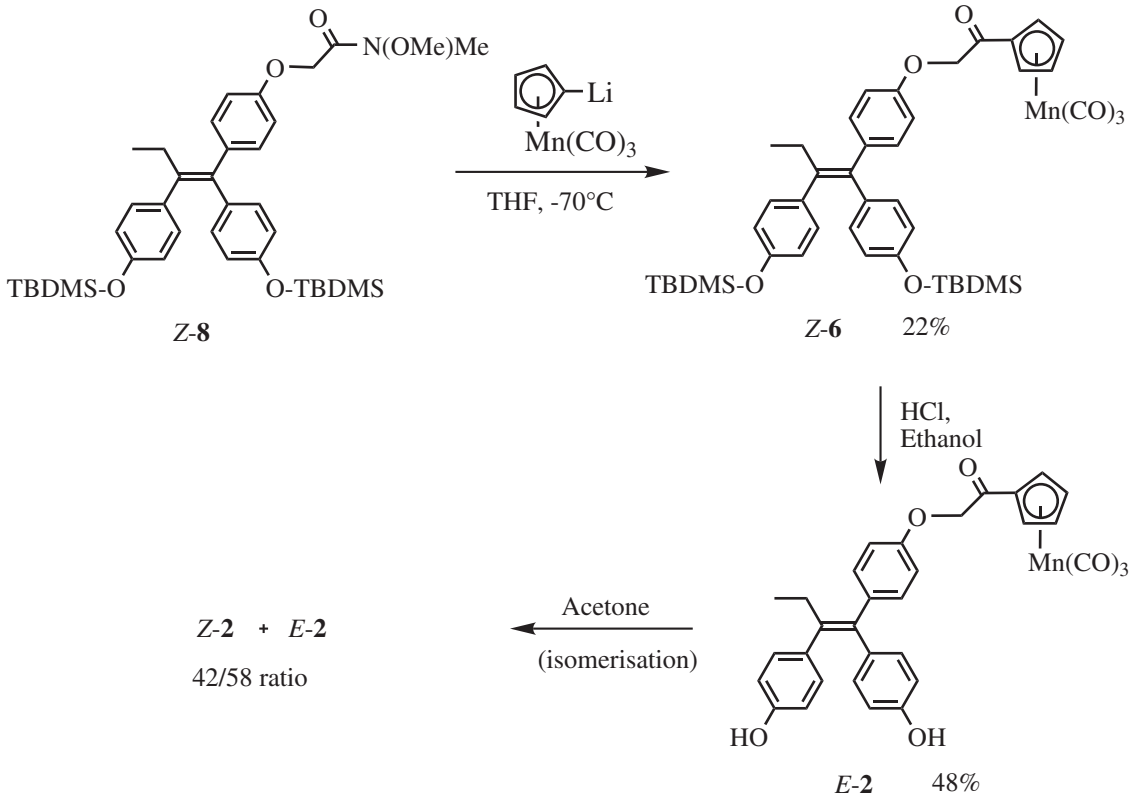
that the good cytotoxic activity of hydroxyferrocifen is due to the presence of the ferrocenyl double-bond phenol motif, which allows for the formation of quinone methide, its active metabolite. The low cytotoxic activity of **2** compared to that of hydroxyferrocifen may be a consequence of the absence of this motif. Interestingly, **2** is more active than the ferrocenyl analogue, in which there is no conjugation between the organometallic unit and the phenol.

Experimental

All air-sensitive reactions were carried out under argon atmosphere, using standard Schlenk and vacuum-line techniques. Anhydrous THF was obtained by distillation from sodium–benzophenone. Thin-layer chromatography (TLC) was performed on silica gel 60 GF254. Flash chromatography was performed on silica gel Merck 60 (40–60 μm). Infrared spectra were recorded on an IR-FT BOMEM Michelson-100 spectrometer equipped with a deuterated triglycine sulfate (DTGS) detector. ^{1}H and ^{13}C NMR spectra were recorded on a 300 MHz Bruker spectrometer. Mass spectrometry was performed with a Nermag R 10-10C spectrometer. Elemental analyses were performed by the microanalysis services of ICSN (Gif sur Yvette, France). High-resolution mass spectra were acquired with an electrospray time-of-flight (ESI-TOF, LCT Premier XE, Waters) mass spectrometer in the positive or negative ion mode. Melting points were measured with a Kofler device. Determination of the cytotoxicity of the complexes was performed at IMAGIF (ICSN, Gif sur Yvette, France).

N-Methoxy-*N*-methyl-bromoacetamide (**3**)

Grounded *N*,*O*-dimethylhydroxylamine hydrochloride (2.14 g, 22 mmol) was partially dissolved in ethanol-free chloroform (25 ml) under argon. Pyridine (3.48 g, 44 mmol) was added and the mixture was stirred for 20 min. In another Schlenk tube, bromoacetylbromide (4.8 g, 22 mmol) was dissolved in chloroform (15 ml) and cooled to 5 °C (ice bath). The first solution was added to the second solution (5 min). The mixture was stirred for 1 h.



Scheme 4. Synthesis of compound *E*-**2**.

Table 1. Cell growth inhibition (%) by **2** for MCF-7 and MDA-MB-231 breast cancer cell lines at 1 and 10 μM, as well as IC_{50} values

Cancer cell lines tested	% inhibition		IC_{50} (μM)
	at 1 μM	at 10 μM	
MCF-7	17 ± 3	83 ± 03	4.80 ± 2.00
MDA-MB-231	6 ± 5	89 ± 2	4.79 ± 0.70

Dichloromethane (100 ml) was added and the mixture was poured into water (100 ml). The products were extracted with dichloromethane (2 × 100 ml). The organic layer was dried over $MgSO_4$ and evaporated under reduced pressure. *N*-Methoxy-*N*-methyl-bromoacetamide (**3**) was obtained as an oil (3.45 g, 86 % yield), $R_f = 0.36$ (diethyl ether–pentane 1:1). ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.22 (s, 3 H, Me); 3.78 (s, 3 H, MeO), 4.00 (s, 2 H, CH_2) ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 25.1 (Me); 32.6 (CH_2), 61.6 (OMe), 167.5 (CO). IR (CH_2Cl_2, cm^{-1}) : 1669.8 (CO). MS (CI): $m/z = 199$ $[M + NH_4]^+$, 182 $[M + H]^+$.

2-Bromoacetyl-cyclopentadienyltricarbonylmanganese (4)

$CpMn(CO)_3$ (206 mg, 1 mmol) was dissolved in 3 ml THF under argon. The solution was cooled to −70 °C, then *n*BuLi (0.48 ml, 1.2 mmol, 2.5 M in hexane) was added. The mixture was stirred at −70 °C for 1 h 30 min. Then, a solution of **3** (273 mg, 1.5 ml) in 2 ml THF was added. The mixture was stirred again for 1 h at −70 °C. 2 ml of 1/10 HCl solution was added and the cold bath was removed. After 5 min, 20 ml of 1/10 HCl solution was added and the product was extracted with 3 × 30 ml diethyl ether. The organic layer was washed with 20 ml water, dried over $MgSO_4$, and the solvent evaporated under reduced pressure. The crude product was purified by silica gel column using diethyl ether–pentane (1:1) as the eluent. Unreacted $CpMn(CO)_3$ was first eluted (74 mg, 36%). The second fraction was identified as compound **4** (yellow solid, 84 mg, 25.8% yield). $R_f = 0.75$ (diethyl ether–pentane, 1:1). ^{1}H NMR (300 MHz, CD_3COCD_3): δ 4.27 (s, 2H, CH_2), 5.08 (t, 2 H, $J = 1.8$ Hz, C_5H_4), 5.68 (t, 2 H, $J = 1.8$ Hz, C_5H_4) ^{13}C NMR (75 MHz, CD_3COCD_3): δ 32.1 (CH_2), 85.9, 88.6 (CH, C_6H_4), 89.8 (C_{ip}, C_5H_4), 190.2 (CO). M.p. 76 °C. MS (ESI): $m/z = 383.3$ $[MCH_3COO]^-$. IR (CH_2Cl_2, cm^{-1}): 2033 and 1952 ν (Mn(CO)), 1681 ν(CO). Elemental analysis calcd for $C_{10}H_6BrMnO_4$: C, 36.96; H, 1.86. Found: C, 37.76; H, 1.84.

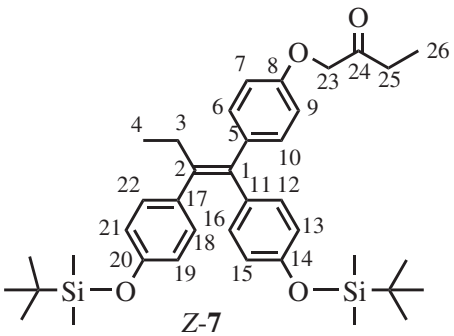
(*Z*)- and (*E*)-1-[4-(2-(Cyclopentadienyltricarbonylmanganese)-2-oxo-ethoxy-phenyl]-1,2-di(*p*-*tert*-butyldimethylsiloxyphenyl) but-1-ene (6)

Cs_2CO_3 (326 mg, 1 mmol) was added to a solution of compound **5** (560.9 mg, 1 mmol) in 15 ml acetone. A solution of **4** (277 mg, 0.85 mmol) in acetone (7 ml) was added dropwise (15 min). The mixture was stirred for 1 h and then filtered over silica gel and evaporated under reduced pressure. TLC of the crude products showed the presence of both starting materials and other compounds. Some of these compounds may come from **5** by the loss of *t*-butyldimethylsilyl protecting group. A first purification by silica gel column, using diethyl ether–pentane (1:5) as the eluent, allowed the isolation of one fraction (140 mg) which mainly contains **6**. After a second purification by column, using the same eluent, 73 mg pure (*Z* + *E*)-**6** was isolated (9% yield). The compound was crystallized from pentane, giving pale-yellow crystals containing almost only one isomer. R_f (diethyl ether–pentane, 1:5) = 0.39. ^{1}H NMR (300 MHz, CD_3COCD_3): δ 0.12 (s,

6 H, $(CH_3)_2Si$), 0.17 (s, 6 H, $(CH_3)_2Si$), 0.91 (t, 3 H, $J = 6.0$ Hz, CH_3), 0.93 (s, 9 H, t-Bu), 0.97 (s, 9 H, t-Bu), 2.44 (q, 2 H, $J = 6.0$ Hz, CH_2), 5.11 (s, 2 H, OCH_2), 5.21 (t, 2 H, $J = 2.2$ Hz, C_5H_4), 5.88 (t, 2 H, $J = 2.2$ Hz, C_5H_4), 6.52 (d, 2 H, $J = 6.5$ Hz, C_6H_4), 6.66 (d, 2 H, $J = 6.5$ Hz, C_6H_4), 6.73 (d, 2 H, $J = 6.5$ Hz, C_6H_4), 6.9 (d, 4 H, $J = 6.5$ Hz, C_6H_4), 7.16 (d, 2 H, $J = 6.5$ Hz, C_6H_4). ^{13}C NMR (75 MHz, $CDCl_3$): δ −4.4 (Si $(CH_3)_2$), 13.6 (CH_3 of C_2H_5), 18.2 (C_q of t-Bu), 25.7 (CH_3 of t-Bu), 28.7 (CH_2 of Et), 71.9 (CH_2 of CH_2CO)), 83.7 (C_5H_4), 87.5 (C_5H_4), 114.1, 118.8, 119.4, 130.6, 130.8 and131.8 (CH_{ar}), 135.3–153.4 (C═C and C_{ar}), 191.3 (CO). IR (CH_2Cl_2, cm^{-1}): 2033 and 1952 (Mn(CO)), 1681 (CO). M.p.118 °C. MS (ESI): *m/z* = 822.6 $[MNH_4]^+$. Elemental analysis calcd for $C_{44}H_{53}MnO_7Si_2$: C, 65.65; H, 6.64. Found: C, 64.88; H, 6.76.

Compound (*Z* + *E*)-7

The reaction was carried out at room temperature. Compound **5** (5.5 g, 9.8 mmol) was dissolved in dry acetone (120 ml) and Cs_2CO_3 (4.5 g, 13.8 mmol) was added in one portion. Then, $BrCH_2CO_2Et$ (2.3 g, 13.8 mmol) was added dropwise to the solution. The reaction mixture was stirred for 1 h, filtered and the solvent was removed under reduced pressure. The crude product was chromatographed on a silica gel column with diethyl ether–petroleum ether (1:20) solution as the eluent. First fraction: *E*-**7** (1.34 g, colourless oil, 22% yield). $R_f = 0.52$, ether–pentane, 1:10). ^{1}H NMR (300 MHz, CD_3SOCD_3): δ 0.12 (s, 6 H, $(CH_3)_2Si$), 0.18 (s, 6 H, $(CH_3)_2Si$), 0.85 (t, 3 H, CH_3), 0.89 (s, 9 H, t-Bu), 0.94 (s, 9 H, t-Bu), 1.14 (t, 3 H, $J = 7.1$ Hz, OCH_2CH_3), 2.35 (q, 2 H, CH_2), 4.10 (q, 2 H, $J = 7.1$ Hz, OCH_2CH_3), 4.60 (s, 2 H, OCH_2), 6.54 (d, 2 H, $J = 8.9$ Hz, C_6H_4), 6.63 (d, 2 H, $J = 8.6$ Hz, C_6H_4), 6.68 (d, 2 H, $J = 8.9$ Hz, C_6H_4), 6.82 (d, 2 H, $J = 8.5$ Hz, C_6H_4), 6.94 (d, 2 H, $J = 8.6$ Hz, C_6H_4), 7.02 (d, 2 H, $J = 8.5$ Hz, C_6H_4). ^{13}C NMR (75.5 MHz, CD_3SOCD_3): δ −4.5 ($Si(CH_3)_2$), 13.3 (CH_3), 13.9 (OCH_2CH_3), 17.9 (C_q of t-Bu), 25.5 (CH_3 of t-Bu), 28.4 (CH_2), 60.5 (OCH_2), 64.5 (OCH_2CH_3), 113.3, 119.4 (2C), 130.2, 130.4 and 131.3 (CH_{arom}), 134.9, 136.0, 137.0, 137.5, 140.2, 153.0, 153.5 and 155.4 (C_q of 3 C_6H_4 and C═C), 168.6 (C═O). IR (CH_2Cl_2, ν cm^{-1}):1755 (CO), 1604 cm^{-1} (C═C). MS (EI): *m/z*: 646 $[M]^{+\cdot}$). Elemental analysis calcd for $C_{38}H_{54}O_5Si_2$ C, 70.54; H, 8.41. Found: C, 70.58; H, 8.46.



Z-**7**

Second fraction: isomer *Z*-**7** (1.758 g, white solid; 27% yield; m.p. 106 °C). $R_f = 0.39$, diethyl ether–pentane, 1:10). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.11 (s, 6 H, $(CH_3)_2Si$), 0.16 (s, 6 H, $(CH_3)_2Si$), 0.92 (t, 3 H, $J = 7.4$ Hz, CH_3), 0.92 (s, 9 H, t-Bu), 0.96 (s, 9 H, t-Bu), 1.31 (t, 3 H, $J = 7.1$ Hz, H26), 2.43 (q, 2 H, $J = 7.4$ Hz, H3), 4.29 (q, 2 H, $J = 7.1$ Hz, H25), 4.63 (s, 2 H, H23), 6.47 (d, 2 H, $J = 8.6$ Hz, H13 + H15), 6.62 (d, 2 H, $J = 8.6$ Hz, H19 + H21), 6.68 (d, 2 H, $J = 8.6$ Hz, H12 + H16), 6.87 (d, 2 H, $J = 8.6$ Hz, H7 + H9), 6.93 (d, 2 H, $J = 8.6$ Hz, H18 + H22), 7.13 (d, 2 H, $J = 8.6$ Hz, H6 + H10). ^{13}C NMR (100.62 MHz, $CDCl_3$): δ −4.4 ($Si(CH_3)_2$), 13.8 (C1), 14.2 (C26), 18.2 and 18.3 (C_q of t-Bu), 25.7 and 25.8 (CH_3 of t-Bu), 28.8 (C3), 61.4 (C25), 65.8 (C23), 114.3 (C7 + C9), 118.9 (C13 + C15), 119.5 (C19 + C21), 130.7 (C18 + C22), 130.8 (C6 + C10), 132.0 (C12 + C16), 135.5 (C17), 136.5 (C11), 137.3 (C1), 137.6 (C5), 140.9 (C2), 153.5 (CC14), 153.8 (C20), 156.5 (C8), 169.1 (C24). IR (CH_2Cl_2, ν cm^{-1}) :1755.7 (CO), 1603.5 (C═C). MS (EI) 646 $[M]^{+\cdot}$). Anal: calcd for $C_{38}H_{54}O_5Si_2$ C,70.54; H,8.41. Found: C,70.72; H, 8.81. The *Z* configuration of this isomer was identified by COSY, HMBC, HMQC and NOESY NMR. The NOESY spectrum showed correlation between protons H3, and also protons H4, with the aromatic protons H6,10 and H18,22.

Compound *Z*-8

The reaction was carried out at −10 °C under argon. Compound *Z*-**7** (647 mg, 1 mmol) was dissolved in distilled dichloromethane (6 ml) and the solution was cooled to −10 °C. HNMe(OMe)–HCl (195 mg, 2 mmol) was added. Then, $AlMe_3$ (0.8 ml, 2 mmol, 2.5 M in toluene) was added dropwise (10 min). The stirring was maintained for 30 min. The mixture was diluted with dichloromethane (50 ml), washed with water (2 × 30 ml), dried over $MgSO_4$ and filtered. After concentration under reduced pressure, the crude product was chromatographed on silica gel column with diethyl ether–pentane (2:1) solution as the eluent to yield compound *Z*-**8** as a yellow solid (474 mg; 72% yield; m.p. 97 °C). $R_f = 0.65$, diethyl ether–pentane, 2:1). ^{1}H NMR (300 MHz, $CDCl_3$): 0.10 (s, 6 H, $(CH_3)_2Si$), 0.15 (s, 6 H, $(CH_3)_2Si$), 0.89 (s, 9 H, CH_3 of t-Bu), 0.91 (t, 3 H, CH_3 of Et), 0.96 (s, 9 H, CH_3 of t-Bu), 2.44 (q, 2 H, CH_2 of Et), 3.25 (s, 3 H, CH_3 of N-CH_3), 3.77 (s, 3 H, OCH_3), 4.63 (s, 2 H, CH_2 of OCH_2), 6.46 (d, 2 H, $J = 8.7$ Hz, C_6H_4), 6.61 (d, 2 H, $J = 8.6$ Hz, C_6H_4), 6.67 (d, 2 H, $J = 8.7$ Hz, C_6H_4), 6.90 (d, 2 H, $J = 8.6$ Hz, C_6H_4), 6.92 (d, 2 H, $J = 8.6$ Hz, C_6H_4), 7.13 (d, 2 H, $J = 8.6$ Hz, C_6H_4). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ −4.4 ($Si(CH_3)_2$), 13.6 (CH_3 of Et), 18.1 and 18.2 (Cq of t-Bu), 25.6 (CH_3 of t-Bu), 28.7 (CH_2 of Et), 29.2 (CH_3 of NCH_3), 61.6 (OCH_2), 65.7 ($NOCH_3$), 114.5, 118.8, 119.4, 130.6 and 131.9 (CH_{arom}), 135.5, 137.3, 140.7, 153.4, 153.7 and 156.9 (C═C and C_{arom}), 170 (C═O). IR (CH_2Cl_2, cm^{-1}): 1692.4 (CO), 1603.7 (C═C). MS (EI): *m/z*: 661 $[M]^{+\cdot}$. Elemental analysis calcd for $C_{38}H_{55}NO_5Si_2$ C,68 .94; H, 8.37. Found: C,68.65; H, 8.62.

Compound *E*-8

The reaction was carried out at −10 °C under argon. Compound *E*-**7** (770 mg, 1.19 mmol) was dissolved in distilled dichloromethane (15 ml) and cooled to −10 °C. HNMe(OMe)–HCl (464 mg, 4.76 mmol) was added. Then, $AlMe_3$ (2.38 ml, 4.76 mmol, 2.5 M in toluene) was added dropwise (10 min). After stirring for 2 h 30 min, the mixture was diluted with CH_2Cl_2 (100 ml), washed with water (2 × 30 ml), dried over $MgSO_4$ and filtered. After concentration under reduced pressure, the crude product was chromatographed on a silica gel column using diethyl ether–pentane (2:1) as the eluent. Compound *E*-**8** was obtained as a colourless oil (562 mg, 71% yield). $R_f = 0.5$, diethyl ether–pentane, 2:1). ^{1}H NMR (300 MHz, $CDCl_3$): 0.16 (s, 6 H, $(CH_3)_2Si$), 0.22 (s, 6 H, $(CH_3)_2Si$), 0.88 (t, 3 H, CH_3 of Et), 0.92 (s, 9 H, CH_3 of t-Bu), 0.96 (s, 9 H, CH_3 of t-Bu), 2.43 (q, 2 H, $J = 7.2$ Hz, CH_2 of Et), 3.23 (s,3 H, CH_3 of N-CH_3), 3.7 (s, 3 H, OCH_3), 4.67 (s, 2 H, CH_2 of OCH_2), 6.58 (d, 2 H, $J = 8.7$ Hz, C_6H_4), 6.64 (d, 2 H, $J = 8.7$ Hz, C_6H_4), 6.72 (d, 2 H, $J = 8.7$ Hz, C_6H_4), 6.79 (d, 2 H, $J = 8.7$ Hz, C_6H_4), 6.94 (d, 2 H, $J = 8.7$ Hz, C_6H_4), 7.06 (d, 2 H, $J = 8.7$ Hz, C_6H_4). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ −4.4 ($Si(CH_3)_2$), 13.6 (CH_3), 18.1 and 18.2 (C_q of t-Bu), 25.7 (CH_3 of t-Bu), 28.9 (CH_2), 32.4 (NMe), 61.6 (NOMe), 65.6 (OCH_2), 113.5, 119.5, 119.6, 130.6, 130.7 and 131.9 (CH_{arom}),

135.5, 136.8, 136.9, 137.2, 140.8, 153.7, 154.2 and 156.0 (C_q of 3 C_6H_4 and C═C), 167.0 (C═O). IR (CH_2Cl_2, cm^{-1}): 1692.4 (CO), 1603.7 (C═C). MS (EI) *m/z* : 661 $[M]^{+\cdot}$. Elemental analysis calcd. for $C_{38}H_{55}NO_5Si_2$ C, 68.94; H, 8.37 ; N, 2.12. Found: C, 68.35; H, 8.47; N, 2.04.

Compound *Z*-6

$CpMn(CO)_3$ (735 mg, 3.6 mmol) was dissolved in anhydrous THF (10 ml). The solution was cooled at −70 °C, then *n*BuLi (1.68 ml, 2.7 mmol, 1.6 M solution in hexane) was added (2 min). The reaction mixture was stirred for 1 h 30 min and the temperature raised from −70 °C to −30 °C. A solution of compound *Z*-**8** (597 mg, 0.9 mmol) in THF (5 ml) was then added (2 min). The solution was stirred for 10 min at −30 °C, after which 30 ml of 1/10 HCl solution was added and the cold bath was removed. After 5 min, 30 ml of 1/10 HCl solution was added and the product was extracted with 3 × 50 ml diethyl ether. The organic layer was washed with 2 × 40 ml water and was dried over $MgSO_4$ and filtered. After concentration under reduced pressure, the crude product was chromatographed on a silica gel column using diethyl ether–pentane (2:1) as the eluent. Unreacted $CpMn(CO)_3$ was first eluted (510 mg). The second fraction was identified as compound *Z*-**6** (160 mg; 22% yield; m.p. 122 °C). R_f = 0.78, dietyl ether:pentane, 2:1). ^{1}H NMR (300 MHz, CD_3COCD_3): δ 0.13 (s, 6 H, $(CH_3)_2Si$), 0.18 (s, 6 H, $(CH_3)_2Si$), 0.92 (t, 3 H, *J* = 5.6 Hz, CH_3), 0.94 (s, 9 H, t-Bu), 0.97 (s, 9 H, t-Bu), 2.45 (q, 2 H, *J* = 5.6 Hz, CH_2), 5.12 (s, 2 H, OCH_2), 5.21 (t, 2 H, *J* = 1.7 Hz, C_5H_4), 5.88 (t, 2 H, *J* = 1.7 Hz, C_5H_4), 6.53 (d, 2 H, *J* = 6.4 Hz, C_6H_4), 6.68 (d, 2 H, *J* = 6.4 Hz, C_6H_4), 6.73 (d, 2 H, *J* = 6.4 Hz, C_6H_4), 6.99 (d, 4 H, *J* = 6.4 Hz, C_6H_4), 7.17 (d, 2 H, *J* = 6.4 Hz, C_6H_4). ^{13}C NMR (75.5 MHz, CD_3COCD_3): δ −4.4 ($Si(CH_3)_2$), 13.8 (CH_3), 18.6 and 18.7 (C_q of t-Bu), 25.9 (CH_3 of t-Bu), 29.3 (CH_2), 71.5 (OCH_2), 85.5 and 88.3 (CH, C_5H_4), 90.0 (C_{ip}, C_5H_4), 115.1, 119.7, 120.3, 131.3, 131.6 and 132.6 (CH_{arom}), 136.3, 137.6, 137.8, 138.5, 141.7, 154.4, 154.8 and 157.8 (C_q of 3 C_6H_4 and C═C), 193.3 (C═O). IR (CH_2Cl_2, cm^{-1}): 2033 and 1952 (Mn(CO)), 1702 and 1679 (CO). MS (CI): *m/z* = 805 $[MH]^{+\cdot}$. Elemental analysis calcd for $C_{44}H_{53}MnO_7Si_2$: C, 65. 65 ; H, 6.64. Found: C, 65.78; H, 6.60.

Compound *Z* + *E*-2

Compound Z-**6** (130 mg, 0.16 mmol) was dissolved in ethanol (10 ml). 0.2 ml concentrated HCl was added. The mixture was stirred at 80 °C for 15 min. The oil bath was removed and 50 ml diethyl ether was added. The mixture was washed with water (3 × 10 ml) and dried over $MgSO_4$. After concentration under reduced pressure, the crude product was chromatographed on silica gel column using diethyl ether–pentane (4:1) as the eluent to furnish *E*-**2** as a yellow solid (48 mg, 48% yield). R_f = 0.50 (diethyl ether–pentane, 4:1). ^{1}H NMR (300 MHz, CD_3COCD_3): δ 0.92 (t, 3 H, *J* = 7.4 Hz, CH_3), 2.45 (q, 2 H, *J* = 7.4 Hz, CH_2), 5.10 (s, 2 H, OCH_2), 5.20 (t, 2 H, *J* = 2.1 Hz, C_5H_4), 5,87 (t, 2 H, *J* = 2.1 Hz, C_5H_4), 6.49 (d, 2 H, *J* = 8.5 Hz, C_6H_4), 6.64 (d, 2 H, *J* = 8.5 Hz, C_6H_4), 6.70 (d, 2 H, *J* = 8.5 Hz, C_6H_4), 6.96 (d, 2 H, *J* = 8.5 Hz, C_6H_4), 6.98 (d, 2 H, *J* = 8.6 Hz, C_6H_4), 7.15 (d, 2 H, *J* = 8.6 Hz, C_6H_4), 8.08 and 8.15 (s, s, OH, OH). ^{13}C NMR (75.5 MHz, CD_3COCD_3): δ 14.0 (CH_3), ≅ 29.8 (CH_2, overlap with CD_3COCD_3), 71.5 (OCH_2), 85.5 and 88.3 (CH, C_5H_4), 90.0 (C_{ip}, C_5H_4), 114.2, 115.1, 115.6, 115.8, 131.3, 131.6 and 132.7 (CH_{arom}), 134.3, 135.8, 138.2, 138.3, 141.3, 156.1, 156.5 and 157.7 (C_q of 3 C_6H_4 and C═C), 193.3 (C═O), 223.0 (MnCO).

Isomerization

E-**2** was dissolved in acetone and left for 4 days at room temperature. The proportion between *E*/*Z* isomers reached a value of 58/42, determined by NMR: ^{1}H NMR (300 MHz, CD_3COCD_3) of *Z*-**2** from the (*E* + *Z*)-**2** spectrum: δ 0.87 (t, 3 H, *J* = 7.4 Hz, CH_3), 2.41 (q, 2 H, *J* = 7.4 Hz, CH_2), 4.94 (s, 2 H, OCH_2), 5.16 (t, 2 H, *J* = 2.1 Hz, C_5H_4), 5.81 (t, 2 H, *J* = 2.1 Hz, C_5H_4), 6.65 (d, 2 H, *J* = 8.8 Hz, C_6H_4), 6.65 (d, 2 H, *J* = 8.8 Hz, C_6H_4), 6.81 (d, 2 H, *J* = 8.8 Hz, C_6H_4), 6.82 (d, 2 H, *J* = 8.4 Hz, C_6H_4), 6.95 (d, 2 H, *J* = 8.8 Hz, C_6H_4), 7.03 (d, 2 H, *J* = 8.4 Hz, C_6H_4), 8.17 and 8.31 (s, s, OH, OH). ^{13}C NMR (75.5 MHz, CD_3COCD_3): δ 14.0 (CH_3), ≅ 29.8 (CH_2, overlap CD_3COCD_3), 71.5 (OCH_2), 85.5 and 88.3 (CH, C_5H_4), 90.0 (C_{ip}, C_5H_4), 114.2, 115.1, 115.6, 115.8, 131.3, 131.6 and 132.7 (CH_{arom}), 134.3, 136.0, 138.2, 138.3, 141.4, 156.1, 156.5 and 157.3 (C_q of 3 C_6H_4 and C═C), 193.3 (C═O), 223.0 (MnCO). (*Z* + *E*)-**2**: m.p. 120 °C. IR (CH_2Cl_2, cm^{-1}): 2033 and 1951 (Mn(CO)), 1701 and1682 (CO), 1608 cm^{-1} (C═C). HRMS (TOF MS ESI, $C_{32}H_{24}MnO_7$: $[M–H]^-$) calcd: 575.0902; found: 575.0920.

Cell Culture and Cell Proliferation Assay

The breast adenocarcinoma cell lines MDA-MB-231 and MCF7 were obtained respectively from ATCC and Dr Matthias Kassack (Bonn, Germany). Cells were grown in RPMI medium supplemented with 10% fetal calf serum, in the presence of penicillin, streptomycin and fungizone in 75 cm^2 flask under 5% CO_2. Cells were plated in 96-well tissue culture plates in 200 µl medium and treated 24 h later with 2 µl stock solution of compounds dissolved in DMSO using a Biomek 3000 (Beckman-Coulter). Controls received the same volume of DMSO (1% final volume). After 72 h exposure, MTS reagent (Promega) was added and incubated for 3 h at 37 °C; absorbance was monitored at 490 nm and results expressed as the inhibition of cell proliferation calculated as the ratio [(1-(OD490 treated/OD490 control)) × 100] in triplicate experiments. For IC_{50} determination (50% inhibition of cell proliferation), cells were incubated for 72 h following the same protocol with compound concentrations ranging from 5 nM to 100 µM in separate duplicate experiments.

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