Antiestrogenically Active 2-benzyl-1,1-diarylbut-2-enes: Synthesis, Structure-Activity Relationships and Molecular Modeling Study for Flexible Estrogen Receptor Antagonists

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Abstract: The nonsteroidal antiestrogen drug tamoxifen is the endocrine treatment of choice for estrogen receptor positive breast cancer, while the related estrogen receptor antagonist raloxifene is an effective therapeutic intervention for osteoporosis. We report the development of a series of hydroxylated 2-benzyl-1,1-diarylbut-2-enes containing a flexible core scaffold structure differing from the 1,1,2-triarylethylene typical of tamoxifen analogues. In this novel structure, a benzylic methylene group acts as a flexible hinge linking the aryl ring C and the ethylene group. The target products were synthesized using a McMurry coupling (titanium tetrachloride/zinc mediated) procedure. In this study, introduction of hydroxyl, ether and ester substitution on ring C was explored in an attempt to correlate possible metabolic activation in Ring C with antiproliferative activity. These Ring C substituted products showed potent antiproliferative activity against the MCF-7 human breast cancer cell line. The compounds were also shown to have high binding affinity for the estrogen receptor (IC_{50} values in the low nanomolar range) together with up to 17 fold selectivity for ERα/β.Some compounds demonstrated antiestrogenic activity in the Ishikawa cells at 40 nM without estrogenic stimulation. The products also displayed a pro-apoptotic effect in MCF-7 cells in a flow cytometry based assay.

In a computational study, docked structures of the active compounds were compared with the X-ray crystal structures for the complexes of ERαwith 4-hydroxytamoxifen and ERβ with raloxifene. The novel ligands are predicted to bind to the ERα and ERβ in an antiestrogenic orientation, with expected differences obtained in the alignment of the benzylic ring C within the ligand binding domain.

Key Words: Estrogen receptor modulators, antiestrogens, structure activity relationships, anticancer drugs.

INTRODUCTION

The estrogen receptor regulates a number of disease processes such as estrogen receptor positive breast cancers, osteoporosis, cardiovascular disease and also controls menopausal symptoms [1,2]. The development of new therapeutic agents capable of modulation of ER physiological function can provide unique understanding of the complex mechanism of action of tissue selectivity and agonist antagonist function of ER ligands [3,4,5]. Selective estrogen receptor modulators (SERMs) as represented by tamoxifen (**1a**) and raloxifene (**2**) display selective agonist or antagonist action on tissues such as breast and bone which express the estrogen receptor, Fig. (**1**). These drugs offer useful options in the clinical treatment of hormone related diseases such as osteoporosis and breast cancer [6]. Tamoxifen and toremiphene are clinically effective as antiestrogens by competing with estradiol at the estrogen binding site in target breast tumour tissue. A number of related SERM drugs such as lasofoxifene [7], arzoxifene [8], EM652 [9] and GW5638 (**1c**) [10] together with the pure antiestrogen fulvestrant [11] are under investigation in various clinical trials for the treatment of breast cancer, osteoporosis and other indications. There is ongoing interest in the beneficial effects of SERMs on the cardiovascular system together with continued speculation on a possible role of SERMs in cognition and memory function.

Protein X-Ray crystallography studies have been completed for a number of ER-ligand complexes, e.g. ERα in antagonistic mode with 4-hydroxytamoxifen (**1b**) [12], raloxifene [13], the pure antiestrogen IC164384 [14] and the acrylic acid GW5638 [15] while the structure of ERβ in antagonistic mode with raloxifene has also been determined [16]. The structural studies for these SERMs demonstrate a characteristic estrogen receptor conformation for each ligand complex. The specific nature of ligand binding to the ligand binding domain (LBD) of the ER can be determined, resulting in reorientation of the helix 12 thus preventing the recruitment of the coregulator proteins necessary for the activation of downstream transcription processes.

Many structurally varied ligands such as bisphenols [17], isoquinolines [18], benzoxepines [19], quinolines e.g. (**3**) [20], naphthalenes e.g. (**4**) [21], furans [22], and ferrocene ligands related to resveratrol [23] have been investigated for ERα and ERβ modulation properties. Ligands for the orphan

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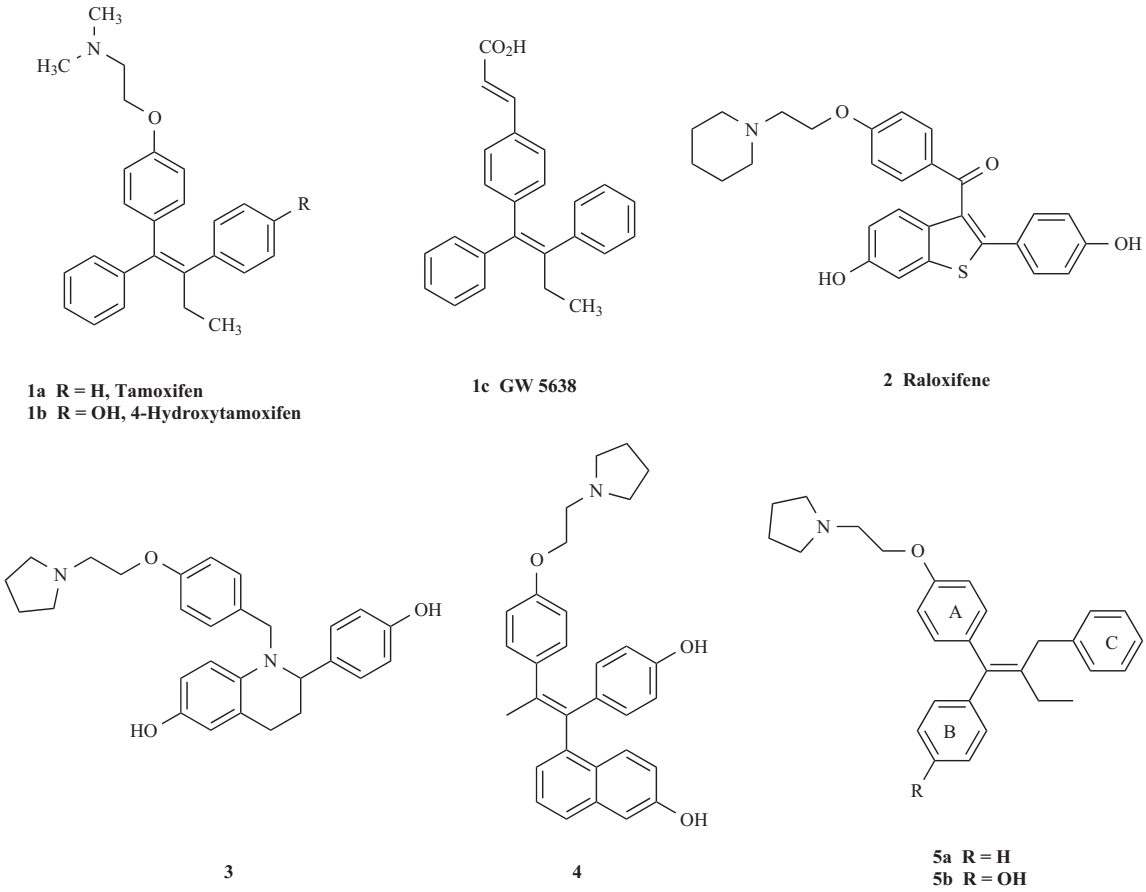


Fig. (1). Structures of SERMs and related compounds.

estrogen related receptor subtypes ERRβ and γ have recently been identified [24]. In our continuing studies on the rational design of ER ligands with improved antiproliferative properties, we have identified the flexible triphenylethylenes such as **5a** and **5b** as lead structures in the design of ligands with ER modulation properties, Fig. (**1**) [25,26]. To optimize the antiproliferative activity and ER binding ability of this series of these compounds, hydroxylation of rings B and C was explored.

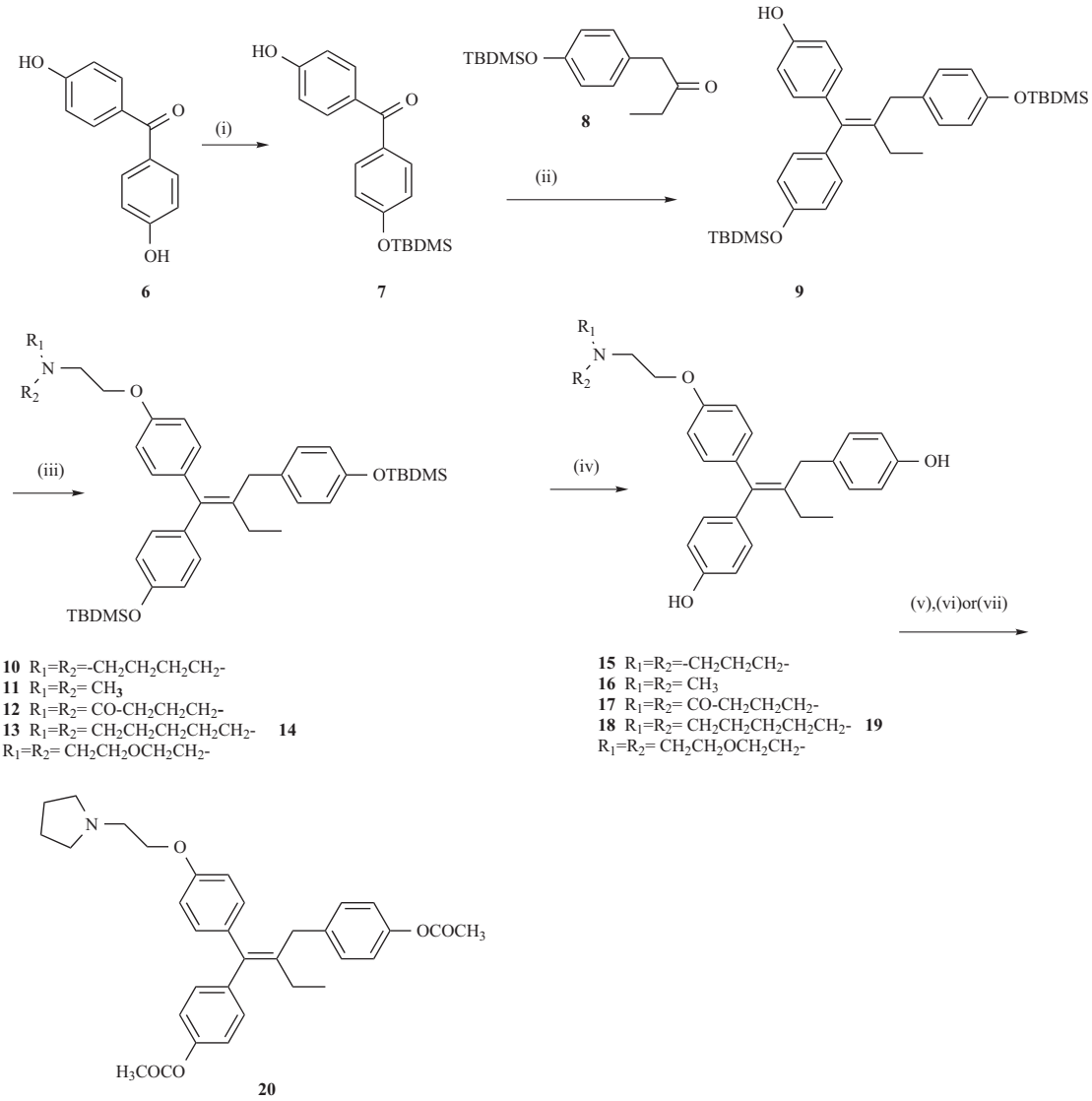
In this work, we report the development of a series of hydroxylated 2-benzyl-1,1-diarylbut-2-enes which contain a flexible core scaffold structure differing from the 1,1,2-triarylethylene typical of tamoxifen analogues. In this novel structure, a benzylic methylene group acts as a flexible hinge linking the aryl ring C and the ethylene group. In the raloxifene structure, the carbonyl group also functions as a similar molecular hinge, between the benzothiophene and the piperidine substituted aromatic Ring A. The introduction of the lipophilic pivaloyl ester [9,27] as an active prodrug of the hydroxylated compounds was also investigated as a method of improving the oral bioavailability and circumventing the rapid metabolic glucuronidation and elimination of potent phenolic ER modulating ligands. The pivaloyl ester is known to be hydrolysed slowly in intact cells to release active phenolic ligands [28].

The antiproliferative and antiestrogenic properties of the new Ring B and C substituted products prepared is determined by appropriate human MCF-7 breast cancer cell-based biochemical assays and competitive binding experiments with recombinant human estrogen receptors ERα and ERβ. The interactions of the new antiestrogenic products with the LBD of the estrogen receptors α and β can be examined through computational modeling with the reported resolved crystal structures of the estrogen receptor α and β. The predicted orientation and interaction of selected compounds within the LBD of the human ERα and ERβ is examined The ability of the ERα and ERβ to accommodate these structurally diverse Ring B and C substituted ligands is demonstrated together with the potential ability of these flexible ligands to demonstrate modulation of the estrogen receptor.

CHEMISTRY

The design of the initial group of compounds (**15-19**) contain free hydroxyl groups in both rings B and C, together with a number of structurally varied basic ether substituents

on ring A. A McMurry reductive carbonyl coupling reaction with titanium tetrachloride/zinc was effective in all cases for the synthesis of the required alkene products from the appropriate carbonyl compounds as illustrated in Scheme **1**, [29,30,31]. The required monoprotected benzophenone (**7**) was obtained by treatment of the dihydroxybenzophenone (**6**) with *tert*butyldimethylsilyl chloride and imidazole in DMF; the ketone (**8**) was similarly obtained from 1-(4-hydroxyphenyl)butan-2-one. Reaction of benzophenone (**7**) with the protected ketone (**8**) afforded the alkene (**9**) which from examination of the ^{1}H NMR spectrum was obtained as a single *E* isomer; confirmation of the stereochemistry was possible by examination of the NOE spectrum. It has been demonstrated that the McMurry coupling of phenolic benzophenones usually results in the formation of the predominantly *trans* product arrangement of the phenolic aryl substituent relative to the ethyl vinylic group, [25,26,31]. The phenol (**9**) was then treated with the appropriate amino alcohol under standard Mitsunobu conditions (triphenylphosphine and diisopropylazidodicarboxylate, DIAD) to afford the basic ethers (**10-14**) in good yield. The products were identified as single isomers in the case of compounds (**10, 11, 13, 14**); E/Z mixture was observed for compound (**12**). The removal of the TBDMS protecting group from



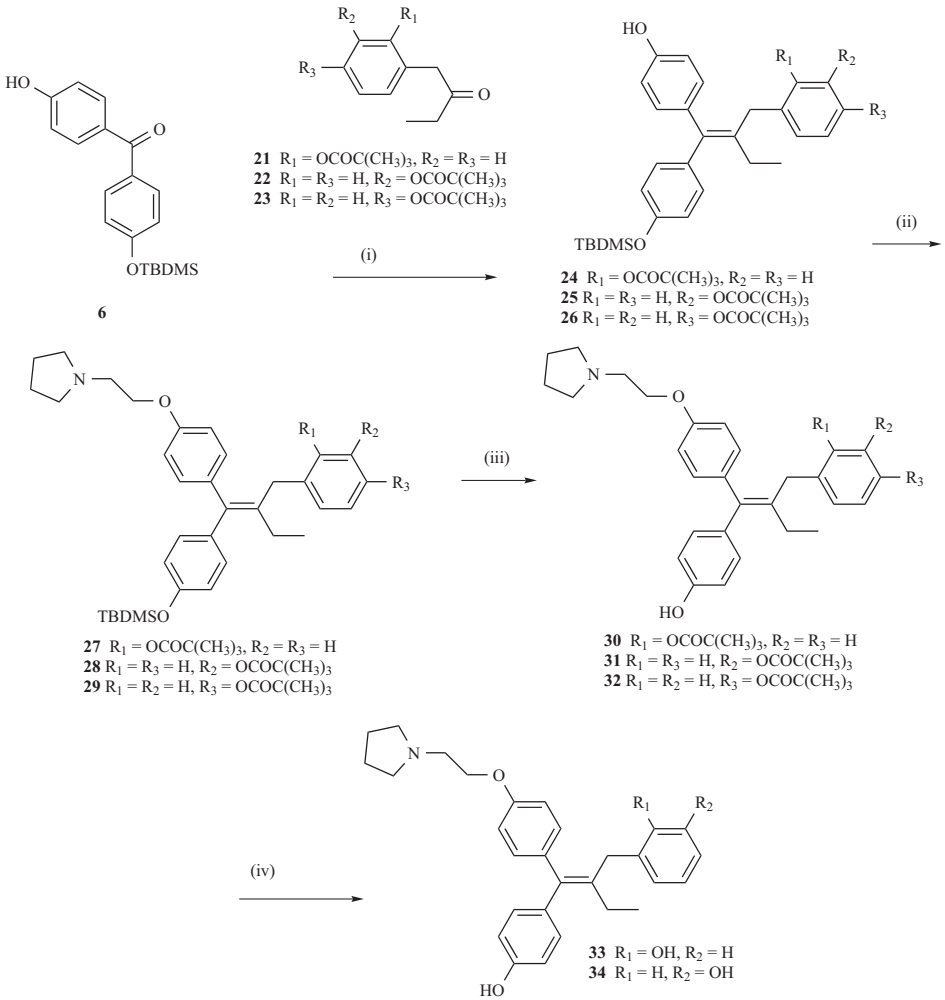
Scheme reagents and conditions: (i) TBDMSCl, imidazole, DMF (ii) $TiCl_4$, Zn, THF, reflux (iii) $R_1R_2NCH_2CH_2Cl$, K_2CO_3, Acetone/H_2O or $R_1R_2NCH_2CH_2OH$, DIAD, PPh_3, CH_2Cl_2 (iv) $(n\text{Bu})_4NF$, THF (v) $(CH_3CO)_2O$, pyridine

Scheme 1.

compounds (**10-14)** was achieved by treatment with TBAF and the phenolic products (**15-19**) were obtained. The yields and isomeric mixture composition obtained are displayed in Table **1**. Partial conversion of the initially formed phenolic Z isomer to the corresponding E isomer following removal of the protecting group is a characteristic feature of these aryl substituted alkenes. The *Z/E* isomer identity and composition of the final products (**15-19**) was determined based on the relative chemical shifts of the Ring A aryl proton signals of the A_2B_2 *para* substituted system and also on the relative chemical shifts of the OCH_2 signal of the basic side chain together with the benzylic CH_2 signal [25,26]. The diacetate ester (**20**) was prepared from (**15**) by treatment with acetic anhydride and pyridine.

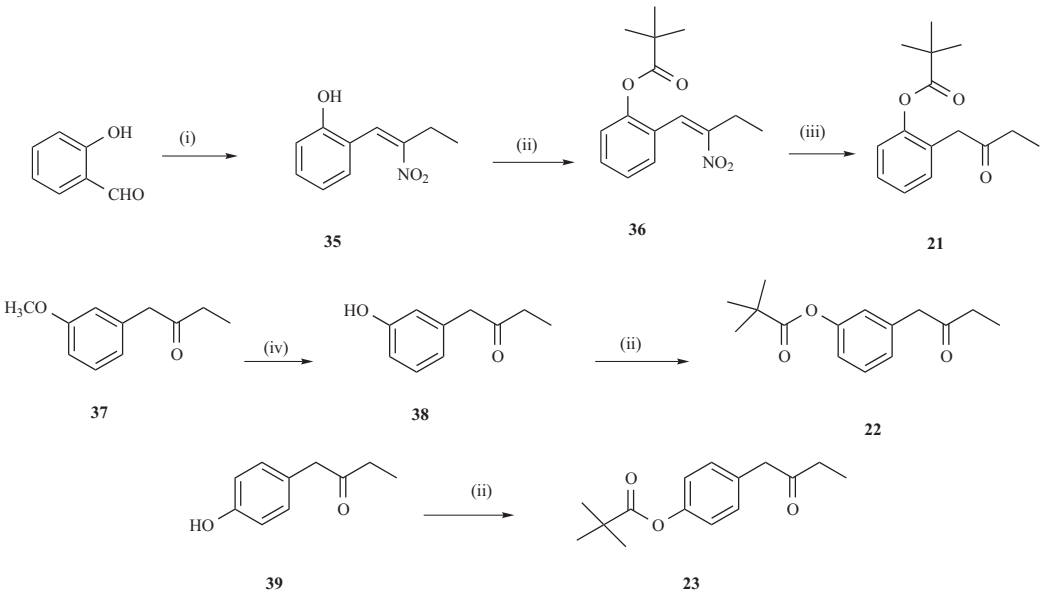
The pivaloyl esters (**30-32**) were prepared by a similar route as illustrated in Scheme **2**. It is reported that the pivaloyl esters are more stable to hydrolysis than simple esters under physiological conditions, and so these esters might be considered as prodrugs of the phenolic compounds (**15),** (**33**) and (**34**). The monoprotected benzophenone (**6**) was reacted under reductive conditions with the appropriately substituted 1-aryl-2-butanones (**21**)**,** (**22**) or (**23**) to afford the protected alkene esters (**24-26).** Subsequent alkylation of the phenols under standard Mitsunobu conditions with the amino alcohol 1-(2-hydroxyethyl)pyrrolidine and DIAD afforded the basic products (**27-29**) which could be easily deprotected with TBAF to yield the phenolic products (**30-32**) as *Z* isomers exclusively. In the case of compounds (**30**) and (**31**), it was also possible to hydrolyse the pivaloyl ester group in base to obtain the corresponding diphenolic products (**33**) and (**34**), (Scheme **2**).

The 1-aryl-2-butanones (**21**)**,** (**22**) and (**23**) were prepared using modifications of the literature procedures for 1-phenyl-2-butanones as illustrated in Scheme **3**. Reaction of salicylaldehyde with 1-nitropropane under Henry conditions afforded the *ortho*-hydroxynitrostyrene (**35**) which was then



Scheme reagents and conditions: (i) $TiCl_4$, Zn, THF, reflux (ii) $R_1R_2NCH_2CH_2OH$, DIAD, PPh_3, CH_2Cl_2 (iii) $(n\text{Bu})_4NF$, THF (iv) NaOH, $EtOH/H_2O$

Scheme 2.

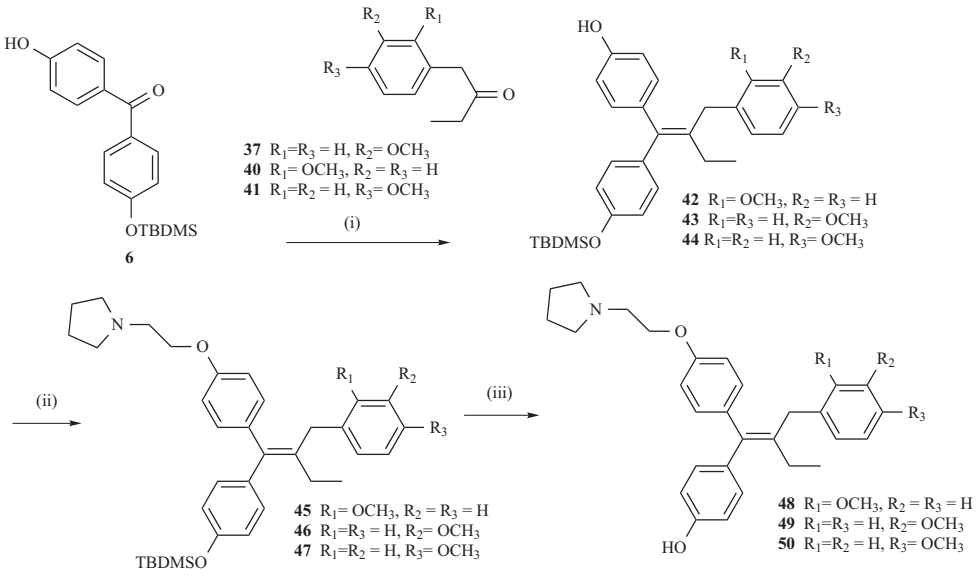


Scheme reagents and conditions: (i) $CH_3CH_2CH_2NO_2$, $(CH_3)_2NH$, KF, Toluene, reflux (ii) $(CH_3)_3COCl$, Et_3N, DMAP (iii) Fe, CH_3COOH, 100°C (iv) $BF_3.Me_2S$, CH_2Cl_2

Scheme 3.

converted to the ester (**36**) with pivaloyl chloride and DMAP. Reduction to the ketone (**21**) was achieved by treatment with iron powder in glacial acetic acid. The most convenient route to the *meta*-substituted ketone (**22**) was found to be *via* the 1-(3-methoxypheny)-2-butanone (**37**) which could be demethylated using boron trifluoride dimethylsulfide complex to afford the phenol (**38**) which could be converted to the ester (**22**) by reaction with pivaloyl chloride. The ester (**23**) was similarly obtained from the 1-(4-hydroxyphenyl)-2-butanone (**39**).

The methoxy ethers (**48-50**) were obtained in a similar fashion by initial reductive coupling of the benzophenone (**51**) with the 1-methoxyphenyl-2-butanones (**37**), (**40**) and (**41**) to afford the alkene products (**42**-44) as the E isomer. (Scheme **4**). Alkylation of the phenols with N-hydroxyethyl-

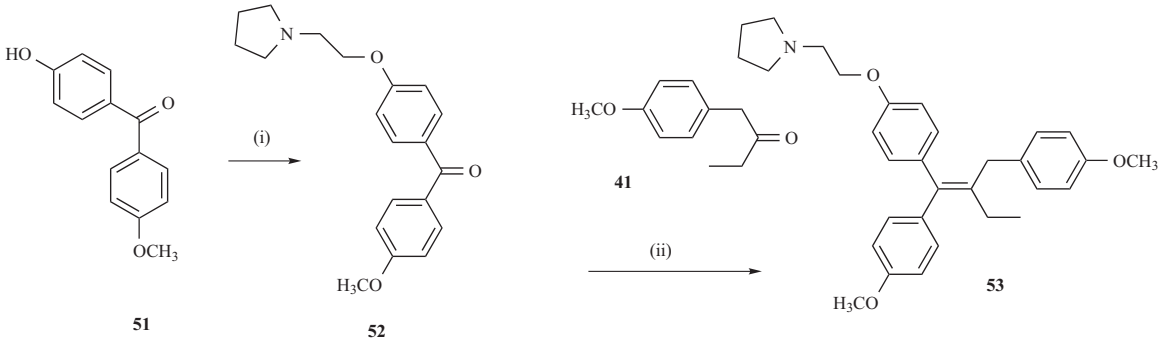


Scheme reagents and conditions: (i) $TiCl_4$, Zn, THF, reflux (ii) $R_1R_2NCH_2CH_2OH$, DIAD, PPh_3, CH_2Cl_2 (iii) $(n\text{Bu})_4NF$, THF

Scheme 4.

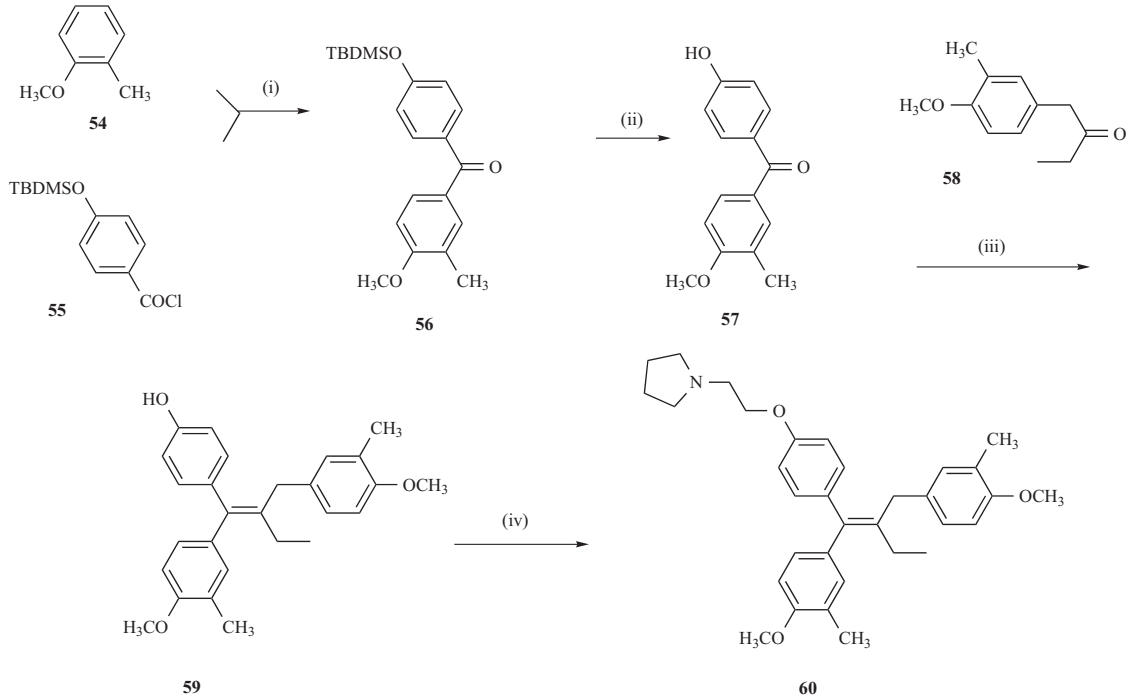
pyrrolidine under the standard Mitsunobu conditions and subsequent deprotection of the TBDMS ethers (**45-47**) afforded the required phenols (**48-50**) in moderate yield; (**49**) and (**50**) being isolated as single Z isomers while(48) was obtained as a 1:1 E/Z mixture. The dimethoxy alkene (**53**) was obtained in a variation of the synthetic route as illustrated in Scheme **5**. The pyrrolidine side chain was first introduced onto the 4-hydroxy-4'-methoxybenzophenone (**6**) to give (**52**) under standard Mitsunobu conditions. Subsequent McMurry coupling of (52) with the 1-(4-methoxyphenyl)-2-butanone (**41**) yielded the dimethoxyalkene (**53**) as a single Z isomer.

Access to the more substituted hindered 2-benzyl-1,1-diarylbut-2-enes (**60**) was also completed as outlined in Scheme **6**. The synthetic route involved Friedel Crafts reaction of 2-methylanisole (**54**) with the acid chloride (**55**). The resulting benzophenone silyl ether (**56**) was then deprotected to give the phenol (**57**) which was subsequently reacted with the 1-(4-methoxy-3-methylphenyl)-2-butanone (**58**) under reductive coupling conditions to afford the phenol (**59**). The required product (**60**) was obtained by alkylation of (**59**) and was isolated as a single *Z* isomer. The yields and E/Z ratios for all final products are provided in Table **1**.



Scheme reagents and conditions: (i) $R_1R_2NCH_2CH_2OH$, DIAD, PPh_3, CH_2Cl_2 (ii) $TiCl_4$, Zn, THF, reflux

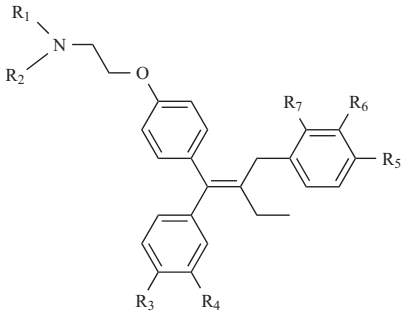
Scheme 5.



Scheme reagents and conditions: (i) $SnCl_4$, TCE (ii) *tert*-Bu_4NF, THF (iii) $TiCl_4$, Zn, THF, reflux (iv) $R_1R_2NCH_2CH_2OH$, DIAD, PPh_3, CH_2Cl_2

Scheme 6.

Table 1. Yield, Isomeric Ratio Data and Mean IC_{50} Values for Antiproliferative Effects on Human MCF-7 Breast Cancer Cell Line[a,b] for Compounds 15-20, 30-34, 48-50, 53, 60



Compound	Yield (%)	Isomer ratio[c] *Z:E*	IC_{50}(μM)
15	87	71:29	45.9
16	76	74:26	7.98
17	72	68:32	9.36
18	84	69:32	30.1
19	33	64:36	35.1
20	62	29:71	10.6
30	87	51:49	0.697
31	77	65:35	14.7
32	54	50:50	1.09
33	41	63:37	20.1
34	36	69:31	14.5
48	59	53:47	4.93
49	55	>100:1	5.19
50	33	>100:1	0.873
53	60	>100:1	36.3
60	43	>100:1	12.0

[a]The values are an average of at least 9-12 replicate experiments. Errors in each case are less than+/-15%.

[b]The value recorded for tamoxifen(**1a**) in this work is in good agreement with IC_{50} values in other works using the MTT assay in human MCF-7 cells[26,44].

[c]Isomer ratio Z:E determined from integration of benzylic methylene signals in ^{1}H NMR spectrum.

BIOCHEMISTRY: INHIBITION OF PROLIFERATION OF HUMAN BREAST CANCER MCF-7 CELLS

The clinically useful antiestrogen tamoxifen is known to achieve its antiproliferative effects through a number of mechanisms including ER modulation, cytotoxic effects and induction of apoptosis. The compounds prepared were initially evaluated for inhibition of proliferation of the human breast cancer MCF-7 cell line using the standard MTT assay to determine their chemotherapeutic potential as outlined in the Experimental section. The results are displayed in Table **1**.

The following structural compound classes were considered:

1. Rings B and C both containing hydroxyl substituents (**15-19), (33,34**)
2. Rings B and C both containing acetate ester substituents (**20**)
3. Ring B containing hydroxyl substituents and Ring C containing pivaloyl ester substituents (**30-32**)
4. Ring B containing hydroxyl substituents and Ring C containing methoxy ether substituents (**48-50**)

5. Rings B and C both containing methoxy ether substituents (**53**)

6. Rings B and C both containing methoxy and methyl substituents (**60**)

The initial group of compounds investigated (**15-19**) all contain phenolic groups at the *para* positions of both aromatic Rings B and C. The basic side chains examined include the following types: N,N-dimethyl, pyrrolidinyl, piperidinyl, morpholinyl, and pyrrolidin-2-onyl. The IC_{50} values for this series were found to be in the range 7.98-45.9μM. In previous work for related flexible compounds in which Ring C was unsubstituted, we had found that the pyrrolidinyl basic group provided the optimum antiproliferative activity. Surprisingly, in the case of the group 1 compounds, the dimethyl and pyrrolidin-2-one compounds (**16**) and (**17**) were found to be the most active (IC_{50} 7.98 and 9.36 μM respectively), the dihydroxylated compounds having increased activity over the lead compound (**5**), (IC_{50} 12.5μM), but showing some reduction in activity over the corresponding Ring B monohydroxylated compounds. The pyrrolidinyl basic group in (**15**) was found to produce an unexpected decrease in antiproliferative activity (IC_{50} 45.9μM), with similar poor activities also observed for compounds (**18**) and (**19**). The positioning of the second phenolic group at the *meta* and *ortho* positions of Ring C in compounds (**33**) and (**34**) resulted in an increase in antiproliferative activity observed. The diacetate ester (**20**) also showed improvement in activity over the corresponding diphenolic compound (**15**) indicating that the requirement for such substitution is not always necessary for optimum antiproliferative activity. The activity of the Ring C pivaloyloxy esters (**30**), (**31**) and (**32**) was next examined. These esters were considered to be sterically hindered and would be expected to be slowly hydrolysed *in vivo* and so could be considered as prodrugs for the release of the free phenolic compounds [9,27]. Surprisingly, the *meta*-pivaloyloxy ester (**31**) showed antiproliferative activity with almost the same potency as the related phenolic compound (**34**). Potent antiproliferative activity was displayed by the *ortho* and *para* substituted Ring C pivaloyloxy esters (**30**) and (**32**)**,** with IC_{50} 0.697 and 1.09μM respectively, with an increase of 20 to 40 fold over the activity of the corresponding diphenolic comounds (**33**) and (**15**) respectively. Ring C *ortho*, *meta* and *para* substituted methoxy ethers (**48**), (**49**) and (**50**) were all potent compounds with the *para* substituted compound (**50**) being the most potent with activity of IC_{50} 0.873μM which is almost 50 fold more potent than the corresponding free phenolic compound (**15**), while compounds (**48**) and (**49**) displayed considerable activity, with IC_{50} values of 4.93 and 5.19μM respectively; again 3-4 times more active than the corresponding free phenolic compounds, (**33**) and (**34**). Introduction of the methyl ether functions onto both Rings B and C as in compound (**53**) resulted in only slight improvement in activity over the diphenolic compound (**15**), indicating that the Ring B phenolic group was essential for activity while the free phenol on Ring C was not as critical for antiproliferative activity. The introduction of methyl groups at the *meta* positions of both Rings B and C was achieved in compound (**60**) and this compound showed moderative antiproliferative activity(IC_{50} 12.0μM) indicating that some sterically hindered substitution can be tolerated in both of these rings.

The inhibition of proliferation and cytotoxicity profiles for the most active compounds (**30**) and (**50**) are displayed in Fig. **2**.

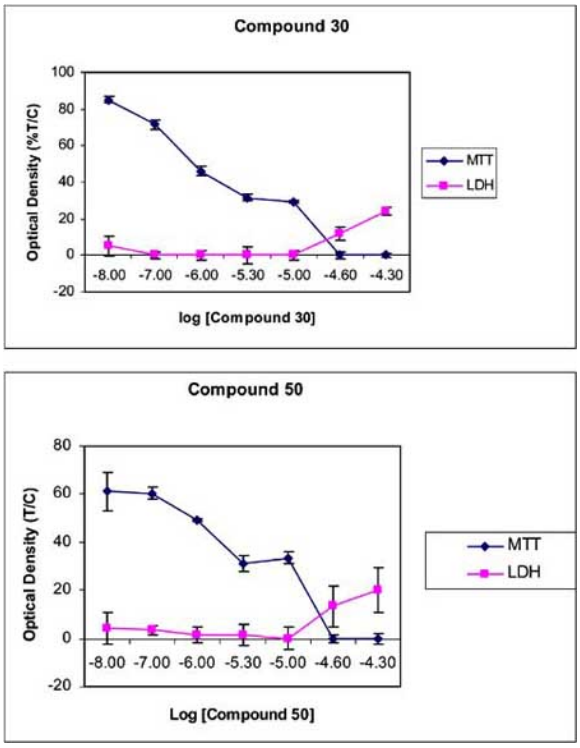


Fig. (2). Antiproliferative and cytotoxic activity of compounds **30** and **50** on estrogen sensitive MCF-7 breast cancer cells . The optical density values are given as a ratio of the treated cells and control cells x100 and are means of 9-12 replicates. The absence of error bars indicates that the error was smaller than the size of the symbol.

CYTOTOXICITY PROFILE

The cytotoxicity of all of the above compounds was determined using the standard LDH assay as we wished to confirm that the antiproliferative potency of the new investigational compounds was attributed to cytostasis rather than promoting cellular necrosis. In the LDH assay, the compounds displayed low cytotoxicity profiles (Fig. **2**), suggesting their mechanism of action to be cytostatic rather than cytotoxic. For example for compound (**12**), the cytotoxicity observed at 10μM was 12%, when compared to tamoxifen, which displays a cytotoxicity value of 24% at 10μM. The cytotoxicity values obtained were less than the value for tamoxifen for all compounds except compound (**50**)(18% at 5μM).

ESTROGEN RECEPTOR BINDING STUDIES

Estrogen receptor binding studies with ERα and ERβ were carried out for the most potent antiproliferative compounds in the series, i.e. compounds **(15),(16),(17),(30),**

(**32**), (**58**), (**59**), (**60**). A fluorescence polarization procedure was used to determine the displacement of fluorescein labeled estradiol (fluoromone) in a competitive binding assay from the human recombinant full length receptor proteins ERα and ERβ expressed from baculovirus –infected insect cells [32,33]. The observed polarization values decrease with the addition of the competitor compounds and the 50% inhibition values (IC_{50}) are calculated from the resultant sigmoidal inhibition curves as illustrated in Table **2**, Fig. (**3**) and Fig. (**4**). The compounds (**16**), (**17**), (**30**), (**32**) and (**50**)were shown to exhibit an IC_{50} binding values for ERα of less than 140nM, with compound (**17**) containing hydroxylated rings B and C together with the pyrrolidin-2-one type basic side chain substituent on ring A being the most potent having an IC_{50} binding value of 15nM for ERα. This value is favourably comparable with the ERα binding result obtained in this assay for tamoxifen (IC_{50} 72nM) and 4-hydroxytamoxifen(IC_{50} 40nM). The presence of the phenolic substituent on Ring C as demonstrated by compounds (**30**), (**32**) and (**50**) (IC_{50} for ERα 81, 95 and 44nM respectively) is not essential for potent binding of these compounds. These binding studies are also broadly consistent with the antiproliferative activity of these compounds observed in the MCF-7 study in which compounds (**30**), (**32**) and (**50**) demonstrate IC_{50} values of less than 1.09 μM. The affinity for the ERβ for this series of compounds was found to be in the region 0.15-9.24μM with most of the compounds clearly showing selectivity for the ERα, (Table **2**, Fig. (**4**)).The highest binding values for the ERβ was also found for compound (**17**) (IC_{50} 150nM) which is very similar to that obtained for tamoxifen (IC_{50} 169nM) and clearly indicated the requirement of the flexible structures for hydroxylation of both aromatic B and C rings for both α and β ER binding to be most potent. Ligand selectivity for ERα and ERβ allows targeting of potential therapeutic agents, [3,34,43]. The ERα/β ligand binding ratio for the compounds evaluated is presented in Table **3** and it is observed that with the exception of compound (**48**) containing an *ortho* methoxy ether substituent in Ring C, there is a selectivity for ERα binding for the ligands studied. The greatest ERα/β selectivity in the series was apparent for compound (**30**) (ERα/β 17.0:1) while compounds (**15**), (**50**), (**17**), (**32**) and (**16**) displayed considerable selectivity, with ERα/β binding ratios of 15.7:1, 11.5:1, 10.1:1, 7.21:1 and 5.86 respectively. The selectivity of these compounds for ERα is considerably greater than the values obtained for tamoxifen and hydroxytamoxifen (ERα/β 2.35:1 and 1:1.67 respectively) and is comparable to typical ERα/β selectivities reported for many ER antagonists e.g. those based on ferrocene [23], and isoquinoline [18] type structures. Compound (**48**) containing *ortho*-methoxy substituent on ring C was the only example in the series which displayed a slight reversal in selectivity for ERα(ERα/β 1:1.40). ERβ selectivity for a number of structurally diverse ligands has been reported e.g. aryl diphenolic azoles and 1,3,5-triazines. [35,36].

The relationship between antiproliferative activity and ERα binding affinity for these flexible ligands is evident from the results obtained above and the inclusion of large lipophilic esters located at the *ortho* or *para* positions in ring C such as in compounds (**30**) and (**32**) might be useful and is of relevance in the rational design of related flexible ER modulators which would be specific for ERα.

ESTROGENIC STIMULATION

The estrogen stimulating and antagonistic properties of the most potent series of the flexible products, compounds

Table 2. Estrogen Receptor Binding Data for Compounds 15, 16, 17, 30, 32, 48-50.

Compound Number	ER Binding assay IC_{50}[a] (μM)		ERα: β Ligand Binding ratio
	α	β	
15	0.588	9.238	15.7:1
16	0.141	0.826	5.86:1
17	0.015	0.151	10.1:1
30	0.081	1.379	17.0:1
32	0.095	0.685	7.21:1
48	0.732	0.524	1:1.40
49	0.223	0.371	1.66:1
50	0.044	0.506	11.5:1
1a	0.072	0.169	2.35:1
1b	0.040	0.024	1:1.67

[a]Values are an average of at least nine replicate experiments for ERα and six replicate experiments for ERβ with typical standard errors below 15%.

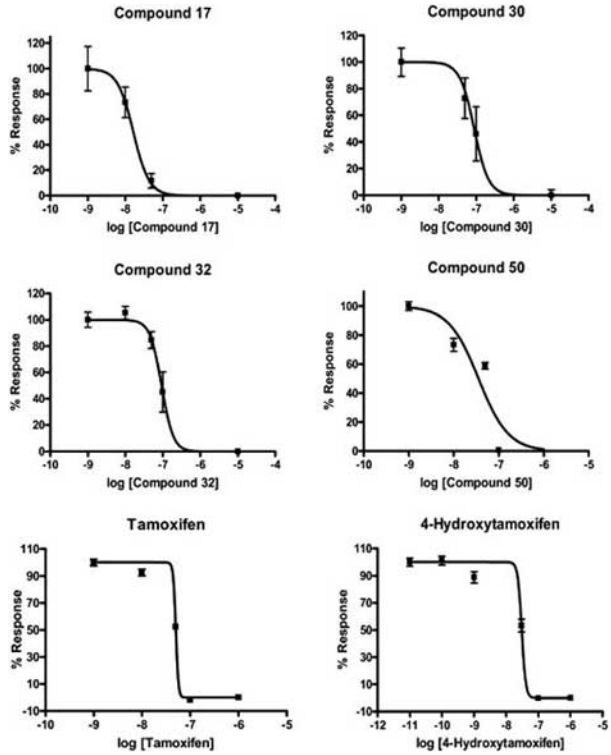


Fig. (3). Estrogen Receptor alpha binding data for compounds **17**, **30**, **32** and **50** together with data for tamoxifen and 4-hydroxytamoxifen and are means of 9-12 replicates. The absence of error bars indicates that the error was smaller than the size of the symbol.

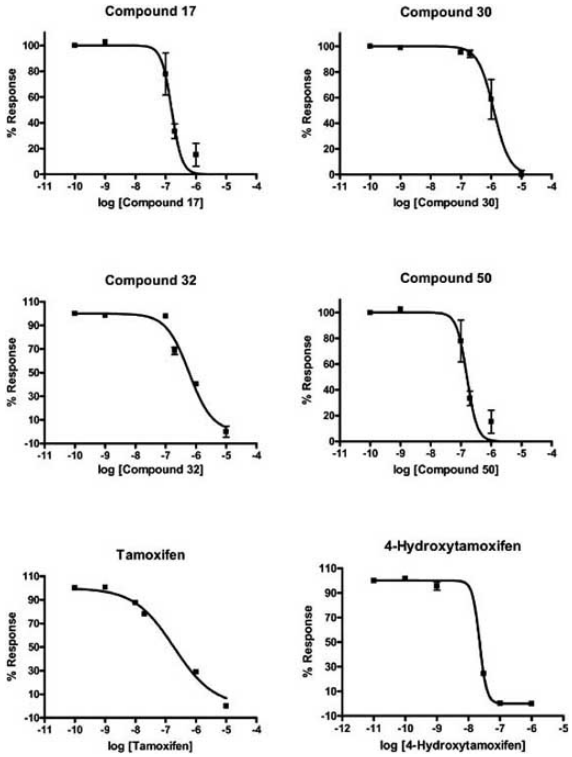


Fig. (4). Estrogen Receptor beta binding data for compounds **17**, **30**, **32** and **50** together with data for tamoxifen and 4-hydroxytamoxifen and are means of 9-12 replicates. The absence of error bars indicates that the error was smaller than the size of the symbol.

Table 3. Antiestrogenic, Estrogenic and Apoptotic Activity for Compounds 15, 16, 17, 30, 32, 48-50.

Compound Number	Antiestrogenic Activity Ishikawa $IC_{50}(\mu M)$	Estrogenic Activity Ishikawa[a] (% stimulation)	Apoptotic Activity MCF-7 Sub-G1(%)[b]
15	0.24 ± 0.1	2	8.30
16	4.86 ± 0.0	0	7.60
17	2.34 ± 0.1	1	4.20
30	0.21 ± 0.1	2.5	18.5
32	0.01 ± 0.0	5	18.3
48	0.14 ± 0.2	10	13.2
49	0.23 ± 0.2	0	21.1
50	1.15 ± 0.1	4.0	22.2
1a	0.17 ±0.0	4	20.5

[a]Relative stimulator activity in comparison with estradiol E2 = 100.

[b]Values are an average of at least twelve replicate experiments with standard errors below 15%.

(**15**),(**16**),(**17**),(**30**), (**32**), (**58**), (**59**), (**60**) were evaluated in an estrogen bioassay which is based on the sensitive stimulation of alkaline phosphatase (AlkP) in the Ishikawa human endometrial adenocarcinoma cell line in which estrogen stimulation of AlkP can be demonstrated as low as 10^{-12}M, [37]. Antiestrogenic activity of potential ER antagonists were determined by inhibition of estradiol stimulation in the Ishikawa cells in a dose dependent manner. The results of the study are summarized in Table **3**. Compound (**32**) containing the *para*-phenolic substituent in Ring B and *para*-pivaloyloxy ester substituent in Ring C together with the pyrrolidine basic side chain in ring A displayed the most potent activity of the series as an estrogen antagonist with an IC_{50} value of 10nM; this compound is considerably more active as an estrogen antagonist than tamoxifen (IC_{50} 170nM). This result also correlates with the potent ERα binding observed for this compound. Compounds (**15**), (**30**), (**48**) and (**49**) also demonstrated good antiestrogenic activity with IC_{50} values in the range 140-240nM. The estrogenic stimulatory properties of these compounds could be determined in the Ishikawa cells by measuring the stimulation of alkaline phosphatase (AlkP) in the absence of estradiol. Many of the compounds showed a low level of stimulation of estradiol, with compounds (**16**) and (**49**) having a zero level of stimulation. The value for tamoxifen was 4%. Compound (**30**) which was the most potent compound in the antiproliferative assay showed a low level of stimulation (2.5%) as illustrated in Fig. (**5**) and Fig. (**6**). Compound (**17**), the most potent compound in the ERα and β binding assay was also found to exhibit a negligible stimulatory effect of 1%.The results from this AlkP assay for estrogen antagonism and stimulation are useful in selecting the appropriate arrangement of structural features in this series of compounds required for optimum antiestrogenic activity without associated adverse estrogenic effect on tissues such as uterus.

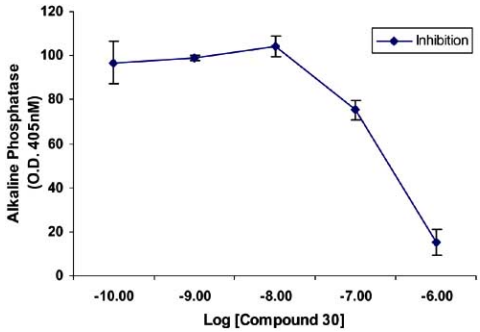


Fig. (5). Effect of increasing concentrations of compound (**30**) on alkaline phosphatase activity in human Ishikawa cells. Alkaline phosphatase activity was measured after a 4 day exposure to increasing concentrations of antiestrogen compound (**30**) in the presence of 10^{-9}M estradiol. The data is expressed as the means +/- SEM of quadruplicates. The absence of error bars indicates that the error was smaller than the size of the symbol.

APOPTOSIS

In order to further investigate the mechanism of action of these flexible estrogen receptor modulators, their ability to modulate apoptosis in MCF-7 cells was measured by determining the cleavage of poly-adenosine diphosphate ribose polymerase(PARP) [38,39,40]. The DNA repair enzyme PARP is known to be a substrate for the apoptosis effector enzyme caspase 9; cleavage of PARP confirms that apoptosis has been induced by the test compounds in the MCF-7 cell line *via* caspase activation; tamoxifen has been shown to induce the process of apoptosis [40,41,42]. PARP cleavage is determined using Western Blot analysis by the disappearance of the 116kDa PARP protein band and the simultaneous appearance of the 87kDa cleavage product. Changes in the distribution of the of cell cycle phases G1, S,

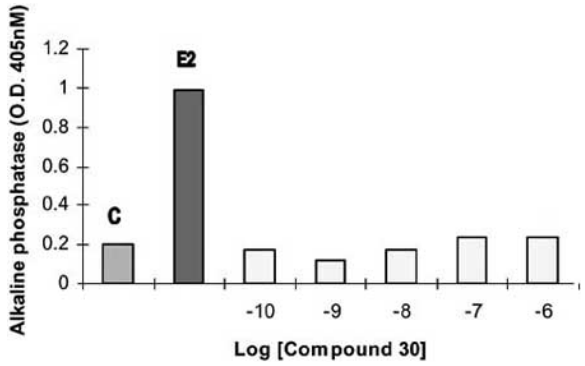


Fig. (6). Effect of increasing concentrations of compound (**30**) on alkaline phosphatase activity in human Ishikawa cells together with data from control **C** (grown without estradiol)and cells grown with 10^{-9}M estradiol **E2**. Alkaline phosphatase activity was measured after a 4 day exposure to increasing concentrations of antiestrogen compound (**30**) in the absence estradiol. The data is expressed as the means +/-SEM of quadruplicates. The absence of error bars indicates that the error was smaller than the size of the symbol.

G2/M together with the sub-G1 apoptotic peak are measured and the results for the more active of the antiproliferative compounds are illustrated in Table **3**. It is evident that compounds (**30**), (**32**), (**48**), (**49**) and (**50**) which are the most active in the antiproliferative assay, also demonstrate considerable apoptotic activity with apoptotic values of 13-22% observed at 50μM after 24h. These results are comparable with the activity determined for known antiestrogenic compounds such as tamoxifen (20.5%) in this assay and confirm that in addition to the antiproliferative mechanism of action of these novel flexible products these compounds also exhibit pro-apoptotic activity which is possibly independent of the ER.

COMPUTATIONAL STUDIES

To rationalize the observed biological activity of the most potent ligands (compounds (**17**), (**30**), (**32**) and (**50**), a computational investigation was undertaken to quantitatively examine the interactions established by the ligands in both isoforms of the ER (α and β). We employed a technique involving receptor conformer generation using FIRST5 software [45] in combination with FRODA recently developed by Thorpe [46]. FIRST determines flexible regions of the protein by assigning topological bars that describe the nature of all the bonds present in the protein (covalent, H-bond, salt bridge, hydrophobic tethers). The network of bars are then analysed *via* graph-theoretical algorithm to provide input for FRODA on whether a bond will participate in a dihedral angle rotation or not. Ghost templates extracted from information about the rigid sections of the protein guide the movement of the flexible areas within FRODA. Noteworthy, to reduce the possibility of steric clashes being present, it is critical that the initial crystal structures used are highly resolved (~ ≤ 2Å).

For this reason, the crystal structure of 4-hydroxytamoxifen co-crystallised with ERα (3ERT) [12] and raloxifene with ERβ (1QKN)[16] were utilized in this study. FRED2.11 [47] was used to dock conformers of the series of ligands generated by Omega1.8.1 [48] into each conformer of the receptor and scored with Chemgauss 2. The top ranked pose over all of the receptor and ligand conformers for each ligand were selected and atomic interactions were analysed by Ligand Protein Contacts (LPC) software [49]. The residues depicted are those that have been previously shown to be crucial in the binding process: Asp351 (interacts with the basic side-chain nitrogen) [57,58], Glu353 and Arg394 (anchor the ligand in the active site), His524 (additionally important in ligand binding process) [50]. Table **4** illustrates

Table 4. Summary of Key Ligand-Protein Contacts[a]

Compd	Isoform	Asp 351 (Asp 258)	Glu 353 (Glu 260)	Arg 394 (Arg 301)	His 524 (His 430)	NC	Chemgauss2
17	α	3.9	3.2	4.1	3.2	0.89	-52.84
17	β	3	3.5	5.5	2.4	0.69	-51.22
30	α	2.8	2.4	3.2	3	0.91	-50.48
30	β	3.2	3.2	5.1	4.1	0.72	-47.65
32	α	3.1	2.3	1.8	5.5	0.76	-54.91
32	β	4.3	5.3	---	---	0.61	-51.22
50	α	3.3	3.9	1.8	5.1	0.9	-56.21
50	β	4.1	3.1	4.9	6.4	0.8	-43.08
OHT	α	3.2	2.4	3	4	0.89	---
RAL	β	3.3	2.6	3	2.6	0.69	---

[a]Data provided as nearest distance (Å) between atoms of ligand and the residue. Residues depicted are those present in crystal structure 3ERT, except those in brackets denoting residues of crystal structure 1QKN. OHT, 4-Hydroxytamoxifen from PDB entry 3ERT; RAL, Raloxifene from PDB entry 1QKN; NC, Normalised Complementarity; Chemgauss2, score attributed to the top ranked solution.

the interactions made by each ligand with both receptor isoforms.

As detailed in Table **4** compounds (**17**), (**30**), (**32**) and (**50**) dock in a typical antiestrogenic manner when compared with OHT and RAL. Fig. (**7**) (A)/(B) clearly illustrate a similar binding mode for compound (**30**) in both receptor isoforms, with only the benzylic pivaloxy moiety differing in position. In addition, the binding data for compound **(17)** clearly confirms the role of the Ring C, showing interaction distances from the para-hydroxy group to His524 (His430) of 3.2 Å (2.4 Å) respectively (Table **4**).

Another feature of this series is their inherent isoform selectivity with compound (**30**) for example, possessing 17-fold preference in binding to ERα over ERβ. Table **5** also depicts the calculated Normalised Complementarity (NC) for each top docked solution, which illustrates the 'buriedness' of a molecule within an active site of a protein. What is evident from the docking studies is that the NC value is always lower for ERβ than ERα reflecting the selectivity observed in ER binding results, (Table **2**). Corroborating this, the Chemgauss2 scores obtained were consistently more negative in the alpha active site versus the beta site.

To further investigate the reasons for the selectivity, the active site of the top ranked solution for compound (**30**) docked in both isoforms was visually examined. Fig. (**7**) shows the two positions of the pivaloxy group substituent attached at the ortho position of the C-ring of compound (**30**) when docked in ERα and β. The movement of flexible residues Ile424, Phe425, His524 and mainly Met342 of ERα compared with Ile331, Phe332, His430 and Met250 of ERβ prevent the pivaloxy substituent from adopting a position directed away from the active site. This small lipophilic cavity allows a dramatic difference in interactions with His524 and Arg394 to be achieved if occupied as observed in Table **4** and corroborated by binding affinity data. Taking the twenty receptor conformers of 3ERT and 1QKN, we determined by an svl script written for MOE.2005.06 [51] the degree of motion by rmsd of Ile, Phe and Met for each receptor. This showed that Ile424, Phe425 and Met342 could move 1.18, 0.86 and 1.57 Å respectively in ERα. However, in ERβ, Ile331, Phe332 and Met250 could move by 3.80, 3.16 and 1.88 Å. This indicated that this particular lipophilic binding cleft was far more restricted in ERα, allowing the benzylic moiety to adopt a different position and holding it in a more favourable position. In agreement with this, Amari

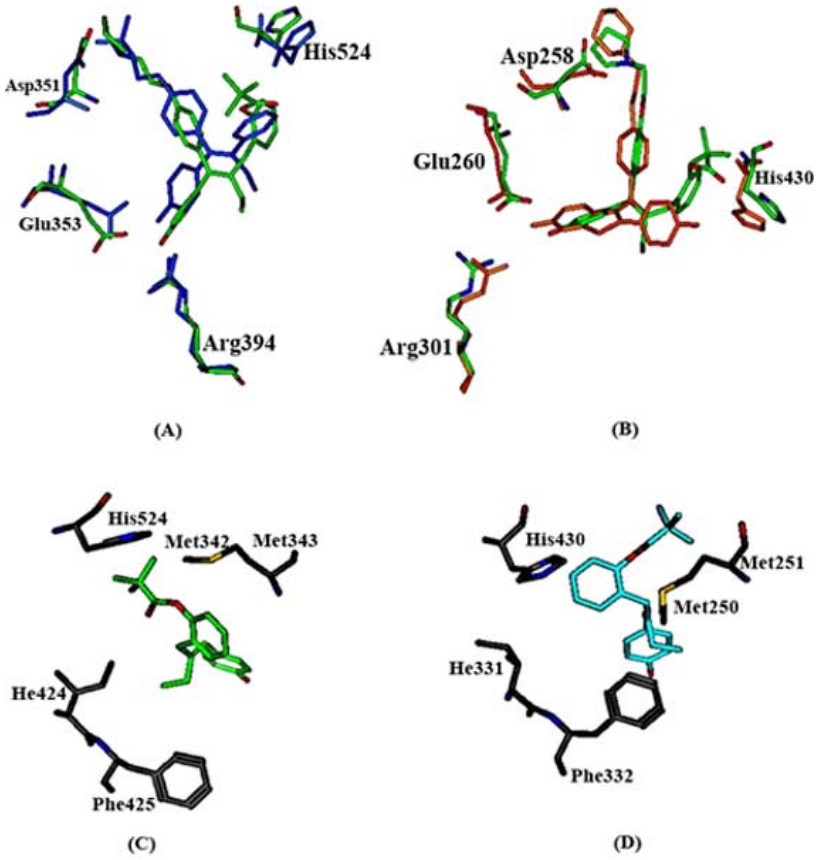


Fig. (7). Top ranked docking solution of compound (**30**) (coloured by atom) superimposed by backbone on 3ERT (blue) (A) and 1QKN (orange) (B). (C) Depicts residues of ERα that form a small lipophilic cavity preventing rotation of the benzylic pivaloxy group away from the active site. (D) Phenyl ring of Phe332 is projected inwards into active site with Met250 further enclosing the section.

et al. also recently recognized the presence of a small lipophilic cavity maintained by Ile424 (Ile376) and His524 (His475) in both receptor isoforms that could accommodate a small substituent [52]. However, the involvement of Met342 (Met250) in the process was not described. Importantly, this cavity difference would not be recognised without incor-porating receptor flexibility into the procedure and exemplifies the necessity to apply both ligand and receptor flexibility to accurately assess binding modes of newly synthesized ligands.

CONCLUSIONS

The design and synthesis of a series of conformationally flexible 2-benzyl-1-phenyl-1-[4-(pyrrolidinylethoxy)phenyl] but-2-enes is investigated. These compounds display potent antiproliferative activity when evaluated against the MCF-7 breast cancer cell line and also show low cytotoxicity, indicating their mode of action to be cytostatic. A number of the products demonstrated high ER binding affinity together with up to 17 fold ERα/β selectivity. One of the products displayed an antiestrogenic effect at 10nM when evaluated in the Ishikawa cell line with negligible estrogenic stimulation. The most potent products were also shown to induce some apoptosis in MCF-7 cells in a flow cytometry based assay. The potential ability of these compounds to inhibit estrogen mediated ERE promoter based transcription using a luciferase reporter assay will be investigated in the future to determine their biochemical mechanism of action. A computational docking study was carried out on the most active ligands which demonstrates how these compounds selectively bind to residues in the LBD of the ERα and ERβ in an antiestrogenic mode. These results further demonstrate the structural possibilities for selective ER ligand binding, particularly in the tolerance in hydrophobic substitution on Ring C, and the potential utility of these flexible modulators for ER positive breast cancers.

ACKNOWLEDGEMENTS

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Experimental

All reagents used were commercial grade chemicals from freshly opened containers. IR spectra were recorded as thin films on NaCl plates on a Perkin-Elmer Paragon 100 FT-IR spectrometer. ^{1}H and ^{13}C NMR spectra were obtained on a Bruker Avance DPX 400 instrument at 20°C, 400.13MHz for ^{1}H spectra, 100.61MHz for ^{13}C spectra, in either $CDCl_3$ (internal standard tetramethylsilane) or CD_3OD. All J values are quoted in Hz. Low resolution mass spectra were run on a Hewlett-Packard 5973 MSD GC–MS system in an electron impact mode, while high resolution accurate mass determinations for all final target compounds were obtained on a Micromass Time of Flight mass spectrometer (TOF) equipped with electrospray ionization (ES) interface operated in the positive ion mode at the High Resolution Mass Spectrometry Laboratory in the Department of Chemistry, Trinity College Dublin. Flash chromatography was carried out using standard silica gel 60 (230-400 mesh) obtained from Merck. All products isolated were homogenous on TLC. Analytical HPLC work was performed on a chromatographic system comprising a Waters 501 pump (flow-rate 2 ml/min / sample loop 20 µl) and a Waters Spherisorb® S5 ODS2 (4.6x250mm) reversed phase C18 analytical column. Detection was on a Waters 486 Tunable Absorbance Detector with λ=241, chart recorder speed at 1/6 cm per min. The mobile phase used was prepared from HPLC grade solvents and comprised ACN:H_2O:THF:18M NH_3 Buffer 30:12.5:7.5:2 respectively. Retention times are given in minutes. Unless otherwise stated all reactions were carried out under a nitrogen atmosphere. Additional spectroscopic details for the products are supplied as supplementary information. The biochemical methodologies employed for the antiproliferative, cytotoxicity, estrogen stimulation and estrogen receptor binding studies are described in previous publications [25,26] and are available in the supplementary information.

1-[4'-(*tert*-Butyldimethyl-silanyloxy)-phenyl]-butan-2-one (8)

1-(4'-Hydroxy-phenyl)-butan-2-one (**7**) (1.32 g, 8.05 mmol) and imidazole (711 mg, 10.5 mmol) were dissolved in DMF (10 ml). *tert*-butyldimethylsilyl chloride (1.455 g, 9.66 mmol) was added in 6 portions over 4 hours. Stirring was continued at room temperature for a further 10 hours. The reaction mixture was diluted with ethyl acetate (150 ml) and quenched with 10% HCl (20 ml). The organic layer was separated, washed with water (40 ml) and brine (40 ml) and dried over Na_2SO_4. The solvent was removed under reduced pressure and the residue was chromatographed on silica gel (hexane: diethyl ether = 20:1) to yield the product **8** (1.54 g, 69%) as a colourless oil. IR: ν_{max} ($CHCl_3$) cm^{-1}: 2944, 2922, 2880(CH), 1708.3 (C=O), 1606 (C=O). ^{1}H-NMR (400MHz, $CDCl_3$): δ 0.21 (s, 6H, $(CH_3)_2$-Si), 1.00 (s, 9H, $(CH_3)_3C$), 1.04 (t, 3H, J = 7.5 Hz, CH_3), 2.47 (q, 2H, J = 7.5 Hz, CH_2), 3.62 (s, 2H, CH_2-Ar), 6.81 (d, 2H, J = 8.0 Hz, H-3', H-5'), 7.08 (d, 2H, J = 8.0 Hz, H-2', H-6'). HRMS (EI): Found 279.1762 ($M+H^+$), $C_{16}H_{27}O_2Si$ requires 279.1780.

4-{2-[4-(*tert*-Butyldimethylsilanyloxy)-benzyl]-1-[4-(*tert*-butyldimethylsilanyloxy)-phenyl]-but-1-enyl}-phenol (9)

Zinc powder (602 mg, 9.26 mmol) was placed in a 3-necked flask into which dry THF (10 ml) was added under nitrogen. Titanium tetrachloride (509 µl, 4.63 mmol) was slowly added *via* syringe over 5 min in the dark. The reaction mixture was maintained at reflux for 1.5 hours, after which time the benzophenone **6** [26] (430 mg, 1.22 mmol) and 1-[4'-(*tert*-butyldimethylsilanyloxy)-phenyl]-butan-2-one **8** (235 mg, 1.22 mmol) in THF (10 ml) were added *via* a syringe. After a further 4 hours at reflux, the solution was cooled to room temperature, diluted with ethyl acetate (50 ml) and quenched with 10% K_2CO_3 solution (20 ml).

Following filtration and separation, the aqueous layer was extracted with ethyl acetate (3 x 40 ml). The combined organic layers were washed with 10% K_2CO_3 solution (20 ml), water (20 ml) and brine (20 ml) and dried over Na_2SO_4. The solvent was removed under reduced pressure and the residue was chromatographed on silica gel (dichloromethane: methanol = 10:1) to yield the product **9** as a colourless oil (87%) which was used in the following reaction without further purification. LRMS (*m/z*): 575 (M^++H, 38%). IR: ν_{max} ($CHCl_3$) cm^{-1}: 3421.1 (OH), 3029, 2944, 2880, 2858 (CH), 1601 (C=C), ^{1}H-NMR (400MHz, $CDCl_3$): δ 0.24 (s, 12H, 2 x $(CH_3)_2$-Si), 0.97 (t, 3H, *J* = 7.5 Hz, CH_3), 1.03 (s, 18H, 2 x $(CH_3)_3$-C), 2.07 (q, 2H, *J* = 7.5 Hz, CH_2), 3.50 (s, 2H, CH_2-Ar), 6.73-6.81 (m, 6H, ArH), 7.06-7.11 (m, 6H, ArH).

1-[2-(4-{2-[4-(*tert*-Butyldimethylsilanyloxy)-benzyl]-1-[4-(*tert*-butyldimethyl silanyloxy)-phenyl]-but-1-enyl}-phenoxy)-ethyl]-pyrrolidine (10)

The phenol **9** (1.74 mmol) was stirred in dichloromethane (10 mL) with triphenylphosphine (3.48 mmol) and 1-(2-hydroxyethyl)pyrrolidine (5.22 mmol) at room temperature. Diisopropylazodicarboxylate (4.35 mmol) was added slowly over 20 minutes. The reaction was stirred for two days. then washed with ammonium chloride solution, extracted with dichloromethane and dried (sodium sulphate). The solvent was removed under reduced pressure to give a brown oil. The pure product was isolated as a colourless oil in 87% yield following chromatography on silica gel (dichloromethane: methanol = 15:1). IR: ν_{max} ($CHCl_3$) cm^{-1}: 3023, 2948, 2915, 2861, 2861 (CH), 1602 (C=C), ^{1}H-NMR (400MHz, $CDCl_3$): δ 0.21 (s, 12H, 2 x $(CH_3)_2$-Si), 0.95 (t, 3H, *J* = 7.5 Hz, CH_3), 0.99 (s, 9H, $(CH_3)_3$-C), 1.00 (s, 9H, $(CH_3)_3$-C), 1.85 (m, 4H, CH_2), 2.04 (q, 2H, *J* = 7.5 Hz, CH_2), 2.69 (m, 4H, $(CH_2)_2$N), 2.95 (m, 2H, CH_2N), 3.47 (s, 2H, CH_2-Ar), 4.13 (m, 2H, CH_2O), 6.72-6.87 (m, 6H, ArH), 7.04-7.13 (m, 6H, ArH). HRMS (EI): Found 672.4252 (M^++H), $C_{41}H_{62}NO_3Si_2$ requires 672.4268.

[2-(4-{2-[4-(*tert*-Butyldimethylsilanyloxy)-benzyl]-1-[4-(*tert*-butyldimethyl-silanyloxy)-phenyl]-but-1-enyl}-phenoxy)-ethyl]-dimethyl-amine (11)

11 was prepared from **9** and *N,N*-dimethylethanolamine in the manner described for **10** above. The pure product was isolated as a colourless oil in 49% yield following chromatography on silica gel (dichloromethane: methanol = 10:1). IR: ν_{max} ($CHCl_3$) cm^{-1}: 3025, 2944, 2922, 2858, 2762(CH), 1606 (C=C), ^{1}H-NMR (400MHz, $CDCl_3$): δ 0.21 (s, 12H, 2 x $(CH_3)_2$-Si), 0.95 (t, 3H, *J* = 7.5 Hz, CH_3), 1.00 (s, 9H, $(CH_3)_3$-C), 1.01 (s, 9H, $(CH_3)_3$-C), 2.05 (q, 2H, *J* = 7.5 Hz, CH_2), 2.35 (s, 3H, CH_3N), 2.37 (s, 3H, CH_3N), 2.75 (m, 2H, CH_2N), 3.48 (s, 2H, CH_2-Ar), 4.07 (m, 2H, CH_2O), 6.72-6.88 (m, 6H, ArH), 7.04-7.13 (m, 6H, ArH). HRMS (EI): Found 646.4160 (M^++H): $C_{39}H_{60}NO_3Si_2$ requires 646.4112.

1-[2-(4-{2-[4-(tert-Butyldimethylsilanyloxy)-benzyl]-1-[4-(*tert*-butyldimethyl-silanyloxy)-phenyl]-but-1-enyl}-phenoxy)-ethyl]-pyrrolidin-2-one (12)

12 was prepared from **9**and 1-(2-hydroxyethyl)-2-pyrrolidinone in the manner described for **10** above. The pure product was isolated as a colourless oil in 66% yield following chromatography on silica gel (dichloromethane: methanol = 10:1). IR: ν_{max} ($CHCl_3$) cm^{-1}: 3025, 2942, 2921, 2858 (CH), 1732, 1690, 1601.9 (C=C), ^{1}H-NMR (400MHz, $CDCl_3$): δ 0.21 (s, 12H, 2 x $(CH_3)_2$-Si), 0.95 (t, 3H, *J* = 7.5 Hz, CH_3), 0.99 (s, 9H, $(CH_3)_3$-C), 1.00 (s, 9H, $(CH_3)_3$-C), 1.97-2.08 (m, 4H, 2 x CH_2), 2.37 (t, 2H, *J* = 8.0 Hz, CH_2CO), 3.47 and 3.48 (2 x s, 2H, CH_2-Ar), 3.56 (t, 2H, *J* = 7.0 Hz, CH_2N), 3.66 (t, 2H, *J* = 5.0 Hz, CH_2N), 4.09 (t, 2H, *J* = 5.0 Hz, CH_2O), 6.73-6.82 (m, 6H, ArH), 7.04-7.13 (m, 6H, ArH). HRMS (EI): Found 688.4208 ($M+H^+$), $C_{41}H_{62}NO_4Si_2$ requires 688.4217.

1-[2-(4-{2-[4-(*tert*-Butyldimethylsilanyloxy)-benzyl]-1-[4-(*tert*-butyldimethyl-silanyloxy)-phenyl]-but-1-enyl}-phenoxy)-ethyl]-piperidine (13)

13 was prepared from **9** and 1-piperidine ethanol in the manner described for **10** above. The pure product was isolated as a colourless oil in 67% yield following chromatography on silica gel (dichloromethane: methanol = 10:1). IR: ν_{max} ($CHCl_3$) cm^{-1}: 3025, 2932, 2928, 2785(CH), 1738, 1601 (C=C). ^{1}H-NMR (400MHz, $CDCl_3$): δ 0.19 (s, 6H, $(CH_3)_2$-Si), 0.21 (s, 6H, $(CH_3)_2$-Si), 0.95 (t, 3H, *J* = 7.5 Hz, CH_3), 0.98 (s, 9H, $(CH_3)_3$-C), 1.01 (s, 9H, $(CH_3)_3$-C), 1.47 (m, 2H, CH_2), 1.63 (m, 4H, CH_2), 2.05 (q, 2H, *J* = 7.5 Hz, CH_2), 2.55 (m, 4H, $(CH_2)_2$N), 2.80 (t, 2H, *J* = 6.0 Hz, CH_2N), 3.48 (s, 2H, CH_2-Ar), 4.12 (t, 2H, *J* = 6.0 Hz, CH_2O), 6.72-6.86 (m, 6H, ArH), 7.04-7.13 (m, 6H, ArH). HRMS (EI): Found 686.4448 (M^++H), $C_{42}H_{54}NO_3Si_2$ requires 686.4425.

4-[2-(4-{2-[4-(tert-Butyldimethylsilanyloxy)-benzyl]-1-[4-(*tert*-butyldimethyl-silanyloxy)-phenyl]-but-1-enyl}-phenoxy)- ethyl]-morpholine (14)

14 was prepared from **9** and 4-(2-hydroxyethyl) morpholine in the manner described for **10** above. The pure product was isolated as a colourless oil in 47% yield following chromatography on silica gel (dichloromethane: methanol = 20:1). IR: ν_{max} ($CHCl_3$) cm^{-1}: 2954, 2925, 2858 (OH), 1601(C=O). ^{1}H-NMR (400MHz, $CDCl_3$): δ 0.21 (s, 12H, 2 x $(CH_3)_2$-Si), 0.94 (t, 3H, *J* = 7.5 Hz, CH_3), 0.99 (s, 9H, $(CH_3)_3$-C), 1.00 (s, 9H, $(CH_3)_3$-C), 2.04 (q, 2H, *J* = 7.5 Hz, CH_2), 2.59 (m, 4H, (2 x CH_2N), 2.80 (t, 2H, *J* = 5.8 Hz, CH_2N), 3.47 (s, 2H, CH_2-Ar), 3.75 (t, 4H, *J* = 4.5 Hz, $(CH_2)_2$O), 4.09 (t, 2H, *J* = 5.8 Hz, CH_2O), 6.72-6.85 (m, 6H, ArH), 7.03-7.13 (m, 6H, ArH). HRMS (EI): Found 688.4208 (M^++H) $C_{41}H_{62}NO_4Si_2$ requires 688.4217.

4-{2-(4-Hydroxy-benzyl)-1-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-but-1-enyl}-phenol (15)

Tetrabutylammonium fluoride (1M, 6.73 ml) was added to **10** (2.0 g, 5.61 mmol) in THF (50 ml) and stirred for 10 min at room temperature. The reaction mixture was diluted with ethyl acetate (200 ml) and quenched with 10% HCl (50 ml). The organic layer was separated and washed with water (50 ml) and brine (50 ml) and dried over Na_2SO_4. The solvent was removed under reduced pressure and the pure product was isolated as a colourless oil in 87% yield following chromatography on silica gel (dichloromethane: methanol = 10:1). HPLC**:** t_R = 3.2, 3.3 min. IR: ν_{max} ($CHCl_3$) cm^{-1}: 3277, 2963, 2921(OH), 1607(C=C). ^{1}H-NMR (400MHz, $CDCl_3$): δ 0.96 (t, 3H, *J* = 7.5 Hz, CH_3), 1.92 (m,

4H, CH_2), 2.02 (q, 2H, *J* = 7.5 Hz, CH_2), 2.94 (m, 4H, $(CH_2)_2N$), 3.33 (m, 2H, CH_2N), 3.42 and 3.43 (2 x s, 2H, CH_2-Ar), 4.16 (m, 2H, CH_2O), 6.70-6.74 (m, 4H, ArH), 6.87-6.89 (m, 2H, ArH), 6.98-7.14 (m, 6H, ArH). HRMS (EI): Found 444.2526 (M^++H), $C_{29}H_{34}NO_3$ requires 444.2539.

4-[1-[4-(2-Dimethylaminoethoxy)-phenyl]-2-(4-hydroxy-benzyl)-but-1-enyl]-phenol (16)

16 was prepared from **11** in the manner described for **15** above. The pure product was isolated as a colourless oil in 76% yield following chromatography on silica gel (dichloromethane: methanol = 10:1). HPLC: t_R = 2.6 min. IR: ν_{max} ($CHCl_3$) cm^{-1}: 3340, 2952, 2921, 2868 (CH), 1601 (C=C). ^{1}H-NMR (400MHz, $CDCl_3$): δ 0.94 (t, 3H, *J* = 7.5 Hz, CH_3), 2.03 (q, 2H, *J* = 7.5 Hz, CH_2), 2.38 (s, 6H, $(CH_3)_2N$), 2.68 (m, 2H, CH_2N), 3.42 and 3.43 (2 x s, 2H, CH_2-Ar), 4.07 (t, 2H, *J* = 5.3 Hz, CH_2O), 6.70-6.73 (m, 4H, ArH), 6.84-6.89 (m, 2H, ArH), 6.98-7.12 (m, 6H, ArH). HRMS (EI): Found 418.2437 (M^++H), $C_{27}H_{32}NO_3$ requires 418.2382.

1-(2-{4-[2-(4-Hydroxybenzyl)-1-(4-hydroxyphenyl)-but-1-enyl]-phenoxy}-ethyl)-pyrrolidin-2-one (17)

17 was prepared from **12** in the manner described for **15** above. The pure product was isolated as a colourless oil in 72% yield following chromatography on silica gel (dichloromethane: methanol = 15:1). HPLC: t_R = 1.8 min. IR: ν_{max} ($CHCl_3$) cm^{-1}: 3193, 2967, 2924, 2870(C=C), 1663, 1604(C=C). ^{1}H-NMR (400MHz, $CDCl_3$): δ 0.77 (t, 3H, *J* = 7.5 Hz, CH_3), 1.81-1.89 (m, 4H, 2 x CH_2), 2.19 (t, 2H, *J* = 8.0 Hz, CH_2CO), 3.25 and 3.27 (2 x s, 2H, CH_2-Ar), 3.40 (t, 2H, *J* = 7.0 Hz, CH_2N), 3.49 (m, 2H, CH_2N), 3.92 (m, 2H, CH_2O), 6.58-6.63 (m, 6H, ArH), 6.82-6.96 (m, 6H, ArH). HRMS (EI): Found 458.2318 (M^++H), $C_{29}H_{32}NO_4$ requires 458.2331.

4-{2-(4-Hydroxybenzyl)-1-[4-(2-piperidin-1-yl-ethoxy)-phenyl]-but-1-enyl}-phenol (18)

18 was prepared from **13** in the manner described for **15** above. The pure product was isolated as a colourless oil in 84% yield following chromatography on silica gel (dichloromethane: methanol = 15:1). IR: ν_{max} ($CHCl_3$) cm^{-1}: 3131, 2967, 2921, 2863 (CH), 1602 (C=C). ^{1}H-NMR (400MHz, $CDCl_3$): δ 0.77 (t, 3H, *J* = 7.5 Hz, CH_3), 1.43-1.51 (m, 6H, 3 x CH_2), 1.86 (q, 2H, *J* = 7.5 Hz, CH_2), 2.53 (m, 4H, $(CH_2)_2N$), 2.75 (t, 2H, *J* = 5.5 Hz, CH_2N), 3.25 and 3.27 (2 x s, 2H, CH_2-Ar), 4.01 (t, 2H, *J* = 5.5 Hz, CH_2O), 6.61-6.68 (m, 6H, ArH), 6.81-6.95 (m, 6H, ArH). HRMS (EI): Found 458.2318 (M^++H), $C_{29}H_{32}NO_4$ requires 458.2331.

4-{2-(4-Hydroxybenzyl)-1-[4-(2-morpholin-4-yl-ethoxy)-phenyl]-but-1-enyl}-phenol (19)

19 was prepared from **14** in the manner described for **15** above. The pure product was isolated as a colourless oil in 33% yield following chromatography on silica gel (dichloromethane: methanol = 10:1). HPLC: t_R = 2.0 min. IR: ν_{max} ($CHCl_3$) cm^{-1}: 3412 (OH), 2956, 2874 (CH), 1602 (C=C). ^{1}H-NMR (400MHz, $CDCl_3$): δ 0.78 (t, 3H, *J* = 7.5 Hz, CH_3), 1.88 (q, 2H, *J* = 7.5 Hz, CH_2), 2.67-2.72 (m, 6H, 3 x CH_2N), 3.27 and 3.29 (2 x s, 2H, CH_2-Ar), 3.75 (m, 4H, $(CH_2)_2O$), 3.99 (m, 2H, CH_2O), 6.56-6.69 (m, 6H, ArH), 6.84-6.97 (m, 6H, ArH). HRMS (EI): Found 460.2515 (M^++H), $C_{29}H_{34}NO_4$ requires 460.2488.

Acetic acid 4-{3-(4-acetoxyphenyl)-2-ethyl-3-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-allyl}-phenyl ester (20)

15 (160 mg, 0.37 mmol) and triethylamine (104 µL, 0.75 mmol) were dissolved in THF (5 ml). Acetyl chloride (40 µL, 0.56 mmol) was added and stirring was continued for 30 min at room temperature. The reaction mixture was diluted with ethyl acetate (40 mL) and quenched with 10% HCl (10 ml). The organic layer was separated, washed with and brine (10 mL) and dried over Na_2SO_4. The solvent was removed under reduced pressure and the residue was chromatographed on silica gel (dichloromethane: methanol = 20:1) to yield the product **20** (62%) as a colourless oil. HPLC: t_R = 3.2, 4.0 min. IR: ν_{max} ($CHCl_3$) cm^{-1}: 3026, 2926, 2921, 2874, 2793 (OH), 1759 (C=O), 1602 (C=C). ^{1}H-NMR (400MHz, $CDCl_3$): δ 0.97 (t, 3H, *J* = 7.5 Hz, CH_3), 1.86 (m, 4H, CH_2), 2.06 (q, 2H, *J* = 7.5 Hz, CH_2), 2.29 (s, 3H, CH_3CO), 2.30 (s, 3H, CH_3CO), 2.74 (m, 4H, $(CH_2)_2N$), 2.97 (t, 2H, *J* = 5.5 Hz, CH_2N), 3.54 and 3.55 (2 x s, 2H, CH_2-Ar), 4.15 (t, 2H, *J* = 5.5 Hz, CH_2O), 6.82-6.88 (m, 2H, ArH), 7.00-7.21 (m, 10H, ArH). HRMS (EI): Found 529.2878 (M^++2H), $C_{33}H_{39}NO_5$ requires 529.2828.

2,2-Dimethylpropionic acid 2-(2'-oxobutyl)-phenyl ester (21)

A suspension of iron powder (18.346 g, 328 mmol) in glacial acetic acid (150 ml) was heated to 100°C for 20 min. **36** (13.60 g, 49.1 mmol) in glacial acetic acid (20 ml) was added over 10 min. The reaction was stirred for a further 2 hours at 100°C. The reaction mixture was cooled and added to ice-water (100 ml) and extracted with dichromethane (3 x 300 ml). The combined organic layers were washed with saturated sodium bicarbonate solution (100 ml), water (100 ml) and brine (100 ml) and dried over Na_2SO_4. The solvent was removed under reduced pressure and the residue was chromatographed on silica gel (hexane: diethyl ether = 4:1) to yield the product **21** (9.9 g, 81%) as a colourless oil which was used in subsequent reactions without further purification. IR: ν_{max} ($CHCl_3$) cm^{-1}: 2976, 2938, 2908, 2877(CH), 1749, 1715.8 (C=O). ^{1}H-NMR (400MHz, $CDCl_3$): δ 1.03 (t, 3H, *J* = 7.5 Hz, CH_3), 1.37 (s, 9H, $(CH_3)_3C$), 2.42 (q, 2H, *J* = 7.5 Hz, CH_2), 3.60 (s, 2H, CH_2-Ar), 7.07 (d, 1H, $J_{6,5}$ =8.2 Hz, H-6), 7.20-7.34 (m, 3H, H-3, H-4, H-5)

2,2-Dimethylpropionic acid 3-(2'-oxo-butyl)-phenyl ester (22)

22 was prepared from **38** in the manner described for **23** below. The pure product was isolated as a colourless oil in 49% yield following chromatography on silica gel (hexane: diethyl ether = 5:1) and used in subsequent reactions without further purification. IR: ν_{max} ($CHCl_3$) cm^{-1}: 2974, 2927, 2880 (CH), 1751.0 (C=O), 1713 (C=O), 1609 (C=C). ^{1}H-NMR (400MHz, $CDCl_3$): δ 1.05 (t, 3H, *J* = 7.5 Hz, CH_3), 1.37 (s, 9H, $(CH_3)_3C$), 2.49 (q, 2H, *J* = 7.5 Hz, CH_2), 3.70 (s, 2H, CH_2-Ar), 6.95 (d, 1H, $J_{2,6}$ =1.5 Hz, H-2), 6.99 (dd, 1H, $J_{6,2}$ = 1.5 Hz, $J_{6,5}$ = 8.0 Hz, H-6), 7.08 (d, 1H, $J_{4,5}$ = 7.5 Hz, H-4), 7.34 (dd, 1H, $J_{5,4}$ = 7.5 Hz, $J_{5,6}$ = 7.5 Hz, H-5).

2,2-Dimethylpropionic acid 4-(2'-oxo-butyl)phenyl ester (23)

1-(4-Hydroxyphenyl)-butan-2-one **(39)**(800 mg, 4.88 mmol), triethylamine (2.036 ml, 14.64 mmol) and DMAP (10 mg, 0.08 mmol) were dissolved in THF (10 ml). Trimethylacetyl chloride (1.196 ml, 9.76 mmol) was added dropwise over 10 min and stirring was continued for a further 16 hours at room temperature. The reaction mixture was diluted with ethyl acetate (50 ml) and quenched with 10% HCl (10 ml). The organic layer was washed with water (10 ml), brine (10 ml) and dried over Na_2SO_4. The solvent was removed under reduced pressure and the residue was chromatographed on silica gel (hexane: diethyl ether = 4:1) to yield the product **23** (646 mg, 53%) as a colourless oil and used in subsequent reactions without further purification. IR: ν_{max} ($CHCl_3$) cm^{-1}: 2976, 2938, 2876 (CH), 1750 (C=O), 1715 (C=O), 1606 (C=C). ^{1}H-NMR (400MHz, $CDCl_3$): δ 1.03 (t, 3H, *J* = 7.5 Hz, CH_3), 1.36 (s, 9H, $(CH_3)_3C$), 2.48 (q, 2H, *J* = 7.5 Hz, CH_2), 3.68 (s, 2H, CH_2-Ar), 7.03 (d, 2H, *J* =8.5 Hz, H-2, H-6), 7.22 (d, 2H, *J* = 8.5 Hz, H-3, H-5).

2,2-Dimethylpropionic acid 2-[3-[4-(*tert*-butyldimethyl-silanyloxy)-phenyl]-2-ethyl-3-(4-hydroxyphenyl)-allyl]-phenyl ester (24)

24 was prepared from **6** and **21** in the manner described for **9** above. The pure product was isolated as a colourless oil in 41% yield following chromatography on silica gel (hexane: diethyl ether = 5:1) and used in subsequent reactions without further purification. IR: ν_{max} ($CHCl_3$) cm^{-1}: 3431 (OH), 3032, 2960, 2932, 2859 (CH), 1749, 1730.0 (C=O), 1604(C=C). ^{1}H-NMR (400MHz, $CDCl_3$): δ 0.18 (s, 3H, CH_3-Si), 0.22 (s, 3H, CH_3-Si), 0.94 (t, 3H, *J* = 7.5 Hz, CH_3), 1.00 (s, 9H, $(CH_3)_3$-C), 1.37 (s, 9H, $(CH_3)_3$-C), 2.01 (q, 2H, *J* = 7.5 Hz, CH_2), 3.43 and 3.45 (2 x s, 2H, CH_2-Ar), 6.67-6.80 (m, 4H, ArH), 6.97-7.23 (m, 8H, ArH).

2,2-Dimethylpropionic acid 3-[3-[4-(*tert*-butyldimethyl-silanyloxy)-phenyl]-2-ethyl-3-(4-hydroxyphenyl)-allyl]-phenyl ester (25)

25 was prepared from **6** and **22** in the manner described for **9** above. The pure product **25** was isolated as a colourless oil in 49% yield following chromatography on silica gel (hexane: diethyl ether = 5:1) and used in subsequent reactions without further purification. IR: ν_{max} ($CHCl_3$) cm^{-1}: 3434, 3012, 2958, 2931, 2858 (CH), 1752(C=O), 1604(C=C). ^{1}H-NMR (400MHz, $CDCl_3$): δ 0.20 (s, 3H, CH_3-Si), 0.22 (s, 3H, CH_3-Si), 0.99 (t, 3H, *J* = 7.5 Hz, CH_3), 1.01 (s, 9H, $(CH_3)_3$-C), 1.39 (s, 9H, $(CH_3)_3$-C), 2.08 (q, 2H, *J* = 7.5 Hz, CH_2), 3.56 and 3.57 (2 x s, 2H, CH_2-Ar), 6.69-7.09 (m, 9H, ArH), 7.28-7.38 (m, 3H, ArH).

2,2-Dimethylpropionic acid 4-[3-[4-(*tert*-butyldimethyl-silanyloxy)-phenyl]-2-ethyl-3-(4-hydroxyphenyl)-allyl]-phenyl ester (26)

26 was prepared from **6** and **23** in the manner described for **9** above. The pure product was isolated as a colourless oil in 67% yield following chromatography on silica gel (hexane: diethyl ether = 5:1) and used in subsequent reactions without further purification. IR: ν_{max} ($CHCl_3$) cm^{-1}: 3427 (OH), 3029, 2959, 2919, 2853(CH), 1747 (C=O), 1722 (C=O), 1602 (C=C). ^{1}H-NMR (400MHz, $CDCl_3$): δ 0.20 (s, 3H, CH_3-Si), 0.21 (s, 3H, CH_3-Si), 0.97 (t, 3H, *J* = 7.5 Hz, CH_3), 1.01 (s, 9H, $(CH_3)_3$-C), 1.39 (s, 9H, $(CH_3)_3$C), 2.06 (q, 2H, *J* = 7.5 Hz, CH_2), 3.54 and 3.55 (2 x s, 2H, CH_2-Ar), 6.71-6.80 (m, 4H, ArH), 6.99-7.22 (m, 8H, ArH).

2,2-Dimethylpropionic acid 2-{3-[4-(*tert*-butyldimethyl-silanyloxy)-phenyl]-2-ethyl-3-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-allyl}-phenyl ester (27)

27 was prepared from **24** in the manner described for **10** above. The pure product was isolated as a colourless oil in 80% yield following chromatography on silica gel (dichloromethane: methanol = 20:1) and used in subsequent reactions without further purification. IR: ν_{max} ($CHCl_3$) cm^{-1}: 2960, 2932, 2859 (CH), 1750.4 (C=O), 1605.1 (C=C). ^{1}H-NMR (400MHz, $CDCl_3$): δ 0.20 (s, 3H, CH_3-Si), 0.23 (s, 3H, CH_3-Si), 0.95 (t, 3H, *J* = 7.5 Hz, CH_3), 0.98 (s, 9H, $(CH_3)_3$-C), 1.39 (s, 9H, $(CH_3)_3$-C), 1.84 (m, 4H, (CH_2), 2.04 (q, 2H, *J* = 7.5 Hz, CH_2), 2.66 (m, 4H, $(CH_2)_2N$), 2.92 (m, 2H, CH_2N), 3.44 (s, 2H, CH_2-Ar), 4.12 (m, 2H, CH_2O), 6.71-7.36 (m, 12H, ArH).

2,2-Dimethylpropionic acid 3-{3-[4-(*tert*-butyldimethyl-silanyloxy)-phenyl]-2-ethyl-3-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-allyl}-phenyl ester (28)

28 was prepared from **25** in the manner described for **10** above. The pure product **28** was isolated as a colourless oil in 39% yield following chromatography on silica gel (dichloromethane: methanol = 20:1) and used in subsequent reactions without further purification. IR: ν_{max} ($CHCl_3$) cm^{-1}: 3015, 2960, 2931, 2944, 2858 (CH), 1753.2 (C=O), 1605(C=C). ^{1}H-NMR (400MHz, $CDCl_3$): δ 0.20 (s, 3H, CH_3-Si), 0.22 (s, 3H, CH_3-Si), 0.98 (t, 3H, *J* = 7.5 Hz, CH_3), 1.00 (s, 9H, $(CH_3)_3$-C), 1.39 (s, 9H, $(CH_3)_3$-C), 1.84 (m, 4H, CH_2), 2.09 (q, 2H, *J* = 7.5 Hz, CH_2), 2.70 (m, 4H, $(CH_2)_2N$), 2.95 (m, 2H, CH_2N), 3.57 (s, 2H, CH_2-Ar), 4.14 (m, 2H, CH_2O), 6.74-6.89 (m, 6H, ArH), 7.04-7.31 (m, 6H, ArH).

2,2-Dimethylpropionic acid 4-{3-[4-(*tert*-butyldimethyl-silanyloxy)-phenyl]-2-ethyl-3-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-allyl}-phenyl ester (29)

29 was prepared from **26** in the manner described for **10** above. The pure product was isolated as a colourless oil in 30% yield following chromatography on silica gel (dichloromethane: methanol = 20:1) and used in subsequent reactions without further purification. IR: ν_{max} ($CHCl_3$) cm^{-1}: 2962, 2927, 2857 (CH), 1749 (C=O), 1600 (C=C). ^{1}H-NMR (400MHz, $CDCl_3$): δ 0.19 (s, 3H, CH_3-Si), 0.21 (s, 3H, CH_3-Si), 0.94 (t, 3H, *J* = 7.5 Hz, CH_3), 0.97 (s, 9H, $(CH_3)_3$-C), 1.37 (s, 9H, $(CH_3)_3$-C), 1.83 (m, 4H, (CH_2), 2.05 (q, 2H, *J* = 7.5 Hz, CH_2), 2.67 (m, 4H, $(CH_2)_2N$), 2.93 (m, 2H, CH_2N), 3.54 (s, 2H, CH_2-Ar), 4.12 (m, 2H, CH_2O), 6.72-7.21 (m, 12H, ArH).

2,2-Dimethylpropionic acid 2-{2-ethyl-3-(4-hydroxyphenyl)-3-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-allyl}-phenyl ester (30)

30 was prepared from **27** in the manner described for **15** above. The pure product was isolated as a colourless oil in 87% yield following chromatography on silica gel (dichloromethane: methanol = 10:1). IR: ν_{max} ($CHCl_3$) cm^{-1}: 3401

(OH), 2963, 2875 (CH), 1746 (C=O), 1608 (C=C). ^{1}H-NMR (400MHz, $CDCl_3$): δ 0.96 (t, 3H, *J* = 7.5 Hz, CH_3), 1.35 (s, 9H, $(CH_3)_3$-C), 1.62 (m, 4H, CH_2), 1.99 (q, 2H, *J* = 7.5 Hz, CH_2), 2.94 (m, 4H, $(CH_2)_2N$), 3.15 (m, 2H, CH_2N), 3.40 and 3.41 (2 x s, 2H, CH_2-Ar), 4.19 (m, 2H, CH_2O), 6.75-7.32 (m, 12H, ArH). HRMS (EI): Found 528.3103 (M^++ 1), $C_{34}H_{42}NO_4$ requires 528.3114.

2,2-Dimethylpropionic acid 3-{2-ethyl-3-(4-hydroxyphenyl)-3-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]- allyl}-phenyl ester (31)

31 was prepared from **28** in the manner described for **15** above. The pure product was isolated as a colourless oil in 77% yield following chromatography on silica gel (dichloromethane: methanol = 10:1). IR: ν_{max} ($CHCl_3$) cm^{-1}: 3394(OH), 2962, 2875, 1750 (C=O), 1608 (C=C). ^{1}H-NMR (400MHz, $CDCl_3$): δ 0.96 (t, 3H, *J* = 7.5 Hz, CH_3), 1.38 (s, 9H, $(CH_3)_3$-C), 1.82 (m, 4H, (CH_2), 2.08 (q, 2H, *J* = 7.5 Hz, CH_2), 2.63 (m, 4H, $(CH_2)_2N$), 2.90 (m, 2H, CH_2N), 3.54 and 3.56 (2 x s, 2H, CH_2-Ar), 4.09 (m, 2H, CH_2O), 6.77-7.28 (m, 12H, ArH). HRMS (EI): Found 528.3124 (M^++H), $C_{34}H_{42}NO_4$ requires 528.3114

2,2-Dimethylpropionic acid 4-{2-ethyl-3-(4-hydroxyphenyl)-3-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-allyl}-phenyl ester (32)

32 was prepared from **29** in the manner described for **15** above. The pure product was isolated as a colourless oil in 54% yield following chromatography on silica gel (dichloromethane: methanol = 10:1). IR: ν_{max} ($CHCl_3$) cm^{-1}: 3402 (OH), 2961, 2875 (CH), 1746 (CO), 1608 (C=C). ^{1}H-NMR (400MHz, $CDCl_3$): δ 0.96 (t, 3H, *J* = 7.5 Hz, CH_3), 1.30 (s, 9H, $(CH_3)_3$-C), 1.74 (m, 4H, (CH_2), 1.97 (q, 2H, *J* = 7.5 Hz, CH_2), 2.56 (m, 4H, $(CH_2)_2N$), 2.83 (m, 2H, CH_2N), 3.46 and 3.47 (2 x s, 2H, CH_2-Ar), 4.03 (m, 2H, CH_2O), 6.71-7.13 (m, 12H, ArH). HRMS (EI): Found 528.3123 (M^++H), $C_{34}H_{42}NO_4$ requires 528.3114.

4-{2-(2-Hydroxybenzyl)-1-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-but-1-enyl}-phenol (33)

33 was prepared from **30** in the manner described for **34** below. The pure product was isolated as a colourless oil in 41% yield following chromatography on silica gel (dichloromethane: methanol = 10:1). IR: ν_{max} ($CHCl_3$) cm^{-1}: 3381 (OH), 2964, 2874, 2728 (CH), 1606 (C=C). ^{1}H-NMR (400MHz, $CDCl_3$): δ 0.88 (t, 3H, *J* = 7.5 Hz, CH_3), 1.90 (m, 4H, CH_2), 2.02 (q, 2H, *J* = 7.5 Hz, CH_2), 3.01 (m, 4H, $(CH_2)_2N$), 3.15 (m, 2H, CH_2N), 3.44 and 3.47 (2 x s, 2H, CH_2-Ar), 4.21 (m, 2H, CH_2O), 6.62-6.78 (m, 6H, ArH), 6.92-7.08 (m, 6H, ArH). HRMS (EI): Found 444.2559 (M^++H), $C_{29}H_{34}NO_3$ requires 444.2539.

4-{2-(3-Hydroxybenzyl)-1-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-but-1-enyl}-phenol (34)

31 (350 mg, 0.664 mmol) and sodium hydroxide (133 mg, 3.32 mmol) were dissolved in 1:4 water/ethanol (5 ml) and stirred at room temperature for 4 hours. The reaction mixture was acidified with 10% HCl (10 ml) and extracted with dichloromethane (4 x 40 ml). The combined organic layers were washed with brine (20 ml) and dried over Na_2SO_4. The solvent was removed under reduced pressure and the residue was chromatographed on silica gel (dichloromethane: methanol = 10:1) to yield the product **34** (106 mg, 36%) as a colourless oil. IR: ν_{max} ($CHCl_3$) cm^{-1}: 3394.0 (OH), 2961, 2929 (CH), 1606 (C=C). ^{1}H-NMR (400MHz, $CDCl_3$): δ 0.90 (t, 3H, *J* = 7.5 Hz, CH_3), 1.90 (m, 4H, CH_2), 2.01 (q, 2H, *J* = 7.5 Hz, CH_2), 2.98 (m, 4H, $(CH_2)_2N$), 3.12 (m, 2H, CH_2N), 3.39 and 3.43 (2 x s, 2H, CH_2-Ar), 4.20 (m, 2H, CH_2O), 6.62-7.06 (m, 12H, ArH). HRMS (EI): Found 444.2528 (M^++H), $C_{29}H_{34}NO_3$ requires 444.2539.

2-(2'-Nitro-but-1'-enyl)-phenol (35)

Salicylaldehyde (8.72 ml, 0.82 mol), nitropropane (15.3 ml, 0.16 mol), dimethyamine HCl (20 g, 0.246 mol) and potassium fluoride (5 g, 0.086 mol) were added to toluene (100 ml) and maintained at reflux with a Dean-Stark trap for 20 hours. The reaction mixture was diluted with ethyl acetate (200 ml) and quenched with 10% HCl (50 ml). The organic layer was separated, washed with water (50 ml), brine (50 ml) and dried over Na_2SO_4. The solvent was removed under reduced pressure and the residue was chromatographed on silica gel (hexane: diethyl ether = 6:1) to yield the product **35** (9.934 g, 63%) as a yellow oil which was used in subsequent reactions without further purification. IR: ν_{max} ($CHCl_3$) cm^{-1}: 3465 (OH), 2977, 2939, 2879 (CH), 1648 (C=C), 1605 (C=C). ^{1}H-NMR (400MHz, $CDCl_3$): δ 1.28 (t, 3H, *J* = 7.3 Hz, CH_3), 2.84 (t, 2H, *J* = 7.3 Hz, CH_2), 6.90 (d, 1H, $J_{6,5}$ =7.9 Hz, H-6), 7.03 (dd, 1H, *J* = 6.7 Hz, *J* = 7.3 Hz, H-4), 7.28-7.35 (m, 2H, H-3, H-5), 8.22 (s, 1H, H-1').

2,2-Dimethylpropionic acid 2-(2'-nitro-but-1'-enyl)-phenyl ester (36)

35 (9.934 g, 52 mmol), triethylamine (21.49 ml, 154 mmol) and DMAP (100 mg, 0.8 mmol) were dissolved in THF (200 ml). Trimethylacetyl chloride (12.625 ml, 103 mmol) was added dropwise over 1 hour and stirring was continued for a further 16 hours at room temperature. The reaction mixture was diluted with ethyl acetate (200 ml) and quenched with 10% HCl (100 ml). The aqeuous layer was separated and extracted with ethyl acetate (2 x 100 ml). The combined organic layers were washed with water (100 ml), brine (100 ml) and dried over Na_2SO_4. The solvent was removed under reduced pressure and the residue was chromatographed on silica gel (hexane: diethyl ether = 4:1) to yield the product **36** (13.60 g, 95%) as a yellow oil which was used in subsequent reactions without further purification. IR: ν_{max} ($CHCl_3$) cm^{-1}: 2977, 2894 (CH), 1789 (C=O), 1775 (C=O), 1667 (C=C). ^{1}H-NMR (400MHz, $CDCl_3$): δ 1.23 (t, 3H, *J* = 7.5 Hz, CH_3), 1.38 (s, 9H, $(CH_3)_3C$), 2.79 (q, 2H, *J* = 7.3 Hz, CH_2), 7.16 (d, 1H, $J_{6,5}$ =8.0 Hz, H-6), 7.28-7.49 (m, 3H, H-3, H-4, H-5).

1'-(3-Hydroxyphenyl)-butan-2'-one (38)

Boron trifluoride-dimethyl sulphide (13.0 ml, 120.36 mmol) was added dropwise over 30 min to 1-(3-methoxy-phenyl)-butan-2-one **37**[53] (2.20 g, 12.36 mmol) in dichoromethane (50 ml). Stirring was continued for a further 10 hours at room temperature. The solvent was removed using a nitrogen purge and the remaining residue was dissolved in ethyl acetate (200 ml) and washed with saturated sodium bicarbonate solution (2 x 50 ml), water (50

ml) and brine (50 ml) and was dried over Na_2SO_4. The solvent was removed under reduced pressure and the residue was chromatographed on silica gel (hexane: ethyl acetate = 4:1) to yield the product **38** (734 mg, 36%) as a colourless oil which was used in subsequent reactions without further purification. IR: ν_{max} ($CHCl_3$) cm^{-1}: 3389 (OH), 2978, 2940, 2901 (CH), 1704 (C=O), 1590 (C=C). ^{1}H-NMR (400MHz, $CDCl_3$): δ 1.05 (t, 3H, *J* = 7.5 Hz, CH_3), 2.51 (q, 2H, *J* = 7.5 Hz, CH_2), 3.67 (s, 2H, CH_2-Ar), 6.72-6.79 (m, 3H, H-2, H-4, H-6), 7.20 (dd, 1H, $J_{5,4}$ = 7.5 Hz, $J_{5,6}$ = 7.5 Hz, H-5).

4-[1-[4-(*tert*-Butyldimethylsilanyloxy)-phenyl]-2-(2-methoxybenzyl)-but-1-enyl]-phenol (42)

42 was prepared from **6** and 1-(2-methoxy-phenyl)-butan-2-one **(40)** in the manner described for **9** above. The pure product was isolated as a colourless oil in 21% yield following chromatography on silica gel (hexane: diethyl ether = 4:1) and used in subsequent reactions without further purification. . IR: ν_{max} ($CHCl_3$) cm^{-1}: 3399 (OH), 2956, 2918, 2861 (CH), 1600 (C=C). ^{1}H-NMR (400MHz, $CDCl_3$): δ 0.20 (s, 6H, $(CH_3)_2$-Si), 0.98 (t, 3H, *J* = 7.5 Hz, CH_3), 1.00 (s, 9H, $(CH_3)_3$-C), 2.08 (q, 2H, *J* = 7.5 Hz, CH_2), 3.59 (s, 2H, CH_2-Ar), 3.83 (s, 3H, CH_3O), 6.72-6.99 (m, 12H, ArH).

4-[1-[4-(*tert*-Butyldimethylsilanyloxy)-phenyl]-2-(3-methoxybenzyl)-but-1-enyl]-phenol (43)

43 was prepared from **6** and 1-(3-methoxy-phenyl)-butan-2-one **(37)** in the manner described for **9** above. The pure product was isolated as a colourless oil in 29% yield following chromatography on silica gel (hexane: diethyl ether = 4:1) and used in subsequent reactions without further purification. IR: ν_{max} ($CHCl_3$) cm^{-1}: 3401 (OH), 2957, 2951, 2858 (CH), 1602.6 (C=C). ^{1}H-NMR (400MHz, $CDCl_3$): δ 0.21 (s, 3H, CH_3-Si), 0.23 (s, 3H, CH_3-Si), 0.98 (t, 3H, *J* = 7.5 Hz, CH_3), 1.02 (s, 9H, $(CH_3)_3$-C), 2.09 (q, 2H, *J* = 7.5 Hz, CH_2), 3.55 (s, 2H, CH_2-Ar), 3.83 (s, 3H, CH_3O), 6.73-7.28 (m, 12H, ArH).

4-[1-[4-(*tert*-Butyldimethylsilanyloxy)-phenyl]-2-(4-methoxybenzyl)-but-1-enyl]-phenol (44)

44 was prepared from **6** and 1-(4-methoxy-phenyl)-butan-2-one **(41)** in the manner described for **9** above. The pure product was isolated as a colourless oil in 54% yield following chromatography on silica gel (hexane: diethyl ether = 4:1) and used in subsequent reactions without further purification. IR: ν_{max} ($CHCl_3$) cm^{-1}: 3409 (OH), 3030, 2943, 2857 (CH), 1605 (C=C). ^{1}H-NMR (400MHz, $CDCl_3$): δ 0.20 (s, 3H, CH_3-Si), 0.23 (s, 3H, CH_3-Si), 0.97 (t, 3H, *J* = 7.5 Hz, CH_3), 1.01 (s, 9H, $(CH_3)_3$-C), 2.06 (q, 2H, *J* = 7.5 Hz, CH_2), 3.50 (s, 2H, CH_2-Ar), 3.82 (s, 3H, CH_3O), 6.73-7.15 (m, 12H, ArH).

1-(2-{4-[1-[4-(*tert*-Butyldimethylsilanyloxy)-phenyl]-2-(2-methoxybenzyl)-but-1-enyl]-phenoxy}-ethyl)-pyrrolidine (45)

45 was prepared from **42** in the manner described for **10** above. The pure product was isolated as a colourless oil in 67% yield following chromatography on silica gel (dichloromethane: methanol = 15:1) and used in subsequent reactions without further purification. IR: ν_{max} ($CHCl_3$) cm^{-1}: 2943, 2931, 2858 (CH), 1604.1 (m). ^{1}H-NMR (400MHz, $CDCl_3$): δ 0.17 (s, 3H, CH_3-Si), 0.21 (s, 3H, CH_3-Si), 0.96 (t, 3H, *J* = 7.5 Hz, CH_3), 0.98 (s, 9H, $(CH_3)_3$-C), 1.81 (m, 4H, CH_2), 2.05 (q, 2H, *J* = 7.5 Hz, CH_2), 2.63 (m, 4H, $(CH_2)_2$N), 2.90 (m, 2H, CH_2N), 3.54 (s, 2H, CH_2-Ar), 3.81 (s, 3H, CH_3O), 4.09 (m, 2H, CH_2O), 6.83-7.23 (m, 12H, ArH).

1-(2-{4-[1-[4-(*tert*-Butyldimethylsilanyloxy)-phenyl]-2-(3-methoxybenzyl)-but-1-enyl]-phenoxy}-ethyl)-pyrrolidine (46)

46 was prepared from **43** in the manner described for **10** above. The pure product was isolated as a colourless oil in 29% yield following chromatography on silica gel (dichloromethane: methanol = 15:1) and used in subsequent reactions without further purification. IR: ν_{max} ($CHCl_3$) cm^{-1}: 3016, 2958, 2868, 2834 (CH), 1728 (C=O), 1604 (C=C). ^{1}H-NMR (400MHz, $CDCl_3$): δ 0.19 (s, 3H, CH_3-Si), 0.21 (s, 3H, CH_3-Si), 0.98 (t, 3H, *J* = 7.5 Hz, CH_3), 1.00 (s, 9H, $(CH_3)_3$-C), 1.83 (m, 4H, CH_2), 2.07 (q, 2H, *J* = 7.5 Hz, CH_2), 2.65 (m, 4H, $(CH_2)_2$N), 2.91 (m, 2H, CH_2N), 3.54 (s, 2H, CH_2-Ar), 3.82 (s, 3H, CH_3O), 4.11 (m, 2H, CH_2O), 6.73-7.07 (m, 12H, ArH).

1-(2-{4-[1-[4-(*tert*-Butyldimethylsilanyloxy)-phenyl]-2-(4-methoxybenzyl)-but-1-enyl]-phenoxy}-ethyl)-pyrrolidine (47)

47 was prepared from 44 in the manner described for 10 above. The pure product was isolated as a colourless oil in 84% yield following chromatography on silica gel (dichloromethane: methanol = 15:1) and used in subsequent reactions without further purification. IR: ν_{max} ($CHCl_3$) cm^{-1}: 2955, 2930, 2857 (CH), 1605 (C=C). ^{1}H-NMR (400MHz, $CDCl_3$): δ 0.15 (s, 3H, CH_3-Si), 0.16 (s, 3H, CH_3-Si), 0.93 (t, 3H, *J* = 7.5 Hz, CH_3), 0.94 (s, 9H, $(CH_3)_3$-C), 1.79-1.82 (m, 6H, 3 x CH_2), 2.67 (m, 4H, $(CH_2)_2$N), 2.92 (m, 2H, CH_2N), 3.40 (s, 2H, CH_2-Ar), 3.75 (s, 3H, CH_3O), 4.09 (m, 2H, CH_2O), 6.69-7.09 (m, 12H, ArH).

4-{2-(2-Methoxybenzyl)-1-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-but-1-enyl}-phenol (48)

48 was prepared from **45** in the manner described for **15** above. The pure product was isolated as a colourless oil in 59% yield following chromatography on silica gel (dichloromethane: methanol = 10:1). IR: ν_{max} ($CHCl_3$) cm^{-1}: 3401 (OH), 2962, 2875 (CH), 1607 (C=C). ^{1}H-NMR (400MHz, $CDCl_3$): δ 0.85 (t, 3H, *J* = 7.5 Hz, CH_3), 1.78 (m, 4H, CH_2), 1.90 (q, 2H, *J* = 7.5 Hz, CH_2), 2.76 (m, 4H, $(CH_2)_2$N), 2.97 (m, 2H, CH_2N), 3.39 and 3.41 (2 x s, 2H, CH_2-Ar), 3.67 (s, 3H, CH_3O), 4.06 (m, 2H, CH_2O), 6.62-7.08 (m, 12H, ArH). HRMS (EI): Found 458.2680 (M^++H), $C_{30}H_{36}NO_3$ requires 458.2695.

4-{2-(3-Methoxybenzyl)-1-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-but-1-enyl}-phenol (49)

49 was prepared from **46** in the manner described for **15** above. The pure product was isolated as a colourless oil in 55% yield following chromatography on silica gel (dichloromethane: methanol = 10:1). IR: ν_{max} ($CHCl_3$) cm^{-1}: 3401 (OH), 2962, 2875, 2834 (CH), 1607 (C=C). ^{1}H-NMR (400MHz, $CDCl_3$): δ 0.92 (t, 3H, *J* = 7.5 Hz, CH_3), 1.95-2.04 (m, 6H, 3 x (CH_2), 3.04 (m, 4H, $(CH_2)_2$N), 3.21 (m, 2H, CH_2N), 3.47 and 3.49 (s, 2H, CH_2-Ar), 3.77 (s, 3H, CH_3O),

4.26 (m, 2H, CH_2O), 6.69-7.19 (m, 12H, ArH). HRMS (EI): Found 458.2687 (M^++H), $C_{30}H_{36}NO_3$ requires 458.2695.

4-{2-(4-Methoxybenzyl)-1-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-but-1-enyl}-phenol (50)

50 was prepared from **47** in the manner described for **15** above. The pure product was isolated as a colourless oil in 33% yield following chromatography on silica gel (dichloromethane: methanol = 15:1). IR: ν_{max} ($CHCl_3$) cm^{-1}: 3414 (OH), 2962, 2876 (CH), 1608 (C=C). ^{1}H-NMR (400MHz, $CDCl_3$): δ 0.96 (t, 3H, *J* = 7.5 Hz, CH_3), 1.93 (m, 6H, 3 x CH_2), 2.99 (m, 4H, $(CH_2)_2N$), 3.17 (m, 2H, CH_2N), 3.42 (s, 2H, CH_2-Ar), 3.76 (s, 3H, CH_3O), 4.22 (m, 2H, CH_2O), 6.75-7.05 (m, 12H, ArH). HRMS (EI): Found 458.2695($M+H^+$), $C_{30}H_{36}NO_3$ requires 458.2695.

(4-Methoxyphenyl)-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-methanone (52)

(4-Hydroxyphenyl)-(4-methoxyphenyl)-methanone**(51)** (840 mg, 3.68 mmol) and K_2CO_3 (4.062 g, 29.44 mmol) were dissolved in DMF (10 ml) and heated to 60°C. 1-(2-Chloroethyl)-pyrrolidine hydrochloride (1.877 g, 11.04 mmol) was added and stirring was continued for 16 hours. The solution was cooled to room temperature, filtered and washed with ethyl acetate (10 ml). The solvent was removed under reduced pressure and the residue was chromatographed on silica gel (dichloromethane: methanol = 10:1) to yield the product **52** (560 mg, 47%) as a colourless oil. HPLC: t_R = 4.6, 4.8 min. LRMS (*m/z*): 326 ($M+H^+$, 10%). IR: ν_{max} ($CHCl_3$) cm^{-1}: 2948, 2864, 2801, 2788 (CH), 1636, 1599 (C=C). ^{1}H-NMR (400MHz, $CDCl_3$): δ 1.84–1.88 (m, 4H, CH_2), 2.71 (m, 4H, $(CH_2)_2N$), 2.99 (t, 2H, *J* = 5.8 Hz, CH_2N), 3.90 (s, 3H, CH_3O), 4.23 (t, 2H, *J* = 5.8 Hz, CH_2O), 6.97-7.00 (m, 4H, ArH), 7.78-7.81 (m, 4H, ArH). HRMS (EI): Found 326.1759 ($M+H^+$), $C_{20}H_{24}NO_3$ requires 326.1756.

1-(2-{4-[2-(4-Methoxybenzyl)-1-(4-methoxyphenyl)-but-1-enyl]-phenoxy}-ethyl)-pyrrolidine (53)

Zinc powder (602 mg, 9.26 mmol) was placed in a 3-necked flask into which dry THF (10 ml) was added under nitrogen. Titanium tetrachloride (509 µl, 4.63 mmol) was slowly added *via* syringe over 5 min in the dark. The reaction mixture was maintained at reflux for 1.5 hours, after which time **52** (430 mg, 1.32 mmol) and 1-(4-methoxy-phenyl)-butan-2-one **(41)** (235 mg, 1.32 mmol) in THF (10 ml) were added *via* syringe. After a further 4 hours at reflux, the solution was cooled to room temperature, diluted with ethyl acetate (50 ml) and quenched with 10% K_2CO_3 solution (20 ml). Following filtration and separation, the aqueous layer was extracted with ethyl acetate (3 x 40 ml). The combined organic layers were washed with 10% K_2CO_3 solution (20 ml), water (20 ml) and brine (20 ml) and dried over Na_2SO_4. The solvent was removed under reduced pressure and the residue was chromatographed on silica gel (dichloromethane: methanol = 10:1) to yield the product **53** (371 mg, 60%) as a colourless oil. HPLC: t_R = 9.1, 9.7 min. LRMS (*m/z*): 471 (M^+, 3%). IR: ν_{max} ($CHCl_3$) cm^{-1}: 2959, 2928, 2865 (CH), 1605 (C=C). ^{1}H-NMR (400MHz, $CDCl_3$): δ 0.97 (t, 3H, *J* = 7.3 Hz, CH_3), 1.84 (m, 4H, CH_2), 2.06 (q, 2H, *J* = 7.3 Hz, CH_2), 2.66 (m, 4H, $(CH_2)_2N$), 2.90 (m, 2H, CH_2N), 3.51 (s, 2H, CH_2-Ar), 3.78 (s, 3H, CH_3O), 3.81 (s, 3H, CH_3O), 4.11 (m, 2H, CH_2O), 6.80-6.88 (m, 6H, ArH), 7.10-7.15 (m, 6H, ArH). HRMS (EI): Found 472.2857 ($M+H^+$), $C_{31}H_{38}NO_3$ requires 472.2852.

[4'-(*tert*-Butyldimethylsilanyloxy)-phenyl]-(4-methoxy-3-methylphenyl)-methanone (56)

1-Methoxy-2-methyl-benzene (2.26 g, 18.5 mmol) and tin tetrachloride (2.60 ml, 22.2 mmol) were stirred in 1,1,2,2-tetrachloroethane (TCE) (50 ml) for 10 min at room temperature. *tert*-Butyldimethylsilanyloxybenzoyl chloride (**55**)[56] (5.0 g, 18.5 mmol) in TCE (50 ml) was added dropwise over 50 min. Stirring was continued for a further 16 hours. The reaction mixture was diluted with dichloromethane (200 ml) and quenched with 10% $NaHCO_3$ (50 ml). The organic layer was washed with water (50 ml) and brine (50 ml) and dried over Na_2SO_4. The solvent was removed under reduced pressure and the residue was chromatographed on silica gel (hexane: diethyl ether = 4:1) to yield the product **56** (4.5 g, 68%) as a colourless oil. LRMS (*m/z*): 356 (M^+, 24%). IR: ν_{max} ($CHCl_3$) cm^{-1}: 2944, 2890, 2858 (CH), 1649 (C=O), 1596 (C=C). ^{1}H-NMR (400MHz, $CDCl_3$): δ 0.28 (s, 6H, $(CH_3)_2$-Si), 1.03 (s, 9H, $(CH_3)_3C$), 2.28 (s, 3H, CH_3-Ar), 3.93 (s, 3H, CH_3O), 6.88-6.93 (m, 3H, H-5, H-3', H-5'), 7.66-7.67 (m, 2H, H-2, H-6), 7.74 (d, 2H, *J* = 8.5 Hz, H-2', H-6'). HRMS (EI): Found 357.1888 (M^++H), $C_{21}H_{29}O_3Si$ requires 357.1886.

(4'-Hydroxyphenyl)-(4-methoxy-3-methylphenyl)-methanone (57)

Tetrabutylammonium fluoride (1M, 6.73 ml) was added to **56** (2.0 g, 5.61 mmol) in THF (50 ml) and stirred for 10 min at room temperature. The reaction mixture was diluted with ethyl acetate (200 ml) and quenched with 10% HCl (50 ml). The organic layer was separated and washed with water (50 ml) and brine (50 ml) and dried over Na_2SO_4. The solvent was removed under reduced pressure and the residue was chromatographed on silica gel (hexane: diethyl ether = 4:1) to yield the product **57** (1.072 g, 79%) as a colourless solid which was used in subsequent reactions without further purification. LRMS (*m/z*): 356 (M^++H, 100%). IR: ν_{max} ($CHCl_3$) cm^{-1}: 3232, 2954 (CH), 1628 (C=O), 1601 (C=C). ^{1}H-NMR (400MHz, $CDCl_3$): δ 2.28 (s, 3H, CH_3-Ar), 3.93 (s, 3H, CH_3O), 6.89 (d, 1H, $J_{5,6}$ = 9.0 Hz, H-5), 6.92 (d, 2H, *J* = 8.5 Hz, H-5', H-3'), 7.66-7.68 (m, 2H, H-2, H-6), 7.77 (d, 2H, *J* = 8.5 Hz, H-2', H-6').

4-[2-(4-Methoxy-3-methylbenzyl)-1-(4-methoxy-3-methyl-phenyl)-but-1-enyl]-phenol (59)

59 was prepared from **57** and 1-(4-methoxy-3-methyl-phenyl)-butan-2-one **(58)** in the manner described for **9** above. The pure product was isolated as a colourless oil in 70% yield following chromatography on silica gel (hexane: diethyl ether = 4:1) and used in the subsequent reaction without further purification.. LRMS (*m/z*): 500 ($M+H^+$, 8%). IR: ν_{max} ($CHCl_3$) cm^{-1}: 3400 (OH), 2956 (CH), 1686 (C=O), 1604 (C=C). ^{1}H-NMR (400MHz, $CDCl_3$): δ 0.99 (t, 3H, *J* = 7.5 Hz, CH_3), 2.09 (q, 2H, *J* = 7.5 Hz, CH_2), 2.22 (s, 3H, CH_3-Ar), 2.25 (s, 3H, CH_3-Ar), 3.49 (s, 2H, CH_2-Ar), 3.82 (s, 3H, CH_3O), 3.85 (s, 3H, CH_3O), 6.38-6.81 (m, 4H, ArH), 6.99-7.13 (m, 6H, ArH).

1-(2-{4-[2-(4-Methoxy-3-methylbenzyl)-1-(4-methoxy-3-methylphenyl)-but-1-enyl]-phenoxy}-ethyl)-pyrrolidine (60)

59 (270 mg, 0.67 mmol) and K_2CO_3 (742 mg, 5.38 mmol) were dissolved in DMF (5 ml) and heated to 60°C. 1-(2-Chloroethyl)-pyrrolidine hydrochloride (343 mg, 2.02 mmol) was added and stirring was continued for 16 hours. The solution was then cooled to room temperature, filtered and washed with ethyl acetate (10 ml). The solvent was removed under reduced pressure and the residue was chromatographed on silica gel (dichloromethane: methanol = 10:1) to yield the product **60** (144 mg, 43%) as a colourless oil. HPLC: t_R = 9.2, 9.8 min. IR: ν_{max} ($CHCl_3$) cm^{-1}: 2942, 2868 (CH), 1701.4 (w), 1601 (C=C). ^{1}H-NMR (400MHz, $CDCl_3$): δ 0.97 (t, 3H, *J* = 7.5 Hz, CH_3), 1.85 (m, 4H, CH_2), 2.07 (q, 2H, *J* = 7.5 Hz, CH_2), 2.20 (s, 3H, CH_3-Ar), 2.23 (s, 3H, CH_3-Ar), 2.70 (m, 4H, $(CH_2)_2$N), 2.94 (m, 2H, CH_2N), 3.47 (s, 2H, CH_2-Ar), 3.79 (s, 3H, CH_3O), 3.83 (s, 3H, CH_3O), 4.14 (m, 2H, CH_2O), 6.72-6.82 (m, 4H, ArH), 6.96-7.15 (m, 6H, ArH). LRMS (*m/z*): 500 ($M+H^+$, 8%). HRMS (EI): Found 500.3192 (M^++H), $C_{33}H_{42}NO_3$ requires 500.3165.

COMPUTATIONAL PROCEDURE

Ligand Preparation

Structures for compounds (**17**), (**30**), (**32**) and (**50**) were drawn using ACD/Chemsketch 8.17 and SMILES [55] strings generated for each. Marvinview 4.0.1 [56] was utilised to determine the protonation states of each ligand at pH 7.4 with each adjusted accordingly in the SMILES string. 100 conformers of each compound were produced using Omega 1.8.1 with all conformers receiving a final MMFF optimisation step. All conformers were saved in mol2 format. ***Receptor Preparation***: PDB entries 3ERT and 1QKN were downloaded from the Protein Data Bank (PDB) and all crystallographic waters removed. Addition and optimisation of hydrogen positions was carried out using MOE.2005.06 ensuring all other atom positions remained fixed. FIRST5 (Floppy Inclusions and Rigid Substructure Topography) [45] in combination with FRODA (Framework Rigidity Optimised Dynamic Algorithm) [46]was utilised to firstly establish flexible regions of both proteins and subsequently, to generate conformers of the receptor. To ensure receptor conformational space was fully explored, a step size of 1.0 was used to displace every mobile atom randomly by a distance of up to 1Å with an energy cut-off of –1.0. 400 conformers were generated with every 20th saved as a PDB. Macromodel 6.5 was utilised to convert all PDB structures to mol2. ***Docking****:* FRED2.11 [47] was utilized in this study to dock each ligand in both estrogen receptor isoforms. All default values were applied with rigid-body optimisation of each ligand pose using Chemgauss2. In internal validation studies we have found Chemgauss2 to be an efficacious method of evaluating the binding affinity of docked poses in a lipophilic binding site such as the ER. Sequential docking of all ligand and receptor conformers was carried out and the optimally docked solutions established by top score. Ligand Protein Contacts (LPC) software was used to calculate all interatomic contacts between ligand and receptor and furnish Normalised Complementarity (NC) values for each docked complex.

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