Flexible Estrogen Receptor Modulators: Synthesis, Biochemistry and Molecular Modeling Studies for 3-Benzyl-4,6-diarylhex-3-ene and 3,4,6-Triarylhex-3-ene Derivatives

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Abstract: Selective estrogen receptor modulators (SERMs) such as tamoxifen and toremifene are clinically useful drugs in the endocrine treatment of estrogen receptor positive breast cancer while raloxifene is an effective intervention for osteoporosis. In an ongoing SERM discovery programme we now report the synthesis of a series of 3-benzyl-4,6-diarylhex-3-enes and 3,4,6-triarylhex-3-enes containing an extended flexible core structure. In these novel structures, the ethylene group acts as a flexible spacing group linking the aryl Ring A or Ring B with the core alkene group. In the benzyl-4,6-diarylhex-3-ene series an additional methylene group is inserted as a spacing group between the aryl ring C and the ethylene core group. These products demonstrated antiproliferative activity against the MCF-7 human breast cancer cell line. The alkene compounds were also shown to have binding affinity for the estrogen receptor alpha (IC_{50} values for the most active compounds in the range 0.110-0.293μM) together with selectivity for ERα/β. The compounds demonstrated antiestrogenic activity in Ishikawa cells with low estrogenic stimulation. The structure-activity relationships for the active ligands were further explored in a computational study where docked structures of the active compounds were compared with the X-ray crystal structures for the complexes of ERα with 4-hydroxytamoxifen and ERβ with raloxifene. The alignment of the aromatic rings B and C of the compounds within the ligand binding domain could then be correlated with their observed ERα/β selectivity.

Key Words: Estrogen receptor modulators, 3-benzyl-4,6-diarylhex-3-ene, 3,4,6-triarylhex-3-ene, antiestrogens, anticancer drugs.

INTRODUCTION

The estrogen receptor is recognized as the single most important target in breast cancer over the last few decades [1]. Selective estrogen receptor modulators (SERMs) such as tamoxifen(**1a**) and toremifene are clinically useful therapeutics for the endocrine treatment of estrogen receptor positive breast cancer [2] while raloxifene(**2**) is an effective intervention for osteoporosis [3-6]. The discovery of new therapeutic agents which are capable of modulation of the various biochemical roles of the ER can provide insight into the nature of the physiological response of the ER to agonist and antagonist ligands [7-9].

The estrogen receptor which exists in two forms (ERα and ERβ) is widely distributed in the body with ERα occurring in breast and uterus and it is the predominant subtype in malignant mammary carcinoma [10]. ERβ occurs in the CNS, cardiovascular system, gastrointestinal tract, kidney and lung tissues. Ligands for the estrogen related subtypes ERRα, β and γ have also been reported [11,12]. The ER is usually found in the nucleus of target cells and is more recently reported in the membrane also [13]. The ERα and ERβ differ both in LBD structure and in their tissue distribution [14], and share only 59% homology within the ligand binding domain. For ligands such as 4-hydroxytamoxifen (**1b**) and raloxifene(**2**), the B ring phenolic hydroxyl interacts *via* a hydrogen bond with Glu 353 (Glu 260 in ERβ). An additional hydrogen bond is formed with His 524 (His 430 in ERβ) for those ligands which contain a phenolic hydroxyl group on the C-ring [14]. The piperazine side chain of raloxifene facilitates the formation of a hydrogen bond with Asp 351(Asp268 in ERβ) [3, 15]. On binding of estradiol or other agonist, Helix 12 then repositions to enclose the ligand in the hydrophobic cavity and coactivator recruitment to the AF-2 site is facilitated allowing transcription to be initiated. However, when raloxifene binding occurs in the ER LBD, the piperazine side chain interaction with the Asp 351 prevents Helix-12 from enclosing the ligand [3, 15]. Structural studies have demonstrated that different ER modulators can induce distinct conformations of both the ERα and ERβ as demonstrated in the recently reported crystal structure of a partial antagonist GW5638 bound to the ERα [16]. A recognized association of estrogen receptor positive invasive breast carcinomas and the use of HRT (Hormone replacement therapy) was confirmed recently in a study relating the biological effects of continuing HRT after a diagnosis of breast carcinoma [7, 17]. Many SERMs have been developed including GW3638(**3a**) [16], GW7604(**3b**) which is the active metabolite of GW3638, lasofoxifene [18], basedoxifene [19], arzoxifene [20], naphthalenes such as LY2066948(**4**) [8], EM-800 [21] and acolbifene which is the active metabolite of EM-800 [22].

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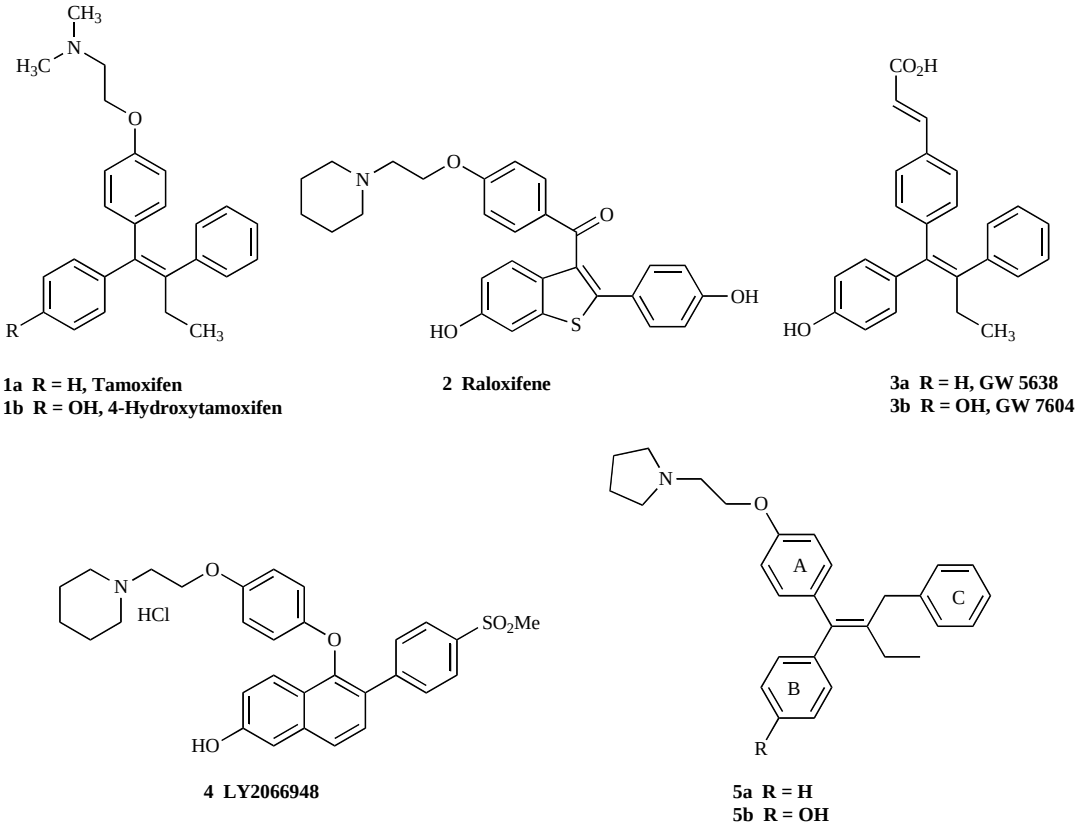
1573-4064/07 $50.00+.00

Among the many structurally varied ligands which have been reported to demonstrate ER modulating properties are isoquinolines [23], furans [24], benzoxepines [25] and indazoles [26]. We have identified a series of flexible hydroxylated 2-benzyl-1,1-diarylbut-2-enes e.g. compounds (**5a**) and (**5b**) containing a scaffold structure in which a benzylic methylene group is positioned between the aryl ring C and the ethylene group to act as a flexible hinge [27, 28]. We now report the synthesis and biochemical evaluation of a series of novel 3-benzyl-4,6-diarylhex-3-enes structures in which the ethylene group acts as a flexible spacing group linking the aryl Ring A or Ring B with the core alkene group. In the related benzyl-4,6-diarylhex-3-ene series we have positioned an additional methylene group as a spacing group between the aryl ring C and the ethylene core group. While it is predicted that the presence of at least one phenolic hydroxyl group in the SERM type structure is required for optimum ligand binding, we have also included the lipophilic pivaloyl ester as an active prodrug of the phenolic compounds in order to improve the oral bioavailability of the compounds by avoiding the rapid metabolism e.g. glucuronidation and subsequent elimination of the hydroxylated ligands. We have previously demonstrated that pivaloyl esters of Ring B and C phenolic groups are intrinsically active as antiproliferative agents as the ester group is slowly hydrolysed in intact cells [29].

CHEMISTRY

The synthesis of the target compound structures requires the initial condensation of the appropriately substituted acetophenones and aryl aldehydes to afford a series of α,β-unsaturated ketones (chalcones)(**6-14**) as illustrated in Scheme **1**. This procedure was found to be efficient for the synthesis of the phenolic chalcones; initial protection of the phenolic acetophenone or aldehyde as tetrahydropyran derivatives could be used but was not found to be necessary [30]. Subsequent hydrogenation of the chalcones resulted in the formation of the phenolic dihydrochalcones (**15-23**) which were then alkylated with 2-chloroethypyrrolidine under basic conditions yielded the aminoalkylketones (**24-32**). The methylethers (**27**), (**28**) and (**31**) were demethylated with boron trifluoride etherate to afford the phenolic ketones (**33-35**). The details of yield for the Series 1 products are displayed in Table **1**.

The initial series of Series 2 target alkenes (**44-60**) were obtained by reaction of the ketones (**18**), (**19**) and (**21-23**) with propiophenones (**42**) and (**43**) in the presence of titanium tetrachloride and zinc under McMurry reductive carbonyl coupling conditions [31, 32] to allow formation of the alkenes (**36-41)** in moderate yield, (Scheme **2**, Table **2**). Some self-coupled propiophenone product was also observed in these reactions. It has been observed that the McMurry coupling of phenolic benzophenones usually results in the formation of the *trans* product arrangement of the phenolic aryl substituent relative to the ethyl vinylic substituent [33, 34]. ^{1}H NMR analysis of the products confirmed the predominantly *trans* nature of the products in some cases while other product examples show a 1:1 *E/Z* mixture. The phenolic products (**36-41**) were then alkylated with 2-chloroethyl-



1a R = H, Tamoxifen
1b R = OH, 4-Hydroxytamoxifen

2 Raloxifene

3a R = H, GW 5638
3b R = OH, GW 7604

4 LY2066948

5a R = H
5b R = OH

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

pyrrolidine or 2-chloroethylpiperidine to afford the aminoalkyl ether products (**44-50**). The ^{1}H NMR spectrum of the product (**45**) clearly shows the pyrrolidine methylenes as singlets at δ2.80 and δ1.90, the ethyl group signals as a triplet (δ0.93) and a quartet (δ2.39, J=7.4Hz), the basic side chain methylenes as triplets at δ3.02 and δ4.17, (J=5.5Hz) and the methylene groups linking Ring B are identified at δ2.55 and δ2.59 as multiplets.

Compounds (**51-53**) were obtained by direct coupling of the ketones (**54-56**) with the aminoalkylated propiophenone (**57**), (Scheme **2**). The phenolic product (**59**), containing the aminoalkyl substituent on Ring A, was also obtained by direct coupling of the ketone (**33**) with the pivaloyloxy substituted propiophenone (**58**) while the diphenolic product (**60**) could be obtained by base hydrolysis of the ester (**59**). The pivaloyl esters are more stable to hydrolysis than simple esters under physiological conditions [35], therefore the ester (**59**) might be considered as a prodrug ester of the phenolic compound (**60**) .

Synthesis of Series 3 products (**68**), (**78**), (**79**) and (**81**) was achieved by direct reaction of the aminoalkylketones (**27**), (**33**) with the substituted phenylbutanones(**61**), (**73**), (**74**) to afford the products (**68**), (**78**), (**79**) and (**81**), (Scheme **3**). Alternatively initial coupling of the ketone (**18**) with the phenylbutanones (**62**)-(**64**) and (**72**) afforded the alkenes (**65**), (**66**), (**67**) and (**75**) respectively which were subsequently alkylated with 2-chloroethylpyrrolidine giving the required products (**69**)-(**71**), (**76**) and (**81**) respectively. The phenolic compounds (**77**), (**80**) and (**82**) were obtained by basic hydrolysis of the pivaloyl esters (**76**), (**79**) and (**81**). The yield data for the product series are displayed in Table **2**.

INHIBITION OF PROLIFERATION OF HUMAN BREAST CANCER MCF-7 CELLS

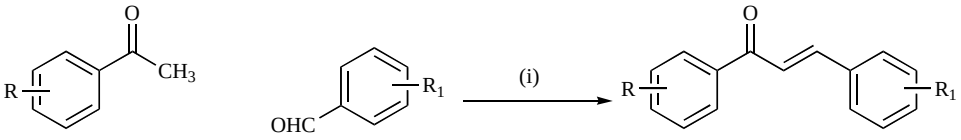
The clinically useful antiestrogen tamoxifen is known to achieve its antiproliferative effects through a number of mechanisms including ER modulation, cytotoxic effects and induction of apoptosis. The compounds prepared were initially evaluated for inhibition of proliferation of the human breast cancer MCF-7 cell line using the standard MTT assay. The results are displayed in Tables **1** and **2**.

The following structural compound classes were examined:

*Series 1:*1,3-Diarylpropan-2-ones: Compounds (**24**)-(**35**)

Series 2: 3-Benzyl-4,6-diarylhex-3-enes: Compounds (**44**)-(**60**)

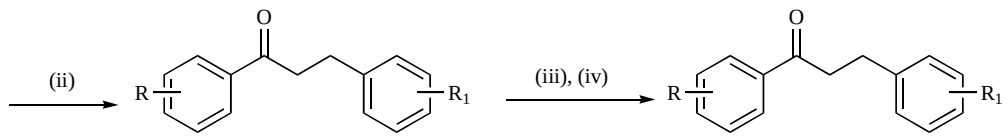
Series 3: 3,4,6-Triarylhex-3-enes: Compounds (**68**)-(**71**) and (**76**)-(**82**)



R = H, 2-OH, 3-OH, 4-OH, 2-OCH_3, 3-OCH_3, 4-OCH_3

R_1 = H, 2-OH, 3-OH, 4-OH, 2-OCH_3, 3-OCH_3, 4-OCH_3

6 R = 3-OH, R_1 = H
7 R = 2-OH, R_1 = H
8 R = 4-OH, R_1 = H
9 R = 4-OH, R_1 = 4-OCH_3
10 R = 4-OH, R_1 = 3-OCH_3
11 R = 4-OH, R_1 = 2-OCH_3
12 R = 3-OCH_3, R_1 = 4-OH
13 R = 2-OCH_3, R_1 = 4-OH
14 R = 4-OCH_3, R_1 = 4-OH



15 R = 3-OH, R_1 = H
16 R = 2-OH, R_1 = H
17 R = 4-OH, R_1 = H
18 R = 4-OH, R_1 = 4-OCH_3
19 R = 4-OH, R_1 = 3-OCH_3
20 R = 4-OH, R_1 = 2-OCH_3
21 R = 3-OCH_3, R_1 = 4-OH
22 R = 2-OCH_3, R_1 = 4-OH
23 R = 4-OCH_3, R_1 = 4-OH

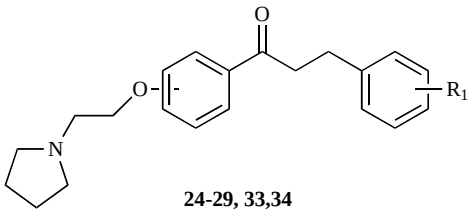
24 R = 3-$OCH_2CH_2NR_2$, R_1 = H
25 R = 2-$OCH_2CH_2NR_2$, R_1 = H
26 R = 4-$OCH_2CH_2NR_2$, R_1 = H
27 R = 4-$OCH_2CH_2NR_2$, R_1 = 4-OCH_3
28 R = 4-$OCH_2CH_2NR_2$, R_1 = 3-OCH_3
29 R = 4-$OCH_2CH_2NR_2$, R_1 = 2-OCH_3
30 R = 3-OCH_3, R_1 = 4-$OCH_2CH_2NR_2$
31 R = 2-OCH_3, R_1 = 4-$OCH_2CH_2NR_2$
32 R = 4-OCH_3, R_1 = 4-$OCH_2CH_2NR_2$
33 R = 4-$OCH_2CH_2NR_2$, R_1 = 4-OH
34 R = 4-$OCH_2CH_2NR_2$, R_1 = 3-OH
35 R = 2-OH, R_1 = 4-$OCH_2CH_2NR_2$

R_2 = -$CH_2CH_2CH_2CH_2$-

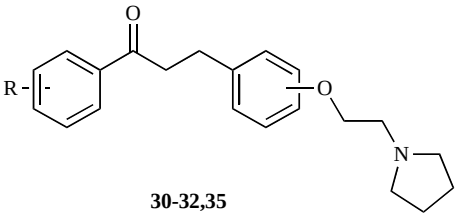
Scheme reagents and conditions: (i) NaOH, EtOH (ii) H_2, Pd/C, EtOH (iii) K_2CO_3, H_2O, 2-Chloroethylpyrrolidine.HCl (iv) $BF_3.(CH_3)_2S$, CH_2Cl_2

Scheme 1.

Table 1. Yield and Antiproliferative Effects of Compounds 24-35 on MCF-7 Cells



24-29, 33,34

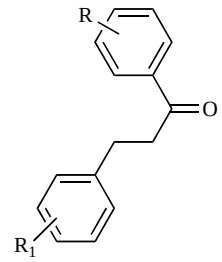


30-32,35

Compound	Yield (%)	MCF-7 Activity IC_{50} value[1,2] (μM)	Compound	Yield (%)	MCF-7 Activity IC_{50} value[1,2] (μM)
24	73	39.9 ± 2.2	30	88	24.3 ± 2.4
25	40	12.2 ± 1.9	31	36	32.9 ± 4.8
26	65	32.4 ± 2.4	32	70	14.3 ± 1.8
27	70	35.5 ± 1.2	33	76	29.8 ± 6.1
28	80	32.2 ± 0.7	34	55	29.1 ± 2.7
29	55	29.9±2.7	35	71	32.5 ± 3.6

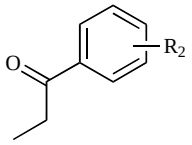
[1]IC_{50} values: the concentration required to inhibit 50% of MCF-7 growth. Values represent the mean ± S.E.M (error values x 10^{-6}) for six replicates.

[2]The value for tamoxifen IC_{50} 4.12±0.38μM is in good agreement with the reported IC_{50} value for tamoxifen using the MTT assay on human MCF-7 cells.



18 R = 4-OH, R_1 = 4-OCH_3
19 R = 4-OH, R_1 = 3-OCH_3
20 R = 4-OH, R_1 = 2-OCH_3
21 R = 3-OCH_3, R_1 = 4-OH
22 R = 2-OCH_3, R_1 = 4-OH
23 R = 4-OCH_3, R_1 = 4-OH
33 R = 4-$OCH_2CH_2NR_3$, R_1 = 4-OH
54 R = R_1 = 4-OCH_3
55 R = 3-OCH_3, R_1 = 4-OCH_3
56 R = 2-OCH_3, R_1 = 4-OCH_3

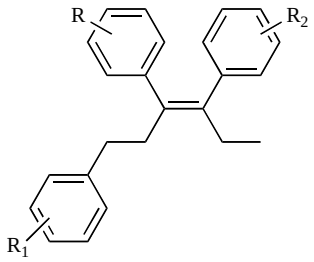
R_3 = -$CH_2CH_2CH_2CH_2$-



42 R_2 = H
43 R_2 = 4-OCH_3
57 R_2 = 4-$OCH_2CH_2NR_3$
58 R_2 = 4-$OCOC(CH_3)_3$

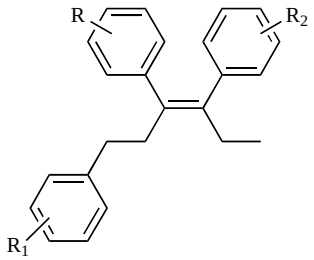
R_3 = -$CH_2CH_2CH_2CH_2$-

(i)



36 R = 3-OCH_3, R_1 = 4-OH, R_2 = H
37 R = 2-OCH_3, R_1 = 4-OH, R_2 = H
38 R = 4-OCH_3, R_1 = 4-OH, R_2 = H
39 R = 4-OH, R_1 = 4-OCH_3, R_2 = H
40 R = 4-OH, R_1 = 3-OCH_3, R_2 = H
41 R = 3-OH, R_1 = R_2 = 4-OCH_3,

(ii), (iii)



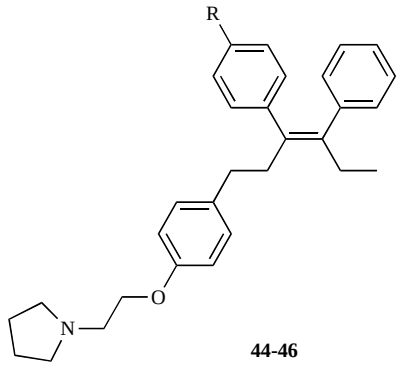
44 R = 3-OCH_3, R_1 = 4-$OCH_2CH_2NR_3$, R_2 = H
45 R = 2-OCH_3, R_1 = 4-$OCH_2CH_2NR_3$, R_2 = H
46 R = 4-OCH_3, R_1 = 4-$OCH_2CH_2NR_3$, R_2 = H
47 R = 4-$OCH_2CH_2NR_3$,R_1 = 4-OCH_3, R_2 = H
48 R = 4-$OCH_2CH_2NR_4$, R_1 = 3-OCH_3, R_2 = H
49 R = 4-$OCH_2CH_2NR_3$, R_1 = 2-OCH_3, R_2 = H
50 R = 4-$OCH_2CH_2NR_3$, R_1 = R_2 = 4-OCH_3
51 R = R_1 = 4-OCH_3, R_2 = 4-$OCH_2CH_2NR_3$
52 R = 3-OCH_3, R_1 = 4-OCH_3, R_2 = 4-$OCH_2CH_2NR_3$
53 R = 2-OCH_3, R_1 = 4-OCH_3, R_2 = 4-$OCH_2CH_2NR_3$
59 R = 4-$OCH_2CH_2NR_3$, R_1 = 4-OH, R_2 = 4-$OCOC(CH_3)_3$
60 R = 4-$OCH_2CH_2NR_3$, R_1 =R_2 = 4-OH

R_3 = -$CH_2CH_2CH_2CH_2$-
R_4 = -$CH_2CH_2CH_2CH_2CH_2$-

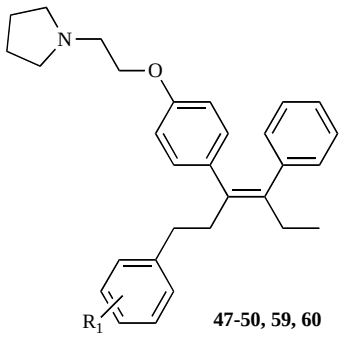
Scheme reagents and conditions: (i) Zn, $TiCl_4$, THF, reflux (ii) K_2CO_3, Acetone/H_2O , 2-Chloroethylpyrrolidine.HCl or 2-Chloroethylpiperidine.HCl (iii) NaOH, EtOH.

Scheme 2.

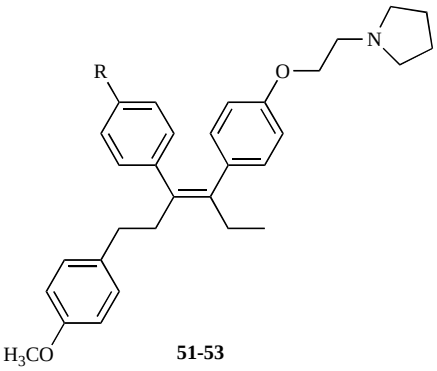
Table 2. Yield and Antiproliferative Effects of Analogues on MCF-7 Cells



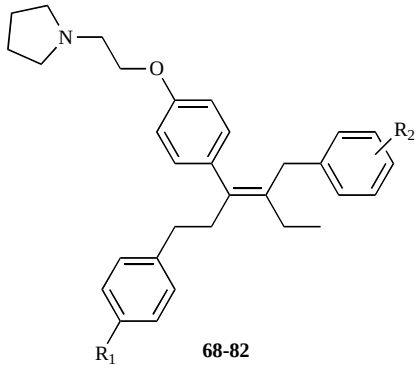
44-46



47-50, 59, 60



51-53



68-82

Compound	Yield (%)	Isomer Ratio[1]	MCF-7 Activity $IC_{50}(\mu M)$[2]	Compound	Yield (%)	Isomer Ratio[1]	MCF-7 Activity $IC_{50}(\mu M)$[2]
44	70	20:1	14.0 ± 2.2	68	85	1;1	5.7 ± 0.1
45	80	3:1	6.0 ± 0.3	69	22	1:1	19.5 ± 0.1
46	75	Z only	29.7 ± 1.8	70	43	1:1	16.0 ± 1.3
47	70	3:1	14.0 ± 1.2	71	34	1:1	27.8 ± 1.3
48	34	3:1	11.8 ± 1.1	76	37	1:1	8.1 ± 1.2
49	38	3:1	4.4 ± 1.2	77	33	1:1	4.6 ± 1.2
50	70	1:6	11.4 ± 1.0	78	44	1:1	7.2 ± 1.2
51	57	10:1	7.1 ± 0.4	79	32	3:2	6.5 ± 0.4
52	42	15:1	6.9 ± 0.1	80	21	1.5:1	6.9 ± 2.2
53	30	4:1	12.2 ± 0.8	81	80	1:1	6.2 ± 1.3
59	43	>100:1	5.4 ± 1.0	82	42	3:1	10.5 ± 0.1
60	31	3:1	0.2 ± 0.1	1a[3]	-	-	4.12 ± 0.4

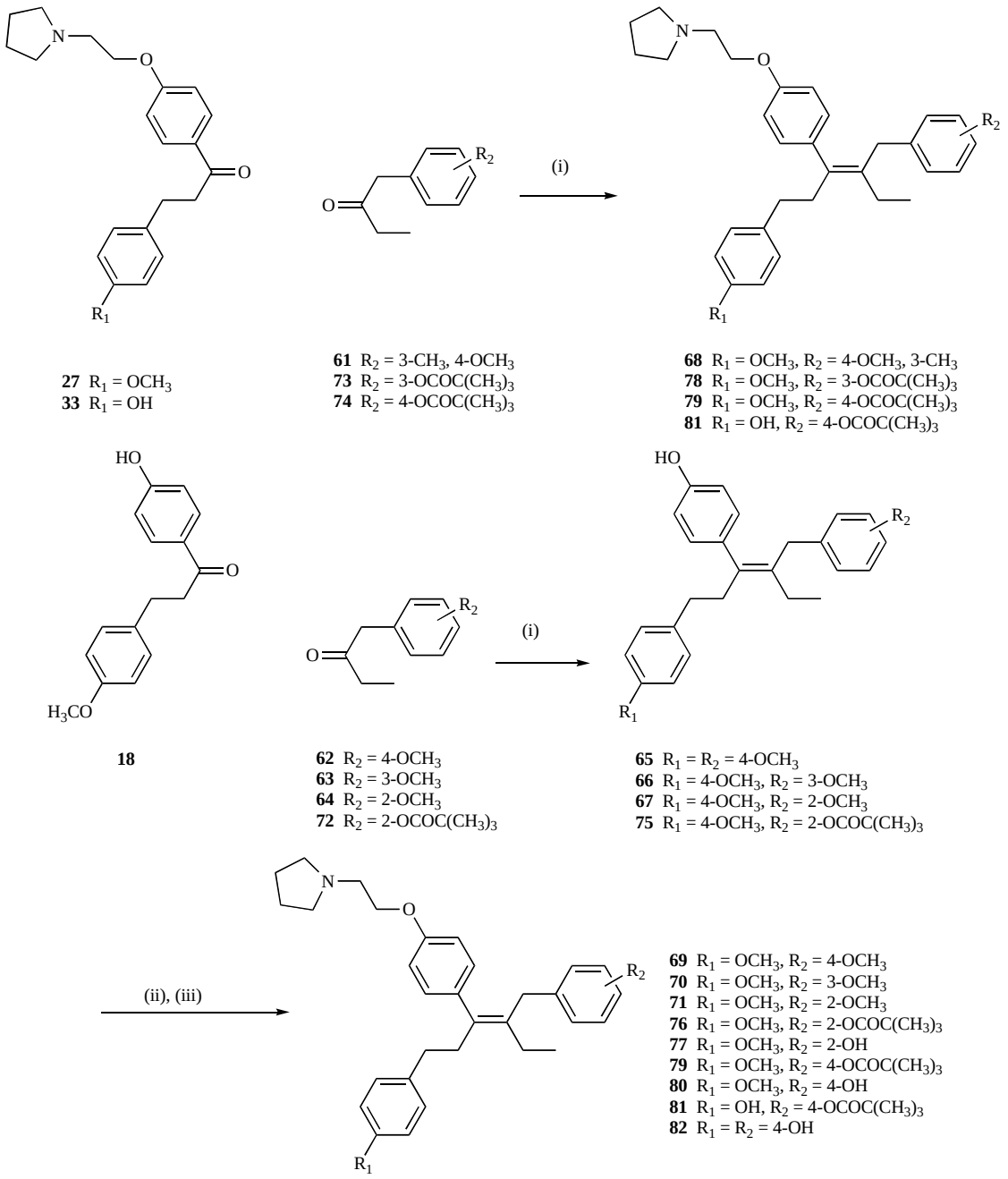
[1] Isomer ratio is determined as major:minor isomer present; *Z* isomer is major isomer for compounds **47-50**, **59**, **60**, **68-82**, *E* isomer is major isomer for compounds **44-46**.

[2] IC_{50} values: the concentration required to inhibit 50% of MCF-7 growth. Values represent the mean ± S.E.M (error values x 10^{-6}) for two experiments performed in triplicate.

[3]The value for tamoxifen IC_{50} 4.12±0.38μM is in good agreement with the reported IC_{50} value for Tamoxifen using the MTT assay on human MCF-7 cells.

Antiproliferative activity for various chalcones and dihydrochalcones has been reported [36, 37] hence the activity of the basic ketones (**24**)-(**35**) was initially examined to determine the contribution of these structural components towards the activity of the products. The dihydrochalcones examined (**24**)-(**35**) (Series1) showed poor antiproliferative activity against the MCF-7 cell line except for compounds (**25**) and (**32**) which demonstrated moderate activity with IC_{50} values 12.2 and 14.3μM respectively, (Table **1**). The placement of the aminoalkyl ether substituent was tolerated on either of the aromatic rings.

The antiproliferative activity of the 3-benzyl-4,6-diarylhex-3-enes (Series 2) compounds (**44**)-(**60**) was next examined (see Table **2** for the details). Compounds (**44**)-(**46**) con-



27 $R_1 = OCH_3$
33 $R_1 = OH$

61 R_2 = 3-CH_3, 4-OCH_3
73 R_2 = 3-$OCOC(CH_3)_3$
74 R_2 = 4-$OCOC(CH_3)_3$

68 $R_1 = OCH_3$, R_2 = 4-OCH_3, 3-CH_3
78 $R_1 = OCH_3$, R_2 = 3-$OCOC(CH_3)_3$
79 $R_1 = OCH_3$, R_2 = 4-$OCOC(CH_3)_3$
81 $R_1 = OH$, R_2 = 4-$OCOC(CH_3)_3$

18

62 R_2 = 4-OCH_3
63 R_2 = 3-OCH_3
64 R_2 = 2-OCH_3
72 R_2 = 2-$OCOC(CH_3)_3$

65 $R_1 = R_2$ = 4-OCH_3
66 R_1 = 4-OCH_3, R_2 = 3-OCH_3
67 R_1 = 4-OCH_3, R_2 = 2-OCH_3
75 R_1 = 4-OCH_3, R_2 = 2-$OCOC(CH_3)_3$

69 $R_1 = OCH_3$, R_2 = 4-OCH_3
70 $R_1 = OCH_3$, R_2 = 3-OCH_3
71 $R_1 = OCH_3$, R_2 = 2-OCH_3
76 $R_1 = OCH_3$, R_2 = 2-$OCOC(CH_3)_3$
77 $R_1 = OCH_3$, R_2 = 2-OH
79 $R_1 = OCH_3$, R_2 = 4-$OCOC(CH_3)_3$
80 $R_1 = OCH_3$, R_2 = 4-OH
81 $R_1 = OH$, R_2 = 4-$OCOC(CH_3)_3$
82 $R_1 = R_2$ = 4-OH

Scheme reagents and conditions: Scheme reagents and conditions: (i) Zn, $TiCl_4$, THF, reflux (ii) K_2CO_3, Acetone/H_2O , 2-Chloroethylpyrrolidine.HCl (iii) NaOH, EtOH.

Scheme 3.

tain the aminoalkyl ether substituent on Ring B, with the most active compound (**45**) (IC_{50}= 6.0μM). Compounds (**47**)-(**50**) and (**59**)-(**60**) contain the aminoalkyl ether in more usual position of Ring A, while also containing methoxy or hydroxyl substituents on Ring B, together with methoxy or pivaloyl ester groups in Ring C. Within this group, effective antiproliferative activity is displayed for compound (**49**) (IC_{50}= 4.4μM). Compound (**60**) was the most potent compound of the entire series with IC_{50} = 0.2μM. Compound (**59**), the pivaloyl prodrug ester of the phenol (**60**), is also active with IC_{50} = 5.4μM. The pivaloyl esters of phenolic type flexible antiestrogens which possessed intrinsic antiproliferative activity have potential use as prodrugs which can be hydrolysed slowly *in vivo* so avoiding rapid initial metabolic glucuronidation of the phenol. The analogues (**51**), (**52**) and (**52**) demonstrated moderate antiproliferative activity with IC_{50} values = 7.1, 6.9 and 12.2μM respectively. This result illustrates that the location of the basic ether substituent in these flexible structures is tolerated on the unexpected location of Ring C where the predicted basic interaction of the pyrrolidine nitrogen with Asp 351 would require an alternative 180^o docking pose for the molecule to be accommodated in the LBD of the ER.

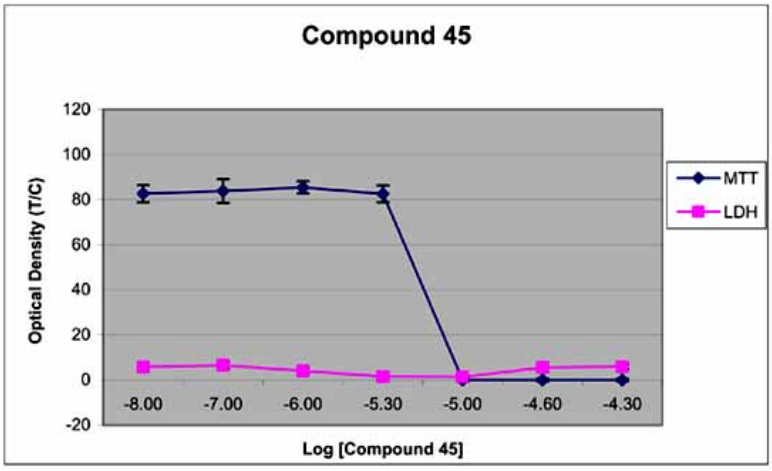
For compounds (**68**)-(**71**), the more sterically hindered compound (**68**) displayed optimum activity, (IC_{50}= 5.7mM), while the dimethoxy ethers (**69**)-(**71**) were weakly active. Compounds (**76**), (**78**) and (**79**) displayed IC_{50} values in the range 6.5-8.1μM. Hydrolysis of the pivaloyl ester (**76**) to afford the phenolic product (**77**), resulted in an improvement in the antiproliferative activity with IC_{50} value of 4.6μM which was the most potent compound in the 3,4,6-triarylhex-3-enes series. Corresponding hydrolysis of the pivaloyl ester group in compound (**79**) to afford compound (**80**) did not result in a significant change in the antiproliferative activity. Compound (**81**) displayed similar activity (IC_{50} = 6.2μM) when compared to compound (**79**), surprisingly indicating that the effect on activity of the 4-methoxy and 4-hydroxy substituent groups in this structural type are equivalent. Compound (**82**), in which both Rings B and C contain phenolic substituents, was moderately active with IC_{50} value of 10.5μM. By comparison, the related compound from the 3-benzyl-4,6-diarylhex-3-ene series, compound (**60**), was the most potent compound of the entire series, (IC_{50} = 0.2μM), indicating that introduction of the ethylene group acting as a flexible spacing group linking the aryl Ring A or Ring B with the core alkene group is well tolerated for antiproliferative activity. The inhibition of proliferation (MTT) and cytotoxicity (LDH) profiles of examples of the most active compounds are illustrated in Fig. (**2**).

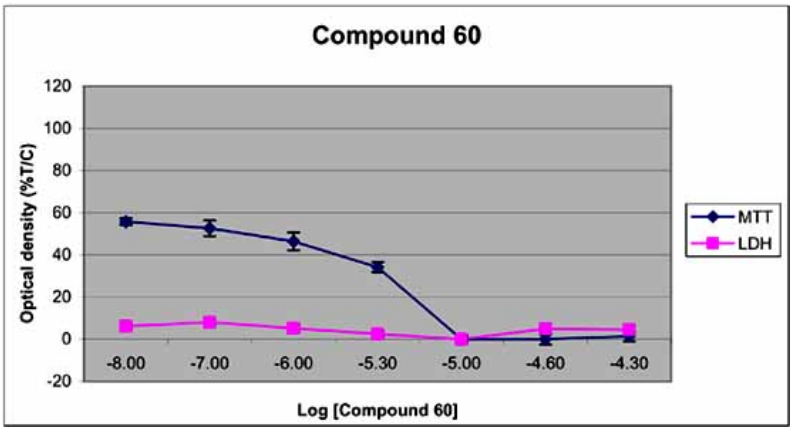
CYTOTOXICITY EFFECTS

The cytotoxicity profile of the novel compounds synthesized was examined using the LDH assay for cytotoxicity. All compounds examined showed low cytotoxicity activity indicating that these products were achieving their antiproliferative action through a cytostatic mechanism rather than a cytotoxic mechanism. For example compounds (**45**), (**60**) and (**80**) demonstrate cytotoxicity values of 1.35, 0 and 2.35% at a concentration of 10μM as illustrated in Fig. (**2**). This result compares favourably with that of tamoxifen which displays a higher cytotoxicity of 12.7% at this concentration.

ESTROGEN RECEPTOR BINDING STUDIES

A selection of the most potent antiproliferative 3-benzyl-4,6-diarylhex-3-ene and 3,4,6-triarylhex-3-ene compounds were evaluated for estrogen receptor binding activity with the human recombinant full length receptor proteins ERα and ERβ expressed from baculovirus infected insect cells. The procedure involves the displacement of fluoromone (a fluorescein labeled estradiol) in a competitive binding assay. The binding results are displayed in Table **3**, Fig. (**3**) and Fig. (**4**). Compounds (**59**), (**60**), (**68**), (**77**), (**80**), and (**82**) all were shown to have IC_{50} values for ERα binding of 290nM or less. The most potent compounds of the 3-benzyl-4,6-





(Fig. 2. Contd….)

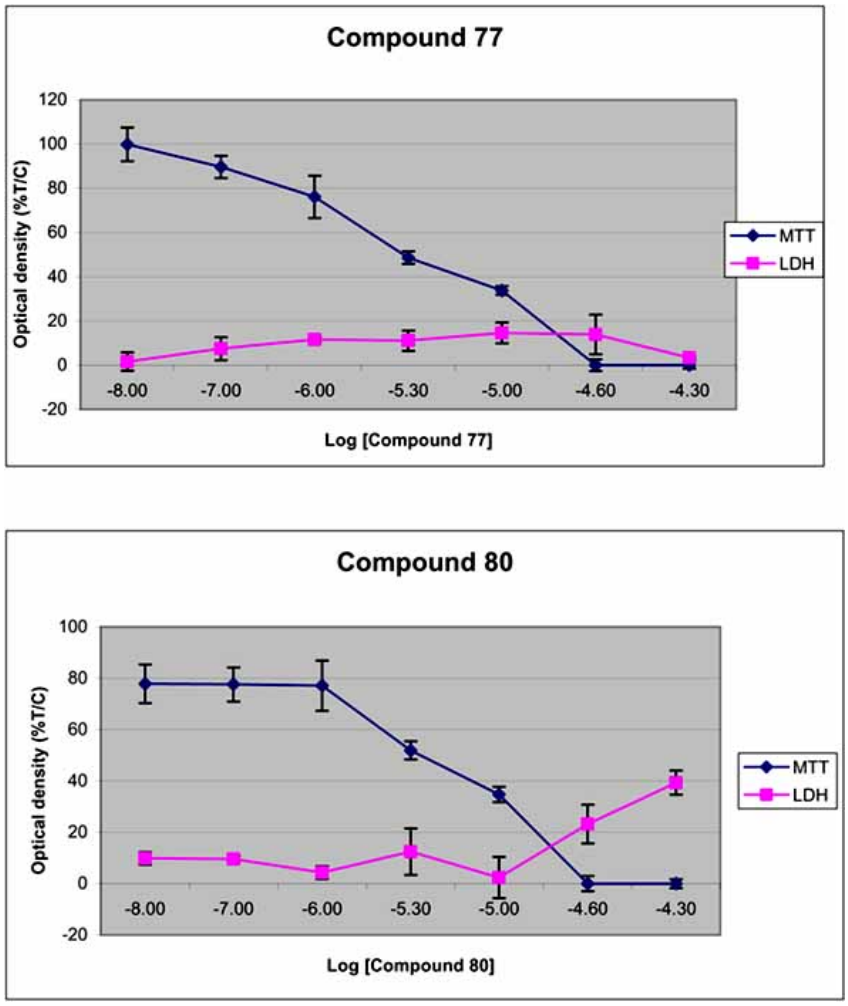


Fig. (2). Antiproliferative and cytotoxic activity of compounds (**45**), (**60**), (**77**) and (**80**) on estrogen sensitive MCF-7 breast cancer cells. The optical density values are given as a ratio of the treated cells and control cells x100% and are means of at least 9 replicates. The absence of error bars indicates that the error was smaller than the size of the symbol.

diarylhex-3-enes (Series 2) was shown to be compounds (**59**) and (**60**), with IC_{50} values of 290 and 220 nM respectively. Both compounds (**59**) and (**60**) contain a *p*-hydroxysubstituted Ring B which is distanced from the core olefin by an ethylene group acting as a flexible hinge, and positioned for optimum interaction with the Arg394 and Glu353 residues of the active binding site. In addition compound (**60**) contains a *p*-hydroxy substituent located on Ring C for interaction with His524 together with the pyrrolidine containing basic ether substituent on Ring A.

The most potent ERα binding for the 3,4,6-triarylhex-3-ene series was observed with compound (**77**), (IC_{50} = 40nM). This binding value is similar to the result observed for 4-hydroxytamoxifen (IC_{50} = 40nM) in this assay. The structural requirement for a phenolic substituent in ring C is also shown in the binding result for compounds (**80**) (IC_{50} = 110nM) and (**82**) (IC_{50} = 290nM) which both contain a *para* substituted phenolic Ring C. The binding values obtained for the compounds with ERα above are also consistent with the antiproliferative results for these compounds in MCF-7 cells in which compounds (**49**), (**59**), (**60**), (**68**), (**77**) and (**80**) all showing IC_{50} values of less than 6.9μM.

The IC_{50} values for ERβ receptor binding for the 3-benzyl-4,6-diarylhex-3-ene compounds were found to be in the region 0.68-2.52μM while the values obtained for the 3,4,6-triarylhex-3-enes tested were in the range 0.13-9.97μM with compound (**77**) identified as the most potent (IC_{50} = 130nM). This value is also comparable with that obtained for tamoxifen binding to ERβ (IC_{50} = 170nM) and demonstrates the structural requirement for *ortho* hydroxyl substitution in Ring C in these flexible compounds for optimum binding at both the ERα and ERβ.

The ERα/ERβ ligand binding ratio for the flexible compounds evaluated is presented in Table **3** and with the one exception of compound (**45**) containing the basic ether substituent in Ring B, the products all display a selectivity for the ERα. The greatest selectivity α/β=13.6:1 was displayed by compound (**68**); while compounds (**49**), (**79**), (**80**) and (**82**) displayed ER binding ratios ERα/β of 7.3:1, 8.7:1, 5.7:1 and 9.5:1 respectively. Examples of ERα selective ligands include the recently reported oxachrysenes for the treatment of postmenopausal symptoms of hot flush [9] and naphthalene type compounds which have application for the treat-

ment of uterine leiomyoma [8]. A fluoroethyl analogue of DPN (2,3-bis-(4-hydroxyphenyl)propanonitrile), a known ERβ selective ligand, has been reported for potential use as a diagnostic imaging agent with 8.3 fold absolute specificity for ERβ [38].

The relationship between ER binding affinity and anti-proliferative activity in MCF-7 cells is demonstrated for the 3-benzyl-4,6-diarylhex-3-enes and 3,4,6-triarylhex-3-enes compounds with the most effective compounds having a free phenolic substituent located on Ring C together with a methoxy or hydroxy substituent on ring B such as compounds (**60**) and (**77**).

Table 3. Estrogen Receptor Binding Data for Compounds

Compound Number	ER Binding assay IC_{50} value[a] (μM)		ERα: β Ligand Binding ratio
	α	β	
45	3.84	1.04	1:3.7
49	0.35	2.52	7.3:1
59	0.29	0.77	2.6:1
60	0.22	0.68	3.1:1
68	0.11	1.44	13.6:1
76	1.45	1.81	1.3:1
77	0.04	0.13	3.2:1
78	9.25	9.97	1.1:1
79	1.14	9.93	8.7:1
80	0.11	0.63	5.7:1
81	0.68	1.25	1.3:1
82	0.29	2.77	9.5:1
Tam	0.07	0.17	2.3:1
4-OHT	0.04	0.02	1:1.7

ESTROGENIC STIMULATION

The stimulatory effect of tamoxifen and hydroxytamoxifen on human breast cancer cell growth is well documented both *in vivo* and *in vitro* [39, 40]. As tamoxifen is usually administered over a long term period for the treatment or prevention of breast cancer, it is critical to evaluate newer antiestrogens for their potential estrogenic effects and so minimize the excess risk of developing uterine carcinomas due to prolonged use of the drugs.

The estrogen antagonistic and stimulating properties of a number of the most potent 3-benzyl-4,6-diarylhex-3-enes and 3,4,6-triarylhex-3-ene compounds were evaluated in an estrogen bioassay carried out with Ishikawa cells. The assay is based on the estrogen sensitive stimulation of alkaline phosphatase (AlkP) in the Ishikawa human endometrial adenocarcinoma cell line which is sensitive to estrogen stimulation as low as 10^{-12}M [41]. The results obtained for these compounds are presented in Table **4**.

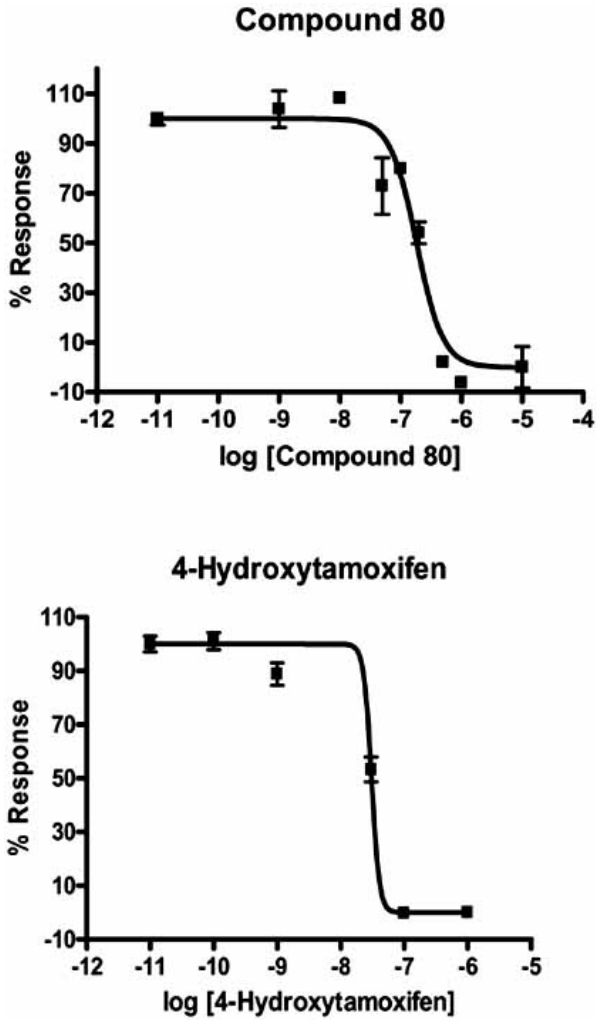


Fig. (3). Estrogen receptor alpha binding data for compound (**80**) together with data for 4-hydroxytamoxifen and are means of at least 9 replicates. The absence of error bars indicates that the error was smaller than the size of the symbol.

Compound (**59**) was found to be the most potent of the 3-benzyl-4,6-diarylhex-3-enes series, with IC_{50} value of 200nM. Compound (**77**) was found to be the most potent in the 3,4,6-triarylhex-3-ene series with IC_{50} value of 260nM as illustrated in Fig. (**5**). These values are in the same range as the value obtained for tamoxifen IC_{50} value of 170 nM and correlate with the ER binding activity of compound (**77**) for the ERα and ERβ. Compounds (**49**), (**76**), (**80**) and (**81**) also showed moderate antiestrogenic activity with IC_{50} values in the range 360-730nM.

The estrogenic stimulatory properties of these compounds on the Ishikawa cells were determined in the absence of estradiol. The results are shown in Table **4** and demonstrate a low level of estrogen stimulation for many of the compounds. Compound (**77**) which was the most potent compound in the ERα and ERβ binding assay, was shown to have a low level of stimulation of 3.2% at a concentration of

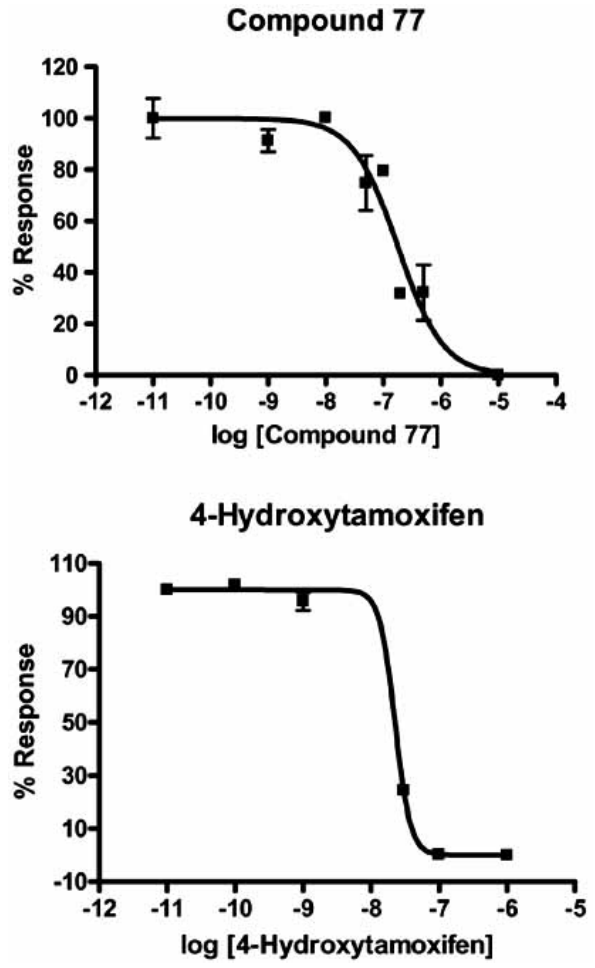


Fig. (4). Estrogen receptor beta binding data for compound (**77**) and 4-hydroxytamoxifen and are means of at least 9 replicates. The absence of error bars indicates that the error was smaller than the size of the symbol.

1μM, as illustrated in Fig. (**5**), whereas the stimulatory effect of tamoxifen was observed to be 4.3% at 0.1μM. Because of the known estrogen stimulatory effects of tamoxifen, 4-hydroxytamoxifen and related compounds in human Ishikawa cells, the AlkP determination of the estrogen antagonistic and stimulatory effects are critical in the selection of appropriate candidate compounds from the 3-benzyl-4,6-diarylhex-3-ene and 3,4,6-triarylhex-3-ene series of compounds for development as antiestrogens without adverse estrogenic effect on the uterus.

MOLECULAR MODELING STUDY

A molecular modeling study was undertaken to examine the interaction of the flexible ligands (**60**), (**80**), and (**82**) with the key residues in the ligand binding domain of the ERα and ERβ. A novel method involving multiple receptor conformation generation using FIRST5 software [42] in combination with FRODA [43] was used to examine the series of flexible antiestrogens and to rationalize the observed ER binding activity. There are currently no X-ray structures of human ERα and ERβ containing the same antagonist co-crystallized.

Table 4. Antiestrogenic and Estrogenic Activity for Compounds

Compound Number	Antiestrogenic Activity in Ishikawa cells $IC_{50}(\mu M)$ [a]	Estrogenic Activity in Ishikawa cells[a, b] (% stimulation)
45	4.40 ± 6.22	2.0
49	0.36 ± 0.0	1[c]
59	0.20 ± 0.14	24.0[d]
60	0.83 ± 0.77	18.5
68	1.29 ± 1.29	0.8
76	0.59 ± 0.07	3.6
77	0.26 ± 0.04	4.4[d]
78	111.4 ± 0.1	2.3
79	3.37±1.73	2
80	0.73±0.27	11.7[d]
81	0.56±0.0	4.3[c]
82	4.94 ±6.90	8.0[d]
1a	0.17 ±0.0	4.3

[a]Values are an average of at least twelve replicate experiments.

[b]Relative initial stimulator activity for compounds at concentrations of 0.01μM[c], 0.1μM and 1μM[d] in comparison with estradiol E2(1nM) = 100%.

The crystal structure of 4-hydroxytamoxifen(**1b**) co-crystallised with ERα (3ERT [44]) and raloxifene(**2**) with ERβ (1QKN [45]) were utilised as before. Docking studies employing the docking engine FRED2.11 [46] were carried out and the optimal scoring binding poses of each conformer of a ligand were retained for analysis with Ligand Protein Contacts (LPC) software [47]. The residues depicted are those that have been previously shown to be crucial in the ER ligand binding process: Asp351 (interacts with the basic side-chain nitrogen), Glu353 and Arg394 (anchor the ligand in the active site), His524 (additionally important in ligand binding process). Table **5** illustrates the interactions made by each ligand with both receptor isoforms indicating that all compounds dock in a similar manner when compared with OHT and RAL. Fig. (**6A/B**) illustrates compound (**82**) binding in an antiestrogenic manner with Rings B and C occupying reversed binding positions from those usually observed for ER antagonists such as 4-hydroxytamoxifen and raloxifene. The compounds when docked in ERα are predicted to make closer contacts with Asp351 than the equivalent Asp258 of ERβ. The reasons for this become clear upon examination of the docked positions of compound (**82**) in both ERα and ERβ as shown in Fig. (**6C**). Differing positions of Ile424, Phe425, Met342/343 in ERα compared with those adopted by Ile331, Phe332 and Met250/251 in ERβ allow these ligands to bind with some selectivity to ERα. A space-filling model of both docked ligands as illustrated in Fig. (**6D**) shows that the position of Met343 does not allow com-

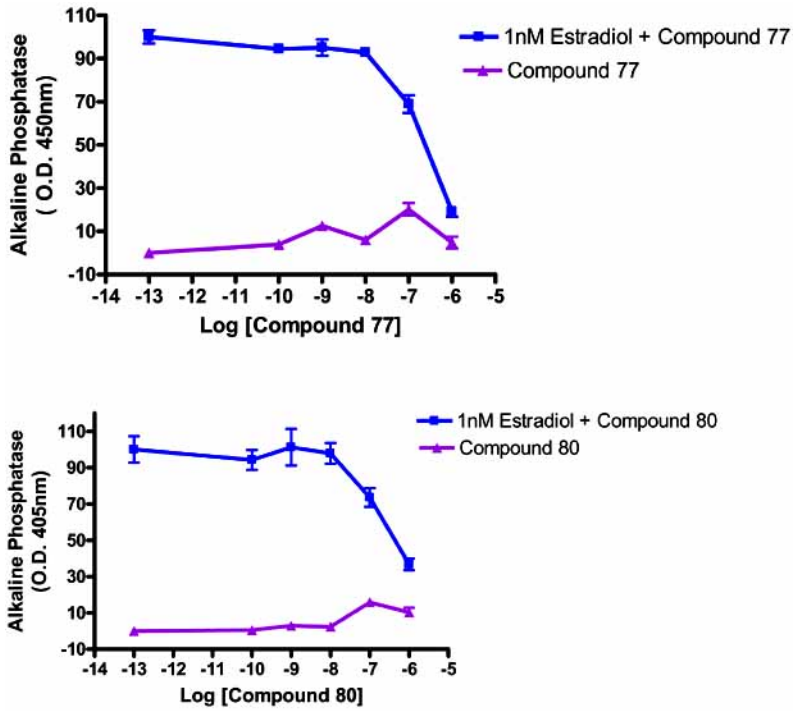


Fig. (5). Effect of increasing concentrations of compounds (**77**) and (**80**) on alkaline phosphatase activity in human Ishikawa cells. Alkaline phosphatase activity was measured after a 4 day exposure to increasing concentrations of antiestrogen compounds (**77**) and (**80**) in the presence and absence 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.

pound (**82**) to take up the same position as in 1QKN. Furthermore, His524 is rotated away from the internal binding cavity ensuring the lengthier chain of the B-ring can occupy this space. The basic side-chain of compound (**82**) can now interact more closely with Asp351 in ERα as shown in the LPC output in Table **5**. 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. Importantly, both the NC value and Chemgauss2 score were always lower for conformers docked in ERβ than ERα and thus corroborate the experimental findings. The subtlety of differences observed in both isoform cavities of the ER cannot be clearly observed by crystal structure alone, and therefore we have highlighted these differences through incorporation of receptor flexibility in our docking procedure.

CONCLUSION

The synthesis of a novel series of antiestrogenic 3-benzyl-4,6-diarylhex-3-enes and 3,4,6-triarylhex-3-enes containing an extended flexible core structure is investigated. In these compounds, the ethylene group acts as a flexible spacing group linking the aryl rings A, B or C with the core alkene structure. The compounds show antiproliferative activity with IC_{50} values up to 20nM against the MCF-7 breast

Table 6. Summary of Key Ligand-Prrotein for Compounds 60, 80, 82[a]

Comp.	Isoform	ASP 351 (Asp 258)	Glu 353 (Glu 260)	Arg 394 (Arg 301)	His 524 (His 430)	NC	Chemgauss2
60	a	2.8	2.5	2.4	2.7	0.89	-54.78
60	b	3.5	2.7	3.6	2.7	0.85	-46.91
80	a	3.4	2.5	2	2.7	0.89	-51.07
80	b	3.9	1.9	3.3	3	0.84	-46.21
82	a	3.1	3.6	2.7	3.2	0.98	-55.98
82	b	5.3	---	3.1	2.9	0.73	-40.85
OHT	a	3.2	2.5	3	4	0.89	---
RAL	b	3.3	2.6	3	2.6	0.69	---

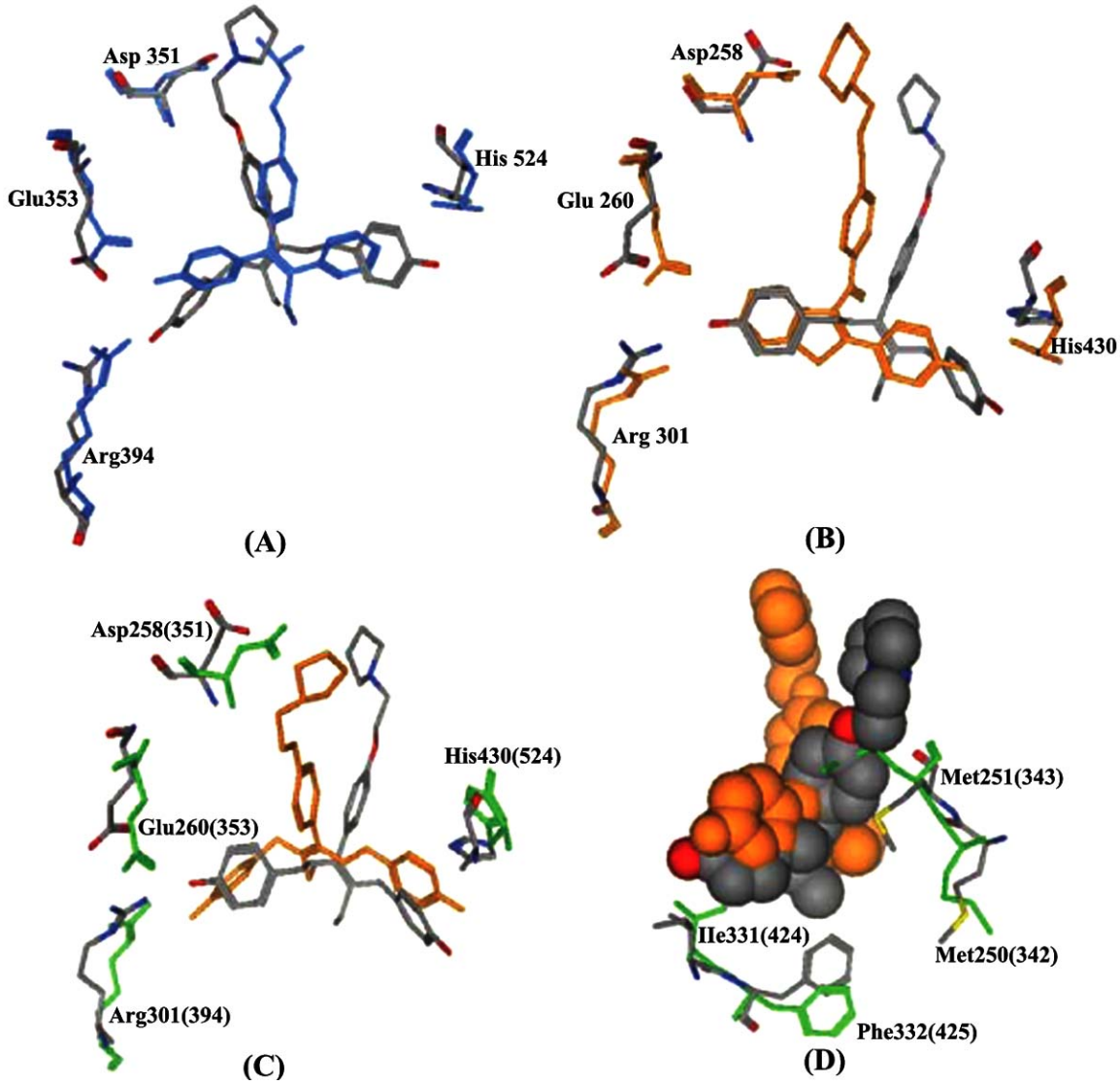


Fig. (6). Top ranked docking solution of compound (**82**) (shaded by atom) superimposed by backbone on 3ERT (A) and 1QKN (B). (C) Depicts compound (**82**) (plain) docked in 3ERT overlayed with compound (**82**) docked in 1QKN (shaded by atom). (D) As in C with 1QKN docked complex shaded by atom and hashed.

cancer cell line and also we have demonstrated low cytotoxicity indicating a cytostatic mechanism of action. Effective ER binding affinity was demonstrated for many of the compounds together with ERα/β selectivity of up to 13 fold. The most potent products displayed antiestrogenic activity in Ishikawa human uterine cell line together with low estrogenic stimulation.

A computational docking study illustrated the selective binding of these compounds to ERα and ERβ and rationalizes the antiestrogenic activity of the products. We envisage that this procedure will guide our synthetic capabilities in the future and allow generation of more potent selective compounds. Structural flexibility is well tolerated in these structures and they offer potential application development as modulators for ER positive breast cancer.

ACKNOWLEDGEMENTS

We are very grateful to Professor Richard Hochberg at Yale University Medical School, for kindly facilitating the alkaline phosphatase experiments and for the generous gift of the Ishikawa cells. We also thank Professor Mike Thorpe and Dr. Brandon Hespenheide of Arizona State University, Tempe, AZ 85287-1504. for use of the software FIRST 5.2. This work was supported through funding from BioResearch Ireland (now the Enterprise Ireland Biotechnology Directorate), The Health Research Board Interdisciplinary Research Programme and the Trinity College IITAC research initiative – part of the Irish Higher Education Authority's program for research in third level institutes (PRTLI), with additional support for our computational facilities gained through funding from the Wellcome Trust.

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^{o}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. The biochemical protocols for the MTT antiproliferative, ER binding and Ishikawa assays were carried out as previously reported [27] and are available in the supplementary information together with ^{13}C NMR data.

1-(4-Hydroxyphenyl)-3-(2-methoxyphenyl)-propan-1-one (20)

1-(4-Hydroxyphenyl)-3-(2-methoxyphenyl)-propenone (**11**) (0.002M) was stirred in ethanol(50ml) with of 10% palladium on charcoal(50mg) under hydrogen. The resulting solution was filtered and the ethanol removed under reduced pressure. The product was purified by flash column chromatography (eluant: hexane:diethyl ether 1:1) and crystallised as clear crystals from hexane:diethyl ether 1:1 (86%), (R_f 0.4 hexane:diethyl ether 1:1), (m.p. 124^{o}C) [48]. IR ν_{max} (KBr) 2939 (OH), 1684 (C=O) cm^{-1}. ^{1}H NMR δ ($CDCl_3$) 3.02-3.06 (2H, t, J=7.16Hz, CH_2), 3.21-3.24 (2H, t, J=7.16Hz, CH_2), 3.84 (3H, s, OCH_3), 6.86-7.95 (8H, m, ArH).

3-(4-Hydroxyphenyl)-1-(2-methoxyphenyl)-propan-1-one (22)

3-(4-Hydroxyphenyl)-1-(2-methoxyphenyl)-propenone (**13**) (0.0048M) was dissolved in ethanol(50ml) and treated with 10% palladium on charcoal(122mg) as above. The product was obtained as a clear oil (67%), (R_f 0.4 hexane:diethyl ether 1:1) and used in subsequent reactions without further purification. IR ν_{max} (film) 3381 (OH), 1668 (C=O)cm^{-1}. ^{1}H NMR δ($CDCl_3$) 2.86-2.90 (2H, t, J=7.54Hz, CH_2), 3.18-3.20 (2H, t, J=7.54Hz, CH_2), 3.81 (3H, s, OCH_3), 6.68-7.62 (9H, m, ArH).

3-Phenyl-1-[3-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-propan-1-one (24)

1-(3-Hydroxyphenyl)-3-phenyl-propan-1-one (**15**) (0.0045 M) was heated at reflux for 5 hours in acetone:water 19:1 (10ml) with potassium carbonate (0.0054M), and 1-(2-chloroethyl)pyrrolidine.HCl (0.009M). The resulting mixture was dried (sodium sulphate), filtered and concentrated under reduced pressure. The product was purified by flash column chromatography (eluant: acetone). The product was obtained as a light brown oil (73%), (R_f 0.3 acetone) and used in subsequent reactions without further purification. IR ν_{max} (KBr) 2926, 2954 (CHs), 1611 (C=O)cm^{-1}. ^{1}H NMR δ($CDCl_3$) 1.82 (4H, s, CH_2CH_2), 1.92-1.96 (2H, t, J=7.52Hz, CH_2), 2.59-2.63 (2H, t, J=7.52Hz, CH_2), 2.64-2.67 (4H, m, CH_2CH_2), 2.90-2.93 (2H, t, J=6.02Hz, H-4), 4.09-4.12 (2H, t, J=6.02Hz, OCH_2), 6.85-7.31 (9H, m, ArH).

3-Phenyl-1-[2-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-propan-1-one (25)

1-(2-Hydroxyphenyl)-3-phenyl-propan-1-one (**16**) (0.0045 M) was treated with potassium carbonate (0.0054M), and 1-(2-chloroethyl)pyrrolidine.HCl (0.009M) for 5 hours as above. The product was purified by flash column chromatography (eluant: acetone) and obtained as an oil (40%), (R_f 0.2 acetone) [49]. IR ν_{max} (film) 2925 (CHs), 1612 (C=O), cm^{-1}. ^{1}H NMR δ($CDCl_3$) 1.89 (4H, s, 2xCH_2), 2.61-2.65 (2H, t, J=7.26, CH_2), 2.73-2.76 (2H, t, J=7.26, CH_2), 2.95 (4H, s, 2xCH_2), 3.11-3.12 (2H, t, J=5.52Hz,CH_2), 4.19-4.21 (2H, t, J=4.26, CH_2), 6.72-7.86 (9H, m, ArH).

3-(4-Methoxyphenyl)-1-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-propan-1-one (27)

1-(4-Hydroxyphenyl)-3-(4-methoxyphenyl)-propan-1-one (**18**)(0.047M) was treated with potassium carbonate (0.094M) and 1-(2-chloroethyl)pyrrolidine.HCl (0.094M) as above. The product was purified by flash column chromatography (eluant: acetone) and isolated as an oil (70%), (R_f 0.2 acetone). IR ν_{max} (film) 2955, 2786 (CHs), 1675 (C=O), cm^{-1}. ^{1}H NMR δ($CDCl_3$) 1.68 (4H, s, 2xCH_2), 2.51 (4H, s, 2xCH_2), 2.77-2.80 (2H, t, J=7.52Hz,CH_2), 2.83-2.86 (2H, t, J=7.52Hz, CH_2), 3.07-3.11 (2H, t, J=5.8Hz, CH_2), 3.65 (3H, s, OCH_3), 4.02-4.06 (2H, t, J=5.8Hz, CH_2), 6.69-7.80 (8H, m, ArH). HRMS calculated for $C_{22}H_{28}NO_3$ 354.2057 (M^++1), observed 354.2069.

3-(4-Methoxyphenyl)-1-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-propan-1-one (28)

1-(4-Hydroxyphenyl)-3-(3-methoxyphenyl)-propan-1-one (19) (0.006M) was treated with potassium carbonate (0.0072 M) and 1-(2-chloroethyl)pyrrolidine.HCl (0.011M) as above. The product was purified by flash column chromatography (eluant: acetone) and obtained as an oil (80%), (R_f 0.2 acetone). IR υ_{max} (film) 2929, 2785 (CHs), 1640 (C=O), cm^{-1}. ^{1}H NMR δ($CDCl_3$) 1.94-1.95 (4H, s, 2xCH_2), 2.95 (4H, s, 2xCH_2), 3.00-3.04 (2H, t, J=7.76Hz, CH_2), 3.15-3.17 (2H, t, J=5.26, CH_2), 3.22-3.27 (2H, t, J=7.52Hz, CH_2), 3.80(3H, s, OCH_3), 4.33-4.35 (2H, t, J=5.52Hz, CH_2), 6.70-7.94 (8H, m, ArH). HRMS calculated for $C_{22}H_{28}NO_3$ 354.2082 (M^++1), observed 354.2069.

3-(2-Methoxyphenyl)-1-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-propan-1-one (29)

1-(4-Hydroxyphenyl)-3-(2-methoxyphenyl)-propan-1-one (20) (0.00045M) was treated with potassium carbonate (0.00054M) and 1-(2-chloroethyl)pyrrolidine.HCl (0.0009M) as above. The product was purified by flash column chromatography (eluant: acetone) and obtained as an oil (55%), (R_f 0.3 acetone). IR ν_{max} (KBr) 2936 (CHs), 1675 (C=O), 1600 (C=C) cm^{-1}. ^{1}H NMR δ($CDCl_3$) 1.72 (4H, s, 2xCH_2), 2.56 (4H, s, 2xCH_2), 2.85 (2H, s, CH_2), 2.97 (2H, s, CH_2), 3.01

(2H, s, CH_2), 3.84 (3H, s, OCH_3), 4.17 (2H, m, CH_2), 6.86-8.00 (8H, d, ArH).HRMS calculated for $C_{22}H_{28}NO_3$ 354.2069 (M^++1), observed 354.2069.

1-(3-Methoxyphenyl)-3-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-propan-1-one (30)

3-(4-Hydroxyphenyl)-1-(3-methoxyphenyl)-propan-1-one (**21**) (0.006M) was treated with potassium carbonate (0.0072 M) and 1-(2-chloroethyl)pyrrolidine.HCl (0.011M) as above. The product was purified by flash column chromatography (eluant:acetone) and obtained as an oil (92%), (R_f 0.2 acetone). IR v_{max} (film) 2932, 2833 (CHs), 1681 (C=O)cm^{-1}. ^1H NMR δ($CDCl_3$) 1.73-1.74 (4H, s, 2xCH_2), 2.57 (4H, s, 2xCH_2), 2.81-2.83 (2H, t, J=5.78Hz,CH_2), 2.85-2.83 (2H, t, J=7.52Hz, CH_2), 2.94-2.98 (2H, t, J=7.52Hz, CH_2), 3.99 (3H, s, OCH_3), 4.01-4.03 (2H, t, J=5.02, CH_2), 6.86-8.00 (8H, m, ArH). HRMS calculated for $C_{22}H_{28}NO_3$ 354.2081 (M^++1), observed 354.2069.

1-(2-Methoxyphenyl)-3-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-propan-1-one (31)

3-(4-Hydroxyphenyl)-1-(2-methoxyphenyl)-propan-1-one (**22**) (0.004M) treated with potassium carbonate (0.0047M), and 1-(2-chloroethyl)pyrrolidine.HCl (0.0078M) as above. The product was purified by flash column chromatography (eluant: dichloromethane:methanol 80:20) and obtained as an oil (36%), (R_f 0.3 dichloromethane:methanol 19:1). IR v_{max} (film) 2942 (CHs), 1606 (C=O)cm^{-1}. ^1H NMR δ($CDCl_3$) 2.03-2.04 (4H, s, 2xCH_2), 2.9-2.98 (2H, t, J=7.78Hz, CH_2), 3.16 (4H, s, 2xCH_2), 3.25-3.27 (2H, t, J=7.78Hz, CH_2), 3.28-3.30 (2H, t, J=5.26, CH_2), 3.90 (3H, s, OCH_3), 4.34-4.36 (2H, t, J=5.26, CH_2), 6.82-7.69 (8H, m, ArH). HRMS calculated for $C_{22}H_{28}NO_3$ 354.2054 (M^++1), observed 354.2069.

1-(4-Methoxyphenyl)-3-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-propan-1-one (32)

3-(4-Hydroxyphenyl)-1-(4-methoxyphenyl)-propan-1-one (**23**) (0.004M) was treated with potassium carbonate (0.0047 M) and 1-(2-chloroethyl)pyrrolidine.HCl (0.0078M) as above. The product was purified by flash column chromatography (eluant: acetone) and obtained as an oil (70%), (R_f 0.2 acetone). IR v_{max} (film) 2955, 2786 (CHs), 1676 (C=O) cm^{-1}. ^1H NMR δ($CDCl_3$) 1.79-1.81 (4H, t, J=7.04Hz, CH_2), 2.56-2.60 (4H, t, J=7.78Hz, 2xCH_2), 2.63-2.64 (4H, s, H-2, 2xCH_2), 2.88-2.91 (2H, t, J=6.26, CH_2), 3.80 (3H, s, OCH_3), 4.08-4.11 (2H, t, J=6.02Hz, CH_2), 6.82-7.11 (8H, m, ArH). HRMS calculated for $C_{22}H_{28}NO_3$ 354.2062 (M^++1), observed 354.2069.

3-(4-Hydroxyphenyl)-1-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-propan-1-one (33)

Boron trifluoride-dimethyl sulphide (0.123M) was added dropwise over 30 min to 3-(4-methoxyphenyl)-1-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-propan-1-one (**27**) (0.0123M) in dichloromethane (30ml). 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 (200ml) and washed with saturated sodium bicarbonate solution (2 x 50ml), water (50ml) and brine (50ml) and was dried over Na_2SO_4. The solvent was removed under reduced pressure and residue was chromatographed on silica gel (eluant: dichloromethane:methanol 19:1) to yield a yellow oil (76%) (R_f 0.2 acetone). IR v_{max} (film) 3400 (OH), 2928, 2815 (CHs), 1673 (C=O), cm^{-1}. ^1H NMR δ($CDCl_3$) 1.72 (4H, s, 2xCH_2), 2.59 (4H, s, 2xCH_2), 2.80-2.84 (2H, t, J=7.78Hz, CH_2), 2.85-2.88 (2H, t, J=5.76Hz, CH_2), 2.83-2.86 (2H, t, J=7.54Hz, CH_2), 4.05 (2H, s, CH_2), 6.60-7.78 (2H, m, ArH). HRMS calculated for $C_{21}H_{25}NO_3$ (M^++1) 340.1907, observed 340.1913.

3-(3-hydroxyphenyl)-1-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-propan-1-one (34)

3-(3-Methoxyphenyl)-1-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-propan-1-one (**28**) (0.0123M) was treated with boron trifluoride-dimethyl sulphide (0.123M) in dichloromethane (8ml) over 30 min. as above. Following chromatography over silica gel (eluant:acetone) the product was obtained as a yellow oil (55%) (R_f 0.2 acetone). IR v_{max} (film) 3436 (OH), 2929, 2864 (CHs), 1674 (C=O)cm^{-1}. ^1H NMR δ($CDCl_3$) 1.89 (4H, s, 2xCH_2), 2.81 (4H, s, 2xCH_2), 2.90-2.94 (2H, t, J=7.78Hz, CH_2), 3.04-3.06 (2H, t, J=5.52Hz, CH_2), 3.10-3.14 (2H, t, J=7.78Hz, CH_2), 4.18-4.21 (2H, t, J=5.52Hz, CH_2), 6.66-7.84 (8H, m, ArH). HRMS calculated for $C_{21}H_{25}NO_3$ (M^++1) 340.1913, observed 340.1926.

1-(2-Hydroxyphenyl)-3-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-propan-1-one (35)

Boron trifluoride-dimethyl sulphide (0.123M) was added dropwise over 30 min to 1-(2-methoxyphenyl)-3-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-propan-1-one (**31**) (0.0123M) in dichloromethane (8ml) as above. Following chromatography over silica gel (eluant:acetone), the product was obtained as a yellow oil (71%) (R_f 0.24 acetone). IR v_{max} (film) 3300 (OH), 2925, 2854 (CHs), 1674 (C=O), 1600 (C=C), cm^{-1}. ^1H NMR δ($CDCl_3$) 1.81 (4H, s, CH_2), 2.62 (4H, s, 2xCH_2), 2.88-2.91 (2H, t, J=4.52, CH_2), 2.99-3.03 (2H, t, J=7.52Hz, CH_2), 3.28-3.32 (2H, t, J=5.28Hz, CH_2), 4.07-4.11 (2H, t, J=5.28Hz, CH_2), 6.80-7.77 (8H, m, ArH). HRMS calculated for $C_{21}H_{25}NO_3$ (M^++1) 340.1899, observed 340.1913.

4-{1-[2-(3-Methoxyphenyl)-ethyl]-2-phenyl-but-1-enyl}-phenol (36)

Zinc dust (0.218M) was refluxed in dry THF (tetrahydrofuran) (80ml) with titanium tetrachloride (0.109M) for 2 hours. 3-(4-Hydroxyphenyl)-1-(3-methoxyphenyl)-propan-1-one (**21**) (0.0137M) and propiophenone (0.0273M) in dry THF (20ml) were then added to the reaction as above. Column chromatography (eluant: hexane:dichloromethane 4:6) afforded the product as a yellow oil (49%), (R_f 0.3 hexane:dichloromethane 4:6) which was used in subsequent reactions without further purification.. IR v_{max} (film) 3408 (OH), 2949 (CHs), 1604 (C=C) cm^{-1}. ^1H NMR δ($CDCl_3$) 0.97-1.00 (3H, t, J=7.52Hz, CH_3), 2.49-2.54 (2H, q, J=7.28Hz, CH_2), 2.63-2.68 (2H, t, J=7.76Hz, CH_2), 2.88-2.90 (2H, t, J=7.78Hz, CH_2), 3.64 (3H, s, OCH_3), 6.84-7.34 (12H, m, ArH).

4-{1-[2-(2-Methoxyphenyl)-ethyl]-2-phenyl-but-1-enyl}-phenol (37)

A mixture of titanium tetrachloride (0.0066M) and zinc dust (0.0013M) in dry THF (tetrahydrofuran) (80ml) was refluxed for 2 hours. 3-(4-Hydroxyphenyl)-1-(2-methoxy-

phenyl)-propan-1-one (**22**) (0.0017M) and propiophenone (0.0033M) were added in dry THF (20ml) and the mixture was refluxed for 5 hours. The solution was washed with 10% K_2CO_3 solution (100ml), brine (100ml) and extracted with ethyl acetate (3 x 100ml). The filtrate was dried (sodium sulphate) and concentrated in vacuo. Column chromatography (eluant: hexane:dichloromethane 40:60) afforded the product as an oil (43%). (R_f 0.4 hexane:dichloromethane 40:60) which was used in subsequent reactions without further purification. IR v_{max} (film) 2930 (CHs), 1610 (C=C), cm^{-1}. ^1H NMR δ($CDCl_3$) 0.99-1.03 (3H, t, J=7.5Hz, CH_3), 2.45-2.48 (2H, q, J=7.28Hz, CH_2), 2.54-2.59 (2H, t, J=7.8Hz, CH_2), 2.83-2.89 (2H, t, J=7.8Hz, CH_2), 3.64 (3H, s, OCH_3), 6.82-7.34 (12H, m, ArH).

4-[3-(4-Methoxyphenyl)-4-phenyl-hex-3-enyl]-phenol (38)

Titanium tetrachloride (0.007M) was added to zinc dust (0.014M) and stirred under reflux in dry THF (80ml) under a nitrogen atmosphere for 2 hours. A solution of 3-(4-hydroxyphenyl)-1-(4-methoxyphenyl)-propan-1-one (**23**) (0.00175 M) and propiophenone (0.0035M) in dry THF (20ml) was added and the reaction was refluxed for 5 hours as above. Flash chromatography (eluant: dichloromethane) afforded the product as a yellow oil (43%), (R_f 0.4 dichloromethane) which was used in subsequent reactions without further purification. IR v_{max} (film) 3368(OH), 2930 (CHs), 1601 (C=C)cm^{-1}. ^1H NMR δ($CDCl_3$) 0.93-0.90 (3H, t, J=7.52Hz, CH_3), 2.41-2.47 (2H, q, J=7.52Hz, CH_2), 2.55-2.59 (2H, t, J=8.04Hz, CH_2), 2.8-2.83 (2H, t, J=8.04Hz, CH_2), 3.74 (3H, s, OCH_3), 6.65-7.12 (13H, m, ArH).

4-{1-[2-(4-Methoxyphenyl)-ethyl]-2-phenyl-but-1-enyl}-phenol (39)

Titanium tetrachloride (0.0016M) was added to zinc (0.0032M) in THF (80ml) and the mixture was refluxed for 2 hours. Then 1-(4-hydroxyphenyl)-3-(4-methoxyphenyl)-propan-1-one (**43**) (0.004M) and propiophenone (0.008M) were dissolved in dry THF (20ml) and added to the reaction vessel refluxed for 5 hours as above. Column chromatography (eluant: dichloromethane) afforded the product as a yellow oil (80%), (R_f 0.6 dichloromethane) which was used in subsequent reactions without further purification. IR v_{max} (film) 3391 (OH), 2961 (CHs), 1610 (C=C) cm^{-1}. ^1H NMR δ($CDCl_3$) 0.78-0.82 (3H, t, J=6.28Hz, H-6, CH_3), 2.21 (4H, m, 2xCH_2), 2.4-2.41 (2H, q, J=7.08, CH_2), 3.76 (3H, s, OCH_3), 6.57-7.35 (13H, m, ArH).

4-{1-[2-(3-Methoxyphenyl)-ethyl]-2-phenyl-but-1-enyl}-phenol (40)

Titanium tetrachloride (0.00313M) was added dropwise to a stirred suspension of zinc dust (0.00625M) in dry THF (tetrahydrofuran) (80ml) under a nitrogen atmosphere. The mixture was refluxed for 2 hours in the dark. 1-(4-Hydroxyphenyl)-3-(3-methoxyphenyl)-propan-1-one (**42**) (0.00078 M) and propiophenone (0.0047M) were dissolved in dry THF (20ml) and added to the reaction in one portion as above. Column chromatography (eluant: dichloromethane) afforded the product as a yellow oil (43%), (R_f 0.7 dichloromethane) which was used in subsequent reactions without further purification. IR v_{max} (film) 3372 (OH), 2951, 2930 (CHs), 1602 (C=C) cm^{-1}. ^1H NMR δ($CDCl_3$) 0.83-0.79 (3H, t, J=7.5Hz, CH_3), 2.45-2.47 (2H, q, J=7.48, CH_2), 2.62-2.64 (2H, t, J=8.84, CH_2), 2.82-2.84 (2H, t, J=8.88, CH_2), 3.73 (3H, s, OCH_3), 6.88-7.37 (13H, m, ArH).

4-{2-(4-Methoxyphenyl)-1-[2-(4-methoxyphenyl)-ethyl]-but-1-enyl}-phenol (41)

Titanium tetrachloride (0.0164M) was added to zinc dust (0.0328M) in THF (80ml) and refluxed at for 2 hours in the dark. 1-(4-Hydroxyphenyl)-3-(4-methoxyphenyl)-propan-1-one (**43**) (0.0042M) and 4-methoxypropiophenone (0.008M) in THF (20ml) were added to the reaction and refluxing was continued for a further 5 hours as above. Column chromatography (eluant: hexane:dichloromethane 40:60) afforded the product as a yellow oil (36%) (R_f 0.5 hexane:dichloromethane 40:60) which was used in subsequent reactions without further purification. IR v_{max} (film) 3040 (OH), 2876 (CHs), 1607 (C=C) cm^{-1}. ^1H NMR δ($CDCl_3$) 0.81-0.83 (3H, t, J=7.52Hz, CH_3), 2.24-2.27 (2H, q, J=7.5Hz, CH_2), 2.54-2.63 (2H, m, CH_2), 2.65 (2H, m, CH_2), 2.80 (2H, m, CH_2), 3.78 (6H, s, 2xOCH_3), 6.63-7.78 (12H, m, ArH).

1-[2-(4-{1-[2-(3-Methoxyphenyl)-ethyl]-2-phenyl-but-1-enyl}-phenoxy)-ethyl]-pyrrolidine (44)

4-{1-[2-(3-Methoxyphenyl)-ethyl]-2-phenyl-but-1-enyl}-phenol (**36**) (0.0033M) in acetone:water 19:1 (10ml) was treated with potassium carbonate (0.004M) and 1-(2-chloroethyl)pyrrolidine.HCl (0.0067M) as above. The product was purified by flash column chromatography (eluant: acetone) to afford an oil (70%), (R_f 0.2 acetone). IR v_{max} (film) 2925, 2854 (CHs), 1606 (C=C) cm^{-1}. ^1H NMR δ($CDCl_3$) 0.89-0.92 (3H, t, J=7.52Hz, CH_3), 1.88 (4H, s, 2xCH_2), 2.40-2.46 (2H, q, J=7.54Hz, CH_2), 2.56-2.60 (2H, t, J=8.04Hz, CH_2), 2.67 (4H, s, 2xCH_2), 2.79-2.83 (2H, t, J=8.02Hz, CH_2), 2.92-2.94 (2H, t, J=6.02Hz, CH_2), 3.60 (3H, s, OCH_3), 4.11-4.14 (2H, t, J=6.02Hz, CH_2), 6.50 -7.11 (13H, d, ArH). HRMS calculated for $C_{31}H_{38}NO_2$ 456.2903 (M^++1), observed 456.2923.

4-{1-[2-(2-Methoxyphenyl)-ethyl]-2-phenyl-but-1-enyl}-phenoxy)-ethyl]-pyrrolidine (45)

4-{1-[2-(2-Methoxyphenyl)-ethyl]-2-phenyl-but-1-enyl}-phenol (**37**) (0.0033M) was treated in acetone:water 19:1 (10ml) with potassium carbonate (0.004M) and 1-(2-chloroethyl)pyrrolidine.HCl (0.0067M) as above. The product was purified by flash column chromatography (eluant: acetone) obtained as an oil (80%), (R_f 0.2 acetone). IR v_{max} (film) 2944, 2929 (CHs), 1597 (C=C) cm^{-1}. ^1H NMR δ($CDCl_3$) 0.93-0.96 (3H, t, J=7.54Hz, CH_3), 1.90 (4H, s, 2xCH_2), 2.39-2.47 (2H, q, J=7.4Hz, CH_2), 2.55 (2H, m, CH_2), 2.59 (2H, m, H-2, CH_2), 2.80 (4H, s, 2xCH_2), 3.02-3.05 (2H, t, CH_2), 3.74 (3H, s, OCH_3), 4.17-4.20 (2H, t, J=5.78Hz, CH_2), 6.70-7.13 (13H, m, ArH). HRMS calculated for $C_{31}H_{38}NO_2$ 456.2903 (M^++1), observed 456.2921.

1-(2-{4-[3-(4-Methoxyphenyl)-4-phenyl-hex-3-enyl]-phenoxy}-ethyl)-pyrrolidine (46)

4-[3-(4-Methoxyphenyl)-4-phenyl-hex-3-enyl]-phenol (**38)**(0.0008M) was treated with potassium carbonate (0.001 M) and 1-(2-chloroethyl)pyrrolidine.HCl (0.00167M) in acetone:water 19:1 (10ml) as above.. The product was obtained

by flash column chromatography (eluant: dichloromethane: methanol 80:20) as an oil (75%), (R_f 0.23 dichloromethane: methanol 9:1). IR v_{max} (film) 2926 (CHs), 1607 (C=C) cm^{-1}. ^1H NMR δ($CDCl_3$) 0.86-0.90 (3H, t, J=7.52Hz, CH_3), 1.9-1.95 (4H, s, 2xCH_2), 2.38-2.43 (2H, q, J=7.52Hz, CH_2), 2.52-2.56 (2H, t, J=7.28Hz, CH_2), 2.75-2.78 (2H, t, J=7.28Hz, CH_2), 2.96 (4H, s, 2xCH_2), 3.13-3.16 (2H, t, J=5.28Hz, CH_2), 3.71 (3H, s, OCH_3), 4.20-4.24 (2H, t, J=5.28Hz, CH_2), 6.60-7.08 (13H, m, ArH). HRMS calculated for $C_{24}H_{24}O_2$ 456.6310 (M^++1), observed 456.2903.

1-[2-(4-{1-[2-(4-Methoxyphenyl)-ethyl]-2-phenyl-but-1-enyl}-phenoxy)-ethyl]-pyrrolidine (47)

4-{1-[2-(4-Methoxyphenyl)-ethyl]-2-phenyl-but-1-enyl}-phenol (**39**) (0.001M) in acetone:water 19:1 (10ml) was treated with potassium carbonate (0.0011M), and 1-(2-chloroethyl)pyrrolidine.HCl (0.002M) and the reaction was refluxed for 5 hours as above. Purification by flash column chromatography (eluant:chloroform) afforded the product as an oil (38%), (R_f 0.2 chloroform). IR v_{max} (KBr) 2962 CHs), 1608(C=C), cm^{-1}. ^1H NMR δ ($CDCl_3$) 0.78-0.80 (3H t, J=7.52Hz, CH_3), 1.84-1.90 (4H, m, 2xCH_2), 2.18-2.24 (2H, m, CH_2), 2.38-2.46 (4H, m, 2xCH_2), 2.79 (4H, s, 2xCH_2), 3.00-3.03 (2H, t, J=5.78Hz, CH_2), 3.76 (3H, s, OCH_3), 4.2-4.24 (2H, t, J=6.02Hz, CH_2), 6.72-7.37 (13H, t, J=7.52Hz, ArH). HRMS calculated for $C_{31}H_{37}NO_2$ 456.2876 (M^++1), observed 456.2880.

1-[2-(4-{1-[2-(3-Methoxyphenyl)-ethyl]-2-phenyl-but-1-enyl}-phenoxy)-ethyl]-piperidine (48)

4-{1-[2-(3-Methoxyphenyl)-ethyl]-2-phenyl-but-1-enyl}-phenol (**40**) (0.0002M) was refluxed for 5 hours in acetone: water 19:1 (20ml) with potassium carbonate (0.00024M), and 1-(2-chloroethyl)pyrrolidine.HCl (0.0004M) as above. The product was obtained by flash column chromatography (eluant: dichloromethane:methanol 80:20) as an oil (34%), (R_f 0.6 dichloromethane:methanol 60:40). IR v_{max} (film) 2931 (CHs), 1605 (C=C) cm^{-1}. ^1H NMR δ($CDCl_3$) 0.78-0.80 (3H, t, J=7.00Hz, CH_3), 1.27 (2H, s, CH_2), 1.65 (4H, s, 2xCH_2), 2.18 (4H, m, 2xCH_2), 2.42-2.44 (2H, q, J=8.88, CH_2), 2.56 (4H, s, 2xCH_2), 2.82-2.85 (2H, t, J=6.26, CH_2), 3.70 (3H, s, OCH_3), 4.2-4.22 (2H, t, J=6.02Hz, CH_2), 6.93-7.37 (13H, m, ArH). HRMS calculated for $C_{32}H_{39}NO_2$ (M^++1) 470.3059, observed 470.3070.

[2-(4-{1-[2-(3-Methoxyphenyl)-ethyl]-2-phenyl-but-1-enyl}-phenoxy)-ethyl]-pyrrolidine (49)

4-{1-[2-(3-Methoxyphenyl)-ethyl]-2-phenyl-but-1-enyl}-phenol (**40**) (0.00056M) was refluxed for 5 hours in acetone:water 19:1 (10ml) with potassium carbonate (0.000672 M), and 1-(2-chloroethyl)pyrrolidine.HCl (0.00112M) as above. The product was obtained by flash column chromatography (eluant: dichloromethane:methanol 80:20) as an oil (73%). IR v_{max} (film) 2952, 2928 (CHs), 1606 (C=C) cm^{-1}. ^1H NMR δ($CDCl_3$) 0.82-0.79 (3H, t, J=7.34, CH_3), 1.88 (4H, s, CH_2), 2.17-2.23 (4H, m, 2xCH_2), 2.45-2.47 (2H, q, J=8.88, CH_2), 2.76 (4H, s, 2xCH_2), 3-3.04 (2H, t, J=5.86, CH_2), 3.73 (3H, s, OCH_3), 4.20-4.23 (2H, t, J=6.02Hz, CH_2), 6.58-7.23 (13H, m, ArH). HRMS calculated for $C_{31}H_{38}NO_2$ 456.2903 (M^++1), observed 456.2925.

1-[2-(4-{2-(4-methoxyphenyl)-1-[2-(4-methoxyphenyl)-ethyl]-but-1-enyl}-phenoxy)-ethyl]-pyrrolidine (50)

4-{2-(4-Methoxyphenyl)-1-[2-(4-methoxyphenyl)-ethyl]-but-1-enyl}-phenol (**41**) (0.00026M) in acetone:water 19:1 (10ml) was treated with potassium carbonate (0.0003M), and 1-(2-chloroethyl)pyrrolidine.HCl (0.000515M) as above. The product was obtained by flash column chromatography (eluant: acetone) as an oil (70%), (R_f 0.2 acetone). IR v_{max} (film) 2926 (CHs), 1608 (C=C) cm^{-1}. ^1H NMR δ($CDCl_3$) 0.78-0.81 (3H, t, J=7.28Hz, CH_3), 1.85 (4H, s, 2xCH_2), 2.27-2.25 (2H, q, J=7.02Hz, CH_2), 2.5-2.60 (2H, m, CH_2), 2.70 (6H, s, 3xCH_2), 2.95-2.98 (2H, t, J=6.02Hz, CH_2), 3.85 (6H, s, 2xOCH_3), 4.15-4.18 (2H, t, J=6.04Hz, CH_2), 6.63-7.18 (12H, m, ArH). HRMS calculated for $C_{32}H_{39}NO_3$ (M^++1) 486.3008, observed 486.3006.

1-(2-{4-[2-Ethyl-5-(4-methoxyphenyl)-3-(4-methoxyphenyl)-pent-2-enyl]-phenoxy}-ethyl)-pyrrolidine (51)

Titanium tetrachloride (0.0325M) was added to zinc (0.0646M) in THF (tetrahydrofuran) (80ml) and the mixture was refluxed for 2 hours. Then 1,3-bis-(4-methoxyphenyl)-propan-1-one (**54**) (0.004M) and 1-[4-(2-cyclopentyl-ethoxy)-phenyl]-propan-1-one (**57**) (0.008M) were dissolved in THF (20ml) and added as above. Column chromatography (eluant: dichloromethane:methanol 19:1)afforded the product as a yellow oil (57%), (R_f 0.3 dichloromethane:methanol 19:1). IR v_{max} (film) 2944 (CHs), 1606 (C=C), cm^{-1}. ^1H NMR δ($CDCl_3$) 0.87-0.91 (3H, t, J=7.54Hz, CH_3), 1.87 (4H, s, 2xCH_2), 2.37-2.43 (2H, q, J=7.28Hz, CH_2), 2.54-2.58 (2H, t, J=8.04Hz, CH_2), 2.79 (4H, s, 2xCH_2), 2.91-2.95 (2H, t, J=7.28Hz, CH_2), 2.97-3.01 (2H, t, J=6.28Hz, CH_2), 3.74 (3H, s, OCH_3), 3.81 (3H, s, OCH_3), 4.07-4.09 (2H, t, J=5.52Hz, CH_2), 6.60-7.11 (12H, m, ArH). HRMS calculated for $C_{33}H_{41}NO_3$ 500.3150 (M^++1), observed 500.3165.

1-(2-{4-[2-Ethyl-5-(4-methoxyphenyl)-3-(3-methoxyphenyl)-pent-2-enyl]-phenoxy}-ethyl)-pyrrolidine (52)

Titanium tetrachloride (0.009M) was added to zinc dust (0.018M) in dry THF (tetrahydrofuran) (80ml) and the mixture was refluxed for 2 hours. Then 3-(4-methoxyphenyl)-1-(3-methoxyphenyl)-propan-1-one (**55**) (0.0023M) and 1-[4-(2-cyclopentyl-ethoxy)-phenyl]-propan-1-one (**57**) (0.00453 M) in THF (20ml) were added as above. Column chromatography (eluant: dichloromethane:methanol 19:1) afforded the product as an oil (42%), (R_f 0.3 dichloromethane:methanol 19:1). IR v_{max} (film) 2933 (CHs), 1607 (C=C)cm^{-1}. ^1H NMR δ($CDCl_3$) 0.88-0.92 (3H, t, J=7.54Hz, CH_3), 1.82 (4H, s, 2xCH_2), 2.39-2.44 (2H, q, J=7.52Hz, CH_2), 2.55-2.59 (2H, t, J=8.02Hz, CH_2), 2.66 (4H, s, 2xCH_2), 2.77-2.81 (2H, t, J=8.02Hz, CH_2), 2.87-2.90 (2H, t, J=6.02Hz, CH_2), 3.63 (3H, s, OCH_3), 3.81 (3H, s, OCH_3), 4.0-4.04 (2H, t, J=6.02Hz, CH_2), 6.52 -7.12 (12H, m,ArH). HRMS calculated for $C_{33}H_{41}NO_3$ 500.3189 (M^++1), observed 500.3165.

1-(2-{4-[2-Ethyl-5-(4-methoxyphenyl)-3-(2-methoxyphenyl)-pent-2-enyl]-phenoxy}-ethyl)-pyrrolidine (53)

Titanium tetrachloride (0.0109M) was added to zinc (0.0218M) in THF (tetrahydrofuran) (80ml) and the mixture was refluxed for 2 hours. Then 3-(4-methoxyphenyl)-1-(2-methoxyphenyl)-propan-1-one (**56**) (0.0027M) and 1-[4-(2-

cyclopentyl-ethoxy)-phenyl]-propan-1-one (**57**) (0.00544M) in THF (20ml) were added as above. Column chromatography (eluant: dichloromethane:methanol 19:1) afforded the product as a yellow oil (30%), (R_f 0.3 dichloromethane: methanol 19:1). IR v_{max} (film) 2967 (CHs), 1608 (C=C) cm^{-1}. ^1H NMR δ($CDCl_3$) 0.91-0.94 (3H, t, J=7.28Hz, CH_3), 1.81 (4H, s, 2xCH_2), 2.42-2.44 (2H, s, CH_2), 2.56 (2H, m, CH_2), 2.65 (4H, s, 2xCH_2), 2.72 (2H, s, CH_2), 2.86-2.89 (2H, t, J=6.02Hz, CH2), 3.74 (3H, s, OCH_3), 3.79 (3H, s, OCH_3), 3.90-4.02 (2H, t, J=5.CH_2), 6.58-7.12 (12H, m, ArH). HRMS calculated for $C_{33}H_{41}NO_3$ 500.3143 (M^++1), observed 500.3165.

1-[4-(2-Pyrrolidin-1-yl-ethoxy)-phenyl]-propan-1-one (57)

4-Hydroxypropiophenone (0.013M) was refluxed for 5 hours in darkness in acetone:water 19:1 (10ml) with potassium carbonate (0.0156M) and 1-(2-chloroethyl)pyrrolidine. HCl (0.027M) as above. The product was obtained by flash column chromatography (eluant: acetone) as a yellow oil (92%), (R_f 0.3 acetone) which was used in subsequent reactions without further purification. IR v_{max} (film) 2927 (CHs), 1607 (C=O), cm^{-1}. ^1H NMR δ($CDCl_3$) 0.77 (3H, t, J=7.52Hz, CH_3), 1.71 (4H, s, 2xCH_2), 2.22-2.28 (2H, q, J=7.52Hz, CH_2), 2.58 (4H, s, 2xCH_2), 2.83-2.86 (2H, t, J=4.5Hz, CH_2), 4.03-4.05 (2H, t, J=4.5Hz, CH_2), 6.77-6.79 (1H, s, H-2', H-3'), 6.91-6.93 (H-1', H-4').

2,2-Dimethylpropionic acid 4-{1-ethyl-4-(4-hydroxyphenyl)-2-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-but-1-enyl}-phenyl Ester (59)

Titanium tetrachloride (0.003M) was added dropwise to zinc dust (0.006M) in dry THF (tetrahydrofuran) and the mixture was then refluxed. Then 3-(4-hydroxyphenyl)-1-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-propan-1-one (**33**) (0.0007 M) and 4-pivaloyloxypropiophenone (**58**) (0.0015M) were dissolved in dry THF (20ml) and added to the reaction and the mixture refluxed for a further 5 hours as above. The product was obtained by flash column chromatography over silica gel (eluant: dichloromethane:methanol 19:1) as an oil (43%) (R_f 0.2 dichloromethane:methanol 19:1). IR v_{max} (KBr) 3290 (OH), 2965, 2930 (CHs), 1747 (C=O,), 1606 (C=C)cm^{-1}. ^1H NMR δ($CDCl_3$) 0.88-0.91 (3H, t, J=7.28Hz, CH_3), 1.32 (9H, s, -C(CH_3)$_3$), 1.88 (4H s, 2xCH_2), 2.41-2.43 (2H, q, J=7.44Hz, CH_2), 2.51-2.56 (2H, t, J=7.78Hz, CH_2), 2.75-2.77 (2H, t, J=7.78Hz, CH_2), 2.82 (4H, s, 2xCH_2), 3.00 (2H, s, CH_2), 4.10 (2H, s, CH_2), 6.57-6.98 (12H, m, ArH). HRMS calculated for $C_{35}H_{44}NO_4$ 542.3270 (M^++1), observed 542.3280.

4-{1-Ethyl-4-(4-hydroxyphenyl)-2-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-but-1-enyl}-phenol (60)

2,2-Dimethylpropionic acid 2,2-dimethylpropionic acid 4-{1-ethyl-4-(4-hydroxyphenyl)-2-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-but-1-enyl}-phenyl ester (**59**) (0.00037M) was stirred with sodium hydroxide (0.0018M) in 1:4 water:ethanol (5ml) at room temperature for 4 hours as above. The product was obtained by chromatography over silica gel (eluant: acetone) as a yellow oil (31%) (R_f 0.2 acetone). IR v_{max} (film) 3340 (OH), 2928 (CHs), 1609 (C=C) cm^{-1}. ^1H NMR δ($CDCl_3$) 0.87-0.91 (3H, t, J=7.52Hz, CH_3), 1.89 (4H, s, 2xCH_2), 2.07-2.15 (2H, q, J=6.26, CH_2), 2.54 (2H, m, CH_2), 2.61 (2H, m, CH_2), 2.82 (4H, s, 2xCH_2), 3.03-3.06 (2H, t, J=5.5Hz, CH_2), 4.16-4.19 (2H, t, J=5.76Hz, CH_2), 6.60-7.09 (12H, m, ArH). HRMS calculated for $C_{30}H_{36}NO_3$ 458.2695 (M^++1), observed 458.2690.

4-{2-(4-Methoxy-benzyl)-1-[2-(4-methoxyphenyl)-ethyl]-but-1-enyl}-phenol (65)

Titanium tetrachloride (0.016M) was added dropwise to zinc dust (0.032M) in THF (80ml). and the mixture was refluxed for 2 hours. 1-(4-Hydroxyphenyl)-3-(4-methoxyphenyl)-propan-1-one (**18**) (0.0042M) and 1-(4-methoxyphenyl)-butan-2-one (**62**) (0.008M) in THF (20ml) were added to the reaction as above. Column chromatography (eluant: hexane:dichloromethane 40:60) afforded the product as a yellow oil (95%) which was used in subsequent reactions without further purification. IR v_{max} (KBr) 3368 (OH), 2933 (CHs), 1610 (C=C)cm^{-1}. ^1H NMR δ($CDCl_3$) 0.94-0.98 (3H, t, J=7.33Hz, CH_3), 2.55-2.60 (2H, t, J=6.12Hz, CH_2), 2.60-2.65 (2H, q, J=8.18Hz, CH_2), 2.68-2.72 (2H, t, J=6.48Hz, CH_2), 3.24 (2H, s, CH_2), 3.81 (3H, s, OCH_3), 6.79-7.18 (12H, m, ArH).

4-{2-(3-Methoxy-benzyl)-1-[2-(4-methoxyphenyl)-ethyl]-but-1-enyl}-phenol (66)

Titanium tetrachloride (0.0229M) was added to zinc dust (0.0458M) in dry THF (tetrahydrofuran) (80ml) and this mixture was refluxed for 2 hours. Then 1-(4-hydroxyphenyl)-3-(4-methoxyphenyl)-propan-1-one (**18**) (0.0057M) and 1-(3-methoxyphenyl)-butan-2-one (**63**) (0.01147M) were dissolved in dry THF (20ml) and added to the reaction mixture as above. The product was obtained by column chromatography (eluant: hexane:dichloromethane 40:60) as a yellow oil (80%) which was used in subsequent reactions without further purification. IR v_{max} (KBr) 3401 (OH), 2945 (CHs), 1609 (C=C)cm^{-1}. ^1H NMR δ($CDCl_3$) 0.96-0.99 (3H, t, J=7.52Hz, CH_3), 2.04-2.10 (2H, q, J=7.18Hz, CH_2), 2.56-2.59 (2H, t, J=6.14Hz, CH_2), 2.7-2.75 (2H, t, J=6.48Hz, CH_2), 3.29 (2H, s, CH_2), 3.82 (3H, s, OCH_3), 6.65-7.11 (11H, m, ArH).

4-{2-(2-Methoxy-benzyl)-1-[2-(4-methoxyphenyl)-ethyl]-but-1-enyl}-phenol (67)

Zinc dust (0.0656M) in dry THF (80ml) and titanium tetrachloride (0.0328M) were refluxed for 2 hours. 1-(4-Hydroxyphenyl)-3-(4-methoxyphenyl)-propan-1-one (**18**) (0.0047M) and 1-(2-methoxyphenyl)-butan-2-one (**64**) (0.0047M) were dissolved in dry THF (20ml) and added to the reaction mixture as above. Flash column chromatography (eluant: hexane:dichloromethane 40:60) afforded the product (49%) as an oil, (R_f 0.4 hexane:dichloromethane 40:60) which was used in subsequent reactions without further purification. IR v_{max} (film) 3396 (OH), 2946 (CHs), 1610 (C=C)cm^{-1}. ^1H NMR δ($CDCl_3$) 0.95-0.99 (3H, t, J=7.52Hz, CH_3), 2-2.05 (2H, q, J=7.16Hz, CH_2), 2.55-2.59 (2H, t, J=6.14Hz, CH_2), 2.7-2.75 (2H, t, J=6.48Hz, CH_2), 3.24 (2H, s, CH_2), 3.82 (3H, s, OCH_3), 6.74-6.76 (2H, d, H-3', H-5'), 6.78-7.10 (12H, m, ArH).

1-[2-(4-{2-(4-Methoxy-3-methyl-phenyl)-1-[2-(4-methoxyphenyl)-ethyl]-but-1-enyl}-phenoxy)-ethyl]-pyrrolidine (68)

A mixture of zinc (0.016M), in dry THF (100ml), and titanium tetrachloride (0.008M) were refluxed for 2 hours. 1-

(3-Methyl-4-methoxyphenyl)-butan-2-one (**61**) (0.002M) and 3-(4-methoxyphenyl)-1-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-propan-1-one (**27**) (0.002M), dissolved in dry tetrahydrofuran (50ml), were then added and the mixture refluxed for a further 4 hours as above. The product was obtained by flash column chromatography on silica gel (eluant: dichloromethane:methanol 19:1) as an oil (85%) (R_f 0.3 dichloromethane:methanol 19:1). IR v_{max} (film) 2925 (CHs), 1607 (C=C) cm^{-1}. ^1H NMR $\delta(CDCl_3)$ 0.86-0.89 (3H, t, J=7.54Hz, CH_3), 1.85 (4H, s, 2xCH_2), 2.02-2.08 (2H, q, J=7.54Hz, CH_2), 2.23 (3H, s, CH_3), 2.49-2.51 (2H, m, CH_2), 2.53-2.55 (2H, m, CH_2), 2.73 (4H, s, 2xCH_2), 2.92-2.94 (2H, t, J=6.04Hz, CH_2), 3.21 (2H, s, CH_2), 3.80 (3H, s, OCH_3), 3.82 (3H, s, OCH_3), 4.12-4.18 (2H, t, J=6.04Hz, CH_2), 6.70-7.15 (12H, m, ArH). HRMS calculated for $C_{33}H_{44}NO_3$ 514.3321 (M^++1), observed 514.3324.

1-[2-(4-{2-(4-Methoxy-benzyl)-1-[2-(4-methoxyphenyl)-ethyl]-but-1-enyl}-phenoxy)-ethyl]-pyrrolidine (69)

4-{2-(4-Methoxy-benzyl)-1-[2-(4-methoxyphenyl)-ethyl]-but-1-enyl}-phenol (**65**) (0.0006M) was heated under refluxed for 5 hours in darkness in acetone:water 19:1 (10ml) with potassium carbonate (0.00072M) and 1-(2-chloroethyl) pyrrolidine.HCl (0.0012M) as above. The product was purified after flash column chromatography (eluant: dichloromethane:methanol 19:1) as an oil (22%), (R_f 0.2 dichloromethane:methanol 19:1). IR v_{max} (film) 2932 (CHs), 1608 (C=C) cm^{-1}. ^1H NMR $\delta(CDCl_3)$ 0.83-0.87 (3H, t, J=7.5Hz, CH_3), 1.81-1.85 (4H, s, 2xCH_2), 2.47-2.51 (2H, q, J=7.52Hz, CH_2), 2.53-2.56 (2H, t, J=7.84Hz, CH_2), 2.59-2.61 (2H, t, J=7.52Hz, CH_2), 2.69-2.73 (4H, s, 2xCH_2), 2.95-3.00 (2H, t, J=5.8Hz, CH_2), 3.42 (2H, s, CH_2), 3.79 (3H, s, OCH_3), 3.81 (3H, s, OCH_3), 4.13-4.19 (2H, t, J=5.8Hz, CH_2), 6.77-7.13 (12H, m, ArH). HRMS calculated for $C_{33}H_{41}NO_3$ 499.31 (M^++1), observed 500.3165.

1-[2-(4-{2-(3-Methoxybenzyl)-1-[2-(4-methoxyphenyl)-ethyl]-but-1-enyl}-phenoxy)-ethyl]-pyrrolidine (70)

4-{2-(3-Methoxybenzyl)-1-[2-(4-methoxyphenyl)-ethyl]-but-1-enyl}-phenol (**66**) (0.0006M) was treated in acetone:water 19:1 (10ml) with potassium carbonate (0.00072 M), and 1-(2-chloroethyl)pyrrolidine.HCl (0.0012M) and the reaction mixture was refluxed for 5 hours as above. The product was obtained by flash column chromatography (eluant: dichloromethane:methanol 19:1) as an oil (43%), (R_f 0.1 dichloromethane:methanol 19:1). IR v_{max} (film) 2931 (CHs), 1606 (C=C), cm^{-1}. ^1H NMR $\delta(CDCl_3)$ 0.84-0.88 (3H, t, J=7.5Hz, CH_3), 1.81-1.85 (4H, s, 2xCH_2), 2.04-2.06 (2H, q, J=7.52Hz, CH_2), 2.46-2.49 (2H, t, J=5.44Hz, CH_2), 2.53-2.57 (2H, t, J=5.44Hz, CH_2), 2.63-2.68 (4H, s, 2xCH_2), 2.91-2.97 (2H, t, J=6.16Hz, CH_2), 3.45 (2H, s, CH_2), 3.77 (3H, s, OCH_3), 3.79 (3H, s, OCH_3), 4.10-4.17 (2H, t, J=6.48Hz, CH_2), 6.79-7.13 (12H, m, ArH). HRMS calculated for $C_{33}H_{41}NO_3$ 499.31 (M^++1), observed 500.3165.

1-[2-(4-{2-(2-Methoxy-benzyl)-1-[2-(4-methoxyphenyl)-ethyl]-but-1-enyl}-phenoxy)-ethyl]-pyrrolidine (71)

4-{2-(2-Methoxy-benzyl)-1-[2-(4-methoxyphenyl)-ethyl]-but-1-enyl}-phenol (**67**) (0.0006M) was refluxed for 5 hours in acetone:water 19:1 (10ml) with potassium carbonate (0.00072M) and 1-(2-chloroethyl)pyrrolidine.HCl (0.0012M) as above. The product was obtained by flash column chromatography (eluant: dichloromethane:methanol 19:1 as an oil (34%), (R_f 0.1 dichloromethane:methanol 19:1). IR v_{max} (film) 2930 (CHs), 1609 (C=C)cm^{-1}. ^1H NMR $\delta(CDCl_3)$ 0.98-0.99 (3H, t, J=7.5Hz, H-6, CH_3), 1.87-1.89 (4H, s, 2xCH_2), 2.47-2.49 (2H, q, J=7.52Hz, CH_2), 2.54-2.61 (4H, m, 2xCH_2), 2.72-2.79 (4H, s, CH_2), 3.02-3.04 (2H, t, J=5.8Hz, CH_2), 3.49 (2H, s, CH_2), 3.75 (3H, s, OCH_3), 3.76 (3H, s, OCH_3), 4.20-4.23 (2H, t, J=5.8Hz, CH_2), 6.74-6.78 (2H, d, H-3'', H-5''), 6.82-7.18 (12H, m, ArH). HRMS calculated for $C_{33}H_{41}NO_3$ 499.3102 (M^++1), observed 500.3165.

2,2-Dimethylpropionic acid 2-[2-ethyl-3-(4-hydroxyphenyl)-5-(4-methoxyphenyl)-pent-2-enyl]-phenyl ester (75)

Titanium tetrachloride (0.008M) was added to zinc (0.016M) in THF (tetrahydrofuran) (80ml) and the reaction was refluxed for 2 hours. 1-(4-Hydroxyphenyl)-3-(4-methoxyphenyl)-propan-1-one (**43**) (0.002M) and 2,2-dimethylpropionic acid 2-(2'-oxo-butyl)-phenyl ester (**72**) (0.004M) were dissolved in dry THF (20ml) and added as above. Column chromatography (eluant: hexane:dichloromethane 3:2), (R_f 0.5 hexane:dichloromethane 3:2). afforded the product (44%) as an oil which was used in subsequent reactions without further purification. IR v_{max} (film) 3390 (OH), 2964 (CHs), 1731 (C=O, OPiv), 1633, 1610 (C=C) cm^{-1}. ^1H NMR $\delta(CDCl_3)$ 1.02-1.05 (3H, t, J=7.04Hz, CH_3), 1.38 (9H, s, -$C(CH_3)_3$), 2.48-2.49 (2H, q, J=7.44Hz, CH_2), 2.5-2.57 (2H, t, J=8.6Hz, CH_2), 2.6-2.63 (2H, t, J=8.5Hz, CH_2), 3.62 (2H, s, CH_2), 3.80 (3H, s, OCH_3), 6.77-7.30 (12H, m, ArH).

2,2-Dimethylpropionic acid 2-{2-ethyl-5-(4-methoxyphenyl)-3-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-pent-2-enyl}-phenyl Ester (76)

2,2-Dimethylpropionic acid 2-[2-ethyl-3-(4-hydroxyphenyl)-5-(4-methoxyphenyl)-pent-2-enyl]-phenyl ester (**75**) (0.0006M) in acetone:water 19:1 (10ml) was treated with potassium carbonate (0.00072M) and 1-(2-chloroethyl)pyrrolidine.HCl (0.0012M) as above. The product was purified by flash column chromatography (eluant: dichloromethane: methanol 19:1) to afford an oil (37%), (R_f 0.3 dichloromethane:methanol 19:1). IR v_{max} (KBr) 2959, 2872 (CHs), 1747 (C=O), 1611 (C=C)cm^{-1}. ^1H NMR $\delta(CDCl_3)$ 0.81-0.85 (3H, t, J=7.5Hz, CH_3), 1.36 (9H, s, -$C(CH_3)_3$), 1.78-1.86 (4H, s, 2xCH_2), 2.48-2.49 (2H, q, J=7.5Hz, CH_2), 2.63-2.68 (4H, m, 2xCH_2), 2.72 (4H, s, 2xCH_2), 2.95-3.0 (2H, t, J=6.12Hz, CH_2), 3.38-3.41 (2H, s, CH_2), 3.74-3.80 (3H, s, OCH_3), 4.11-4.17 (2H, t, J=6.12Hz, CH_2), 6.70-7.20 (13H, m, ArH). HRMS calculated for $C_{37}H_{48}NO_4$ 570.3583 (M^++1), observed 570.3598.

2-{2-Ethyl-5-(4-methoxyphenyl)-3-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-pent-2-enyl}-phenol (77)

2,2-Dimethylpropionic acid 2-{2-ethyl-5-(4-methoxyphenyl)-3-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-pent-2-enyl}-phenyl ester (**76**) (0.000664M) was stirred with sodium hydroxide (0.00332M) in 1:4 water:ethanol (5ml) at room temperature for 4 hours as above. The product was obtained by chromatography over silica gel (eluant: dichloromethane: methanol 19:1) as a yellow oil (21%) (R_f 0.2 dichloromethane:methanol 19:1). IR v_{max} (film) 2926, 2855 (CHs),

1610 (C=C) cm^{-1}. ^1H NMR δ($CDCl_3$) 0.81-0.84 (3H, t, J=7.5Hz, CH_3), 1.88 (4H, s, 2xCH_2), 2.45-2.49 (2H, q, J=7.5Hz, CH_2), 2.62-2.65 (4H, m, H-1, 2xCH_2), 2.79 (4H, s, 2xCH_2), 3.01-3.04 (2H, t, J=5.46Hz,), 3.38 (2H, s, CH_2), 3.77 (3H, s, OCH_3), 4.16-4.19 (2H, t, J=5.8Hz, CH_2), 6.74-7.09 (12H, m, ArH). HRMS calculated for $C_{32}H_{40}NO_3$ 486.3008 (M^++1), observed 486.3010.

2,2-Dimethylpropionic acid 3-{2-ethyl-5-(4-methoxyphenyl)-3-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-pent-2-enyl}-phenyl Ester (78)

Titanium tetrachloride (0.008M) was added to zinc dust (0.016M) in THF (tetrahydrofuran) (20ml) and the reaction was refluxed for 2 hours. 3-(4-Methoxyphenyl)-1-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-propan-1-one (**27**) (0.002M) and 2,2-dimethylpropionic acid 3-(2'-oxo-butyl)-phenyl ester (**73**) (0.004M) were dissolved in dry THF (80ml) and added to the reaction mixture as above. Column chromatography (eluant: hexane:dichloromethane 3:2) afforded the product (44%) as an oil, (R_f 0.5 hexane:dichloromethane 3:2). IR v_{max} (film) 2946 (CHs), 1755 (C=O), 1609 (C=C) cm^{-1}. ^1H NMR δ($CDCl_3$) 0.93-0.97 (3H, t, J=7.52Hz, CH_3), 1.38 (9H, s, -C(CH_3)$_3$), 2.04 (4H, s, 2xCH_2), 2.06-2.08 (2H, q, J=7.52Hz, CH_2), 2.53-2.57 (2H, t, J=8.04Hz, CH_2), 2.67-2.71 (2H, t, J=7.78Hz, CH_2), 2.85 (4H, s, 2xCH_2), 3.46 (2H, s, CH_2), 3.67-3.69 (2H, t, J=8.04Hz, CH_2), 3.80 (3H, s, OCH_3), 4.20-4.24 (2H, t, J=8.78, CH_2), 6.75-7.25 (11H, m, ArH). HRMS calculated for $C_{37}H_{47}NO_4$ 570.3583 (M^++1), observed 570.3542.

2,2-Dimethylpropionic acid 4-{2-ethyl-5-(4-methoxyphenyl)-3-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-pent-2-enyl}-phenyl Ester (79)

The general method 5.6 was applied to this reaction. Titanium tetrachloride (0.0057M) was added to zinc dust (0.01132M) in dry THF (tetrahydrofuran) (80ml) and the mixture was refluxed for 2 hours. 3-(4-Methoxyphenyl)-1-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-propan-1-one (**27**) (0.00142M) and 2,2-dimethylpropionic acid 4-(2'-oxo-butyl)-phenyl ester (**64**) (0.00283M) were dissolved in dry THF (20ml) and added to the reaction mixture as before. The product was obtained after column chromatography (eluant: dichloromethane:methanol 19:1) as a yellow oil (32%), (R_f 0.2 dichloromethane:methanol 19:1). IR v_{max} (film) 2946 (CHs), 1748 (C=O), 1607 (C=C), cm^{-1}. ^1H NMR δ($CDCl_3$) 0.82-0.86 (3H, t, J=7.52Hz, CH_3), 1.36 (9H, s, -C(CH_3)$_3$), 2.05-2.07 (4H, s, 2xCH_2), 2.46-2.50 (2H, q, J=7.52Hz, CH_2), 2.52-2.56 (2H, t, J=6.16Hz, CH_2), 2.65-2.70 (2H, t, J=6.12Hz, CH_2), 3.24 (4H, s, 2xCH_2), 3.34-3.37 (2H, t, J=6.12Hz, CH_2), 3.38 (2H, s, CH_2), 3.77 (3H, s, OCH_3), 4.4-4.42 (2H, t, J=5.12, CH_2), 6.77-7.18 (12H, m, ArH). HRMS calculated for $C_{37}H_{47}NO_4$ 569.7734 (M^++1), observed 570.3583.

4-{2-Ethyl-5-(4-methoxyphenyl)-3-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-pent-2-enyl}-phenol (80)

2,2-Dimethylpropionic acid 4-{2-ethyl-5-(4-methoxyphenyl)-3-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-pent-2-enyl}-phenyl ester (79) (0.000664M) was stirred with sodium hydroxide (0.00332M) in 1:4 water:ethanol (5ml) at room temperature for 4 hours. The reaction mixture was acidified with 10% HCl (10ml) and extracted with dichloromethane (4 x 40ml). The combined organic layers were washed with brine (20ml) and dried over sodium sulphate. The solvent was removed under reduced pressure and the residue was chromatographed over silica gel (eluant: dichloromethane:methanol 19:1) to yield the product as a yellow oil (33%) (R_f 0.2 dichloromethane:methanol 19:1). IR v_{max} (film) 3403 (OH), 2927 (CHs), 1608 (C=C) cm^{-1}. ^1H NMR δ($CDCl_3$) 0.92-0.95 (3H, t, J=7.5Hz, CH_3), 1.90 (4H, s, 2xCH_2), 2.01-2.06 (2H, q, J=7.84Hz, CH_2), 2.51-2.55 (2H, t, J=8.26, CH_2), 2.65-2.70 (2H, t, J=7.52Hz, CH_2), 2.86 (4H, s, 2xCH_2), 3.03-3.17 (2H, t, J=5.46Hz, CH_2), 3.79 (2H, s, CH_2), 3.79 (3H, s, OCH_3), 4.2-4.20 (2H, t, J=5.46Hz, CH_2), 6.68-7.08 (12H, m, ArH). HRMS calculated for $C_{32}H_{40}NO_3$ 486.3008 (M^++1), observed 486.3020.

2,2-Dimethylpropionic acid 4-{2-ethyl-5-(4-hydroxyphenyl)-3-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-pent-2-enyl}-phenyl Ester (81)

Titanium tetrachloride (0.003M) was added to zinc dust (0.006M) in dry THF (tetrahydrofuran) (100ml) and the mixture was refluxed for 2 hours. 3-(4-Hydroxyphenyl)-1-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-propan-1-one (**33**) (0.0074 M) and 2,2-dimethylpropionic acid 4-(2'-oxo-butyl)-phenyl ester (**64**) (0.0015M) were dissolved in dry THF (20ml) and added to the reaction mixture as above. Column chromatography (eluant: dichloromethane:methanol 19:1) afforded the product as an oil (80%). (R_f 0.27 dichloromethane:methanol 19:1). IR v_{max} (film) 1607(C=C)cm^{-1}. ^1H NMR δ($CDCl_3$) 0.92-0.95 (3H, t, J=7.54Hz, CH_3), 1.36 (9H, s, -C(CH_3)$_3$), 1.84 (4H, s, 2xCH_2), 1.98-1.99 (2H, q, J=7.52Hz, CH_2), 2.50-2.55 (2H, t, J=7.78Hz, CH_2), 2.68-2.70 (6H, m, 3xCH_2), 2.95-2.97 (2H, t, J=6.00Hz, CH_2), 3.24 (2H, s, H-7, 2xCH_2), 4.10-4.14 (2H, t, J=6.02Hz, CH_2), 6.69-7.05 (12H, m, ArH). HRMS calculated for $C_{36}H_{45}NO_4$ 556.3427 (M^++1), observed 556.3439.

4-{2-Ethyl-5-(4-hydroxyphenyl)-3-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-pent-2-enyl}-phenol (82)

4-{2-Ethyl-5-(4-hydroxyphenyl)-3-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-pent-2-enyl}-phenyl ester (**81**) (0.00037M) was stirred with sodium hydroxide (0.00018M) in 1:4 water:ethanol (5ml) at room temperature for 4 hours. The product was obtained by chromatography on silica gel (eluant: dichloromethane:methanol 19:1) as a yellow oil (42%) (R_f 0.1 dichloromethane:methanol 19:1). IR v_{max} (film) 3308 (OH), 2924, 2854 (CHs), 1609 (C=C) cm^{-1}. ^1H NMR δ($CDCl_3$) 0.85-0.90 (3H, m, CH_3), 1.85 (4H, s, 2xCH_2), 2.34-2.38 (2H, m, CH_2), 2.49-2.52 (4H, m, 2xCH_2), 2.72 (4H, s, 2xCH_2), 2.97-2.98 (2H, m, CH_2), 3.52 (2H, s, CH_2), 4.12-4.15 (2H, m, CH_2), 6.67-7.18 (13H, m, ArH). HRMS calculated for $C_{31}H_{37}NO_3$ 472.2852 (M^++1), observed 472.2872.

Computational Procedure

Ligand Preparation

Structures for compounds (**60**), (**80**), and (**82**) were drawn using ACD/Chemsketch 8.17 and SMILES [50] strings generated for each. Marvinview 4.0.1 [51] 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) [42] in combination with FRODA (Framework Rigidity Optimised Dynamic Algorithm) [43] 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 20^{th} saved as a PDB. Macromodel 6.5 was utilised to convert all PDB structures to mol2.

Docking

FRED2.11 [46] 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. We have previously validated this procedure for a different series of flexible antiestrogens and thus apply the same procedure here to evaluate the differences in ER isoform selectivity for each compound. 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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Received: 03 April, 2006 **Revised: 03 October, 2006** **Accepted: 04 October, 2006**