Amide Derivatives of 2,3-diarylacrylophenone as Estrogen Receptor Binding Ligands[#]

Atul Gupta[a,¶], Resmi Raghunandan[b], Atul Kumar[a], Prakash R. Maulik[b], Anila Dwivedy[c], Govind Keshri[c], Man Mohan Singh[c] and Suprabhat Ray[a,*]

[a]*Medicinal and Process Chemistry Division,* [b]*Molecular and Structural Biology Division,* [c]*Endocrinology Division, Central Drug Research Institute, Lucknow-226001, India*

Abstract: Substituted amidoalkyl derivatives of 2,3-diarylacrylophenones carrying the amide chain on the 3-aryl residue have been prepared by reacting corresponding phenolic 2,3-diarylacrylophenones with haloalkyl carboxylic acid esters, their hydrolysis and subsequent treatment with different alkyl amines. Compounds thus prepared were evaluated for their relative binding affinity (RBA) towards estrogen receptors (ER), estrogen agonistic and antagonistic activities. Out of eleven amide derivatives thus prepared, compounds **7**, **13**, **15-19**, **23**, **24** showed significant estrogen antagonistic activity. Interestingly the phenolic compound **7** and the acid ester **18** also exhibited estrogen inhibiting property. Majority of the dimethoxy derivatives (R = OCH_3) showed significantly high estrogenic activity. In order to throw light on their SAR, In silico docking of the acrylophenone derivatives in the ligand binding site of the ERα and their comparison with pure steroidal estrogen antagonist ICI-164,384 and the non-steroidal antiestrogen raloxifene, was carried out. Crystal structure of compound **6** revealed relative trans-geometry of the 2(B) and 3(C) phenyl rings.

Key Words: Antiestrogens, estrogen antagonists, antifertility, crystal structure, docking studies, *RBA*.

1. INTRODUCTION

A common feature associated with non-steroidal estrogen antagonists is the presence of an aminoalkyl substituent generally connected with an aromatic moiety. This residue has been shown to bind to a sub site on the estrogen receptor, termed as antiestrogen binding site and thereby interfere with the initiation of estrogenic response [1-5]. However, such compounds usually elicit partial antagonistic property. Most of the published selective estrogen receptor modulators (SERMs) contain aminoalkyl side chain [6-8]. Interestingly, it was observed that when estradiol is substituted with an appropriate amide chain at 7α- position, it led to pure estrogen antagonist [9]. It was therefore, considered worthwhile to investigate the effect of incorporation of a substituted amido alkyl chain in nonsteroidal compounds for modulation of its estrogenic profile. 2,3-Diarylacrylophenones are known to interact with estrogen receptor. Some tert-aminoalkyl substituted diaryl acrylophenones show partial estrogen antagonistic activity depending upon the position of the substituents [10, 11]. In the present study different substituted amidoalkyl chains were introduced at position 4 of the 3-phenyl residue of the 2,3-diarylacrylophenones (**1B**) to simulate 7α-position of estradiol as present in ICI-164,384 & ICI-163,984 (**1A**) (Fig. (**1**)).

Receptor conformation plays a key role in determining the transcriptional potency and agonists/antagonists character of a given ligand. A recent X-ray crystallographic study of the ligand-binding domain of estrogen receptor (ER) complexed with estrogen and raloxifene has suggested a molecular basis for agonism and antagonism in ERα [12]. Although molecular requirement for a ligand to interact with ER has been reasonably delineated, but finer aspects which make them tissue selective is poorly understood. Using this atomic level structural knowledge, we have described recently in-silico docking studies of some newly synthesized non-steroidal estrogens [13]. These structure-based investigations together with *in vivo* activity and structural studies have been correlated to demonstrate a practical approach for suitable ligand design. It is for this reason; synthesis of the title compounds and study of their structural features with respect to their biological activity has been under taken.

2. CHEMISTRY

The desired amide derivatives were prepared starting from known 2,3-diarylacrylophenones, their condensation with bromo carboxylic acid esters followed by alkaline hydrolysis and subsequent treatment with amines as shown in Scheme **1**.

All the synthesized compounds were identified by ^{1}H NMR, Fab Mass & IR spectroscopy. Compounds showed IR peaks at 3445-3305 cm^{-1} (OH), 1650-1598 cm^{-1} (C=O, conjugated), 1755-1729 cm^{-1} (C=O, ester), 1715-1705 cm^{-1} (C=O, acid) and 1722-1660 cm^{-1} (C=O, amide).

In NMR, a singlet at 3.80 ppm showed the presence of the methoxy grouping, olefinic proton of the acrylophenone appeared at 7.08 ppm and the protons the phenyl rings at the aromatic region (6-8 ppm). Methylene protons of the ethyl ester group appeared as quarted at 4.23 ppm whereas the methyl group as triplet at 1.25 ppm.

*Address correspondence to Dr. Atul Gupta C/o Dr. S. Ray, 16/3 kha Sarojni Naidu Marg, Behind- Masonic Lodge, Hazratganj, Lucknow-226001, India; Tel: +91-9335626703; E-mail: atisky2001@yahoo.co.in, suprabhatray@yahoo.co.in

¶ Present address: Department de chimie-biologie, Université du Québec a Trois-Riviéres, C.P. 500, Trois-Reviéres, Quebec, Canada/G9A 5H7

[#]CDRI communication No. 6402

1573-4064/07 $50.00+.00

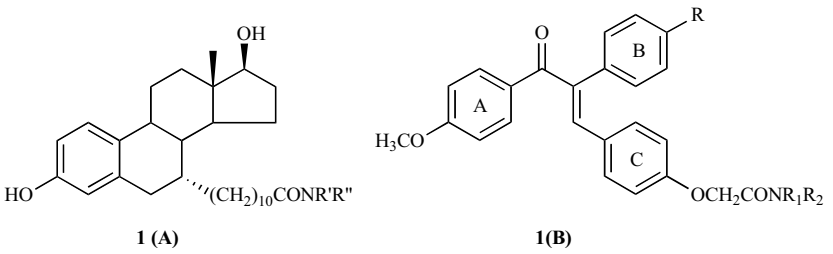
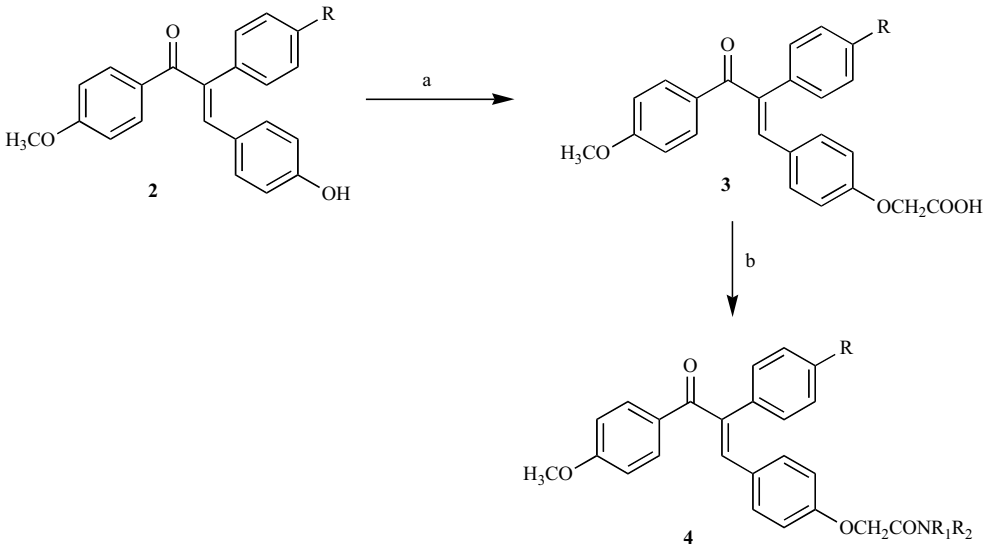


Fig. (1).



Scheme (1). (a) i. Ethyl bromoacetate, K2CO3, dry acetone, ii. NaOH, MeOH (b) N,N'-Dicyclohexylcarbodiimide, 1-hydroxybenzotriazole, Et3N, alkyl amines, dichloromethane.

FAB mass of compounds displayed M or M+1 peaks corresponding to studied.

Novel compounds thus synthesized and evaluated for biological activities are represented by structure **5** (Fig. (**5**)).

3. BIOLOGY

3.1. Estrogen Receptor Binding Affinity [14]

The relative binding affinity (RBA) of the compounds for estrogen receptor was determined by competition assay, employing radio labeled estradiol (^{3}H-E_2) as the reference compound. The test ligands and (^{3}H-E_2) were incubated (4 °C) with cytosol estrogen receptors obtained from immature 20–21 days old rat uteri. Aliquots of the uterine cytosol (200 µL concd 1 uterus per mL) prepared in TEA buffer (10 mM TRIS, 1.5 mM EDTA, 0.02% sodium azide, pH 7.4) were incubated in triplicate with a fixed concentration of radio labeled estradiol with or without various concentrations of the competitor substance dissolved in 60 µL of the TEA buffer containing DMF as co solvent (final concentration of DMF in the incubation medium never exceeded 5%) for 18 h at 4 °C. At the end of this period, dextran coated charcoal (DCC) (5% Norit 0.5% dextran) suspension in 100 µL of TEA buffer was added into each tube, which were briefly vortexed and allowed to stand for 15 min. DCC was precipitated by centrifugation (800 g×10 min) and the supernatants counted for radioactivity in 10 mL of a dioxane-based scintillation fluid. RBA of the text compound was computed from a graph plotted between percent bound radioactivity verses log concentration of the test substance. At 50% inhibition, log of the competitor concentration relative to that of estradiol, gave the affinity of the test compound to estrogen receptor relative to estradiol. This when multiplied with 100 gave the percentage value designated as RBA.

3.2. Estrogen Agonistic Activity [15]

Twenty one day old immature female Sprague–Dawley rats were bilaterally ovariectomized under light ether anesthesia and after post-operative rest for 7 days were randomized into different treatment groups. Each rat received the compound of the invention once daily for 3 consecutive days on days 28–30 of age by oral route. A separate group of animals received only the vehicle for similar duration served as control. At autopsy 24 h after the last treatment on day 31 of age, vaginal smear of each rat was taken and uterus was carefully excised, gently blotted, weighed. Premature open-

ing of vagina, cornification of vaginal epithelium and increase in uterine fresh weight were taken as parameters for evaluation of estrogen agonistic activity in comparison to rats of vehicle control group. The objective was to evaluate estrogen agonistic effect of the compounds on the uterus and vagina.

3.3. Estrogen Antagonistic Activity [15]

Twenty-one-day-old immature female Sprague–Dawley rats were bilaterally ovariectomized under light ether anesthesia and after post-operative rest for 7 days were randomized into different treatment groups. Each rat received the compound of the invention and 0.02 mg kg^{-1} dose of 17 α-ethynylestradiol in 10% ethanol-distilled water once daily for 3 consecutive days on days 28–30 of age by oral route. A separate group of animals receiving only 17 α -ethynylestradio l (0.02 $mgkg^{-1}$) in 10% ethanol-distilled water for similar duration were used for comparison. At autopsy on day 31 of age, vaginal smear of each rat was taken and uterus was carefully excised, gently blotted, weighed and fixed for histology. Inhibition in ethynylestradiol induced cornification of vaginal epithelium and increase in uterine fresh weight were taken as parameters for evaluation of estrogen antagonistic effect of the compounds.

RBA, estrogen agonistic and antagonistic activities are shown in Table **1**.

4. X-RAY AND COMPUTATIONAL STUDY

4.1. Ligand's Stuctural Study[1]

Compound **6**, hydroxy acrylophenone derivative, picked up for X-ray analysis to assign structural geometry of basic skeleton of the newly synthesized compounds. Diffraction quality crystal of **6** was prepared by its slow crystallization from methanol. The molecular structure of **6** as assigned by single crystal X-ray analysis is shown in (Fig. ((**2a**). The molecule contains three non-planner rings A (1), B (2), and C (3). Rings B and C are disposed in trans form across the double bond. The crystal packing (Fig. (**2 b/c**)) reveals that there are two intermolecular H-bonding interactions of the type C-H…O and O-H…O. The hydroxyl O341 atom form strong hydrogen bond with carbonyl O11 atom [O341-H341…O11 (x, 1+y, z); O341-O11: 2.739(4) Å; H341…O11: 1.93Å; O341-H341-O11: 171.3°]. The methoxy oxygen O141, form hydrogen bond with C26 atom [C26–H26…O141 (1-x, -y. -z); C26-O141: 3.3604 Å; H26-O141: 2.57 Å; C26-H26-O141: 143°]. The involvement of O141 in H-bonding, although weak in nature, but is an interesting fact that the 3-hydroxy of estradiol forms three H-bonding interactions at the receptor site[12]. The crystal packing further reveals the presence of intermolecular C-H…π interaction. Methyl carbon C17 is brought close to the phenyl ring A of other molecule (1-x, 1-y, -z) with methyl H…phenyl centroid distance 3.17 Å.

[1] Crystal data of 6: $C_{22}H_{18}O_3$, M = 330.36, monoclinic, P2/c, a = 11.973(2), b = 9.131(10), c = 16.286(2) Å, β = 90.63(1)°, V = 1786.4(4) $Å^3$, Z = 4, D_c = 1.233 gcm^{-1}, μ (Mo-K_α) = 0.081mm^{-1}, F(000) = 696.0, colourless crystal, size 0.7×0.35×0.75mm, 4114 reflections measured (1350 unique), R_w = 0.1356, conventional R = 0.0577 on F values of reflections 3137 with I > 2σ (I), S = 0.937 for all data and 229 parameters. Unit cell determination and intensity data collection (2θ = 50°) was performed on a Bruker P4 diffractometer at 293(2) K. Structure solutions by direct methods and refinements by full-matrix least-squares methods on F^2. Programs: XSCANS (Siemens Analytical X-ray Instrument Inc.: Madision, WI, USA, 1997).

4.2. Docking Studies Protocol

Docking, molecular dynamics, energy minimization and molecular graphics works were performed on a silicon graphics octane workstation. All the ligand structures were built and optimized using Builder module of Insight II (M/S Accelyrs Inc.). Minimizations were carried out by Discover software that used molecular mechanical method. The minimization was completed using the default parameters (1000 iterations, derivative 0.01 and charges on). Reference protein coordinates used for docking were taken from X-ray structure deposited in protein data bank (www.rcsb.org) like the crystal structure of ligand binding domain (LBD) of estrogen receptor in complex with raloxifene (1ERR), with endogenous estrogen, 17β-estradiol (1A52), rER-β-ICI 164,384 (1HJ1), rER-β-raloxifene(1QKN). The genetic algorithm of AutoDock 3.0.5 has been employed for docking the acrylophenone derivatives into the active sites of the receptor. Autodock helps to narrow the conformational possibilities and to identify the structure. The original procedure developed for AutoDock used Monte Carlo simulated annealing (SA) technique for configurational exploration with the rapid energy evaluation using grid based molecular affinity potentials. Docking procedure involves the following steps-: The protein target and the ligand were prepared for docking using the AutoDock 3.0.5 and autodocktools. All the "heteroatoms", including water molecules and ions were removed from the original files. The macromolecule first needs polar hydrogens to be added and then partial atomic charges to be assigned (Kollman charges). The atomic solvation parameters were assigned using ADDSOL utility in AutoDock 3.0.5 program. The ligand molecules were checked for polar hydrogens and assigned for Gasteiger-Huckel partial atomic charges. Flexible torsion was defined with the help of Autotors. This allowed the conformational search of ligand during the process of docking. The PDBQ file was created for the ligand. Using Autogrid Algorithm the 3-D maps of 0.375 Å spacing was centered on the active site for whole protein using Autogrid algorithm to evaluate the interaction energies between the ligand and the ER-LBD. In this Autogrid program the protein is embedded in a 3D grid and a probe atom is placed at each grid point. The affinity and electrostatic potential grid were calculated for each type of atom in the ligands. A series of docking parameters were set on. Atom types, generations, energy evaluations, and GA runs were set of 27000, 250000 and 100 respectively. The Lamarckian genetic algorithm (LGA) method was used with the default parameters as suggested by Autodock. Finally the docked of ligand-receptor complexes were selected according to the criteria of interacting energies combined with the geometrical matching quality. These complexes were used to have a comparative account of activity and their structural conformations. The total binding energy and corresponding inhibition constant between the compound and receptor was calculated according to the algorithm in the AutoDock 3.0.5 program.

4.3. *In-Silico* Docking Studies

The ligand-binding cavity of ER is buried deep within the hydrophobic core of the ligand binding domain (LBD) and surrounded by parts of helices 3, 6, 8, 11 and 12 respectively. Raloxifene, a potent SERM, binds at the same site as

Table 1. RBA, Estrogen Agonistic and Antagonistic Activities. Compound Described in Table 1 are Based on Structure 5 (Fig. (5))

Compd. No.	R	R_3	Dose (oral) mg/kg/day	Estrogen antagonistic activity		Estrogen agonistic activity		RBA % of E_2
				Uterine weight[a] (mg/10g body weight)	Inhibition[b] %	Uterine weight[a] (mg/10g body weight)	Gain[c] %	
Vehicle			10	0.53± 0.01		0.53± 0.01		
EE			0.02	2.78±0.04		2.78±0.04		
6	H	H	10	2.41±0.13[d]	16.45	0.55±0.05	0.89	0.173
7	OCH_3	H	10	1.88±0.04[e]	40	1.56±0.01[g]	46.22	0.21
Vehicle			10	0.40±0.01		0.40±0.01		
EE			0.02	2.32±0.09		2.32±0.09		
8	H	CH_2COOH	10	2.24±0.08	4.19	0.57±0.02[g]	8.9	0.03
9	OCH_3	CH_2COOH	10	2.11±0.05	11	0.56±0.04[g]	4.19	0.02
10	H	CH_2CONHn-propyl	10	2.32±0.21	-	1.20±0.15[g]	41.88	<0.001
11	H	CH_2CONHn-butyl	10	2.60±0.19	-	0.85±0.24[g]	23.56	0.03
12	H	CH_2COOEt	10	2.25±0.16	-	0.81±0.08[g]	20.94	ND
Vehicle			10	0.36±0.02		0.36±0.02		
EE			0.02	2.16±0.13		2.16±0.13		
13	OCH_3	CH_2CONHn-butyl	10	1.35±0.13[e]	44.69	1.11±0.08[g]	41.90	ND
14	H	CH_2CONHn-nonyl	10	2.42±0.14	-	0.59±0.05[g]	12.29	ND
Vehicle			10	0.39±0.01		0.39±0.01		
EE			0.02	2.19±0.28		2.19±0.28		
15	H	$CH_2CONHethylhexyl$	10	1.47±0.13[e]	40.00	0.78±0.05[g]	22.22	ND
16	OCH_3	CH_2CONHn-octyl	10	1.59±0.02	32.78	1.37±0.11	54.44	0.044
17	H	$CH_2CON(Me)n$-butyl	10	1.67±0.07	28.34	0.55±0.03[g]	8.89	<0.001
18	OCH_3	CH_2COOEt	10	1.66±0.05	44.44	1.11±0.10	40.00	0.03
Vehicle			10	0.79±0.07		0.79±0.07		
EE			0.02	2.36±0.07		2.36±0.07		
19	H	$(CH_2)_4COOEt$	10	1.83±0.07[e]	34.39	1.20±0.00[g]	25.48	0.02
20	H	$(CH_2)_5COOEt$	10	2.22±0.06	9.55	0.66±0.02	-	<0.001
21	OCH_3	CH_2CONHn-dodecyl	10	2.27±0.12	6.37	0.39±0.02	-	<0.001
22	OCH_3	$CH_2CON(Me)n$-butyl	10	2.50±0.12	-	0.54±0.01	-	ND
Vehicle			10	0.46±0.02		0.46±0.02		
EE			0.02	1.96±0.11		1.96±0.11		
23	H	CH_2CONHn-octyl	10	0.95± 0.10[e]	32.67	1.01±0.04[g]	36.67	ND
Vehicle			10	0.53±0.01		0.53±0.01		
EE			0.02	2.78±0.04		2.78±0.04		
24	H	CH_2CONHn-dodecyl	10	1.07±0.05[e]	76.34	0.97±0.07[g]	19.20	<0.001
Vehicle			10	0.37±0.01		0.37±0.01		

(Table 1. Contd….)

Compd. No.	R	R_3	Dose (oral) mg/kg/day	Estrogen antagonistic activity		Estrogen agonistic activity		RBA % of E_2
				Uterine weight[a] (mg/10g body weight)	Inhibition[b] %	Uterine weight[a] (mg/10g body weight)	Gain[c] %	
EE			0.02	1.47±0.04		1.47±0.04		
25	H	$(CH_2)_2N(CH_2)_5$	10	1.64±0.05	-	0.85±0.00[g]	43.24	0.06
Vehicle			10	0.42±0.02		0.42±0.02		
EE			0.02	2.51±0.09		2.51±0.09		
26	H	$(CH_2)_3COOEt$	10	2.79±0.06	-	1.35±0.07[g]	44.98	<0.001
27	OCH_3	$(CH_2)_4COOEt$	10	2.71±0.11	-	0.76±0.05[g]	16.27	0.02

ND = Not determined; EE= 17α-Ethynylestradiol; E_2= Estradiol-17β, [a]Values represents mean±SEM of minimum of six observations in each group; [b]Percent of 17α-Ethynylestradiol *per se* treated group; compounds at nos. 10-12, 14, 25-27 exhibited an additive effect on ethynylestradiol induced uterine weight gain, but lacked any estrogen antagonistic activity. while compound 22 did not exihibit either estrogenic or antiestrogenic activity. [c]Percent of vehicle control group; [d]P<0.05, [e]P<0.01 versus corresponding EE *per se* treated group, [f]P<0.05, [g]P<0.01, *versus* corresponding vehicle control group, all other relevant comparisons were statistically not significant. Estrogenic and antiestrogenic activity calculated using following formula:

Estrogenic effect = $[(E_t - E_v)/(E_s - E_v)]\times 100$,

Antiestrogenic effect = $100-[(E_{s,t}-E_v)/(E_s - E_v)\times 100]$,

E_t= effect of test compound, E_v= effect of vehicle, E_s= effect of EE standard, E_{s+t}= effect of standard under simultaneous administration of test substance.

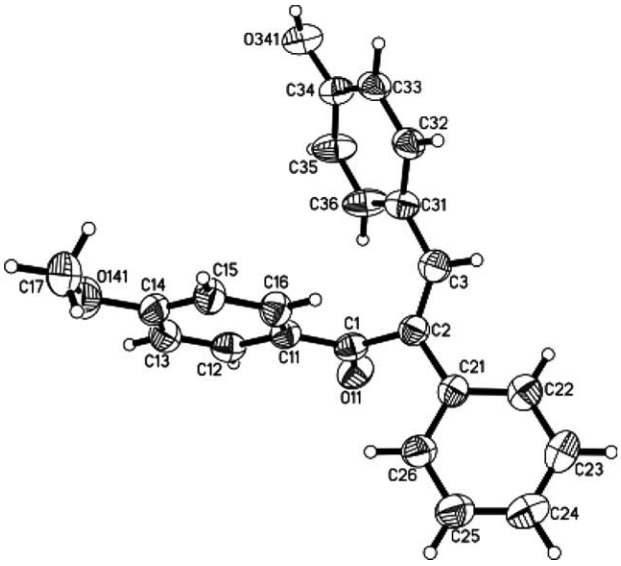


Fig. (2a). ORTEP diagram showing the crystal structure of 6 with atomic numbering scheme.

estradiol within the LBD with hydroxyl group of benzothiophene moiety mimicking the A ring phenolic hydroxyl of the estradiol. The 3-benzoyl group with its amino alkoxy residue is oriented along the 11β-axis of estradiol. The docking values for the compounds studied here were calculated as described in the experimental section are summarized in Tables **2** and **3**. Superposition of the Autodock predicted conformation of raloxifene (blue) with the X-ray crystallographically obtained conformation (red) is shown in Fig. **3**. The root mean square deviation (RMSD) between these two conformations is ~0.68 Å indicating that the parameter set for the Autodock simulation is reasonable to reproduce the X-ray structure. The Autodock method and the parameter set were extended to search the binding conformations for the present compounds. Likewise the 3-D model for binding of ligands with estrogen receptor, the monomethoxy and dimethoxy acrylophenone derivatives could bind to the receptor in a fashion such that phenyl ring bearing long chain amido alkyl residue would occupy a similar position to that of pure antiestrogen's long chain alkyl amide (ICI-164 384) and raloxifene's basic benzoyl group with its amino alkoxy residue.

The estrogenic effect of a molecule is mediated through its binding to estrogen receptor (ERα & ERβ) [3]. The binding of the molecule to the ligand binding domain (LBD) of the ER results in a conformational change in the receptor that

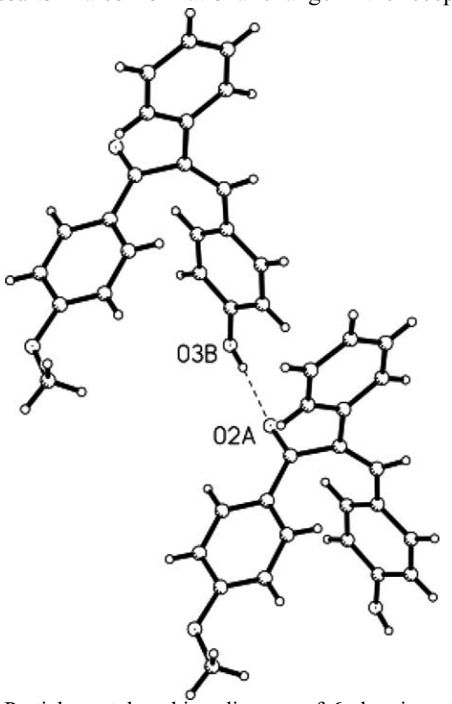


Fig. (2b). Partial crystal-packing diagram of **6** showing strong H-bonding (dotted line) involving O341 and O11.

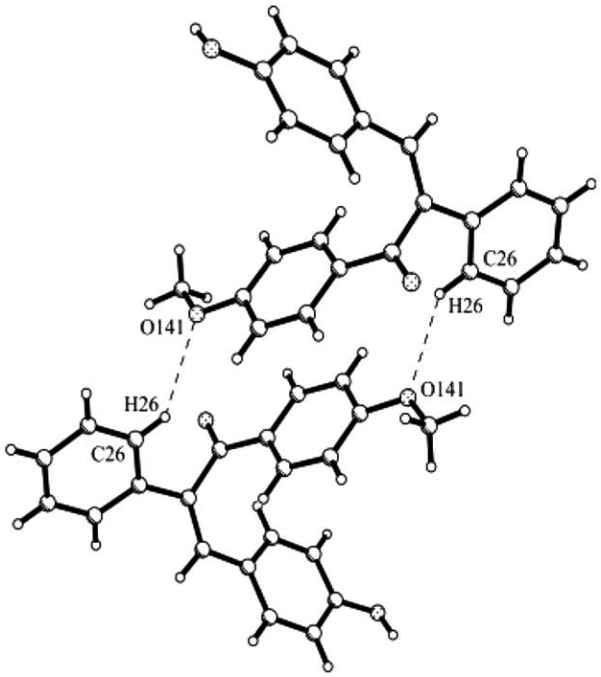


Fig. (2c). Partial crystal-packing diagram showing the dimerization of molecules through C-H…O bond involving methoxy oxygen atom (O11).

allows dimerization, DNA binding interaction with co-regulators and ultimately the gene transcription resulting in initiation of the estrogenic response.

Hormonal recognition is achieved through structural complimentarity of the ligand to the binding cavity of LBD and specific hydrogen bondings. When estrogen agonists like estradiol-17β and diethylstilbestrol bind with ER, helix-12 of LBD folds to reveal a groove formed by helices 3,4,5 and 11 providing a binding site for co-activatiors [10]. In the case of non-steroidal estrogen antagonists, carrying an aryl group, bearing an -tertiary amino alkoxy residue, this chain interferes with recruitment of co-activators, thereby inhibiting with cellular transcription, giving a tissue selective action to the molecule.

In the case of steroidal pure antiestrogen ICI 164,384, the long chain amide residue takes up the position of the amino alkyl chain of raloxifene or any other SERM. However, the flexible side chain of ICI 164,384 adopts a conformation that is different from that of raloxifene. This side chain is longer than the side chain of raloxifene and thus allows it to extend deep into the hydrophobic groove between helices 3 and 5. Although the side chain of ICI 164,384 has some positional resemblance to SERM chains, but they interact with different amino acid residues for receptor binding.

In order to study resemblance between our newly synthesized compounds, raloxifene and ICI 164,384, in-silico docking studies have been carried out with estrogen receptor-α (ER α). Superposition of the raloxifene and ICI 164,384 with the representative compound **17** of the synthesized series in its possible cis and trans configurations shows some similarity among molecules Fig. (**4a**, **4b**, **4c** and **4d**) which is in agreement with its observed high estrogen antagonistic activity and relatively low estrogenecity. However, some of the

Table 2. Autodock Results of Compounds. Compound Described in Table 2 are Based on Structure 5 (Fig. (5))

Compound No.	R	R_3	Cluster rank	Final intermolecular energy	Final binding energy	Final docking energy
6	H	H	1	-10.69	-9.13	-10.11
7	OCH_3	H	1	-10.69	-8.82	-10.22
8	H	CH_2COOH	1	-10.56	-8.07	-10.83
9	OCH_3	CH_2COOH	1	-11.07	-8.15	-11.07
10	H	CH_2CONHn-propyl	1	-10.79	-7.36	-10.33
11	H	CH_2CONHn-butyl	1	-11.16	-7.42	-10.82
12	H	CH_2COOEt	1	-9.59	-6.48	-8.33
13	OCH_3	CH_2CONHn-butyl	1	-11.93	-7.88	-11.69
14	H	CH_2CONHn-nonyl	1	-8.60	-3.31	-7.04
15	H	CH_2CONHethylhexyl	1	-11.04	-6.37	-11.24
16	OCH_3	CH_2CONHn-octyl	1	-11.76	-6.47	-9.86
17	H	$CH_2CON(Me)n$-butyl	1	-11.99	-8.25	-11.94
18	OCH_3	CH_2COOEt	1	-11.50	-11.43	-8.08
19	H	$(CH_2)_4COOEt$	1	-10.67	-6.62	-9.85
20	H	$(CH_2)_5COOEt$	1	-12.06	-7.70	-11.12

(Table 2. Contd….)

Compound No.	R	R_3	Cluster rank	Final intermolecular energy	Final binding energy	Final docking energy
21	OCH_3	CH_2CONHn-dodecyl	1	-13.48	-6.94	-8.84
22	OCH_3	$CH_2CON(Me)n$-butyl	1	-11.85	-7.80	-9.92
23	H	CH_2CONHn-octyl	1	-4.45	-9.43	-9.52
24	H	CH_2CONH-n-dodecyl	1	-12.09	-5.86	-9.87
25	H	$(CH_2)_2NC_5H_{10}$	1	-11.44	-8.64	-11.27
26	H	$(CH2)_3COOEt$	1	-11.43	-7.69	-11.39
27	OCH_3	$(CH_2)_4COOEt$	1	-11.55	-7.19	-11.45

Table 3. Hydrogen Bonding Interactions of Monomethoxy Compounds. Compound Described in Table 3 are Based on Structure 5 (Fig. (5))

Compound No.	Conformer number	Interacting atoms of the source	Interacting atoms of the target	Distance
6	65	O141 O341 O341	ARG B394:NH2 GLY B521:O HIS B524:ND1	2.54 2.77 2.87
7	44	O141 O341 O341	ARG B394:NH2 GLY B521:O HIS B524:NH2	2.58 2.56 2.85
8	7	O141	ARG B394:NH2	2.80
9	64	O141	ARG B394:NH2	2.80
10	54	O141	ARG B394:NH2	2.68
11	31	O141	ARG B394:NH2	2.68
12	25	O141 O(esters carbonyl)	ARG B394:NH2 TYR B526:OH	2.93 2.96
13	51	O141 O241	ARG B394:NH2 HIS B524:NH2	2.52 2.84
14	6	O141	ARG B394:NH2	2.67
15	54	O141	TYR B526:OH	2.65
16	-	-	No interaction	-
17	77	O141 O141	ARG B394:NH2 GLU B353:O	2.60 2.97
18	80	O141	ARG B394:NH2	2.66
19	6	O11 O(esters carbonyl)	ARG B394:NH2 THR B347:OG1	2.62 2.48
20	99	O11	THR B347:OG1	2.70
21	-	-	No interaction	-
22	-	-	No interaction	-
23	21	O141	ARG B394:NH2	2.71

(Table 3. Contd….)

Compound No.	Conformer number	Interacting atoms of the source	Interacting atoms of the target	Distance
24	71	-	No interaction	-
25	68	O11	THR B347:OG1	2.83
26	93	O11	THR B347:OG1	2.85
27	49	O11	THR B347:OG1	2.47

Geometry of H-bond mediated by two oxygen atoms in the title compound

D…H	d(D….H)	d(H…A)	<DHA	A	D…..A
O3-H3	0.82	1.93	171	O2 x, 1+y, z	2.739(4)
C14-H14	0.93	2.57	143	O1 1-x,-y,-z	3.3604

members of the series turned out to be inactive or possessed poor antagonistic activity. The cis-trans isomerisation energies of **17** were obtained by quantum chemical methods and the energy values are nearly equal.(energy for trans isomer = 160.89 kcal/mol and that for cis isomer =157.93kcal/mol). quantum chemical calculations were done by using discover software (1000 iteration, derivative 0.01 and charges on).

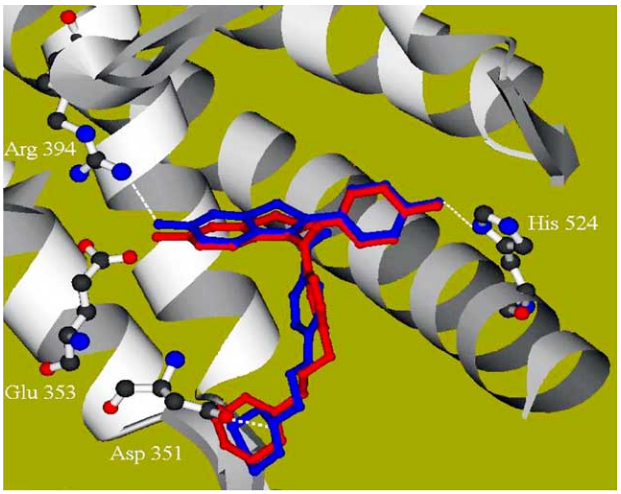


Fig. (3). Superimposion of the docked raloxifene molecule (light) with crystallographic raloxifene molecule (dark) complexed in the binding cavity of ER. The H-bonding are shown by broken white lines.

5. RESULT AND DISCUSSION

Relative binding affinity (RBA), estrogen agonistic and antagonistic activities of compounds prepared is listed in Table **1**. Compounds described in Table **1** are based on structure **5** Fig. (**5**). Lack of free hydroxyl groups at appropriate places in most of the compounds is responsible for the observed poor RBA values. Compounds **7**, **13**, **15-19**, **23** and **24** elicited > 20% estrogen inhibiting activity. Highest inhibition (76.34%) was shown by compound **24.** Compounds **7**, **10**, **13**, **16**, **18**, **23**, **25 and 26** showed > 30 % uterine weight gain.

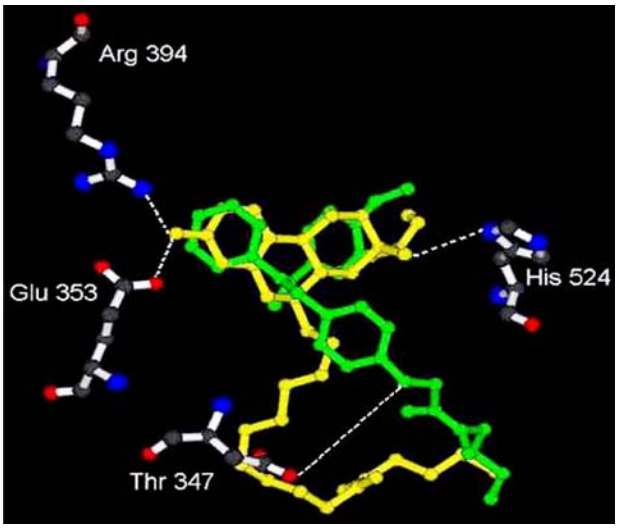


Fig. (4a). Superposition of the docked **17** (trans) with pure anti estrogen ICI 164,384.

Although some of the compounds possessed significant estrogen antagonistic activity but SAR is lacking in this series. This anomalous behavior could be due to molecular flexibility and metabolism.

Both estrogen agonistic as well as antagonistic activities of a molecule are initiated on its binding to estrogen receptor (ER). Whereas estrogen binding sub sites on ER is common for both agonists and antagonists, interaction of the molecule with an additional sub site (anti estrogen binding site), on the receptor results in antagonistic activity [3].

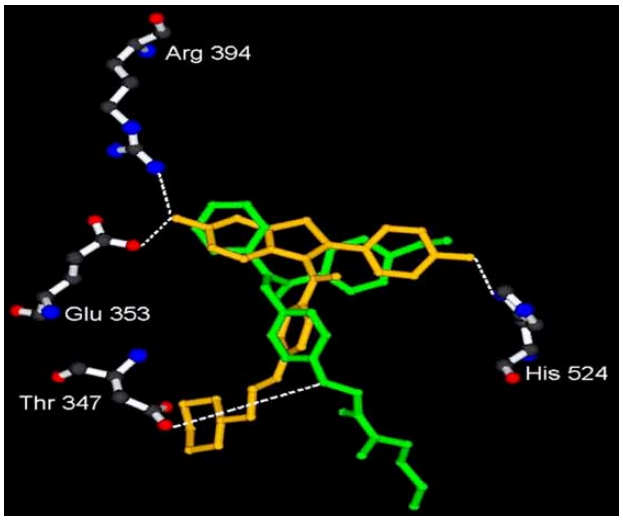


Fig. (4b). Superposition of the docked **17** (trans) with raloxifene.

Molecular framework simulating estradiol & diethyl stilbestrol, possessing OH groups, bind to agonist binding sub site. Such OH groups are often generated on molecular metabolism.

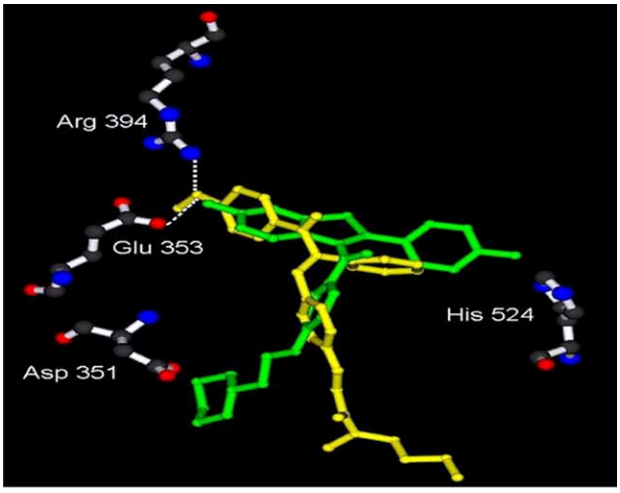


Fig. (4c). Superposition of the docked **17** (cis) with raloxifene.

Due to the flexible nature of the 2,3-diarylacrylophenone molecule, it may interact with ER in different orientations depending upon the substituents on the aryl residues as such or on its metabolism, affecting biological activity.

It is likely that compound **24** interacts with ER with its aryl residues A & B (Fig. (**1**)) occupying estrogen binding

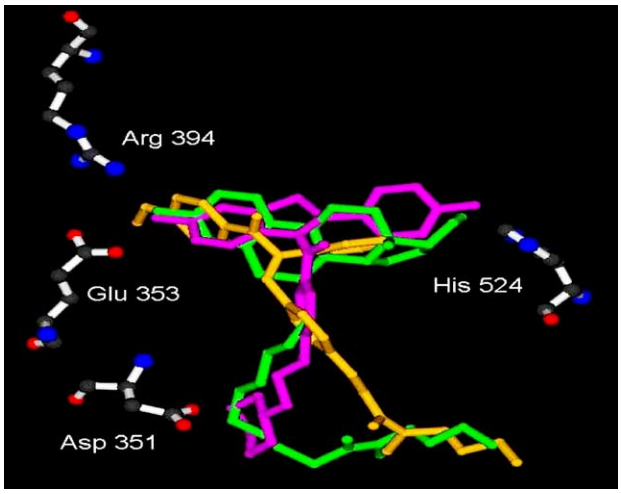
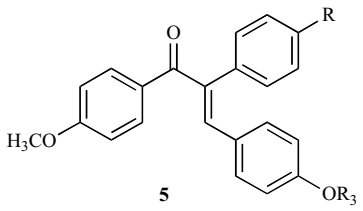


Fig. (4d). Superposition of the docked **17** (cis) with raloxifene and with pure anti estrogen ICI 164,384.

sites corresponding to DES or rings A & D of estradiol and the dodecyl amide group interacts with the anti estrogen binding subsite corresponding to 7α- position of estradiol as present in ICI-164,384 & ICI-163,984, resulting in high order of estrogen antagonistic activity.



5

Fig. (5).

6. CONCLUSION

An important consideration in this study points to the fact that unlike reasonably rigid configurations of raloxifene and ICI 164,384, the 2,3-diaryl acrylophenones possess a flexible structure. Although X-ray study shows a trans geometry of B & C rings of 2,3-diaryl acrylophenones, but its *in vivo* inversion to cis-geometry can not be ruled out. The energy barrier of isomerisation, obtained by quantum chemical methods, is very low, thus permitting the cis-trans isomerisation across the double bond. Thus change in the coformation/configuration of the molecules and their likely *in vivo* metabolism could be the reason for the observed anomalous behavior of some of the derivatives. RBA, estrogen agonistic and antagonistic data (Table **1**), discussed above, suggests that whereas rings A & B possibly occupies the estrogen binding site, ring C is projected into the antiestrogen binding site of LBD.

ACKNOWLEDGEMENT

AG is thankful to the Ministry of Health and Family Welfare, Government of India and CSIR (India) for SRF. The authors thank Miss Mohini Chhabra for efficient technical assistance.

7. EXPERIMENTAL

The reported melting points (ºC) are the uncorrected ones. The infrared spectra were recorded in KBr and neat on a Perkin Elmer model 881. NMR spectra were obtained in $CDCl_3$ (with Me_4Si internal standard, Aldrich) and are reported on Bruker Advance DRX 2000 instrument. Mass spectra were recorded on Jeol JMS-D-300 spectrometer. Elemental analyses were carried out on a Carlo-Erba EA 1108 instrument.

7.1. General Procedure for the Synthesis of 6 & 7

7.1.1. 1-(4-Methoxyphenyl)-2-phenyl-3-(4-hydroxyphenyl)-3-propen-1-one (6)

To a solution of 4-methoxydeoxybenzoin (22.6 g, 0.1mol) and 4-hydroxybenzaldehyde (12.2 g, 0.1mol) in dry benzene (100 ml) was added piperidine (1 ml) and CH_3COOH (0.5 ml). The reaction mixture was refluxed for 30 hr, removing water azeotropically. The reaction mixture was then cooled and washed with water. The organic layer was separated, dried over anhydrous sodium sulphate and concentrated. The residue was chromatographed over a column of silica gel, eluting with the ethyl acetate-hexane to afford compound **6**.

Yield: 80 %, m.p.131-133^{0}C.

FABMS: 331; IR (cm^{-1}): 3445, 1650, 1501; ^{1}H NMR(δ): 3.08 (s, 3H, OCH_3), 6.63 (d, J = 8.60 Hz, 2H, ArH), 6.83 (d, J = 8.90 Hz, 2H, ArH), 7.08 (s, 1H, CH), 7.23 (m, 5H, ArH), 7.40 (d, J = 7.00 Hz, 2H, ArH), 7.97(d, J = 8.90 Hz, 2H, ArH). Anal. Calcd. for $C_{22}H_{18}O_3$; C, 80.00; H, 5.45. Found: C, 80.21; H, 5.63.

7.1.2. 1-(4-Methoxyphenyl)-2-(methoxyphenyl)-3-(4-hydroxyphenyl)-3-propen-1-one (7)

Yield: 78 %, m.p.143-144^{0}C.

FABMS: 361; IR (cm^{-1}): 3305, 1642, 1596, 1509, 1245, 1167; ^{1}H NMR (δ): 3.78 (s, 3H, OCH_3), 3.80 (s, 3H, OCH_3), 6.12 (bs, 1H, OH), 6.57 (d, J = 8.40 Hz, 2H, ArH), 6.83 (d, J = 7.80 Hz, 4H, ArH), 7.07 (s, 1H, CH), 7.20 (d, J = 8.40 Hz, 2H, ArH), 7.32 (d, J = 8.70 Hz , 2H, ArH), 7.96 (d, J = 8.70 Hz , 2H, ArH). Anal. Calcd. for $C_{23}H_{20}O_4$; C, 76.67; H, 5.55. Found: C, 76.81; H, 5.72.

7.2. General Procedure for the Synthesis of 12, 18-20, 26 & 27

7.2.1. Ethyl[4-{3-(4-methoxyphenyl)-3-oxo-2-phenyl-propenyl}phenoxy]-acetate (12)

A mixture of hydroxy acrylophenone **6** (8.25 g, 0.025 mol) and ethyl bromoacetate (5.04 g, 0.03 mol) and K_2CO_3 (10 g) in dry acetone (100 ml) was heated under reflux for 24 hr. Reaction mixture was then filtered and concentrated. The crude oil thus obtained was taken in ethyl acetate, washed with water, dried over sodium sulphate and chromatographed through silica gel using ethyl acetate-hexane as eluent. Pure fraction was collected and concentrated to give the desired compound.

Yield: 80 %, m.p. 124-125ºC.

FABMS: 417; IR (cm^{-1}): 1755, 1650, 1508, 1258, 1224; ^{1}H NMR (δ): 1.25 (t, 3H, CH_3), 3.81 (s, 3H, OCH_3), 4.23 (q, 2H, CH_2), 4.53 (s, 2H, CH_2), 6.72 (d, J = 7.33 Hz, 2H, ArH), 6.83 (d, J = 8.90 Hz, 2H, ArH), 7.08 (s, 1H, CH), 7.29 (m, 5H, ArH), 7.43 (d, J = 7.33 Hz, 2H, ArH), 7.96 (d, J = 8.90 Hz, 2H, ArH). Anal. Calcd. for $C_{26}H_{24}O_5$; C, 75.00; H, 5.77. Found: C, 75.23; H, 5.74.

7.2.2. Ethyl[4-{3-(4-methoxyphenyl)-3-oxo-2-(4-methoxyphenyl)-propenyl}phenoxy]-acetate (18)

Yield: 76 %, m.p 96-97°C.

FABMS: 447; IR (cm^{-1}): 3450, 1757, 1647, 1598, 1256, 1166; ^{1}H NMR (δ): 1.40 (t, 3H, CH_3), 3.79 (s, 3H, OCH_3), 3.81 (s, 3H, OCH_3), 4.23 (q, 2H, CH_2), 4.53 (s, 2H, CH_2), 6.71 (d, J = 9.00 Hz, 2H, ArH), 6.83 (d, J = 9.00 Hz, 2H, ArH), 6.88 (d, J = 9.00 Hz, 2H, ArH), 6.92 (s, 1H, CH), 7.19 (d, J = 9.00 Hz, 2H, ArH), 7.36 (d, J = 8.90 Hz, 2H, ArH), 7.95 (d, J = 9.00 Hz, 2H, ArH). Anal. Calcd. for $C_{27}H_{26}O_6$; C, 72.65; H, 5.83. Found: C, 72.83; H, 5.71.

7.2.3. Ethyl 5-{4-[3-(4-Methoxy-phenyl)-3-oxo-2-phenyl-propenyl]-phenoxy)-pentanoic acid ester (19)

Yield: 80 %, m.p Oil.

FABMS: 458; IR (cm^{-1}): 3017, 2937, 1729, 1599, 1509, 1254, 1168, 1029, 758; ^{1}H NMR (δ): 0.90 (t, 3H, CH_3), 1.40 (m, 4H, $2CH_2$), 2.20 (t, 2H, CH_2), 3.81 (s, 3H, OCH_3), 4.48 (q, 2H, CH_2), 4.60 (t, 2H, CH_2), 6.71 (d, J = 9.00 Hz, 2H, ArH), 6.80 (d, J = 9.00 Hz, 2H, ArH), 6.85 (d, J = 9.00 Hz, 2H, ArH), 6.92 (s, 1H, CH), 7.19 (d, J = 9.00 Hz, 2H, ArH), 7.36 (m, 5H, ArH), 7.90 (d, J = 8.90 Hz, 2H, ArH). Anal. Calcd. for $C_{29}H_{30}O_5$; C, 75.98: H, 6.55. Found: C, 75.82; H, 6.59.

7.2.4. Ethyl 6-{4-[3-(4-Methoxy-phenyl)-3-oxo-2-phenyl-propenyl]-phenoxy)-hexanoic acid ester (20)

Yield: 82 %, m.p. Oil.

FABMS: 473; IR (cm^{-1}): 3018, 2981, 1729, 1600, 1251, 1220, 1170, 759. ^{1}H NMR (δ): 0.89 (t, 3H, CH_3), 1.42 (m, 6H, CH_2), 2.20 (t, 2H, CH_2), 3.81 (s, 3H, OCH_3), 4.25 (q, 2H, CH_2), 4.52 (s, 2H, CH_2), 6.73 (d, J = 8.60 Hz, 2H, ArH), 6.83 (d, J = 9.00 Hz, 2H, ArH), 6.78 (d, J = 9.10 Hz, 2H, ArH), 6.92 (s, 1H, CH), 7.19 (m, 5H, ArH), 7.36 (d, J = 8.90 Hz, 2H, ArH). Anal. Calcd. for $C_{30}H_{32}O_5$; C, 76.27: H, 6.78. Found: C, 76.52; H, 6.68.

7.2.5. Ethyl 4-{4-[3-(4-Methoxy-phenyl)-3-oxo-2-phenyl-propenyl]-phenoxy)-butyric acid ester (26)

Yield: 82 %, m.p. Oil.

FABMS: 444; IR (cm^{-1}): 2976, 1731, 1654, 1598, 1508, 1307, 1255, 1170, 1029, 833, 795; ^{1}H NMR (δ): 0.90 (t, 3H, CH_3), 1.40 (m, 2H, CH_2), 2.20 (t, 2H, CH_2), 3.81 (s, 3H, OCH_3), 4.23 (q, 2H, CH_2), 4.53 (t, 2H, CH_2), 6.71 (d, J = 9.00 Hz, 2H, ArH), 6.83(d, J = 9.00 Hz, 2H, ArH), 6.88 (d, J = 9.00 Hz, 2H, ArH), 6.92 (s, 1H, CH), 7.19 (d, J = 9.00 Hz,

2H, ArH), 7.36 (d, J = 8.90 Hz, 2H, ArH). Anal. Calcd. for $C_{28}H_{28}O_5$; C, 75.67: H, 6.31. Found: C, 75.81; H, 6.58.

7.2.6. Ethyl 5-{4-[3-(4-Methoxy-phenyl)-3-oxo-2-(4-methoxy-phenyl)-propenyl]-phenoxy)-pentanoic acid ester (27)

Yield: 77 %, m.p. Oil.

FABMS: 489; IR (cm^{-1}): 2940, 1731, 1653, 1600, 1510, 1252, 1165, 1069, 1030, 833, 758;. ^{1}H NMR (δ): 0.91 (t, 3H, CH_3), 1.52 (m, 4H, CH_2), 2.20 (t, 2H, CH_2), 3.80 (s, 3H, OCH_3), 3.92 (s, 3H, OCH_3), 4.20 (q, 2H, CH_2), 4.53 (t, 2H, CH_2), 6.71 (d, J = 9.00 Hz, 2H, ArH), 6.83 (d, J = 8.90 Hz, 2H, ArH), 6.983 (d, J = 9.00 Hz, 2H, ArH), 7.05 (s, 1H, CH), 7.19 (d, J = 9.00 Hz, 2H, ArH), 7.36 (d, J = 8.90 Hz, 2H, ArH). Anal. Calcd. for $C_{30}H_{32}O_6$; C, 73.77: H, 6.56. Found: C, 74.00; H, 6.60.

7.3. General Procedure for the Synthesis of 8 & 9

7.3.1. [4-{3-(4-Methoxyphenyl)-3-oxo-2-phenyl-propenyl} phenoxy]-acetic acid (8)

Ester **12** (0.50 g, 0.001 mol) was taken into methanolic NaOH (0.08 g, 0.002 mol) and refluxed for 1 hr. The reaction mixture was concentrated, taken in ethyl acetate, acidified with 10 % HCI and subsequently washed with water to neutral. The organic extract was dried over Na_2SO_4 and concentrated to give the title compound **8** which was crystallized from ethyl acetate-hexane.

Yield: 87 %, m.p. 184-185°C.

FABMS: 389; IR (cm^{-1}): 3453, 1715, 1653, 1602, 1234; ^{1}H NMR (δ): 3.80 (s, 3H, OCH_3), 4.59 (s, 2H, CH_2), 6.73 (d, J = 8.57 Hz, 2H, ArH), 6.83 (d, J = 8.60 Hz, 2H, ArH), 7.08 (s, 1H, CH), 7.28 (m, 5H, ArH), 7.43 (d, J = 7.40 Hz, 2H, ArH), 7.96 (d, J = 8.56 Hz, 2H, ArH). Anal. Calcd. for $C_{24}H_{20}O_5$; C, 74.23; H, 5.15. Found: C, 74.42; H, 5.03.

7.3.2. [4-{3-(4-Methoxyhenyl)-3-oxo-2-(4-methoxyphenyl)-propenyl}phenoxy]-acetic acid (9)

Yield: 84 %, m.p. 155-156°C.

FABMS: 419; IR (cm^{-1}): 3450, 1705, 1650, 1600, 1239; ^{1}H NMR (δ): 3.79 (s, 3H, OCH_3), 3.81 (s, 3H, OCH_3), 4.59 (s, 2H, CH_2), 6.73 (d, J = 9.00 Hz, 2H, ArH), 6.84 (m, 4H, ArH), 7.00 (s, 1H, CH), 7.36 (d, J = 9.00 Hz, 2H, ArH), 7.42 (d, J = 8.40 Hz, 2H, ArH), 7.95 (d, J = 9.00 Hz, 2H, ArH). Anal. Calcd. for $C_{25}H_{22}O_6$; C, 71.77; H, 5.26. Found: C, 71.58; H, 5.15.

7.4. General Procedure for the Synthesis of 10, 11, 13, 14-17, 21-24.

7.4.1. N-propyl-2-[4-{3-(4-methoxyphenyl)-3-oxo-2-phenyl-propenyl}-phenoxy] acetamide (10)

To a solution of the acid **8** (1.0 g, 0.003 mol) in DCM (60 ml), HOBt (0.35 g, 0.003 mol) and DCC (0.74 g, 0.004 mol) were added. The reaction mixture was stirred at room temperature for 1 hr; n-propyl amine (0.18 g, 0.003 mol), triethylamine (4 drops) were added to it and refluxed for 4 hr. Dicyclohexyl urea formed on cooling, was separated. The compound **10** was purified by column chromatography using silica gel and ethyl acetate-hexane as eluent.

Yield: 75 %, m.p. 105-106°C.

FABMS: 430; IR (cm^{-1}): 3436, 3015, 1665, 1598, 1542, 1226; ^{1}HNMR (δ): 0.89 (t, 3H, CH_3), 1.57 (m, 2H, CH_2), 3.27 (t, 2H, CH_2), 3.80 (s, 3H, OCH_3), 4.40 (s, 2H, CH_2), 6.50 (bs, 1H, NH), 6.73 (d, J = 8.76 Hz, 2H, ArH), 6.84 (d, J = 8.80 Hz, 2H, ArH), 7.09 (s, 1H, CH), 7.31 (m, 5H, ArH), 7.44 (d, J = 9.50 Hz, 2H, ArH), 7.96 (d, J = 8.82 Hz, 2H, ArH). Anal. Calcd. for $C_{27}H_{27}NO_4$; C, 75.52; H, 6.29; N, 3.26. Found: C, 75.30; H, 6.18; N, 3.45.

7.4.2. N-Butyl-2-[4-{3-(4-methoxyphenyl)-3oxo-2-phenyl-propenyl}phenoxy]-acetamide (11)

Yield: 83 %, m.p. 76-78°C.

FABMS: 444; IR (cm^{-1}): 3432, 1667, 1598, 1538, 1222; ^{1}H NMR (δ): 0.90 (t, 3H, CH_3), 1.43 (m, 4H, 2×CH_2), 3.31 (t, 2H, CH_2), 3.80 (s, 3H, OCH_3), 4.40 (s, 2H, CH_2), 6.46 (s, 1H, NH), 6.74 (d, J = 8.69 Hz, 2H, ArH), 6.79 (d, J = 8.79 Hz, 2H, ArH), 7.09 (s, 1H, CH), 7.30 (m, 5H, ArH), 7.46 (d, J = 7.00 Hz, 2H, ArH), 7.92 (d, J = 8.76 Hz, 2H, ArH). Anal. Calcd. for $C_{28}H_{29}NO_4$; C, 75.85: H, 6.55; N, 3.16. Found: C, 76.23; H, 6.14; N, 3.30.

7.4.3. N-Butyl-2-[4-{3-(4-methoxyphenyl)-3-oxo-2-(4-methoxyphenyl)-propenyl}-phenoxy] acetamide (13)

Yield: 75 %, m.p. Oil.

FABMS: 474; IR (cm^{-1}): 3431, 3014, 1663, 1599, 1511, 1252, 1166; ^{1}H NMR (δ): 0.87 (t, 3H, CH_3), 1.26 (bs, 4H, CH_2), 3.29 (t, 2H, CH_2), 3.78 (s, 3H, OCH_3), 3.80 (s, 3H, OCH_3), 4.39 (s, 2H, CH_2), 6.40 (bs, 1H, NH), 6.72 (d, J = 8.60 Hz, 2H, ArH), 6.84 (m, 4H, ArH), 6.99 (s, 1H, CH), 7.24 (d, J = 7.50 Hz, 2H, ArH), 7.36 (d, J = 8.60 Hz, 2H, ArH), 7.96 (d, J = 8.73 Hz, 2H, ArH). Anal. Calcd. for $C_{29}H_{31}NO_5$; C, 73.57; H, 6.55; N, 2.96. Found: C, 73.43; H, 6.42; N, 3.01.

7.4.4. N-Nonyl-2-[4-{3-(4-methoxyphenyl)-3-oxo-2-phenyl-propenyl}-phenoxy] acetamide (14)

Yield: 72 %, m.p. Oil.

FABMS: 514; IR (cm^{-1}): 3432, 3018, 1668, 1599, 1510, 1219, 1168; ^{1}HNMR (δ): 0.87 (t, 3H, CH_3), 1.25 (bs, 14H, 7 × CH_2), 3.29 (t, 2H, CH_2), 3.81 (s, 3H, OCH_3), 4.40 (s, 2H, CH_2), 6.45 (bs, 1H, NH), 6.73 (d, J = 8.60 Hz, 2H, ArH), 6.84 (d, J = 8.81 Hz, 2H, ArH), 7.08 (s, 1H, CH), 7.43 (d, J = 7.44 Hz, 2H, ArH), 7.31 (m, 5H, ArH), 7.96 (d, J = 8.30 Hz, 2H, ArH). Anal. Calcd. for $C_{33}H_{39}NO_4$; C, 77.19: H, 7.60; N, 2.73. Found: C, 77.28; H, 7.88; N, 2.53.

7.4.5. N-(2-Ethylhexyl)-2-[4-{3-(4-methoxyphenyl)-3-oxo-2-phenyl-propenyl}-phenoxy] acetamide (15)

Yield: 80 %, m.p. Oil.

FABMS: 500; IR (cm^{-1}): 3434, 3014, 2932, 1665, 1598, 1510, 1251; ^{1}HNMR (δ): 0.86 (t, 6H, 2×CH_3), 1.25 (m, 9H, 4 × CH_2 & CH), 3.25 (m, 2H, CH_2), 3.81 (s, 3H, OCH_3), 4.42 (s, 2H, CH_2), 6.43 (bs, 1H, NH), 6.76 (d, J = 9.20 Hz, 2H, ArH), 6.84 (d, J = 9.20 Hz, 2H, ArH), 7.09 (s, 1H, CH), 7.33 (m, 5H, ArH), 7.44 (d, J = 9.00 Hz, 2H, ArH), 7.84 (d, J = 9.25 Hz, 2H, ArH). Anal. Calcd. for $C_{32}H_{37}NO_4$; C, 76.95: H, 7.41; N, 2.80. Found: C, 77.13; H, 6.98; N, 2.96.

7.4.6. N-Octyl-2-[4-{3-(4-methoxyphenyl)-3-oxo-2-(methoxy-phenyl)-propenyl}-phenoxy] acetamide (16)

Yield: 78 %, m.p. Oil.

FABMS: 530; IR (cm^{-1}): 3432, 3012, 1666, 1600, 1512, 1250; ^{1}HNMR (δ): 0.89 (t, 3H, CH_3), 1.24 (bs, 12H, 6 × CH_2), 3.29 (t, 2H, CH_2), 3.79 (s, 3H, OCH_3), 3.81 (s, 3H, OCH_3), 4.39 (s, 2H, CH_2), 6.47 (bs, 1H, NH), 6.72 (d, J = 8.74 Hz, 2H, ArH), 6.83 (m, 4H, ArH), 6.99 (s, 1H, CH), 7.24 (d, J = 7.42Hz, 2H, ArH), 7.36 (d, J = 8.76, 2H, ArH), 7.96 (d, J = 8.46 Hz, 2H, ArH). Anal. Calcd. for $C_{33}H_{39}NO_5$; C, 74.85: H, 7.37; N, 2.65. Found: C, 74.78; H, 7.58; N, 2.67.

7.4.7. N-Butyl-N-methyl-2-[4-{3-(4-methoxyphenyl)-3-oxo-2-phenyl-propenyl}-phenoxy] acetamide (17)

Yield: 80 %, m.p Oil.

FABMS: 458; IR (cm^{-1}): 3013, 2933, 1653, 1599, 1507, 1249; ^{1}HNMR (δ): 0.90 (t, 3H, CH_3), 1.30 (m, 2H, CH_2), 1.57 (m, 2H, CH_2), 2.95 (m, 2H, CH_2), 3.30 (s, 3H, CH_3), 3.83 (s, 3H, OCH_3), 4.40 (s, 2H, CH_2), 6.75 (d, J = 8.50 Hz, 2H, ArH), 6.83 (d, J = 8.80 Hz, 2H, ArH), 7.07 (s, 1H, CH), 7.29 (m, 5H, ArH), 7.43 (d, J = 7.00 Hz, 2H, ArH), 7.96 (d, J = 8.86 Hz, 2H, ArH). Anal. Calcd. for $C_{29}H_{31}NO_4$; C, 76.15; H, 6.78; N, 3.06. Found: C, 76.33; H, 6.48; N, 3.32.

7.4.8. N-Dodecyl-2-[4-{3-(4-methoxyphenyl)-3-oxo-2-(4-methoxyphenyl)-propenyl}-phenoxy] acetamide (21)

Yield: 71 %, m.p. Oil.

FABMS: 585; IR (cm^{-1}): 3342, 2926, 2854, 1661, 1600, 1512, 1252, 1171, 1031, 834, 754; ^{1}HNMR (δ): 0.87 (t, 3H, CH_3), 1.25 (bs, 20H, 10×CH_2), 3.29 (t, 2H, CH_2), 3.79 (s, 3H, OCH_3), 3.81 (s, 3H, OCH_3), 4.39 (s, 2H, CH_2), 6.49 (bs, 1H, NH), 6.73 (d, J = 8.74 Hz, 2H, ArH), 6.85 (m, 4H, ArH), 7.00 (s, 1H, CH), 7.25 (d, J = 8.43 Hz, 2H, ArH), 7.37 (d, J = 8.78 Hz, 2H, ArH), 7.96 (d, J = 8.82 Hz, 2H, ArH). Anal. Calcd. for $C_{37}H_{47}NO_5$; C, 75.89: H, 8.03; N, 2.39. Found: C, 75.98; H, 8.12; N, 2.53.

7.4.9. N-Butyl-N-methyl-2-[4-{3-(4-methoxyphenyl)-3-oxo-2-(4-methoxy-phenyl)-propenyl}-phenoxy] acetamide (22)

Yield: 80 %, m.p. Oil.

FABMS: 488; IR (cm^{-1}): 3009, 2931, 1656, 1599, 1511, 1249, 1146, 765; ^{1}HNMR (δ): 0.90 (t, 3H, CH_3), 1.30 (m, 2H, CH_2), 1.57 (m, 2H, CH_2), 2.95 (t, 2H, CH_2), 3.30 (s, 3H, CH_3), 3.79 (s, 3H, OCH_3), 3.81 (s, 3H, OCH_3), 4.50 (s, 2H, CH_2), 6.75 (d, J = 8.50 Hz, 2H, ArH), 6.84 (m, 4H, ArH), 6.99 (s, 1H, CH), 7.21 (d, J = 8.76 H, 2H, ArH), 7.36 (d, J = 8.81 Hz, 2H, ArH), 7.96 (d, J = 8.84 Hz, 2H, ArH). Anal. Calcd. for $C_{30}H_{33}NO_5$; C, 73.92: H, 6.78; N, 2.87. Found: C, 74.23; H, 6.52; N, 3.01.

7.4.10. N-Octyl-2-[4-{3-(4-methoxyphenyl)-3-oxo-2-phenyl-propenyl}-phenoxy] acetamide (23)

Yield: 76 %, m.p. Oil.

FABMS: 500; IR (cm^{-1}): 3429, 3014, 2932, 1722, 1665, 1599, 1510, 1256; ^{1}HNMR (δ): 0.88 (t, 3H, CH_3), 0.98 (q, 2H, CH_2), 1.25 (bs, 10H, 5 × CH_2), 3.29 (t, 2H, CH_2), 3.81 (s, 3H, OCH_3), 4.40 (s, 2H, CH_2), 6.48 (bs, 1H, NH), 6.73 (d, J = 8.76 Hz, 2H, ArH), 6.84 (d, J = 8.88 Hz, 2H, ArH), 7.08 (s, 1H, CH), 7.43 (d, J = 9.50 Hz, 2H, ArH), 7.61 (m, 5H, ArH), 7.96 (d, J = 8.82 Hz, 2H, ArH). Anal. Calcd. for $C_{32}H_{37}NO_4$; C, 76.95: H, 7.41; N, 2.80. Found: C, 76.81; H, 7.18; N, 2.96.

7.4.11. N-Dodecyl-2-[4-{3-(4-methoxyphenyl)-3-oxo-2-phenyl-propenyl}-phenoxy] acetamide (24)

Yield: 72 %, m.p. Oil.

FABMS: 556; IR (cm^{-1}): 3336, 3010, 1660, 1598,1510, 1254; ^{1}HNMR (δ): 0.87 (t, 3H, CH_3), 1.25 (bs, 20H, 10×CH_2), 3.29 (t, 2H, CH_2), 3.81 (s, 3H, OCH_3), 4.40 (s, 2H, CH_2), 6.45 (bs, 1H, NH), 6.74 (d, J = 8.72 Hz, 2H, ArH), 6.84 (d, J = 8.86 Hz, 2H, ArH), 7.09 (s, 1H, CH), 7.44 (d, J = 7.50 Hz, 2H, ArH), 7.29 (m, 5H, ArH), 7.97 (d, J = 8.86 Hz, 2H, ArH). Anal. Calcd. for $C_{36}H_{45}NO_4$; C, 77.84: H, 8.10; N, 2.52. Found: C, 77.81; H, 7.98; N, 2.48.

7.5. Synthesis of 1-(4-Methoxy-phenyl)-phenyl-3-[4-(3-piperidin-1-yl-ethoxy)-phenyl]-propenone (25)

To a solution of hydroxy acrylophenone **6** (0.33 g, 0.001 mol) in dry acetone, anhydrous potassium carbonate and chloroethylpiperidine hydrochloride (0.39 g, 0.002 mol) was added. The solution was taken to reflux for 6 hr. The reaction mixture was filtered and was evaporated to dryness. The residue was taken into ethyl acetate, washed with water dried over anhydrous sodium sulfate and concentrated. The crude material was then chromatographed over a column of silica gel eluting with the methanol-chloroform to afford compound **25**.

Yield: 80%, m.p. Oil.

FABMS: 442; IR (cm^{-1}): 1702, 1650, 1599, 1253, 1171,1029, 755; ^{1}HNMR (δ): 1.61 (bs, 6H, CH_2), 2.51 (m, 4H, CH_2), 2.78 (t, 2H, CH_2), 4.10 (t, 2H, CH_2), 3.81 (s, 3H, OCH_3), 6.73 (d, J = 8.76 Hz, 2H, ArH), 6.84 (d, J = 8.88 Hz, 2H, ArH), 7.08 (s, 1H, CH), 7.43 (d, J = 9.50 Hz, 2H, ArH), 7.61 (m, 5H, ArH), 7.96 (d, J = 8.82 Hz, 2H, ArH). Anal. Calcd. for $C_{29}H_{31}NO_3$; C, 78.91; H, 7.03; N, 3.17. Found: C, 78.81; H, 7.18; N, 2.96.

REFERENCES

[1] Jordan, V.C. *J. Med. Chem.,* **2003**, *46(6),* 883.
[2] Jordan, V.C. *J. Med. Chem.*, **2003**, *46(7)*, 1081.
[3] Ray, S.; Rastogi, R.; Kumar, A. *Progress Drug Res.,* **2002**, *59*, 201.
[4] Ray, S.; Dwivedy, I. *Adv. Drug Res.,* **1997**, *29*, 171.
[5] Geistlinger, T. R.; McReynolds, A. C.; Guy, R. K. *Chem. Biol.,* **2004**, *11,* 273.
[6] Grese, T. A.; Adrian, M. D.; Philips, D. L.; Schetle, P. K.; Short, L. L.;Glasebrook, A. L.; Bryant, H. U. *J. Med. Chem.,* **2001**, *44*, 2857.
[7] Labrie, F.; Labrie, C.; Belanger, A.; Simard, J.; Gauthier, S.; Luu-The, V.; Merand, Y.; Giguere, V.; Candas, B.; Luo, S.; Martel, C.; Singh, S. M.; Fournier, M.; Coquet, A.; Richard, V.; Charbonneau, R.; Charpenet, G.; Tremblay, A.; Tremblay, G.; Cusan, L.; Veilleux, R. J. *Steroid Biochem. Mol. Biol.,* **1999**, *69,* 51.
[8] Blizzard, T. A.; DiNinno, F.; Morgan II., J. d.; Chen, H. y.; Wu, J. Y.; Gude, C.; Kim, S.; Chan, W.; Birzin, E. T.; Yang, Y. T.; Pai, L. Y.; Zhang, Z.; Hayes, E. C.; DaSilva, C. A.; Tang, W.; Rohrer, S. P.; Schaeffer, M.; Hammond, M. L. *Bioorg. Med. Chem. Lett*., **2004**, *14,* 3861 & 3865.
[9] Bowler, J.; Lilley, T. J.; Pitlam, J. D.; Wakeling, A. E. *Steroids*, **1989**, *54*, 71.
[10] Iyer, R. N.; Gopalachari, R. *J. Pharmacy*, **1969**, *31,* 49.
[11] Mittal, S.; Durani, S.; Kapil, R. S. *J. Med. Chem.,* **1985**, *28,* 492.

[12] Brzozowski, A.M.; Pike, A.C.W.; Dauter, Z.; Hubbard, R.E.; Bonn, T.; Engström, O.; Öhman, L.; Greene, L.; Gustafsson, J.A.; Carlquist, M. *Nature,* **1997**, *386*(6652), 389.
[13] Sarkhel, S.; Sharon, A.; Trivedi, V.;. Maulik, P. R.; Singh, M.M.; Venugopalan, P.; Ray, S. *Bioorg Med. Chem.,* **2003**, *11*, 5025.
[14] Dhar, J. D.; Dwivedi, A.; Srivastava, A.; Setty, B. S. *Contraception,* **1994**, *49,* 609.
[15] Ghosh, R.; Kamboj, V. P.; Singh, M. M. *Contraception,* **2001**, *64*, 261.

Received: 08 May, 2006 **Revised: 23 August, 2006** **Accepted: 31 October, 2006**