ChemPubSoc Europe

DOI: 10.1002/cmdc.201400003

Bis-arylidene Oxindoles as Anti-Breast-Cancer Agents Acting via the Estrogen Receptor

Abhishek Pal,[c] Anirban Ganguly,[a] Avijit Ghosh,[b] Md Yousuf,[b] Bhowmira Rathore,[a] Rajkumar Banerjee,*[a] and Susanta Adhikari*[b]

We report a new family of bis-arylidene oxindole derivatives that show highly selective estrogen receptor (ER)-mediated anticancer activity at low-nanomolar concentrations in ER-positive (ER+) breast cancer cells. In terms of cell growth inhibition, IC_{50} values for these compounds in ER+ breast cancer cells are two to three orders of magnitude lower than in ER-negative (ER−) breast cancer cells and non-cancer cells. In comparison with known bis-arylidene drugs, these compounds are at least three orders of magnitude more toxic than tamoxifen and 1.5–4-fold more toxic than 4-hydroxytamoxifen in ER+ MCF-7 cancer cells. These oxindoles inhibit ER transactivation, and their anticancer activities are inhibited in ER-depleted MCF-7 cells. Some of these nonsteroidal molecules also exhibit essential properties of selective ER down-regulation. From the development of two series of bis-arylidene oxindole-based compounds, we report a new series of anticancer agents for estrogen-responsive breast cancer.

Estrogen and its receptor are classically involved in a majority of cancers of gynecological origin.[1] The estrogen receptor (ER), a nuclear hormone receptor, is expressed in estrogen-responsive organs such as ovary, uterus, and mammary glands. Estrogen and estrogen-bound ER possess both genomic and non-genomic functions.[2–6] Because the functional expression of the ER is confined to the initial stages of neoplastic transformation, the design of compounds that interfere with ER function is expected to be an effective strategy in preventing these types of cancer.

17β-Estradiol (ES), the chief endogenous steroid hormone ligand of the ER, primarily regulates a broad range of physiological processes such as growth, differentiation, and physiology of reproductive processes. Estrogen-bound ER classically acts as a transcription factor, regulating cell proliferation and influencing the pathological processes of hormone-dependent breast, endometrial, prostate, ovarian, and thyroid cancers.[7,8] This makes the ER one of the principal targets for cancer therapy. We have shown that ES can be conveniently used as a ligand for targeting bioactive cargoes to ER-positive (ER+) breast cancer cells; if appropriately modified, ES can also be used for the targeted delivery of kinase inhibitors for treating breast cancer.[9] Estrogens, both natural and synthetic, have various levels of agonistic or antagonistic character in different target tissues.[10,11] For example, ES stimulates responses in the uterus, breast, bone, and liver, whereas the nonsteroidal estrogen reloxifene blocks estrogen action in uterus and breast, but has agonistic activity in bone and liver. There are several ER antagonists, aromatase inhibitors, and selective ER modulators (SERMs) that alone, or in combination, provide several clinical options for breast cancer therapy with limited efficacy.[12–16]

Small polyphenolic molecules such as stilbenes, flavonoids, pro-anthocyanidins, and their derivatives found throughout the vegetable world, are the most acknowledged antioxidants working against free radicals associated with diseases related to certain cancers and cardiac, ocular, and degenerative problems.[17] They also act as protein function modulators. For example, resveratrol is an endocrine modulator, whereas a structurally similar stilbene, *trans*-diethylstilbesterol, acts as a powerful estrogen via ERα and ERβ. Similarly, the synthetic diphenolethylene, 1,1-bis(4′-hydroxyphenyl)-2-phenylbut-1-ene (**1c**), shows estrogenic effects.[17] Accordingly, in our design, we sought to modify the biological properties of organic polyphenols, originally inefficacious at therapeutic levels, through covalent addition of an oxindole group to the organic skeleton. In this work we show that by grafting the oxindole moiety to organic diphenol, the estrogenic properties can be profoundly modified, in fact, reversed. Oxindole was chosen as the key molecular fragment in the present design because it is a privileged heterocyclic motif that is known to possess a wide range of biological activities and medicinal applications.[18,19] Earlier we reported a new synthesis of enantiopure 3,3-disubstituted oxindoles directed toward the total synthesis of the complex antitumor pyrrolidinoindoline alkaloid, laptosin D, by starting from isatin.[20] In our quest to develop more potent anticancer molecules with potential anti-estrogenic properties, we incorporated an oxindole unit into the bisphenolic or bis-arylidene moieties. These have the base skeleton of the active metabolite, 4-hydroxytamoxifen (**1b**), bearing the crucial hydroxy and dimethylamino groups, which are known to maintain excellent ER recognition (Scheme 1). We also intended for the designed

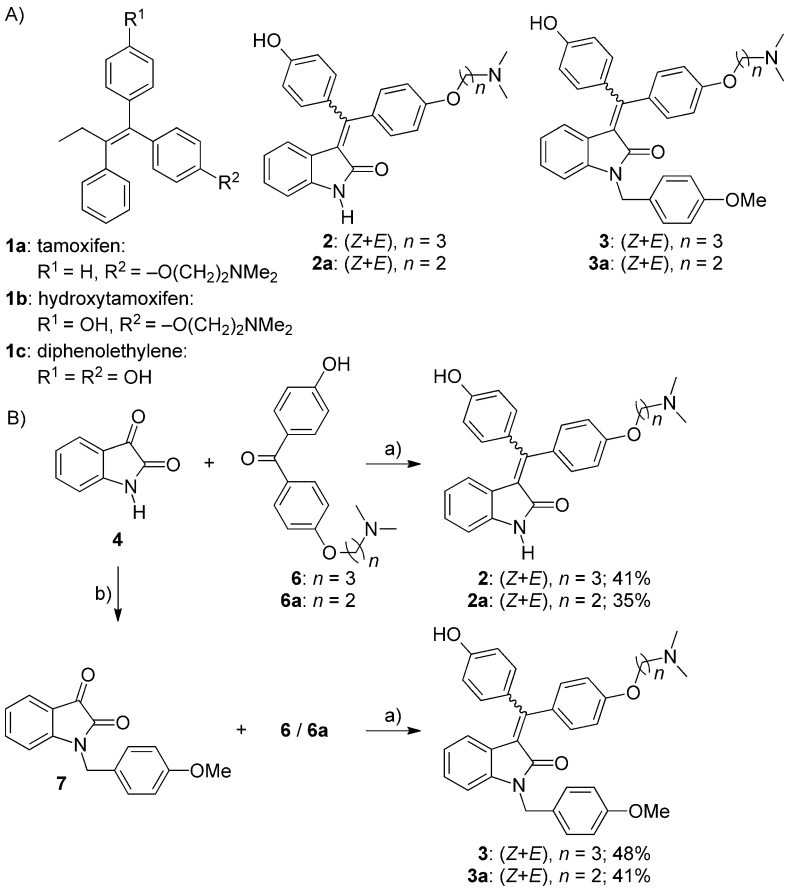
[a] *A. Ganguly,[+] B. Rathore, Dr. R. Banerjee*
Biomaterials Group, Division of Lipid Science & Technology
CSIR-Indian Institute of Chemical Technology, Hyderabad 500607 (India)
E-mail: rkbanerjee@yahoo.com
banerjee@iict.res.in

[b] *A. Ghosh, M. Yousuf, Dr. S. Adhikari*
Department of Chemistry, University of Calcutta
92, A.P.C. Road, Kolkata 700 009 (India)
E-mail: adhikarisusanta@yahoo.com

[c] *A. Pal[+]*
Department of Organic Chemistry, IACS, Jadavpur, Kolkata 700032 (India)

[+] *These authors contributed equally to this work.*

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/cmdc.201400003.



Scheme 1. Synthesis of bis-arylidineoxindoles. *Reagents and conditions*: a) Zn, $TiCl_4$, THF, reflux, 3 h; b) 1. NaH, THF, RT, 1 h, 2. PMB-Br, DMF, RT, 5 h, 78%.

compounds to have higher selectivity for cancer cells with minimal toxicological liability.

Initially, we undertook the synthesis of **2** and **2a** with the respective basic *N*,*N*-dimethylaminoalkoxy side chain in tamoxifen-based molecules (**1a**,**b**), as it is readily available. It is well documented that increasing the number of methylene units in the alkylamino side chain of various nonsteroidal anti-estrogens such as tamoxifen,[21] nafoxidine, idoxifene,[22] and organometallic hormones[23] allows maintenance of antagonistic potency and antiproliferative effects toward breast-cancer cell lines. To verify the significance of the oxindole nucleus in determining antagonistic potency with increased cytotoxic effects in MCF-7 breast cancer cells, we also synthesized *N*-4-methoxybenzylated analogues **3** and **3a** by incorporation of hydrophobic substituents such as 4-methoxybenzyl at the oxindole nitrogen atom, based on computational modeling and published precedent.[24] While assuming a possible binding of ligand to ER, we preliminarily studied the interaction by molecular modeling. We found that among the various N-alkylating groups on the oxindole nitrogen, "4-methoxybenzyl group" with an acceptor atom "O" has an additional stabilizing electrostatic interaction with the neighboring Ile 424 residue of human ERα (data not shown).

The synthetic procedure is illustrated in Scheme 1B. It appeared that a McMurry coupling reaction[25] would be most effective for preparing the desired alkene derivatives. Compounds **2** and **2a** were obtained as a mixture of *Z* and *E* isomers via a cross-coupling reaction between isatin (**4**) and 4,4′-dihydroxybenzophenone derivatives **6** and **6a**, respectively. Compounds **6** and **6a** were obtained by mono-alkylation of 4,4′-dihydroxybenzophenone (**5**) with respective *N*,*N*-dimethylaminoalkyl chlorides in the presence of ethanolic sodium ethoxide. Compound **2** and **2a** were present as respective mixtures of *Z* and *E* isomers, and could not be separated by flash column chromatography or fractional crystallization. This can be explained from the consequence of the interaction between the central double bond and the hydroxy group of the phenol, leading to interconversion between *Z* and *E* isomers, as observed in other instances in the stilbene series.[23a] Similarly, compounds **3** and **3a** were also obtained as *Z*/*E* isomeric mixtures from the subsequent coupling between **7** and **6**/**6a**.

The compounds were first evaluated for their cytotoxic effects in ER+ MCF-7 breast cancer cells. The molecules with promising cytotoxicity were further tested against ER-negative (ER−) MDA-MB-231 cells and non-cancer cells, CHO and HEK 293 (Table 1). Compounds **2**/**2a** and **3**/**3a** showed antiproliferative effects against ER+ breast cancer cells only. Compounds **2**/**2a** and **3** were 1.5–4-fold more potent than the most active tamoxifen metabolite 4-hydroxytamoxifen (**1b**) and 1000-fold more potent than tamoxifen (**1a**), whereas **3a** exhibited activity similar to that of **1b** in MCF-7 cells. The introduction of an oxindole group to the diphenol moiety classically associated with tamoxifen, with or without *para*-methoxybenzyl (PMB) protection, did not change its selective toxicity against ER+ cancer cells. This ER selectivity was also unaffected by the change in side chain carbon number, which differed from tamoxifen derivatives. It appears that the oxindole moiety imparted a strong influence on ERα recognition. Incidentally, compound **3a**, with PMB protection and a two-carbon side chain, showed a 2–4-fold lower anticancer effect than **2**, **2a**, and **3** in ER+ breast cancer cells. It seems that the ER-targeting ability is slightly more tolerated and stabilized in

Table 1. Growth inhibition elicited by test compounds against various cell lines.

Compound	IC_{50} [μM] MCF-7[a]	MDA-MB-231[b]	CHO[a]	HEK 293[a]
1a	>35	ND	>35	ND
1b	0.096±0.004	>35	>35	>35
ICI 182780	0.023±0.003	ND	ND	ND
2	0.032±0.004	5.764±0.005	>35	>35
2a	0.037±0.004	>35	>35	>35
3	0.033±0.006	5.896±0.005	>35	>35
3a	0.112±0.006	>35	>35	>35
8	0.602±0.033	>35	>35	>35
8a	>35	ND	ND	ND
8b	>35	ND	ND	ND
8c	>35	ND	ND	ND
9	>35	ND	ND	ND
13	>35	ND	>35	ND
13a	0.672±0.005	ND	ND	ND
13b	0.069±0.021	26.324±0.005	>35	>35
13c	0.055±0.031	20.251±0.005	>35	>35

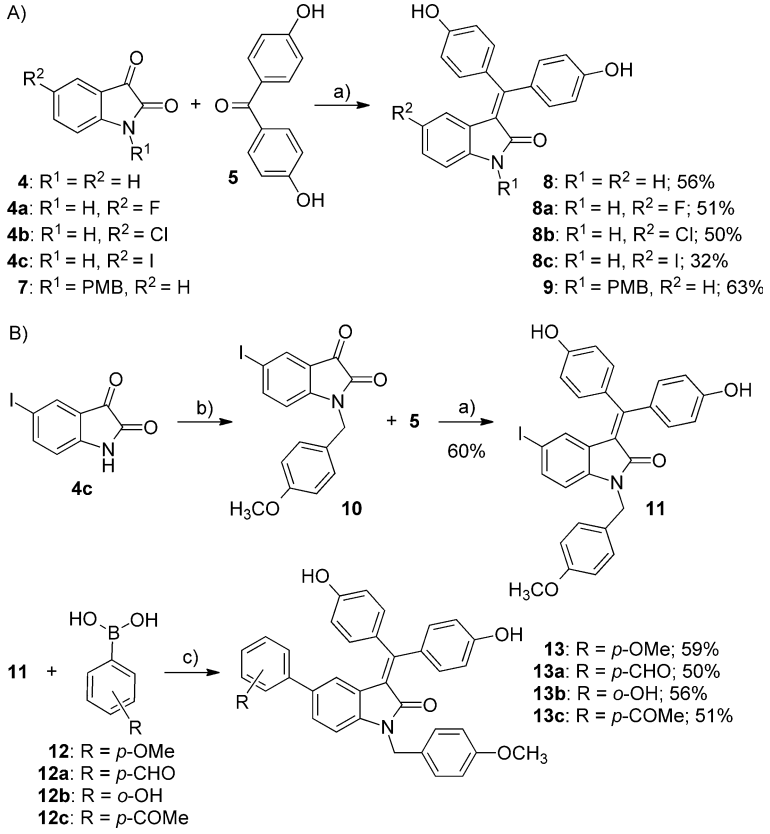
[a] Values are the mean±SD for $n=3$ replicates performed in triplicate on different days. [b] Values are the mean±SD for $n=2$ replicates performed in duplicate on different days.

PMB-protected, three-carbon side chain oxindoles than in two-carbon side chain oxindoles.

Encouraged by the above result, we proceeded to examine the biological efficacy of diphenols (without an alkylamino-terminated side chain) as shown in Scheme 2. The desired molecule **8** was obtained in 56% yield (Scheme 2A) by an analogous McMurry coupling of isatin (**4**) and 4,4′-dihydroxybenzophenone (**5**). We also prepared the 5-halo derivatives (F, Cl, I), as precursors are commercially available. The PMB-protected iodoisatin derivative **10** was subjected to McMurry coupling to yield **11**, which in turn provided access to a number of C5-substituted aryl derivatives through Suzuki–Miyaura coupling using the appropriate commercially available boronic acids (Scheme 2B) under microwave irradiation.[26]

The cytotoxicities of these new compounds **8**, **8a**–**c**, **9**, **13**, and **13a**–**c** were again tested. Moreover, they were also tested against MDA-MB-231 (ER−) and non-cancer cell lines, CHO and HEK 293. The selective anticancer effects against ER+ breast cancer cells exhibited by these compounds are clearly evident in Table 1. The parent molecule **8**, in comparison with its halogenated analogues (**8a**–**c**) showed the highest anticancer effect against MCF-7 cells. Although the PMB-protected derivative **9** did not exhibit anticancer activity, compounds **13a**–**c**, which are all PMB protected, exhibited significant and selective anticancer effects against MCF-7 cells. However, among these PMB-protected molecules, the C5-substituted *para*-methoxyphenyl analogue **13** surprisingly showed no activity. That the PMB group per se is not detrimental to biological activity was independently proven by similar, low-level IC_{50} values between compounds **2**/**2a** and **3**/**3a**, as described above. Notably, IC_{50} values for **13b**,**c** are 4–5-fold greater than those for **2** and **3** in ER− MDA-MB-231 cells. This indicates that as far as selectivity toward ER-associated breast cancer cells is concerned, compounds **13b**,**c** are possibly even more potent than **2**/**2a** and **3**/**3a**.

The most potent molecules, **2**, **3**, **13b**, and **13c**, and one of the potent parent oxindoles, **8**, were further tested to determine if their anticancer effects could be antagonized by the ER endogenous ligand, 17β-estradiol (ES). This was done to corroborate the ER-specificity of these molecules. Figure 1 shows that the anticancer effect of all the compounds tested against MCF-7 were significantly inhibited ($p<0.001$) if cells were pre-treated and co-treated with 10 μM ES. The results indicate that ES, the most potent natural ER ligand possessing the highest ER binding affinity, could restrict and antagonize the anticancer effect of oxindoles. This could be due to possible restriction of oxindole binding to the ES binding site in the ER. These data were corroborated independently when we observed similar inhibition of cytotoxicity with MCF-7 cells pre-treated with the ER antagonist/anti-estrogen ICI 182780 (fulvestrant) for 4 h followed by prolonged treatment with our new oxindoles (Supporting Information figure S1). This ER antagonist also has binding affinity for the ES binding site of the ER, and hence indicates further for a possible ER-binding capacity of these molecules. However, it is clear that whether or not these oxindoles



Scheme 2. Synthesis of bis-phenol analogues. *Reagents and conditions*: a) Zn, $TiCl_4$, THF, reflux, 3 h; b) 1. NaH, THF, RT, 1 h, 2. PMB-Br, DMF, RT, 5 h, 72%; c) K_2CO_3, $Pd(PPh_3)_4$, microwave, 120 °C, 30 min.

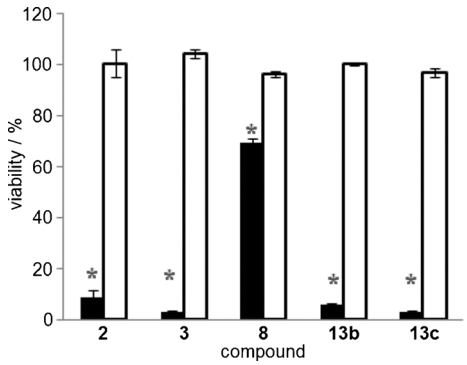


Figure 1. ER-mediated anticancer activity of oxindoles: Viability of MCF-7 cells treated with indicated test compounds (0.1 μM) in the presence (□) and absence (■) of the endogenous ER ligand, 17β-estradiol (*$p < 0.001$).

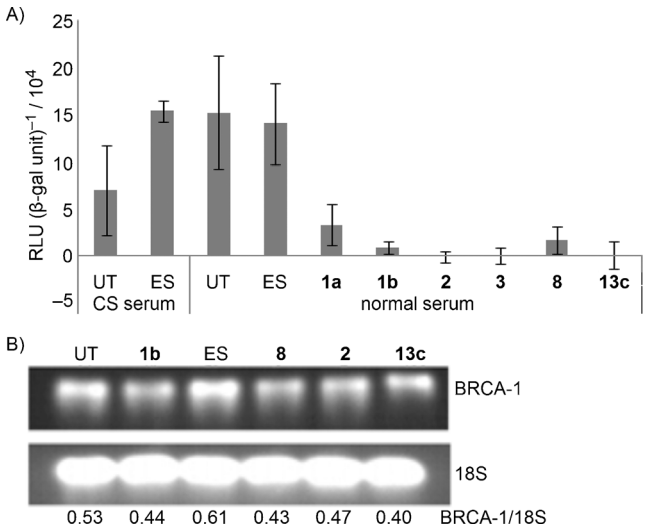


Figure 2. A) *Trans*-repression of ERE-promoted protein expression by oxindoles: Relative light units (RLU) of luciferase expression per unit of β-galactosidase (β-gal) expression obtained from MCF-7 cell lysates, preconditioned in either hormone-free (i.e., charcoal stripped, CS) serum or normal serum, then pre-transfected with ERE–luciferase and β-gal plasmids and respectively kept untreated (UT) or treated with 17β-estradiol (ES) and indicated test compounds (*$p < 0.001$). B) RT-PCR analyses of the *BRCA-1* gene: The ERE-regulated *BRCA-1* gene in MCF-7 cells was analyzed following treatment with the indicated compounds (UT: untreated control; 18S: internal control). The ratios of increased or decreased *BRCA-1* expression levels with respect to 18S are indicated below.

have ER binding affinity, the anticancer activities of these compounds are clearly mediated by the ER.

Toward elucidating any potential anti-estrogenic effects that may be linked to the anticancer effects of these oxindoles, MCF-7 cells were treated with the potent compounds **2**, **3**, **8**, and **13b**,**c**. Two more compounds were incorporated into this study: compound **1a** is a well-known ER antagonist that binds to the ER and inhibits any further estrogen-responsive gene transactivation; **1b** acts as an anti-estrogen and binds to the ER, repressing estrogen-responsive genes. Following the binding of an estrogenic or anti-estrogenic compound, the ER dimerizes and acts on the estrogen responsive element (ERE) promoter region in chromosomal DNA to regulate the transcription and expression of various genes (ERE genes).[8,10] To quantitatively assess the effect of lead oxindoles on the expression of ERE-promoted genes, we pre-transfected MCF-7 cells with an ERE–luciferase plasmid and a CMV–β-gal plasmid. It is known that only the estrogenic/anti-estrogenic molecules influence the expression of the ERE-promoted reporter luciferase gene, whereas β-gal expression should remain independent of any such treatment. Following plasmid transfection, treatment with test compounds was performed. Figure 2A shows that under normal serum conditions, in which all the endogenous hormones in cell culture serum are present, the known antagonist **1a**, anti-estrogen **1b**, and test compounds **2**, **3**, **8**, and **13c** clearly down-regulated luciferase expression under ERE promoter control. Notably, under hormone-free conditions (charcoal-stripped serum), ES indeed induced the up-regulation of luciferase expression with respect to untreated control (UT), thereby indicating that the luciferase expression in these ERE–luciferase transfected cells was truly estrogen sensitive. Generally, upon binding with a pro-estrogenic molecule such as natural estrogen ES, the ER favorably up-regulates expression of the pro-proliferative, ERE-promoted genes. On the other hand, anti-estrogens, following ER binding, tend to down-regulate ERE genes, whereas ER antagonists, upon ER binding, oppose any further up-regulation of these genes. Our data show that the tested oxindoles behave more like anti-estrogens, the ERE-*trans*-repression activities of which remain undeterred even in the presence of natural hormonal conditions.

The repression of ERE gene translation was not an isolated effect. We observed similar effects on ERE gene transcription. For these, we chose to monitor the gene transcription (mRNA expression) levels of two ERE-promoted genes, *BRCA-1* and *Bcl-2*, by RT-PCR techniques in the presence of these compounds. The 18S gene was selected as a homing control gene whose mRNA expression should remain undeterred by any treatment. MCF-7 cells were treated with key molecules **2**, **8**, and **13c**, which showed ER-mediated anticancer activity in MCF-7 cells. Other control treatment groups such as pro-estrogenic ES and anti-estrogenic **1b** were also kept along with untreated control (UT). Following treatment, cells were isolated for their respective RNAs, which were converted into cDNA by reverse transcriptase. This was followed by PCR using individual primers for BRCA-1 and 18S for their respective amplification. The respective DNAs were separated by gel electrophoresis for determining their respective levels of expression (Figure 2B and Supporting Information figure S2). The trend in the mRNA expression of these ERE genes corroborated this luciferase protein expression pattern, indicating that the compounds do indeed exhibit a down-regulating effect on ERE-promoted genes at both transcriptional and translational levels, as particularly exhibited by known anti-estrogens.

To demonstrate that the induced cytotoxicity of these newly designed lead molecules are indeed mediated by the ER, we performed a silencing RNA (siRNA)-based ER knock-down study. The hypothesis is that if compound-induced cytotoxicity in MCF-7 (ER+) cells is significantly inhibited following ER depletion, then the given compound induces ER-mediated cyto-

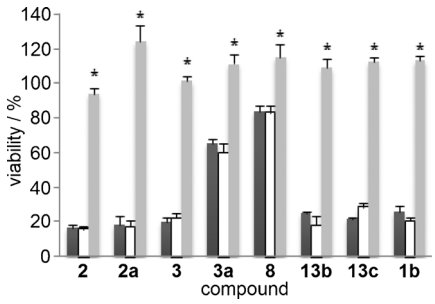


Figure 3. Reversal of cytotoxicity in ERα-depleted MCF-7 cells: Percent viability of either siRNA-untreated (UT, ■), control siRNA (□), or ERα siRNA-pretreated (■) MCF-7 cells under 36 h continuous treatment with lead compounds **2**, **2a**, **3**, **3a**, **8**, **13b**, and **13c**, as well as known anti-estrogen **1b** (*$p < 0.001$).

toxicity in ER+ cells. Toward understanding this, MCF-7 cells were pre-treated with either ERα siRNA or with control (non-targeted) siRNA. Cells were then treated with lead oxindoles. Figure 3 shows that the most potent oxindoles **2**, **2a**, **3**, **3a**, **8**, and **13b**,**c** exhibited maximum toxicity (i.e., minimum viability) in siRNA-untreated or control-siRNA-treated MCF-7 cells. However, there were significant, if not complete reversal of cytotoxicities (or in other words, exhibition of 100% viability) mediated by these molecules in ERα-depleted MCF-7 cells. ERα depletion was evident from its protein level following ERα-targeted siRNA treatment (Supporting Information figure S3). The 100% reversal of toxicity in ERα-depleted cells indicates the possibility that the cellular toxicities of these oxindoles are predominantly mediated via ERα and not via ERβ.

Further to this, we sought to find the ERα expression status in MCF-7 cells treated with lead molecules **2**, **2a**, **3**, **13b**, and **13c** compared with levels obtained by treating cells with known selective ER down-regulators (SERDs), **1b** and ICI 182780. SERDs are functionally different from selective ER modulators (SERMs). Unlike SERMs, SERDs degrade ER levels, while exhibiting anti-estrogenic properties, regulation of ERE genes, etc.[27] Figure 4 indicates that under the same subclass, compounds **2**, **2a**, and **3** behave as SERDs in MCF-7 cells, in which ERα levels are clearly degraded relative to those in untreated cells. Interestingly, among the other subclass, **13c** degrades ERα, but **13b** maintains ERα levels. So, despite exhibiting similar cytotoxicities, these molecules under the same broad class, individually exhibit either SERM- or SERD-type properties. Further experiments are certainly warranted to ascertain the specific and important roles of these oxindoles.

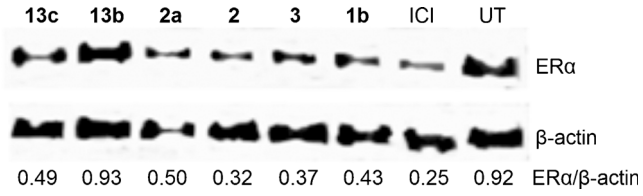


Figure 4. Western blot analysis of cell lysates for ERα content: Differential expression of ERα along with the housekeeping control gene β-actin in cell lysates of MCF-7 kept untreated (UT) or treated with various test compounds as indicated (ICI = ICI 182780).

We also performed molecular docking analyses with the complex between human the ERα ligand binding site and **1b** as the template. Preliminarily, the analysis showed that all the synthesized molecules are not only potent in binding to the ER ligand binding site, but also maintain a trend in the docking score among the respective series, such as **2**/**3** or **8** or **13** oxindole groups, roughly equivalent to their trend in cellular toxicity (data not shown). Detailed modeling studies may further help elucidate the tentative ER binding capability, if any, of these oxindoles.

In summary, we report herein the development of highly potent, ER-selective, anti-breast-cancer oxindole-conjugated bisphenols. Our data show that the ER-targeted anticancer activities of arylidenes are not compromised by changing or replacing basic alkylaminoethoxy side chains present in highly anti-estrogenic 4-hydroxytamoxifen (**1b**). The uncompromised ER-targeting ability and anti-estrogenic or reverse agonistic properties are equivocally strengthened by the introduction of oxindole moieties in arylidenes. Serendipitously, *N*-4-methoxybenzyl protection of oxindoles rather reinforced ER-mediated anti-breast-cancer activity. Some of these compounds down-regulate the ER and hence behave as SERDs. The in vivo evaluation in breast cancer models for the most active compound is currently in progress. Toward further structure–activity relationship studies, the syntheses of additional oxindole derivatives are underway to expand our knowledge in optimizing the pharmacophore with improved anti-breast-cancer potential.

Experimental Section

General

Chemicals and solvents were purchased from commercial suppliers and used as received. ^{1}H and ^{13}C NMR spectra were recorded on Bruker Avance III HD (300 MHz) or Avance III 500 (500 MHz) spectrometers. Chemical shifts (δ) are reported in parts per million (ppm), and the residual solvent peak was used as an internal reference: ^{1}H ($CHCl_3$ $\delta = 7.26$ ppm), ^{13}C ($CHCl_3$ $\delta = 77.16$ ppm), or tetramethylsilane (TMS $\delta = 0.00$ ppm) was used as a reference. Multiplicity is indicated as follows: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), dd (doublet of doublets), brs (broad singlet), appt (apparent triplet). Coupling constants are reported in Hertz (Hz). High-resolution MS data were obtained on a Micromass/Q-ToF microspectrometer. Melting points were determined on a Thomas Hoover capillary melting point apparatus. IR spectra were measured on Thermo Scientific Nicolet 380 instrument. A CEM Discover (MN-542470) microwave was used for Suzuki–Miyaura cross-coupling reactions. Merck pre-coated TLC plates (Merck 60 F_{254}) were used for thin-layer chromatography, and compounds were visualized with UV light at λ 254 nm. Further visualization was achieved by staining with iodine. Flash chromatography separations were performed on SRL 230–400 mesh silica gel.

Starting materials

Compounds **4**, **4a**, **4b**, and **4c** were purchased from Avra Synthesis Pvt. Ltd. (India), compound **5** was brought from Sigma (India), and compounds **12**, **12a**, **12b**, and **12c** were purchased from Frontier Scientific (India). Compounds **6** and **6a** were prepared according to published methods.[23a]

Representative procedure for McMurry coupling

To a suspension of Zn^0 dust (314 mg, 4.8 mmol) in THF (10 mL), $TiCl_4$ (0.26 mL, 2.4 mmol) was added at −10 °C. The cooling bath was removed, and the mixture was held at reflux for 1 h. After cooling to room temperature, anhydrous pyridine (0.38 mL, 4.8 mmol) was added, and the mixture was stirred for 5 min. A solution of the appropriate oxindole (**4**/**4a**/**4b**/**4c**/**7**; 0.8 mmol) and 4,4′-dihydroxybenzophenone (**5**) (171 mg, 0.8 mmol) in THF (4 mL) was added, and the mixture was held at reflux for 2 h (monitored by TLC). After cooling to room temperature, the mixture was hydrolyzed with 10 mL of 8% aqueous K_2CO_3. The reaction mixture was extracted with several 10-mL portions of EtOAc. The organic phase was washed with H_2O (2×20 mL) and brine (20 mL), dried over Na_2SO_4, and evaporated to dryness. The crude product was purified by flash chromatography on silica gel (230–400 mesh) with petroleum ether/EtOAc (3:2) as eluent to obtain the first unreacted oxindole moiety [$R_f \approx 0.7$; ~10–15%] followed by the product (**8**/**8a**/**8b**/**8c**/**9**) [$R_f \approx 0.4$; ~32–63%] and finally the homo-coupled product derived from the corresponding benzophenone moiety [$R_f \approx 0.35$; ~15–20%]. It was observed that going from **8** to **8c**, the product yield decreases due to additional formation of homo-coupled product from the oxindole motif.

Representative procedure for Suzuki–Miyaura coupling

A septum-sealed microwave tube charged with **11** (100.0 mg, 0.174 mmol), appropriate arylboronic acid (**12**/**12a**/**12b**/**12c**; 0.261 mmol), $Pd(PPh_3)_4$ (4.0 mg, 0.0035 mmol), and K_2CO_3 (72.0 mg, 0.52 mmol) in dioxane (1.0 mL) was irradiated in a microwave cavity (160 W, 120 °C, 30 min). The reaction mixture was quenched with dilute AcOH and extracted with EtOAc. The combined organic extracts were dried over anhydrous Na_2SO_4, then filtered off, and the solvent was evaporated under reduced pressure. The crude product was purified by flash chromatography on silica gel (230–400 mesh) with petroleum ether/EtOAc (3:2) as eluent to give first unreacted starting material [$R_f \approx 0.45$; ~20–25%] followed by the cross-coupled products (**13**/**13a**/**13b**/**13c**) [$R_f \approx 0.4$; ~50–59%].

Acknowledgements

We acknowledge Prof. Amitabha Sarkar (IACS, Kolkata) for his generous suggestions and support. We also thank Priyanka Sharma (IICT, Hyderabad) for technical assistance in cell culture experiments. A.P. and M.Y. acknowledge fellowship support from CSIR, New Delhi. A. Ghosh is thankful to UGC for a fellowship. R.B. acknowledges the DST (project no. SR/S1/OC-64/2008) and IICT (in-house project OLP0003). S.A. acknowledges the DST (project no. SR/S1/OC-101/2012).

Keywords: anticancer agents · estrogen response element · McMurry coupling · oxindoles · receptors · transactivation

[1] C. K. Osborne, *Breast Cancer Res. Treat.* **1998**, *51*, 227–238.
[2] P. Thomas, Y. Pang, E. J. Filardo, J. Dong, *Endocrinology* **2005**, *146*, 624–632.
[3] M. Razandi, A. Pedram, G. L. Greene, E. R. Levin, *Mol. Endocrinol.* **1999**, *13*, 307–319.
[4] Z. Chen, I. S. Yuhanna, Z. Galcheva-Gargova, R. H. Karas, M. E. Mendelsohn, P. W. Shaul, *J. Clin. Invest.* **1999**, *103*, 401–406.
[5] R. X. D. Song, R. A. McPherson, L. Adam, Y. Bao, M. Shupnik, R. Kumar, R. J. Santen, *Mol. Endocrinol.* **2002**, *16*, 116–127.
[6] P. Monje, R. Boland, *Mol. Cell. Endocrinol.* **1999**, *147*, 75–84.
[7] P. Ascenzi, A. Bocedi, M. Marino, *Mol. Aspects Med.* **2006**, *27*, 299–402.
[8] S. T. Pearce, V. C. Jordan, *Crit. Rev. Oncol. Hematol.* **2004**, *50*, 3–22.
[9] a) B. S. Reddy, R. Banerjee, *Angew. Chem. Int. Ed.* **2005**, *44*, 6723–6727; *Angew. Chem.* **2005**, *117*, 6881–6885; b) S. Sinha, S. Roy, B. S. Reddy, K. Pal, G. Sudhakar, S. Iyer, S. Dutta, E. Wang, P. K. Vohra, K. R. Roy, P. Reddanna, D. Mukhopadhyay, R. Banerjee, *Mol. Cancer Res.* **2011**, *9*, 364–374.
[10] D. P. McDonnell, *Trends Endocrinol. Metab.* **1999**, *10*, 301–311.
[11] D. P. McDonnell, *J. Soc. Gynecol. Invest.* **2000**, *7*, S10–S15.
[12] L. W. Wattenberg, *Cancer Res.* **1985**, *45*, 1–8.
[13] A. Fugh-Berman, S. Epstein, *Lancet* **1992**, *340*, 1143–1145.
[14] J. F. Robertson, *Cancer Treat. Rev.* **2004**, *30*, 695–706.
[15] K. L. Jones, A. U. Buzdar, *Endocr.-Relat. Cancer* **2004**, *11*, 391–406.
[16] I. C. Henderson, *Semin. Oncol.* **2004**, *31*, 31–34.
[17] A. Vessières, S. Top, P. Pigeon, E. Hillard, L. Boubeker, D. Spera, G. Jaouen, *J. Med. Chem.* **2005**, *48*, 3937–3940, and references cited therein.
[18] N. V. Hanhan, Y. C. Tang, N. T. Tran, A. K. Franz, *Org. Lett.* **2012**, *14*, 2218–2221, and references cited therein.
[19] C. R. Reddy, E. Jithender, G. Krishna, G. V. Reddy, B. Jagadeesh, *Org. Biomol. Chem.* **2011**, *9*, 3940–3947, and references cited therein.
[20] S. Adhikari, S. Caille, M. Hanbauer, V. X. Ngo, L. E. Overman, *Org. Lett.* **2005**, *7*, 2795–2797.
[21] D. W. Robertson, J. A. Katzenellenbogen, J. R. Hayes, B. S. Katzenellenbogen, *J. Med. Chem.* **1982**, *25*, 167–171.
[22] L. Jin, N. Legros, G. Leclercq, I. R. Hardcastle, M. Jarman, *Cancer Chemother. Pharmacol.* **1998**, *41*, 339–342.
[23] a) S. Top, A. Vessières, G. Leclercq, J. Quivy, J. Tang, J. Vaissermann, M. Huché, G. Jaouén, *Chem. Eur. J.* **2003**, *9*, 5223–5236; b) E. Hillard, A. Vessieres, L. Thouin, G. Jaouen, C. Amatore, *Angew. Chem. Int. Ed.* **2006**, *45*, 285–290; *Angew. Chem.* **2006**, *118*, 291–296.
[24] K. L. Vine, J. M. Locke, M. Ranson, S. G. Pyne, J. B. Bremner, *J. Med. Chem.* **2007**, *50*, 5109–5117.
[25] J. E. McMurry, *Acc. Chem. Res.* **1983**, *16*, 405–411.
[26] A. K. Sharma, K. Gowdahalli, J. Krzeminski, S. Amin, *J. Org. Chem.* **2007**, *72*, 8987–8989.
[27] a) J. Hoffmann, R. Bohlmann, N. Heinrich, H. Hofmeister, J. Kroll, H. Künzer, R. B. Lichtner, Y. Nishino, K. Parczyk, G. Sauer, H. Gieschen, H.-F. Ubrich, M. R. Schneider, *J. Natl. Cancer Inst.* **2004**, *96*, 210–218; b) B. M. Wittmann, A. Sherk, D. P. McDonnell, *Cancer Res.* **2007**, *67*, 9549–9560.

Received: January 1, 2014
Published online on January 31, 2014