Benzoxepin-Derived Estrogen Receptor Modulators: A Novel Molecular Scaffold for the Estrogen Receptor

David G. Lloyd,[†] Rosario B. Hughes,[‡] Daniela M. Zisterer,[†] D. Clive Williams,[†] Caterina Fattorusso,[§] Bruno Catalanotti,[§] Giuseppe Campiani,[||] and Mary J. Meegan*[,‡]

Department of Biochemistry, Trinity College Dublin, Dublin 2, Ireland, School of Pharmacy, Trinity College Dublin, Dublin 2, Ireland, Dipartimento di Chimica delle Sostanze Naturali, Universita' degli Studi di Napoli "Federico II", vi a D. Montesano, 49. 80131 Napoli, Italy, and Dipartimento Farmaco Chimico Tecnologico, Facolta' di Farmacia, Universita' degli Studi di Siena, Via Aldo Moro, 53100 Siena, Italy

Received June 2, 2004

Abstract: We present and examine the efficacy of a novel benzoxepin-based scaffold for modulation of the human estrogen receptor. Receptor tolerance of this new molecular scaffold is examined through presentation of experimentally determined antiproliferative effects on human MCF-7 breast tumor cells and measured binding affinities. The effect of functional group substitution on the benzoxepin scaffold is explored through a brief computational structure−activity relationship investigation with molecular simulation.

The estrogen receptor (ER) is a ligand-inducible nuclear receptor which mediates the physiological effects of binding estrogen steroid hormones.[1] Transcription, facilitated through the activation or inactivation of particular genes, is initiated through hormone binding to the ligand-binding domain of the receptor.[2] There remains considerable interest, both industrial and academic, in the modulation of the known isoforms of the ER, primarily due to their role in numerous disease states.[3,4] In any undertaking involving the preparation of novel ligands for the ER due consideration must be given to the eclectic liganding behavior of the target.[5] Tamoxifen **1**, ((*Z*)-1-[4-(2-dimethylaminoethoxy)phenyl]-1,2-diphenyl-1-butene), is a well established estrogen antagonist, and traditionally the design approach for the creation of new modulators has been one of preparing triarylethylene analogues of this parent structure (Figure 1).[6−8]

In recent years much attention has been given to the production of novel alternate scaffolds for estrogen receptor modulation through 'scaffold hopping' exercises to suggest novel structures de novo.[3,4,9,10] One proven approach to the design of novel estrogen antagonists is the inclusion of conformational restriction within the molecular skeleton so as to 'freeze' the geometry of the ligand in an appropriate antiestrogenic configuration.[11−17] Such an approach avoids any complication inherent in the production of isomerically pure tamoxifen-like compounds, particularly as the *E* or 'cis' geometry of tamoxifen is a known estrogen agonist. Stemming from our previous examinations of ER-ligand binding tolerance and behavior,[18,19] it was decided in this instance to employ a heterocyclic ring system as the central molecular scaffold which would afford the desired molecular geometry while avoiding the formation of mixed geometric isomers in synthesis. With this in mind we purported the rational design of a novel estrogen receptor modulator core containing a central seven-membered benzoxepin scaffold in which appropriate substitution would further challenge the liganding behavior of this therapeutically relevant target. A number of nonisomerizable heterocyclic tamoxifen-like compounds incorporating benzopyran structures (e.g. centchroman and related compounds) have been reported which display antiestrogenic activity.[4,11,15,20−22] Until the current work, benzoxepin systems have not been explored or demonstrated as viable ER modulating scaffolds.

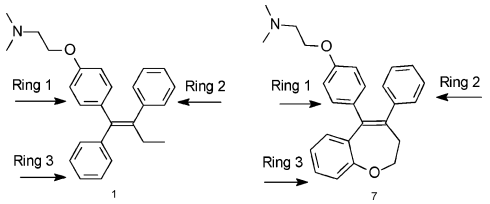
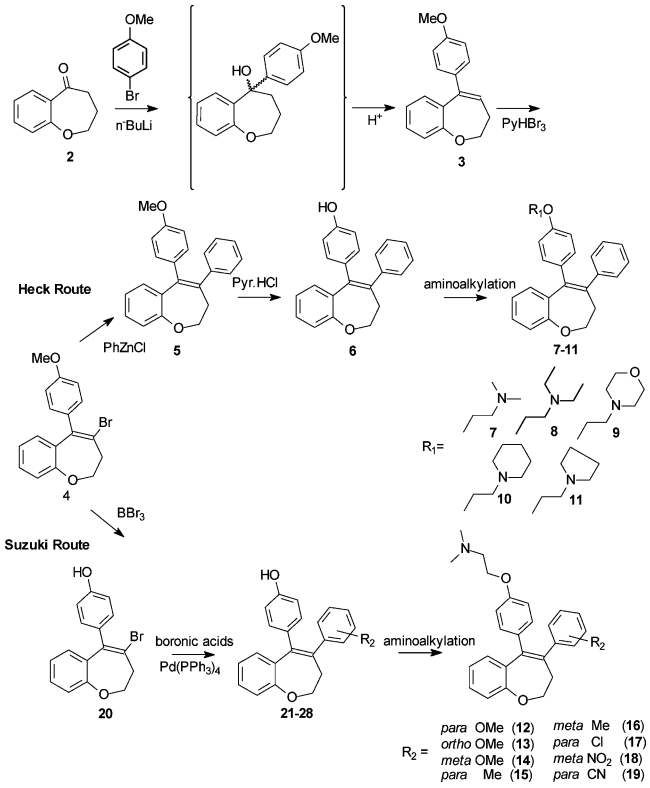


Figure 1. The parent tamoxifen and the novel benxoxepin scaffold.

Scheme 1. Route to Target Compounds via Suzuki or Heck Coupling Reactions



A facile multistep route to the desired benzoxepin scaffold (Scheme 1) proceeded via the synthesis of 2,3,4,5-tetrahydro-1-benzoxepin-5-one, **2**, utilizing polyphosphoric acid-mediated cyclization of 4-phenoxy-

* To whom correspondence should be addressed. Phone: +353-1-6082798; Fax: +353-1-6082973; e-mail: mmeegan@tcd.ie.
[†] Department of Biochemistry, Trinity College Dublin.
[‡] School of Pharmacy, Trinity College Dublin.
[§] Universita' degli Studi di Napoli "Federico II".
[||] Universita' degli Studi di Siena.

10.1021/jm0495834 CCC: $27.50
Published on Web 10/02/2004

Table 1. Mean IC_{50} Values of Compounds for Their Antiproliferative Effects on a Human MCF-7 Breast Cancer Cell Line

compd	R_1	R_2	IC_{50} (μM) ± SD
1	-	-	11.3 ± 0.1
7	dimethylamino	H	16.2 ± 6.8
8	diethylamino	H	14.2 ± 1.6
9	morpholinyl	H	7.6 ± 0.6
10	piperidyl	H	10.6 ± 0.6
11	pyrrolidinyl	H	6.1 ± 2.6
12	dimethylamino	*p*-OMe	11.1 ± 4.8
13	dimethylamino	*o*-OMe	11.7 ± 2.1
14	dimethylamino	*m*-OMe	56.6 ± 6.5
15	dimethylamino	*p*-Me	15.7 ± 4.7
16	dimethylamino	*m*-Me	33.6 ± 3.5
17	dimethylamino	*p*-Cl	18.3 ± 1.7
18	dimethylamino	*m*-NO_2	64.6 ± 1.5
19	dimethylamino	*p*-CN	74.3 ± 5.1

butyric acid.[23] **2** was arylated with 4-bromoanisole using *n*-butyllithium and dehydrated in situ with polyphosphoric acid to furnish the desired 5-(*p*-methoxyphenyl)-2,3-dihydro-1-benzoxepin core structure **3**. This compound was readily brominated under standard conditions with pyridine hydrobromide perbromide[24] to yield 4-bromo-5-(4-methoxyphenyl)-2,3-dihydro-1-benzoxepin, **4**, a key intermediate in the synthetic strategy. Intermediate **4** was converted to the target compounds through one of two metal-mediated coupling strategies employing either Heck or Suzuki reactions. Compounds **8**−**12** were afforded using the Heck (Zn-mediated) coupling reaction.[25] Phenylzinc chloride was employed to introduce an aromatic ring, forming 5-(4-methoxyphenyl)-4-phenyl-2,3-dihydro-1-benzoxepin, **5**, which was subsequently demethylated using pyridine hydrochloride to yield the phenolic species 5-(4-hydroxyphenyl)-4-phenyl-2,3-dihydro-1-benzoxepin, **6**. Basic side chains were readily introduced to **6** using standard alkylating conditions to yield the final products **7**−**11**. The strategy for production of what we term *ring-2 probes* (**12**−**19**), each a variation on the parent benzoxepin compound **7**, was facilitated through Suzuki (Pd-mediated) coupling reactions utilizing intermediate **4** and appropriate boronic acids.[26] Demethylation to **20** was readily afforded by BBr_3, and the demethylated product was treated with the boronic acid of choice, catalyzed by $Pd(PPh_3)_4$ so as to afford a phenolic product (**21**−**28**) which was readily alkylated under basic conditions to afford the target compounds **12**−**19** in moderate to high yields. Boronic acids prepared for use in the Suzuki coupling reactions were accessible using established methods.[27,28]

The individual IC_{50} results obtained for inhibition of proliferation in an ER-expressing (ER-dependent) human breast tumor (MCF-7) cell line are recorded in Table 1. Activity is compared to that of a tamoxifen reference standard.

The data may be examined in two groupings, compounds **7**−**11**, representing the novel benzoxepin scaffold with variations in the basic side chain and compounds **12**−**19** examining the effects of aromatic 'ring 2' substitutions on the activity of compounds derived from the parent compound **7**. It can be seen from side chain variation in compounds **7**−**11** that the bulkier heterocyclic basic side chains (pyrrolidinyl, morpholinyl, piperidyl) impart increased activity, with the pyrrolidinyl side-chain-containing compound **11** exhibiting the most potent IC_{50} of the five types investigated, echoing earlier findings by this group.[19]

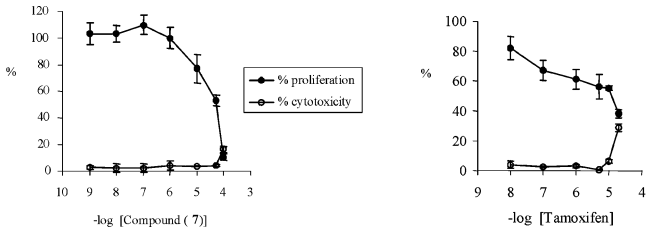


Figure 2. Percent cytotoxicity and percent inhibition of proliferation recorded in MCF-7 cells upon treatment with varying concentrations of compound **7** and tamoxifen controls.

Compounds were concurrently tested to assess the extent of their cytotoxicity using a standard LDH assay and demonstrated low cytotoxicity, indicating their mode of action to be cytostatic rather than cytotoxic. Cytotoxic-induced antiproliferation effects for the parent benzoxepin compound **7** rise to only a maximum of 11% at a 100 μM testing (Figure 2) concentration whereas the level observed for tamoxifen in the same system is approximately 29% at a testing concentration of 20 μM.

To confirm our hypothesis that these compounds were acting through the estrogen receptor, an initial binding investigation[19,29,30] was carried out using compounds **7** and **15** as representative candidates. The study indicates the compounds are competitive antagonists with moderate binding affinity for the ER (binding K_i 3.2 ± 2.1 μM (**7**) and 828 ± 106 nM (**15**)), marginally weaker than that measured for tamoxifen (**1**) and the structurally related benzocycloheptenes.[14] Preliminary rigid[31] and flexible computational docking studies[32] performed on the parent benzoxepin compound **7** indicate it to orient in a typical 'antiestrogenic' binding mode within the ligand binding domain of the human ER alpha (PDB entry 3ERT).[4] In vivo metabolic activation of tamoxifen through para-hydroxylation in ring 3 is well-known to increase antiproliferative potency and receptor binding affinity of the parent through hydrogen bonding interactions with Glu 353 and Arg 394.[4] Similar metabolic activation is computationally predicted for the benzoxepin system.

We therefore focused our initial investigations on ring 2 variation in the benzoxepin system, paying particular attention to the predicted interactions with His 524. Of particular interest to us was the potential for structure−activity relationship exploration invoked through variation in the aromatic substitution pattern in ring 2 of the structure—specifically the marked drop in compound efficacy when moving from ortho → meta position with methoxy substituents in compounds **13** and **14**, and also the low activity recorded for **19** with its *p*-cyano substitution, given that the ER is usually tolerant of polar functional groups in this position—natural estrogens and some synthetic ER modulators (e.g. raloxifene) contain hydroxyl functionality here. To facilitate an initial investigation of these phenomena, a flexible docking study of these ER−ligand systems was undertaken[33] in which both an area within the receptor and the ligand were treated as fully flexible entities during the docking procedure (for details, see Supporting Information). Figure 3 illustrates the predicted binding mode for the parent benzoxepin scaffold-containing ligand, **7**, and indicates it to bind in an antiestrogenic

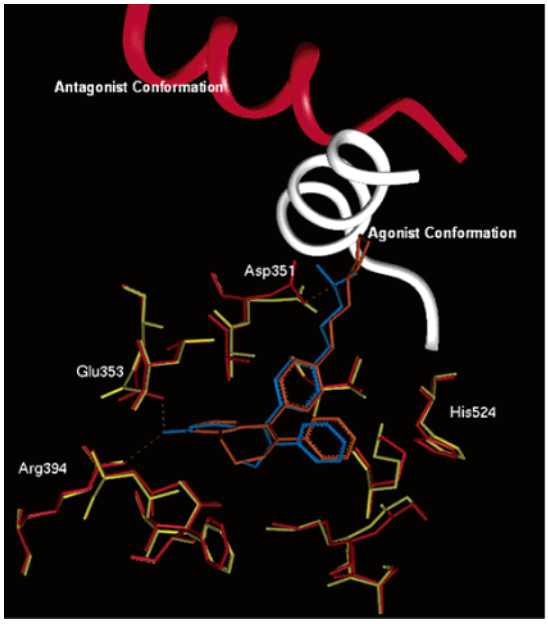


Figure 3. Docking orientation and predicted receptor interactions for the parent benzoxepin structure **7** (brown ligand/yellow protein) compared to the crystal solution for hydroxytamoxifen (blue/red). The relative locations of helix 12 on agonist (white) and antagonist (red) liganding are illustrated for comparative purposes. (Hydrogens are omitted for clarity, and H-bond interactions are illustrated as broken lines.)

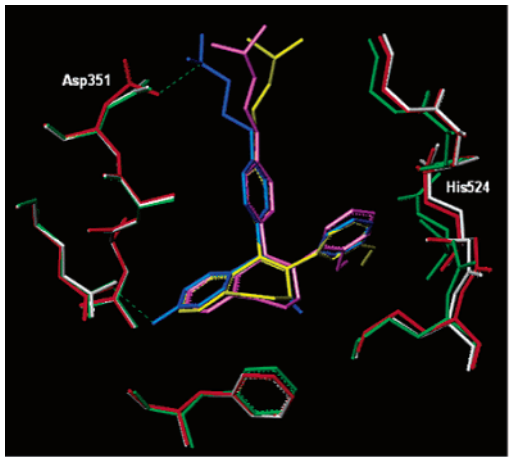


Figure 4. Comparative docking orientations and predicted receptor interactions for **13** (pink)/ERα(white) and **14** (yellow)/ERα(green) compared with the crystal solution for hydroxytamoxifen (OHT(blue)/ERα(red) (PDB ID 3ERT).

manner within the ligand binding domain of the human estrogen receptor alpha. The scaffold is well oriented in the binding site and analysis of the pose immediately suggests the potential for optimization through para hydroxylation (as in the case of tamoxifen to hydroxytamoxifen activation).

Figure 4 illustrates a superimposition of the crystal (OHT/ERα) and docked **13** and **14** complex structures. It can be clearly seen through the disturbance of the backbone region in the green rendered solution (corresponding to liganding of **14**) relative to those of the cocrystal structure (red backbone) and docked structure for **13** (white backbone) that the receptor tolerance for aromatic substitution on ring 2 of the benzoxepin scaffold is lost when moving from ortho (**13**) to meta (**14**) substituents. Such substitution induces perturbation of

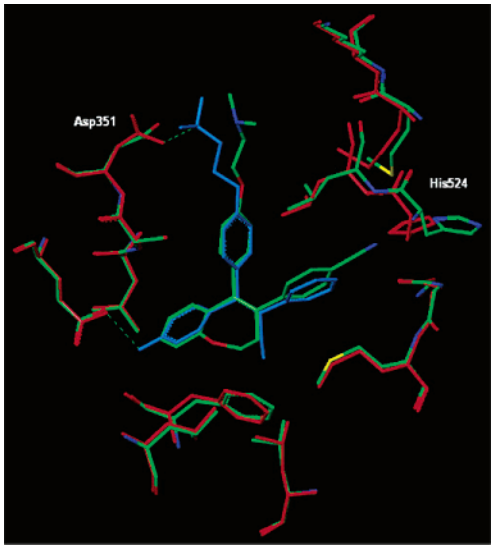


Figure 5. Comparative docking orientations and predicted receptor interactions for **19** (colored by atom type) and the crystal solution for hydroxytamoxifen OHT(blue)/ERα(red) (PDB ID 3ERT).

the ERα backbone, decreasing the overall stability of the complex in the region of His 524 (most likely through steric bulk clashing) and is accompanied by a marked drop in biological efficacy (Table 1).

When we examine Figure 5, which illustrates a superimposition ofthe crystal complex with calculated structure of **19**/ERα docked complex, a similar perturbation of the receptor backbone becomes apparent. The *p*-cyano group, the only para substitution explored for ring 2 in this first series of modulators which is tied to significantly decreased biological activity (Table 1), again induces a predicted receptor backbone perturbation by steric and electronic unfavorable contacts—this is very clearly seen through significant side chain and backbone displacement in the region of His 524 in the ER binding pocket on liganding this compound.

These data from docking studies will be used in future optimization of the benzoxepin scaffold to enhance potency through the next iteration of synthesis on these ligands.

In summary, a novel molecular scaffold for modulation of the ER, the conformationally restrained benzoxepin system, is presented. The compounds prepared deviate from the traditional triphenylethylene structure of tamoxifen analogues through incorporation of an isomerically constraining heterocyclic ring system, and through variation in the nature of basic side-chain systems and the introduction of aromatic ring substituents. These compounds have demonstrated competitive ER binding and exhibit antiestrogenic potency through their inhibition of the proliferation of human MCF-7 breast cancer cells.

In particular, we note that compound members with the unsubstituted benzoxepin-containing ER scaffold (**7**−**11**) exhibit similar, and better, antiproliferative potencies than tamoxifen with lower inherent cytotoxicity, indicating the potential for this novel scaffold class and their activated metabolites in future investigations aimed at the identification of tissue and subtype selective estrogen receptor modulators.

Acknowledgment. This work was supported through funding from BioResearch Ireland (now the Enterprise Ireland Biotechnology Directorate) 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 equipment funding from the Wellcome Trust. D.G.L. and M.J.M. are most grateful to Enterprise Ireland's International Collaboration Scheme for facilitating an inter-institutional research visit during the course of this program.

Supporting Information Available: Experimental details, analytical and spectral data for final compounds, and biochemical and computational procedures employed. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Katzenellenbogen, B. S. Estrogen receptors: bioactivities and interactions with cell signaling pathways. *Biol. Reprod.* **1996**, *54*, 287−293.
(2) Beato, M.; Sanchez-Pacheco, A. Interaction of steroid hormone receptors with the transcription initiation complex. *Endocr. Rev*. **1996**, *17*, 587−609.
(3) Lloyd, D. G.; Meegan, M. J. Recent advances in estrogen receptor antagonists. *IDrugs* **2000**, *3*, 343−353.
(4) Meegan, M. J.; Lloyd, D. G. Advances in the Science of Estrogen Receptor Modulators. *Curr. Med. Chem.* **2003**, *10*, 181−210.
(5) Shiau, A. K.; Barstad, D.; Loria, P. M.; Cheng, L.; Kushner, P. J.; Agard, D. A.; Greene, G. L. The structural basis of estrogen receptor/coactivator recognition and the antagonism of this interaction by tamoxifen. *Cell* **1998**, *95*, 927.
(6) Lerner, L. J.; Jordan, V. C. Development of antiestrogens and their use in breast cancer: Eighth Cain Memorial Award Lecture. *Cancer Res*. **1990**, *50*, 4177−4189.
(7) Shani, J.; Gazit, A.; Licshitz, T.; Biran, S. Synthesis and receptor binding affinity of fluorotamoxifen, a possible estrogen-receptor imaging agent. *J. Med. Chem.* **1985**, *28*, 1504−1511.
(8) Itami, K.; Kamei, T.; Yoshida, J. Diversity-oriented synthesis of tamoxifen-type tetrasubstituted olefins. *J. Am. Chem. Soc.* **2003**, *125*, 14670−14671.
(9) Lloyd, D. G.; Buenemann, C. L.; Todorov, N. P.; Manallack, D. T.; Dean, P. M. Scaffold hopping in de novo design − ligand generation in the absence of receptor information. *J. Med. Chem.* **2004**, *47*, 493−496.
(10) Schmidt, J. M.; Mercure, J.; Tremblay, G. B.; Page, M.; Kalbakji, A.; Feher, M.; Dunn-Dufault, R.; Peter, M. G.; Redden, P. R. De novo design, synthesis, and evaluation of novel nonsteroidal phenanthrene ligands for the estrogen receptor. *J. Med. Chem.* **2003**, *46*, 1408−1418
(11) Wolf, D. M.; Langan-Fahey, S. M.; Parker, C. J.; McCague, R.; Jordan, V. C. Investigation of the mechanism of tamoxifen-stimulated breast-tumor growth with nonisomerizable analogues of tamoxifen and metabolites. *J. Natl. Cancer Inst.* **1993**, *85*, 806−812.
(12) Leichtl, S.; von Angerer, E. 2-Phenylbenzo[*b*]thiophene-based antiestrogens with mammary tumour inhibiting activity. *Arch. Pharm.* **1998**, *331*, 283−289.
(13) Teo, C. C.; Kon, O. L.; Sim, K. Y.; Ng, S. C. Synthesis of 2-(para-chlorobenzyl)-3-aryl-6-methoxybenzofurans as selective ligands for antiestrogen-binding sites − effects on cell proliferation and cholesterol synthesis. *J. Med. Chem.* **1992**, *35*, 1330−1339.
(14) McCague, R.; Kuroda, R.; Leclercq, G.; Stoessel, S. Synthesis and Estrogen Receptor Binding of 6,7-Dihydro-8-phenyl-9-[4-[2-(dimethylamino)ethoxy]phenyl]-5H-benzocycloheptene, a Non-isomerisable Analogue of Tamoxifen. X-ray Crystallographic Studies. *J. Med. Chem.* **1986**, *29*, 2053−2059.
(15) McCague, R.; LeClercq, G.; Jordan, V. C. Nonisomerizable Analogues of (Z)- and (E)-4-Hydroxytamoxifen. Synthesis and Endocrinological Properties of Substituted Diphenylbenzocycloheptenes. *J. Med. Chem.* **1988**, *31*, 1285−1290.
(16) McCague, R. An analogue of the antioestrogen tamoxifen of sufficient rigidity to exist as distinct enantiomers: synthesis and conformational dynamics studies. *Tetrahedron Asymm.* **1990**, *11*, 97−110.
(17) Henke, B. R.; Consler, T. G.; Go, N.; Hale, R. L.; Hohman, D. R.; Jones, S. A.; Lu, A. T.; Moore, L. B.; Moore, J. T.; Orband-Miller, L. A.; Robinett, R. G.; Shearin, J.; Spearing, P. K.; Stewart, E. L.; Turnbull, P. S.; Weaver, S. L.; Williams, S. P.; Wisely, G. B.; Lambert, M. H. A new series of estrogen receptor modulators that display selectivity for estrogen receptor beta. *J. Med. Chem.* **2002**, *45*, 5492−5505.
(18) Meegan, M. J.; Hughes, R. B.; Lloyd, D. G.; Williams, D. C.; Zisterer, D. M. Ethyl side-chain modifications in novel flexible antiestrogens − design, synthesis and bilogical efficacy in assay against the MCF-7 breast tumor cell line. *Anti-Cancer Drug Des.* **2001**, *16*, 57−69.
(19) Meegan, M. J.; Hughes, R. B.; Lloyd, D. G.; Williams, D. C.; Zisterer, D. M. Flexible estrogen receptor modulators − design, synthesis and antagonistic effects in human MCF-7 breast cancer cells. *J. Med. Chem.* **2001**, *44*, 1072−1084.
(20) Singh, M. M. Centchroman, a selective estrogen receptor modulator, as a contraceptive and for the management of hormone related disorders. *Med. Res. Rev.* **2001**, *21*, 302−347.
(21) Catherino, W. H.; Jordan, V. C. Increasing the number of tandem estrogen response elements increases the estrogenic activity of a tamoxifen analogue. *Cancer Lett.* **1995**, *92*, 39−47.
(22) Blizzard, T. A.; Morgan, J. D.; Mosley, R. T.; Birzin, E. T.; Frisch, K.; Rohrer, S. P.; Hammond, M. L. 2-Phenylspiroindenes: A Novel Class of Estrogen Receptor Modulators (SERMs). *Bioorg. Med. Chem. Lett.* **2003**, *13*, 479−483.
(23) Tandon, V. K.; Khanna, J. M.; Arand, N.; Chandra, A. Phenethylamine in a rigid framework: synthesis, stereochemistry and reactions of cis and trans-4-amino-2,3,4,5-tetrahydro-1-benzoxepin-5-ols and their derivatives. *Tetrahedron* **1990**, *46*, 2871−2882.
(24) Feiser, L. F.; Feiser, M. *Reagents for Organic Chemistry*; Wiley & Sons: London, 1987; Vol. 1, pp 967−970.
(25) Miyaura, N.; Suzuki, A. Palladium-catalyzed cross-coupling reactions of organoboron compounds. *Chem. Rev.* **1995**, *95*, 2457−2483.
(26) Brown, A. G.; Crimmin, M. J.; Edwards, P. D. Application of the Suzuki biphenyl synthesis to the natural products biphenomycin and vancomycin, *J. Chem. Soc., Perkin Trans. 1* **1992**, 123−130.
(27) Morgan, J.; Hambley, T. W.; Pinley, J. T. Mechanism of arylation of nucleophiles by aryllead triacetates. Part 3. Concerning their reaction with phenols and X-ray molecular structure of p-methoxyphenyllead triacetate. *J. Chem. Soc., Perkin Trans. 1* **1996**, 2173−2176.
(28) Cundy, D. J.; Forsyth, S. A. Cupric acetate mediated N-arylation by arylboronic acids: a preliminary investigation into the scope of application. *Tetrahedron Lett.* **1998**, *39*, 7979−7982.
(29) Yang, D. J.; Wallace, S.; Tansey, W.; Wright, K. C.; Wuang, L. R.; Tilbury, R. S.; Diego, I.; Lim, J. L.; Emran, A. M.; Kim, E. E. Synthesis and in vitro receptor-binding studies of fluorotamoxifen analogues. *Pharm. Res.* **1991**, *8*, 174−177.
(30) Fishman, J. H. Stabilization of estradiol-receptor complexes by elimination of cytosolic factors. *Biochem. Biophys. Res. Commun.* **1983**, *110*, 713−718.
(31) Sobolev, V.; Wade, R. C.; Vriend, G.; Edelman, M. Molecular docking using surface complementarity. *Proteins* **1996**, *25*, 120−129.
(32) Rarey, M.; Kramer, B.; Lengauer, T.; Klebe, G. A fast flexible docking method using an incremental construction algorithm *J. Mol. Biol.* **1996**, *261*, 470−489.
(33) Discover module in InsightII, Accelrys Inc., 2000.

JM0495834