Published in final edited form as:

J Med Chem. 2017 July 27; 60(14): 6321–6336. doi:10.1021/acs.jmedchem.7b00585.

Adamantyl Antiestrogens with Novel Side Chains Reveal a Spectrum of Activities in Suppressing Estrogen Receptor (ER)-Mediated Activities in Breast Cancer Cells

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Abstract

To search for new antiestrogens more effective in treating breast cancers, we explored alternatives to the acrylic acid side chain used in many antiestrogens. To facilitate our search, we used a simple adamantyl ligand core that by avoiding stereochemical issues enabled rapid synthesis of acrylate ketone, ester, and amide analogs. All compounds were high affinity estrogen receptor- α (ER α) ligands, but displayed a range of efficacies and potencies as antiproliferative and ER α -downregulating agents. There were large differences in activity between compounds having minor structural changes, but antiproliferative and ER α -downregulating efficacies generally paralleled one another. Some compounds with side chain polar groups had particularly high affinities. The secondary carboxamides had the best cellular activities, and the 3-hydroxypropylamide was as efficacious as fulvestrant in suppressing cell proliferation and gene expression. This study has produced structurally novel antiestrogens based on a simple adamantyl core structure with acrylate side chains optimized for cellular antagonist activity.

TOC Graphic

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Supporting Information Availability

Supporting Information. ¹H and ¹³C NMR spectra, normal and reversed phase HPLC chromatograms, list of molecular formula strings.

Conflict of interest:

JAK – consultant at Radius Health, Inc.

Nothing to Disclose: JM, VSG, AS, YZ, YZ, ML, PG, SHK, KEC, KWN, BSK. JAK is a consultant at Radius Health, Inc.

Keywords

estrogen receptor; novel antiestrogens; breast cancer; endocrine therapy resistance

INTRODUCTION

Many patients with estrogen receptor alpha (ER α) positive breast cancers that had been treated effectively with tamoxifen or aromatase inhibitors may later experience a recurrence of their breast cancer in a form that has become resistant to these treatments. These observations clearly highlight the need for developing new antiestrogens that are effective on this form of endocrine therapy-resistant cancer.^{1, 2} About one-third of these recurrent breast cancers harbor activating mutations that convey constitutive activity and relative resistance to antiestrogens, whereas many contain wild type ER (WT-ER α) but have undergone cellular changes that make cells less responsive to certain standard-of-care treatments.³⁻⁸ A clearer understanding of the molecular mechanisms by which antiestrogens are able to suppress the agonist activity of ER α is needed so that better compounds can be developed. It is likely that new agents that are more potent and efficacious inhibitors of WT-ER α will also show greater effectiveness in inhibiting the growth of breast cancers driven by ER α with activating mutations that display resistance to current treatments.

Conceptually, antiestrogens can be thought to consist of a ligand *core element* that is accommodated within the ligand-binding pocket and provides robust binding affinity, onto which is appended a *side chain* that extends outward from the ligand binding domain (LBD) and whose function is to disrupt the agonist activity of the receptor by mispositioning helix-12 (h12) of the LBD in various ways.⁹ Historically, remarkably few side chains have been studied thoroughly. Antiestrogens of the selective ER modulator (SERM) class, like tamoxifen, raloxifene, lasofoxifene, and bazedoxifene, have structurally distinct aromatic-rich, non-steroidal *cores* but very similar positively charged basic *side chains* (Figure 1). By forming strong charge-charge interactions with D351, these basic side chains displace h12, causing it to be repositioned into the hydrophobic groove where coactivators bind in ER-agonist complexes. An alternative antiestrogen design, first exemplified in 1994 on the tamoxifen core structure in compounds from Glaxo-SmithKline (GSK), GW-5638 and GW-7604, has an acrylic acid as a functional side chain (Figure 1).^{10, 11} This side chain, which also moves h12 to block the coactivator binding groove, has been replicated without any change in two orally active antiestrogens currently in clinical development, GDC-0810 (32) and AZD-9496 (33) (Figure 1).^{12, 13}

In addition, some antiestrogens regulate ER α levels: those that downregulate ER α , termed SERDs (for selective ER downregulators), were initially exemplified by fulvestrant, a steroid onto which is appended a long, largely hydrophobic side chain at the 7 α position, but SERD activity is also found with the GSK, AZD, and GDC compounds mentioned above (Figure 1). Because of its very poor oral bioavailability, fulvestrant needs to be administered by painful intramuscular injection, and even this does not provide sufficiently high blood levels of drug to fully occupy ER in tumors.¹⁴⁻¹⁶ These pharmaceutical problems are avoided by the GDC and AZD compounds (**32** and **33**, respectively) because they are orally active and can be dosed at higher levels, providing high internal exposure to the drug.^{12, 13, 17} Importantly, GW-7604 and the newer generation SERDs proved efficacious in tamoxifen-resistant models,¹⁷ suggesting that altering the side chain provides a route to altering the efficacy profile of antiestrogen treatments. Nevertheless, despite their promise so far, it is not yet clear that these newer antiestrogens will have a safety profile consistent with their widespread use in the treatment of breast cancers and prevention of disease recurrence.¹⁸

Because overcoming the development of resistance to endocrine therapies in breast cancers is a large unmet clinical need, we were motivated to explore a broad range of side chain structures in antiestrogens in a search for new compounds that might have high potency and efficacy as antiproliferative agents and possibly as ER α downregulators. To expedite our investigation, we chose to use a structurally and stereochemically simple, rigid core unit, derived from the cyclofenil class of ER ligands, but having the bridged tricyclic adamantane system in place of the cyclohexane ring of cyclofenil.¹⁹ This adamantane-based ligand core and its evolution are shown in Figure 1, together with other compounds for comparison. In place of the acrylic acid side chain, we explored a considerable number and variety of carboxylate analogs (ketones, esters, and amides). Our side chain design was influenced in part by a crystal structure of GW-5638 bound to the ER LBD showing that the acidic side chain interaction with the N-terminus of h12 was assisted by a structural water molecule (Figure 2).²⁰ In various other protein-small molecule systems, inhibitors that were designed to include a polar atom that replaced a structural water molecule had particularly elevated affinity.²¹⁻²³ Thus, to explore this potential opportunity, we included oxygen or nitrogen atoms in the side chain of some of our compounds.

For each new compound, we evaluated ER α binding affinity, and efficacy in suppressing proliferation of ER-positive breast cancer cells and in downregulating ER α levels. We also explored the potency of the most efficacious compounds in ER target gene regulation. In the process, we uncovered some rather striking structure-activity relationships (SARs) whereby small structural changes resulted in large changes in activity, and in a number of cases, the inclusion of a polar atom at the terminus of the side chain gave a substantial boost to binding affinity. With this set of compounds, we also made comparisons of the degree to which antiproliferative activity is related to ER α -downregulating activity. The best members of this series are as efficacious as fulvestrant in suppressing breast cancer cell proliferation and are nearly as complete in downregulating ER α levels, and they have low nanomolar potencies for both activities.

RESULTS

Compound Selection and Synthesis

The ligand core we selected is based on the well-known SERM cyclofenil in which we have replaced the monocyclic cyclohexane with the much bulkier tricyclic adamantane, generating a series of ER ligands that we term “adamantyls” (Figure 1). We had identified this adamantane-based core from an earlier exploration of substitutions for the cyclohexane ring of cyclofenil.¹⁹ Major advantages of this structurally simple adamantane-based core are its planar symmetry, which avoids problems with geometric isomerism (present in the antiestrogens GW-5638/7604 and in **32**), and its lack of chirality and stereoisomers, which avoids stereochemical issues (present in **33**) that can complicate synthesis and purifications. In addition, ligands having the 4,4'-dihydroxybenzhydrylidine substructure (two *p*-hydroxyphenyl groups attached to one end of a double bond) almost universally have very high binding affinities for ER α ;^{19, 24} the parent bisphenolic adamantyl-core, in fact, has an affinity ca. 3 fold higher than that of estradiol (E2), which corresponds to a *K*_i value of 70 pM.¹⁹ The issue regarding geometric isomerism of hindered 4-hydroxystilbene compounds such as GW-7604 is no small matter because there are, in fact, no reports that the presumed active, E isomer has ever been isolated in isomerically pure form, and consequently the E-form of GW-7604 has been tested only as an E/Z mixture.^{25, 26} Other non-steroidal ER ligands having similar 4-hydroxystilbene substructures, such as hydroxytamoxifen and diethylstilbestrol, are known to undergo facile, spontaneous cis-trans isomerism and hence do not have a stable stereoisomeric form in vivo.²⁷

Our selected adamantyl core system **3** was easily prepared by a McMurray coupling between 4,4'-dihydroxybenzophenone (**1**) and 2-adamantanone (**2**) (Scheme 1A).²⁵ The monotriflate **4** could be obtained efficiently by treatment with *p*-nitrophenyl trifluoromethanesulfonate,²⁸ and then various acryloyl groups were appended by a Heck reaction.²⁵ The ketone **6**, the various esters **7-9**, **11** and **13**, as well as the nitrile **14**, sulfonamide **15**, and simple carboxamide **16**, were prepared in one step by a Heck reaction with the corresponding acrylate derivative (Scheme 1A). The acrylic acid **5** was obtained by hydrolysis of the ethyl ester **8**; ester **10** was obtained from acrylic acid **5** by reaction with 1-bromo-6-methylheptane, and ester **12** by esterification of acrylic acid **5** with propane-1,3-diol (Scheme 1B).²⁹

Reaction conditions and reagents: a) TiCl₄/Zn, THF, reflux; b) K₂CO₃, DMF, rt; c) Pd(PPh₃)₂Cl₂, TEA, DMF, 120 °C; d) KOH, CH₃OH; e) 1-bromo-6-methylheptane, Cs₂CO₃, DMF, rt; f) (i) Isobutyl chloroformate, DMAP, Et₃N, DCM, propane-1,3-diol, -15 °C to 0 °C, 3 h; (ii) piperidine, 1 h, rt.

The secondary amides **17-23**, **24a-26a**, **27-28** and tertiary amides **29-31** were prepared by HATU-mediated coupling³⁰ of the primary (Scheme 2A) and secondary amines (Scheme 2B),³¹ respectively. Boc deprotection of **24a-26a** with TFA afforded secondary amides **24-26** (Scheme 2A). With the acrylic acid core structure **5**, the couplings with secondary amines required somewhat elevated temperatures (50 °C) (Scheme 2B). To explore the possible role of a water molecule in mediating the side chain-h12 interaction, we prepared a

few isostructural esters and amides in which the side chains had the same number of carbon atoms but were terminated with either a hydrogen atom or an OH or NH₂ group.

Reaction conditions and reagents: a) HATU, DMF, rt; b) TFA/DCM, 0° C; c) HATU, DMF, 50 °C.

ER α Binding Affinities

The affinities of all compounds for purified, full-length, human ER α were determined using a competitive radiometric binding assay, with [³H]estradiol as tracer.³² The affinities are given as relative binding affinity (RBA) values (E2 = 100), and they are presented in Table 1. By selecting the high affinity adamantyl core as the building block for our new antiestrogens, we have ensured that essentially all of these compounds bind with high affinities; most of the RBA values are in the range of 10-60, and some rival (**26** (62) and **16** (71)) or even exceed (**20** (109) and **31** (115)) the affinity of E2 (RBA = 100). Considering that the K_d of E2 for ER α is 0.2 nM, all of these compounds bind to ER α with affinities in the single digit nanomolar range or below.

One structure-affinity trend we explored was related to the potential replacement of a structural water linking the side chain to the N-terminus of h12 that was noted on one crystal structure with GW-5638 (Figure 2); this involved the replacement of a chain-terminal hydrogen atom (Figure 3, open bars) with an OH or NH₂ polar group (Figure 3, stippled or dark bars). In the amide and ester series where direct comparisons can be made between isostructural alkyl vs. hydroxyl- or amino-alkyl side chains, this addition of a terminal polar group provides a substantial and consistent (2 to 5-fold) improvement in affinity, at least in the 2-4 methylene group range.

Initial Screen of Antiproliferative and ER α Downregulating Activities

Because of the large number of compounds evaluated, we initially screened them in ER-positive MCF-7 breast cancer cells for their suppression of cell proliferation (CP) and for their effects on ER α levels, using a single, saturating concentration of 3 μ M. Relationships between the results of these initial screening assays and compound structures are displayed graphically in a series of star plots (Figures 4 A-C).³³ On these plots, the levels of suppression of proliferation and downregulation of ER α , plotted on percent scales, are displayed in a radial manner, each spoke of which is associated with the structure of a compound. The antiestrogen and SERD, fulvestrant (Fulv), is included for comparison in each star plot. The activity in suppressing cell proliferation (CP, shown in blue) is given as percent of vehicle control; maximum suppression is ca. 20% (corresponding to essentially no increase in cell number after 6 days). For ER α levels (ER α , shown in red), 20% is the ER α level after 24 h with 3 μ M fulvestrant treatment, with 100% being the ER α level in vehicle control cells. Representative compounds were also screened in ER α -negative MDA-MB-231 breast cancer cells, and they had no effect on proliferation of cells lacking ER α (not shown).

Comparison of the Antiproliferative and ER α Downregulating Efficacies of Core Structures and of Ketone, Ester, and Other Variant Side Chains—A

comparison between our parent adamantyl acrylic acid analog **5** and the GSK antiestrogen GW-7604 (Figure 4A) indicates that our parent compound has somewhat better ER downregulating and antiproliferative efficacies.

Among the side chains, the one ketone we prepared **6** had some efficacy in terms of both antiproliferative and ER downregulating activities. Because its efficacy was less than that found for some of the esters and amides (see below and Figure 4B), further ketone analogs were not explored in this study. The series of acrylate esters **7-10** and ω -hydroxyl esters **11-13** showed a broad span in both dimensions of efficacy. The best in terms of antiproliferative activity was **9** at 40%, but none were better than **5**. The ER α downregulating activity of this series varies a great deal, with the butyl **9**, iso-octyl **10**, and hydroxybutyl **13** esters being good to very good, but the methyl and ethyl esters **7** and **8**, and the ω -hydroxy esters **11** and **12** were non-efficacious in reducing cell ER α levels.

The two other variant side chains shown in this star plot again reveal marked differences in both antiproliferative and ER downregulating efficacies. The acrylonitrile **14** is neither anti- or pro-proliferative, with proliferation matching that of vehicle control; nevertheless, it is very effective in reducing the cellular ER α level. The propyl sulfonamide **15** is only modest as an ER downregulator and an antiproliferative agent.

Comparison of the Antiproliferative and ER α Downregulating Activities of Various Carboxamide Analogs—Because we prepared a significant number of adamantyl carboxamides, their activities are displayed in two separate star plots (Figures 4B and C). The amides presented in Figure 4B are all secondary amides except for the primary amide **16**. The alkyl amides **17-19** and ω -hydroxyalkyl amides **20-23** all have relatively comparable antiproliferative efficacies, with the best two (**21** and **23**) being equivalent to that of the parent acid **5**; the ER downregulating activities of these two are also good and similar to that of the parent **5**. (Further distinction among some of these compounds based on their potencies in dose-response assays is shown below in Figures 6 and 7.) Much more variable are the activities of the primary amide **16**, the ω -aminoalkyl amides **24-26**, and the other amides **27** and **28**. Of these, only the 3-aminopropyl amide **25** rivals the antiproliferative efficacies of the amides discussed above; both **25** and the butyl homolog **26** have good ER α downregulating activity. Least good are the primary amide **16** and the trifluoroethyl amide **28**. The compounds shown in Figure 4B show overall quite a detailed concordance between antiproliferative and ER α -downregulating efficacies, more so than those in Figure 4A.

In evaluating the amides, we noted a marked difference in both antiproliferative and ER α downregulating efficacies of secondary vs. tertiary amides. Rather striking comparisons between sets of related compounds in these two structural series are illustrated in Figure 4C. The good efficacies of the N-butyl secondary amide **18** can be contrasted with the very poor efficacies of the N-methyl tertiary amide analog **29**. Similarly, the impressive efficacies of the hydroxyethyl secondary amide **20** are markedly better than those of the N-methyl analog **30** and the bis-hydroxyethyl analog **31**. This last compound, the symmetrically substituted tertiary amide **31**, has the highest ER binding affinity of the compounds we have studied, yet it is relatively unimpressive in both antiproliferative and ER downregulating activities. In another comparison among secondary amides, the 2-aminoethyl amide **24** has better

antiproliferative and ER α downregulating efficacies than both the N,N-dimethyl aminoethyl amide **27** and the trifluoroethyl amide **28**. As was the case in Figure 4B, the compounds in Figure 4C show a good concordance between antiproliferative and ER α downregulating activities.

Antiproliferative and ER α Downregulating Potencies, and Suppression of Estrogen Target Gene Expression by the Most Efficacious Adamantyl Compounds—

Our initial examination of compounds in the adamantyl series was based on their *efficacies* at a single, high concentration of 3 μ M. Therefore, to identify from among the best compounds those that would also be most *potent* in terms of their antiproliferative and ER α downregulating activities, we conducted dose-response assays on a number of compounds selected from those that had excellent anti-proliferative *efficacies*, including a few others for comparison.

As seen in Figure 5, the hydroxypropyl **21** (IC₅₀ = 1.9 nM) and hydroxyethyl **20** (IC₅₀ = 2.4 nM) amides are clearly more potent than the parent carboxylic acid **5** (IC₅₀ = 31 nM), with the hydroxypropyl analog **21** being slightly better than the hydroxyethyl analog **20** both in terms of potency and in maximum efficacy (24% vs 30%, see also Figure 4B). Earlier, we noted the markedly greater antiproliferative efficacy of the secondary vs. tertiary amides; this is evident here from the dose-response curves showing that the two tertiary amide analogs of **20**, the N-methyl **30** and the bis-hydroxyethyl **31** compounds, have limited antiproliferative efficacy despite having good potencies (IC₅₀ = 5.4 nM and 2.1 nM, respectively). (In similar dose-response studies not shown in Figure 5, IC₅₀ values were obtained for additional secondary amide compounds: **18** (IC₅₀ = 9.1 nM), **22** (IC₅₀ = 4.3 nM), **23** (IC₅₀ = 10 nM), **24** (IC₅₀ = 10 nM), **25** (IC₅₀ = 12 nM) and **27** (IC₅₀ = 2.9 nM). The IC₅₀ value for Fulvestrant is 0.3 nM).

Dose-response curves for ER α downregulation by **5**, **20**, and **21**, as well as fulvestrant, derived from in-cell western immunoassays for ER α , are shown in Figure 6A. Of the three new compounds, the hydroxypropyl amide **21** is clearly the most potent (IC₅₀ = 0.53 nM), with the hydroxyethyl amide **20** and carboxylic acid **5** being considerably less potent (IC₅₀ = 5.6 nM and 17 nM, respectively). These ER downregulating activities were confirmed by direct Western blot assays (Figure 6B). The maximum levels of ER α downregulation from both the ICW dose-response assays and the western blots are equivalent to those from single-dose assays, previously shown in Figure 4, and are close to that of fulvestrant in efficacy.

We selected the most efficacious antiproliferative adamantyl compounds **5** and **21** to assay for their suppression of E2-stimulated gene expression (Figure 7). Expression of the ER-target genes, GREB1, progesterone receptor (PgR), and pS2, was monitored in cells grown in full media (with 5% fetal bovine serum) and with added (3×10^{-10} M) estradiol (E2). The dose-response studies revealed that **21** was more potent than **5** in inhibiting these estrogen-stimulated genes (IC₅₀ of 50 nM for **21** and 500 nM for **5**) and that fulvestrant was 10 to 20-fold more potent than **21**.

DISCUSSION

In this study, we explored structure-activity relationships in adamantyl antiestrogens having variations on the acrylic acid side chain. This side chain, which is clearly an important functional element in conveying the effectiveness of ER antagonism, has survived over several decades in structurally unaltered form in antiestrogens from GSK,^{10, 11} Genentech,¹² AstraZeneca,^{13, 34} and other pharma companies.³⁵ In contrast, while many variations have been made in the structures of the core elements (Figure 1), only limited alterations have been made in the acrylate side chain, and none have been explored in a systematic fashion.^{10, 26, 36, 37} Starting with a structurally simple biaryl adamantyl core structure, which provides very high ER α binding affinity and stereochemical simplicity for ease of synthesis, we surveyed a considerable number of compounds having systematic acrylic acid side chain variants. These were evaluated for their ER α binding affinities, and nearly all proved to have K_i values in the single-digit nanomolar range or below. All compounds were then assayed for their antiproliferative and ER α downregulating (SERD) *efficacies*, and in several cases we found that relatively small structural changes resulted in marked alterations in efficacies and potencies. In particular, extension of the acrylic acid side chain as secondary amides provided a favorable combination of high affinity, high efficacy and high potency in suppressing E2-driven proliferation of MCF-7 cells and in downregulating ER α levels. Closely related tertiary amides, however, were much less effective. Several of those compounds having promising antiproliferative efficacies were evaluated further in terms of their *potencies* and gene-regulating activities. The best secondary amides, **20** and **21**, showed higher potency than the parent carboxylic acid **5** in breast cancer cells.

Relationship Between Suppression of MCF-7 Cell Proliferation and ER α Downregulation

There has been considerable discussion of how antagonism of breast cancer proliferation may be related to or indeed dependent upon the downregulation of the level of ER α (i.e., SERD activity).³⁸ Clearly, ER α downregulation does not predict antagonism of proliferation because agonists such as E2, and in our case the acrylonitrile compound **14**, elicit strong ER α downregulation but are either stimulatory or essentially neutral on proliferation. On the other hand, among most of the compounds we have studied, we did find a reasonable overall correlation between the maximum level of proliferation suppression and ER α downregulation; this is evident from the similar shape of the cell proliferation and ER α level lines in the last two star plots (Figures 4B and C). However, some compounds are more efficacious ER α downregulators than antiproliferative compounds, such as the esters **7** and **10** (Figure 4A) and the primary amide **16** (Figure 4B). This is indicated by the line for cell proliferation (blue) being substantially outside of the line for ER α level (red).

Since agonists can also degrade ER α through potentially different mechanisms, we examined whether degradation predicted the anti-proliferative response in the compounds with at least 25% growth inhibition (Figure 8). An r^2 of 0.59 demonstrates that degradation may account for 59% of the variance in proliferation. This type of analysis does not determine whether anti-proliferative effects are a consequence of degradation, or whether both degradation and anti-proliferative effects are downstream of the same structural perturbation, but it does indicate that there are other significant contributors to efficacy of

the ligands besides degradation. It is notable as well that some SERMs, such as tamoxifen and raloxifene, are effective in suppressing proliferation yet have no effect on cellular ER α level or can even stabilize the receptor and upregulate ER α levels, as we observed in Figure 6B for hydroxytamoxifen and as observed by others.³⁹

One should note that when ER α is occupied by a SERM or a SERD, its overall effect on gene expression is not only a suppression of pro-proliferative and anti-apoptotic activity but also an upregulation of anti-proliferative and pro-apoptotic factors.⁴⁰⁻⁴² The potential benefits of such effects would be lost in the complete absence of ER α ^{43, 44} and might, in fact, engender the development of more aggressive forms of breast cancer that are independent of ER α or fully resistant to ER α -targeted endocrine therapy agents.¹ Hence, while the importance of ER downregulation for effective antiestrogenic activity is far from being settled, it would appear illogical to discard interest in a particularly potent and complete antiproliferative antiestrogen just because it may not be fully efficacious in reducing cellular ER α levels.

Partitioning the Functional Characteristics of Antiestrogens: The Core-Side Chain Paradigm and Structural Features of the Acrylate Side Chain

The approach we have taken in our study was predicated on the concept that an antiestrogen can be thought of as a core-side chain construct, each part of which operates rather independently: The core provides the binding energy and the side chain the functional regulation, with side chains being essentially interchangeable among various cores.⁹ The interchangeability of antiestrogen side chains onto different core elements is certainly well illustrated in the literature, and while it may not apply strictly in all cases⁴⁵ it supports our selection of a simplified adamantyl core element.¹⁹ (Contrary examples are generally compounds having more bulky cores or altogether lacking side chains, which are classified as indirect antagonists⁴⁶⁻⁴⁹. These include compounds in which the core can distort the shape of the coregulator binding surface to alter coregulator preferences⁵⁰ and produces anti-proliferative effects independent of a side chain.⁴⁷) With the adamantyl core we selected, we were able to search in an expeditious fashion for side chains that might afford more effective and/or potent antiproliferative and ER α downregulating activities, the expectation being that once found, these side chains might likely be transferrable to other, possibly more complex ligand cores. The core element, of course, also contributes to the drug-like attributes of the overall molecule that defines its pharmacokinetic behavior, such as oral bioavailability.

Our choice of the acrylic acid side chain as the starting point for our side chain exploration was first based on our recognition that the rigidity of the double bond is important in its ER α antagonizing function. With the SERMs, the amino group in the side chain serves to localize the positively charged side chain through an attractive interaction with Asp351.⁵¹ Here, we are using the double bond to enforce the positioning of a neutral or negatively charged side chain. In an earlier study, we explored a variety of other acid side chains on a related core element, and we found that although many of them bound to ER α with excellent affinity, only those having the rigid acrylic acid unit attached directly to the phenyl proved to be effective antagonists and ER downregulators.²⁵ Thus, we limited this study to groups that maintained side chain rigidity through direct attachment of the double bond to the ring.

Possible Role of a Structural water Molecule and Conformational Preferences of Amides

Earlier, we noted that a crystal structure of GW-5638 bound to the ER α LBD showed a structured or ordered water molecule, or possibly an ion, linking the carboxylic acid group to the N-terminus of h12 when it was positioned to obstruct coactivator binding (Figure 2).²⁰ In our ligand design we included terminal polar atoms in the side chains of some of our compounds to explore whether they might replace this water molecule. In other systems, replacement of a structural water has resulted in a large increase in ligand potency or binding affinity, presumed to be due to the gain in entropy in releasing the water from the bound or ordered state,²¹⁻²³ or in our case by potentially providing more direct electrostatic interactions between the side chain and the h11-h12 loop or h12 itself. While we have no direct structural evidence that we have achieved such a replacement, in a number of amides and esters, substitution of a terminal hydrogen atom with an OH or NH₂ group caused a systematic two to five-fold increase in binding affinity (Figure 3). It was not apparent, however, that this increase in affinity translated into greater antiproliferative or ER α -downregulating efficacies or potencies, nor did binding affinity in general correlate with measures of efficacy or potency. This is a common theme for targeting allosteric proteins, where it is not just necessary to achieve efficient binding, but also to induce specific conformational changes that regulate activity.

A striking feature of members of the amide class is that the secondary amides are much more efficacious than the tertiary amides, particularly in terms of their antiproliferative activity. While it is possible that adding a second alkyl group on the nitrogen might cause steric hindrance that diminishes affinity and efficacy, there is certainly no consistent effect of the second substituent on binding affinity (2° **18** ~ 3° **29**; but 2° **20** > 3° (**30** << **31**)). Both esters and secondary amides are notable in having an extended (Z or s-cis) conformation, which is energetically favored due to n- σ^* or σ - σ^* π overlap, respectively,^{52, 53} that would project the single substituent directly outward, away from the ring and presumably in a productive direction, toward h12 (Figure 9). The two possible geometries of tertiary amides, on the other hand, are of essentially equal energy, so the smaller group could be extended in the s-cis orientation. It is also possible that the secondary amide is participating in an h-bonding interaction that contributes to both binding and the conformational changes that control efficacy.

The Compounds Most Promising for Further Investigation

From the results of this study, we have identified two compounds that work well on breast cancer cells with WT ER α . The 3-hydroxypropyl amide **21** is the most promising compound with a new side chain; it has antiproliferative and ER α downregulating efficacy comparable to that of fulvestrant, and although its potency is less, it is still in the low nM range. This compound, together with the parent compound having the parent carboxylic acid side chain **5**, are promising candidates to study further in breast cancer cell lines where ER α has activating mutations, such as Y537S and D538G, as well as in vivo with xenografts of such cells, where pharmacokinetic factors can play a dominant role in compound potency and preferred routes of administration.

EXPERIMENTAL SECTION

I. Materials, General Methods, and Statement of Purity

All reagents and solvents were obtained from Sigma-Aldrich, Acros, TCI and Matrix Scientific. Tetrahydrofuran, dimethylformamide, trimethylamine and dichloromethane were obtained from a solvent dispensing system (SDS).⁵⁴ Glassware was oven-dried, assembled while hot, and cooled under an inert atmosphere. Unless otherwise noted, all reactions were conducted in an inert atmosphere. Reaction progress was monitored using analytical thin-layer chromatography (TLC) on 0.25 mm Merck F-254 silica gel glass plates. Visualization was achieved by either UV light (254 nm) or potassium permanganate indicator spray. Flash chromatography was performed with Woelm silica gel (0.040-0.063 mm) packing using standard procedures, or with a Teledyne-Isco CombiFlash Rf purification system using Silica gel 60 Å (230-400 or 400-632 mesh size).

¹H NMR and ¹³C NMR spectra were obtained on a 400 or 500 MHz instrument. The chemical shifts are reported in ppm and are referenced to either tetramethylsilane or the solvent. Mass spectra were recorded under electron impact conditions at 70 eV. High-resolution mass spectra (HRMS) were obtained at the University of Illinois Mass Spectrometry Laboratory using a Micromass Q-ToF instrument. Melting points are uncorrected.

All compounds that were tested biologically were shown to be >95% pure by HPLC analysis on both normal and reversed phase columns.

General procedure for McMurry coupling (3)—Zinc powder (8.0 equiv) was suspended in dry THF at 0 °C in a three-neck round bottom flask under a nitrogen atmosphere. Titanium tetrachloride (4.0 equiv) was added dropwise via a syringe while stirring. The reaction mixture was then refluxed for 2 h. After cooling to rt, a THF solution containing 4,4'-dihydroxy benzophenone (**1**, 1.0 equiv) and 2-Adamantanone (**2**, 1.0 equiv) was added dropwise to the slurry. The mixture was refluxed for an additional two hours and then was cooled, poured into NaHCO₃ solution, and kept stirring until the dark color disappeared. After filtration through Celite, the filtrate was extracted with ethyl acetate, dried with anhydrous Na₂SO₄ and concentrated under vacuum. The resulting residue (**3**) was used for the next step without any purification.

General procedure for Mono-triflate synthesis (4)—At rt, potassium carbonate (K₂CO₃, 2 equiv) was added to the DMF solution of compound **3** (1.0 equiv) 4-Nitrophenyl trifluoromethanesulfonate (1.2 equiv) was then slowly added to the suspension.²⁸ The reaction mixture was stirred at rt overnight and extracted with ethyl acetate, washed with water and brine. The organic layer was dried with anhydrous Na₂SO₄ and concentrated under vacuum. The resulting residue was purified by flash chromatography on silica gel to give the desired product (**4**).

General procedure for Heck reaction—A mixture of triflate derivative **4** (1.0 equiv), corresponding alkene (1.2 equiv), Pd(PPh₃)₂Cl₂ (10 mol%), Et₃N (3 equivalents) in DMF was heated under N₂ at 120 °C for 12 h. The reaction mixture was cooled and extracted with

ethyl acetate, washed with water and brine. The organic layer was dried with anhydrous Na₂SO₄ and concentrated under vacuum. The resulting residue was purified by flash chromatography (10-80% Ethyl acetate/Hexane gradient) on silica gel to give the desired products **6-9** and **13-16**.

General procedure for Amide Synthesis using HATU—O-(7-Azabenzotriazole-1-yl)-N,N,N,N'-tetramethyluronium hexafluorophosphate (HATU, 1.5 equiv) was added to the DMF solution of the carboxylic acid (1.0 equiv). The mixture was stirred at rt for 10 min, and then the corresponding amine (3.0 equiv) was injected dropwise. The resulting yellow solution was stirred for 20 min before diisopropylethylamine (DIPEA, 3.0 equiv) was added by syringe. For reaction with primary amines (to prepare the secondary amides), the mixture was stirred at rt while for reaction with secondary amines (to prepare tertiary amides), temperature was elevated to 50 °C. The reaction was monitored by TLC. After complete consumption of starting material, ethyl acetate was added, and the resulting solution was washed with brine. The organic layer was dried with anhydrous Na₂SO₄ and concentrated under vacuum. The resulting residue was purified by flash chromatography on silica gel to give the desired products **17-23**, **24a-26a**, **27-31**.

General Procedure for the deprotection of Boc-group—The Boc-protected compounds (**24a-26a**) were dissolved in dry DCM and cooled to 0 °C. TFA (equal amount as the solvent) was added dropwise, and the solution was stirred at rt. After complete consumption of starting material (as monitored by TLC), the solution was concentrated under vacuum. The residue was extracted with ethyl acetate, washed with water and sat. NaHCO₃ solution. The combined organic layers were dried with anhydrous Na₂SO₄ and evaporated under vacuum. The resulting residue was purified by flash chromatography on silica gel to give the desired products (**24-26**).

II. Chemical Synthesis

(E)-3-(4-(((1r,3r,5R,7S)-Adamantan-2-ylidene)(4-hydroxyphenyl)methyl)phenyl)acrylic acid (5)—To the solution of the ester **8** (1.0 equiv) in CH₃OH was added 2N KOH (2.0 mL). The mixture was stirred at rt and monitored by TLC. After complete consumption of starting material, the reaction mixture was poured into 1 N HCl (4.0 mL) and extracted from the aqueous phase with ethyl acetate. The organic layer was dried with anhydrous Na₂SO₄ and concentrated under vacuum. Flash column chromatography (10% CH₃OH/CH₂Cl₂) gave the acid product **5** as white solid (Yield 92%, mp 228 °C). ¹H NMR (500 MHz, Acetone-*d*₆) δ 7.66 (d, *J* = 16.0 Hz, 1H), 7.61 (d, *J* = 8.2 Hz, 2H), 7.20 (d, *J* = 8.2 Hz, 2H), 6.98 (d, *J* = 8.5 Hz, 2H), 6.78 (d, *J* = 8.5 Hz, 2H), 6.50 (d, *J* = 16.0 Hz, 1H), 2.81 (s, 1H), 2.76 (s, 1H), 1.99 (s, 2H), 1.88 (d, *J* = 13.1 Hz, 10H). ¹³C NMR (126 MHz, Acetone-*d*₆) δ 167.14, 156.17, 146.59, 145.87, 144.65, 133.78, 132.56, 130.71, 130.46, 130.16, 128.09, 117.82, 115.10, 39.44, 39.42, 37.04, 34.75, 34.62, 28.37. HRMS (ESI) calcd for C₂₆H₂₇O₃ (M+H⁺) 387.1960, found 387.1971.

(E)-1-(4-(((1r,3r,5R,7S)-Adamantan-2-ylidene)(4-hydroxyphenyl)methyl)phenyl)oct-1-en-3-one (6)—Following the general procedure for Heck reaction using corresponding triflate **4** and 1-Octen-3-one, compound **6** was

obtained as yellow solid (Yield 47%, mp 158 °C). ¹H NMR (500 MHz, CDCl₃) δ 7.51 (d, *J* = 16.2 Hz), 7.44 – 7.39 (m, 2H), 7.15 – 7.10 (m, 2H), 6.98 – 6.90 (m, 2H), 6.83 – 6.75 (m, 2H), 6.68 (d, *J* = 16.2 Hz, 1H), 2.77 (m, 1H), 2.66 – 2.60 (m, 2H), 1.98 (m, 2H), 1.83 (m, 10H), 1.66 (m, 2H), 1.31 (m, 4H), 0.93 – 0.83 (m, 3H). ¹³C NMR (125 MHz, CDCl₃) δ 201.6, 154.7, 147.6, 146.2, 142.9, 134.7, 132.2, 131.0, 130.9, 130.4, 129.8, 128.2, 128.0, 125.6, 115.2, 115.1, 41.0, 39.8, 39.8, 37.3, 36.7, 34.8, 34.7, 34.7, 31.7, 31.2, 28.4, 28.3, 24.5, 24.2, 22.7, 19.9, 14.2, 13.8. HRMS-ESI: *m/z* [M+H]⁺ for C₃₁H₃₇O₂, calculated 441.2794; observed 441.2801.

Methyl (E)-3-(4-(((1*r*,3*r*,5*R*,7*S*)-Adamantan-2-ylidene)(4-hydroxyphenyl)methyl)phenyl) acrylate (7)—

Following the general procedure for Heck reaction using corresponding triflate **4** and methyl acrylate, compound **7** was obtained as white solid (Yield 68%, mp 209 °C). ¹H NMR (500 MHz, CDCl₃) δ 7.68 (d, *J* = 16.0 Hz, 1H), 7.44 (d, *J* = 8.2 Hz, 2H), 7.15 (d, *J* = 8.2 Hz, 2H), 7.00 (d, *J* = 6.5 Hz, 2H), 6.77 (d, *J* = 8.6 Hz, 2H), 6.41 (d, *J* = 16.0 Hz, 1H), 3.82 (s, 3H), 2.80 (d, *J* = 10.4 Hz, 2H), 2.02 (s, 2H), 1.87 (d, *J* = 12.4 Hz, 11H). ¹³C NMR (126 MHz, CDCl₃) δ 167.92, 154.20, 147.82, 145.90, 145.11, 135.32, 132.24, 131.12, 130.38, 129.64, 128.05, 117.10, 115.16, 51.95, 39.85, 39.82, 37.31, 34.81, 34.75, 28.36. HRMS (ESI) calcd for C₂₇H₂₉O₃ (M+H⁺) 401.2117, found 401.2129.

Ethyl (E)-3-(4-(((1*r*,3*r*,5*R*,7*S*)-Adamantan-2-ylidene)(4-hydroxyphenyl)methyl)phenyl) acrylate (8)—

Following the general procedure for Heck reaction using corresponding triflate **4** and ethyl acrylate, compound **8** was obtained as white solid (Yield 65%, mp 135-137 °C). ¹H NMR (500 MHz, CDCl₃) δ 7.67 (d, *J* = 16.0 Hz, 1H), 7.44 (d, *J* = 8.2 Hz, 2H), 7.15 (d, *J* = 8.2 Hz, 2H), 7.00 (d, *J* = 8.6 Hz, 2H), 6.77 (d, *J* = 8.6 Hz, 2H), 6.40 (d, *J* = 16.0 Hz, 1H), 4.28 (q, *J* = 7.1 Hz, 2H), 2.80 (d, *J* = 9.8 Hz, 2H), 2.02 (s, 2H), 1.87 (d, *J* = 12.4 Hz, 11H), 1.35 (t, *J* = 7.1 Hz, 3H). ¹³C NMR (126 MHz, CDCl₃) δ 167.55, 154.29, 147.76, 145.83, 144.86, 135.25, 132.30, 131.11, 130.36, 128.02, 117.55, 115.17, 60.74, 39.85, 39.82, 37.31, 34.81, 34.76, 28.36, 14.58. HRMS (ESI) calcd for C₂₈H₃₁O₃ (M+H⁺) 415.2273, found 415.2264.

Butyl (E)-3-(4-(((1*r*,3*r*,5*R*,7*S*)-Adamantan-2-ylidene)(4-hydroxyphenyl)methyl)phenyl) acrylate (9)—

Following the general procedure for Heck reaction using corresponding triflate **4** and butyl acrylate, compound **9** was obtained as yellow solid (Yield 54%, mp 140 °C). ¹H NMR (500 MHz, CDCl₃) δ 7.64 (d, *J* = 15.9 Hz, 1H), 7.43 – 7.35 (m, 2H), 7.12 (m, 2H), 6.98 – 6.94 (m, 2H), 6.76 (m, 2H), 6.38 (d, *J* = 15.9 Hz, 1H), 4.19 (t, *J* = 6.3 Hz, 2H), 2.77 (d, *J* = 19.4 Hz, 2H), 1.98 (m, 2H), 1.83 (d, *J* = 13.2 Hz, 10H), 1.68 (m, 2H), 1.41 (m, 2H), 0.95 (m, 3H). ¹³C NMR (125 MHz, CDCl₃) δ 167.9, 154.5, 147.6, 145.9, 145.1, 134.9, 133.0, 132.1, 131.0, 130.3, 129.7, 128.0, 117.3, 115.2, 77.5, 77.2, 64.8, 39.8, 39.8, 37.3, 34.8, 34.7, 30.9, 28.3, 28.3, 19.4, 14.0. HRMS-ESI: *m/z* [M-H]⁻ for C₃₀H₃₃O₃, calculated 441.2430; observed 441.2420.

6-Methylheptyl (E)-3-(4-(((1*r*,3*r*,5*R*,7*S*)-Adamantan-2-ylidene)(4-hydroxyphenyl)methyl) phenyl)acrylate (10)—

The mixture of compound **5** (14.3 mg, 0.037 mmol), 1-bromo-6-methylheptane (9.7 mg, 0.05 mmol), and cesium carbonate (7.0

mg, 0.036 mmol) in DMF (100 μ L) was stirred for 6 h at rt. Once starting material had disappeared on silica gel TLC (15% ethyl acetate in n-hexane), the reaction mixture was poured into water (1.5 mL) and extracted with ethyl acetate (1 mL $\times$ 3), followed by drying over Na₂SO₄ before concentrating it to load onto a preparative TLC plate (1 mm thickness, 5 $\times$ 20 cm SiO₂, R_f = 0.6, 15% ethyl acetate in n-hexane). The developed band was scraped off from Prep. TLC and placed in a sintered glass funnel. Extraction with the mixture of ethyl acetate and dichloromethane (1:1, v/v) afforded compound **10** (9.2 mg, 51%) as a colorless sticky semi-solid. ¹H NMR (499 MHz, CDCl₃) δ 7.67 (d, *J* = 16.0 Hz, 1H), 7.44 (d, *J* = 8.0 Hz, 2H), 7.15 (d, *J* = 8.0 Hz, 2H), 7.00 (d, *J* = 8.5 Hz, 2H), 6.77 (d, *J* = 8.5 Hz, 2H), 6.41 (d, *J* = 16.0 Hz, 1H), 4.21 (t, *J* = 7.0 Hz, 2H), 2.81 (s, 1H), 2.79 (s, 1H), 2.02 (brs, 2H), 1.88 (ts, 10H), 1.72 (quintet, *J* = 7.5 Hz, 2H), 1.55 (nonatet, *J* = 7.0 Hz, 1H), 1.39 – 1.28 (m, 4H), 1.22 (q, *J* = 7.0 Hz, 2H), 0.89 (d, *J* = 6.5 Hz, 6H). ¹³C NMR (125 MHz, CDCl₃) δ 167.65, 154.27, 147.80, 145.83, 144.83, 135.33, 132.37, 131.13, 130.37, 129.72, 128.04, 117.64, 115.19, 64.98, 39.86, 39.12, 37.33, 34.83, 34.77, 29.01, 28.38, 27.30, 26.48, 22.88. HRMS-ESI: *m/z* [M-H][–] for C₃₄H₄₁O₃, calculated 497.3056; observed 497.3052.

2-Hydroxyethyl (E)-3-(4-(((1*r*,3*r*,5*R*,7*S*)-Adamantan-2-ylidene)(4-hydroxyphenyl)methyl) phenyl)acrylate (11)—Following the general procedure for Heck reaction using corresponding triflate **4** and 2-hydroxyethyl acrylate, compound **11** was obtained as white solid (Yield 46%, mp 158 °C). ¹H NMR (500 MHz, CDCl₃) δ 7.67 (d, *J* = 16.0 Hz, 1H), 7.39 (d, *J* = 8.0 Hz, 2H), 7.11 (d, *J* = 7.9 Hz, 2H), 6.95 (d, *J* = 8.4 Hz, 2H), 6.73 (d, *J* = 8.5 Hz, 2H), 6.40 (d, *J* = 16.0 Hz, 1H), 4.36 – 4.31 (m, 2H), 3.92 – 3.87 (m, 2H), 2.76 (d, *J* = 11.6 Hz, 2H), 1.98 (m, 2H), 1.83 (m, Hz, 10H); ¹³C NMR (126 MHz, CDCl₃) δ 167.8, 154.5, 147.7, 145.9, 145.0, 135.0, 133.3, 132.2, 131.0, 130.3, 129.9, 129.7, 128.5, 128.0, 117.4, 115.2, 77.5, 69.4, 64.2, 60.8, 39.8, 39.8, 37.3, 34.8, 34.7, 28.3, 14.5. HRMS-ESI: *m/z* [M-H][–] for C₂₈H₂₉O₄, calculated 429.2066; observed 429.2057.

3-Hydroxypropyl (E)-3-(4-(((1*r*,3*r*,5*R*,7*S*)-Adamantan-2-ylidene)(4-hydroxyphenyl)methyl) phenyl)acrylate (12)—To a solution of cinnamic acid **5** (1 equiv) and DMAP (2 mol%) in DCM at –15 °C, Et₃N (1 equivalent) and isobutyl chloroformate (2.2 equiv) were added sequentially and the reaction mixture was allowed to reach rt over 3 h. Thereafter, piperidine (15 equiv) was added and the resulting mixture further stirred for 1 h at rt. The reaction mixture was evaporated under vacuum and ethyl acetate was added followed by washing with KHSO₄ (10%). The organic component was dried with Na₂SO₄ and vacuum evaporated. The resulting residue was purified by flash chromatography (10-80% Ethyl acetate/Hexane gradient) on silica gel to give the desired product **12** as white solid (Yield 49%, mp 165-168 °C). ¹H NMR (500 MHz, CDCl₃) δ 7.65 (d, *J* = 16.0 Hz, 1H), 7.42 – 7.37 (m, 2H), 7.14 – 7.09 (m, 2H), 6.98 – 6.93 (m, 2H), 6.76 – 6.71 (m, 2H), 6.37 (d, *J* = 16.0 Hz, 1H), 4.36 (t, *J* = 6.1 Hz, 2H), 3.72 (t, *J* = 6.0 Hz, 2H), 2.76 (d, *J* = 16.2 Hz, 2H), 2.02 – 1.96 (m, 2H), 1.93 (p, *J* = 6.0 Hz, 2H), 1.89 – 1.78 (m, 10H). ¹³C NMR (125 MHz, CDCl₃) δ 168.0, 154.4, 147.8, 146.1, 145.5, 135.1, 132.1, 131.1, 130.4, 129.7, 128.1, 117.0, 115.2, 77.5, 61.6, 59.4, 39.8, 39.8, 37.3, 34.8, 34.7, 32.1, 28.3. HRMS-ESI: *m/z* [M-H][–] for C₂₉H₃₁O₄, calculated 443.2222; observed 443.2215.

4-Hydroxybutyl (E)-3-(4-(((1r,3r,5R,7S)-Adamantan-2-ylidene)(4-hydroxyphenyl)methyl)phenyl)acrylate (13)—Following the general procedure for Heck reaction using corresponding triflate **4** and 4-hydroxybutyl acrylate, compound **13** was obtained as yellow solid (Yield 57%, mp 180-181 °C). ¹H NMR (500 MHz, CDCl₃) δ 7.63 (d, *J* = 15.9 Hz, 1H), 7.41 – 7.34 (m, 2H), 7.10 (m, *J* = 2.1, 8.4 Hz, 2H), 6.96 – 6.91 (m, 2H), 6.76 – 6.70 (m, 2H), 6.36 (d, *J* = 15.9 Hz, 1H), 4.22 (t, *J* = 6.5 Hz, 2H), 3.70 (t, *J* = 6.4 Hz, 2H), 2.76 (d, *J* = 18 Hz, 2H), 2.00 – 1.95 (m, 2H), 1.89 – 1.74 (m, 12H), 1.67 (m, 2H); ¹³C NMR (125 MHz, CDCl₃) δ 167.7, 154.5, 147.7, 146.0, 145.2, 134.9, 132.1, 131.0, 130.3, 129.7, 128.0, 117.2, 115.2, 77.0, 64.6, 62.7, 39.8, 39.8, 37.3, 34.8, 34.7, 29.3, 28.3, 25.4. HRMS-ESI: *m/z* [M-H][−] for C₃₀H₃₃O₄, calculated 457.2379; observed 457.2377.

(E)-3-(4-(((1r,3r,5R,7S)-Adamantan-2-ylidene)(4-hydroxyphenyl)methyl)phenyl)acrylonitrile (14)—Following the general procedure for Heck reaction using corresponding triflate **4** and acrylonitrile, compound **14** was obtained as yellow solid (Yield 55%, mp 156 °C). ¹H NMR (500 MHz, CDCl₃) δ 7.42 – 7.35 (m, 3H), 7.17 (d, *J* = 8.2 Hz, 2H), 6.99 (d, *J* = 8.5 Hz, 2H), 6.77 (d, *J* = 8.5 Hz, 2H), 5.84 (d, *J* = 16.6 Hz, 1H), 2.81 (s, 1H), 2.76 (s, 1H), 2.03 (s, 2H), 1.93 – 1.83 (m, 10H). ¹³C NMR (126 MHz, CDCl₃) δ 154.29, 150.71, 148.29, 146.89, 135.06, 131.43, 131.12, 130.58, 129.40, 127.33, 118.70, 115.22, 95.46, 39.84, 39.80, 37.26, 34.87, 34.77, 28.31. HRMS (ESI) calcd for C₃₀H₃₇N₂O₂ (M+H⁺) 457.2855, found 457.2864.

(E)-2-(4-(((1r,3r,5R,7S)-Adamantan-2-ylidene)(4-hydroxyphenyl)methyl)phenyl)-N-propylethene-1-sulfonamide (15)—Following the general procedure for Heck reaction using corresponding triflate **4** and N-propylethene sulfonamide, compound **15** was obtained as yellow solid (Yield 46%, mp 205-207 °C). ¹H NMR (500 MHz, CDCl₃) δ 7.46 (d, *J* = 15.4 Hz, 1H), 7.40 (d, *J* = 8.2 Hz, 2H), 7.17 (d, *J* = 8.2 Hz, 2H), 6.99 (d, *J* = 8.6 Hz, 2H), 6.78 (d, *J* = 8.6 Hz, 2H), 6.70 (d, *J* = 15.4 Hz, 1H), 3.03 (q, *J* = 6.9 Hz, 2H), 2.82 (s, 1H), 2.77 (d, *J* = 3.8 Hz, 1H), 2.03 (s, 2H), 1.87 (d, *J* = 11.5 Hz, 10H), 1.64 – 1.57 (m, 2H), 0.95 (t, *J* = 7.4 Hz, 3H). ¹³C NMR (126 MHz, CDCl₃) δ 154.41, 148.04, 146.46, 141.88, 135.02, 131.09, 130.55, 130.40, 129.51, 128.18, 124.28, 115.22, 45.04, 39.85, 39.81, 37.28, 34.85, 34.75, 28.33, 23.52, 11.43. HRMS (ESI) calcd for C₃₀H₃₇N₂O₂ (M+H⁺) 457.2855, found 457.2864.

(E)-3-(4-(((1r,3r,5R,7S)-Adamantan-2-ylidene)(4-hydroxyphenyl)methyl)phenyl)acrylamide (16)—Following the general procedure for Heck reaction using corresponding triflate **4** and acrylamide, compound **16** was obtained as white solid (Yield 56%, mp 221 °C). ¹H NMR (500 MHz, DMSO-*d*₆) δ 9.32 (s, 1H), 7.45 (d, *J* = 8.1 Hz, 2H), 7.35 (d, *J* = 15.8 Hz, 1H), 7.07 (d, *J* = 7.9 Hz, 2H), 6.86 (d, *J* = 8.4 Hz, 2H), 6.67 (d, *J* = 8.4 Hz, 2H), 6.53 (d, *J* = 15.8 Hz, 1H), 2.70 (s, 1H), 2.64 (s, 1H), 1.94 (s, 2H), 1.80 (s, 10H). ¹³C NMR (126 MHz, DMSO-*d*₆) δ 167.37, 156.48, 146.21, 144.85, 139.58, 133.29, 130.89, 130.32, 128.00, 122.35, 115.59, 37.19, 34.69, 34.53, 28.17. HRMS (ESI) calcd for C₂₆H₂₈NO₂ (M+H⁺) 386.2120, found 386.2110.

(E)-3-(4-(((1r,3r,5R,7S)-Adamantan-2-ylidene)(4-hydroxyphenyl)methyl)phenyl)-N-propylacrylamide (17)—Following the general procedure for amide synthesis between

5 and propylamine, compound **17** was obtained as white solid (Yield 82%, mp 250 °C). ¹H NMR (500 MHz, DMSO-*d*₆) δ 8.07 (t, *J* = 5.4 Hz, 1H), 7.44 (d, *J* = 7.7 Hz, 2H), 7.34 (d, *J* = 15.8 Hz, 1H), 7.06 (d, *J* = 7.6 Hz, 2H), 6.85 (d, *J* = 7.8 Hz, 2H), 6.67 (d, *J* = 7.7 Hz, 2H), 6.57 (s, 1H), 3.10 (q, *J* = 6.5 Hz, 2H), 2.69 (s, 1H), 2.64 (s, 1H), 1.94 (s, 2H), 1.79 (s, 11H), 1.44 (q, *J* = 7.2 Hz, 2H), 0.85 (t, *J* = 7.4 Hz, 3H). ¹³C NMR (126 MHz, DMSO-*d*₆) δ 165.54, 156.48, 146.19, 144.74, 138.77, 133.38, 133.30, 130.87, 130.34, 130.30, 127.90, 122.41, 115.59, 37.19, 34.67, 34.54, 28.18, 23.09, 12.17. HRMS (ESI) calcd for C₂₉H₃₄NO₂ (M+H⁺) 428.2590, found 428.2581.

(E)-3-(4-(((1*r*,3*r*,5*R*,7*S*)-Adamantan-2-ylidene)(4-hydroxyphenyl)methyl)phenyl)-N-butylacrylamide (18)—Following the general procedure for amide synthesis between **5** and butylamine, compound **18** was obtained as white solid (Yield 86%, mp 304 °C). ¹H NMR (500 MHz, DMSO-*d*₆) δ 8.04 (t, *J* = 5.6 Hz, 1H), 7.44 (d, *J* = 8.2 Hz, 2H), 7.34 (d, *J* = 15.7 Hz, 1H), 7.06 (d, *J* = 8.1 Hz, 2H), 6.85 (d, *J* = 8.5 Hz, 2H), 6.66 (d, *J* = 8.5 Hz, 2H), 6.54 (d, *J* = 15.8 Hz, 1H), 3.14 (q, *J* = 6.8 Hz, 2H), 2.69 (s, 1H), 2.64 (s, 1H), 1.94 (s, 2H), 1.79 (s, 11H), 1.41 (p, *J* = 7.1 Hz, 2H), 1.32 – 1.25 (m, 2H), 0.86 (t, *J* = 7.3 Hz, 3H). ¹³C NMR (126 MHz, DMSO-*d*₆) δ 165.50, 156.48, 146.19, 144.74, 138.76, 133.39, 133.31, 130.88, 130.34, 130.31, 127.90, 122.40, 115.59, 37.19, 34.54, 31.93, 28.18, 20.32, 14.36. HRMS (ESI) calcd for C₃₀H₃₆NO₂ (M+H⁺) 442.2746, found 442.2739.

(E)-3-(4-(((1*r*,3*r*,5*R*,7*S*)-Adamantan-2-ylidene)(4-hydroxyphenyl)methyl)phenyl)-N-pentylacrylamide (19)—Following the general procedure for amide synthesis between **5** and pentylamine, compound **19** was obtained as white solid (Yield 82%, mp 301–303 °C). ¹H NMR (500 MHz, DMSO-*d*₆) δ 8.05 (t, *J* = 5.7 Hz, 1H), 7.44 (d, *J* = 8.2 Hz, 2H), 7.34 (d, *J* = 15.7 Hz, 1H), 7.06 (d, *J* = 8.2 Hz, 2H), 6.85 (d, *J* = 8.5 Hz, 2H), 6.67 (d, *J* = 8.5 Hz, 2H), 6.54 (d, *J* = 15.8 Hz, 1H), 3.13 (q, *J* = 6.8 Hz, 2H), 2.69 (s, 1H), 2.64 (s, 1H), 1.94 (s, 2H), 1.79 (s, 11H), 1.42 (p, *J* = 7.2 Hz, 2H), 1.33 – 1.13 (m, 6H), 0.85 (t, *J* = 7.0 Hz, 3H). ¹³C NMR (126 MHz, DMSO-*d*₆) δ 165.49, 156.49, 146.18, 144.74, 138.74, 133.39, 133.30, 130.87, 130.34, 130.30, 127.90, 122.42, 115.59, 37.19, 34.68, 34.54, 29.51, 29.37, 28.18, 22.55, 14.61. HRMS (ESI) calcd for C₃₁H₃₈NO₂ (M+H⁺) 456.2903, found 456.2897.

(E)-3-(4-(((1*r*,3*r*,5*R*,7*S*)-Adamantan-2-ylidene)(4-hydroxyphenyl)methyl)phenyl)-N-(2-hydroxyethyl)acrylamide (20)—Following the general procedure for amide synthesis between **5** and ethanolamine, compound **20** was obtained as white solid (Yield 76%, mp 259 °C). ¹H NMR (500 MHz, DMSO-*d*₆) δ 9.32 (s, 1H), 8.11 (t, *J* = 5.6 Hz, 1H), 7.44 (d, *J* = 8.0 Hz, 2H), 7.36 (d, *J* = 15.7 Hz, 1H), 7.07 (d, *J* = 7.9 Hz, 2H), 6.86 (d, *J* = 8.2 Hz, 2H), 6.67 (d, *J* = 8.4 Hz, 2H), 6.60 (d, *J* = 15.8 Hz, 1H), 4.73 (t, *J* = 5.4 Hz, 1H), 3.45 (d, *J* = 5.9 Hz, 2H), 3.22 (q, *J* = 5.8 Hz, 2H), 2.70 (s, 1H), 2.64 (s, 1H), 1.94 (s, 2H), 1.79 (s, 10H). ¹³C NMR (126 MHz, DMSO-*d*₆) δ 165.79, 156.49, 146.19, 144.77, 138.88, 133.38, 133.31, 130.87, 130.34, 130.31, 127.92, 122.39, 115.59, 60.58, 42.35, 37.19, 34.67, 34.54, 28.18. HRMS (ESI) calcd for C₂₈H₃₂NO₃ (M+H⁺) 430.2382, found 430.2374.

(E)-3-(4-(((1*r*,3*r*,5*R*,7*S*)-Adamantan-2-ylidene)(4-hydroxyphenyl)methyl)phenyl)-N-(3-hydroxypropyl)acrylamide (21)—Following the general procedure for amide synthesis between **5** and 3-amino-1-propanol, compound **21** was obtained as white solid

(Yield 74%, mp 155 °C). ¹H NMR (500 MHz, DMSO-*d*₆) δ 7.44 (d, *J* = 8.1 Hz, 2H), 7.35 (d, *J* = 15.7 Hz, 1H), 7.07 (d, *J* = 8.1 Hz, 2H), 6.85 (d, *J* = 8.4 Hz, 2H), 6.66 (d, *J* = 8.5 Hz, 2H), 6.54 (d, *J* = 15.8 Hz, 1H), 4.46 (t, *J* = 5.1 Hz, 1H), 3.41 (q, *J* = 6.0 Hz, 2H), 3.19 (q, *J* = 6.7 Hz, 2H), 2.69 (s, 1H), 2.64 (s, 1H), 1.94 (s, 2H), 1.79 (s, 10H), 1.58 (p, *J* = 6.5 Hz, 2H). ¹³C NMR (126 MHz, DMSO-*d*₆) δ 165.67, 156.48, 146.19, 144.76, 138.80, 133.37, 133.30, 130.87, 130.34, 130.30, 127.91, 122.33, 115.59, 59.09, 37.19, 36.59, 34.67, 34.54, 33.11, 28.18. HRMS (ESI) calcd for C₂₉H₃₄NO₃ (M+H⁺) 444.2540, found 444.2533.

(E)-3-(4-(((1*r*,3*r*,5*R*,7*S*)-Adamantan-2-ylidene)(4-hydroxyphenyl)methyl)phenyl)-N-(4-hydroxybutyl)acrylamide (22)—Following the general procedure for amide synthesis between **5** and 4-amino-1-butanol, compound **22** was obtained as white solid (Yield 72%, mp 250 °C). ¹H NMR (500 MHz, DMSO-*d*₆) δ 8.06 (t, *J* = 5.6 Hz, 1H), 7.44 (d, *J* = 8.2 Hz, 2H), 7.35 (d, *J* = 15.7 Hz, 1H), 7.07 (d, *J* = 8.2 Hz, 2H), 6.85 (d, *J* = 8.5 Hz, 2H), 6.67 (d, *J* = 8.5 Hz, 2H), 6.55 (d, *J* = 15.8 Hz, 1H), 3.38 (q, *J* = 5.6 Hz, 2H), 3.14 (q, *J* = 6.4 Hz, 2H), 2.69 (s, 1H), 2.64 (s, 1H), 1.94 (s, 2H), 1.79 (s, 11H), 1.52 – 1.35 (m, 4H). ¹³C NMR (126 MHz, DMSO-*d*₆) δ 165.50, 146.19, 144.74, 138.77, 133.38, 133.31, 130.87, 130.34, 130.31, 127.90, 122.41, 115.57, 61.09, 39.28, 37.19, 34.67, 34.54, 30.66, 28.18, 26.53. HRMS (ESI) calcd for C₂₉H₃₄NO₃ (M+H⁺) 458.2699, found 458.2690.

(E)-3-(4-(((1*r*,3*r*,5*R*,7*S*)-Adamantan-2-ylidene)(4-hydroxyphenyl)methyl)phenyl)-N-(5-hydroxypentyl)acrylamide (23)—Following the general procedure for amide synthesis between **5** and 5-amino-1-pentanol, compound **23** was obtained as white solid (Yield 78%, mp 180 °C). ¹H NMR (500 MHz, DMSO-*d*₆) δ 8.05 (t, *J* = 5.6 Hz, 1H), 7.44 (d, *J* = 8.2 Hz, 2H), 7.34 (d, *J* = 15.7 Hz, 1H), 7.07 (d, *J* = 8.2 Hz, 2H), 6.85 (d, *J* = 8.5 Hz, 2H), 6.66 (d, *J* = 8.5 Hz, 2H), 6.54 (d, *J* = 15.8 Hz, 1H), 4.35 (t, *J* = 5.1 Hz, 1H), 3.40 – 3.35 (m, 2H), 3.13 (q, *J* = 6.7 Hz, 2H), 2.69 (s, 1H), 2.64 (s, 1H), 1.94 (s, 2H), 1.79 (s, 11H), 1.41 (dq, *J* = 14.7, 7.0, 6.6 Hz, 5H), 1.29 (q, *J* = 8.4 Hz, 2H). ¹³C NMR (126 MHz, DMSO-*d*₆) δ 165.49, 156.48, 146.19, 144.74, 138.76, 133.38, 133.31, 130.88, 130.33, 130.31, 127.91, 122.41, 115.59, 61.29, 39.41, 37.19, 34.67, 34.54, 32.91, 29.74, 28.17, 23.74. HRMS (ESI) calcd for C₃₁H₃₈NO₃ (M+H⁺) 472.2852, found 472.2848.

(E)-3-(4-(((1*r*,3*r*,5*R*,7*S*)-Adamantan-2-ylidene)(4-hydroxyphenyl)methyl)phenyl)-N-(2-aminoethyl)acrylamide (24)—Following the general procedure for amide synthesis between **5** and N-Boc-ethylenediamine, and then following the general procedure for Boc deprotection, compound **24** was obtained as brown solid (Yield 46%, mp 163 °C). ¹H NMR (500 MHz, Acetone-*d*₆) δ 7.53 (d, *J* = 15.7 Hz, 1H), 7.48 (d, *J* = 8.1 Hz, 3H), 7.15 (d, *J* = 8.2 Hz, 2H), 6.96 (d, *J* = 11.3 Hz, 2H), 6.77 (d, *J* = 9.3 Hz, 2H), 6.64 (d, *J* = 15.7 Hz, 1H), 3.40 – 3.33 (m, 2H), 3.12 (s, 2H), 2.80 (s, 1H), 2.74 (s, 1H), 1.98 (s, 2H), 1.85 (s, 11H). ¹³C NMR (126 MHz, Acetone-*d*₆) δ 146.46, 141.15, 133.83, 132.90, 130.69, 130.12, 127.72, 120.03, 115.06, 39.45, 39.42, 37.04, 34.73, 34.61, 28.37. HRMS (ESI) calcd for C₂₈H₃₃N₂O₂ (M+H⁺) 429.2542, found 429.2531.

(E)-3-(4-(((1*r*,3*r*,5*R*,7*S*)-Adamantan-2-ylidene)(4-hydroxyphenyl)methyl)phenyl)-N-(3-aminopropyl)acrylamide (25)—Following the general procedure for amide synthesis between **5** and N-Boc-1,3-propanediamine, and then following the general

procedure for Boc deprotection, compound **25** was obtained as yellow solid (Yield 42%, mp 187 °C). ¹H NMR (500 MHz, DMSO-*d*₆) δ 7.45 (dd, *J* = 8.1, 5.3 Hz, 2H), 7.36 (dd, *J* = 15.7, 13.1 Hz, 1H), 7.07 (dd, *J* = 8.2, 3.8 Hz, 2H), 6.85 (d, *J* = 11.1 Hz, 2H), 6.67 (d, *J* = 8.5 Hz, 2H), 6.55 (dd, *J* = 15.8, 2.3 Hz, 1H), 3.25 – 3.17 (m, 2H), 3.12 (s, 1H), 2.72 (s, 2H), 2.64 (s, 1H), 1.94 (s, 2H), 1.79 (s, 11H), 1.72 – 1.62 (m, 2H). ¹³C NMR (126 MHz, DMSO-*d*₆) δ 165.98, 165.57, 156.48, 146.19, 144.75, 138.74, 133.38, 133.29, 130.86, 130.31, 127.98, 127.91, 122.41, 122.03, 115.59, 48.91, 37.18, 34.67, 34.54, 31.38, 31.06, 28.17. HRMS (ESI) calcd for C₂₉H₃₅N₂O₂ (M+H⁺) 443.2699, found 443.2689.

(E)-3-(4-(((1*r*,3*r*,5*R*,7*S*)-Adamantan-2-ylidene)(4-hydroxyphenyl)methyl)phenyl)-N-(4-aminobutyl)acrylamide (26)—Following the general procedure for amide synthesis between **5** and N-Boc-1,4-butanediamine, and then following the general procedure for Boc deprotection, compound **26** was obtained as yellow solid (Yield 45%, mp 191 °C). ¹H NMR (500 MHz, DMSO-*d*₆) δ 7.44 (t, *J* = 7.5 Hz, 2H), 7.36 (d, *J* = 15.7 Hz, 1H), 7.08 (d, *J* = 8.2 Hz, 2H), 6.86 (d, *J* = 8.4 Hz, 2H), 6.67 (d, *J* = 8.2 Hz, 2H), 6.56 (d, *J* = 15.8 Hz, 1H), 3.56 (t, *J* = 7.0 Hz, 1H), 3.19 (t, *J* = 6.7 Hz, 2H), 2.70 (s, 1H), 2.64 (s, 1H), 2.35 – 2.24 (m, 2H), 1.94 (s, 2H), 1.80 (s, 10H), 1.63 (d, *J* = 7.3 Hz, 2H), 1.51 (t, *J* = 10.8 Hz, 2H). ¹³C NMR (126 MHz, DMSO-*d*₆) δ 164.78, 156.52, 146.14, 144.56, 138.10, 133.56, 133.30, 130.83, 130.37, 130.27, 127.80, 123.86, 115.61, 110.00, 51.53, 41.69, 36.73, 35.80, 34.55, 29.49, 28.98, 28.18. HRMS (ESI) calcd for C₃₀H₃₇N₂O₂ (M+H⁺) 457.2855, found 457.2847.

(E)-3-(4-(((1*r*,3*r*,5*R*,7*S*)-Adamantan-2-ylidene)(4-hydroxyphenyl)methyl)phenyl)-N-(2-(dimethylamino)ethyl)acrylamide (27)—Following the general procedure for amide synthesis between **5** and N,N-dimethylethylenediamine, compound **27** was obtained as white solid (Yield 75%, mp 217 °C). ¹H NMR (500 MHz, DMSO-*d*₆) δ 8.00 (t, *J* = 5.6 Hz, 1H), 7.45 (d, *J* = 8.1 Hz, 2H), 7.35 (d, *J* = 15.7 Hz, 1H), 7.07 (d, *J* = 7.9 Hz, 2H), 6.85 (d, *J* = 8.2 Hz, 2H), 6.67 (d, *J* = 8.4 Hz, 2H), 6.63 – 6.55 (m, 1H), 3.25 (q, *J* = 6.3 Hz, 2H), 2.70 (s, 1H), 2.64 (s, 1H), 2.32 (t, *J* = 6.5 Hz, 2H), 2.14 (s, 6H), 1.93 (s, 2H), 1.79 (s, 10H). ¹³C NMR (126 MHz, DMSO-*d*₆) δ 165.61, 156.50, 146.19, 144.78, 138.90, 133.40, 133.31, 130.87, 130.36, 130.30, 127.94, 122.37, 115.60, 58.92, 45.83, 37.50, 37.20, 34.57, 28.20. HRMS (ESI) calcd for C₃₀H₃₇N₂O₂ (M+H⁺) 457.2855, found 457.2864.

(E)-3-(4-(((1*r*,3*r*,5*R*,7*S*)-Adamantan-2-ylidene)(4-hydroxyphenyl)methyl)phenyl)-N-(2,2,2-trifluoroethyl)acrylamide (28)—Following the general procedure for amide synthesis between **5** and 2,2,2-trifluoroethylamine, compound **28** was obtained as yellow solid (Yield 65%, mp 207-209 °C). ¹H NMR (500 MHz, DMSO-*d*₆) δ 9.32 (s, 1H), 8.72 (t, *J* = 6.4 Hz, 1H), 7.51 – 7.44 (m, 3H), 7.09 (d, *J* = 8.2 Hz, 2H), 6.86 (d, *J* = 8.5 Hz, 2H), 6.72 – 6.58 (m, 3H), 4.08 – 3.95 (m, 2H), 2.70 (s, 1H), 2.64 (s, 1H), 1.95 (s, 2H), 1.80 (s, 10H). ¹³C NMR (126 MHz, DMSO-*d*₆) δ 146.46, 141.15, 133.83, 132.90, 130.69, 130.12, 127.72, 120.03, 115.06, 39.45, 39.42, 37.04, 34.73, 34.61, 28.37. HRMS (ESI) calcd for C₂₉H₃₄NO₂F₃ (M+H⁺) 468.2150, found 468.2144.

(E)-3-(4-(((1*r*,3*r*,5*R*,7*S*)-Adamantan-2-ylidene)(4-hydroxyphenyl)methyl)phenyl)-N-butyl-N-methylacrylamide (29)—Following the general procedure for amide

synthesis between **5** and N-methylbutylamine, compound **29** was obtained as white solid (Yield 66%, mp 225-228 °C). ¹H NMR (500 MHz, DMSO-*d*₆) δ 9.32 (s, 1H), 7.65 – 7.51 (m, 2H), 7.41 (dd, *J* = 15.3, 11.9 Hz, 1H), 7.11 – 7.02 (m, 3H), 6.85 (d, *J* = 11.0 Hz, 2H), 6.67 (d, *J* = 8.5 Hz, 2H), 3.47 (t, *J* = 7.3 Hz, 1H), 3.34 (d, *J* = 7.8 Hz, 1H), 3.09 (s, 1H), 2.88 (s, 2H), 2.71 (s, 1H), 2.64 (s, 1H), 1.94 (s, 2H), 1.79 (s, 10H), 1.54 – 1.40 (m, 2H), 1.25 (dq, *J* = 14.6, 7.5 Hz, 2H), 0.87 (t, *J* = 7.3 Hz, 3H). ¹³C NMR (126 MHz, DMSO-*d*₆) δ 166.05, 165.87, 156.48, 146.15, 144.88, 141.34, 133.68, 133.63, 133.26, 130.88, 130.38, 130.15, 128.38, 119.00, 118.45, 115.57, 49.38, 47.53, 37.20, 35.61, 34.71, 34.52, 34.36, 31.51, 29.61, 28.18, 20.26, 19.96, 14.47. HRMS (ESI) calcd for C₃₁H₃₈NO₂ (M+H⁺) 456.2903, found 456.2904.

(E)-3-(4-(((1*r*,3*r*,5*R*,7*S*)-Adamantan-2-ylidene)(4-hydroxyphenyl)methyl)phenyl)-N-(2-hydroxyethyl)-N-methylacrylamide (30)—Following the general procedure for amide synthesis between **5** and 2-(methylamino)ethanol, compound **30** was obtained as white solid (Yield 71%, mp 219 °C). ¹H NMR (500 MHz, DMSO-*d*₆) δ 9.33 (s, 1H), 7.57 (dd, *J* = 15.0, 8.1 Hz, 2H), 7.40 (dd, *J* = 15.3, 9.1 Hz, 1H), 7.15 – 7.02 (m, 3H), 6.86 (d, *J* = 8.4 Hz, 2H), 6.67 (d, *J* = 8.4 Hz, 2H), 3.53 (s, 3H), 3.41 (t, *J* = 5.9 Hz, 1H), 3.16 (s, 1H), 2.92 (s, 2H), 2.71 (s, 1H), 2.64 (s, 1H), 1.94 (s, 2H), 1.79 (s, 10H). ¹³C NMR (126 MHz, DMSO-*d*₆) δ 166.47, 166.17, 156.47, 146.13, 144.91, 144.80, 140.81, 133.77, 133.25, 130.89, 130.38, 130.14, 128.40, 128.29, 119.24, 118.91, 115.57, 59.92, 59.40, 52.09, 37.20, 34.85, 34.73, 34.51, 28.18. HRMS (ESI) calcd for C₂₉H₃₄NO₃ (M+H⁺) 444.2539, found 444.2519.

(E)-3-(4-(((1*r*,3*r*,5*R*,7*S*)-Adamantan-2-ylidene)(4-hydroxyphenyl)methyl)phenyl)-N,N-bis(2-hydroxyethyl)acrylamide (31)—Following the general procedure for amide synthesis between **5** and diethanolamine, compound **31** was obtained as yellow solid (Yield 78%, mp 283 °C). ¹H NMR (499 MHz, DMSO-*d*₆) δ 7.57 (d, *J* = 8.1 Hz, 2H), 7.42 (d, *J* = 15.4 Hz, 1H), 7.14 – 7.03 (m, 3H), 6.87 (d, *J* = 8.2 Hz, 2H), 6.68 (d, *J* = 8.2 Hz, 2H), 4.84 (t, *J* = 5.3 Hz, 1H), 4.72 (t, *J* = 5.3 Hz, 1H), 3.64 – 3.48 (m, 6H), 3.44 (t, *J* = 6.0 Hz, 2H), 2.72 (s, 1H), 2.65 (s, 1H), 1.96 (s, 2H), 1.81 (s, 10H). ¹³C NMR (126 MHz, DMSO-*d*₆) δ 171.55, 166.46, 156.47, 153.20, 146.16, 144.84, 141.07, 134.92, 133.75, 133.26, 130.87, 130.37, 130.14, 128.31, 119.32, 115.58, 114.33, 60.52, 59.65, 54.22, 51.34, 50.01, 37.20, 34.72, 34.51, 28.18. HRMS (ESI) calcd for C₃₀H₃₆NO₄ (M+H⁺) 474.2644, found 474.2648.

III. Biological Methods

Cell cultures, reagents and ligands—17β-Estradiol (E2), 4-hydroxytamoxifen (4-OHT) and fulvestrant (ICI 182,780, Fulv) were from Sigma-Aldrich. Tritiated estradiol was obtained from Perkin Elmer and purified, full-length human estrogen receptor α from Invitrogen. MCF7 cells from the ATCC were maintained and cultured as described.⁵⁵ For some studies, cells were cultured in phenol-red free media supplemented with 5% charcoal stripped FBS for 5 days prior to experiments, to be in an E2-deprived condition. All cells were tested for mycoplasma using Real-Time PCR Mycoplasma Detection Kit (Akron Biotech, Boca Raton, FL).

Binding Assays—Competitive radiometric binding assays were performed on 96-well microtiter filter plates (Millipore), using full length human estrogen receptor α , with tritiated estradiol as tracer, as previously described.³² After incubation on ice for 18-24 h, ER α -bound tracer was absorbed onto hydroxyapatite (BioRad), washed with buffer, and measured by scintillation counting. RBA values are the average $\pm$ SD of 2-3 determinations.

Cell proliferation assay—WST-1 assay (Roche, Basel, Switzerland) was used to quantify cell viability after a 6-day exposure to compounds, as described.⁵⁶ Absorbance was measured at 450 nm using a VICTOR X5 PerkinElmer 2030 Multilabel Plate Reader, and cell proliferation values represent signal from compound-treated samples relative to vehicle-treated controls. All assays were performed in triplicate, and the values shown in Figure 4 are the average of 2-3 independent experiments.

In-cell western assay—Cells were cultured in 96-well plates at 3000 cells/well, and treated with compound for 24 h. Cells were washed twice in PBS, fixed with 4% formaldehyde (Fisher Scientific) solution in PBS, permeabilized in 0.1% Triton X-100 in PBS, blocked with Odyssey Blocking Buffer (LI-COR), and incubated with rabbit HC-20 ER α antibody (Santa Cruz, Cat# SC-543) at 4 °C overnight. Both IRDye 800 CW goat anti-rabbit secondary antibody (LI-COR, Cat# 926-32211) and Cell Tag 700 (LI-COR, Cat# 926-41090) were diluted (1:600) for incubation with cells. Plates were washed and ER α staining signals were quantified and normalized with Cell Tag signals using LI-COR Odyssey infrared imaging system. The ER α protein levels were calculated relative to the vehicle-treated samples. The values shown in Figure 4 are the average from at least three independent experiments.

Western blot analysis—Western blot was performed as described.⁵⁷ Briefly, whole-cell protein extracts were prepared using 1 $\times$ complete protease inhibitor and phosphatase inhibitor (Roche). Proteins were separated on 10% SDS-PAGE gels, transferred to nitrocellulose membranes and incubated with primary ER α antibody (F10, Santa Cruz Cat# 8002) and β -actin antibody (Sigma-Aldrich).

RNA isolation and real-time PCR—Total RNA was isolated using TRIzol (Invitrogen) and reverse transcribed using MMTV reverse transcriptase (New England BioLabs). Real-time PCR was performed using SYBRgreen PCR Master Mix (Roche) as described.⁵⁸ Relative mRNA levels of genes were normalized to the housekeeping gene 36B4, and fold change calculated relative to the vehicle-treated samples. Results are the average $\pm$ SD from at least two independent experiments carried out in triplicate.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Sources of Research Support: Grants from the Breast Cancer Research Foundation (BCRF082854 to BSK and JAK) and the National Institutes of Health (NIH DK015556 to JAK and NIH CA132022 and DK077085 to KWN).

Abbreviations

CP	cell proliferation
E2	estradiol
ERα	estrogen receptor alpha
FBS	fetal bovine serum
Fulv	fulvestrant (ICI 182,780)
GREB1	gene regulated by estrogen in breast cancer 1
GSK	GlaxoSmithKline
ICW	in-cell western
LBD	ligand binding domain
MTT assay	a cell viability assay
PgR	progesterone receptor
qPCR	quantitative polymerase chain reaction
SERD	selective estrogen receptor downregulator
SERM	selective estrogen receptor modulator
Tam	4-hydroxytamoxifen

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Figure 1.

Evolutionary Design of Antiestrogen Cores and Side Chains. Starting with the middle structures of the original SERMs and SERDs, we have taken the upward direction in this study (modifying the side chain on a simple adamantyl core, which is a tricyclic analog of cyclofenil, a well-known SERM); Genentech and AstraZeneca have taken the downward direction (modifying the core structure) to produce compounds **32** and **33**.

Figure 2.

Crystal Structure of GW-5638-ERα LBD Complex. **a.** Overlay of the ERα LBD complex with hydroxytamoxifen (OHT) (blue color for h12, OHT, and arrow) and with GW-5638 (yellow color for h12, GW, and arrow). The differing orientation of h12 is evident in the two structures. **b.** Detail showing the interaction of the carboxylate in GW-5638 with the N-terminus of h12. One of the interactions between these two units is mediated through a structural water molecule. (PDB code: 1R5K)

Figure 3.
Binding affinities of isostructural esters and amides terminated either with a hydrogen (open bars), a hydroxyl (stippled bars) or an amino group (dark bars).

Figure 4.

Star Plots of the Antiproliferative Efficacy (blue line) and ER α Downregulating Efficacies (red lines) of fulvestrant (Fulv), GW-7604, and Compounds **5-31**. All compounds tested at 3 μ M, and values plotted as percent of vehicle control. As noted in the text, GW-7604 is tested as an E/Z isomer mixture. **A.** Parent compounds, ketone, esters, and other functional groups (**5-15**). **B.** Primary and secondary amides (**16-28**). **C.** Comparison of secondary amides and structurally related tertiary or other amides (**18** vs. **29**; **20** vs. **30** and **31**; **24** vs. **27** and **28**). Except for Fulv and GW-7604, all compounds have the adamantyl core and are related to **5**. Note, the scale in Plot **A** is different from the scales in Plots **B** and **C**.

Figure 5. Dose Response Curves for Antiproliferative Activities of Selected Compounds. Compounds with high efficacy are **5**, **20**, and **21**, and low efficacy are **30** and **31**. IC_{50} values are extracted from this or similar experiments.

Figure 6.

ERα Downregulating Activities of Selected Compounds. (A) Dose-response results from in-cell Western blots and (B) Western blots. Cells were treated with Fulv, **5**, or **21** (at 3×10^{-10} M, 3×10^{-8} M, or 3×10^{-6} M) or with E2 (1×10^{-8} M) or Tam (3×10^{-6} M) for 24 h. Values as percent of vehicle are given below the blots. IC₅₀ values are extracted from the experiments in (A). Tam is 4-hydroxtamoxifen.

Figure 7. Dose-Response Suppression of E2-Stimulated Expression of (A) GREB1, (B) PgR and (C) pS2 Genes by Adamantyl Antiestrogen Ligands. Treatment was for 24 h at the concentrations indicated.

Figure 8. Correlation between Suppression of Cell Proliferation and ERα Downregulation. Values are measured at 3 μ M and are the same as in Figure 4. Dots are color coded by structural class of the acrylate functional group. Three compounds that had no anti-proliferative activity (**7**, **10**, and **14**) were omitted from this correlation. Significance was calculated from an F test for non-zero slope (GraphPad Prism).

Figure 9. Conformational Preferences of Esters, and Secondary and Tertiary Amides. Both esters and secondary amides prefer an extended conformation due to n-σ* or σ-σ* π-overlap, respectively. The tertiary amides have no such stereoelectronic preference.

A. Synthesis of compounds 6-11, 13-16

B. Synthesis of compounds 5, 10 and 12

Scheme 1.
Synthesis of compound 5-16

A. Secondary Amides

Comp. No.	R_2
17	$-(\text{CH}_2)_2\text{CH}_3$
18	$-(\text{CH}_2)_3\text{CH}_3$
19	$-(\text{CH}_2)_4\text{CH}_3$
20	$-(\text{CH}_2)_2\text{OH}$
21	$-(\text{CH}_2)_3\text{OH}$
22	$-(\text{CH}_2)_4\text{OH}$
23	$-(\text{CH}_2)_5\text{OH}$
24a	$-(\text{CH}_2)_2\text{NHBoc}$
25a	$-(\text{CH}_2)_3\text{NHBoc}$
26a	$-(\text{CH}_2)_4\text{NHBoc}$
27	$-(\text{CH}_2)_2\text{N}(\text{CH}_3)_2$
28	$-\text{CH}_2\text{CF}_3$

Comp. No.	R_3
24	$-(\text{CH}_2)_2\text{NH}_2$
25	$-(\text{CH}_2)_3\text{NH}_2$
26	$-(\text{CH}_2)_4\text{NH}_2$

B. Tertiary Amides

Comp. No.	R_4	R_5
29	$-\text{CH}_3$	$-(\text{CH}_2)_3\text{CH}_3$
30	$-\text{CH}_3$	$-(\text{CH}_2)_2\text{OH}$
31	$-(\text{CH}_2)_2\text{OH}$	$-(\text{CH}_2)_2\text{OH}$

Scheme 2.
Synthesis of amides **17-31**.

Table 1

Relative Binding Affinities of Adamantyl Compounds and GW-7604 for full-length human estrogen receptor alpha (ER α).

Compd #	R	RBA ER α (%) ^a	K _i (nM) ^a
	Reference compounds		
	Estradiol	(100)	0.2 (K _d value)
	GW-7604 ^b	20 $\pm$ 6	1.0 $\pm$ 0.3
	Adamantyl Compounds		
	Acid		
5	-COOH	51 $\pm$ 12	0.40 $\pm$ 0.09
	Ketone		
6	-CO(CH ₂) ₄ CH ₃	12 $\pm$ 2	1.7 $\pm$ 0.3
	Esters		
7	-COOCH ₃	46 $\pm$ 14	0.43 $\pm$ 0.13
8	-COOCH ₂ CH ₃	13 $\pm$ 1	3.3 $\pm$ 0.3
9	-COO(CH ₂) ₃ CH ₃	11 $\pm$ 2	1.8 $\pm$ 0.3
10	-COO(CH ₂) ₅ CH(CH ₃) ₂	2.8 $\pm$ 0.4	7.1 $\pm$ 1.0
11	-COO(CH ₂) ₂ OH	54 $\pm$ 11	0.37 $\pm$ 0.08
12	-COO(CH ₂) ₃ OH	28 $\pm$ 2	0.71 $\pm$ 0.05
13	-COO(CH ₂) ₄ OH	44 $\pm$ 9	0.45 $\pm$ 0.09
	Other groups		
14	-CN	14 $\pm$ 4	1.4 $\pm$ 0.4
15	-SO ₂ NH(CH ₂) ₂ CH ₃	52 $\pm$ 1	0.38 $\pm$ 0.01
	Amides		
16	-CONH ₂	71 $\pm$ 18	0.27 $\pm$ 0.07
17	-CONH(CH ₂) ₂ CH ₃	18 $\pm$ 0.1	1.1 $\pm$ 0.0
18	-CONH(CH ₂) ₃ CH ₃	28 $\pm$ 3	0.71 $\pm$ 0.08
19	-CONH(CH ₂) ₄ CH ₃	27 $\pm$ 7	0.74 $\pm$ 0.18
20	-CONH(CH ₂) ₂ OH	109 $\pm$ 17	0.18 $\pm$ 0.03
21	-CONH(CH ₂) ₃ OH	47 $\pm$ 8	0.43 $\pm$ 0.07
22	-CONH(CH ₂) ₄ OH	56 $\pm$ 12	0.36 $\pm$ 0.08
23	-CONH(CH ₂) ₅ OH	31 $\pm$ 7	0.65 $\pm$ 0.15
24	-CONH(CH ₂) ₂ NH ₂	35 $\pm$ 9	0.57 $\pm$ 0.15
25	-CONH(CH ₂) ₃ NH ₂	54 $\pm$ 7	0.37 $\pm$ 0.05
26	-CONH(CH ₂) ₄ NH ₂	62 $\pm$ 1	0.32 $\pm$ 0.0
27	-CONH(CH ₂) ₂ N(CH ₃) ₂	57 $\pm$ 14	0.35 $\pm$ 0.09
28	-CONHCH ₂ CF ₃	15 $\pm$ 3	1.33 $\pm$ 0.27

Compd #	R	RBA ER α (%) ^a	K _i (nM) ^a
29	-CON(CH ₃)(CH ₂ CH ₂ CH ₂ CH ₃)	28 ± 8	0.71 ± 0.20
30	-CON(CH ₃)(CH ₂ CH ₂ OH)	54 ± 12	0.37 ± 0.08
31	-CON(CH ₂ CH ₂ OH) ₂	115 ± 33	0.13 ± 0.04

^aRelative binding affinity values, determined by a competitive radiometric binding assay with [³H]estradiol and purified, full-length human ER α are reported as percent relative to E₂ = 100%, and expressed as the mean ± SD of 2 or more independent experiments. K_i values were calculated from the formula: K_i = (K_d[estradiol]/RBA) × 100. The K_d for estradiol is 0.2 nM.

^bAs noted in the text, GW-7604 was tested as an E/Z isomer mixture.