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Synthesis and Evaluation of Derrubone and Select Analogues

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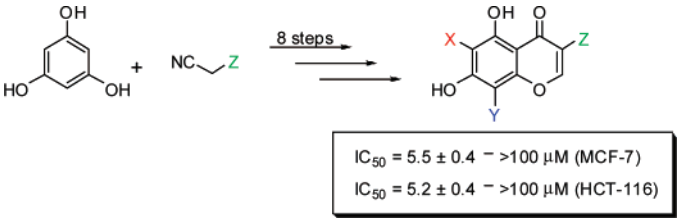

Recently, we reported that the natural product derrubone exhibits Hsp90 inhibitory activity. Due to its unique architectural scaffold and proposed rapid assembly, the synthesis of this natural product was pursued with the aim of identifying structure−activity relationships. Synthesis of the natural product was accomplished in eight highly convergent steps, which led to a facile method for the construction of related compounds. Biological evaluation of derrubone and its analogues identified several compounds that exhibit low micromolar inhibitory activity against breast and colon cancer cell lines.

Introduction

The 90 kDa heat shock proteins (Hsp90) continue to emerge as promising chemotherapeutic targets for the development of anti-tumor agents.[1] As a molecular chaperone, Hsp90 is responsible for the maturation of nascent polypeptides into their biologically active, three-dimensional structures.[2,3] This protein folding process is dependent upon the hydrolysis of ATP, which provides the energy necessary for conformational reorientation of the polypeptide substrate and client protein release.[4] Studies have revealed two nucleotide binding pockets in the Hsp90 protein, one at the N-terminus and the other in the C-terminal domain.[5,6] Only the N-terminal ATP binding pocket manifests ATPase activity, while the C-terminus appears to exhibit allosteric properties.[7]

Although the N-terminal ATP binding site is the only pocket that manifests ATPase activity, inhibitors of both nucleotide binding pockets induce the degradation of client proteins dependent upon the Hsp90 protein folding machinery. In collaboration with the Matts research laboratory, we recently reported an assay that was capable of identifying inhibitors of both the N- and C-terminal ATP binding pockets.[8] This assay was optimized to identify small molecules that prohibit the maturation and activation of firefly luciferase in rabbit reticulocyte. High-throughput screening of a small library of natural products with this assay resulted in the identification of derrubone as an Hsp90 inhibitor. Subsequent studies in vitro determined that derrubone exhibits a novel mechanism of action for Hsp90 inhibition.[9]

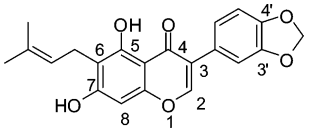

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FIGURE 1. Derrubone.

Derrubone (Figure 1) is a prenylated isoflavone that was first isolated from *Derris robusta* in 1972.[10] To date, no biological activity has been reported for this molecule, and only two

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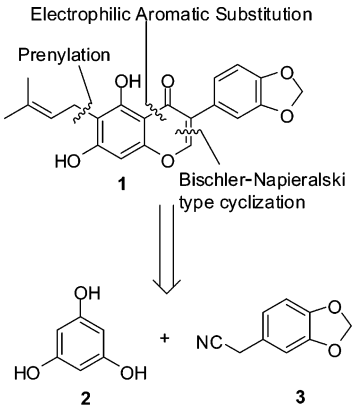
synthetic routes have been disclosed, both of which required 14 linear steps.[11,12] Therefore, our goal was to provide a succinct synthetic protocol for this natural product from which the preparation of analogues could be easily accomplished and structure−activity relationships revealed.

Results and Discussion

Synthesis of Derrubone. Retrosynthetic analysis of derrubone presented three primary disconnections that provided ample opportunity for diversification without a lengthy protection−deprotection strategy (Scheme 1). Installation of the aryl ketone moiety via electrophilic aromatic substitution followed by cyclization allowed access to the isoflavone core in three steps from commercially available starting materials. Literature precedence has shown that a variety of nitriles can serve as precursors to the aryl ketone, thus allowing for introduction of structural diversity.[13,14]

SCHEME 1. Retrosynthetic Analysis of Derrubone

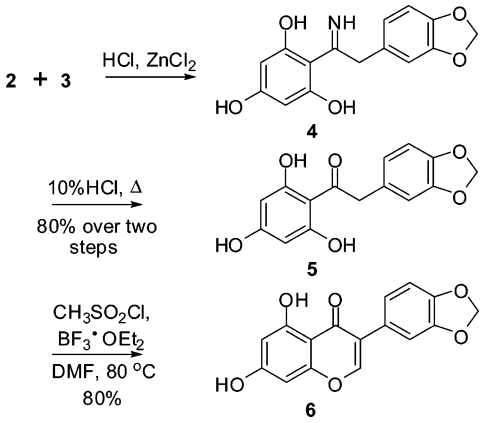


From the isoflavone core, two routes were proposed for attachment of the prenyl side chain: (1) nucleophilic addition to 1-bromo-3-methyl-2-butene, or (2) Claisen rearrangement and subsequent olefin cross-metathesis. Both routes were envisioned to produce derrubone via a succinct method that is amenable to a diversity-oriented protocol for the rapid assembly of derrubone analogues and elucidation of the first structure−activity relationships.

In practice, the isoflavone backbone was obtained in three steps from (3,4-methylenedioxy)phenylacetonitrile via electrophilic aromatic substitution enlisting a zinc chloride catalyst. Subsequent hydrolysis of the resulting imine (**4**) afforded phenyl ketone **5** in good yield (Scheme 2). Utilizing the cyclization procedure reported by Pelter and Foot,[15] the isoflavone scaffold (**6**) was obtained in excellent yield upon exposure to DMF under Bischler−Napieralski-type conditions[16] without protection of the free phenols.

Direct prenylation at the 6-position of the isoflavone backbone proved problematic as the 7-*O*-prenyl derivative was the predominant product obtained under various conditions. To circumvent formation of the undesired O-alkylated products,

SCHEME 2. Synthesis of the Isoflavone Core



we followed a procedure recently reported by our laboratory[17] that allowed for C-prenylation of phenols. Following this protocol, the 7-hydroxyl of **6** was selectively allylated with allyl bromide in the presence of potassium carbonate in a solution of DMF (Scheme 3). Subsequent Claisen rearrangement led to a 3:1 mixture of regioisomers, **8** and **9**, with the unnatural regioisomer **8** predominating. The regioisomers were inseparable at this stage via flash chromatography. Therefore, the hydroxyl groups of the crude mixture were masked as acetates **10** and **11**, and the prenyl group was introduced via cross-metathesis with 2-methyl-2-butene using Grubbs' second generation catalyst.[18] Hydrolysis of acetates **12** and **13** afforded the unnatural 8-prenyl isomer **14** of derrubone in good yield. Unfortunately, the yield of derrubone was less than desirable (<5%) and required investigation of alternate methods for regioselective prenylation of the aromatic ring.

To avoid formation of the undesired regioisomer, the 7-hydroxyl of **6** was protected as the methoxy methyl ether to afford phenol **15** (Scheme 4). Subsequent allylation of the free phenol followed by Claisen rearrangement at 160 °C gave the desired 6-allyl isoflavone **16** in good yield. As before, cross-metathesis with 2-methyl-2-butene provided the prenyl moiety. Completion of the natural product was ultimately accomplished upon hydrolysis of the methoxymethyl ether to give derrubone in eight linear steps and overall yield of 16%.

Synthesis of Derrubone Analogues. Upon completion of the natural product, the synthetic route was utilized for the preparation of analogues with the purpose of revealing structure−activity relationships (Figure 2). In choosing constituents of the library, we aimed to probe both the size and nature of the binding pocket into which the 3-aryl side chain projects.

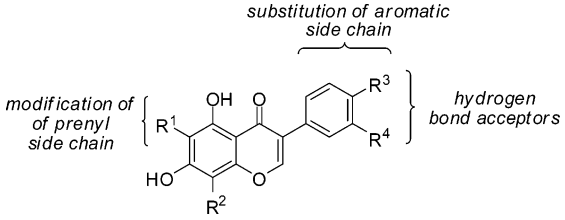


FIGURE 2. A Library of derrubone analogues.

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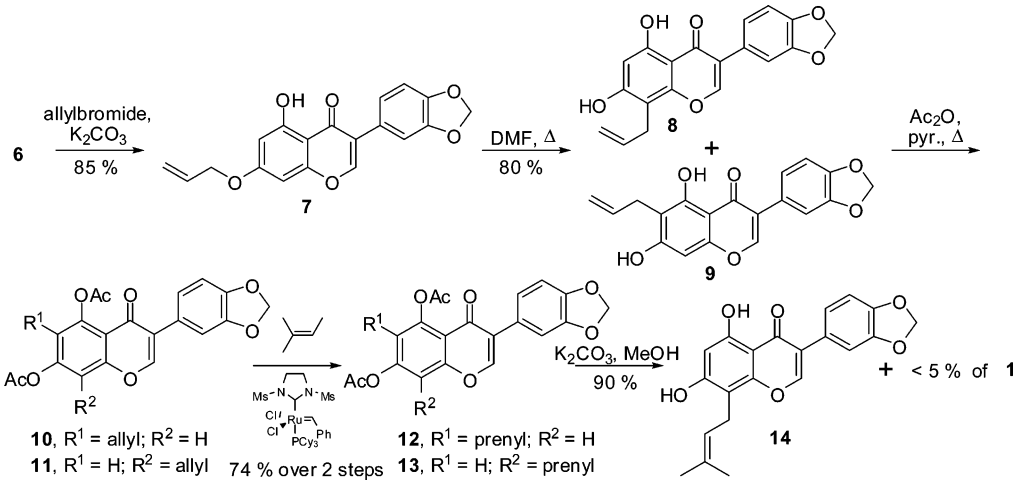
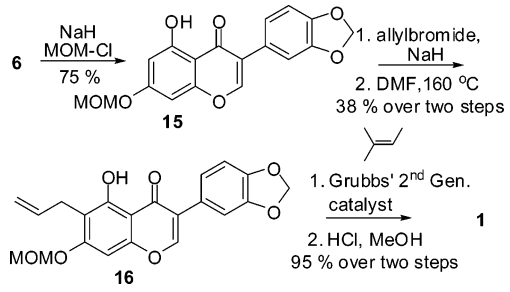
(13) Hakala, U.; Waehaelae, K. *Tetrahedron Lett.* **2006**, *47*, 8375−8378.
(14) Bolek, D.; Guetschow, M. *J. Heterocycl. Chem.* **2005**, *42*, 1399−1403.
(15) Pelter, A.; Foot, S. *Synthesis* **1976**, 326.
(16) Bischler, A.; Napieralski, B. *Ber.* **1893**, *26*, 1891−1903.
(17) Burlison, J. A.; Neckers, L.; Smith, A. B.; Maxwell, A.; Blagg, B. S. J. *J. Am. Chem. Soc.* **2006**, *128*, 15529−15536.
(18) Scholl, M.; Ding, S.; Lee, C. W.; Grubbs, R. H. *Org. Lett.* **1999**, *1*, 953−956.

SCHEME 3. Synthesis of the 8-Prenyl Regioisomer



SCHEME 4. Completed Synthesis of Derrubone



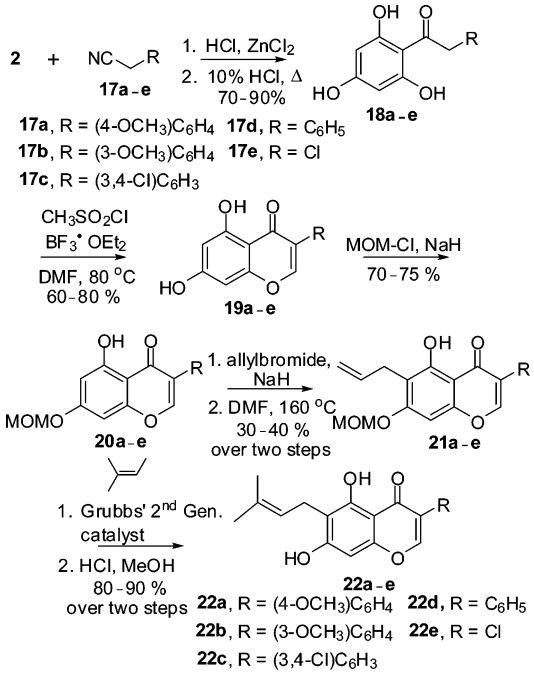
To explore the necessity of having an aromatic substituent at C3, we proposed replacement of the aryl group with chlorine. In addition, it is unknown whether one or both of the oxygen atoms of the dioxolane serve as hydrogen-bond acceptors with the putative binding pocket. To elucidate the role of the 3′- and 4′-oxygen atoms, a series of analogues were sought beginning with an unsubstituted phenyl side chain and extending to a 3′,4′-dichloro analogue. Substitution of the prenyl side chain was proposed to explore the effects of C6 modification. As proposed, alteration of the synthetic route was not required to obtain the desired analogues.

Beginning from commercially available nitriles, aryl ketones **18a**−**e** were obtained in good yields (Scheme 5). Cyclization under Bischler−Napieralski conditions as described earlier also worked well for these substrates and gave isoflavones **19a**−**e**, which were subsequently protected before O-allylation. Claisen rearrangement of the allylic ethers gave the C-allylated products, which underwent olefin cross-metathesis in the presence of Grubbs' second generation catalyst and 2-methyl-2-butene to afford the prenyl side chain. Removal of the methoxy methyl ether afforded analogues **22a**−**e** in overall yields comparable to that of the natural product.

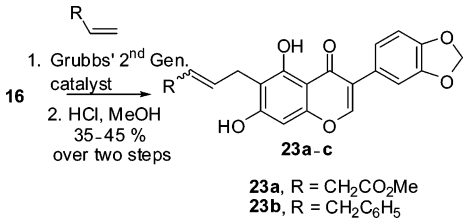
Replacement of the prenyl moiety was readily accomplished via cross-metathesis between **16** and the requisite olefin (Scheme 6). Subsequent hydrolysis of the methoxy methyl ether as described previously afforded analogues **23a**,**b** (35−45%).

Growth Inhibitory Activity by Derrubone and Analogues. Data shown in Table 1 present the anti-proliferative activity of derrubone and analogues against MCF-7 (breast) and HCT-116 (colon) cancer cell lines. The structure−activity relationships revealed that modification at C6 has a dramatic effect on inhibitory activity. When the prenyl moiety is removed or

SCHEME 5. Synthesis of C3 Analogues



SCHEME 6. Synthesis of C6 Analogues



replaced by a more polar functionality such as **6** or **23a**, respectively, activity is significantly decreased. When a lipophilic side chain is present in this position as in compounds **9** and **23b**, anti-proliferative activity is comparable to that of the natural product. Interestingly, when the prenyl side chain is transposed from the 6-position to the 8-position (**14**), a small increase in activity was observed against both cell lines.

Replacement of the aromatic ring at C3 with a chlorine atom (**22e**) resulted in almost complete loss of activity (IC_{50} = 91.6 μM in MCF-7 and IC_{50} >100 μM in HCT-116 cells). Removal

of the 3′,4′-methylenedioxy moiety (**22d**) did not result in substantial effects in cell growth assays. However, replacement of the aromatic side chain with the 3′,4′-dichloro analogue resulted in increased anti-proliferative activity, suggesting that electron-withdrawing substituents at these positions could improve activity. Interestingly, substitution at either C3′ or C4′ of the aromatic side chain greatly influenced activity. The 4′-methoxy analogue (**22a**) demonstrated a 2-fold increase in inhibitory activity versus derrubone, whereas a single C3′-methoxy (**22b**) completely abolished anti-proliferation activity at concentrations below 100 μM. When considered in tandem with the data obtained from the unsubstituted phenyl analogue (entry **22d**), these results clearly suggest that substitution at C3′ is detrimental to anti-proliferative activity, while C4′ substitution is beneficial but not essential.

TABLE 1. Anti-Proliferative Activities and Derrubone and Analogues

entry (IC_{50}, μM)	MCF-7	HCT-116
6	>100[a]	>100
9	14.1 ± 1.7	14.2 ± 1.8
14	10.6 ± 0.7	10.2 ± 0.8
22a	5.5 ± 0.4	7.3 ± 1.8
22b	>100	>100
22c	7.3 ± 2.5	5.2 ± 0.4
22d	12.3 ± 1.2	13.9 ± 1.3
22e	91.6 ± 1.1	>100
23a	62.6 ± 4.1	55.5 ± 11.3
23b	12.6 ± 0.9	10.8 ± 0.9
derrubone	11.9 ± 0.6	13.7 ± 3.4

[a] Values represent mean ± standard error for at least two separate experiments performed in triplicate.

On the basis of the results obtained from anti-proliferative studies, two compounds were chosen that contained modifications at each location, **14** and **22c**, and tested for their ability to induce Hsp90 client protein degradation (Figure 3). Both compounds caused a decrease in Her2 and Raf levels, two well-characterized proteins that depend upon Hsp90. Since actin is not a Hsp90 substrate, it was used as a negative control to verify that all protein levels were not affected by these inhibitors.

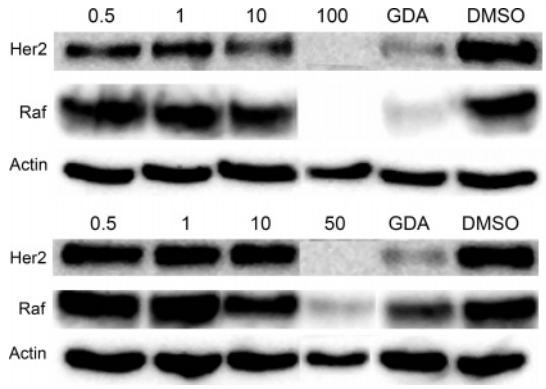


FIGURE 3. Western blot analysis of **14** (top) and **22c** (bottom) in MCF-7 cells (μM). Geldanamycin (500 nM) was used as the positive control.

Conclusion

In conclusion, we have completed the total synthesis of derrubone in eight steps and an overall yield of 16%. In addition, we have successfully applied this synthetic strategy toward the preparation of derrubone analogues in an effort to elucidate structure−function relationships for derrubone via evaluation against breast and colon cancer cell lines. Anti-proliferative data from two distinct cancer cell lines were obtained, identifying several compounds that exhibit increased activity over the natural product. In addition, these two compounds induced the degradation of Hsp90-dependent client proteins in human breast cancer cells, supporting their Hsp90 inhibitory activity in vitro. Continued synthesis of derrubone analogues is currently underway to identify compounds that exhibit optimal inhibitory activity.

Experimental Section

2-(2-(Benzo[*d*][1,3]dioxol-5-yl)-1-iminoethyl)benzene-1,3,5-triol (4).[12] A solution of phloroglucinol (5 g, 39.7 mmol) and (3,4-methylenedioxy)phenylacetonitrile (7 g, 43.6 mmol) in anhydrous Et_2O was prepared under an Ar atmosphere before zinc(II) chloride (1.08 g, 7.9 mmol) was added to the mixture. The solution was cooled to 0 °C. HCl gas was bubbled through the reaction mixture for 10 min. The reaction was allowed to slowly warm to rt and was stirred for 14 h. The solvent was decanted from the orange oil. The residue was rinsed with cold Et_2O then dissolved in EtOAc. The organic layer was washed with saturated aqueous $NaHCO_3$, dried (Na_2SO_4), filtered, and evaporated to give **4** (9.7 g, 85%) as a yellow, amorphous solid: ^{1}H NMR (DMSO-d_6, 400 MHz) δ 11.64 (s, 1H), 11.40 (br s, 1H), 10.73 (s, 1H), 6.85 (d, 2H, J = 7.96 Hz), 6.81 (s, 1H), 6.03 (s, 2H), 5.98 (s, 2H), 4.42 (s, 2H) ppm.

2-(Benzo[*d*][1,3]dioxol-5-yl)-1-(2,4,6-trihydroxyphenyl)ethanone (5).[12] Compound **4** (9.7 g, 33.7 mmol) was suspended in aqueous HCl (50% v/v, 100 mL). The suspension was warmed to reflux and stirred for 3 h. The resulting solid was filtered and washed with water and cold Et_2O to afford **5** (9.2 g, 95%) as a white powder: ^{1}H NMR (DMSO-d_6, 400 MHz) δ 12.22 (s, 1H), 10.40 (s, 1H), 6.83 (s, 1H), 6.80 (d, 1H, J = 5.32 Hz), 6.68 (d, 1H, J = 7.92 Hz), 5.97 (s, 2H), 5.82 (s, 2H), 4.25 (s, 2H) ppm; ^{13}C (DMSO-d_6, 125 MHz) δ 202.6, 164.8, 164.5, 146.9 (2C), 145.7 (2C), 129.5, 122.6, 109.8, 107.9, 103.6, 100.6, 94.7, 48.5 ppm; IR (film) ν_{max} 3297, 3202, 3082, 3026, 2920, 2853, 1655, 1612, 1502, 1371, 1323, 1250, 1197, 1036 cm^{-1}; HRMS (ESI+) m/z 289.0700 ([M + H]$^+$, $C_{15}H_{13}O_6$, requires 289.0712).

3-(Benzo[*d*][1,3]dioxol-5-yl)-5,7-dihydroxy-4*H*-chromen-4-one (6).[12,19] Boron trifluoride etherate (1.7 mL, 2 g, 13.9 mmol) was added dropwise to a solution of **5** (1 g, 3.5 mmol) in anhydrous DMF (11 mL) under an Ar atmosphere at rt. The solution was stirred for 10 min before the dropwise addition of CH_3SO_2Cl (0.8 mL, 1.18 g, 10.5 mmol). The resulting solution was warmed to 80 °C and stirred for 2 h. The reaction was cooled to rt before H_2O (25 mL) was added. The aqueous mixture was extracted three times with EtOAc. The combined organic extracts were washed with saturated aqueous NaCl, dried (Na_2SO_4), filtered, and evaporated under reduced pressure. The residue was purified by column chromatography (SiO_2, 96:4 CH_2Cl_2/MeOH) to afford **6** (0.83 g, 80%) as a pale yellow, amorphous solid: ^{1}H NMR (DMSO-d_6, 400 MHz) δ 12.89 (s, 1H), 10.91 (s, 1H), 8.38 (s, 1H), 7.14 (d, 1H, J = 1.64 Hz), 7.02 (m, 2H), 6.40 (d, 1H, J = 2.16 Hz), 6.24 (d, 1H, J = 2.08 Hz), 6.06 (s, 2H) ppm; ^{13}C (DMSO-d_6, 125 MHz) δ 179.9, 164.3, 162.0, 157.5, 154.6, 147.1 (2C), 124.4, 122.6, 121.9, 109.4, 108.2, 104.4, 101.1, 99.0, 93.7 ppm; HRMS (ESI+) m/z 299.0564 ([M + H]$^+$, $C_{16}H_{11}O_6$, requires 299.0556).

3-(Benzo[*d*][1,3]dioxol-5-yl)-5-hydroxy-7-(methoxymethoxy)-4*H*-chromen-4-one (15). Compound **6** (1 g, 3.4 mmol) and NaH (160 mg, 4.8 mmol, 60% disp. w/w) were dissolved in DMF (10 mL) and stirred for 10 min at rt. Chloromethyl methoxy ether (0.65 mL, 4.8 mmol) was added dropwise, and the mixture was stirred

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for 9 h at rt. The reaction was quenched by the addition of saturated aqueous $NaHCO_3$ (50 mL) to the mixture. The resulting slurry was further diluted with H_2O (50 mL) and extracted three times with EtOAc. The combined organic layers were washed with saturated aqueous NaCl, dried (Na_2SO_4), filtered, and evaporated. The residue was purified by column chromatography (SiO_2, 4:1 hexanes/EtOAc) to give **15** (870 mg, 75%) as a pale yellow, amorphous solid: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 12.79 (s, 1H), 7.89 (s, 1H), 7.07 (d, 1H, J = 1.64 Hz), 6.94 (m, 2H), 6.60 (d, 1H, J = 2.24 Hz), 6.53 (d, 1H, J = 2.20 Hz), 6.02 (s, 2H), 5.26 (s, 2H), 3.52 (s, 3H) ppm; ^{13}C NMR ($CDCl_3$, 125 MHz) δ 180.8, 163.1, 162.6, 157.8, 153.0, 147.9, 147.8, 124.3, 123.8, 122.5, 109.6, 108.6, 106.9, 101.3, 100.2, 94.3, 94.2, 56.5 ppm; IR (film) ν_{max} 3078, 2904, 1655, 1612, 1574, 1504, 1489, 1435, 1366, 1300, 1248, 1146, 1078, 1036, 1001, 939, 920, 822 cm^{-1}; HRMS (ESI+) m/z 343.0801 ([M + H]$^+$, $C_{18}H_{15}O_7$, requires 343.0818).

6-Allyl-3-(benzo[*d*][1,3]dioxol-5-yl)-5-hydroxy-7-(methoxymethoxy)-4*H*-chromen-4-one (16). DMF (1.0 mL) was added to a mixture of NaH (40 mg, 0.52 mmol) and **15** (89 mg, 0.26 mmol). The resulting suspension was stirred for 10 min. Allyl bromide (63 mg, 0.52 mmol) was added, and the reaction was stirred for 10 h at rt. The reaction was quenched by addition of H_2O. The solvent was evaporated under reduced pressure, and the oily residue was dissolved in EtOAc. The organic layer was extracted twice with H_2O, washed with saturated aqueous NaCl, and dried (Na_2SO_4). The solvent was decanted from the drying agent and evaporated under reduced pressure. The yellow solid was dissolved in DMF (1 mL) and warmed to 160 °C in a focused microwave reactor at 300 W for 45 min. The solution was then diluted with EtOAc. The mixture was washed three times with H_2O. The organic layer was then washed with saturated aqueous NaCl, dried (Na_2SO_4), filtered, and evaporated under reduced pressure. The residue was purified by column chromatography (SiO_2, 3:1 hexanes/acetone) to give **16** (51 mg, 51%) as a pale yellow, amorphous solid: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 12.99 (s, 1H), 7.88 (s, 1H), 7.06 (d, 1H, J = 1.60 Hz), 6.79 (m, 2H), 6.68 (s, 1H), 6.00 (m, 3H), 5.30 (s, 2H), 5.03 (m, 2H), 3.51 (m, 5H) ppm; ^{13}C NMR ($CDCl_3$, 125 MHz) δ 178.0, 157.9, 156.6, 153.5, 150.4, 147.6, 145.1, 133.2, 121.8, 121.0, 119.7, 111.9, 109.1, 107.2, 106.9, 105.4, 103.8, 98.5, 91.6, 89.2, 53.6, 23.8 ppm; IR (film) ν_{max} 3078, 2997, 2959, 2908, 2829, 1651, 1612, 1580, 1502, 1487, 1439, 1408, 1367, 1323, 1294, 1275, 1252, 1223, 1155, 1130, 1072, 1045, 987, 943, 920, 829 cm^{-1}; HRMS (ESI+) m/z 383.1121 ([M + H]$^+$, $C_{21}H_{19}O_7$, requires 383.1131).

Derrubone (1). Grubbs' second generation catalyst (1 mg, 0.0012 mmol) was added to a solution of **16** (10 mg, 0.026 mmol) in CH_2Cl_2 (0.1 mL) and 2-methyl-2-butene (0.3 mL) under an Ar atmosphere. The solution was stirred for 24 h at rt. The mixture was concentrated under reduced pressure to give a green solid. The solid was dissolved in MeOH (0.5 mL) and transferred to a screw-cap vial. Concentrated HCl (50 μL) was added to the solution. The mixture was warmed to 70 °C and stirred for 1 h. The reaction was quenched by the addition of a saturated aqueous $NaHCO_3$ solution (5 mL). The aqueous solution was extracted with EtOAc. The organic layer was washed with saturated aqueous NaCl, dried (Na_2SO_4), filtered, and evaporated under reduced pressure. The resulting solid was purified by chromatography (SiO_2, 6:1 hexanes/EtOAc) to afford **1** (9 mg, 95%) as a colorless, amorphous solid: ^{1}H NMR (acetone-d_6, 500 MHz) δ 13.27 (s, 1H), 9.75 (br s, 1H), 8.20 (s, 1H), 7.16 (d, 1H, J = 1.65 Hz), 7.08 (dd, 1H, J = 8.03, 1.65 Hz), 6.92 (d, 1H, J = 8.00 Hz), 6.51 (s, 1H), 6.06 (s, 2H), 5.29 (tt, 1H, J = 7.10, 1.35 Hz), 3.38 (d, 2H, J = 7.20 Hz), 1.79 (s, 3H), 1.66 (s, 3H) ppm; ^{13}C (acetone-d_6, 125 MHz) δ 180.6, 161.8, 159.7, 155.9, 153.6, 147.6, 147.5, 130.9, 125.1, 122.8, 122.5, 122.2, 111.6, 109.6, 108.0, 105.1, 101.3, 93.0, 25.0, 21.1, 17.0 ppm; IR (film) ν_{max} 3580, 3524, 3433, 3412, 3005, 2964, 2924, 1701, 1620, 1580, 1433, 1365, 1321, 1250, 1229, 1094, 1038, 824 cm^{-1}; HRMS (ESI+) m/z 367.1174 ([M + H]$^+$, $C_{21}H_{19}O_6$, requires 367.1182).

Anti-Proliferation Assays. MCF-7 cells were maintained in a 1:1 mixture of Advanced DMEM/F12 (Gibco) supplemented with non-essential amino acids, L-glutamine (2 mM), streptomycin (500 μg/mL), penicillin (100 units/mL), and 10% FBS. HCT-116 cells were maintained in Iscove's Modified Dulbecco's Medium (Sigma) supplemented with L-glutamine (2 mM), streptomycin (500 μg/mL), penicillin (100 units/mL), and 10% FBS. Cells were grown to confluence in a humidified atmosphere (37 °C, 5% CO_2), seeded (2000/well, 100 μL) in 96-well plates, and allowed to attach overnight. Compound or GDA at varying concentrations in DMSO (1% DMSO final concentration) was added, and cells were returned to the incubator for 72 h. At 72 h, the number of viable cells was determined using an MTS/PMS cell proliferation kit (Promega) per the manufacturer's instructions. Cells incubated in 1% DMSO were used as 100% proliferation, and values were adjusted accordingly. IC_{50} values were calculated from separate experiments performed in triplicate using GraphPad Prism.

Western Blot Analysis of 14 and 22c. MCF-7 cells were cultured as described above and treated with various concentrations of drug or GDA in DMSO (1% DMSO final concentration) for 24 h. The cells were collected in cold PBS and lysed in RIPA lysis buffer containing 1 mM PMSF, 2 mM sodium orthovanadate, and protease inhibitors on ice for 1 h. Lysates were clarified at 14 000*g* for 10 min at 4 °C. Protein concentrations were determined using the Pierce BCA protein assay kit per the manufacturer's instructions. Equal amounts of protein (20 μg) were electrophoresed under reducing conditions, transferred to a nitrocellulose membrane, and immunoblotted with the corresponding specific antibodies. Membranes were incubated with an appropriate horseradish peroxidase labeled secondary antibody, developed with a chemiluminescent substrate, and visualized.

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Supporting Information Available: Experimental procedures and characterization for all compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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