Identification of New Mono/Dihydroxynaphthoquinone as Lead Agents That Inhibit the Growth of Refractive and Triple-Negative Breast Cancer Cell Lines

Richard Schroeder,†,∥ Mary Sfondouris,§,⊥ Navneet Goyal,† Rajesh Komati,‡ Achira Weerathunga,† Cory Gettridge,† Cheryl L. Klein Stevens,‖ Frank E. Jones,§ and Jayalakshmi Sridhar‡,*

†Department of Chemistry, Xavier University of Louisiana, 1, Drexel Dr., New Orleans, Louisiana 70125, United States
‡Department of Chemistry, Nicholls State University, 129 Beauregard Hall, 906 E. 1st Street, Thibodaux, Louisiana 70301, United States
§Department of Cell and Molecular Biology, Tulane University, 6400 Freret Street, 2000 Percival Stern Hall, New Orleans, Louisiana 70118, United States
⊥Ogden College of Science and Engineering, Western Kentucky University, 1906 College Heights Boulevard #11075, Bowling Green, Kentucky 42101-1075, United States

ABSTRACT: Human epidermal growth factor receptor 2 (HER2) is overexpressed in nearly 20–30% of breast cancers and is associated with metastasis resulting in poor patient survival and high recurrence. The dual EGFR/HER2 kinase inhibitor lapatinib has shown promising clinical results, but its limitations have also led to the resistance and activation of tumor survival pathways. Following our previous investigation of quinones as HER2 kinase inhibitors, we synthesized several naphthoquinone derivatives that significantly inhibited breast tumor cells expressing HER2 and trastuzumab-resistant HER2 oncogenic isoform, HER2Δ16. Two of these compounds were shown to be more effective than lapatinib at the inhibition of HER2 autophosphorylation of Y1248. Compounds 7 (5,8-dihydroxy-2-methylnapthalene-1,4-dione) and 9 (2-(bromomethyl)-5,8-dihydroxynaphthalene-1,4-dione) inhibited HER2-expressing MCF-7 cells (IC50 0.29 and 1.76 μM, respectively) and HER2Δ16-expressing MCF-7 cells (IC50 0.51 and 1.76 μM, respectively). Compound 7 was also shown to promote cell death in multiple refractory breast cancer cell lines with IC50 values ranging from 0.12 to 2.92 μM. These compounds can function as lead compounds for the design of a new series of nonquinonoid structural compounds that can maintain a similar inhibition profile.

INTRODUCTION

Breast cancer (BC) is the second-most common cause of cancer-related deaths in women with ~40 000 deaths per year in the United States.1 Of the BC patients, 20–30% have human epidermal growth factor receptor 2 (HER2)-overexpressed BC, which has been shown to result in poor prognosis with high recurrence and decreased overall survival rates.2 In 2006, FDA approved the clinical use of trastuzumab, a monoclonal antibody that targets the extracellular domain of the HER2 receptor, which dramatically improved the survival rate for patients with HER2-positive BC.3,4 The initial treatment strategy for HER2-positive breast cancer typically consists of trastuzumab (an IgG1-class monoclonal antibody),5–8 in combination with chemotherapy5,9 or lapatinib (a small molecule EGFR/HER2 kinase inhibitor).10–12 Trastuzumab was shown to increase the overall patient survival and progression-free survival and showed higher response rates when used in conjunction with chemotherapy.13–16 However, despite the efficacy of trastuzumab, acquired or intrinsic resistance remains a major clinical issue and has been evidenced to occur within a year of treatment.17–19

There are several implicating factors responsible for trastuzumab resistance. Among them, a common phenomenon found in HER2-positive breast cancer is the presence of a truncated HER2 (p95HER2), which has shed its extracellular domain while retaining active kinase functionality.20 Additionally, an oncogenic isoform of HER2 containing an in-frame deletion of exon 16 (HER2Δ16) was shown to enhance transformation activity over wild-type HER2, leading to node-positive breast cancer and trastuzumab resistance.21,22 Further, HER2Δ16 was shown to promote estrogen-independent growth in ERα-positive breast tumor cells and induced tamoxifen resistance through multiple mechanisms including the upregulation of BCL-2 through miR-15a/16 suppression.23 Tyrosine kinase inhibitors may be more effective in treating HER2-positive breast cancer, due to their ability to block downstream-signaling pathways in p95HER2, HER2Δ16, and...
full-length HER2. Treatment with lapatinib was shown to delay tumor progression by 4 months as a single-agent treatment and 8 months when used in conjunction with chemotherapy in advanced HER2-positive breast cancer.24 However, lapatinib has its limitations, and resistance remains a major challenge.25 One of the primary mechanisms linked to lapatinib resistance is an ER-dependent survival pathway occurring as a result of the upregulation of ER signaling and the parallel upregulation of the antiapoptotic BCL-2 protein.26

We previously demonstrated that analogs of the natural product emodin could be used as HER2 kinase inhibitors.27,28 To further explore and optimize the structure–activity relationships, we designed and synthesized several quinone compounds that were highly effective at growth inhibition of HER2 and HER2Δ16 overexpressed in MCF-7 breast tumor cells. Western blot assays were performed on these compounds to determine their effectiveness in the inhibition of autophosphorylation of Y1248. Additionally, the most potent inhibitor was subjected to high-throughput assays in multiple breast cancer cell lines expressed in triple-negative breast cancer, as well as trastuzumab-sensitive and -resistant cells. Many of the tyrosine kinase inhibitors being investigated as EGFR or HER2 inhibitors have its limitations, and resistance remains a major challenge.25 One of the primary mechanisms linked to lapatinib resistance is the presence of Rose Bengal catalyst and green light-emitting diode (LED). The photooxidation method 31 was used to prepare 5-hydroxy-8-methylnaphthalene-1,4-dione 22 from 1,5-dihydroxynaphthalene 12 (Scheme 2). Compounds 14−17 were prepared using reported methods.30 The preparation of ethyl (E)-3-(5,8-dihydroxy-1,4-dioxo-1,4-dihydropyrazine-2-yl)acrylate 20 was achieved through a three-step process involving the Wittig reaction of 2-formyl-1,4,5,8-tetramethoxynaphthalene 12 and ethyl (triphenylphosphoranylidene)acetate in dichloromethane (DCM) at room temperature. The phosphonium ylide was prepared through the nucleophilic substitution of ethyl 2-bromoacetate with triphenylphosphine under refluxing conditions in toluene. The precipitated phosphonium bromide was filtered and then deprotonated with sodium hydroxide to afford ethyl (triphenylphosphoranylidene)acetate. The obtained Wittig product ethyl (E)-3-(1,4,5,8-tetramethoxynaphthalene-2-yl)acrylate 18 was then oxidized using aqueous ammonium cerium(IV) nitrate at room temperature, which afforded a mixture of isomers that were easily separated through silica gel chromatography and showed distinct chemical shifts in proton NMR. Demethylation of ethyl (E)-3-(5,8-dimethoxy-1,4-dihydropyrazine-2-yl)acrylate was achieved through a boron tribromide adduct, which was hydrolyzed by the addition of water to afford product 20 (41% yield from 12). The photooxidation method 31 was used to prepare 5-hydroxynaphthalene-1,4-dione (juglone) 11 from 1,5-dihydroxynaphthalene by bubbling molecular oxygen in methanol in the presence of Rose Bengal catalyst and green light-emitting diode (LED). Compounds 5-hydroxy-8-methylnaphthalene-1,4-dione 22 and 5-methylnaphthalene-1,4-dione 23 were prepared (Scheme 3) through a Diels−Alder [4 + 2] cycloaddition of 2-methylthiophene and 1,4-benzoquinone, with m-chloroperox-

**RESULTS AND DISCUSSION**

Our earlier work28 on the identification of lead compounds as growth inhibitors of the trastuzumab-resistant MCF-7/HER2Δ16 cell lines gave us three effective compounds. All of them were mono/dihydroxynaphthoquinone derivatives 1−3 (Figure 1).

![Figure 1](image)

**Figure 1.** Growth inhibitors of trastuzumab-resistant MCF-7/HER2Δ16 breast cancer cell line reported earlier.

**Scheme 1. Synthesis of 5,8-Dihydroxynaphthalene-1,4-dione Derivatives Friedel–Crafts Acylation Reaction**

![Scheme 1](image)

“Reagents and conditions: (a) NaCl, AlCl₃; (b) HCl, 180−200 °C; (c) N-bromosuccinimide, azobisisobutyronitrile (AIBN), CCl₄.”
ybenzoic acid in chloroform for 48 h followed by purification using silica gel chromatography.32

Compound 5-hydroxy-7-methylnaphthalene-1,4-dione

was prepared through a Diels−Alder [4 + 2] cycloaddition using 3-methyl-1-methoxy-1-trimethylsiloxy-1,4-diene and 1,4-benzoquinone as a dienophile at 0 °C for 20 h (Scheme 4). The

resulting 7-methyl 5-(trimethylsiloxy)naphthalene-1,4-dione adduct was hydrolyzed using 1 N hydrochloric acid in methanol to afford product 25.33 Compound 5-methoxy-7-methylnaphthalene-1,4-dione 27 was obtained by refluxing 25 in silver(II) oxide with iodomethane followed by filtration over celite and flash chromatography.33 Side-chain bromination of compound 25 to form compound 26 was accomplished using NBS in the presence of the radical initiator azobisisobutyronitrile (AIBN) in carbon tetrachloride under refluxing conditions.

Our laboratory is also working on a class of phthalimides as cyclin-dependent kinase inhibitors (unpublished work). Some of the commercially available compounds were purchased and tested for their growth inhibition ability of the MCF-7/HER2 and MCF-7/HER2Δ16 cell lines (Figure 2).

**Growth Inhibition of HER2- and HER2Δ16-Overexpressed MCF-7 Breast Cancer Cell Lines.** The naphthoquinone series of compounds that were synthesized in our laboratory and the phthalimide compounds purchased from Timtec Chemicals were initially subjected to high-throughput

**Scheme 2. Synthesis of 5,8-Dihydroxynaphthalene-1,4-dione Derivatives and 5-Methylnaphthalene-1,4-dione**

**Scheme 3. Synthesis of 5-Hydroxy-8-methylnaphthalene-1,4-dione and 5-Methylnaphthalene-1,4-dione**

**Scheme 4. Synthesis of 5-Hydroxy-7-methylnaphthalene-1,4-dione, 5-Methoxy-7-methylnaphthalene-1,4-dione, and 5-Hydroxy-7-bromomethylnaphthalene-1,4-dione**

**Figure 2. Structures of the phthalimide derivatives that were tested for the growth inhibition of MCF-7 cells.**

"Reagents and conditions: (a) O2, LED, Rose Bengal, MeOH, 15 h; (b) NaBH4, MeOH; (c) ceric ammonium nitrate, MeCN; (d) AlCl3, DCM; (e) Ph3P=CHCO2Et, DCM; (f) BBr3, DCM, H2O, 0 °C.

"Reagents and conditions: (a) benzoquinone, MCPBA, CHCl3.

"Reagents and conditions: (a) benzoquinone, DCM; (b) HCl, MeOH; (c) N-bromosuccinimide, AIBN, CC14; (d) silver oxide, iodomethane.

Figure 2. Structures of the phthalimide derivatives that were tested for the growth inhibition of MCF-7 cells.
screening against the MCF-7 breast cancer cell lines expressing HER2, HER2Δ16, and empty vector. Lapatinib was used as positive control. The cells were treated with the compounds at 10 μM concentration for 48 h. After treatment, cell viability was tested using the CellTiter-Glo Assay (Promega). Out of a total of 24 compounds, 5 compounds suppressed cell viability potently in all three cell lines. After 48 h, these five compounds decreased cell viability by >90% when compared to lapatinib with a cell viability of <70% at the same concentration. Eight compounds showed moderate suppression of cell viability with many of them showing a greater inhibition of the MCF-7-HER2 cell line than that of the MCF-7-HER2Δ16 cell line in a pattern that was similar to that of lapatinib (Figure 3). The six best compounds 7, 9, the mixture of 8a + 8b, 25, and 27 were then taken up for further studies.

The IC_{50} values of the six best compounds from the high-throughput screening were measured by treating each of the cell lines with different drug concentrations for 48 h followed by the CellTiter-Glo Assay to detect cell viability (Table 1). Lapatinib, a known HER2/EGFR inhibitor in clinical use, was taken as the positive standard. Lapatinib inhibited the three cell lines MCF-7/pcDNA, MCF-7/HER2, and MCF-7/HER2Δ16 with IC_{50} values of 15.71, 15.79, and 19.22 μM. Other than compound 25, most of our compounds (7, 9, the mixture of 8a + 8b, and 27) showed higher potency of inhibition of the three breast cancer cell lines than lapatinib. The dose–response curves for the three compounds 7, the mixture of 8a + 8b, and 9 with the lowest IC_{50} values for MCF-7/pcDNA (0.32, 1.28, and 1.66 μM), MCF-7/HER2 (0.29, 1.30, and 1.78 μM), and MCF-7/HER2Δ16 (0.51, 0.51, and 3.61 μM) are represented in Figure 4. Compound 7 showed the best inhibition potency for all three cell lines.

HER2 is a member of the erbB family of tyrosine kinases, which is composed of four partial homologous transmembrane receptors: EGFR/HER1 (erbB1), HER2 (erbB2/neu), HER3 (erbB3), and HER4 (erbB4). With the exception of HER2, these receptors exhibit ligand specificity. Ligand binding induces homo- or heterodimerization through a disulfide bond linkage and leads to receptor activation and tyrosine autophosphorylation. HER2 is a preferred dimerization partner due to its high catalytic activity and forms potent heterodimers with EGFR and HER3. HER2 with its intrinsic kinase activity can transphosphorylate other members of the erbB family. HER2 and HER2Δ16 are overexpressed in the breast cancer cell lines that have been used in this study, leading to the kinases being constitutively active. Upon dimerization, the constitutively active HER2 and HER2Δ16 receptors can transphosphorylate coexpressed EGFR. The levels of phosphorylated HER2 in the parental MCF-7 cell line were less, resulting in its lack of dimerization with EGFR and its transphosphorylation. Western blots were performed to determine the ability of the compounds to inhibit the activation of the HER2 receptor (Figure 5).

The total and phosphorylated protein were detected upon the treatment of each of the cell lines with compounds 6, 7, 8a + 8b, 9, 12, 25, 26, 27, and 28 along with the positive control lapatinib at 10 μM concentration for 2 h. Compound 28 did not show notable growth inhibition in the high-throughput assay and was used as a negative control. Compounds 6, 7, 8a + 8b, and 9 significantly decreased HER2-activating phosphorylation to the same extent as lapatinib at the autophosphorylation site Y1284. Compounds 12, 25, 26, and 27 did not show such an effect on autophosphorylation. The ability of these compounds to inhibit the breast cancer cell lines and not the HER2 autophosphorylation at Y1284 indicates that the mechanism of growth

Table 1. Inhibition of MCF-7/pcDNA, MCF-7/HER2, and MCF-7/HER2Δ16 Breast Cancer Cell Lines by the Six Compounds Identified from the High-Throughput Assay

| compound | IC_{50} in μM |
|----------|--------------|
|          | MCF-7 pcDNA  | MCF-7 HER2 | MCF-7 HER2Δ16 |
| 7        | 0.32         | 0.29       | 0.51          |
| 8a + 8b  | 1.28         | 1.30       | 0.51          |
| 9        | 1.66         | 1.78       | 3.61          |
| 22       | 2.93         | 4.61       | 9.39          |
| 25       | 30.88        | ND         | ND            |
| 27       | 4.94         | 3.18       | 7.30          |

Figure 3. Cell viability after 48 h treatment of each compound at a concentration of 10 μM.
inhibition in cancer cells might involve some other alternate pathways. Compounds 6, 7, (8a + 8b), and 9 that showed a decreased autophosphorylation in HER2 were then tested for the ability to decrease the transphosphorylation of the EGFR receptor at the residue Y1068 (Figure 6). Compounds 26 and 28 were used as negative controls. The transphosphorylation at
Y1068 of EGFR receptor was decreased by compounds 6, 7, 8a + 8b, and 9 in levels comparable to that of lapatinib. Compound 7 was taken up for further analysis of its ability to inhibit the growth of a panel of breast cancer cell lines of varied types. The panel included the triple-negative cell lines BT20, MDA-MB-468, and MDA-MB-231, the trastuzumab-sensitive cell lines SKBR3 and BT474, and the trastuzumab-resistant cell lines SUM 190PT and SUM 225CWN (Figure 7). Compound 7 inhibited the growth of these breast cancer cell lines with IC50 values ranging from 0.1231 to 2.923 μM. The potency of inhibition was comparable for most of the cell lines that were tested except for MDA-MB-231 where a 3- to 4-fold decrease in potency was evidenced.

Compound 7 was then subjected to a cross kinase panel high-throughput assay at 10 μM concentration, at Life Technologies, to determine the selectivity of kinase inhibition. A panel of 100 disease-relevant kinases (assay performed by Thermo Fisher Scientific’s SelectScreen Profiling Service) was studied (see the Supporting Information). Kinases (11) were inhibited at 80% or more, protein kinase B 1/2 (also known as AKT1/2), Aurora kinases A/B (AurK A/B), checkpoint kinase 2 (CHEK2), feline sarcoma kinase, fibroblast growth factor 1 (FGFR1), I kappa B kinase B/E (IKK B/E), mitogen-activated protein kinase kinase 1 (MAP2K1), mitogen-activated protein kinase kinase 8 (MAP3K8), never in mitosis (NIMA)-related kinase 2 (NEK2), polo-like kinase 1/3 (PLK1/3), serum/glucocorticoid-regulated kinase 1 (SGK1), and TEK tyrosine kinase. All of these kinases except for CHEK2 are reported to have tumorigenic roles in breast cancer.35−42

Docking studies were performed on the active compounds with the HER2 X-ray crystal structure 3RCD.pdb,43 using the molecular operating environment software’s docking module. The binding pocket of the HER2 protein is L-shaped with two large cavities that are connected to each other. The cavity where the base of the ATP molecule binds to the hinge region has a width of ~13.11 Å and a height of 8.30 Å. The second cavity behind the first cavity has the invariant Lys753 and Asp853 residues outlining it with a width of 22.13 Å and a height of 9.20 Å. Compound 7 is a planar molecule with the dimensions 8.07 Å × 6.63 Å. Docking studies of this compound revealed that it preferred to reside in the second cavity where it made more hydrogen bonds with the side-chain hydroxyl group of Thr862, the backbone −NH of Asp863, and the backbone carbonyl of Leu796. The side-chain methyl group of compound 7 depicted hydrophobic interactions with the side chain of Leu852 and the side-chain methyl group of Thr862. On the other hand, compound 3 with one less phenolic group preferred to reside in the first cavity. The additional hydrogen bonds made by compound 7 might have contributed to its greater efficacy in the growth inhibition of the breast cancer cell lines MCF-7/HER2 and MCF-7/HER2Δ16 (Figure 8).

**CONCLUSIONS**

Our search for new inhibitors that can have good efficacy against HER2Δ16- and HER2-overexpressed breast cancer cell lines has yielded several compounds with sub-micromolar growth inhibition potency. The compounds with greater growth inhibition profile also showed HER2 autophosphorylation and transphosphorylation repression. Some of the compounds that showed potential for growth inhibition of the HER2Δ16- and HER2-overexpressed breast cancer cell lines did not exhibit inhibiting effects on the HER2 autophosphorylation and transphosphorylation. One can safely conclude that these agents might be affecting the growth inhibition of these breast cancer cell lines through other mechanisms. Additionally, we found that

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**Figure 7.** Inhibition of various breast cancer cell lines by compound 7.
the most effective compound showed good growth inhibition potency for several types of breast cancer cell lines with an IC_{50} range of 0.1231–2.923 μM. These results demonstrate the ability of these compounds to effectively inhibit the growth of refractory breast cancer cells. This could be due to the inhibition of several key kinases that play a detrimental role in cancer. With several of the highly selective kinase inhibitors in clinical settings as breast cancer therapeutics leading to the development of resistance within a year,^{44–47} the thought arises whether the inhibition of more than one key tumorigenic kinase would be the path to take for achieving success in the fight against aggressive and refractory breast cancer. Such an approach will also require better success at the targeted delivery of these agents to minimize any side effects due to multiple kinase targeting. The problems with the quinone structural moiety are recognized for issues on redox cycling and chelating. The future research work will involve mitigating these issues by modifying the quinone ring and designing a new series of compounds that are structurally acceptable therapeutic series. The goal will be to maintain similar breast cancer growth inhibition profile and improved kinase inhibition profile as compound 7.

**EXPERIMENTAL SECTION**

**Synthetic Materials and Methods.** All reagents were used as received from the manufacturer, with the exception of NBS, which was recrystallized from water and allowed to dry before use. All NMR spectra were recorded on a Agilent 400 MHz using tetramethylsilane as an internal solvent reference. Aluminum-backed 60F254 silica plates were used for thin-layer chromatography. Flash chromatography was performed using the Teledyne Isco CombiFlash automated column machine using ethyl acetate and hexane as the mobile solvents. Melting points were obtained from a Mel-Temp melting point apparatus. High-resolution MS was performed at the University of Texas at Austin on the Micromass Autospec Ultima, giving high-resolution chemical ionization.

**General Procedure for Synthesis of Compounds (6).** A mixture of aluminum chloride (13.1 mmol) and sodium chloride (6.50 mmol) was allowed to heat to 180 °C under a nitrogen atmosphere until melt occurred, after which 1,4-dimethoxybenzene (1.31 mmol) and 2-methylmaleic anhydride (3.93 mmol) were added. The resulting reaction mixture was allowed to heat to 190–200 °C and then allowed to cool down to room temperature. An ice-cold solution of 10% HCl was added, and the reaction mixture was allowed to stir overnight, and then 5% oxalic acid (20 mL) was added. The reaction mixture was filtered through celite, extracted with dichloromethane, dried over sodium sulfate, and concentrated under reduced pressure. Purification via flash chromatography (10% EtOAc/Hex) yielded pure products. **5,8-Dihydroxy-2-methylnaphthalene-1,4-dione (6).**

**Flash chromatography.** Flash chromatography was performed using the Teledyne Isco CombiFlash automated column machine using ethyl acetate and hexane as the mobile solvents. Melting points were obtained from a Mel-Temp melting point apparatus. High-resolution MS was performed at the University of Texas at Austin on the Micromass Autospec Ultima, giving high-resolution chemical ionization.

**General Procedure for Synthesis of Compounds (6), (7), (8a), and (8b).** A mixture of aluminum chloride (13.1 mmol) and sodium chloride (6.50 mmol) was allowed to heat to 180 °C under a nitrogen atmosphere until melt occurred, after which 1,4-dimethoxybenzene (1.31 mmol) and 2-methylmaleic anhydride (3.93 mmol) were added. The resulting reaction mixture was allowed to heat to 190–200 °C and then allowed to cool down to room temperature. An ice-cold solution of 10% HCl was added, and the reaction mixture was allowed to stir overnight, and then 5% oxalic acid (20 mL) was added. The reaction mixture was filtered through celite, extracted with dichloromethane, dried over sodium sulfate, and concentrated under reduced pressure. Purification via flash chromatography (10% EtOAc/Hex) yielded pure products. **5,8-Dihydroxy-2-methylnaphthalene-1,4-dione (6).** Re-crystallization from hexane afforded green needlelike crystals with 55% yield. **1H NMR (CDCl3) δ 12.53 (s, 1H), 12.42 (s, 1H), 7.18 (s, 2H), 6.88 (s, 1H), 2.22 (s, 3H); 13C NMR (CDCl3) δ 183.26, 182.85, 162.74, 162.14, 148.18, 135.15, 131.17, 130.72, 111.72, 16.19; HRMS (CI-magnetic sector) (M + H) calcd for C_{11}H_{8}O_{4} 204.0423; found: 204.0423.**
1H NMR (CDCl3) δ 6.86 (d, J = 8.0 Hz, 1H), 12.88 (s, 1H), 12.38 (s, 1H), 7.81 (d, J = 8.0 Hz, 3H); 13C NMR (CDCl3) δ 151.06, 150.64, 149.18, 139.32, 124.60, 122.87, 121.79, 118.78, 56.88, 14.19; HRMS (CI-magnetic sector) (M + H) calcd for C15H12O6 138.77, 135.65, 135.13, 131.13, 126.38, 112.12, 61.13, 14.25; HRMS (CI-magnetic sector) (M + H) calcd for C15H12O6 139.03.

5-Hydroxy-8-methylnaphthalene-1,4-dione (25) (0.100 g, 0.53 mmol) and silver oxide (0.180 g, 1.48 mmol) in iodomethane (2.65 mL) was refluxed for 1.5 h. After completion, it was filtered through celite and washed with DCM. The solvent was removed under reduced pressure, and the obtained crude product was purified using flash chromatography (40% EtOAc/hexane) to get the pure product 27 (0.040 g, 52%) as a bright yellow solid.

7-(Bromomethyl)-5-hydroxynaphthalene-1,4-dione (26). To a stirring solution of 25 (0.050 g, 0.265 mmol) was dissolved in 5.0 mL of carbon tetrachloride. A few crystals of AIBN were then added to the solution. The reaction mixture was immersed immediately in a preheated oil bath and stirred at 80 °C for 1 h. N-Bromosuccinimide (0.056 g, 0.318 mmol) was added to the reaction mixture and then it was stirred overnight at 80 °C. The reaction mixture was concentrated on rotavaporator and purified using column chromatography to get pure product 26 (0.042 g, 61%). 1H NMR (CDCl3) δ 1.88 (s, 1H), 7.64 (s, 1H), 7.31 (s, 1H), 6.97 (s, 2H), 4.46 (s, 2H); 13C NMR (CDCl3) δ 175.36, 173.94, 170.02, 169.59, 143.92, 131.92, 133.93, 133.61, 112.09, 111.85, 24.75. HRMS (CI-magnetic sector) (M + H) calcd for C17H14BrO4 316.0746; found: 316.0751.

5-Methoxy-7-methylnaphthalene-1,4-dione (19). To a stirred solution of ethyl (E)-3-(5,8-dimethoxy-1,4-dioxo-1,4-dihydronaphthalene-2-yl) acrylate (18) (0.332 g, 0.96 mmol) in acetonitrile (10 mL) was added dropwise ammonium cerium(IV) nitrate (1.321 g, 2.41 mmol) dissolved in water (3 mL). The reaction mixture was stirred at room temperature for 45 min, diluted with water (30 mL), and extracted with chloroform. The combined organic layers were dried over sodium sulfate, concentrated under reduced pressure, and the dark solid residue was purified via flash chromatography (30% EtOAc/Hex). The first fraction provided the desired product as a bright yellow solid (0.360 g, 91%). 1H NMR (CDCl3) δ 8.26 (d, J = 8.0 Hz, 1H), 6.98 (s, 1H), 6.92 (q, J = 8.0 Hz, 2H), 6.50 (d, J = 16.0 Hz, 4.32 (q, J = 8.0 Hz, 2H), 3.96 (s, 6H), 3.90 (s, 3H), 3.75 (s, 3H), 1.37 (s, 2H); 13C NMR (CDCl3) δ 167.20, 153.31, 151.36, 152.16, 150.64, 149.18, 139.32, 124.60, 122.87, 121.79, 118.78, 110.35, 108.65, 103.85, 83.63, 60.45, 57.89, 57.10, 56.95, 14.36; HRMS (CI-magnetic sector) (M + H) calcd for C19H22O6 316.0957; found: 316.0954.

5-Hydroxy-7-methylnaphthalene-1,4-dione (20). To a stirred solution of ethyl (E)-3-(5,8-dihydroxy-1,4-dioxo-1,4-dihydronaphthalene-2-yl) acrylate (18) (0.332 g, 0.96 mmol) in dichloromethane (10 mL) at 0 °C under nitrogen atmosphere was slowly added boron tribromide (36 μL, 0.24 mmol) in dichloromethane. The reaction mixture was vigorously stirred for 2 h and then was slowly added dropwise to an ice/water mixture (20 mL). The resulting suspension was stirred vigorously for 30 min, and the organic layer was washed with brine, dried over sodium sulfate, and concentrated to afford a dark solid, which was purified by flash column chromatography (40% EtOAc/Hex). The first fraction afforded the product as a red solid (0.015 g, 41%). Mp 131–133 °C. 1H NMR (CDCl3) δ 12.88 (s, 1H), 12.38 (s, 1H), 7.81 (d, J = 8.0 Hz, 1H), 7.32 (s, 1H), 6.86 (d, J = 16 Hz, 1H), 4.31 (m, 2H), 1.39 (s, J = 4.0 Hz, 3H); 13C NMR (CDCl3) δ 174.07, 173.39, 171.33, 165.94, 138.77, 135.65, 135.13, 131.13, 126.38, 112.12, 61.13, 14.25; HRMS (CI-magnetic sector) (M + H) calcd for C19H17O6 288.0634; found: 288.0640.

5-Hydroxy-8-methylnaphthalene-1,4-dione (22) and 5-Methylnaphthalene-1,4-dione (23). Previously reported.

5-Hydroxy-7-methylnaphthalene-1,4-dione (25). Previously reported.

Cell Culture. Generation of MCF-7/pcDNA3, MCF-7/HER2, and MCF-7/HER2Δ16 cell lines has been described previously. All MCF-7 cell lines were cultured according to the American Type Culture Collection (ATCC) recommendations. The human breast cancer cell lines BT20, MDA-MB-468, MDA-MB-231, SKBR3, and BT474 were purchased from ATCC and cultured according to their instructions. The SUM 190PT and SUM 225CWN cell lines were procured from Asterand Bioscience and cultured according to their protocol.

Cell Viability Assay. Cell lines were plated in duplicate wells using a white 96-well plate (VWR) at 3000 cells per well. After 24 h, the cells were treated with the listed drug concentrations for 48 h. The plates were then assayed for cell viability using the CellTiter-Glo Luminescent Cell Viability Assay (Promega) according to the manufacturer’s instructions. Luminescence was recorded using a Victor X3 Multilabel Plate Reader (PerkinElmer).

Protein Extraction and Western Blot. MCF-7 cell lines were plated at 3 × 104 cells per well in a 6-well plate and cultured for 48 h followed by a 10 μM drug treatment for 2 h. Cells were lysed using a 50 μL radioimmunoprecipitation assay buffer (10 mM NaPO4 pH 7.2, 150 mM NaCl, 1 mM EDTA, 0.1% SDS, 1% Na-deoxycholate, 1% Nonidet P40) per well. Lysates were collected and protein concentrations were measured by a plate reader using a Coomassie Protein Assay kit (Thermo Scientific). Samples were then combined with a 4X LDS loading buffer (Thermo Fisher) and DTT (Thermo Fisher) and then boiled at 95 °C for 10 min. Sodium dodecyl sulfate polyacrylamide gel electrophoresis was performed by loading 30 μg of protein sample onto a NuPage 4–12% gradient Bis-Tris gel (Thermo Fisher). Samples were transferred to a PVDF Immobilon-FL membrane (Millipore) using a trans-blot semidry electrophoretic transfer cell (Bio-Rad) and blocked for 1 h using 5% BSA in TBST (10 mM Tris, pH 7.5, 150 mM NaCl, 0.1% Tween-20). Membranes were probed with the following...
antibodies overnight: e-ERBB-2 ABB e2-4001 (MS-325, Neo-markers), HER2-Y1248 (ab47755, Abcam), EGFR D38B1 (4267, Cell Signaling), EGFR-Y1068 (ab5644, Abcam), or α-tubulin (05-829, Millipore). All washes were performed using TBST. After incubation with IR dye-conjugated secondary antibodies goat antirabbit 680RD (926-68071, Li-Cor) and goat antimouse 800CW (926-32210, Li-Cor), membranes were scanned using the Odyssey Infrared Imaging System (Li-Cor Biosciences).

**ASSOCIATED CONTENT**

2 Supporting Information
The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomega.9b00929.

High-throughput assay of compound 7 against a panel of 100 disease-relevant kinases at a concentration of 10 μM performed by Thermo Fisher Scientific’s SelectScreen Profiling Service (Table S1) (PDF)

**AUTHOR INFORMATION**

Corresponding Author
*E-mail: jsridhar@xula.edu. Phone: 1-504-520-7519.

**ORCID**
Rajesh Komati: 0000-0002-9940-0690
Jayalakshmi Sridhar: 0000-0002-4424-9874

**Author Contributions**
†R.S. and M.S. contributed equally.

**Notes**
The authors declare no competing financial interest.

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