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Improved Dose-Response Relationship of (+)-Discodermolide-Taxol Hybrid Congeners

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Abstract

(+)-Discodermolide is a microtubule-stabilizing agent with potential for the treatment of taxol-refractory malignancies. (+)-Discodermolide congeners containing the C-3'-phenyl side chain of taxol (paclitaxel) were synthesized based on computational docking models predicting this moiety would fill an aromatic pocket of β -tubulin insufficiently occupied by (+)-discodermolide, thereby conferring improved ligand–target interaction. It was recently demonstrated, however, that the C-3'-phenyl side chain occupied a different space, instead extending toward the M-loop of β -tubulin, where it induced a helical conformation, hypothesized to improve lateral contacts between

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Notes

The authors declare no competing financial interest.

DEDICATION

The other coauthors of this article dedicate it to Dr. Susan Band Horwitz, of Albert Einstein College of Medicine, Bronx, NY, for her pioneering work on bioactive natural products.

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnat-prod.8b00111.

Excel spreadsheet (XLSX)

Table S2 (PDF)

adjacent microtubule protofilaments. This insight led us to evaluate the biological activity of hybrid congeners using a panel of genetically diverse cancer cell lines. Hybrid molecules retained the same tubulin-polymerizing profile as (+)-discodermolide. Since (+)-discodermolide is a potent inducer of accelerated senescence, a fate that contributes to drug resistance, congeners were also screened for senescence induction. Flow cytometric and transcriptional analysis revealed that the hybrids largely retained the senescence-inducing properties of (+)-discodermolide. In taxol-sensitive cell models, the congeners had improved dose-response parameters relative to (+)-discodermolide and, in some cases, were superior to taxol. However, in cells susceptible to senescence, E_{Max} increased without concomitant improvements in EC_{50} such that overall dose-response profiles resembled that of (+)-discodermolide.

Graphical Abstract

(+)-Discodermolide (Figure 1) was isolated from the Caribbean sponge *Discodermia dissoluta* and, like taxol (paclitaxel), also binds to β -tubulin and stabilizes microtubules.¹ We previously characterized (+)-discodermolide as a potent inducer of senescence^{2,3} (defined as prolonged exit from proliferation that is distinct from quiescence) and hypothesize that the pneumotoxicity observed in the phase I clinical trial of this molecule was likely due to senescence-associated toxicity, thereby justifying the need for improved congeners. Photoaffinity (+)-discodermolide analogues indicated binding in the taxane binding pocket of β -tubulin,⁴ while additional studies showed differential effects on the M-loop,⁵ a domain thought to be important for stabilizing lateral protofilament interactions.⁶ We hypothesized that taxol and (+)-discodermolide could exert complementary stabilizing effects on microtubules; therefore computational docking was used to design novel (+)-discodermolide congeners containing the C-3'-phenyl side chain of taxol (Figure 1).⁷ We posited that this moiety would fill an aromatic pocket of β -tubulin insufficiently occupied by (+)-discodermolide, thereby improving the ligand-target interaction. However, recent data indicate that the taxol moiety does not occupy the predicted space, extending instead toward the M-loop of β -tubulin, where it induces a helical conformation not observed for (+)-discodermolide.⁸ This conformation is predicted to improve lateral contacts between

adjacent microtubule protofilaments and was associated with a reduction in the critical concentration required for tubulin assembly, relative to the parent drug.

These recent insights into the binding of (+)-discodermolide-taxol hybrids in β -tubulin and effects on the M-loop led us to perform an extended screen of congener activity utilizing multiparameter dose-response modeling across a genetically heterogeneous panel of cancer cell lines. Furthermore, since senescence is central to the mechanistic action of (+)-discodermolide, we also determined the ability of hybrid congeners to induce the phenotype. Senescence is a common outcome of many FDA-approved interventions and occurs in both tumor and nontumor cells, thus limiting the anticancer efficacy of cycle-dependent cancer therapeutics.⁹ Furthermore, spontaneous reversion of senescent cancer cells can propel tumors toward an aggressive, recalcitrant state,^{2,10,11} while recent studies have demonstrated that chemotherapy-mediated senescence in nonmalignant cells contributes to cumulative toxicity.¹² However, contemporary drug screening programs do not typically screen for senescence as a clinically relevant end point. We report here that (+)-discodermolide hybrids generally exhibit improved dose-response relationships in taxol-sensitive cancer cell lines; however, in senescent-prone cancer cell lines, hybrids largely act similarly to the parent drug. Overall, hybrid congeners largely retain a similar mechanism of action to the parent compound (+)-discodermolide including senescence induction, despite the known helical conformation of the M-loop.

RESULTS AND DISCUSSION

Properties of (+)-Discodermolide–Taxol Hybrid Congeners

The synthesis and initial testing of the hybrid congeners is described elsewhere.⁷ The chemical structures of (+)-discodermolide–taxol hybrids are shown (Figure 1), denoted (+)-**3**, **4**, **5**, **6**, and **7** [referred to hereafter without the (+)-]. Congeners **4**, **6**, and **7** have a tether length of two carbons and suppress cancer cell proliferation more effectively than congeners **3** and **5**, which have one and three carbon tether lengths, respectively.⁷

Hybrid congeners largely share the same tubulin-polymerizing properties as (+)-discodermolide (Figure 2), as determined by calculating the relative proportion of polymerized versus soluble tubulin in congener-treated extracts of purified bovine brain tubulin. Although congeners **4** and **6** demonstrate slightly enhanced polymerization relative to (+)-discodermolide, these differences are not reproducibly significant. Similar results were obtained when polymerization in supernatants was analyzed, and in intact cells (data not shown), such that overall the polymerization properties of the hybrid congeners are consistent with previously published data.⁷ It is possible that this assay lacks the sensitivity required to detect small increases in polymerization, particularly since (+)-discodermolide is such a potent inducer of microtubule stabilization.

Therefore, the helical conformation of the M-loop induced by congener **6**⁸ does not translate to significant increases in tubulin polymerization. The M-loop is a structural domain of β -tubulin implicated in the mechanism of action of taxol, whereby a folded M-loop conformation is hypothesized to promote lateral contacts between microtubule protofilaments, thereby stabilizing the microtubule lattice.^{6,13} However, the impact of

conformational alterations in the M-loop on the mechanism of action of tubulin-polymerizing agents is disputed,^{14,15} and an alternate hypothesis proposes that conformational changes to the M-loop impart a negligible role on microtubule stability.¹⁵ Instead, it is speculated that the mechanism of action of drugs such as taxol is via effects on longitudinal contacts and conformational strain within tubulin monomers.¹⁵

Multiparameter Dose-Response Analysis of Hybrid Congeners

Cancer drug discovery studies largely report cancer-cell-based analyses as potency, or EC_{50} , which is the drug concentration corresponding to the half-maximal effect. However, EC_{50} doses are associated with proliferative arrest rather than tumor cell death and fail to account for other features of the dose–response relationship, specifically E_{Max} , a metric of efficacy closely associated with cell death, and AUC (area under the curve), a metric that combines potency and efficacy into a single measure.¹⁶ The goal in drug design is to select lead compounds that have increased E_{Max} and AUC coupled with a homogeneous response in a wide array of genetically and histologically diverse cancer models.

With that in mind, we tested the efficacy of the congeners in a panel of cancer cell lines representing malignancies that are treated with taxanes as first-line therapy. These included basal triple negative breast cancer (TNBC) (MDA-MB-468, HCC1143), mesenchymal TNBC (HS578T, BT549, MDA-MB-157), an unclassified TNBC (BT-20), and a non-small-cell lung cancer adenocarcinoma (A549). Three additional cell lines were also selected based on their propensity to undergo (+)-discodermolide-mediated senescence (Figure 3 and Table S1, Supporting Information), including the ER+ breast cancer line (MCF-7), serous ovarian cancer cells (HEY), and a (+)-discodermolide-resistant variant of A549.² All cell models were exposed to compounds for three cell doublings, since E_{Max} and EC_{50} are strongly influenced by doubling time, particularly for microtubule-interacting and DNA-damaging compounds.¹⁷

Figure 3 illustrates examples of dose-response curves for taxol, (+)-discodermolide, and one of the more biologically active hybrid congeners (**6**), across all cancer cell lines (Figure 3A), versus senescent-prone cancer cell lines (Figure 3B). Metrics that reflect features of the dose–response relationship are illustrated for a taxol curve (Figure 3A), namely, E_{Max} , EC_{50} , and AUC. Dose-response curves were highly variable for (+)-discodermolide and congener **6**, whereas taxol demonstrated a more uniform response (Figure 3A). Most notable was low E_{Max} and AUC, with high variability for (+)-discodermolide, consistent with its known senescence-inducing properties. This was even more apparent in cell lines highly susceptible to (+)-discodermolide-mediated senescence (Figure 3B). Dose-response curves clearly illustrate the enhanced activity of hybrid congener **6** relative to (+)-discodermolide in senescent-prone lines (Figure 3B), evident primarily as increased E_{Max} and AUC.

Subsequently, data were subject to sigmoidal dose-response curve-fitting simulations and multiparametric analysis to generate E_{Max} , AUC, and EC_{50} values for all cell lines (Table S1, Supporting Information). Consistent with the dose-response curves from Figure 3, (+)-discodermolide had the lowest median E_{Max} and AUC in all cell lines (Figure 4A). E_{Max} for taxol (Tx) and congeners **4** through **7** was highest; however, AUC for these congeners was much more variable, particularly for congener **6**. This suggests that congener **6** has cell-

killing activity comparable to taxol, but with considerably more variability in E_{Max} and EC_{50} across heterogeneous cancer cell lines.

We sought to clarify whether the variability in dose-response parameters observed for hybrid congeners (particularly congener **6**) was due to weak activity in senescent-prone cancer cell lines. Drug-resistant cell lines, including those that are prone to senescence, typically have shallow dose-response curves; therefore, using this criterion we identified three cancer cell lines prone to (+)-discodermolide-mediated senescence and recomputed dose-response parameters and box plots for sensitive versus senescent-prone cohorts (Figure 4B). Within the sensitive group of cancer cell lines, all congeners had improved parameters relative to (+)-discodermolide. In fact, for congeners **4**, **6**, and **7**, values for all three parameters were superior or comparable to taxol (specifically decreased EC_{50} and increased E_{Max} and AUC: right side of Figure 4B). Conversely, in senescent-prone cancer cell lines, EC_{50} and AUC for all congeners generally tracked with (+)-discodermolide values. However, congeners **6** and **7** had EC_{Max} values that were slightly superior and less variable than taxol, indicating improved dose-response profiles, even in recalcitrant, senescent-prone cell lines.

Therefore, in-depth analysis indicates that the addition of a C-3'-phenyl side chain of taxol to (+)-discodermolide utilizing a tether length of two carbons (congeners **4**, **6**, and **7**) favorably improves EC_{50} , consistent with our original findings.⁷ Moreover, congener **6** has an improved dose-response relationship relative to taxol in the sensitive cell lines, including increased AUC and E_{Max} and reduced EC_{50} . However, these improvements are subtle in senescent-prone cancer cell lines, although congeners **6** and **7** have improved E_{Max} . Despite these favorable findings, the large variability in dose-response parameters across the multiple cancer cell lines for congeners **4**, **6**, and **7** (Figure 4A) tempers interest for continued development.

The improved dose-response profiles do not correlate with enhanced microtubule stabilization. It is plausible that the hybrid congeners have increased intracellular transport or duration of the ligand-target interaction, and these and other factors account for the enhancement of E_{Max} observed in senescent prone models. Alternatively, the differences in congener activity between sensitive and senescent-prone cohorts may be due to differential expression of tubulin isotypes in these cell lines and preferential binding of congeners to distinct tubulins.

Flow-Cytometric-Based Analysis of Cell Fate

Chemotherapy can result in an array of cell fates, including cell death, senescence, and mitotic catastrophe. The proportion of each phenotype varies widely among cancer cells, as does the time for these fates to fully emerge and stabilize, typically 6–14 days in the case of senescence. Taken the strong senescence induction by (+)-discodermolide, it was necessary to contrast this characteristic for hybrid congeners.

Detection of senescence remains an ongoing challenge in cancer since one of the most commonly used methods, senescence-associated β -gal (SA- β -gal) activity,¹⁸ indicative of lysosomal β -gal activity,¹⁹ is noninformative for many cancer cell lines, particularly TNBC, due to high basal expression of SA- β -gal. Thus, we devised a flow-cytometry approach to

quantify senescent versus dead cells in live cell populations following exposure to cytotoxic molecules. This method uses the cell permeant DNA dye DAPI (4',6-diamidino-2-phenylindole) to identify dead cells, based on rapid uptake into live cells when membrane permeability is compromised. Conversely, stably senescent cells exhibiting increased cell volume and cytoplasmic granularity can be detected as populations with high forward scatter (FSC, increased volume) and side scatter (SSC, increased cytoplasmic granularity) that have low DAPI uptake.

The method was optimized using the TNBC mesenchymal cells HS578T that were treated for 10 days with 100 nM doxorubicin, a strong senescence inducer in this cell line. As depicted in Figure 5A, a large population of cells exhibited both increased FSC and SSC, indicative of senescence, whereas cells undergoing mitotic catastrophe (largely absent in this case) exhibited only an increase in FSC. As shown in Figure 5B, dead cells were easily identified as populations with increased DAPI uptake. Cells were sorted for high side scatter (SSC+) and low DAPI staining (DAPI-) indicated by the gated quadrant. Subsequently, qRT-PCR of sorted versus nonsorted doxorubicin-treated HS578T cells was performed to evaluate the expression of transcripts associated with the senescence-associated secretory phenotype (SASP), a robust indicator of senescence.²⁰ As shown in Figure 5C, IL-6, IL-8, and CXCL1 expression was statistically significantly higher in sorted cells ($p < 0.001$; unpaired t test), confirming enrichment for senescence, based on both morphologic changes and elevated expression of SASP-associated genes.

This method was subsequently used to measure cell death and senescence, whereby cells were treated with approximately EC₈₀ doses of compounds (determined from dose-response data) for 3 to 6 days, depending on the cell line. These doses were chosen to induce cell death and mimic clinically relevant doses. Figure 6A illustrates representative population distributions for (+)-discodermolide and taxol-treated HEY cells, a senescent-prone cancer cell line. The gated rectangle indicates the senescent population (SSC+, DAPI-), while the triangle represents dead cells (SSC-, DAPI+). Hybrid congeners induced senescence at levels comparable to (+)-discodermolide and taxol in HEY (~10–15%, Figure 6B) and HS578T (~15–18%, Figure 6C), suggesting that inherent tumor biology predominately influences development of the phenotype. HS578T cells were resistant to cell death by (+)-discodermolide and hybrid congeners, under the conditions tested. Conversely, BT549 (Figure 6D) and MDA-MB-468 (Figure 6E) had higher levels of cell death generally, with lower senescence induction (<10%), and congeners **4**, **6**, and **7** induced the most senescence, although statistical significance was not achieved (one-way ANOVA, $p > 0.05$). Thus, hybrid congeners induce senescence at levels comparable to (+)-discodermolide.

Transcriptional Analysis of SASP mRNA Species in Congener-Treated Cancer Cells

Senescent cells impart detrimental effects on the local tumor microenvironment, primarily due to a hyperactive secretory phenotype, termed the SASP,²¹ whereby a secreted, pro-inflammatory milieu drives resistance and metastatic progression.^{10,11} We evaluated the transcription of three well-characterized SASP-associated genes, IL8, CXCL1, and PAI1. CYCLA2 (cyclin A) was also evaluated to confirm proliferative arrest. In HEY cells, all

three SASP genes were induced to comparable levels, consistent with these cells being generally susceptible to senescence.

MDA-MB-468 cells demonstrated higher induction of SASP genes with (+)-discodermolide and hybrid congeners (**4**, **6**, and **7**), consistent with data presented in Figure 6. Thus, (+)-discodermolide-taxol congeners retain the senescence-inducing properties of the parent compound (+)-discodermolide.

Target-based analysis of tubulin–ligand interactions has provided insight into the binding of microtubule-stabilizing drugs such as taxol,^{6,13–15} although there is a difference of opinion regarding the pharmacodynamic impact of M-loop stabilization.^{8,15} Clearly, the addition of the taxol side chain to (+)-discodermolide improves pharmacology, evidenced by improved dose–response relationships; however there is no evidence to support that this is due to altered tubulin polymerization. Tubulins are a ubiquitous and multifunctional cellular protein family comprising several isoforms; therefore it is plausible that perturbations of tubulin function via ligand–target binding induces diverse downstream biological effects depending on the type of tubulin targeted, intracellular localization, and specific cellular specialization. These unknowns serve to remind us that our knowledge of tubulin isoforms and their cellular functions is limited and that vastly more research in this field is required and justified from a drug discovery perspective. We also note that contemporary drug development fails to adequately screen against senescence induction as a clinically relevant therapeutic outcome. Thus, we advocate for a more intensive, holistic approach to phenotypic drug screening that incorporates multiparameter dose–response modeling in genetically diverse cancer cell lines, coupled with more target-ligand-based approaches such as crystallography and molecular modeling to continue to push the translational impact of discovery for this class of molecules.

EXPERIMENTAL SECTION

Congener Synthesis and Formulation

(+)-Discodermolide and hybrid congeners were synthesized as described previously.⁷ Taxol was obtained from the U.S. National Cancer Institution. All were dissolved in DMSO and diluted in cell culture media such that the DMSO concentration was less than 0.01%.

Tubulin Polymerization Assays

BBT (2 M) was incubated at 37 °C for 30 min in the presence of 3 μ M (+)-discodermolide or its analogues in MEM–glycerol buffer containing 2 mM GTP, pH 6.9. Samples were centrifuged at 120000g at 37 °C for 1 h. The pellet containing the polymerized tubulin was dissolved in sodium dodecyl sulfate (SDS) sample buffer. Both supernatant fractions and the solubilized pellets were analyzed by SDS-PAGE. Alpha-tubulin in these two fractions was determined by Western blot analysis using anti- α -tubulin antibody.

Cell Culture

Ten cell lines were used in this study as detailed: MDA-MB-468, BT20, HCC1143, HS578T, BT549, MDA-MB-157 (TNBC breast), MCF7 (ER+ breast), HEY (serous ovarian), A549,

and its (+)-discodermolide-resistant variant, AD32² (K-RAS mutant NSCLC). All cell lines were purchased from the American Type Culture Collection, unless otherwise stated, and maintained in RPMI medium (HyClone) supplemented with 10% fetal bovine serum (Gibco). All cell lines were mycoplasma negative, and only early passages (<25) were used in experiments.

Cell Proliferation Assays

Standard cell proliferation assays were performed using sulforhodamine B (SRB) to measure total protein in adherent cells.²² A prerequisite for highly quantitative data analysis is that a sigmoidal dose–response curve be generated for all compounds analyzed. Cells were seeded into 96-well plates at densities varying between 900 and 4000 cells per well, depending on the characteristics of each cell line. After 24 h to permit adherence, six replicate wells of cells were treated with different concentrations of drugs for three cell doublings (corresponding to 3 to 7 days). After drug exposure, cells were processed according to the SRB assay. All experiments were replicated at least three times.

Multiparameter Dose–Response Analysis

Data were analyzed as described previously,¹⁶ whereby values were fitted to the logistical sigmoidal model using nonlinear least-squares regression performed in the R statistical software suite (<http://www.R-project.org/>). Dose-response parameters, EC₅₀, E_{Max} , and AUC were subsequently computed in R. Efficacy, E_{Max} , is the maximum response achievable from a dosed molecule. Potency, EC₅₀, is the drug concentration corresponding to the half-maximal effect. Area under the curve, AUC, is a metric that combines potency and efficacy into a single measure. Data were plotted as box and whisker plots with median (horizontal line) and interquartile range (boxes) using R.

Fluorescence-Activated Cell Sorting (FACS)

Cells were exposed to taxol, (+)-discodermolide, or hybrid congeners at EC₈₀ doses (determined from dose–response data), for 3 to 6 days, depending on each cell line. Nonadherent cells were collected by centrifuging media and combining with trypsinized monolayers containing adherent cells. Cell pellets were washed three to five times with sterile, warm PBS using gentle centrifuging between washes. Cell pellets were resuspended in 1 mL of FACS reagent (1× PBS, 4% fetal bovine serum) containing DAPI at 1/10,000 dilution. Cells were kept on ice and immediately analyzed by FACS (Direct X 10 Calibur), counting 10 000 cells per gated region per sample. All experiments were performed in duplicate.

Evaluation of Gene Expression in Drug-Treated Cancer Cells

Total RNA was isolated from surviving, adherent cells treated with various compounds using RNeasy and subsequently cDNA synthesized using the Superscript VILO reverse transcriptase. Real-time qRT-PCR was performed using the SYBRGreen I Master (Roche) and run on a LightCycler 480 (Roche) to determine expression of the indicated genes. Expression was normalized to cyclophilin B and expressed relative to vehicle-treated control. Experiments were performed in triplicate. Primers were designed using primerbank

(<http://pga.mgh.harvard.edu/primerbank>), and primer sequences are provided in Table S2, Supporting Information.

Statistics

With the exception of multiparameter dose–response analyses, all statistical analyses were performed using GraphPad Prism 7.0. Differences were considered statistically significant at a value of $p < 0.05$.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.
Designed (+)-discodermolide hybrid analogues.

Figure 2.

Polymerization of bovine brain tubulin by (+)-discodermolide and hybrid congeners. Bovine brain tubulin was incubated with (+)-discodermolide (D), taxol (T), or hybrid congeners for 1 h. Supernatant (S) and pellet (P) fractions were extracted, and % polymerized tubulin was calculated from α -tubulin expression by immunoblotting as $P/(S + P)$. A graphical representation of % polymerization for the hybrid congeners is shown, indicating that hybrids **4** and **6** had the most potent effect on bovine brain tubulin (BBT) polymerization. C = DMSO control.

Figure 3.

Selected examples of dose-response data for taxol, (+)-discodermolide, and hybrid congener 6. (A) Examples of dose-response curves generated from SRB assays across 10 cancer cell lines. Each line represents a dose-response data set for one cell line. Among all lines, the least variation was observed for taxol. E_{Max} , EC_{50} , and AUC (gray, shaded area) are illustrated for one taxol dose-response curve. (B) Examples of dose-response relationships for three cancer cell lines susceptible to (+)-discodermolide-mediated senescence. Low E_{Max} and absence of an upper dose-response plateau at high doses are indicative of cell lines prone to senescence induction.

Figure 4.

Improved dose-response parameters of hybrid congeners relative to (+)-discodermolide. (A) Variation in dose-response parameters for taxol (T), (+)-discodermolide (D), and hybrid congeners (3–7) across 10 cancer cell lines, computed from sigmoidal dose-response simulations followed by multiparameter dose-response analysis. EC_{50} (potency), E_{Max} (efficacy), and AUC (potency and efficacy combined) are represented as box and whisker plots showing median (horizontal line) and interquartile range (boxes). Bars extending to one-and-a-half times the interquartile range indicate variance for each parameter, while outliers are shown as nonconnected data points. (B) Hybrid congeners have generally superior E_{Max} and AUC relative to (+)-discodermolide but inferior to taxol in senescent-prone cancer cell lines. Congener 6 has values superior or comparable to taxol in cancer cell lines sensitive to tubulin-polymerizing agents (right side of graph), although highly variable among cell lines.

Figure 5.

Selection and enrichment of senescent cells by live cell sorting for SSC+ and DAPI- populations. (A) Increase in FSC (forward scatter) and SSC (side scatter) in doxorubicin-treated HS578T breast cancer cells (100 nM, 10 days). (B) Cell sorting for SSC+ DAPI- cells (gated quadrant). The region encompassing DAPI+ dead cells is indicated. (C) Sorted and unsorted populations were processed for qRT-PCR and expression of senescence associated secretory phenotype (SASP)-associated cytokines determined. Expression of the SASP-associated genes IL6, IL8, and CXCL1 were statistically significantly higher in doxorubicin-treated, senescent-enriched SSC+ DAPI- populations (gray) versus unsorted populations (black) ($p < 0.001$: unpaired t test).

Figure 6.

Determination of cell fate (death versus senescence) in congener-treated cancer cell lines. Cancer cell lines were treated with EC₈₀ doses of (+)-discodermolide, taxol, or hybrid congeners for 3 to 6 days, depending on the cell line, and subsequently analyzed by flow cytometry to determine the relative proportion of dead (DAPI+) versus senescent (SSC+ DAPI-) cells. (A) Representative FSC versus SSC distribution of (+)-discodermolide or taxol-treated HEY ovarian cancer cells. Regions demarcated by the rectangle and triangle indicate gates established to quantify senescent or dead cells, respectively. Relative quantitation of dead versus senescent cells in drug-treated (B) HEY ovarian cancer cells, (C) HS578T mesenchymal TNBC cells, (D) BT549 mesenchymal TNBC cells, and (E) MDA-MBA-468 basal TNBC cells. Bars represent mean ± SEM ($n = 2$). Note that the left y-axis represents % dead cells, while the right y-axis represents % senescent cells. Overall, hybrid congeners retained the senescent-inducing properties of (+)-discodermolide (one-way ANOVA, $p > 0.05$), although high variability was noted between cell lines.

Figure 7.

Increased expression of senescence-associated secretory genes indicative of senescence following treatment with hybrid congeners. Transcriptome analysis (qRT-PCR) of SASP-related gene expression (IL8, CXCL1, and PAI1) and proliferation (cyclin A2, CYCLA2) in (A) HEY ovarian cancer cells and (B) MDA-MB-468 basal breast cancer cell populations treated with EC₈₀ doses of the indicated compounds for 6 days. The dashed line (at 1) represents baseline expression in vehicle-only treated asynchronous cells. Note that the gray bars correspond to the bottom y-axis, while black bars correspond to the scale on the top y-axis. Bars represent mean ± SEM (n = 2). Hybrid congeners induce SASP gene expression to a comparable or greater extent than either taxol or (+)-discodermolide (disco), suggesting retention of senescence-inducing properties.