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Solid-Phase Synthesis and Biological Activity of a Combinatorial Cross-Conjugated Dienone Library

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Abstract: The solid-phase synthesis of a combinatorial cross-conjugated dienone library based on the structure of clavulones and their biological activity are reported. Clavulones are a family of marine prostanoids, and are composed of a cross-conjugated dienone system bearing two alkyl side-chains. The cross-conjugated dienone system irreversibly reacted with two nucleophiles. Our strategy for the solid-phase synthesis of the cross-conjugated dienones involves the Sonogashira-coupling reaction of a solid-supported cyclopentenone **10** bearing an acetylene group, followed by aldol condensation with aldehydes. The diphenyl derivative **7aA** was prepared from the solid-supported cyclopentenone **10** in 56% total yield. Combinatorial synthesis of a small library using twelve halides and eight aldehydes resulted in the production of 74 desired compounds from 98 candidates, and were detected by their mass spectra. Antiproliferative effects of the crude compounds against HeLaS3 cells showed that eleven samples showed strong antitumor activity ($IC_{50} < 0.05$ μM). Further biological examination of four purified compounds by using five tumor cell lines (A549, HeLaS3, MCF7, TMF1, and P388) revealed strong cytotoxicity comparable to that of adriamycin.

Keywords: alkylation · chemical biology · clavulone · combinatorial chemistry · prostanoids · solid-phase synthesis

Introduction

Biologically active natural products have served as effective biochemical probes for the discovery of not only new drug targets but also new biomarkers.[1,2] The synthesis of small molecules based upon the structure of biologically active natural products would be an effective and promising way for the identification of new biochemical probes.[3,4] Combinatorial chemistry can assist the high-speed synthesis of these focused libraries. The combinatorial approach might not be an economic route when compared with the traditional approach based on elucidating the best fragment at each diverse site because fully combinatorial libraries contain many redundant compounds. However, in the traditional approach the compounds composed of the best fragments would not often exhibit the strongest biological activity in cell-based assay since the cell permeability of the small molecules would largely influence their biological activity.

Mammalian cross-conjugated dienone prostanoids such as Δ^7-prostaglandin A_2 (Δ^7-PGA_1) (**2**) and 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 (15d-PGJ_2) (**3**) are metabolites of cyclopentenone prostanoids PGA_2, PGA_1, and PGJ_2 (Scheme 1).[5] They display varied biological activities, the biological mechanisms of which, would be based on reversible and selective

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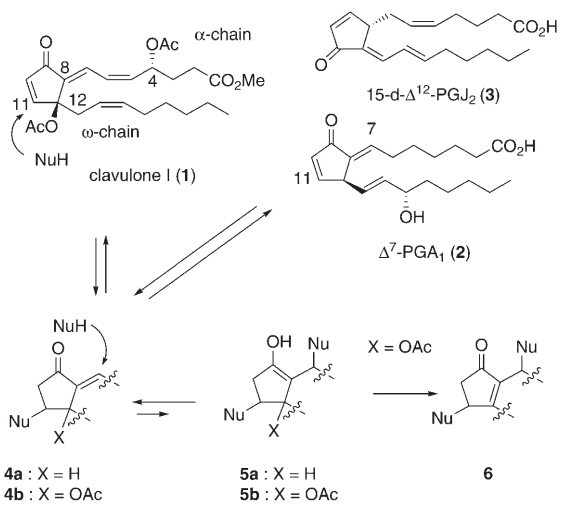
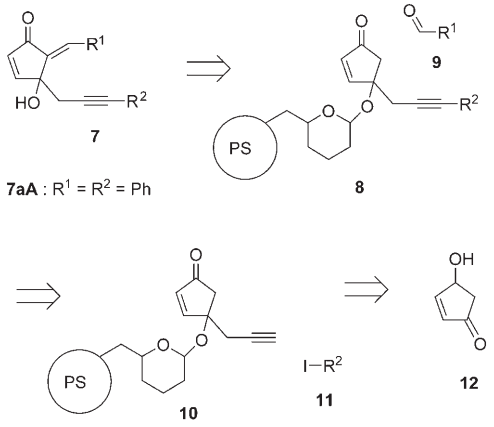


Scheme 1. Structure of cross-conjugated dienone prostanoids and their proposed biological mechanism of action.



Scheme 2. Strategy for the solid-phase synthesis of cross-conjugated dienone library **7**.

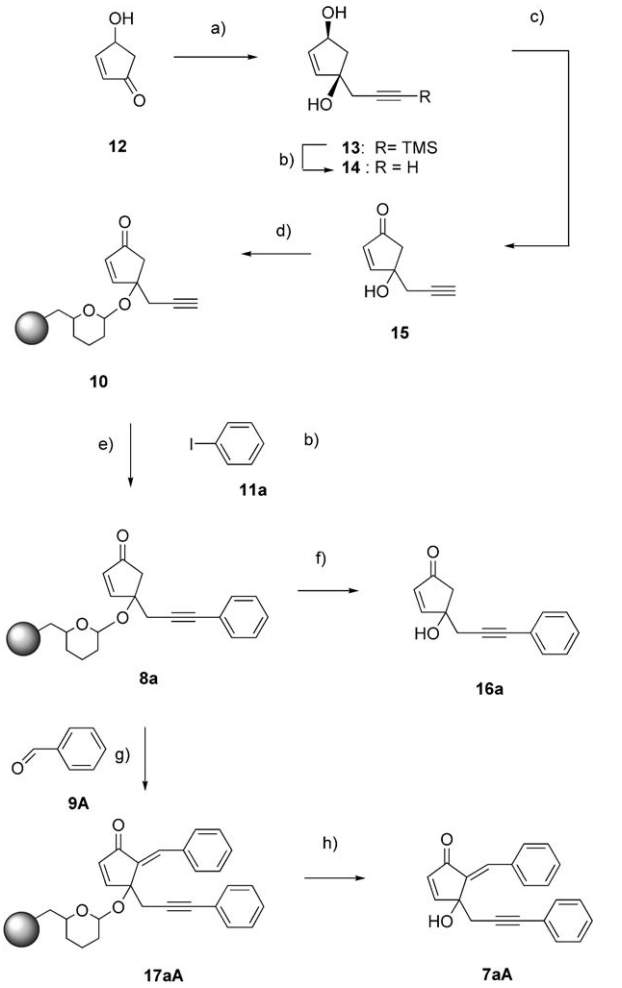
alkylation with specific proteins at their C11 position to give thermodynamically stable adducts **4a**.[6,7] For example, in 1995, the 15-deoxy-$\Delta^{12,14}$-PGJ_2 (**2**) ligand was found to have a high affinity for the nuclear receptor PPARγ, and to modulate gene transcription by binding to this receptor via alkylation with the highly reactive cross-conjugated dienone system.[6a] On the other hand, marine prostanoid clavulone I (**1**) isolated from the Okinawan soft coral features the same cross-conjugate dienone system along with a *tert*-acetoxyl group at the C12 position and shows strong cytotoxicity.[8,9] The cross-conjugated dienone **1** could undergo sequential alkylation to provide the dicoupling product **5b**, followed by irreversible β-elimination of the C12 acetoxyl group to afford enone **6**. The irreversible alkylation is expected to be effective for inducing stronger biological activity than such mammalian prostanoids. Therefore, we started a project involving the combinatorial synthesis of cross-conjugated dienones based on the structure of clavulones. There have been many reports on the synthesis of clavulones as a single target.[9,10] However, there are few examples of the synthesis of their libraries. We have previously reported an effective solid-phase synthesis of clavulones involving two carbon–carbon bond-forming reactions.[11] Herein we report the solid-phase synthesis of a combinatorial library of cross-conjugated dienones and the biological activity of the library members.

Results and Discussion

The cross-conjugated dienones **7** attached to two aromatic side-chains was designed as a scaffold in the library synthesis (Scheme 2). We have already reported that the diphenyl derivative **7aA** possessing a hydroxyl group at the C12 position irreversibly reacted with two nucleophiles under mildly basic conditions, and showed strong antitumor activity (IC_{50} = 15 nM) in HeLaS3 cells.[12] Lower steric hindrance of the hydroxyl group at the C12 position compared with that of the the acetoxyl group improved reactivity of enone **7aA** towards electrophilic addition. Tuning the steric and electronic parameters of the two aromatic side-chains would be effective not only for further improvement of the biological activity, but also inducing selectivity in the alkylation reaction.

Our strategy for the solid-phase synthesis of cross-conjugated dienones **7** is described in Scheme 2. We designed the solid-supported 4-propynyl-4-hydroxylcyclopentenone **10** as a key intermediate. Sonogashira-coupling reaction of cyclopentenone **10** with the aryl iodide **11** and aldol condensation with aldehyde **9** would enable the incorporation of the α- and ω-chains, respectively. In the previous report, the aldol reaction involved β-elimination of the *tert*-alkyloxy group as an undesired side-reaction. In the solid-phase synthesis, the corresponding β-elimination does not reduce the purity of the products because the β-eliminated products would be released from the resin. The cyclopentenone core **10** was immobilized at the *tert*-hydroxyl group through a tetrahydropyranyl (THP) linker,[13] that is stable to the two carbon–carbon bond formations. Cleavage from the solid-support under mildly acidic conditions yields the cross-conjugated dienones **7** without decomposition.

Preparation of solid-supported cyclopentenone **15** bearing a terminal acetylene is shown in Scheme 3. Treatment of cyclopentenone **12** with 3-trimethylsilyl-2-propynyl lithium in THF at −78 °C gave stereoselective diol **13** in 85% yield as a single isomer. Removal of the TMS group under basic conditions gave the terminal acetylene **14** in 85% yield, followed by selective oxidation of the secondary alcohol to afford cyclopentenone **15** in 65% yield. The latter was attached to the resin through the THP linker. Exposure of 3,4-dihydro-2*H*-pyran (DHP) polystyrene (1.10 mmol g^{-1}) to a solution of diol **13** and pyridinium *p*-toluenesulfonate (PPTS) in CH_2Cl_2 at 40 °C for 24 h provided the solid-supported terminal acetylene group of cyclopentenone **10**. The IR spectra of **10** show a 3293 cm^{-1} absorption derived from the terminal acetylene group. The loading of **10** was estimat-

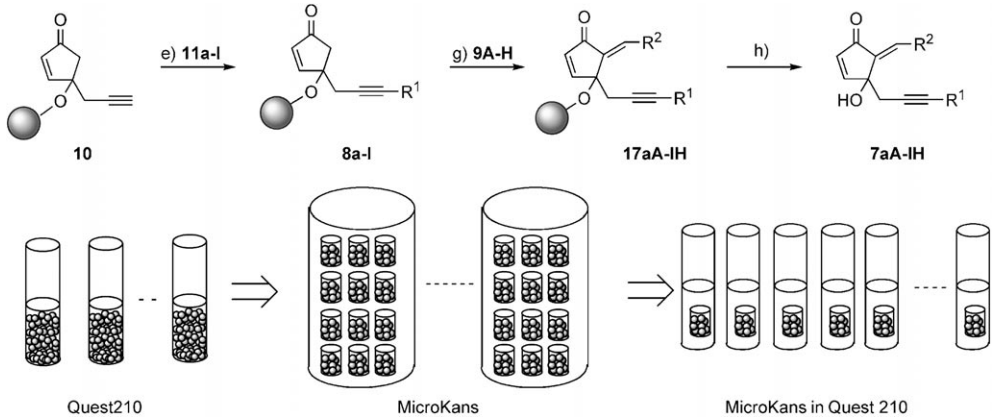


Scheme 3. a) $TMSCCCH_2Li$, THF, −78 °C, 85 %; b) K_2CO_3, MeOH, RT, 85 %; c) MnO_2, CH_2Cl_2, RT, 65 %; d) HM-DHP resin, PPTS, CH_2Cl_2, 40 °C, 24 h, 54 % based on the resin; e) $[Pd(Ph_3P)_4]$, CuI, NEt_3, DMF, 40 °C, 24 h; f) 5 % TFA/CH_2Cl_2, quant, from **10**; g) KHMDS, THF, −78 °C, then **9A**, −78 °C; h) 5 % TFA/CH_2Cl_2, 56 % from **10**.

ed by acidic cleavage followed by purification using column chromatography on silica gel to be 54 % yield based on the resin.

Solid-phase synthesis of the diphenyl derivative **7aA** was examined. Incorporation of the ω-chain was achieved by treatment of compound **10** with phenyl iodide (**11a**), $[Pd(PPh_3)_4]$, CuI, and diisopropylethylamine in DMF for 24 h at 40 °C[14] to give the solid-supported phenyl acetylene **8a**. Cleavage from the resin under acidic conditions provided the phenyl acetylene **16a** in quantitative yield based on **10**. Next, we explored the aldol reaction using phenyl acetylene **8a**. The resin was packed into Irori MicroKans. The solid-supported ketone **8a** was treated with potassium hexamethyldisilazane (KHMDS) in THF at −78 °C for 1 h to generate the enolate that was subsequently treated with benzaldehyde **9A** to provide the solid-supported cross-conjugated dienone **17aA**. The cross-conjugated dienone **17aA** was cleaved from the resin under acidic conditions, followed by purification through column chromatography on silica gel, to give the cross-conjugated dienone **7aA** in 56 % yield based on compound **10**. The analytical data (^1H NMR, ^{13}C NMR, and IR) of **7aA** was identical with our previously reported data.[12] Surprisingly, the yield of the products suggested that the β-elimination of the *tert*-alkyloxy group did not occur during the aldol reaction of **8a**, presumably because the large steric hindrance of the solid-support could hinder the attack of the base at the propargyl position.

Combinatorial synthesis of cross-conjugated dienones was then investigated (Scheme 4). Eleven aryl iodides **11a–k** and a vinyl bromide **11l** for the ω-chain, and eight aldehydes **9A–H** for the α-chain were used as building blocks for the preparation of a 96-member library (Figure 1). Sonogashira-coupling reaction of the solid-supported acetylene group with each of the twelve halides **11a–l** was achieved utilizing an Argonaut Quest 210 Parallel Organic synthesizer. Use of the Quest 210 synthesizer was effective for minimization of solvent and reagent quantities in the coupling reactions. The resulting resins coupled with each building block were packed into eight MicroKans to provide 96 MicroKans. Aldol condensation of the twelve solid-supported ketones **8a–l** in MicroKans with each of the eight aldehydes **9A–H** was achieved in the same vessel to provide the solid-supported cross-conjugated dieneones **17aA–lH**. Cleavage of the compounds from each resin was achieved by using the



Scheme 4. Combinatorial synthesis of a combinatorial library **7**: e), g), and h) as in Scheme 3.

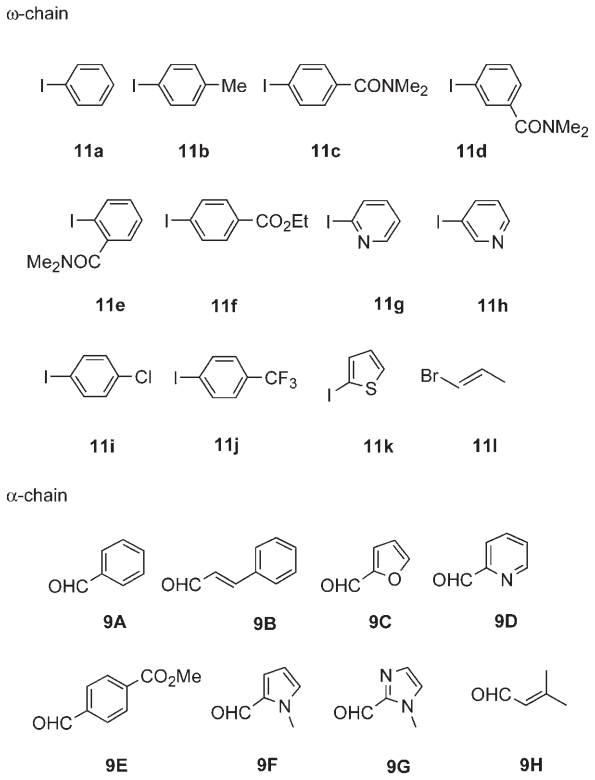


Figure 1. Building blocks for the synthesis of a combinatorial library **7**.

Quest 210 synthesizer. The cleaved solution was neutralized with 2.0 equivalents of piperidinomethyl polystyrene (3.48 mmol g^{-1}), followed by concentration in vacuo. It should be noted that concentration of the crude products without notarization resulted in decomposition of the cross-conjugated dienones **7**. The purity of the library compounds was estimated by HPLC-MS analysis using the UV absorption at 254 nm (Figure 2). In 98 trials, 76 compounds **7** were

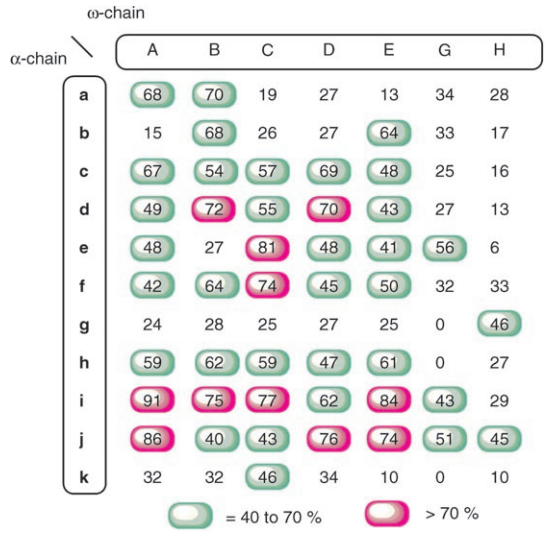


Figure 2. Purity of the combinatorial library **7**, as estimated by HPLC-MS analysis based on the UV absorption at 254 nm.

detected by MS spectra. Aldehyde **9F** and the vinyl bromide **11l** did not perform well in the library synthesis because of the low solubility of **9F** in the reaction solvent and low reactivity of **9l** in the coupling reaction. Further HPLC analysis based on the UV absorption at 254 nm shows that there are 12 compounds with over 70% purity and 33 compounds with 40–70% purity (Figure 2).

Biological evaluation: The alkylation reaction with biomolecules in cells can result in antiproliferative effects. We first examined the cytotoxicity of all unpurified library compounds in HeLaS3 cells (Figure 3).[15] Figure 4 shows the library members **7jA**, **7kA**, **7aD**, **7dD**, **7hD**, **7kD**, **7hE**, **7kE**, **7iG**, **7jG**, and **7iH** exhibiting very strong antitumor activity ($IC_{50} < 0.05$ μM). The phenyl, 2-pyridyl, 4-methoxylcarbonyl-

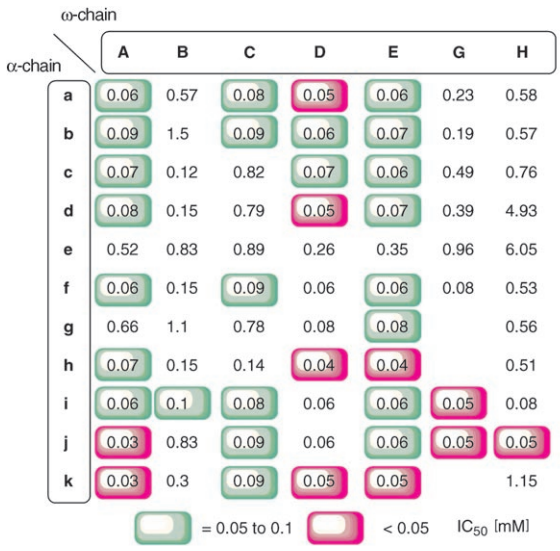
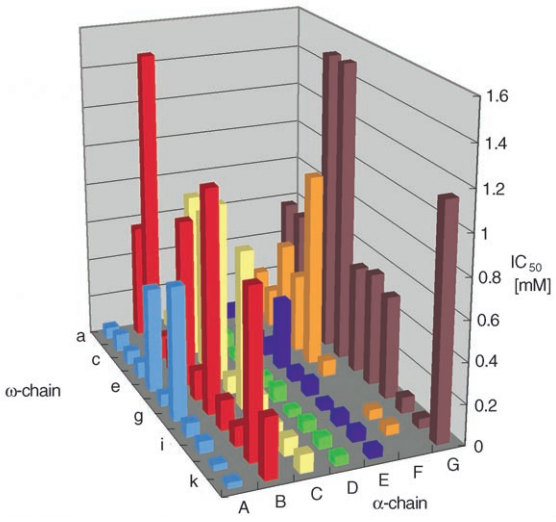


Figure 3. Cytotoxicity of crude library compounds **7** in HeLaS3 cells.



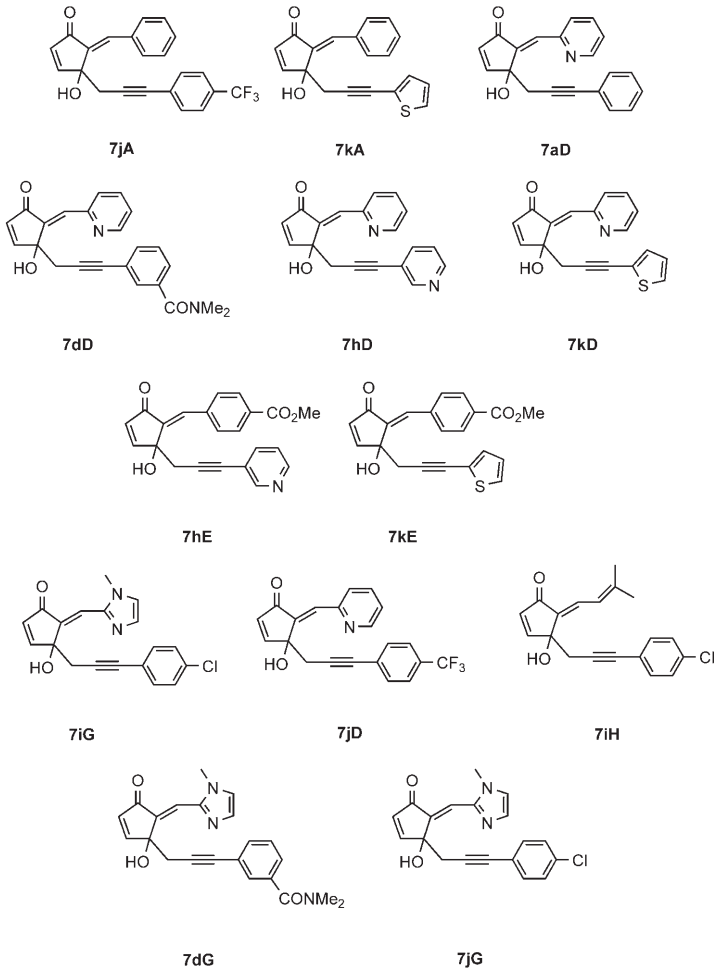


Figure 4. Structures of the members of the library **7** showing strong biological activity.

phenyl, pyrazoyl substituents at the α-chain would improve cytotoxicity. On the other hand, although *trans*-cinnamaldehyde (**9B**) and furfural (**9C**) were converted to the desired cross-conjugated dienones with good purity, the cross-conjugated dienones **7aB**–**7kB** and **7aC**–**7kC** exhibited relatively low cytotoxicity. These results suggested that electron-withdrawing groups at the α-chain could be effective for the strong biological activity.

Further biological testing of the purified compounds against five tumor cell lines (A549, HeLaS3, MCF7, TMF1, and P388) was examined (Table 1). We selected four compounds **7jA**, **7dD**, **7dG**, and **7jG** from the library on the basis not only of their biological activity, but also their hydrophilicity as hydrophobic compounds often result in nonspecific interaction with biomolecules. 5-Fluorouracil (5-FU) and adriamycin (ADM) were used as positive controls. All compounds showed very strong biological activity comparable to adriamycin against four tumor cells except for TMF1. Especially, the cytotoxicity of **7jG** against HeLaS3 was fourfold stronger than the lead compound **7aA**. At this stage, it is not clear if the cytotoxicity is caused by alkylation of specific targets or by random alkylation. However, the difference of cytotoxicity against TMF1 and the other cell lines is promising, and we plan to elucidate the mechanism of action in subsequent work.

Table 1. Cytotoxicity of purified cross-conjugated dienones **7jA**, **7dD**, **7dG**, and **7jG** in various tumor cells.

Entry	Compound	IC_{50} [μM]				
		A549	HeLaS3	MCF7	TMF1	P388
1	**7jA**	0.058	0.009	0.020	0.112	0.014
2	**7dD**	0.048	0.016	0.030	0.220	0.009
4	**7dG**	0.077	0.040	0.057	0.419	0.061
3	**7jG**	0.086	0.004	0.040	0.200	0.055
5	5-FU	1.44	13.3	0.52	4.72	0.887
6	ADM	0.067	0.021	0.021	0.083	0.015

Conclusion

We demonstrated the solid-phase synthesis of a cross-conjugated dienone library using the Sonogashira-coupling reaction and aldol condensation. A 96-member combinatorial synthesis using twelve aryl iodides **11a**–**l** and eight aldehydes **9A**–**H** provided 76 cross-conjugate dienones **7** with good purity. From the library, eleven compounds showed very strong cytotoxicity in HeLaS3 cells. Further biological examination using four selected and purified compounds against several tumor cell lines showed that all compounds have strong cytotoxicity comparable to that of adriamicin, except against TMF1 cells. Combinatorial synthesis of larger libraries and identification of the target molecules are in progress.

Experimental Section

General procedure: NMR spectra were obtained by using a JEOL Model EX-270 (270 MHz for ^{1}H, 67.8 MHz for ^{13}C NMR spectra) or a JEOL Model ECP-400 (400 MHz for ^{1}H, 100 MHz for ^{13}C NMR spectra) instrument in the indicated solvent. ^{1}H NMR spectral data are reported as follows: Chemical shifts are reported relative to tetramethylsilane (0.00 ppm) or chloroform (7.26 ppm). ^{13}C signals are reported relative to $CDCl_3$ (77.0 ppm) or [D_6]DMSO (39.7 ppm). FTIR spectra were recorded on a JASCO FT/IR-610 spectrometer and only significant diagnostic bands are reported. Reverse-phase column chromatography was performed using ODS-AM120-S50 resin (YMC). Silica gel thin-layer chromatography (TLC) was performed on plates precoated with Kieselgel 60F254 (E. Merck AG, Darmstadt). High Performance Liquid Chromatography (HPLC) was performed on a Hewlett–Packard1100 series instrument equipped with an XTerra MS C18 column (Waters, 2.5 mm, 2.1×20 mm). Mass spectra were provided by a Mariner Biospectrometry Workstation (ESI-TOF) from PE Science or a Micromass LCT (ESI-TOF) system.

(1*R,4*S**)-1-(1-Trimethylsilylpropynyl)-cyclopent-2-en-1,4-diol (13)**: *n*-Butyllithium (14.2 mL, 1.59 M in hexane, 22.5 mmol) was added to a stirred solution of diisopropylamine (3.4 mL, 24.5 mmol) in dry tetrahydrofuran (40 mL) at 0 °C under argon. After stirring for 20 min, the mixture was cooled to −20 °C and 1-trimethylsilyl-

propyne (3.3 mL, 22.5 mmol) in dry tetrahydrofuran (5.0 mL) was added. After 20 min, a solution of 4-hydroxy-2-cyclopentenone (**12**) (950 mg, 9.79 mmol) in dry tetrahydrofuran (10 mL) was added at −78 °C to the mixture. After stirring at −78 °C for 10 min, the reaction mixture was diluted with Et_2O and poured into saturated aqueous NH_4Cl (50 mL) at 0 °C. The aqueous layer was extracted with Et_2O (3×50 mL) and the combined extracts were washed with brine (50 mL), and dried over anhydrous $MgSO_4$. After removal of the solvent, the residue was purified by column chromatography on silica gel (hexane/ethyl acetate 60:40) to afford acetylene **13** (1.74 g, 8.29 mmol, 85 %) as a white solid. ^{1}H NMR (270 MHz, $CDCl_3$): δ = 6.00 (dd, J = 2.0, 5.6 Hz, 1H), 5.93 (d, J = 5.6 Hz, 1H), 4.72 (m, 1H), 2.56 (dd, J = 6.9, 14.2 Hz, 1H), 2.54 (s, 2H), 1.81 (dd, J = 3.6, 14.2 Hz, 1H), 0.17 ppm (s, 9H; Me_3Si); ^{13}C NMR (67.8 MHz, $CDCl_3$): δ = 138.4, 136.2, 102.3, 82.5, 81.4, 75.5, 48.0, 32.4 ppm; IR (KBr): $\tilde{\nu}$ = 3724, 2180, 1353, 1306, 1248, 1082 cm^{-1}.

(1*R,4*S**)-1-(1-Propynyl)-cyclopent-2-en-1,4-diol (14)**: K_2CO_3 (108 mg, 0.667 mmol) was added to a stirred solution of diol **12** (140 mg, 0.667 mmol) in dry MeOH (10 mL) at room temperature under argon. After being stirred at the same temperature for 6 h, the reaction mixture was filtered through Celite. After removal of the solvent in vacuo, the residue was purified by column chromatography on silica gel (hexane/ethyl acetate 90:10) to afford terminal acetylene **14** (78.5 mg, 0.568 mmol, 85 %) as a white solid. ^{1}H NMR (270 MHz, $CDCl_3$): δ = 6.01 (dd, J = 2.0, 5.6 Hz, 1H), 5.96 (d, J = 5.6 Hz, 1H), 4.76 (br s, 1H), 2.56 (dd, J = 6.9, 14.2 Hz, 1H), 2.52 (s, 2H), 2.05 (t, J = 2.6 Hz, 1H), 1.83 ppm (dd, J = 3.3, 14.2 Hz, 1H); ^{13}C NMR (67.8 MHz, $CDCl_3$): δ = 138.4, 136.0, 82.2, 80.4, 75.2, 70.5, 47.4, 30.8 ppm; IR (solid): $\tilde{\nu}$ = 3295, 2119, 1642, 1422, 1354, 1091 cm^{-1}; HRMS (ESI-TOF): m/z: calcd for $C_8H_{12}NaO_2$: 161.0573, found: 161.0572 $[M+Na]^+$.

4-Hydroxy-4-(prop-1-ynyl)-cyclopent-2-en-1-one (15): MnO_2 (26.3 g, 303 mmol) was added to a stirred solution of diol **14** (4.18 g, 30.3 mmol) in dry CH_2Cl_2 (30 mL) at room temperature under argon. After being stirred at the same temperature for 60 h, the mixture was filtered through Celite. After removal of the solvent in vacuo, the residue was purified by column chromatography on silica gel (-hexane/ethyl acetate 50:50) to afford enone **15** (2.71 g, 19.9 mmol, 65 %) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.50 (d, J = 5.8 Hz, 1H), 6.20 (d, J = 5.8 Hz, 1H), 2.69–2.67 (m, 2H), 2.66 (d, J = 18.5 Hz, 1H), 2.55 (d, J = 18.5 Hz, 1H), 2.14 ppm (t, J = 2.4 Hz, 1H); ^{13}C NMR (67.8 MHz, $CDCl_3$): δ = 206.8, 164.5, 134.1, 78.9, 77.6, 71.9, 48.2, 30.6 ppm; IR (neat): $\tilde{\nu}$ = 3407, 3291, 2931, 2120, 1715, 1590 cm^{-1}.

Solid-supported hydroxycyclopentenone (10): 3,4-Dihydro-2*H*-pyran-2-ylmethoxymethyl polystyrene (2.30 g, 3.30 mmol, 1.10 mmol g^{-1}) was added into a 50 mL reaction vessel in a Quest 205 synthesizer. To the reaction vessel was added a solution of 4-hydroxy-4-propargyl-2-cyclopentenone (**15**) (1.90 g, 52.1 mmol) and pyridinium *p*-toluenesulfonate (226 mg, 0.900 mmol) in dry dichloromethane (18 mL) at room temperature under argon. After agitation at 40 °C for 24 h, the reaction mixture was drained. The remaining beads were washed with tetrahydrofuran (3×20 mL), tetrahydrofuran/water 1:1 (3×20 mL), methanol (3×20 mL), tetrahydrofuran/water 1:1 (3×20 mL), and methanol (3×20 mL), and were dried in vacuo to afford the solid-supported cyclopentenone **10**. IR (KBr): $\tilde{\nu}$ = 3293, 3024, 2858, 1719, 1601 cm^{-1}.

A part of the resin **10** (119 mg) was treated with a solution of trifluoroacetic acid (0.1 mL) in dichloromethane (2.0 mL) for 30 min at room temperature. The resulting resin was rinsed with dry dichloromethane (3×5.0 mL). The filtrate was washed with saturated $NaHCO_3$ solution and brine, and dried over $MgSO_4$. After removal of the solvent, the residue was purified by column chromatography on silica gel ($MeOH/CH_3Cl$ 5:95) to give the recovered-cyclopentenone **15** (9.9 mg, 72 mmol, 56 % based on the resin).

Solid-supported phenylacetylene (8a): The acetylene resin **10** (300 mg, 0.185 mmol) and CuI (95.0 mg, 0.500 mmol) were added into 20 mL reaction vessels in a Quest 210 organic synthesizer. To the reaction vessel, a solution of phenyl iodide (**11a**) (0.279 mL, 2.50 mmol) and diisopropylethylamine (0.61 mL, 3.50 mmol) in DMF (15 mL), and $[Pd(PPh_3)_4]$ (289 mg, 0.25 mmol) were added under argon. After agitation at 40 °C for 24 h, the reaction mixture was drained. The remaining beads were washed with tetrahydrofuran (2×50 mL), tetrahydrofuran/water 1:1 (2×50 mL), *N*,*N*-dimethylformamide (2×50 mL), methanol (2×50 mL), tetrahydrofuran (2×50 mL), and were dried in vacuo to afford the solid-supported cyclopentenones **8a** (1.56 g). IR (KBr): $\tilde{\nu}$ = 3026, 2939, 1729, 1600 cm^{-1}.

A part of the resin **8** (21 mg) was treated with a solution of trifluoroacetic acid (0.1 mL) in dichloromethane (2.0 mL) for 30 min at room temperature. The mixture was filtered. The resulting resin was rinsed with dry dichloromethane (3×3.0 mL). The filtrate was washed with saturated $NaHCO_3$solution and brine, and dried over $MgSO_4$. After removal of the solvent, the residue was purified by column chromatography on silica gel ($MeOH/CH_3Cl$ 5:95) to provide phenylacetylene **16a** (2.5 mg, 0.012 mmol, quant). ^{1}H NMR (270 MHz, $CDCl_3$): δ = 7.54 (d, J = 5.6 Hz, 1H), 7.27–7.41 (m, 5H; aromatic), 6.23 (d, J = 5.6 Hz, 1H), 2.90 (s, 2H), 2.74 (d, J = 18.2 Hz, 1H), 2.58 ppm (d, J = 18.2 Hz, 1H); ^{13}C NMR (67.8 MHz, $CDCl_3$): δ = 206.2, 164.3, 134.2, 131.7, 128.4, 122.6, 84.1, 83.9, 78.2, 48.5, 31.9 ppm; IR (KBr): $\tilde{\nu}$ = 3378, 3059, 2927, 1716, 1598 cm^{-1}; HRMS (ESI-TOF): m/z: calcd for $C_{14}H_{12}NaO_2$: 235.0730, found: 235.0729 $[M+Na]^+$.

Solid-phase synthesis of 7aA: Two MicroKans containing resins **8a** supported with phenylacetylene (30 mg×2) were added to a reaction vessels under argon. Tetrahydrofuran (4.0 mL) was added to the reaction vessel to swell the resins. Subsequently, a solution of potassium bis(trimethylsilyl)amide (1.20 mL, 0.600 mmol) in toluene (0.5 M) at −78 °C under argon was added to the reaction vessel. The reaction mixtures were stirred for 1 h at the same temperature. To the mixture, benzaldehyde (**11A**) (0.305 mL, 3.00 mmol) in tetrahydrofuran (0.80 mL) was added to the reaction vessel at −78 °C. After stirring for 1 h at the same temperature, the reaction mixture was warmed at −20 °C and stirred for 30 min at this temperature. The MicroKans were isolated by filtration and washed with cooled tetrahydrofuran/saturated aqueous NH_4Cl 1:1 (2×10 mL), methanol (2×10 mL), tetrahydrofuran (2×10 mL), dichloromethane (2×30 mL), and methanol (2×10 mL), and dried in vacuo to afford solid-supported dienone **14aA**.

Solid-supported dienone **14aA** was treated with a solution of trifluoroacetic acid (0.1 mL) in dichloromethane (2.0 mL) for 30 min at room temperature. The resulting resins were rinsed with dry dichloromethane (3×5.0 mL). The filtrate was washed with saturated $NaHCO_3$ solution and brine, and dried over $MgSO_4$. After removal of the solvent, the residue was purified by column chromatography on silica gel to give enone **7aA** (7.2 mg, 0.024 mmol, 73 % yield based on compound **10**). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.81(br s, 1H), 2.90 (d, J = 17.0 Hz, 1H), 3.28 (d, J = 17.0 Hz, 1H), 6.51 (d, J = 6.8 Hz, 1H), 7.27–7.30 (m, 3H), 7.33–7.39 (m, 2H), 7.33–7.39 (m, 2H), 7.41–7.45 (m, 3H), 7.52 (s, 1H), 7.66 (d, J = 6.8 Hz, 1H), 7.98 ppm (br d, J = 8.7 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 195.2, 160.8, 136.1, 135.3, 134.4, 133.4, 132.1, 131.6, 130.0, 128.8, 128.3, 128.2, 122.8, 84.1, 84.0, 78.1, 27.4 ppm; IR (neat): $\tilde{\nu}$ = 3418, 3074, 2910, 1681, 1589 cm^{-1}; MS (ESI-TOF): m/z: 301 $[M+H]^+$; HRMS (ESI-TOF): m/z: calcd for $C_{21}H_{16}NaO_2$: 323.1043; found: 323.1044 $[M+Na]^+$.

Solid-phase synthesis of library 7

Sonogashira-coupling to provide 8a–l: Resin **10** supported with terminal acetylene (300 mg) and CuI (0.3 mmol) were added into twelve 100 mL reaction vessels in the Quest 205 synthesizer. To the reaction vessels, solutions of aryl iodide **11a–l** (1.5 mmol) in *N*,*N*-dimethylformamide (3.0 mL) and diisopropylethylamine (130 mL, 0.75 mmol), and $[Pd(PPh_3)_4]$ (347 mg, 0.30 mmol) were added under argon. After agitation at 40 °C for 36 h, the reaction mixture was drained. The remaining beads were washed with tetrahydrofuran (2×3.0 mL), tetrahydrofuran/water 1:1 (2×3.0 mL), *N*,*N*-dimethylformamide (2×3.0 mL), methanol (2×3.0 mL), tetrahydrofuran (2×3.0 mL), and were dried in vacuo to afford the solid-supported cyclopentenones **8a–l**.

Solid-phase synthesis of solid-supported cross-conjugated dienones 14aA–14lH: Each of the resins **8a–l** (30 mg) was packed into eight MicroKans encoded with an Rf Tag to provide a total of 96 MicroKans. Eight reaction vessels involving the twelve different MicroKans **8a–l** were prepared. Tetrahydrofuran (36 mL) was added to the reaction vessels to swell the resins. Subsequently, a solution of potassium bis(trime-

thylsilyl)amide (7.60 mL, 3.60 mmol; 0.50 M in toluene) was added to the reaction vessels at −78 °C under argon. The reaction mixtures were stirred at −78 °C for 1 h. The eight aldehydes **11A–H** (18.0 mmol) in tetrahydrofuran (5.0 mL) were added to the different reaction vessels at −78 °C. After stirring for 1 h at that temperature, the reaction mixture was gradually warmed at −20 °C and stirred for 30 min at this temperature. The MicroKans were isolated by filtration and washed with cooled tetrahydrofuran/saturated aqueous NH_4Cl 1:1 (2× 30 mL), methanol (2×30 mL), tetrahydrofuran (2×30 mL), dichloromethane (2×30 mL), methanol (2×30 mL), and were dried in vacuo to afford 96 solid-supported cross-conjugated dienones **14aA–14lH** in MicroKans.

Cleavage of compounds 7aA–7lH: The MicroKans **14aA–14lH** were separately treated with a solution of trifluoroacetic acid (0.1 mL) in dichloromethane (2.0 mL) for 30 min at room temperature in a different vessel of the Quest210 synthesizer. After addition of dichloromethane (3.0 mL), the reaction mixture was neutralized with a piperidinomethyl polystyrene (316 mg, 1.1 mmol, 3.48 mmol g^{-1}) for 30 min. The mixture was filtered and the resulting resins were rinsed with dry dichloromethane (3×5.0 mL). The combined filtrate was concentrated in vacuo to give crude enones **7aA–7lH** as yellow oils. Purity of the crude enones **7aA–7lH** was analyzed by HPLC-MS based on the UV absorption at 254 nm by using a YMC-Pack Pro C18 (5 μm, 4.6×50 mm column; flow rate: 10 mL min^{-1}, temperature: 40 °C, mobile phase: 0.1 % HCOOH in H_2O/0.1 % HCOOH in MeCN 70:30 (0 min), 10:90 (5–8 min), for **7aA–7lF** and **7aH–7lH**; 0.1 % HCOOH in H_2O/0.1 % HCOOH in MeCN 70:30 (0 min), 10:90 (5–8 min) for **7aG–7kG** (Table 2) Further purification of the selected compounds was achieved by column chromatography on silica gel.

Compound 7jA: ^{1}H NMR (270 MHz, $CDCl_3$): δ = 2.98 (d, J = 17.1 Hz, 1H), 3.25 (d, J = 17.1 Hz, 1H), 6.51 (d, J = 6.8 Hz, 1H), 7.40–7.54 (m, 7H), 7.63 (d, J = 6.8 Hz, 1H), 7.98 ppm (m, 2H); ^{13}C NMR (67.8 MHz, CDCl3): δ = 195.3, 160.7, 136.1, 135.5, 134.6, 133.5, 133.2, 132.1, 131.9, 130.2, 129.7, 128.8, 126.7, 125.2, 86.9, 82.7, 78.1, 27.2 ppm; IR (neat): $\tilde{\nu}$ = 3404, 3067, 1695, 1626 cm^{-1}; MS (ESI-TOF): m/z: 369 $[M+H]^+$.

Compound 7dG: ^{1}H NMR (270 MHz, $CDCl_3$): δ = 2.95 (s, 3H), 3.10 (s, 3H), 3.13 (d, J = 16.5 Hz, 1H), 3.32 (d, J = 16.5 Hz, 1H), 3.32 (s, 3H), 6.58 (d, J =

Table 2. Purity and cytotoxicity against HeLaS3 cells of 76 compounds in the combinatorial library **7**.

Entry	R^2X	R^1CHO	Product	t_R [min]	MS $[M+H]^+$	Yield [mg] (%) /Purity (HPLC area %)
1	**11a**	**9A**	**7aA**	4.62	301	4.2 (56)/73
2	**11b**	**9A**	**7bA**	5.0	315	4.9 (62)/31
3	**11c**	**9A**	**7cA**	3.36	372	6.0 (65)/86
4	**11d**	**9A**	**7dA**	3.39	372	5.8 (63)/77
5	**11e**	**9A**	**7eA**	3.34	372	5.1 (55)/66
6	**11f**	**9A**	**7fA**	5.03	373	5.1 (55)/78
7	**11g**	**9A**	**7gA**	2.85	302	6.8 (90)/46
8	**11h**	**9A**	**7hA**	2.66	302	5.1 (68)/97
9	**11i**	**9A**	**7iA**	5.17	335	5.2 (62)/94
10	**11j**	**9A**	**7jA**	5.36	369	5.5 (60)/92
11	**11k**	**9A**	**7kA**	4.43	307	4.6 (60)/39
12	**11a**	**9B**	**7aB**	4.87	327	8.2 (101)/79
13	**11b**	**9B**	**7bB**	5.21	341	7.5 (88)/82
14	**11c**	**9B**	**7cB**	3.62	398	10.3 (104)/82
15	**11d**	**9B**	**7dB**	3.66	398	10.4 (105)/81
16	**11e**	**9B**	**7eB**	3.71	398	7.7 (78)/61
17	**11f**	**9B**	**7fB**	5.21	399	9.0 (91)/78
18	**11g**	**9B**	**7gB**	3.26	328	11.2 (137)/64
19	**11h**	**9B**	**7hB**	3.03	328	11.0 (135)/82
20	**11i**	**9B**	**7iB**	5.44	361	9.2 (102)/90
21	**11j**	**9B**	**7jB**	5.53	395	11.5 (117)/76
22	**11k**	**9B**	**7kB**	4.72	333	8.5 (102)/71
23	**11a**	**9C**	**7aC**	3.92	291	9.0 (124)/83
24	**11b**	**9C**	**7bC**	4.38	305	7.9 (104)/74
25	**11c**	**9C**	**7cC**	2.60	362	11.0 (122)/83
26	**11d**	**9C**	**7dC**	2.66	362	10.1 (112)/81
27	**11e**	**9C**	**7eC**	2.56	362	8.4 (93)/71
28	**11f**	**9C**	**7fC**	4.41	363	8.9 (98)/83
29	**11g**	**9C**	**7gC**	1.80	292	10.4 (143)/47
30	**11h**	**9C**	**7hC**	1.58	292	10.7 (147)/82
31	**11i**	**9C**	**7iC**	4.57	325	9.7 (120)/90
32	**11j**	**9C**	**7jC**	4.75	359	8.8 (98)/76
33	**11k**	**9C**	**7kC**	3.77	297	6.8 (92)/71
34	**11a**	**9D**	**7aD**	4.26	302	9.2 (122)/79
35	**11b**	**9D**	**7bD**	4.68	316	9.2 (117)/66
36	**11c**	**9D**	**7cD**	2.94	373	12.1 (130)/79
37	**11d**	**9D**	**7dD**	2.94	373	9.0 (97)/86
38	**11e**	**9D**	**7eD**	2.84	373	11.2 (121)/70
39	**11f**	**9D**	**7fD**	4.74	374	10.5 (113)/64
40	**11g**	**9D**	**7gD**	2.12	303	11.5 (152)/60
41	**11h**	**9D**	**7hD**	2.02	303	10.5 (139)/92
42	**11i**	**9D**	**7iD**	4.88	336	10.3 (123)/85
43	**11j**	**9D**	**7jD**	5.09	370	8.6 (93)/87
44	**11k**	**9D**	**7kD**	4.05	308	9.6 (125)/41
45	**11a**	**9E**	**7aE**	4.56	359	9.1 (102)/61
46	**11b**	**9E**	**7bE**	4.20	373	9.7 (104)/84
47	**11c**	**9E**	**7cE**	3.32	430	11.0 (103)/80
48	**11d**	**9E**	**7dE**	3.35	430	10.7 (100)/62
49	**11e**	**9E**	**7eE**	3.39	430	8.1 (76)/73
50	**11f**	**9E**	**7fE**	4.94	431	8.9 (83)/76
51	**11g**	**9E**	**7gE**	2.96	360	10.4 (116)/30
52	**11h**	**9E**	**7hE**	2.72	360	10.0 (112)/92
53	**11i**	**9E**	**7iE**	5.14	393	7.1 (72)/89
54	**11j**	**9E**	**7jE**	5.26	427	8.0 (75)/89
55	**11k**	**9E**	**7kE**	4.37	365	8.7 (96)/25
56	**11a**	**9G**	**7aG**	2.99	305	5.6 (74)/50
57	**11b**	**9G**	**7bG**	3.53	319	5.5 (69)/42
58	**11c**	**9G**	**7cG**	1.46	376	8.6 (92)/23
59	**11d**	**9G**	**7dG**	1.49	376	8.1 (86)/32
60	**11e**	**9G**	**7eG**	1.37	376	7.3 (78)/18
61	**11f**	**9G**	**7fG**	3.76	377	7.1 (76)/43
62	**11i**	**9G**	**7iG**	3.85	339	7.9 (93)/51
63	**11j**	**9G**	**7jG**	4.22	373	7.8 (84)/58
64	**11a**	**9H**	**7aH**	4.24	279	4.1 (59)/33
65	**11b**	**9H**	**7bH**	4.65	293	6.6 (90)/18

Table 2. (Continued)

Entry	R^2X	R^1CHO	Product	t_R [min]	MS $[M+H]^+$	Yield [mg] (%) /Purity (HPLC area %)
66	**11c**	**9H**	**7cH**	2.93	350	6.2 (71)/17
67	**11d**	**9H**	**7dH**	2.97	350	6.9 (79)/15
68	**11e**	**9H**	**7eH**	2.89	350	5.3 (61)/11
69	**11f**	**9H**	**7fH**	4.69	351	6.0 (69)/35
70	**11g**	**9H**	**7gH**	2.33	280	4.6 (66)/45
71	**11h**	**9H**	**7hH**	2.03	280	4.7 (67)/32
72	**11i**	**9H**	**7iH**	4.84	313	5.0 (64)/38
73	**11j**	**9H**	**7jH**	5.05	347	4.8 (56)/49
74	**11k**	**9H**	**7kH**	4.07	285	4.7 (66)/14

5.9 Hz, 1H), 7.01 (d, $J=1.0$ Hz, 1H), 7.17 (s, 1H), 7.27–7.32 (m, 4H), 7.63 (br d, $J=5.9$ Hz, 1H), 8.76 ppm (br s, 1H); IR (neat): $\tilde{\nu}=3412$, 2932, 1700, 1635 cm^{-1}; MS (ESI-TOF): m/z: 376 $[M+H]^+$.

Compound 7iA: ^1H NMR (270 MHz, $CDCl_3$): $\delta=7.99$ (m, 2H), 7.64 (d, $J=5.9$ Hz, 1H), 7.53 (s, 1H), 7.50–7.38 (m, 2H), 7.35–7.20 (m, 5H), 6.53 (d, $J=5.9$ Hz, 1H), 3.28 (d, $J=17.0$ Hz, 1H), 2.91 ppm (d, $J=17.0$ Hz, 1H); ^{13}C NMR (67.8 MHz, $CDCl_3$): $\delta=195.3$, 161.0, 137.5, 137.2, 135.4, 134.6, 133.0, 132.2, 130.2, 128.9, 128.7, 121.4, 85.2, 78.2, 29.8, 27.5 ppm; FTIR (neat): $\tilde{\nu}=3374$, 2925, 1693, 1626, 1489, 826 cm^{-1}; HRMS (ESI-TOF): m/z: calcd for $C_{21}H_{15}ClNaO_2$: 357.0653, found: 357.0661 $[M+Na]^+$.

Compound 7kE: ^1H NMR (270 MHz, $CDCl_3$): $\delta=8.12$–8.01 (m, 4H), 7.68 (d, $J=5.9$ Hz, 1H), 7.51 (s, 1H), 7.21 (dd, $J=1.0$, 5.0 Hz, 1H), 7.12 (dd, $J=1.0$, 3.6 Hz, 1H), 6.93 (dd, $J=3.6$, 5.0 Hz, 1H), 6.53 (d, $J=5.9$ Hz, 1H), 3.94 (s, 3H; Me), 3.26 (d, $J=17.1$ Hz, 1H), 2.86 ppm (d, $J=17.1$ Hz, 1H); ^{13}C NMR (67.8 MHz, $CDCl_3$): $\delta=194.7$, 166.6, 161.0, 138.0, 137.9, 134.7, 134.0, 132.1, 131.8, 130.0, 127.0, 126.9, 87.7, 78.0, 52.4, 28.0 ppm; FTIR (neat): $\tilde{\nu}=3329$, 1924, 1719, 1628, 826 cm^{-1}; HRMS (ESI-TOF): m/z: calcd for $C_{21}H_{16}NaO_4S$: 387.0662; found: 387.0669 $[M+Na]^+$.

Compound 7aD: ^1H NMR (270 MHz, $CDCl_3$): $\delta=8.71$ (br dd, 1H), 7.86 (dt, $J=2.0$, 7.5 Hz, 1H), 7.67 (br d, 1H), 7.63 (br d, 1H), 7.39–7.22 (m, 4H), 7.31 (s, 1H), 6.61 (d, 1H), 3.16 (d, $J=16.8$, 22.8 Hz, 1H), 3.08 ppm (d, $J=16.8$, 22.8 Hz, 1H); ^{13}C NMR (67.8 MHz, $CDCl_3$): $\delta=195.1$, 161.4, 153.1, 148.9, 144.8, 138.4, 135.3, 131.6, 128.7, 128.3, 128.0, 127.6, 123.8, 85.3, 83.7, 78.5, 30.3 ppm; FTIR (neat): $\tilde{\nu}=3060$, 2917, 1705, 1646, 1589, 1087, 692 cm^{-1}; HRMS (ESI-TOF): m/z: calcd for $C_{20}H_{15}NO_2$: 302.1176 $[M+H]^+$, found: 302.1178.

Compound 7kD: ^1H NMR (270 MHz, $CDCl_3$): $\delta=8.71$ (br d, 1H), 7.87 (dt, $J=6.0$, 7.5 Hz, 1H), 7.68 (d, $J=6.2$ Hz, 1H), 7.64 (br d, $J=7.5$ Hz, 1H), 7.36 (dd, $J=5.0$, 12.5 Hz, 1H), 7.31 (s, 1H), 7.16 (dd, J=5.3 Hz, 1H), 7.05 (br d, $J=3.6$ Hz, 1H), 6.90 (dd, $J=3.6$, 5.3 Hz, 1H), 6.60 (d, $J=6.2$ Hz, 1H), 3.13 ppm (s, 2H); ^{13}C NMR (67.8 MHz, $CDCl_3$): $\delta=195.0$, 161.4, 153.1, 148.9, 144.7, 138.4, 135.3, 131.7, 128.8, 127.8, 126.9, 126.5, 123.8, 89.3, 78.4, 30.6, 29.8 ppm; FTIR (neat): $\tilde{\nu}=2918$, 1700, 1644, 1589, 1190, 830 cm^{-1}; HRMS (ESI-TOF): m/z: calcd for $C_{18}H_{13}NO_2S$: 308.0740, found: 308.0742 $[M+H]^+$.

Compound 7jD: ^1H NMR (270 MHz, $CDCl_3$): $\delta=8.71$ (br d, $J=3.7$ Hz, 1H), 7.88 (dt, $J=1.7$, 7.9 Hz, 1H), 7.66 (d, $J=5.9$ Hz, 1H), 7.64 (d, $J=7.9$ Hz, 1H), 7.50 (d, $J=8.3$ Hz, 2H), 7.37 (d, $J=8.3$ Hz, 2H), 7.34–7.40 (m, 1H), 7.32 (s, 1H), 6.61 (d, $J=5.9$ Hz, 1H), 3.18 (dd, $J=16.8$ Hz, 2H), 3.09 ppm (dd, $J=16.8$ Hz, 2H); ^{13}C NMR (67.8 MHz, $CDCl_3$): $\delta=195.0$, 161.2, 153.1, 148.9, 144.7, 138.4, 135.3, 131.8, 128.8, 127.8, 125.2, 123.9, 88.9, 82.6, 78.4, 30.3 ppm; FTIR (solid): $\tilde{\nu}=3081$, 2920, 1701, 1643, 1320, 842 cm^{-1}; HRMS (ESI-TOF): m/z: calcd for $C_{21}H_{14}F_3NO_2$: 370.1049; found: 370.1049 $[M+H]^+$.

Compound 7dD: ^1H NMR (270 MHz, $CDCl_3$): $\delta=8.71$ (br d, 1H), 7.87 (dt, $J=7.9$, 2.0 Hz, 1H), 7.66 (d, $J=5.9$ Hz, 1H), 7.64 (d, $J=7.9$ Hz, 1H), 7.36 (dd, $J=5.4$, 7.9 Hz, 1H), 7.33–7.27 (m, 4H), 7.30 (s, 1H), 6.60 (d, $J=5.9$ Hz, 1H), 3.16 (d, $J=1.68$ Hz, 1H), 3.10 (s, 3H), 3.08 ppm (d, $J=16.8$ Hz, 1H); ^{13}C NMR (67.8 MHz, $CDCl_3$): $\delta=195.1$, 170.8, 161.3, 153.1, 148.9, 144.8, 138.4, 136.5, 135.3, 132.6, 130.1, 128.7, 128.4, 127.7, 126.6, 123.8, 123.5, 86.3, 83.0, 78.5, 39.6, 35.4, 30.3 ppm; FTIR (solid): $\tilde{\nu}=2926$, 1705, 1639, 1087, 747 cm^{-1}; HRMS (ESI-TOF): m/z: calcd for $C_{23}H_{20}N_2O_3$: 373.1547; found: 373.1556 $[M+H]^+$.

Compound 7iG: ^1H NMR (270 MHz, $CDCl_3$): $\delta=7.63$ (d, $J=5.9$ Hz, 1H), 7.37–7.15 (m, 4H), 7.17 (s, 1H), 7.01 (s, 1H), 6.59 (d, $J=5.9$ Hz, 1H), 3.85 (s, 3H), 3.32 (d, $J=16.8$ Hz, 1H), 3.11 ppm (d, $J=16.8$ Hz, 1H); ^{13}C NMR (67.8 MHz, $CDCl_3$): $\delta=$ 194.9, 161.2, 142.8, 142.5, 135.4, 133.9, 132.8, 19.9, 128.6, 123.5, 112.8, 86.8, 82.5, 78.5, 33.7, 29.3 ppm; FTIR (solid): $\tilde{\nu}=2922$, 1697, 1646, 1477, 1224, 826, 755 cm^{-1}; HRMS (ESI-TOF): m/z: calcd for $C_{19}H_{15}ClN_2O_2$: 339.0895; found: 339.0899 $[M+H]^+$.

Compound 7jG: ^1H NMR (270 MHz, $CDCl_3$): $\delta=7.63$ (d, $J=5.9$ Hz, 1H), 7.50 (d, $J=8.2$ Hz, 2H), 7.37 (d, $J=8.2$ Hz, 2H), 7.18 (s, 1H), 7.02 (s, 1H), 6.60 (d, $J=5.9$ Hz, 1H), 3.85 (s, 3H), 3.35 (d, $J=16.8$ Hz, 1H), 3.14 ppm (d, $J=16.8$ Hz, 1H); ^{13}C NMR (67.8 MHz, $CDCl_3$): $\delta=194.8$, 161.1, 142.7, 142.3, 135.4, 131.8, 130.0, 125.2, 123.6, 112.9, 88.5, 82.4, 78.5, 33.7, 29.3 ppm; FTIR (neat): $\tilde{\nu}=$ 3115, 2928, 2225, 1703, 1647, 1323 cm^{-1}; HRMS (ESI-TOF): m/z: calcd for $C_{20}H_{15}F_3N_2O_2$: 373.1158; found: 373.1155 $[M+H]^+$.

Biological assay: Each cell line (A549, HeLaS3, MCF7, TMF1 and P388) was seeded into a 96-multiwell plate and was incubated for 24 h. The cells were treated with **7jA**, **7dD**, **7jG**, **7dD**, 5-FU or Adriamycin for 72 h. In the case of A549, HeLaS3, MCF7, and TMF1 cell lines, the cells were fixed with glutaraldehyde and stained with crystal violet for estimation of cell population. For the P388 cell lines, 20 μL per well of WST-8 solution was added and the plate was further incubated for 1.5 h. After incubation, the absorbance was measured at 450 nm for an estimation of the cell population.

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