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Optimized synthesis and antiproliferative activity of desTHPdactylolides

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ABSTRACT

Dactylolide and certain analogues are attractive targets for study due to their structural resemblance to zampanolide, a very promising anticancer lead compound and a unique covalent-binding microtubule stabilizing agent. The primary goal of this project is identification and synthesis of simplified analogues of dactylolide that would be easier to prepare and could be investigated for antiproliferative activity in comparison with zampanolide. Extension of Almann's concept of a simplified zampanolide analogue to dactylolide in the form of desTHPdactylolide was attractive not only for reasons of synthetic simplification but also for the prospect that analogues of dactylolide could be prepared in both (17*S*) and (17*R*) configurations. Since Altmann's overall yield for the six-step procedure leading to the C9–C18 fragment of desTHPdactylolide was only 8.7%, a study focused on optimized synthesis and antiproliferative evaluation of each enantiomer of desTHPdactylolide was initiated using Altmann's route as a framework. To this end, two optimized approaches to this fragment C9–C18 were successfully developed by us using allyl iodide or allyl tosylate as the starting material for a critical Williamson ether synthesis. Both (17*S*) and (17*R*) desTHPdactylolides were readily synthesized in our laboratory using optimized methods in yields of 37–43%. Antiproliferative activity of the pair of enantiomeric desTHPdactylolides, together with their analogues, was evaluated in three docetaxel-sensitive and two docetaxel-resistant prostate cancer cell models using a WST-1 cell proliferation assay. Surprisingly, (17*R*) desTHPdactylolide was identified as the eutomer in the prostate cancer cell models. It was found that (17*S*) and (17*R*) desTHPdactylolide exhibit equivalent antiproliferative potency towards both docetaxel-sensitive (PC-3 and DU145) and docetaxel-resistant prostate cancer cell lines (PC-3/DTX and DU145/DTX).

1. Introduction

Dactylolide was isolated in 2001 from the marine sponge *Dactylospongia* sp. by Cutignano and was assigned structure **1** (Fig. 1) except for configuration at C11, C15, and C19.[1] On the supposition that the twenty-membered lactone of dactylolide is identical in configuration with the same unit in zampanolide (**2**, Fig. 1) of established relative and absolute configuration[2] and that dactylolide is a likely biogenetic precursor of **2**, we have provisionally assigned the (11*S*, 15*S*, 19*S*) configuration to natural dactylolide.[3] Unfortunately, total syntheses of dactylolide have failed to remove this uncertainty since natural dactylolide is reported to possess $[\alpha]_D$ + 30.0[1] whereas specific rotations for synthetic enantiomers of dactylolide range from +134 to +235 and from −128 to −258.[4–11] These discrepancies have been attributed to (a) the tendency for the aldehyde residue of dactylolide to form a hydrate and/or an acetal with methanol, and (b) a propensity for the aldehyde and/or ketone functions in dactylolide to enolize. Nevertheless, the structural resemblance of dactylolide to zampanolide, a unique microtubule stabilizing agent that covalently binds to tubulin[12] and which has superior cytotoxic potency in both drug-sensitive and multi-drug resistant cancer cell models,[12,13] makes **1** and certain analogues attractive targets for study. Since published synthetic routes to dactylolide appear unlikely to produce sufficient material for the comprehensive *in vivo* studies we envision, a primary goal of this project is identification and synthesis of simplified analogues of **1** that would be easier to prepare and could be investigated for antiproliferative activity in comparison with zampanolide.

Altmann and co-workers have reported that deletion of the tetrahydropyran moiety from zampanolide and its replacement by a direct ether linkage within the macrolactone produces a structure (desTHPzampanolide, **3**) that retains submicromolar cytotoxicity.[8] This simplification of the zampanolide core not only removes two of three

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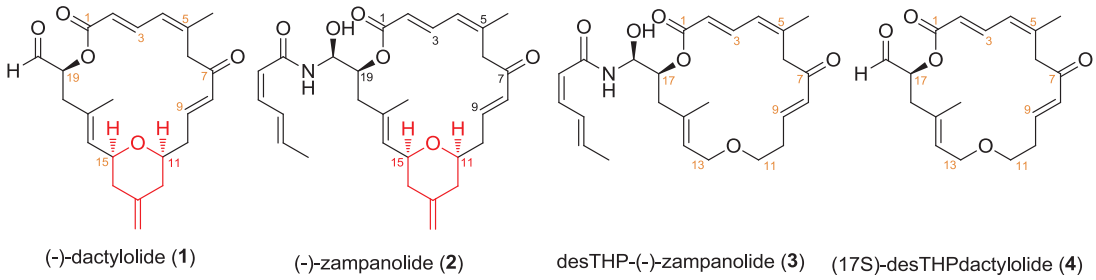


Fig. 1. Structures of dactylolide, zampanolide and simplified analogues.

stereocenters from the macrolactone portion but substantially reduces the total number of synthetic steps from that required for zampanolide itself. Extension of Altmann's concept to dactylolide (**1**) in the form of desTHPdactylolide (**4**, Fig. 1) was attractive not only for reasons of synthetic simplification but also for the prospect that analogues of **1** could be prepared in both (17*S*) and (17*R*) configurations. Therefore, a study focused on optimized synthesis and antiproliferative evaluation of each enantiomer of desTHPdactylolide (**4**) was initiated using Altmann's route to **4** as a framework.

2. Results and discussion

2.1. Optimized syntheses of fragment C9–C18

A key step in Altmann's synthesis of desTHPdactylolide (**4**) is Yamaguchi esterification of acid **5** (fragment C1–C8) with alcohol **6** (fragment C9–C18) to yield an acyclic precursor **7** for macrocyclization (Scheme 1). Ring closure of **7** was accomplished by intramolecular Horner-Wadsworth-Emmons condensation. Construction of the 18-membered macrolactone **4** by this route required a total of 22 steps from commercially available material.[8] Although the C1–C8 fragment **5** can be synthesized in multi-gram quantities from 2-butyn-1-ol with the yield of each step exceeding 70%, Altmann's overall yield for the six-step procedure leading to the C9–C18 fragment **6** was only 8.7%. This places a limitation on the availability of material needed for *in vivo* testing. Our goal was to establish an efficient route to **6** from a readily available starting material that significantly improved the overall yield of this intermediate. Since the low yield of **6** by Altmann's route is mainly attributable to an inefficient synthesis of C9-C15 fragment **8**, we focused initially on modifications to the pathway shown in Scheme 2 which employed a Williamson ether synthesis of iodo alcohol **9** with allyl bromide followed by hydroboration-oxidation of **10** and final protection of primary alcohol **11** to give **8**.

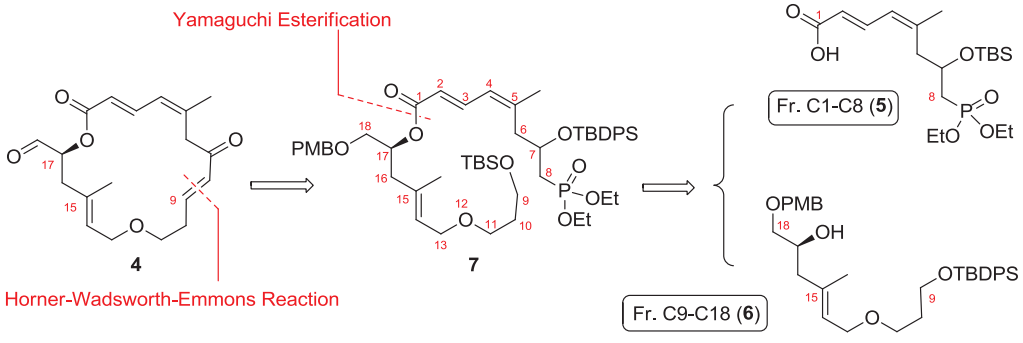
An attractive strategy for improving access to **8** appeared to be reversal of reactant roles in a Williamson ether approach, and to this end iodo alcohol **9** was converted to diiodide **12** (Scheme 3). Treatment of the latter with mono-protected 1,3-propanediols **13** and **14** afforded iodo ethers **8** and **15** in 58–60% yield. Although this sequence represents a substantial improvement over Altmann's synthesis, a disadvantage of the route is the high volatility of diiodide **12**. In order to circumvent the problem of handling this material on a large scale, a second modification of the synthesis of **15** was investigated which first converted iodo alcohol **9** to iodo tosylate **16** (Scheme 4). Williamson ether synthesis with this substrate and alcohol **14** in the presence of sodium hydride gave **15** in an optimized yield of 75% after chromatographic purification. Diiodo ether byproduct **17** formed in this reaction by displacement of tosylate **16** with iodo alcohol **9** was minimized by adding a mixture of **9** and sodium hydride (1.5–1.8 eq) in ether to a solution of tosyl chloride (1.1–1.6 eq) in ether and then adding alcohol **14** (2 eq). This protocol did not diminish formation of a second byproduct, tosylate **18**, from tosylation of alcohol **14**, but this is readily removed upon chromatographic separation.

2.2. Preparation of (17S) and (17R) desTHPdactylolides

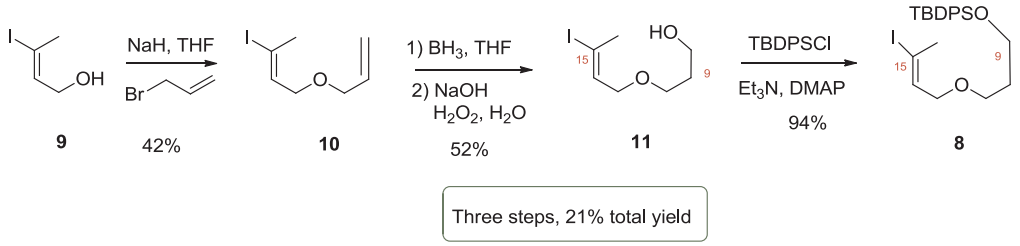
With an efficient synthesis of iodo ether **15** in hand, its lithiation with *tert*-butyllithium followed by reaction with PMB-protected (*R*)-glycidol[14,15] in toluene at −90 °C in the presence of boron trifluoride etherate proceeded smoothly to give **19** in 61% yield. The overall yield of **19** from **9** by this three-step sequence was improved from the literature value of 8.7% to 37–43%. Subsequent transformations towards (17*S*) desTHPdactylolide (**4**) via macrolides **20** and **21** were patterned on Altmann's synthesis[8] which used a TBDPS-masked C9 ether rather than our TBS-protected version **19** (Scheme 5). Our route resulted in an overall yield of (17*S*) **4** from **9** of 30%. A parallel series from **15** using PMB-protected (*S*)-glycidol led to (17*R*) **22** and then to (17*R*) desTHPdactylolide **23** in 32% overall yield via the six-step sequence through macrolactone intermediates **24** and **25**. Note that spectral data for known compounds (final and intermediates) are consistent with data reported by Altmann.[8]

2.3. The antiproliferative activity of the dactylolide analogues

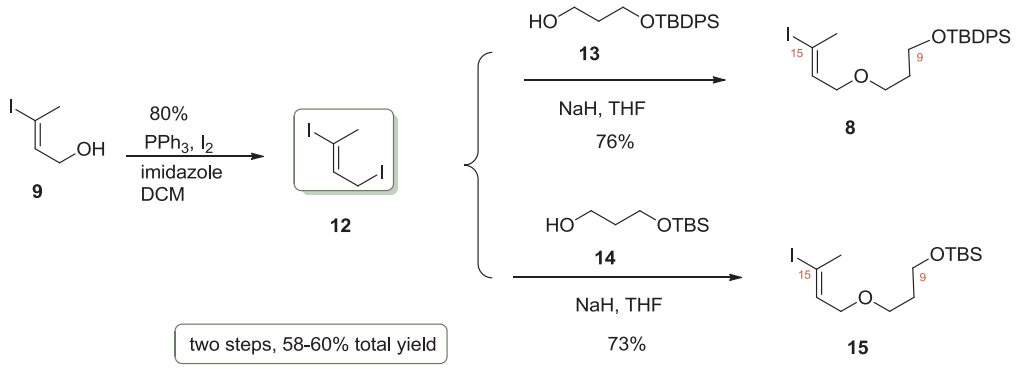
An IC_{50} value of 0.75 ± 0.07 μM has been reported by Altmann and co-workers[8] for (−)-dactylolide towards the PC-3 prostate cancer cell line, which is about 260-fold less potent than (−)-zampanolide (cells



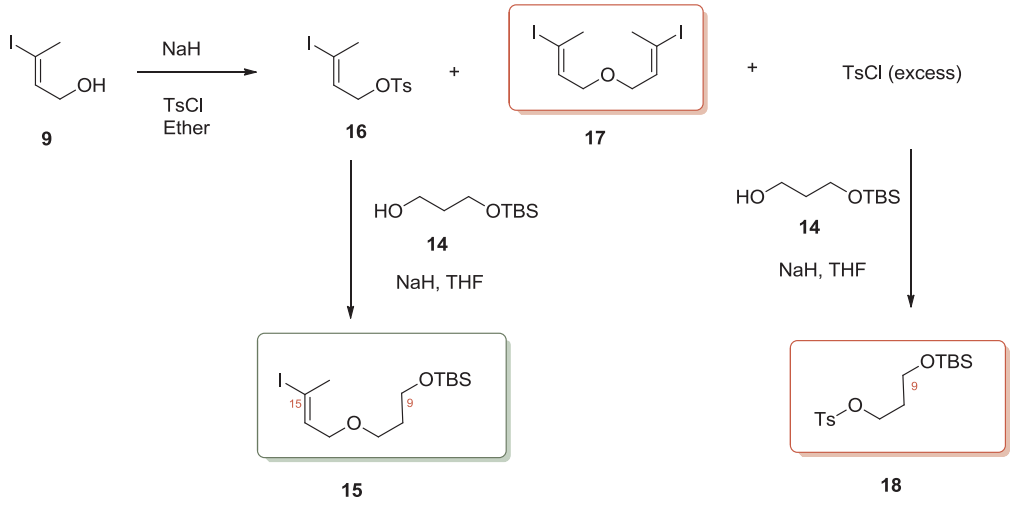
Scheme 1. Retrosynthetic analysis of desTHPdactylolide (**4**).



Scheme 2. Synthesis of fragment C9–C15 (**8**) reported by Zurwerra et. al[8].



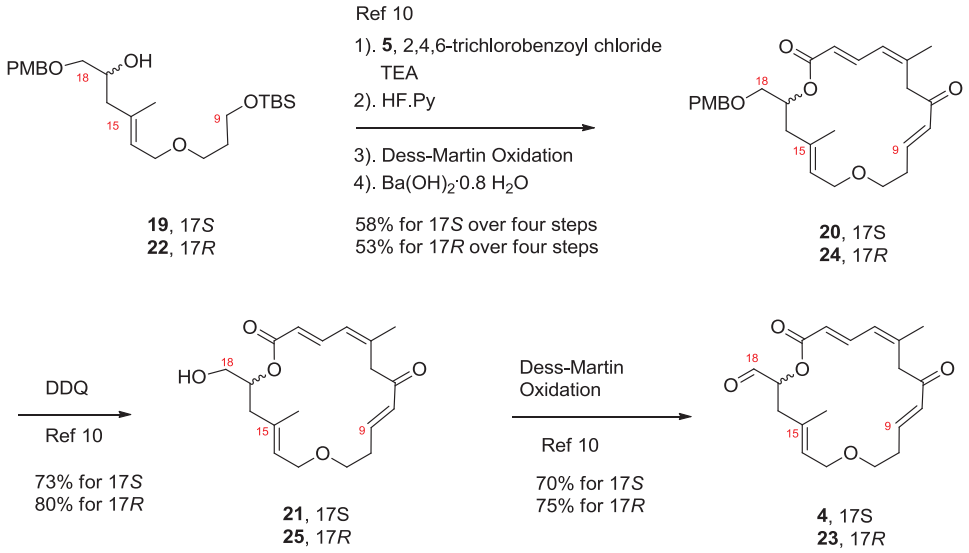
Scheme 3. Optimized synthesis of C9–C15 fragments **8** and **15**.



Scheme 4. Optimized synthesis of fragment C9–C15 **15**.

were exposed to the compound for 72 h). An IC_{50} value of 3.05 ± 0.18 μM for **21** and an IC_{50} value of 4.02 ± 0.10 μM for (17*S*) desTHPdactylolide (**4**), respectively, have been reported towards PC-3 cells in the literature,[8] indicating that **21** and **4** are about 4- to 5-fold less potent than (−)-dactylolide (**3**).

In this work, the antiproliferative activity of three pairs of enantiomeric macrolides, **20** and **24**, **21** and **25**, and (17*S*) desTHPdactylolide (**4**) and (17*R*) desTHPdactylolide (**23**), has been evaluated in three docetaxel-sensitive prostate cancer cell models (PC-3, DU145, and LNCaP) and two docetaxel-resistant prostate cancer cell lines (PC-3/DTX and DU145/DTX) using the WST-1 cell proliferation assay with docetaxel as a positive control. As illustrated in Table 1, two (17*S*) macrolides (**21** and **4**) show almost equivalent antiproliferative potency towards both androgen-sensitive prostate cancer cells (LNCaP) and androgen-insensitive prostate cancer cell lines (PC-3 and DU145), while two (17*R*) macrolides (**24** and **23**) are slightly more potent against androgen-sensitive LNCaP cells. The IC_{50} values for **21** and **4** against PC-3 prostate cancer cells are slightly higher than those reported in the literature, a variation which may be caused by the different assay methods and conditions. Interestingly, two (17*R*) dactylolide analogues (**24** and **23**) are eutomers because they are generally more potent than their enantiomers towards the five prostate cancer cell lines. The eudismic ratios[16,17] for compound (17*R*) **24** over compound (17*S*) **21** are 1.2, 2.9, and 2.9 against PC-3, DU145, and LNCaP cell lines. Similarly, the eudismic ratios for (17*R*) **23** over (17*S*) **4** are 2.3, 3.5, 2.1. 1.9, and 4.2 towards PC-3, PC-3/DTX, DU145, DU145/DTX, and LNCaP cell lines. It is worth noting that the antiproliferative activity of (+)-zampanolide and (+)-dactylolide on prostate cancer cell models has not yet been reported, even though the C_{20}-epimer of (−)-zampanolide is reported to be one order of magnitude less potent than (−)-zampanolide.[8] Our data imply that inverting the C19 chiral center might be a way to develop improved dactylolide analogues. However, it is not clear whether this activity as well as the activity of dactylolide is biologically related to that of zampanolide.



Scheme 5. Preparation of (17*S*) desTHPdactylolide (**4**) and (17*R*) desTHPdactylolide (**23**).

Table 1
Anti-proliferative activity of dactylolide analogues against both docetaxel-sensitive and docetaxel-resistant prostate cancer cells.

Compounds	IC_{50}: (μM)[a]	R/S[d]	IC_{50}: (μM)[a]		R/S[c]	IC_{50}: (μM)[a]	
	PC-3[b]	PC-3/DTX[c]		DU145[e]	DU145/DTX[f]		LNCaP[g]
Docetaxel	0.0019 ± 0.0006	2.34 ± 0.25	1232.0	0.0012 ± 0.0003	8.58 ± 0.39	7150.0	0.0002 ± 0.0001
20	7.66 ± 0.69	> 20.00	> 2.6	9.21 ± 0.13	> 20.00	> 2.2	8.27 ± 0.66
21	16.08 ± 0.44	14.40 ± 1.01	0.9	17.76 ± 1.35	14.07 ± 0.45	0.8	19.33 ± 2.36
4	11.91 ± 3.68	15.42 ± 1.23	1.3	11.58 ± 0.21	15.33 ± 1.24	1.3	13.46 ± 0.9
24	6.31 ± 0.22	3.77 ± 0.84	0.6	3.16 ± 0.25	3.20 ± 0.15	1.0	2.85 ± 0.71
25	> 30.00	> 30.00	–	> 30.00	> 30.00	–	> 30.00
23	5.27 ± 0.65	4.41 ± 0.44	0.8	5.41 ± 0.11	8.03 ± 0.13	1.5	3.22 ± 0.31

[a] IC_{50} is the drug concentration effective in inhibiting 50% of the cell viability measured by WST-1 cell proliferation assay after 3 days of exposure. The data were presented as the mean ± standard deviation of the mean.
[b] Human androgen-insensitive prostate cancer cell line derived from bone metastasis of prostate tumor.
[c] Docetaxel-resistant PC-3 prostate cancer cell line.
[d] The relative resistance of the two cell lines obtained by dividing the IC_{50} value of the resistance cell line by that of the parental cell line.
[e] Human androgen-insensitive prostate cancer cell line derived from brain metastasis of prostate tumor.
[f] Docetaxel-resistant DU145 prostate cancer cell line.
[g] Human androgen-sensitive prostate cancer cell line.

Additionally, four dactylolide mimics (**21**, **4**, **24**, and **23**) were found to exhibit equivalent antiproliferative potency towards both docetaxel-sensitive (PC-3 and DU145) and docetaxel-resistant prostate cancer cell lines (PC-3/DTX and DU145/DTX) (Table 1). The relative resistance of PC-3/DTX over PC-3 ranges from 0.6 to 1.3 while the relative resistance of DU145/DTX over DU145 falls in the range of 0.8 to 1.48. In contrast, the docetaxel-resistant PC-3 cells are 1232 fold more resistant to docetaxel compared to the parent PC-3 cell line while the docetaxel-resistant DU145 cells are 7150 fold more resistant to docetaxel compared to the parent DU145 cell line.

3. Conclusions

The yield reported in the literature for synthesis of fragment C9-C18 of desTHPdactylolides was significantly improved in this work from 8.7% to 36.6–43.0%, which makes the gram-scale synthesis of desTHPdactylolide (**4**) far more efficient. To this end, two optimized methods have been successfully developed by us using allyl iodide or allyl tosylate as the electrophile for the critical Williamson ether synthesis of the C9-C18 unit. With these two methods, both (17*S*)-desTHPdactylolide (**4**) and (17*R*)-desTHPdactylolide (**23**) have been synthesized. The antiproliferative activity of this pair of enantiomers, together with that of the structurally related compounds **20**, **21**, **24**, and **25**, has been evaluated in three docetaxel-sensitive and two docetaxel-resistant prostate cancer cell models using the WST-1 cell proliferation assay, with docetaxel as a positive control. Two (17*S*) dactylolide analogues (**22** and **4**) exhibit almost equivalent potency towards both androgen-sensitive and androgen-insensitive prostate cancer cells while two (17*R*)-dactylolide analogues (**24** and **23**) are more potent against androgen-sensitive LNCaP cells. For the first time, we investigated the effect of configuration at C-17 of desTHPdactylolides (C-19 of dactylolides) on prostate cancer cell proliferation and identified (17*R*) desTHPdactylolide (**23**) and the structurally related compound **24** as the eutomers. Our data imply that inverting the C19 chiral center may be a way to develop improved dactylolide analogues.

4. Experimental section

4.1. General methods

Optical rotations were measured on a RUDOLPH Research Analytical Autopol III Automatic Polarimeter. IR spectra were recorded on a Nicolet Nexus 470 FTIR spectrophotometer. HRMS were obtained on an Orbitrap mass spectrometer with electrospray ionization (ESI). NMR spectra were obtained on a Bruker Fourier 300 spectrometer in

$CDCl_3$. The chemical shifts are given in ppm referenced to the solvent peak, and coupling constants are reported in Hz. Anhydrous THF and dichloromethane were purified by a PureSolv MD 7 Solvent Purification System from Innovative Technologies (MB-SPS-800). All other reagents and solvents were purchased from commercial sources and were used without further purification. Silica gel column chromatography was performed using silica gel (32–63 μM). Preparative thin-layer chromatography (PTLC) separations were carried out on thin layer chromatography plates precoated with silica gel GF254 (EMD Millipore Corporation).

4.2. Preparation of fragment C9-C15 (**8** & **15**)

4.2.1. Method 1

*4.2.1.1. Preparation of (E)-1,3-diiodobut-2-ene (**12**).* To a solution of triphenylphosphine (1.38 g, 5.3 mmol) and imidazole (394 mg, 5.8 mmol) in dichloromethane (10 mL) were sequentially added a solution of iodine (1.21 g, 4.75 mmol) in dichloromethane (10 mL) and a solution of (*E*)-3-iodobut-2-en-1-ol purchased from Fisher Scientific (**9**, 525 mg, 2.65 mmol) in dichloromethane (5 mL) at 4 °C (ice bath), and the reaction was allowed to proceed at room temperature with stirring for 1 h. The reaction was quenched by adding saturated sodium thiosulfate (50 mL), and the resulting mixture was extracted with dichloromethane (3 × 30 mL). The combined organic extracts were dried over anhydrous sodium sulfate and concentrated *in vacuo* at less than 20 °C. The crude product was subjected to PTLC purification eluting with hexane/ethyl acetate (30:1, v/v) to furnish the title compound as a red oil in 91% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.48 (tq, J = 9.0, 1.5 Hz, 1H, vinyl H), 3.76 (d, J = 9.0 Hz, 2H, allylic CH_2), 2.40 (d, J = 1.2 Hz, 3H, allylic CH_3).

*4.2.1.2. Preparation of fragment C9-C15 (**8** and **15**).* To a suspension of sodium hydride (404 mg, 60% in mineral oil, 10.1 mmol) in anhydrous THF (68 mL) at 4 °C was added a solution of alcohol **13** or **14** (10.1 mmol) in THF (5 mL), and the reaction mixture was stirred at 4 °C for 2 h prior to addition of a solution of (*E*)-1,3-diiodobut-2-ene (**12**, 2.39 g, 7.76 mmol) in THF (5 mL). The subsequent reaction mixture was stirred in a cold room (4 °C) overnight before the addition of saturated ammonium chloride (200 mL). The mixture was extracted with dichloromethane (150 mL × 3), the combined organic extracts were dried over anhydrous sodium sulfate, and volatile solvent was removed under vacuum. The crude product was subjected to column chromatography using hexane/ethyl acetate (30:1, v/v) as eluent to yield the desired ether **8** or **15**, respectively. Ether **8** was obtained as a pale yellow oil in 76% yield. The ^{1}H and ^{13}C NMR data of ether **8** are consistent with those reported in the literature.[8] Ether **15** was obtained as a pale yellow oil in 73% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.31 (t, J = 6.9 Hz, 1H, H-14), 3.89 (d, J = 6.8 Hz, 2H, H_2-13), 3.67 (t, J = 6.0 Hz, 2H, H_2-11), 3.48 (t, J = 6.3 Hz, 2H, H_2-9), 2.42 (s, 3H, 15-CH_3), 1.75 (quin, J = 6.3 Hz, 2H, H_2-10), 0.88 (s, 9H, TBS), 0.03 (s, 6H, TBS). ^{13}C NMR (75 MHz, $CDCl_3$) δ 138.0, 98.4, 67.7, 67.1, 59.9, 33.0, 28.3, 26.1, 18.5, −5.2.

4.2.2. Method 2

*4.2.2.1. Preparation of tosylate **16**.* To a suspension of sodium hydride (60 mg, in 60% mineral oil, 1.5 mmol) in diethyl ether (3 mL) at room temperature under argon was added a solution of alcohol **9** (198 mg, 1 mmol) in diethyl ether (5 mL), and the resulting mixture was stirred for 30 min until no gas evolution occurred. The reaction mixture was then cooled down to 0 °C and a solution of *p*-toluenesulfonyl chloride (210 mg, 1.1 mmoL) in diethyl ether (5 mL) was added. The reaction was allowed to proceed with stirring at room temperature overnight before being quenched by adding aqueous saturated ammonium chloride (30 mL). The subsequent mixture was extracted with diethyl ether (20 mL × 3), and the combined ether extracts were rinsed with brine (5 mL × 3), dried over anhydrous sodium sulfate, and concentrated *in vacuo*. The crude tosylate was used directly for the next step reaction.

*4.2.2.2. Preparation of fragment C9-C15 (**15**).* To a suspension of sodium hydride (52 mg, 1.3 mmol) in anhydrous THF (4 mL) at 4 °C under argon was added a solution of alcohol **14** (247 mg, 1.3 mmol) in anhydrous THF (3 mL), and the reaction mixture was stirred at 4 °C for 2 h prior to addition of a solution of crude tosylate (**16**, ~1 mmol) prepared above. The reaction was allowed to proceed in a cold room (4 °C) overnight before being quenched with a saturated solution of ammonium chloride (30 mL). The subsequent mixture was extracted with dichloromethane (20 mL × 3), and the combined organic extracts were dried over anhydrous sodium sulfate and concentrated *in vacuo* to remove the volatile solvent. The crude product was subjected to PTLC purification using hexane/ethyl acetate (30:1, v/v) to yield the desired ether **15** in 27% yield, as well as by products **17** and **18**.

4.2.3. Method 3

*4.2.3.1. Preparation of tosylate **16**.* To a suspension of sodium hydride (360 mg, in 60% mineral oil, 9.0 mmol) in diethyl ether (25 mL) at room temperature under argon was added a solution of (*E*)-3-iodobut-2-en-1-ol (**9**, 990 mg, 5 mmol) in diethyl ether (10 mL), and the resulting mixture was stirred for 30 min until no gas evolution occurred. The reaction mixture was transferred by cannula to a reaction flask charged with a solution of *p*-toluenesulfonyl chloride (1.52 g, 8.0 mmoL) in diethyl ether (15 mL) at 4 °C. The reaction was allowed to proceed with stirring at room temperature for 1 h before being quenched by adding aqueous saturated ammonium chloride (150 mL). The subsequent mixture was stirred for 30 min and then extracted with diethyl ether (60 mL × 3), and the combined ether extracts were rinsed with brine (15 mL × 3), dried over anhydrous sodium sulfate, and concentrated *in vacuo*. The crude tosylate was used directly for the next step.

*4.2.3.2. Preparation of fragment C9-C15 (**15**).* To a suspension of sodium hydride (400 mg, 10 mmol) in anhydrous THF (420 mL) at 4 °C under argon was added a solution of alcohol **14** (1.9 g, 10 mmol) in anhydrous THF (15 mL), and the reaction mixture was stirred at 4 °C for 2 h prior to addition of a solution of crude tosylate (**16**, ~1 mmol) prepared above in anhydrous THF (15 mL). The reaction was allowed to proceed in a cold room (4 °C) overnight before being quenched with saturated solution of ammonium chloride (150 mL). The subsequent mixture was extracted with dichloromethane (60 mL × 3), and the combined organic extracts were dried over anhydrous sodium sulfate and concentrated in *vacuum* to remove the volatile solvent. The crude product was subjected to column chromatographic purification over silica gel using hexane/ethyl acetate (50:1, v/v) to yield the desired ether **15** in 75% yield.

4.3. Synthesis of fragment C9-C18 (compound **19**)

To a solution of vinyliodide **15** (942 mg, 2.546 mmol; co-evaporated twice with pentance) in toluene (16 mL) at −78 °C was added *tert*-BuLi (2.68 mL, 1.9 M, 5.09 mmol), and the mixture was stirred at −78 °C for 45 min prior to being cooled down to −90 °C. PMB-protected (*S*)-glycidol[8] (1.33 g, 6.87 mmol; coevaporated twice with pentance) in toluene (10 mL) was added dropwise to maintain an interior temperature below −78 °C. The reaction solution was re-cooled to −90 °C before $BF_3{\cdot}OEt_2$ (759 mg, 5.35 mmol) was added dropwise. The resulting solution was then stirred at −78 °C overnight prior to being quenched with EtOAc (40 mL) and saturated aqueous $NaHCO_3$ (100 mL) was added. The organic layer was separated and the aqueous layer was extracted with ethyl acetate (100 mL × 3). The combined organic layers were dried over anhydrous sodium sulfate and concentrated *in vacuo* to remove ethyl acetate. The crude mass was subjected to column chromatographic purification over silica gel, using hexane/ethyl acetate 4:1 as eluent, to yield secondary alcohol **19** in 61% yield as a

colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.26 (d, J = 8.7 Hz, 2H, phenyl-H), 6.88 (d, J = 8.7 Hz, 2H, Phenyl-H), 5.43 (t, J = 5.7 Hz, 1H, H-14), 4.48 (s, 2H, benzylic CH_2), 3.98 (d, J = 6.6 Hz, 2H, H_2-18), 3.99–3.91 (m, 1H, H-17), 3.81 (s, 3H, OCH_3), 3.69 (t, J = 6.0 Hz, 2H, H_2-11), 3.49 (t, J = 6.6 Hz, 2H, H_2-9), 3.49–3.44 (overlapped, 1H, H-13), 3.32 (dd, J = 9.3, 7.2 Hz, 1H, H-13), 2.19 (d, J = 6.6 Hz, 2H, H_2-16), 1.78 (quin, J = 6.3 Hz, 2H, H_2-10), 1.70 (s, 3H, 15-CH_3), 0.89 (s, 9H, TBS), 0.05 (s, 6H, TBS).

4.4. Synthesis of (17S) macrolide **20**

The (17*S*) macrolide **20** was prepared from compound **19** and **5** through a four-step procedure as described in the literature.[8] $[\alpha]_D^{20}$: −38.7 (c = 0.46, MeOH). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.60 (dd, J = 15.0, 11.4 Hz, 1H, H-3), 7.25 (d, J = 8.7 Hz, 2H, phenyl-H), 6.87 (d, J = 8.7 Hz, 2H, phenyl-H), 6.89–6.78 (overlapped, 1H, H-9), 6.12 (d, J = 11.4 Hz, 1H, H-4), 6.04 (d, J = 15.9 Hz, 1H, H-8), 5.90 (d, J = 15.0 Hz, 1H, H-2), 5.42–5.34 (m, 1H, H-17), 5.28 (t, J = 5.7 Hz, 1H, H-14), 4.53 (d, J = 11.7 Hz, 1H, benzylic H), 4.47 (d, J = 11.7 Hz, 1H, benzylic H), 4.01 (dd, J = 12.0, 8.1 Hz, 1H, H-18), 3.88 (dd, J = 12.0, 4.8 Hz, 1H, H-18), 3.80 (s, 3H, OCH_3), 3.76 (d, J = 12.0 Hz, 1H, H-6), 3.60–3.36 (m, 4H, H_2-13 & H_2-11), 3.26 (d, J = 12.6 Hz, 1H, H-6), 2.44–2.23 (m, 4H, H_2-10 & H_2-16), 1.83 (s, 3H, 5-CH_3), 1.69 (s, 3H, 15-CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ 197.1, 166.7, 159.5, 146.8, 142.4, 139.5, 134.7, 130.3, 130.2, 129.5, 126.0, 124.9, 121.4, 114.0, 73.1, 71.6, 69.8, 67.9, 67.8, 55.4, 45.9, 42.1, 33.0, 24.1, 16.8. IR (film) ν_{max}: 2914, 2857, 1708, 1668, 1635, 1513 cm^{-1}. HRMS (ESI): m/z calculated for $C_{27}H_{35}O_6$ $[M+H]^+$: 455.2434. Found: 455.2431.

4.5. Synthesis of (17S) macrolide **21**

To a solution of PMB ether **20** (35 mg, 0.077 mmol) in dichloromethane (2 mL) was sequentially added water (0.4 mL) and 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (DDQ, 35 mg, 0.154 mmol), and the reaction mixture was vigorously stirred at room temperature for 1 h. The reaction was quenched by adding aqueous saturated sodium bicarbonate aqueous solution (10 mL), and the mixture was extracted with dichloromethane (10 mL × 4). The combined extracts were dried over anhydrous sodium sulfate and concentrated *in vacuo*. The crude product was subjected to PTLC purification using hexanes/ethyl acetate (1:1, v/v) as eluent to give alcohol **21** as a pale yellow syrup in 73% yield. $[\alpha]_D^{20}$: −86.0 (c = 0.23, MeOH); −79.7 (c = 0.20, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.62 (dd, J = 15.0, 11.7 Hz, 1H, H-3), 6.85 (dt, J = 16.2, 6.6 Hz, 1H, H-9), 6.13 (d, J = 11.1 Hz, 1H, H-4), 6.06 (d, J = 15.9 Hz, 1H, H-8), 5.91 (d, J = 15.0 Hz, 1H, H-2), 5.35–5.25 (m, 2H, H-17 & H-14), 4.02 (dd, J = 12.0, 7.8 Hz, 1H, H-18), 3.89 (dd, J = 12.6, 4.5 Hz, 1H, H-18), 3.79–3.67 (m, 3H, H_2-13 & H-6), 3.53–3.37 (m, 2H, H_2-11), 3.29 (d, J = 12.6 Hz, 1H, H-6), 2.43–2.36 (m, 2H, H_2-10), 2.35 (d, J = 13.8 Hz, 1H, H-16), 2.22 (d, J = 13.5 Hz, 1H, H-16), 1.85 (s, 3H, 5-CH_3), 1.72 (s, 3H, 15-CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ 197.0, 167.1, 146.8, 142.8, 139.8, 134.4, 130.3, 126.0, 125.0, 121.1, 72.2, 68.0, 67.8, 65.5, 46.0, 41.5, 33.0, 24.2, 16.8. IR (film) ν_{max}: 3435, 2921, 2863, 1693, 1667, 1631, 1436 cm^{-1}. HRMS (ESI): m/z calculated for $C_{19}H_{27}O_5$ $[M+H]^+$: 335.1859. Found: 335.1848.

4.6. Synthesis of (17S) desTHPdactylolide (**4**)

To a solution of alcohol **21** (15 mg, 0.045 mmol) in dichloromethane (0.5 mL) at room temperature was added Dess-Martin periodinane (29 mg, 0.0675 mmol), and the reaction mixture was stirred for 10 min before a further quantity of Dess-Martin periodinane (29 mg, 0.0675 mmol) was added. The reaction was allowed to proceed at room temperature for an additional 30 min prior to being quenched with saturated sodium bicarbonate (5 mL) and saturated sodium thiosulfate (5 mL). The resulting mixture was stirred for 15 min, and then extracted with dichloromethane (5 mL × 3). The combined extracts were dried over anhydrous sodium sulfate and concentrated in vacuum. PTLC purification of the crude product eluting with hexane/acetyl acetate (1:1, v/v) furnished the desired dactylolide analogue **4** as a pale yellow syrup in 70% yield. $[\alpha]_D^{20}$: −37.4 (c = 0.065, MeOH). ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.65 (s, 1H, H-20), 7.66 (dd, J = 15.3, 11.7 Hz, 1H, H-3), 6.87 (dt, J = 15.9, 6.9 Hz, 1H, H-9), 6.17 (d, J = 11.7 Hz, 1H, H-4), 6.11 (d, J = 16.2 Hz, 1H, H-8), 5.93 (d, J = 15.0 Hz, 1H, H-2), 5.39 (t, J = 7.2 Hz, 1H, H-14), 5.31 (dd, J = 10.8, 2.7 Hz, 1H, H-17), 4.01 (dd, J = 11.7, 7.5 Hz, 1H, H-13), 3.91 (dd, J = 11.7, 4.5 Hz, 1H, H-13), 3.57 (d, J = 13.2 Hz, 1H, H-6), 3.53–3.46 (m, 3H, H_2-11 & H-6), 2.60 (d, J = 13.8 Hz, 1H, H-16), 2.46–2.38 (m, 3H, H_2-10 & H-16), 1.89 (s, 3H, 5-CH_3), 1.71 (s, 3H, 15-CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ 199.0, 196.6, 166.3, 146.8, 143.8, 140.6, 133.1, 130.6, 126.00, 125.99, 120.1, 75.9, 68.0, 67.6, 45.8, 39.2, 32.9, 24.5, 16.5. IR (film) ν_{max}: 2923, 2855, 1711, 1666, 1631, 1436 cm^{-1}. HRMS (ESI): m/z calculated for $C_{19}H_{25}O_5$ $[M+H]^+$: 333.1702. Found: 333.1696.

4.7. Synthesis of fragment C9–C18 (compound **22**)

A similar procedure to that used to prepare **19** from iodide **15** was employed to prepare **22**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.25 (d, J = 8.4 Hz, 2H, phenyl-H), 6.87 (d, J = 8.7 Hz, 2H, phenyl-H), 5.42 (t, J = 6.0 Hz, 1H, H-14), 4.47 (s, 2H, benzylic CH_2), 3.96 (d, J = 6.6 Hz, 2H, H_2-18), 3.97–3.89 (m, 1H, H-17), 3.79 (s, 3H, OCH_3), 3.68 (t, J = 6.3 Hz, 2H, H_2-11), 3.48 (t, J = 6.3 Hz, 2H, H_2-9), 3.48–3.43 (overlapped, 1H, H-13), 3.32 (dd, J = 9.3, 7.2 Hz, 1H, H-13), 2.18 (d, J = 6.6 Hz, 2H, H_2-16), 1.77 (quin, J = 6.3 Hz, 2H, H_2-10), 1.69 (s, 3H, 15-CH_3), 0.89 (s, 9H, TBS), 0.04 (s, 6H, TBS). ^{13}C NMR (75 MHz, $CDCl_3$) δ 159.4, 136.2, 130.2, 129.5, 124.5, 114.0, 79.1, 74.0, 73.2, 68.4, 67.3, 60.1, 55.4, 43.7, 33.1, 26.1, 18.5, 16.7, −5.2.

4.8. Synthesis of (17R) macrolide **24**

Lactone **24** was prepared from **22** and **5** through a four-step reaction sequence as described in the literature.[8] $[\alpha]_D^{20}$: +38.6 (c = 0.68, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.61 (dd, J = 15.0, 11.4 Hz, 1H, H-3), 7.26 (d, J = 8.1 Hz, 2H, phenyl-H), 6.88 (d, J = 8.7 Hz, 2H, phenyl-H), 6.91–6.80 (overlapped, 1H, H-9), 6.13 (d, J = 11.4 Hz, 1H, H-4), 6.05 (d, J = 15.9 Hz, 1H, H-8), 5.91 (d, J = 15.0 Hz, 1H, H-2), 5.43–5.35 (m, 1H, H-17), 5.29 (t, J = 5.7 Hz, 1H, H-14), 4.54 (d, J = 11.7 Hz, 1H, benzylic H), 4.48 (d, J = 11.7 Hz, 1H, benzylic H), 4.02 (dd, J = 12.0, 8.1 Hz, 1H, H-18), 3.89 (dd, J = 12.0, 4.5 Hz, 1H, H-18), 3.81 (s, 3H, OCH_3), 3.77 (d, J = 12.9 Hz, 1H, H-6), 3.61–3.36 (m, 4H, H_2-13 & H_2-11), 3.27 (d, J = 12.9 Hz, 1H, H-6), 2.45–2.24 (m, 4H, H_2-10 & H_2-16), 1.84 (s, 3H, 5-CH_3), 1.70 (s, 3H, 15-CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ 197.1, 166.7, 159.4, 146.9, 142.4, 139.5, 134.6, 130.3, 130.2, 129.5, 126.0, 124.9, 121.4, 114.0, 73.0, 71.6, 69.7, 67.9, 67.8, 55.4, 45.9, 42.1, 33.0, 24.1, 16.8. IR (film) ν_{max}: 2923, 2854, 1708, 1668, 1633, 1513, 1462 cm^{-1}. HRMS (ESI): m/z calculated for $C_{27}H_{35}O_6$ $[M+H]^+$: 455.2434. Found: 455.2430.

4.9. Synthesis of (17R) macrolide **25**

Lactone **25** (31 mg, 80%, pale yellow oil) was synthesized from lactone **24** using a procedure similar to that employed for conversion of **20** to **21**. $[\alpha]_D^{20}$: +70.4 (c = 0.19, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.61 (dd, J = 15.0, 11.4 Hz, 1H, H-3), 6.84 (dt, J = 16.2, 6.9 Hz, 1H, H-9), 6.12 (d, J = 11.4 Hz, 1H, H-4), 6.04 (dt, J = 16.2, 1.2 Hz, 1H, H-8), 5.90 (d, J = 15.0 Hz, 1H, H-2), 5.31–5.25 (m, 2H, H-14 & H-17), 4.01 (dd, J = 12.0, 7.8 Hz, 1H, H-18), 3.88 (dd, J = 12.0, 4.8 Hz, 1H, H-18), 3.77–3.72 (m, 3H, H_2-13 & H-6), 3.52–3.38 (m, 2H, H_2-11), 3.28 (d, J = 12.6 Hz, 1H, H-6), 2.45–2.37 (m, 2H, H_2-10), 2.32 (d, J = 13.5 Hz, 1H, H-16), 2.21 (d, J = 13.5 Hz, 1H, H-16), 1.83 (s, 3H, 5-CH_3), 1.70 (s, 3H, 15-CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ 197.1, 167.1, 146.9, 142.8, 139.8, 134.4, 130.3, 125.9, 125.0, 121.1, 72.2, 68.0, 67.8, 65.4, 46.0, 41.5, 33.0, 24.2, 16.8. IR (film) ν_{max}: 3446, 2920,

2851, 1705, 1632, 1437 cm^{-1}. HRMS (ESI): *m/z* calculated for $C_{19}H_{27}O_5$ $[M+H]^+$: 335.1859. Found: 335.1851.

4.10. Synthesis of (17R) desTHPdactylolide (23)

(17*R*) DesTHPdactylolide (**23**) (14 mg, 75%, pale yellow oil) was synthesized according to the oxidation procedure employed for the conversion of **21** to (17*S*) desTHPdactylolide (**4**). $[\alpha]_D^{20}$: +38.4 (*c* = 0.06, MeOH). ^1H NMR (300 MHz, $CDCl_3$) δ 9.65 (s, 1H, H-18), 7.65 (dd, *J* = 15.3, 11.7 Hz, 1H, H-3), 6.85 (dt, *J* = 15.9, 6.9 Hz, 1H, H-9), 6.17 (d, *J* = 11.7 Hz, 1H, H-4), 6.10 (dt, *J* = 15.9, 1.5 Hz, 1H, H-8), 5.93 (d, *J* = 15.3 Hz, 1H, H-2), 5.38 (t, *J* = 5.4 Hz, 1H, H-14), 5.31 (dd, *J* = 10.8, 3.0 Hz, 1H, H-17), 4.01 (dd, *J* = 12.0, 7.8 Hz, 1H, H-13), 3.91 (dd, *J* = 12.0, 5.1 Hz, 1H, H-13), 3.54 (d, *J* = 5.4 Hz, 1H, H-6), 3.49–3.44 (m, 3H, H_2-11 & H-6), 2.59 (d, *J* = 13.8 Hz, 1H, H-16), 2.46–2.37 (m, 3H, H_2-10 & H-16), 1.88 (s, 3H, 5-CH_3), 1.71 (s, 3H, 15-CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ 199.1, 196.7, 166.3, 146.9, 143.8, 140.6, 133.1, 130.5, 125.99, 125.96, 120.0, 75.8, 68.0, 67.5, 45.8, 39.2, 32.9, 24.5, 16.5. IR (film) ν_{max}: 2919, 1733, 1706, 1669, 1635, 1558, 1521 cm^{-1}. HRMS (ESI): *m/z* calculated for $C_{19}H_{25}O_5$ $[M+H]^+$: 333.1702. Found: 333.1700.

4.11. Cell culture

All cell lines were initially purchased from American Type Culture Collection (ATCC™). The PC-3 and LNCaP prostate cancer cell lines were routinely cultured in RPMI-1640 medium supplemented with 10% FBS and 1% penicillin/streptomycin. Cultures were maintained in a high humidity environment supplemented with 5% carbon dioxide at a temperature of 37 °C. The DU145 prostate cancer cells were routinely cultured in Eagle's Minimum Essential Medium (EMEM) supplemented with 10% FBS and 1% penicillin/streptomycin.

Docetaxel-resistant prostate cancer cell lines were established based on the procedure illustrated in the literature.[18,19] Docetaxel-resistant DU145 and PC-3 cell lines (DU145/DTX and PC-3/DTX) were developed over a period of one year by stepwise increased concentrations of docetaxel. Cells were continuously maintained in docetaxel, with treatments beginning at the initial IC_{50} of the respective parent cell lines. Media containing docetaxel will be changed every 2–3 days. As cells displayed resistance to treatments of docetaxel the concentration was subsequently increased.

4.12. WST-1 cell proliferation assay[20]

PC-3, LNCap, or DU145 cells were plated in 96-well plates at a density of 3200 each well in 200 μL of culture medium. The cells were then treated with the positive control, or the synthesized compounds at different doses for 3 days, while equal treatment volumes of DMSO were used as vehicle control. The cells were cultured in a CO_2 incubator at 37 °C for three days. 10 μL of the premixed WST-1 cell proliferation reagent (Clontech) was added to each well. After mixing gently for one minute on an orbital shaker, the cells were incubated for additional 3 h at 37 °C. To ensure homogeneous distribution of color, it is important to mix gently on an orbital shaker for one minute. The absorbance of each well was measured using a microplate-reader (Synergy HT, BioTek) at a wavelength of 430 nm. The IC_{50} value is the concentration of each compound that inhibits cell proliferation by 50% under the experimental conditions and is the average from at least triplicate determinations that were reproducible and statistically significant. The IC_{50} values were calculated from the dose-response curves based on at least five dosages for each compound.

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A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2018.05.026.

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