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Protecting-Group Directed Diastereoselective Nozaki-Hiyama-Kishi (NHK) Reaction: Total Synthesis and Biological Evaluation of Zeaenol, 7-epi-Zeaenol and its Analogues

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The stereoselective total synthesis of zeaenol and 7-epi-zeaenol was achieved in a convergent manner by using Julia-Kocienski olefination, protecting group-directed intermolecular diastereoselective Nozaki–Hiyama–Kishi (NHK) reaction, De Brabander’s lactonization reaction and CBS reduction as the key steps. In this article, we have observed the most suitable protecting groups with respect to selectivity during the protecting group directed intermolecular asymmetric Nozaki-Hiyama-Kishi reaction. The Zeaenol, 7-epi-Zeaenol and its derivatives were analyzed for their biological activity and screened in four cancer cell lines.

Introduction

Zeaenol was first isolated and reported by Sugawara et al.1 from ethyl acetate extracts of the culture fluid Drechslera portulacea. The relative and absolute configuration of zeaenol was confirmed by its single X-ray crystallographic and spectroscopic analysis. Later, Nicholas and co-workers reported the isolation of 15-O-desmethyl-(5Z)-7-oxozaenol and 7-epi-zeaenol, along with known other resorcylic acid lactones (RALs) (Figure 1), from filamentous fungus MSX 63935 available from leaf litter in Nigeria.2 All these compounds exhibit potent antibacterial activity as well as mitochondrial transmembrane potential activity. The zeaenol (1) and 7-epi-zeaenol (2) show similar cytotoxic activity against human tumor cell lines3,4 and inhibition activity towards NF-κB (IC50 values >50 μM).

Figure 1. Structures of resorcylic acid lactones (RALs) (1-6).

The first total synthesis of zeaenol and its isopropylidene protected compound cochliomycin A were reported by Nanda et al.5 using RCM strategy. Recently, Yuguó Du and co-workers6 reported the total synthesis of zeaenol along with cochliomycin B using late stage RCM strategy. Their curious skeletal connectivities and potent biological properties attracted our attention to develop a flexible and general synthetic approach for the total synthesis of zeaenol, 7-epi-zeaenol whose synthesis was not reported so far and its derivatives for biological activity. As part of our ongoing research on the total synthesis of macrolides and RALs by protecting group-directed Nozaki-Hiyama-Kishi reaction as the key step.7 In this report, we have applied the protecting group-directed diastereoselective intermolecular Nozaki-Hiyama-Kishi reaction for a convergent and concise synthesis of zeaenol and 7-epi-zeaenol.

Our retrosynthetic strategy of zeaenol and 7-epi-zeaenol is depicted in Scheme 1. The target molecule was anticipated to be derived from the macro lactonization of 7 by using De Brabander’s conditions, which could be obtained by protecting group-directed intermolecular Diastereoselective Nozaki-Hiyma-Kishi reaction of 8 and 9. The advanced fragment 8 could be prepared from 10 and 11 which in turn could be synthesized from dioxinode (13) and D-mannitol (14). The vinyl iodide fragment 9 could be derived from a known epoxide 12.

Results and Discussion

With the retrosynthetic blueprint in mind, our initial focus was on the synthesis of the iodo fragment 9, which commenced with the known chiral epoxide 12.8 The epoxide 12 was prepared by using Jacobsen’s hydrolytic kinetic resolution protocol.9 Reductive opening of epoxide 12 with LiAlH4 in THF afforded alcohol 15 in 92% yield. The resulting secondary alcohol was protected as its TBS-ether9 using TBSCI and imidazole in CH2Cl2.
to yield TBS protected compound, which on treatment with DDQ in CH$_2$Cl$_2$/H$_2$O (19:1) furnished primary hydroxyl compound 16 in 88% yield over two steps. The Dess-Martin periodinane oxidation of primary alcohol 16 followed by Takai olefination afforded the required trans-vinyl iodide compound 9 (E/Z = 95:5, by NMR) in 81% yield over two steps.

Scheme 1. Retrosynthetic approach for 7-epi-zeaenol (1) and zeaenol (2)

For the synthesis of another key fragment 10, we started from 17 which was prepared from commercially available 2,2,6-trimethyl-4H-1,3-dioxin-4-one (13) by following a known protocol. Subsequently protection of the phenolic hydroxyl functionality present in 17 was converted to methyl ether under Mitsunobu conditions to obtain 18 in 87% yield. Bromination of benzylic position of 18 was achieved by NBS and benzoyl peroxide in CHCl$_3$ under reflux conditions to afford benzyl bromide derivative 19 in 79% yield. Treatment of benzyl bromide derivative 19 with 1-phenyl-1H-tetrazole-5-thiol and Et$_3$N in THF gave thiourea ether 20, which was on further treatment with m-CPBA in CH$_2$Cl$_2$ furnished the corresponding sulfone fragment in 88% yield over two steps.

Synthesis of the fragment 11 was initiated from compound 21, which was synthesized from commercially available D-mannitol following a known protocol. The free hydroxyl group was protected as its benzyl ether by using benzyl bromide and NaH to give 22 in 93% yield. The deprotection of cyclohexylidene group was easily achieved by camphorsulfonic acid (CSA) in MeOH to afford diol in which the primary alcohol was selectively protected as its TBS-ether by treatment with TBS-Cl and imidazole in CH$_2$Cl$_2$ to give silyl ether compound 23 in 86% yield over two steps. The resultant secondary hydroxyl group of 23 was protected as its benzyl ether with benzyl bromide in presence of NaNH to afford 24 in 91% yield. The oxidative cleavage of compound 24 under Jin’s one-pot conditions using Os$_2$O$_3$/NaIO$_4$ and 2,6-lutidine in dioxane-water (3:1) furnished aldehyde fragment 11 in 85% yield.
Scheme 4. Reagents and conditions: (a) BnBr, NaH, TBAI, THF 0 °C-r.t., 4 h, 93%; (b) CSA, MeOH, 0-rt 0°C, 12 h, 90%; (c) TBSCl, Imidazole, CH₂Cl₂, 0 °C, 0.5 h, 96%; (d) BnBr, NaH, TBAI, THF, 0 °C-r.t., 6 h, 91%; (e) OsO₄, NaIO₄, 2.6-lutidine, dioxane/water (3:1), rt, 10 h, 85%.

Having both fragments 10 and 11 in hand, we proceeded for Julia-Kocienski olefination using KHMDS and 18-crown-6 ether in DME at -78 °C to afford desired olefin 25 exclusively in 84% yield. The TBS-ether protection in 25 was smoothly removed with CSA in MeOH to obtain 26 in 94% yield.

Scheme 5. Reagents and conditions: (a) KHMDS, 18-crown-6, DME, -78 °C, 12 h, 84%; (b) CSA, MeOH, 0 °C-r.t., 0.5 h, 94%; (c) Dess-Martin periodinane, NaHCO₃, CH₂Cl₂, 0 °C-r.t., 3 h, 92%; (d) 9, CrCl₃, NiCl₂, DMF, rt, 24 h, 81%; (e) MOM-Cl, DIPEA, CH₂Cl₂, 0 °C-r.t., 10 h, 96%; (f) TBAF, THF, 0 °C-r.t., 10 h, 91%; (g) NaH, THF, 0 °C-r.t, 4 h, 78%; (h) TiCl₄, CH₂Cl₂, 0 °C 0.5 h, 88%; (i) NaH, BnBr, THF, 0 °C-r.t., 5 h, 89%; (j) 2N HCl, THF, 15 h, 91%; (k) H₂, Pd/C, THF, rt, 24 h, 87%.

Resulting primary alcohol 26 was treated with Dess-Martín periodinane reagent to afford the corresponding aldehyde 8 in 92% yield. The aldehyde was coupled vinyl iodo fragment 9 under mild conditions using Cr(II)/Ni(II)-mediated protecting group-directed asymmetric intermolecular Nozaki-Hiyama-Kishi (N HK)-reaction to furnish the alkyl alcohol 27 (9:1; separated by column chromatography) as a major isomer in 81% combined yield. The stereochemistry of the major isomer was ascertained by using modified Mosher’s ester method at the later stage as at this stage esterification was leading to an intractable mixture of products. The major isomer was taken forward towards the total synthesis of the target molecule. The secondary alcohol obtained in NHK-reaction was protected as its MOM-ether by using MOM-Cl and DIPEA to give 28 in 96% yield. The TBS group was deprotected with TBAF in THF to obtain compound 7 in 91% yield. The intramolecular macrolactonization of 7 under De Brabander’s conditions using NaH in THF furnished the macrolactone core 29 in 78% yield. At this stage, the phenolic OH group was protected as its benzyl ether and MOM group was selectively deprotected to afford 36. According to the modified Mosher’s ester method, the free hydroxyl group present in 36 was converted to its (R)- and (S)-2-methoxy-2-(trifluoromethyl)-2-phenylacetic acid (MTPA) ester with corresponding 2-methoxy-2-(trifluoromethyl)-2-phenylacetic acid which showed negative chemical shift differences (Δδ = δS - δR) for protons on C8 through C12 while protons on C3 through C6 showed positive differences, which is consistent with C7 bearing an (S)-configuration which was in accord to the stereochemistry of 7-epi-zeaenol (2). Finally, global deprotection of benzyl and MOM groups was achieved by TiCl₄ to afford 7-epi-zeaenol (2) in 88% yield representing the first total synthesis of 7-epi-zeaenol. The spectral (¹H and ¹³C NMR) and analytical data of 7-epi-zeaenol was in good agreement with the reported values. To take the advantage of SAR studies, the 7-epi-azeaenol (2) was treated with Pd/C under hydrogen atmosphere to furnish tetrahydro-7-epi-zeaenol 30 in 87% yield.
For the synthesis of zeaenol, compound 29 was treated with 2N HCl to give secondary alcohol 31 in 88% yield. The resulting secondary hydroxy group was oxidized under Dess–Martin periodinane conditions to obtain \( \alpha,\beta \)-unsaturated ketone 32 in 90% yield, which on asymmetric reduction using Corey-Bakshi-Shibata\(^{22} \) (CBS) reagent \([ (S)-2\text{-methyl oxazaborolidine} \]) provided compound 33 with required C7 stereocenter (97:3 dr, by HPLC, separated by column chromatography) in 82% yield.

Deprotection of the benzyol group was achieved by treating compound 33 with excess TiCl\(_4\) in CH\(_2\)Cl\(_2\) at 0 °C to obtain zeaenol (1) in 86% yield. Similarly, treatment of zeaenol (1) with Pd/C under hydrogen atmosphere afforded tetrahydro-zeaenol (34) in 89% yield. The spectral (\(^1\)H and \(^{13}\)C NMR) and analytical data of zeaenol was in accord with the reported values.\(^{2,5,6}\) The natural products zeaenol (1), 7-epi-zeaenol (2) and analogues 30 and 34 were tested for their cytotoxic activity and the results are summarised in Table 1.

Table 1: Cell lines were treated with different concentration of compounds for 48 h as mentioned in “materials and methods section. Cell viability was measured employing SRB assay. GI\(_{50}\), TGI and IC\(_{50}\) (in \( \mu \)M) values are indicated as mean ± SD (standard deviation) of three independent experiments.

<table>
<thead>
<tr>
<th>code</th>
<th>AS49</th>
<th>HeLa</th>
<th>DU 145</th>
<th>MDA MB 231</th>
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<td>TGI</td>
<td>IC(_{50})</td>
<td>GI(_{50})</td>
</tr>
<tr>
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<td>368 ± 1.7</td>
<td>1207 ± 4.6</td>
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<tr>
<td>2</td>
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<td>1145 ± 1.5</td>
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<tr>
<td>30</td>
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<td>154 ± 1.52</td>
<td>263 ± 2.3</td>
<td>0.05</td>
</tr>
<tr>
<td>34</td>
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<td>162 ± 2.15</td>
<td>241±3.12</td>
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<tr>
<td>Nocodazole</td>
<td>&lt;0.01</td>
<td>0.16 ± 0.02</td>
<td>6.6 ± 0.1</td>
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</tbody>
</table>

Biological studies:

In vitro cytotoxic activity:
The lead compounds inhibit the NF-kB albeit at 50\( \mu \)M, nevertheless we investigated their ability to inhibit growth of cancer cells. To evaluate this possibility, HeLa, AS49, DU145 and MDA-MB-231 cancer cell lines were challenged with these compounds. We followed the protocol set by NCI-60 cell screen and performed the dose-response at five concentrations (0.01, 0.1, 1, 10 and 100 \( \mu \)M) of compounds. The cells were incubated with Nocodazole as standard agent. Based on linear interpolation, we arrived at the values of GI\(_{50}\), with compounds for 48 h. Nocodazole was employed as standard agent. Based on linear interpolation, we arrived at the values of GI\(_{50}\), TGI and IC\(_{50}\). Interestingly, our anti-proliferative assays reveal that the congeners 30 and 34 demonstrate significant growth inhibition effect. In particular, 34 manifested GI\(_{50}\) of 1 \( \mu \)M in AS49 and HeLa cells. Moreover in MDA-MB-231 and DU145 growth was inhibited at 4 \( \mu \)M concentrations of 34. In contrast, 30 exhibited GI\(_{50}\) of 5 \( \mu \)M in HeLa cells. Similarly the GI\(_{50}\) values of 2 were at 1 \( \mu \)M and 1 at 0.44 \( \mu \)M in HeLa cells. Based on these observations, the congeners contain potent pharmacophores that elicit significant growth inhibitory response in cancer cells. Thus further delineating these pharmacophores from the molecules will possibly improve the potency of the pounds.

Conclusions

In conclusion, we have developed an efficient and concise route for the total synthesis of both zeaenol and 7-epi-zeaenol in convergent manner. The key steps involved in this synthesis are Takai olefination, Julia-Kocienski olefination, protecting group-directed asymmetric intermolecular Nozaki–Hiyama–Kishi (NHK) reaction, and De Brabander’s lactonization. The obvious and remarkable advantages of our protocol lie in high overall yield. In addition, we identified that tetrahydro-zeaenol demonstrated significant growth inhibitory activity and these compounds are amenable for further structural modifications to improve their efficacy for anticancer therapy.
Experimental section

General Remarks: All reactions were performed under inert atmosphere, if argon mentioned. All glassware apparatus used for reactions are perfectly oven/flame dried. Anhydrous solvents were distilled prior to use: THF from Na and benzophenone; CH₂Cl₂, DMF from CaH₂; MeOH from Mg cake. Commercial reagents were used without purification. Column chromatography was carried out by using silica gel (60–120 mesh) unless otherwise mentioned. Analytical thin layer chromatography (TLC) was run on silica gel 60 F254 pre-coated plates (25 μm thickness). Optical rotations [α]D were measured on a polarimeter and given in 10° deg cm² g⁻¹. Infrared spectra were recorded in CHCl₃/KBr (as mentioned) and reported in wave number (cm⁻¹).

Mass spectral data were obtained using MS (EI) ESI, HRMS (ESI) spectra recorded in KBr (as mentioned) and reported in wave number (cm⁻¹). Mass spectral data were obtained using MS (EI) ESI, HRMS (ESI) spectra recorded in KBr (as mentioned) and reported in wave number (cm⁻¹). Mass spectral data were obtained using MS (EI) ESI, HRMS (ESI) spectra recorded in KBr (as mentioned) and reported in wave number (cm⁻¹).

To a stirred solution of primary alcohol (3 x 30 mL), was added LiAlH₄ (731 mg, 19.24 mmol) at 0 °C. The solution was warmed to room temperature and stirred for additional 30 min. After completion of the reaction (monitored by TLC), the reaction mixture was cooled to 0 °C and quenched with saturated solution of Na₂SO₄ (20 mL). The residue was filtered through Celite pad and the filtrate was concentrated under reduced pressure. The crude product was purified by silica gel column chromatography (ethyl acetate/hexane = 1:9) to afford the desired alcohol (1.84 g, 92%) as a colorless liquid. [α]D = –4.5 (c 1.1, CHCl₃); IR (neat): 3416, 2964, 2832, 2933, 1613, 1586, 1514, 1248 cm⁻¹; ¹H NMR (300 MHz, CDCl₃); δ 7.21 (d, J = 8.5 Hz, 2H), 6.82 (d, J = 8.5 Hz, 2H), 4.43 (s, 2H), 3.94 (s, 3H), 3.68-3.51 (m, 2H) 2.77 (br s, 1H), 1.75-1.60 (m, 2H), 1.16 (d, J = 6.2 Hz, 3H) ppm; ¹³C NMR (75 MHz, CDCl₃); δ 159.1, 129.9, 129.1, 131.6, 72.7, 68.5, 67.2, 55.1, 37.9, 23.2 ppm; HRMS (ESI): calcd. for C₃H₇O₂Na [M + Na]⁺ 233.1142, found: 233.1142.

(S)-3-(tert-Butylmethylsilyl)butan-1-ol (16): To a stirred solution of alcohol 15 (1.5 g, 7.14 mmol) in CH₂Cl₂ (30 mL), was added imidazole (1.2 g, 17.85 mmol) and TBSCl (2.14 g, 14.28 mmol) at 0 °C and the reaction mixture was stirred for 30 min at the same temperature. After completion of the reaction (monitored by TLC), it was quenched with water (30 mL) and extracted with CH₂Cl₂ (3 x 30 mL). The combined organic layer was dried over anhydrous Na₂SO₄ and the solvent removed under reduced pressure. The crude mass was purified by silica gel column chromatography (ethyl acetate/hexane = 2:5) to afford TBS protected compound (2.2 g, 95%) as a colorless liquid and immediately used for the next step. To a solution of above PMB ether compound (1.7 g, 5.25 mmol) in CH₂Cl₂ (38 mL) and water (2 mL) at room temperature was added DDQ (1.78 g, 8.77 mmol) and the reaction mixture was stirred for 2 h at the same temperature. After completion of the reaction (monitored by TLC), it was quenched with saturated NaHCO₃ (40 mL). The organic layer was extracted with CH₂Cl₂ (2 x 50 mL) and the combined organic layer was washed with brine (60 mL), dried over anhydrous Na₂SO₄. The solvent was removed under reduced pressure and the crude mass was purified by silica gel column chromatography (ethyl acetate/hexane = 1:9) to give 16 (1.0 g, 93%) as a colorless liquid. [α]D = 21.2 (c 1.14, CHCl₃); IR (neat): 3376, 2957, 2932, 2859, 1739, 1615, 1467, 1375, 1254 cm⁻¹; ¹H NMR (300 MHz, CDCl₃); δ 4.09 (m, 1H), 3.79 (m, 1H), 3.68 (m, 1H), 2.3 (br s, 1H), 1.74 (m, 1H), 1.60 (m, 1H), 1.20 (m, J = 6.8 Hz, 3H), 0.91 (s, 9H), 0.09 (s, 3H), 0.08 (s, 3H) ppm; ¹³C NMR (75 MHz, CDCl₃); δ 68.3, 60.4, 40.4, 25.7, 23.4, 17.9, –4.4, –5.0 ppm; HRMS (ESI): calcd. for C₅H₁₀O₂NaSi [M + Na]⁺ 251.1281, found: 251.1261.

(S,E)-tert-Butyl 5-iodopent-4-en-2-yl)methyldisilane (9): To a stirred solution of primary alcohol 16 (0.9 g, 4.41 mmol) and solid anhydrous NaHCO₃ (1.0 g) in CH₂Cl₂ (25 mL) was added Dess–Martin periodinane (4.0 g, 6.65 mmol) at 0 °C. The resulting reaction mixture was stirred at 0 °C for 3 h. After completion of the reaction (monitored by TLC), the mixture was filtered through a bed of Celite. The filtrate was washed with saturated NaHCO₃ (2 x 25 mL). The aqueous layer was extracted with CH₂Cl₂ (2 x 40 mL). The combined organic layer was dried over anhydrous Na₂SO₄ and the solvent was removed under reduced pressure. Purification of the crude mass over silica gel column chromatography (ethyl acetate/hexane = 1:25) to afford corresponding aldehyde (0.82 g, 93%) as a pale-yellow liquid which was immediately used for next step. To a stirred suspension of CrCl₂ (2.89 g, 23.76 mmol) in THF (25 mL) was added the above aldehyde (0.8 g, 3.96 mmol) and CH₃I (4.67 g, 11.88 mmol) dissolved in THF (25 mL) at ambient temperature. The reaction mixture was protected from light and stirred at ambient temperature for 16 h. After completion of the reaction (monitored by TLC), the green reaction mass was quenched with water (30 mL). The reaction mixture was extracted with ethyl acetate (3 x 40 mL). The combined organic extract was washed with saturated aqueous Na₂SO₄ (2 x 50 mL) followed by brine (50 mL), dried over anhydrous Na₂SO₄ and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography (hexane) afforded 9 (1.12 g, 87%) as a pale-yellow oil. [α]D = +9.7 (c 1.5, CHCl₃); IR (neat): 2955, 2928, 2856, 1607, 1464, 1375, 1253 cm⁻¹; ¹H NMR (300 MHz, CDCl₃); δ 6.48 (m, 1H), 6.02 (dd, J = 14.4, 1.5 Hz, 1H), 3.83 (m, 1H), 2.17-2.11 (m, 2H), 1.13 (d, J = 6.0 Hz, 3H), 0.88 (s, 9H), 0.04 (s, 6H) ppm; ¹³C NMR (75 MHz, CDCl₃); δ 143.6, 76.5, 67.5, 45.9, 25.8, 23.5, 18.1, –4.6, –4.7 ppm. 7-Methoxy-2,2,5-trimethyl-4H-benz[d][1,3]dioxin-4-one (18): Compound 17 (3.5 g, 16.83 mmol) was taken in THF (40 mL) and MeOH (1.0 mL, 25.25 mmol) was added followed by Ph₃P (6.6 g, 25.25 mmol) at 0 °C. After being stirred for 5 minutes at the same temperature, disopropyl azodicarboxylate (4.9 mL, 25.25 mmol) was added drop wise to the reaction mixture and stirred for an additional 4 h at room temperature. The reaction mixture was concentrated under reduced pressure and purified by silica gel column chromatography (ethyl acetate/hexane = 1:20) to obtain 18 (3.2 g, 87%) as a white solid. Mp = 147–149 °C; IR (neat): 2927, 2852, 1730, 1614, 1559, 1454, 1205, 1160 cm⁻¹; ¹H NMR (300 MHz, CDCl₃); δ 6.23 (d, J = 2.3 Hz, 1H), 1.62 (d, J = 2.3 Hz, 1H), 3.82 (s, 3H), 2.63 (s, 3H), 1.69 (s, 6H) ppm; ¹³C
NMR (75 MHz, CDCl$_3$): $\delta$ 164.7, 160.4, 158.8, 145.3, 112.7, 104.9, 99.2, 98.1, 55.4, 25.6, 22.2 ppm; HRMS (ESI): calcd. for C$_{10}$H$_{19}$O$_4$Na [M + Na$^+$] 245.0784; found: 245.0803.

5-(Bromomethyl)-7-methoxy-2,2-dimethyl-4H-benzo[d][1,3]dioxin-4-one (19): To a solution of 18 (3.0 g, 13.51 mmol) in CCl$_4$ (40 mL) was added NBS (1.3 g, 7.43 mmol) followed by benzyloxide (40 mg) and the reaction mixture heated under reflux. After 3 h, another portion of NBS (1.3 g, 7.43 mmol) and benzyloxide (40 mg) was added to the above reaction mixture and heated under reflux condition for an additional 3 h. The reaction was cooled to room temperature, the solid succinimide filtered off and the solvent removed under reduced pressure. The residue was purified by silica gel column chromatography (ethyl acetate/hexane = 1:9) to afford compound 19 (3.18 g, 79%) as a white solid. Mp = 89–92 °C; IR ( neat): 2992, 2920, 2853, 1728, 1612, 1580, 1435, 1205, 1163 cm$^{-1}$; $^1$H NMR (500 MHz, CDCl$_3$): $\delta$ 6.69 (s, 1H), 6.37 (s, 1H), 4.96 (s, 2H), 3.83 (s, 3H), 1.68 (s, 6H) ppm; $^13$C NMR (75 MHz, CDCl$_3$): $\delta$ 164.9, 159.6, 159.2, 143.3, 112.7, 105.5, 101.4, 98.2, 55.8, 31.1, 25.5 ppm; HRMS (ESI): calcd. for C$_{12}$H$_{14}$O$_4$Br [M + H$^+$] $^301.0070$ found, 301.0065.

7-Methoxy-2,2-dimethyl-5-[[1-phenyl-1H-tetrazol-5-ylthio]methyl]-4H-benzo[d][1,3]dioxin-4-one (20): To a stirred solution of 19 (2.2 g, 9.6 mmol) in dry THF (30 mL), was added Et$_3$N (1.74 mL, 12.9 mmol) and the mixture was stirred at room temperature. After 40 min, compound 19 (2.6 g, 8.67 mmol) was added and the reaction mixture was refluxed for 6 h. The reaction mixture was diluted with water (30 mL) and extracted with ethyl acetate (3 x 20 mL). The combined organic layers were washed over anhydrous Na$_2$SO$_4$ and evaporated under reduced pressure to give the crude triether which was purified by flash chromatography (ethyl acetate/hexane = 1:7) to afford compound 20 (3.3 g, 96%) as a white solid. Mp = 144–146 °C; IR ( neat): 3306, 3000, 2924, 2853, 1717, 1611, 1579, 1499, 1386, 1289 cm$^{-1}$; $^1$H NMR (500 MHz, CDCl$_3$): $\delta$ 7.59–7.45 (m, 5H), 7.13 (d, J = 2.5 Hz, 1H), 6.33 (d, J = 2.5 Hz, 1H), 4.96 (s, 2H), 3.87 (s, 3H), 1.68 (s, 6H) ppm; $^13$C NMR (75 MHz, CDCl$_3$): $\delta$ 165.1, 160.6, 159.0, 154.8, 142.7, 133.5, 129.9, 129.7, 123.7, 113.5, 105.6, 103.6, 101.6, 55.8, 35.8, 25.5 ppm; HRMS (ESI): calcd. for C$_{18}$H$_{19}$O$_5$SNa [M + Na$^+$] $^399.2012$ found, 399.1124.

(2RS,3S)-3-(Benzyloxy)-1-(tert-butyldimethylsilyloxy)hex-5-en-2-ol (23): To a stirred solution of 22 (9.0 g, 29.8 mmol) in MeOH (20 mL) was added CSA (0.28 g, 2.98 mmol) at 0 °C and the reaction mixture was stirred for 12 h at rt. After completion of the reaction (monitored by TLC), it was quenched with saturated solution of NaHCO$_3$ (100 mL) and MeOH was removed under reduced pressure. The residue was extracted with ethyl acetate (3 x 150 mL) and the combined organic layer was washed with brine (150 mL), dried over anhydrous Na$_2$SO$_4$. The solvent was removed under reduced pressure and purification of the crude product by silica gel column chromatography (ethyl acetate/hexane = 1:1) furnished the desired diol (5.95 g, 90%) as a viscous colorless liquid that was immediately used for next step. To a stirred solution of above diol (5.5 g, 24.77 mmol) in CH$_2$Cl$_2$ (60 mL), was added imidazole (1.85 g, 27.25 mmol) and TBSOCl (3.71 g, 24.77 mmol) at 0 °C. The reaction was stirred for 30 min at the same temperature. After completion of the reaction (monitored by TLC), it was quenched with water (50 mL) and extracted with CH$_2$Cl$_2$ (3 x 50 mL). The combined organic layer was dried over anhydrous Na$_2$SO$_4$ and the solvent removed under reduced pressure. The crude mass was purified by silica gel column chromatography (ethyl acetate/hexane = 2:5) to afford 23 (7.75 g, 96%) as a colorless liquid. [a]$_D^{25}$ +15.9 (c 1.0, CHCl$_3$); IR ( neat): 3343, 3069, 2936, 2861, 1723, 1450, 1278 cm$^{-1}$; $^1$H NMR (500 MHz, CDCl$_3$): $\delta$ 7.36–7.31 (m, 5H), 5.94 (m, 1H), 5.21–5.06 (m, 2H), 4.65 (d, J = 11.0 Hz, 1H), 4.52 (d, J = 11.0 Hz, 1H), 3.77 (q, J = 6.6 Hz, 1H), 3.72–3.66 (m, 2H), 3.53 (q, J = 6.6 Hz, 1H), 2.54–2.39 (m, 2H), 0.91 (s, 9H), 0.08 (s, 6H) ppm; $^13$C NMR (75 MHz, CDCl$_3$): $\delta$ 138.4, 134.7, 128.3, 127.8, 127.6, 117.2, 78.7, 72.4, 72.1, 63.6, 34.7, 25.8, 18.2, –5.4 ppm; HRMS (ESI): calcd. for C$_{18}$H$_{32}$O$_3$Si [M + Na$^+$] 359.2012, found: 359.2010.
2R,3S)-2,3-Bis(benzyloxy)hex-5-en-2-yl[(tert-butyl)dimethylsilane (24): To a suspension of NaH (60%) in mineral oil, 1.6 g, 40.07 mmol) in dry THF (40 mL), was added alcohol 23 (6.85 g, 20.38 mmol) in THF (30 mL) at 0 °C. The suspension was stirred for 1 h at room temperature. The benzyl bromide (3.6 mL, 30.57 mmol) was added slowly to the above reaction mixture at 0 °C. The reaction mixture was stirred at room temperature for 4 h and quenched with water at 0 °C. The reaction mixture was extracted with ethyl acetate (2 x 100 mL). The combined organic layer was washed with brine solution (75 mL), dried over anhydrous Na2SO4 and the solvent removed under reduced pressure. The residue was purified by silica gel column chromatography (ethyl acetate/hexane = 1:19) to give 24 (7.9 g, 91%) as a light-yellow liquid. [α]D26.3 = 2.1 (c 1.3, CHCl3); IR (neat): 3066, 3032, 2953, 2858, 1732, 1641, 1458, 1254 cm−1; 1H NMR (300 MHz, CDCl3): δ 7.42-7.23 (m, 5H), 5.88 (m, 1H), 5.15-5.01 (m, 2H), 4.78-4.55 (m, 4H), 3.87-3.74 (m, 2H), 3.66 (m, 1H), 3.59 (m, 1H), 2.43 (t, J = 6.0 Hz, 2H), 0.96 (s, 9H), 0.05 (s, 6H) ppm; 13C NMR (75 MHz, CDCl3): δ 138.7, 138.5, 135.2, 128.1, 127.7, 127.6, 127.4, 127.3, 116.8, 80.7, 78.4, 72.6, 72.1, 62.7, 34.9, 25.9, 18.2, 5.4 ppm; HRMS (ESI): calcld. for C32H36O5NaSi [M + Na]+ 549.2482 found: 549.2469.

5-((4S,5R,E)-4,5-Bis(benzyloxy)-6-(tert-butyldimethylsilyloxy)-1-ethyl-1-enyl)-7-methoxy-2,2-dimethyl-4H-benzof[1,3]dioxin-4-one (25): To a stirred solution of the compound 24 (2.2 g, 5.16 mmol) in 1,4-dioxane (25 mL) was added 2.6-lutidine (2.4 mL, 20.64 mmol) and NaNO2 (4.78 g, 60.64 mmol) in water (10 mL) followed by OsO4 (0.51 mL, 0.51 mmol, 1 M solution in toluene) at room temperature. The reaction mixture was stirred under dark at room temperature for 6 h. After completion of the reaction (monitored by TLC), it was quenched with saturated aq. Na2SO4 (30 mL) solution. Organic solvent was removed under reduced pressure and the residual aqueous layer was extracted with ethyl acetate (3 x 75 mL). The combined organic layers was washed with brine (2 x 30 mL), dried over anhydrous Na2SO4 and concentrated under reduced pressure to give a colorless oil which was purified by silica gel column chromatography (ethyl acetate/hexane = 1:19) to obtain aldehyde 11 (1.88 g, 85%) as a colorless liquid which was immediately used for next step without further purification and characterization. To a stirred solution of 10 (2.36 g, 5.55 mmol) and 18-crown-6 (2.1 g, 8.2 mmol) in DME (25 mL) was added KHMD (0.5 M in toluene, 11.0 mL, 5.5 mmol) at −78 °C. After 20 min, a solution of 11 (1.57 g, 3.67 mmol) in DME (6 mL) was added and the mixture was stirred at −78 °C for 1 h. The reaction mixture was gradually warmed to −10 °C. After being stirred overnight at −10 °C, the mixture was quenched with saturated NH4Cl (30 mL) and extracted with ethyl acetate (3 x 50 mL). The extract was washed with brine (75 mL), dried over anhydrous Na2SO4 and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (ethyl acetate/hexane = 1:20) to give 25 (1.94 g, 84%) as a colorless oil. [α]D25 = 12.5 (c 1.65, CHCl3); IR (neat): 2928, 2854, 1728, 1607, 1572, 1278, 1159, 1097, 778 cm−1; 1H NMR (300 MHz, CDCl3): δ 7.75 (d, J = 15.9 Hz, 1H), 7.37-7.22 (m, 10H), 6.68 (d, J = 3.0 Hz, 1H), 6.33 (d, J = 2.3 Hz, 1H), 6.23 (m, 1H), 4.82-4.45 (m, 4H), 3.87 (dd, J = 10.6, 3.8 Hz, 1H), 3.81 (s, 3H), 3.76 (m, 1H), 3.69-3.49 (m, 2H), 2.65 (t, J = 6.0 Hz, 1H), 2.58 (t, J = 6.0 Hz, 1H), 1.69 (s, 6H), 0.90 (s, 9H), 0.06 (s, 6H) ppm; 1C NMR (75 MHz, CDCl3): δ 164.6, 160.1, 158.6, 143.9, 138.6, 131.6, 130.1, 129.9, 128.2, 127.9, 127.8, 127.5, 127.4, 108.2, 104.8, 100.1, 80.8, 78.5, 72.7, 72.1, 62.8, 55.5, 34.1, 25.9, 25.6, 18.2, −5.4 ppm; HRMS (ESI): calcld. for C32H32O7Si [M + Na]+ 565.3061, found: 565.3065.
(neat): 3448, 2925, 2854, 1725, 1606, 1575, 1458, 1378, 1283 cm⁻¹; ¹H NMR (300 MHz, CDCl₃): δ 7.53 (d, J = 15.9 Hz, 1H), 7.39-7.29 (m, 10H), 6.67 (d, J = 3.0 Hz, 1H), 6.34 (d, J = 3.0 Hz, 1H), 6.26 (m, 1H), 5.81-5.61 (m, 2H), 4.75-4.59 (m, 4H), 4.35 (m, 1H), 3.86-3.72 (m, 4H), 3.59 (m, 1H), 2.82-2.65 (m, 2H, 2.35 (t, J = 7.6 Hz, 2H), 1.70 (s, 6H), 1.10 (d, J = 6.0 Hz, 3H), 0.88 (s, 9H), 0.94 (s, 6H) ppm; ¹³C NMR (75 MHz, CDCl₃): δ 164.8, 160.2, 158.7, 143.9, 138.3, 137.9, 131.1, 130.9, 130.7, 130.0, 128.4, 128.3, 128.0, 127.9, 127.7, 127.6, 108.4, 104.9, 103.6, 100.1, 79.8, 74.0, 73.8, 71.7, 68.5, 55.6, 42.8, 33.7, 29.7, 25.8, 25.6, 23.4, 18.1, -4.6, -4.7 ppm; HRMS (ESI): calcld. for C₃₉H₆₀O₆Na [M + Na]⁺ 793.3696, found: 793.3697.

5-(1E,4S,5S,6R,7E,10S)-4,5-Bis(benzyloxy)-10-(tert-butyl dimethylsiloxyl)-6-(methoxy methoxy)undeca-1,7-dienyl-7-methoxy-2,2-dimethyl-4H-benzo[d][1,3]dioxin-4-one (28): To a stirred solution of compound 27 (0.855 g, 1.19 mmol) in CH₂Cl₂ (20 mL), was added disopropyl ethylamine (0.63 mL, 3.5 mmol) and stirred for 30 min at 0 °C under argon atmosphere. Methoxymethyl chloride (0.23 mL, 2.98 mmol) was added to the above reaction mixture in CH₂Cl₂ (10 mL) at same temperature. The resultant mixture was stirred at room temperature for additional 10 h. After completion of the reaction (monitored by TLC), it was quenched with water (20 mL) and extracted with CH₂Cl₂ (3 x 30 mL). The combined organic layer was dried over anhydrous Na₂SO₄ and solvent removed under reduced pressure.

The crude mass was purified by silica gel column chromatography (ethyl acetate/hexane = 2:3) to afford 29 (0.56 g, 86% yield) as a colorless liquid. [α]D25 -49.2 (c 1.7, CHCl₃); IR (neat): 2923, 2853, 1729, 1647, 1608, 1572, 1458, 1286, 1256 cm⁻¹; ¹H NMR (500 MHz, CDCl₃): δ 11.71 (s, 1H), 7.31-7.20 (m, 10H), 7.09 (d, J = 15.8 Hz, 1H), 6.38 (br s, 1H), 6.35 (d, J = 2.9 Hz, 1H), 5.89 (m, 1H), 5.79 (m, 1H), 5.61 (m, 1H), 5.12 (m, 1H), 4.80 (s, 2H), 4.63 (d, J = 6.9 Hz, 1H), 4.52-4.43 (m, 3H), 4.02 (m, 1H), 2.92 (m, 1H), 3.81 (s, 3H), 3.30 (s, 3H), 2.75-2.56 (m, 3H), 2.37 (m, 1H), 1.42 (d, J = 6.9 Hz, 3H) ppm; ¹³C NMR (75 MHz, CDCl₃): δ 171.6, 164.9, 163.9, 143.5, 138.9, 138.3, 133.2, 132.2, 128.3, 128.1, 127.7, 127.5, 127.2, 107.3, 104.1, 99.7, 92.7, 83.6, 81.3, 78.4, 73.4, 73.1, 71.8, 55.4, 55.3, 38.6, 34.9, 20.5 ppm; HRMS (ESI): calcld. for C₃₉H₆₀O₆Na [M + Na]⁺ 711.6215, found: 711.6211.

7-epi-Zeaenol (2): To a stirred solution of 29 (50 mg, 0.09 mmol) in CH₂Cl₂ (5 mL) was added TiCl₄ (1.8 mL, 18 mmol, 1 M in CH₂Cl₂) at 0 °C, and the mixture was stirred for 30 min at 0 °C. After completion of the reaction (monitored by TLC), it was quenched with a saturated solution of NaHCO₃ (5 mL), extracted with CH₂Cl₂ (2 x 10 mL) and washed with brine (15 mL). The organic layer was dried over anhydrous Na₂SO₄ and concentrated to afford a yellowish liquid, which was purified by silica gel column chromatography (acetone/hexane 1:1) to obtain compound 28 (28 mg, 88%) as a white powder. [α]D25 -87 (c 1.2, MeOH); IR (neat): 3424, 2926, 2854, 2738, 2489, 1607, 1637, 1385, 1254 cm⁻¹; ¹H NMR (500 MHz, DMSO-d₆): δ 10.56 (s, 1H), 6.64 (d, J = 15.8 Hz, 1H), 6.43 (d, J = 2.2 Hz, 1H), 6.31 (d, J = 2.2 Hz, 1H), 6.09 (m, 1H), 5.67-5.52 (m, 2H), 5.10 (m, 1H), 4.84 (s, 1H), 4.79 (s, 1H), 4.48 (d, J = 3.9 Hz, 1H), 4.05 (m, 1H), 3.74 (s, 3H), 3.58 (m, 1H), 3.45 (m, 1H), 2.48-2.31 (m, 3H), 2.17 (m, 1H), 1.31 (d, J = 5.9 Hz, 3H) ppm; ¹³C NMR (125 MHz, DMSO-d₆): δ 168.9, 161.6, 158.8, 139.5, 132.9, 132.4, 128.5, 126.9, 110.2, 102.6, 99.8, 77.4, 74.6, 72.8, 71.7, 55.2, 38.5, 36.7, 20.0 ppm; HRMS (ESI): calcld. for C₃₉H₆₀O₆Na [M + Na]⁺ 783.3890, found: 783.3890.

1,2,7,8-Tetrahydro-7-epi-zeaenol (30): The compound 2 (15...
mg, 0.04 mmol) was in MeOH (5 mL) and commercial Pd/C (10 mg, 10% w/w) was added in one portion. The resulting suspension was stirred under an atmosphere of H₂ for 24 h until complete disappearance of starting material occurred (indicated by TLC). The suspension was filtered through Celite pad and washed with ethyl acetate (10 mL). The combined filtrates were washed with brine (10 mL), dried over anhydrous Na₂SO₄ and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography (acetone/hexane = 1:1) to afford 2,7,8-tetrahydro-7-epi-Zeaenol (12 mg, 87%) as white powder. [α]D25 58.6 (c 0.5, CHCl₃); IR (neat): 3448, 2924, 2854, 1727, 1645, 1459, 1255 cm⁻¹; ¹H NMR (500 MHz, CDCl₃): δ 11.83 (s, 1H), 7.35-7.18 (m, 10H), 7.00 (m, 1H), 6.94 (d, J = 14.9 Hz, 1H), 6.59 (d, J = 15.9 Hz, 1H), 6.41 (s, 1H), 6.40 (s, 1H), 5.82 (m, 1H), 5.35 (m, 1H), 4.72-4.66 (m, 2H), 4.60-4.46 (m, 3H), 3.86-3.78 (m, 4H), 2.75-2.66 (m, 2H), 2.58-2.48 (m, 2H), 1.36 (d, J = 5.9 Hz, 3H) ppm; ¹³C NMR (75 MHz, CDCl₃): δ 199.0, 170.9, 165.4, 164.0, 143.6, 142.9, 141.8, 138.1, 137.4, 133.3, 132.9, 130.8, 129.8, 128.3, 128.2, 127.8, 127.7, 127.6, 108.6, 103.7, 100.1, 83.4, 80.3, 73.5, 71.7, 71.2, 55.4, 37.7, 35.3, 19.5 ppm; HRMS (ESI): calcd. for C₁₃H₁₂O₂Na [M + Na]⁺ 656.2202, found: 656.2190.

Zeaenol (I): To a stirred solution of 33 (45 mg, 0.08 mmol) in CH₂Cl₂ (5 mL) was added TiCl₄ (1.6 mL, 1.6 mmol, 1M in CH₂Cl₂) at 0 °C and the mixture was stirred for 30 min at 0 °C. After completion of the reaction (monitored by TLC), it was quenched with a saturated solution of NaHCO₃ (5 mL), extracted with CH₂Cl₂ (2 x 10 mL) and washed with brine (15 mL). The organic layer was dried over anhydrous Na₂SO₄ and concentrated under reduced pressure. The crude oil was purified by silica gel column chromatography (acetone/hexane = 1:1) to afford the desired compound 33 (82 mg, 82%) as a pale-yellow liquid. [α]D25 55.6, 391.2 (c 0.6, MeOH); IR (neat): 3424, 2926, 2854, 2738, 2489, 1607, 1637, 1385, 1254 cm⁻¹; ¹H NMR (500 MHz, CDCl₃): δ 11.83 (s, 1H), 7.12 (d, J = 14.9 Hz, 1H), 6.44 (d, J = 2.9 Hz, 1H), 6.39 (d, J = 2.9 Hz, 1H), 5.83 (dd, J = 11.0, 4.0 Hz, 1H), 5.70 (dd, J = 14.9, 7.9 Hz, 1H), 5.32 (m, 1H), 4.26 (t, J = 6.9 Hz, 1H), 3.98 (t, J = 6.9 Hz, 1H), 3.81 (s, 3H), 3.59 (d, J = 7.9 Hz, 1H), 2.60 (br s, 3 H), 2.55-2.25 (m, 4H), 1.46 (d, J = 6.9 Hz, 3H) ppm; ¹³C NMR
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To a solution of the desired esters (35) in MeOH (5 mL) and commercial Pd/C (10 mg, 10% w/w) was added in one portion. The resulting suspension was stirred under an atmosphere of H2 for 2 h and quenched with water solution (2 x 15 mL) followed by brine solution (15 mL), dried over anhydrous Na2SO4 and concentrated under reduced pressure.

The residue was purified by silica gel column chromatography (ethyl acetate/hexane = 1:8) to give 36 (15 mg, 0.023 mmol), MTPA (8 mg, 0.035 mmol) and DMAP (9 mg, 0.071 mmol) in CH2Cl2 (3 mL) was treated with DCC (24 mg, 0.118 mmol) and the reaction mixture was stirred for 12 h. After completion of the reaction (monitored by TLC), the reaction mixture was quenched with saturated aqueous NaHCO3. The aqueous layer was extracted with CH2Cl2. The combined organic layer was washed with brine, dried over anhydrous Na2SO4, concentrated under reduced pressure and the residue was purified by silica gel column chromatography (ethyl acetate/hexane = 1:8) to give 36 (15 mg, 0.023 mmol), MTPA (8 mg, 0.035 mmol) and DMAP (9 mg, 0.071 mmol) in CH2Cl2 (3 mL) was treated with DCC (24 mg, 0.118 mmol) and the reaction mixture was stirred for 12 h. After completion of the reaction (monitored by TLC), the reaction mixture was quenched with saturated aqueous NaHCO3. The aqueous layer was extracted with CH2Cl2.

The combined organic layer was washed with brine, dried over anhydrous Na2SO4, concentrated under reduced pressure and the residue was purified by silica gel column chromatography (ethyl acetate/hexane = 1:8) to give 36 (15 mg, 0.023 mmol), MTPA (8 mg, 0.035 mmol) and DMAP (9 mg, 0.071 mmol) in CH2Cl2 (3 mL) was treated with DCC (24 mg, 0.118 mmol) and the reaction mixture was stirred for 12 h. After completion of the reaction (monitored by TLC), the reaction mixture was quenched with saturated aqueous NaHCO3. The aqueous layer was extracted with CH2Cl2.

The combined organic layer was washed with brine, dried over anhydrous Na2SO4, concentrated under reduced pressure and the residue was purified by silica gel column chromatography (ethyl acetate/hexane = 1:8) to give 36 (15 mg, 0.023 mmol), MTPA (8 mg, 0.035 mmol) and DMAP (9 mg, 0.071 mmol) in CH2Cl2 (3 mL) was treated with DCC (24 mg, 0.118 mmol) and the reaction mixture was stirred for 12 h. After completion of the reaction (monitored by TLC), the reaction mixture was quenched with saturated aqueous NaHCO3. The aqueous layer was extracted with CH2Cl2.

The combined organic layer was washed with brine, dried over anhydrous Na2SO4, concentrated under reduced pressure and the residue was purified by silica gel column chromatography (ethyl acetate/hexane = 1:8) to give 36 (15 mg, 0.023 mmol), MTPA (8 mg, 0.035 mmol) and DMAP (9 mg, 0.071 mmol) in CH2Cl2 (3 mL) was treated with DCC (24 mg, 0.118 mmol) and the reaction mixture was stirred for 12 h. After completion of the reaction (monitored by TLC), the reaction mixture was quenched with saturated aqueous NaHCO3.
Biological evaluation Materials and methods Cell Cultures, Maintenance and Antiproliferative Evaluation: The cell lines, A549, HeLa, DU 145 and MDA MB 231 (lung, cervical, prostate and breast cancer) which were used in this study were procured from American Type Culture Collection (ATCC), United States. The synthesized test compounds were evaluated for their in vitro antiproliferative activity in these six different human cancer cell lines. A protocol of 48 h continuous drug exposure was used, and a cell proliferation assay was used to estimate cell viability or growth. All the cell lines were grown in Dulbecco’s modified Eagle’s medium (containing 10% FBS in a humidified atmosphere of 5% CO₂ at 37 °C). Cells were trypsinized when sub-confluent from T25 flasks/60 mm dishes and seeded in 96-well plates in 100 µl aliquots at plating densities depending on the doubling time of individual cell lines. The microtiter plates were incubated at 37 °C, 5% CO₂, 95% air, and 100% relative humidity for 24 h prior to addition of experimental drugs and were incubated for 48 hrs with different doses (0.01, 0.1, 1, 10, 100 µM) of prepared derivatives. After 48 hours incubation at 37 °C, cell monolayers were fixed by the addition of 10% (w/v) cold trichloroacetic acid and incubated at 4 °C for 1 h and were then stained with 0.057% SRB dissolved in 1% acetic acid for 30 min at room temperature. Unbound SRB was washed with 1% acetic acid. The protein bound dye was dissolved in 10 mM Tris base solution for OD determination at 510 nm using a microplate reader (Enspire, Perkin Elmer, USA).

After 48 hr drug incubation, the drug concentration resulting in total growth increase (as measured by SRB staining) in control cells during the drug treatment as compared to the untreated control cells was calculated at each of the drug concentrations levels. Percentage growth inhibition was calculated as:

\[ \left( \frac{\text{Ti}}{\text{Tz}} \right) \times 100 \]

for concentrations for which Ti < Tz

\[ \left( \frac{\text{Ti}}{\text{Tz}} \right) \times 100 \]

for concentrations for which Ti >/= Tz

where Ti is the dose of the experimental agent. Growth inhibition of 50 % (GI₅₀) was calculated from [(Ti/Tz)/(C-Tz)] x 100 for concentrations for which Ti > Tz and [(Ti/Tz)/Tz] x 100 for concentrations for which Ti < Tz.

Three dose response parameters were calculated for each experimental agent. Growth inhibition of 50 % (GI₅₀) was calculated from [(Ti-Tz)/(C-Tz)] x 100 = 50, which is the drug concentration resulting in a 50% reduction in the net protein increase (as measured by SRB staining) in control cells during the drug incubation. The drug concentration resulting in total growth inhibition (TGI) was calculated from Ti = Tz. The IC₅₀ (concentration of drug resulting in a 50% reduction in the measured protein at the end of the drug treatment as compared to that at the beginning) indicating a net loss of cells following treatment was calculated from [(Ti-Tz)/Tz] x 100 = −50. Values were calculated for each of these three parameters if the level of activity is reached; however, if the effect is not reached or is exceeded, the value for that parameter was expressed as greater or less than the maximum or minimum concentration tested.

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Notes and references


