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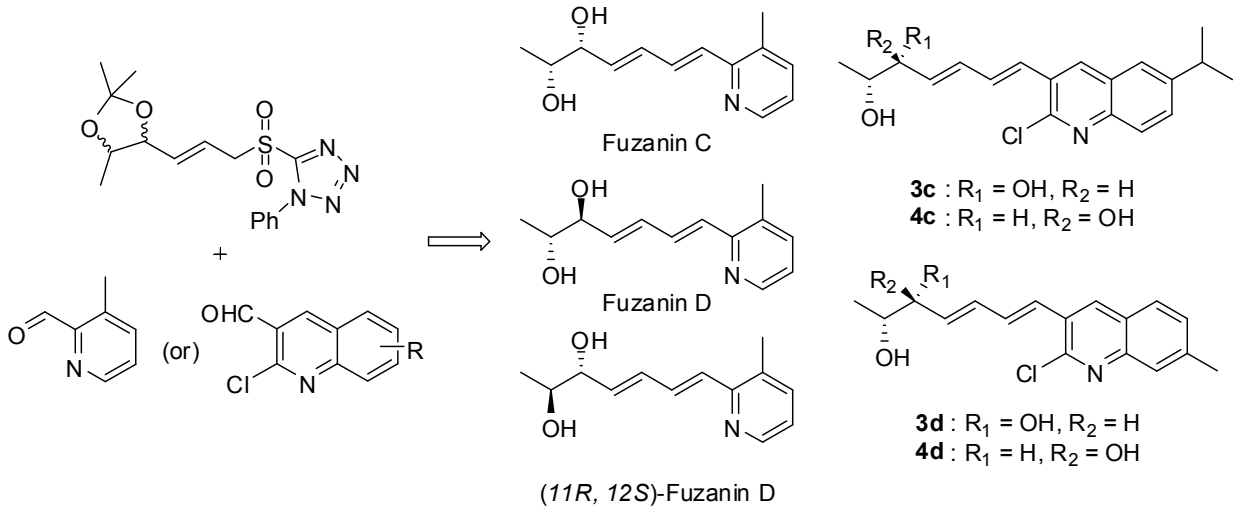
Total synthesis of fuzanins C, D and their quinoline analogues has been accomplished from readily available starting materials. Synthesis of fuzanin D described here also serves to establish its absolute configuration. All compounds were screened for anticancer activity on four cancer cell lines. Quinoline nucleus containing analogs **4d, 4c, 3c** are relatively more potent.



First total synthesis of Fuzanins C, D and their analogues as anticancer agents

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Abstract

The first total synthesis of fuzanins C and D, isolated from the culture supernatant of *Kitasatospora* sp. IFM10917, is described. Key features of this synthetic strategy involve use of Sharpless asymmetric epoxidation, dihydroxylation, Mitsunobu reaction and Julia-Kocienski olefination. The total synthesis reported herein also confirmed the absolute configuration of fuzanin D. The optical rotations of synthesized fuzanin D and natural product were opposite in sign, leading to a revision of the reported structure as its enantiomer which is further confirmed by molecular modeling studies. In addition, we also synthesized the analogues of fuzanins C, D containing quinoline nucleus. All the synthesized compounds were screened for anticancer activity on four cell lines and found to be potent against HT29 colon cancer cell lines, whereas less potent against cervical and breast cancer cell lines.

Keywords: Total synthesis, Natural products, fuzanin C, fuzanin D, Optical rotation, Molecular modeling, Anti cancer activity.

Introduction

Organic synthesis is the art of building organic molecules through chemical reactions. The synthesis of natural products is one of the most fascinating and challenging areas of research in chemistry. Further, most of the natural products have been at the spearhead of melding the fields of organic chemistry and biology, and the fields will only continue to come together in the future. Nitrogen containing heterocycles such as pyridine, quinoline derivatives are among the most omnipresent azaheterocycles found in many natural products and have been claimed to be

the most prevalent heterocycles in pharmaceutically active compounds.[1,2] Micrococcin P1, Streptonigrin and Nemerelline, Etoricoxib, Rosuvastatin and Imatinib are few examples of commercialized drugs bearing pyridine motif. The pyridine ring is also ubiquitous in agrochemicals.[3] Quinine, Chloroquine, Pamaquine, Tafenoquine, Bulaquine are some of the active pharmaceuticals containing quinoline nucleus. Quinoline derivatives exhibit broad range of biological activities such as antimalarial,[4] antimicrobial,[5] anticancer,[6] antifungal,[7] antileishmanial,[8] anti-inflammatory,[9, 10] and analgesic activity.[10]

Recently, new carbamate or pyridine containing natural products, fuzanins A (**1**), B (**2**), C (**3a**), and D (**4a**) were isolated by Ishibashi et al. [11] from the culture supernatant of *Kitasatospora* sp. IFM10917 (active strain from 323 actinomycete strains) obtained from a soil sample collected at Toyama city, Japan (Figure 1). In addition, they have also evaluated cytotoxicity against human colon carcinoma DLD-1 cells, and inhibition of Wnt signal transcription for the four compounds.

Figure 1

As part of our continuing synthetic efforts towards synthesis of biologically and pharmaceutically favored heterocycles and natural products,[12] now we became interested in synthesis of biologically active fuzanin D and its stereo isomer fuzanin C. In this regard, we report the first total synthesis of fuzanin C (**3a**), D (**4a**) along with their analogues and also confirmed the absolute configuration of fuzanin D. Some compounds are selectively potent against HT29 colon cancer cell line.

Results and discussion

Fuzanins C (**3a**) and D (**4a**) are stereoisomers and differ in stereochemistry at 11^{th} position. The compounds are similar with scaffolds, hence similar synthetic strategies were applied to synthesize them. Retrosynthesis for fuzanins C (**3a**), D (**4a**) is depicted in Scheme 1. The analysis revealed that **3a** and **4a** could be prepared efficiently by a Julia-Kocienski olefination protocol using aldehyde **5a** with sulfone **6** and **7** respectively, The intermediate **5a** could be prepared from 2, 3-dimethyl pyridine and compounds **6**, **7** from ethyl sorbate (**8**).

Scheme 1

The synthetic strategy for fuzanin C (**3a**) is described in Scheme 2. The commercially available starting material ethyl sorbate **8**, was subjected to Sharpless dihydroxylation conditions

using osmium tetraoxide as oxidant and $(DHQD)_2PHAL$ as chiral ligand, giving the diol **9**.[13, 12a] Diol **9** was protected as acetonide and the ester functionality was reduced using DIBAL-H to obtain alcohol **10**. Mitsunobu reaction of **10** with 1-phenyl-1H-tetrazole-5-thiol to get sulfide, and subsequent oxidation with H_2O_2 in presence of a molybdenum(VI) catalyst furnished the desired sulfone **6** in 82% yield. The vital coupling reaction of **6** and **5a** (**5a** prepared from SeO_2 oxidation of 2,3-dimethyl pyridine[14]) was performed by the Julia-Kocienski olefination protocol[15] using KHMDS as base to afford **11** as mixture of *E* and *Z* isomers.

The geometrical isomers i.e., *E* and *Z* isomers (7:3 ratio by ^1H NMR) were inseparable at this stage. However, final acetonide deprotection of **11** with *p*-TSA afforded fuzanin C (**3a**) and its *Z*-isomer **3b** respectively (Scheme 2). All characterization data for synthesized fuzanin C (**3a)** (Figs. S2, ESI†) were in good agreement with those reported by Ishibashi *et al*.[11]

Scheme 2

Synthetic strategy for fuzanin D (**4a**) is described in Scheme 3. This synthesis begins with selective protection of C(5)-OH group of **9** with TBS-Cl in presence of triethylamine and DMAP, provided the desired ether **12**.[16] Mitsunobu inversion of hydroxyl group in **12** with formic acid in the presence of PPh_3, DEAD and subsequent hydrolysis of formyl ester using diluted NH_4OH/MeOH afforded compound **13** in 55% overall yield.[17] TBS deprotection of **13** in presence of tetrabutylammoniumfluoride (TBAF) results *trans*-1,2-diol **14**. *Trans*-1,2-diol **14** is protected as acetonide, DIBAL-H reduction of ester functionality, Julia-Kocienski olefination protocol, and hydrolysis or deprotection (The same synthetic sequence was followed as in scheme 2) provided fuzanin D (**4a**) and its *Z*-isomer **4b** in 6:4 ratio (Scheme 3).

Scheme 3

Characterization data for the synthesized fuzanin D (**4a**) (Figs. S6, ESI†) were in good agreement with those reported by Ishibashi *et al*. except optical rotation. The $[\alpha]_D$ of the natural product is -32.9 ($CHCl_3$), and synthesized fuzanin D (**4a**) has +29.47. The original work by Ishibashi *et al*.[11] mentioned that fuzanin D as a stereoisomer to fuzanin C and further there was no attempt made to substantiate the stereochemistry of fuzanin D. The fuzanin D synthesized in this work exhibited optical rotation +29.47, whereas the sign of optical rotation is found to be opposite to the natural product reported by Ishibashi *et al*. In order to investigate the absolute configuration of fuzanin D, we further synthesized the other enantiomer of fuzanin D as given in scheme 5.

The retrosynthesis of other enantiomer fuzanin D i.e., (11*R*, 12*S*)-fuzanin D [**(11*R*, 12*S*)-4a]** is depicted in Scheme 4. We envisioned the two stereocenters in **(11*R*, 12*S*)-4a** and this could be constructed by the Sharpless asymmetric epoxidation. In this regard, the synthesis was started from 2-buten-1-ol, which was subjected to Sharpless epoxidation to afford the epoxy alcohol **18** (Scheme 5).[18]

Scheme 4

Swern oxidation of epoxy alcohol **18** and C2-Wittig homologation with [(ethoxycarbonyl)methylene]triphenyl-phosphorane, afforded requisite epoxy ester **19**.[19] The *trans* regioselective opening of epoxide of **19** was accomplished with benzyl alcohol in presence of $BF_3.OEt_2$ as lewis acid catalyst to get benzyl ether **20**,[20] which was deprotected using $AlCl_3$ to get diol **21**.[20, 21] Acetonide protection of 21, DIBAL-H reduction, Julia-Kocienski olefination protocol, and hydrolysis or deprotection (The same synthetic sequence was followed as in Scheme 2) provided **(11*R*, 12*S*)-4a** and its *Z*-isomer **(11*R*, 12*S*)-4b** in 6:4 ratio (Scheme 5) (Figs. S11, ESI†).

Scheme 5

The optical rotation of **(11*R*, 12*S*)-4a** -26.9, which is in good accordance with isolated fuzanin D. This lead unambiguously to the conclusion that the natural product isolated has opposite configuration to that of reported fuzanin D (Figure 2).

Figure 2

This observation was further corroborated by computational study. Optical rotations (at 589.3 nm) of fuzanin C (**3a**), fuzanin D (**4a**) and (11*R*, 12*S*)–fuzanin D [**(11*R*, 12*S*)-4a]** were computed using B3LYP/Aug-CC-pVDZ method for geometries obtained at B3LYP/6-31G(d) basis set (Figure 3).[22] Calculated $[\alpha]_D$ for (**3a**), (**4a**) and **(11*R*, 12*S*)-4a** are +66.5, +22.4 and -8.5 degrees$[dm(g/cm^3)]^{-1}$ respectively (Table 1). Optical rotations of computationally calculated fuzanins and synthesized fuzanins were having same sign (Table 1). The comparison of experimental and calculated optical rotations allows us to define the stereochemistry fuzanin D reported by Ishibashi *et al*. as (11*R*,12*S*)-fuzanin D (figure 2).

Table 1

Figure 3

Intrigued by this, we also synthesized different analogues (**3c**, **3d**, **4c** and **4d**) using substituted 2-chloroquinoline-3-carboxaldehydes (**5b** and **5c**)[23] by same synthetic strategy of Julia-Kocienski olefination protocol, and hydrolysis (Scheme 6) (Figs. S4-S5, S8-S9, ESI†).

Scheme 6

Furthermore, all the synthesized compounds were subjected to anticancer activity on various cell lines such as HT29 (Colon cancer), ME-180 (Cervical cancer), MCF-7 and MDA-MB-453 (Breast cancer) by employing MTT assay (details of bio assay are presented in experimental section). For comparison purpose, the cytotoxicity of salinomycin was evaluated under the same experimental conditions. The compounds that exhibiting ≥50% cell inhibition at 100 μM were considered for determination of IC_{50} value, which was calculated from the % cell viability (from control) versus concentration curves obtained after 24 h drug treatment from MTT assay, are shown in the Table 2.

Table 2

Figure 4

All compounds were found to be relatively potent against colon cancer cell line and less potent against cervical and breast cancer cell lines. Among all the compounds, quinoline derivatives of fuzanin D (**4c, 4d**) were found to be potent with an IC_{50} value of 35.3±0.83, 27.4±0.12 μM respectively. Compound **3c** has exhibited moderate activity. Remaining compounds were found to be less potent against HT-29 cell lines. The reference compound salinomycin has IC_{50} value of 20.5±1.26 μM.

Conclusion

In conclusion, we have unveiled the first stereoselective total synthesis of fuzanins C, D and their analogues. Synthesis of fuzanin D described here also serves to establish its absolute configuration. Specific optical rotations of synthesized compound and reported fuzanin D indicated opposite signs. This was confirmed by total synthesis of its enantiomer **(11*R*,12*S*)-4a**. Stereochemistry of reported fuzanin D (**4a**) should be 11*R*, 12*S* instead of 11*S*, 12*R* configuration. Molecular modeling studies also supported this observation. Further, fuzanins C, D and their analogues were screened for anticancer activity in four cancer cell lines. The compounds were found to exert cytotoxicity selectively on HT29 cancer cell lines. Quinoline nucleus containing analogues **3c**, **4c** and **4d** are relatively more potent.

Experimental Section

All reactions were carried out under an inert atmosphere unless mentioned otherwise, and standard syringe-septa techniques were followed. Solvents were freshly dried and purified by conventional methods prior to use. The progress of all reactions were monitored by TLC, using TLC aluminium backed sheets precoated with silicagel 60 F_{254} to a thickness of 0.25 mm (Merck). Column chromatography was performed on silica gel (60-120 mesh and 100-200 mesh), and EtoAc, hexane were used as eluents. Optical rotation values were measured with a Perkin-Elmer P241 polarimeter and a JASCO DIP-360 digital polarimeter, and IR spectra were recorded with a Perkin-Elmer FT-IR spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded with Varian Gemini 200 MHz, Bruker Avance 300 MHz, Varian Unity 400 MHz or Varian Inova 500 MHz spectrophotometers. TMS was used as an internal standard in $CDCl_3$. Mass spectra were recorded with a VG micromass 7070H (EI), QSTAR XL high-resolution mass spectrophotometer, a Thermo Finnigan ESI Ion trap Mass spectrophotometer.

(4R,5R,E)-ethyl 4,5-dihydroxyhex-2-enoate (9)

To a mixture of $K_3Fe(CN)_6$ (28.22 g, 85.71 mmol), K_2CO_3 (11.80 g, 85.71 mmol), and $(DHQD)_2PHAL$ (0.41 g, 0.57 mmol) in 45 mL of *t*-BuOH/H_2O (1:1), was added OsO_4 (70.56 mg, 0.28 mmol) followed by methane sulfonamide (2.71 g, 28.57 mmol) at 0 ^oC. After stirring for 15 min at 0 ^oC, ethyl sorbate **8** (4 g, 28.57 mmol) was added and stirred vigorously at 0 ^oC for 12 h, and then quenched with sat. sodium sulfite (35 mL). The reaction mixture was extracted with ethyl acetate (3 × 40 mL). The organic layer washed with 2N KOH (20 mL), brine solution and dried over Na_2SO_4. Solvent removed under *vacuo*. The crude residue was purified by silica gel column chromatography (EtOAc:Hexane, 7:3) to afford diol **9** (3.97 g, 84%) as light yellow oil; $[\alpha]_D^{25}$ +50.21 (*c* 0.1, EtOH); IR (Neat): 3396, 2924, 1704, 1371, 1281, 1036 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 6.89 (dd, 1H, *J* = 15.5, 5.2 Hz), 6.10 (dd, 1H, *J* = 15.5, 1.5 Hz), 4.17 (q, 2H, *J* = 7.5 Hz), 4.02 (brt, 1H, *J* = 5.2 Hz), 3.75-3.63 (m, 1H), 1.27 (t, 3H, *J* =7.5 Hz), 1.20 (d, 3H, *J* = 6.0 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 166.5, 146.6, 122.4, 75.6, 70.2, 60.7, 19.0, 14.1; Anal. Calcd for $C_8H_{14}O_4$ (174.19): C, 55.16; H, 8.10. Found: C, 55.15; H, 8.17.

(E)-3-((4R,5R)-2,2,5-trimethyl-1,3-dioxolan-4-yl)prop-2-en-1-ol (10)

To the solution of **9** (3 g, 17.24 mmol) in 30 mL CH_2Cl_2, 2,2-dimethoxypropane (5.37 g, 51.72 mmol), catalytic amount of *p*-TSA were added and stirred for 15 min at rt. The reaction mixture was washed with saturated aqueous $NaHCO_3$ solution (10 mL), dried over Na_2SO_4, concentrated under *vacuo*. The crude residue was purified by silica gel column chromatography

(EtOAc:Hexane, 1:9) to afford cyclic acetonide (3.46 g, 94 %) as colour less oil. To the solution cyclic acetonide (3.2 g, 14.9 mmol) in 30 mL of dry CH_2Cl_2 at 0 ^oC, DIBAL-H (21.01 mL, 37.25 mmol, 25% solution in THF) was added, and stirred for 1 h at rt. The reaction mixture was quenched by slow addition of aq. sodiumpotassiumtartate and stirred for 2 h. Organic layer was separated and aqueous layer was extracted by chloroform (20 mL), combined organic layer was dried over Na_2SO_4, solvent removed under *vacuo*. The crude residue was purified by silica gel column chromatography (EtOAc:Hexane, 6:4) to afford **10** (2.4 g, 81%) as light yellow, viscous oil; $[\alpha]_D^{24}$ +46.01 (*c* 0.1, $CHCl_3$); IR (Neat): 3395, 2923, 2851, 1449, 1259, 998 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ 5.99 (dtd, 1H, *J* = 15.5, 5.1, 0.7 Hz), 5.70 (tdd, 1H, *J* = 15.5, 7.5, 1.5 Hz), 4.19 (d, 2H, *J* = 5.1 Hz), 3.9 (t, 1H, *J* = 7.5 Hz), 3.84-3.75 (m, 1H), 1.42 (s, 3H), 1.41 (s, 3H), 1.26 (d, 3H, *J* = 6.0 Hz); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 134.2, 127.0, 108.2, 83.1, 76.5, 62.3, 27.2, 26.8, 16.3; Anal. Calcd for $C_9H_{16}O_3$ (172.10): C, 62.77; H, 9.36. Found: C, 62.58; H, 9.57.

1-phenyl-5-(((E)-3-((4R,5R)-2,2,5-trimethyl-1,3-dioxolan-4-yl)allyl)sulfonyl)-1H-tetrazole (6)

To a solution of alcohol **10** (0.6 g, 3.48 mmol) and 1-phenyl-1*H*-tetrazole-5-thiol (0.74 g, 4.15 mmol) in 20 mL THF at 0 ^oC, DIAD (0.82 mL, 4.16 mmol) and triphenyl phosphine (0.84 g, 4.15 mmol) were added, and stirred for 1 h at same temperature. The reaction was quenched by addition of saturated aqueous NH_4Cl solution, extracted with ethyl acetate (2 × 20 mL), dried over Na_2SO_4, concentrated under *vacuo*. The crude residue was purified by silica gel column chromatography (EtOAc:Hexane, 3:7) to afford sulphide (1.0 g, 90%) as colour less, viscous oil; $[\alpha]_D^{24}$ -24.80 (*c* 0.1, $CHCl_3$); IR (Neat): 2923, 1679, 1618, 1185, 1001, 741 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz): δ 7.61-7.55 (m, 5H), 6.05-5.95 (m, 1H), 5.81 (dd, 1H, *J* = 15.1, 6.7 Hz), 4.13-3.97 (m, 2H), 3.90 (t, 1H, *J* = 8.3 Hz), 3.79-3.70 (m, 1H), 1.40 (s, 3H), 1.39 (s, 3H), 1.22 (d, 3H, *J* = 6.7 Hz); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 153.3, 133.4, 132.2, 130.0, 129.6, 127.5, 123.6, 108.4, 82.5, 76.4, 34.4, 27.1, 26.7, 16.2; MS (ESI): *m/z* 355 $(M+Na)^+$; HRMS (ESI): *m/z* 355.1200 $(M+Na)^+$ (calcd for $C_{16}H_{21}N_4O_2S$ 355.1199).

To a solution of sulphide (0.50 g, 1.50 mmol) and ammonium heptamolybdate tetrahydrate (0.55 g, 0.45 mmol) in 12 mL of ethanol at 0 ^oC, H_2O_2 (30 % solution in water, 2.04 mL, 18.07 mmol) was added and stirred for 8 h at rt. The reaction mixture was poured in to saturated aqueous $NaHCO_3$ solution (40 mL) and extracted with ethyl acetate (3 × 30 mL), dried over Na_2SO_4, concentrated under *vacuo*. The crude residue was purified by silica gel column chromatography (EtOAc:Hexane, 3:7) to afford sulphone **4** (0.5 g, 92%) as colour less, viscous oil; $[\alpha]_D^{24}$ -28.70 (*c* 0.1, $CHCl_3$); IR (Neat): 2922, 1679, 1618, 1222, 741 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz): δ

7.68-7.59 (m, 5H), 5.98 (dd, 1H, *J* = 15.8, 6.0 Hz), 5.93-5.83 (m, 1H), 4.45 (m, 2H), 3.93 (dd, 1H, *J* = 8.3, 6.0 Hz), 3.77-3.68 (m, 1H), 1.41 (s, 3H), 1.37 (s, 3H), 1.22 (d, 3H, *J* = 6.0 Hz); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 152.8, 140.0, 132.8, 131.4, 129.5, 125.0, 116.6, 108.9, 82.1, 76.4, 59.0, 27.1, 26.6, 16.3; MS (ESI): *m/z* 365 $(M+H)^+$; HRMS (ESI): *m/z* 387.1093 $(M+Na)^+$ (calcd for $C_{16}H_{20}O_4N_4NaS$ 387.1097).

3-methyl-2-((3E)-4-((4R,5R)-2,2,5-trimethyl-1,3-dioxolan-4-yl)buta-1,3-dien-1-yl)pyridine (11)

To a solution of sulphone **6** (0.40 g, 1.09 mmol) in 10 mL THF at -78 ^oC, Potassium bis(trimethylsilyl)amide (0.5 M in THF solution, 2.85 mL, 1.42 mmol) was added. After 30 min of stirring at same temperature, the solution of aldehyde **5a** (0.14 g, 1.19 mmol) in 2 mL THF was added. The reaction mixture was stirred for further 1.5 h at -78 ^oC, the reaction allowed to warm to rt and was stirred for additional 2 h. Brine solution was added to the reaction mixture and extracted with ethyl acetate (3 × 10 mL), dried over Na_2SO_4, concentrated under *vacuo*. The crude residue was purified by silica gel column chromatography (EtOAc:Hexane, 1:4) to afford **11** (0.23 g, 81%) (inseparable cis, trans mixture, 3:7) as viscous, light yellow oil; IR (Neat): 2984, 2932, 1581, 1498, 1380, 1028, 858 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.45 (d, 0.3H, *J* = 4.7 Hz), 8.40 (d, 0.7H, *J* = 4.1 Hz), 7.45-7.35 (m, 2H), 7.07-7.01 (m, 1H), 6.79 (d, 0.7H, *J* = 15.1 Hz), 6.60-6.65 (m, 1.5H), 5.92-5.76 (m, 1H), 4.02 (brt, 1H, *J* = 7.5 Hz), 3.84-3.75 (m, 1.5H), 2.36 (s, 2.1H), 2.31 (s, 0.9H), 1.44 (s, 4.2H), 1.41 (s, 1.8H), 1.29-1.26 (m, 3H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 155.0, 153.4, 147.3, 146.8, 138.3, 137.8, 133.7, 132.8, 132.3, 131.7, 130.9, 129.1, 127.0, 122.3, 122.0, 116.8, 108.7, 108.5, 83.7, 77.7, 77.0, 27.5, 27.1, 19.3, 18.9, 16.8, 16.7; MS (ESI): *m/z* 260 $(M+H)^+$; HRMS (ESI): *m/z* 260.1641 $(M+H)^+$ (calcd for $C_{16}H_{22}O_2N$ 260.1645).

(2R,3R,4E,6E)-7-(3-methylpyridin-2-yl)hepta-4,6-diene-2,3-diol (3a)

To a solution of **11** (0.10 g, 0.38 mmol) in 10 mL methanol, was added *p*-toluenesulphonic acid (1.32 g, 0.77 mmol) and the reaction mixture stirred for 12 h at rt, and solvent removed under *vacuo*. The crude residue was diluted with CH_2Cl_2 and washed with saturated aqueous $NaHCO_3$ solution and the solvent removed under *vacuo*. The crude compound was purified by silica gel column chromatography (EtOAc:Hexane, 1:1) to afford fuzanin C (**3a**) (0.05 g) as viscous, colour less oil, and cis isomer **3b** (0.02 g) as viscous, colour less oil, (total yield 82%); $[\alpha]_D^{24}$ +39.89 (*c* 0.055, $CHCl_3$); IR (Neat): 3395, 2923, 1679, 1618, 772 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 8.39 (d, 1H, *J* = 4.7 Hz), 7.42 (d, 1H, *J* = 7.7 Hz), 7.37 (dd, 1H, *J* = 15.0, 11.0 Hz), 7.04 (dd, 1H, *J* = 7.7, 4.7 Hz), 6.78 (d, 1H, *J* = 15.0 Hz), 6.54 (dd, 1H, *J* = 15.3, 11.0 Hz), 5.94 (dd,

1H, *J* = 15.3, 6.9 Hz), 3.95 (t, 1H, *J* = 6.9 Hz), 3.68 (q, 1H, *J* = 6.6 Hz), 2.34 (s, 3H), 1.18 (d, 3H, *J* = 6.6 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 153.2, 146.8, 138.2, 135.7, 133.3, 132.1, 130.8, 128.0, 122.0, 77.1, 70.7, 18.8, 18.6; MS (ESI): *m/z* 220 $(M + H)^+$; HRMS (ESI): *m/z* 242.1148 $(M + Na)^+$ (calcd for $C_{13}H_{17}O_2NNa$ 242.1151).

(2R,3R,4E,6Z)-7-(3-methylpyridin-2-yl)hepta-4,6-diene-2,3-diol (3b)

Yield: 0.02 g; $[\alpha]_D^{24}$ +82.23 (*c* 0.051, $CHCl_3$); IR (Neat): 3421, 2924, 1619, 1449, 772 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 8.42 (d, 1H, *J* = 4.7 Hz), 7.45 (d, 1H, *J* = 7.3 Hz), 7.29 (dd, 1H, *J* = 15.2, 11.5 Hz), 7.06 (dd, 1H, *J* = 7.3, 4.7 Hz), 6.46 (d, 1H, *J* = 11.5 Hz), 6.38 (t, 1H, *J* = 11.5 Hz), 5.87 (dd, 1H, *J* = 15.2, 6.6 Hz), 3.93 (t, 1H, *J* = 6.6 Hz), 3.66 (quin, 1H, *J* = 6.2 Hz), 2.31 (s, 3H), 1.17 (d, 3H, *J* = 6.2 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 154.5, 146.2, 137.8, 136.9, 132.9, 131.9, 129.2, 126.0, 121.8, 76.7, 70.4, 19.0, 18.8; MS (ESI): *m/z* 220 $(M + H)^+$; HRMS (ESI): *m/z* 220.1329 $(M + H)^+$ (calcd for $C_{13}H_{18}O_2N$ 220.1332).

(2R,3R,4E,6E)-7-(2-chloro-6-isopropylquinolin-3-yl)hepta-4,6-diene-2,3-diol (3c)

According to the procedure **3a**, the sulphone **6** (0.10 g, 0.27 mmol) and aldehyde **5b** (0.07 g, 0.32 mmol), gave cyclic acetonide (inseparable cis, trans mixture, 6:94 by NMR) as light yellow oil. Cyclic acetonide dissolved in 10 mL of methanol, catalytic amount of *p*-TSA was added and stirred for 30 min at rt, and solvent removed under *vacuo*. The crude residue was diluted with CH_2Cl_2 and washed with saturated aqueous $NaHCO_3$ solution and the solvent removed under *vacuo*. The crude compound was purified by silica gel column chromatography (EtOAc:Hexane, 1:1) to afford compound **3c** (0.06 g, 70%, for two steps) as white solid; mp 120-122 ^{o}C; $[\alpha]_D^{24}$ -89.56 (*c* 0.022, $CHCl_3$); IR (KBr): 3409, 2963, 2926, 1584, 1047, 768 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 8.19 (s, 1H), 7.89 (d, 1H, *J* =9.0 Hz), 7.60-7.56 (m, 2H), 6.98 (d, 1H, *J* =15.9 Hz), 6.83 (dd, 1H, *J* = 15.9, 10.9 Hz), 6.59 (dd, 1H, *J* = 14.9, 10.9 Hz), 5.91 (dd, 1H, *J* = 14.9, 5.9 Hz), 4.01 (t, 1H, *J* = 5.9 Hz), 3.73 (quin, 1H, *J* = 5.9 Hz), 3.07 (sep, 1H, *J* = 6.9 Hz), 1.33 (d, 6H, *J* = 6.9 Hz), 1.24 (d, 3H, *J* = 5.9 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 149.0, 148.0, 145.5, 134.7, 133.3, 132.3, 131.9, 130.3, 129.5, 127.8, 127.6, 127.3, 123.5, 77.0, 70.8, 34.0, 23.6, 19.0; MS (ESI): *m/z* 332 $[M + H]^+$; HRMS (ESI): *m/z* 332.1410 $(M + H)^+$ (calcd for $C_{19}H_{23}O_2NCl$ 332.1411).

(2R,3R,4E,6E)-7-(2-chloro-7-methylquinolin-3-yl)hepta-4,6-diene-2,3-diol (3d)

According to the procedure **3c**, the sulphone **6** (0.10 g, 0.27 mmol) and requisite aldehyde **5c** (0.07 g, 0.35 mmol) gave the compound **3d** (0.06 g, 71%, for two steps) as white solid; mp 130-131 ^{o}C; $[\alpha]_D^{24}$ -91.18 (*c* 0.024, $CHCl_3$); IR (KBr): 3397, 2925, 1623, 1049, 756 cm^{-1}; ^{1}H NMR

($CDCl_3$, 500 MHz): δ 8.19 (s, 1H), 7.73 (br s, 1H), 7.68 (d, 1H, *J* = 8.3, Hz), 7.37 (dd, 1H, *J* = 8.3, 1.13 Hz), 6.99 (d, 1H, *J* = 15.5 Hz), 6.83 (dd, 1H, *J* = 15.5, 10.3 Hz), 6.60 (dd, 1H, *J* = 15.3, 10.3 Hz), 5.91 (dd, 1H, *J* = 15.3, 6.8 Hz), 4.01 (t, 1H, *J* = 6.8 Hz), 3.73 (quin, 1H, *J* = 6.4 Hz), 2.54 (s, 3H), 1.24 (d, 3H, *J* = 6.4 Hz)); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 149.8, 146.9, 141.0, 134.5, 133.3, 132.4, 131.7, 129.5, 128.8, 127.8, 127.2, 127.1, 125.4, 77.1, 70.8, 21.9, 19.0; MS (ESI): *m/z* 304 $(M + H)^+$; HRMS (ESI): *m/z* 304.1096 $[M+H]^+$ (calcd for $C_{17}H_{19}O_2NCl$ 304.1098).

(4R,5R,E)-ethyl 5-((tert-butyldimethylsilyl)oxy)-4-hydroxyhex-2-enoate (12)

To a solution of the diol **9** (3.0 g, 17.5 mmol) in CH_2Cl_2 (30 mL) at 0 °C, Et_3N (4.1 mL, 29.8 mmol), DMAP (1.06 g, 0.88 mmol) and TBSCl (3.96 g, 26.3 mmol) were added simultaneously, and the mixture was stirred for 24 h at rt. The reaction mixture was diluted with CH_2Cl_2, successively washed with 10% aq. HCl and sat. $NaHCO_3$, dried over Na_2SO_4, filtered, and concentrated in *vacuo*. The resulting oil was purified by silicagel column chromatography (EtOAc:Hexane, 1:4) to afford the TBS ether (**12**) (3.29 g, 65%) as a colorless oil; $[\alpha]_D^{25}$ -0.71 (*c* 0.051, EtOH); IR (Neat): 3475, 2932, 2858, 1721, 1257, 838 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 6.88 (dd, 1H, *J* = 15.8, 4.5 Hz), 6.10 (dd, 1H, *J* = 15.8, 1.8 Hz), 4.19 (q, 2H, *J* = 7.1 Hz), 4.02-3.98 (m, 1H), 3.79-3.71 (m, 1H), 2.57 (brs, 1H), 1.29 (t, 3H, *J* = 7.1 Hz), 1.20 (d, 3H, *J* = 6.0 Hz), 0.88 (s, 9H), 0.07 (s, 3H), 0.05 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 166.2, 147.2, 121.9, 75.2, 71.0, 60.3, 25.7, 20.1, 17.9, 14.2, -4.3, -4.9; MS (ESI): *m/z* 289 $(M + H)^+$.

(4S,5R,E)-ethyl 5-((tert-butyldimethylsilyl)oxy)-4-hydroxyhex-2-enoate (13)

Under inert atmosphere, to a solution of PPh_3 (0.62 g, 2.38 mmol) in 5 mL benzene at 0 °C, DEAD (0.16 mL, 2.38 mmol), TBS ether (**12**) (0.274 g, 0.95 mmol) and formic acid (0.11 mL, 2.85 mmol) were added simultaneously and the mixture was stirred for 2 h at rt. The solvent was evaporated in *vacuo*, and the crude residue was dissolved in NH_4OH/MeOH (1:1, 3.0 mL) at 0 °C and stirred for 3 h at rt. This reaction mixture was diluted with Et_2O, successively washed with 10% HCl solution and sat. $NaHCO_3$, dried over Na_2SO_4, and concentrated in *vacuo*. The resulting oil was purified by silica gel column chromatography (EtOAc:Hexane, 1:4) to afford alcohol **13** (0.15 g, 55%) as a colorless oil; $[\alpha]_D^{25}$ -27.1 (*c* 0.051, EtOH); IR (Neat): 3466, 2930, 2857, 1720, 1259, 836 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 6.87 (dd, 1H, *J* = 15.6, 4.6 Hz), 6.08 (dd, 1H, *J* = 15.6, 2.3 Hz), 4.24-4.21 (m, 1H), 4.19 (q, 2H, *J* = 7.0 Hz), 3.93-3.88 (m, 1H), 2.43 (brs, 1H), 1.28 (t, 3H, *J* = 7.0 Hz), 1.08 (d, 3H, *J* = 6.2 Hz), 0.89 (s, 9H), 0.07 (s, 6H); ^{13}C NMR

($CDCl_3$, 70 MHz): δ 166.3, 145.5, 121.7, 74.8, 70.7, 60.3, 25.7, 17.9, 17.7, 14.2, -4.4, -4.9; MS (ESI): *m/z* 289 $(M + H)^+$.

(4S,5R,E)-ethyl 4,5-dihydroxyhex-2-enoate (14)

To a solution of **13** (0.5 g, 1.73 mmol) in 10 mL THF at 0 °C, was added TBAF (1.0 M, 5.2 mL, 5.19 mmol) and the resulting mixture was stirred for 1 h. The reaction was quenched with water (25 mL) and extracted with CH_2Cl_2 (2 x 25 mL). The combined organic extracts were dried over Na_2SO_4, concentrated in *vacuo*. The crude reside was purified by silica gel column chromatography to yield Diol **14** (0.25 g, 85%); $[\alpha]_D^{24}$ -9.15 (*c* 0.047, $CHCl_3$); IR (Neat): 3429, 2981, 2931, 1714, 1659, 1278, 984 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ 6.94 (dd, 1H, *J* = 15.1, 4.5 Hz), 6.11 (d, 1H, *J* = 15.1 Hz), 4.30 (brs, 1H), 4.20 (q, 2H, *J* = 6.8 Hz), 3.99-3.91 (m, 1H), 1.28 (t, 3H, *J* = 6.8 Hz), 1.16 (d, 3H, *J* = 6.0 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 166.6, 145.8, 122.37, 74.5, 69.9, 60.6, 17.4, 14.1; Anal. Calcd for $C_8H_{14}O_4$ (174.19): C, 55.16; H, 8.10. Found: C, 55.19; H, 8.14.

(E)-3-((4S,5R)-2,2,5-trimethyl-1,3-dioxolan-4-yl)prop-2-en-1-ol (15)

According to the procedure **10**, compound **14** (2.4 g, 13.79 mmol) gave alcohol **15** (1.90 g, 80%); $[\alpha]_D^{24}$ -15.1 (*c* 0.1, $CHCl_3$); IR (Neat): 3431, 2984, 1380, 1219, 772 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz): δ 5.90 (td, 1H, *J* = 15.4, 5.1 Hz), 5.68 (tdd, 1H, *J* = 15.4, 7.7, 1.5 Hz), 4.52 (brt, 1H, *J* = 7.7 Hz), 4.32 (quin, 1H, *J* = 6.4 Hz), 4.17 (dd, 2H, *J* = 5.1, 1.5 Hz), 1.47 (s, 3H), 1.35 (s, 3H), 1.13 (d, 3H, *J* = 6.4 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 133.5, 127.1, 108.0, 79.0, 74.0, 62.8, 28.2, 25.5, 16.1; Anal. Calcd for $C_9H_{16}O_3$ (172.10): C, 62.77; H, 9.36. Found: C, 62.61; H, 9.59.

1-phenyl-5-(((E)-3-((4S,5R)-2,2,5-trimethyl-1,3-dioxolan-4-yl)allyl)sulfonyl)-1H-tetrazole (7)

According to the procedure **6**, the alcohol **15** (0.5 g, 2.9 mmol) gave sulphone **7** (0.86 g, 82%); $[\alpha]_D^{24}$ -3.14 (*c* 0.05, $CHCl_3$); IR (Neat): 2922, 1679, 1618, 1524, 1347, 741 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ 7.68-7.56 (m, 5H), 5.96 (dd, 1H, *J* = 15.8, 6.8 Hz), 5.86-5.76 (m, 1H), 4.54-4.38 (m, 3H), 4.32 (dd, 1H, *J* = 12.8, 6.7 Hz), 1.45 (s, 3H), 1.34 (s, 3H), 1.03 (d, 3H, *J* = 6.7 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 152.9, 140.3, 132.9, 131.4, 129.6, 125.1, 116.1, 108.5, 78.1, 73.8, 59.0, 28.0, 25.3, 15.8; MS (ESI): *m/z* 365 $(M+H)^+$; HRMS (ESI): *m/z* $[M+H]^+$ 387.1094 $(M+Na)^+$ (calculated for $C_{16}H_{20}N_4O_4NaS$ 387.1097).

3-methyl-2-((3E)-4-((4S,5R)-2,2,5-trimethyl-1,3-dioxolan-4-yl)buta-1,3-dien-1-yl)pyridine (16)

According to the procedure **11**, the sulphone **6** (0.38 g, 1.04 mmol) and **5a** (0.13 g, 1.14 mmol), gave **16** (0.22 g, 81%) (inseparable cis, trans mixture, 4:6) as colourless, viscous oil; IR (Neat): 2923, 2851, 1667, 1382, 1048, 771 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ 8.45 (d, 0.4H, *J* = 4.5 Hz), 8.42 (d, 0.6H, *J* = 4.5 Hz), 7.48-7.36 (m, 2H), 7.08-7.02 (m, 1H), 6.81 (d, 0.6H, *J* =15.1 Hz) 6.56-6.39 (m, 1.6H), 5.96-5.81 (m, 1H), 4.65-4.59 (m, 1H), 4.41-4.30 (m, 1H), 2.36 (s, 1.8H), 2.30 (s, 1.2H), 1.53 (s, 1.8H), 1.50 (s, 1.2H), 1.39 (s, 1.8H), 1.36 (s, 1.2H), 1.16 (t, 3H, *J* = 6.0 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 154.7, 153.2, 146.9, 146.4, 138.1, 137.6, 133.3, 132.8, 132.6, 132.3, 130.9, 128.3, 126.3, 122.0, 121.7, 108.0, 107.9, 79.4, 79.1, 74.37, 74.32, 28.2, 25.4, 19.0, 18.7, 16.17; MS (ESI): *m/z* 260 $(M+H)^+$; HRMS (ESI): *m/z* 260.1640 $(M+H)^+$ (calcd for $C_{16}H_{22}O_2N$ 260.1645).

(2R,3S,4E,6E)-7-(3-methylpyridin-2-yl)hepta-4,6-diene-2,3-diol (4a)

According to the procedure **3a**, compound **16** (0.1 g, 0.38 mmol) gave fuzanin D (**4a**) (0.05 g) as viscous, colour less oil, and cis isomer **4b** (0.02 g) as viscous, colour less oil, (total yield 82%); $[\alpha]_D^{24}$ +29.47 (*c* 0.045, $CHCl_3$); IR (Neat): 3424, 2924, 2854, 1621, 1458, 1078, 771 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz): δ 8.40 (d, 1H, *J* = 4.1 Hz), 7.45-7.36 (m, 2H), 7.04 (dd, 1H, *J* = 7.2, 4.1 Hz), 6.79 (d, 1H, *J* = 14.5 Hz), 6.54 (dd, 1H, *J* = 14.5, 10.4 Hz), 6.01 (dd, 1H, *J* = 14.5, 6.2 Hz), 4.20 (dd, 1H, *J* = 6.2, 4.1 Hz), 3.90 (dd, 1H, *J* = 6.2, 4.1 Hz), 2.34 (s, 3H), 1.16 (d, 1H, *J* = 6.2 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 153.3, 146.9, 138.2, 134.6, 133.2, 132.3, 130.7, 128.1, 122.0, 75.9, 70.3, 18.7, 17.6; MS (ESI): *m/z* 242 $(M + Na)^+$; HRMS (ESI): *m/z* 242.1148 $(M + Na)^+$ (calcd for $C_{13}H_{17}O_2NNa$ 242.1151).

(2R,3S,4E,6Z)-7-(3-methylpyridin-2-yl)hepta-4,6-diene-2,3-diol (4b)

Yield: (0.02 g, 90% overall yield); $[\alpha]_D^{24}$ +59.15 (*c* 0.04, $CHCl_3$); IR (Neat): 3405, 2924, 1449, 995 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz): δ 8.45 (d, 1H, *J* = 5.2 Hz), 7.46 (d, 1H, *J* = 7.2 Hz), 7.39 (dd, 1H, *J* = 15.6, 11.4 Hz), 7.07 (dd, 1H, *J* = 7.2, 5.2 Hz), 6.52 (d, 1H, *J* =11.4 Hz), 6.44 (t, 1H, *J* = 11.4 Hz), 5.97 (dd, 1H, *J* = 15.6, 7.2 Hz), 4.18 (dd, 1H, *J* = 7.27, 4.1 Hz), 3.9 (dd, 1H, *J* = 6.2, 4.1 Hz), 2.31 (s, 3H), 1.16 (d, 3H, *J* = 6.2 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 154.5, 146.2, 138.0, 135.7, 133.1, 132.0, 129.9, 125.9, 121.9, 75.9, 70.1, 19.0, 17.7; MS (ESI): *m/z* 220 $(M + H)^+$; HRMS (ESI): *m/z* 220.1329 $(M + H)^+$ (Calcd for $C_{13}H_{18}O_2N$ 220.1332).

(2R,3S,4E,6E)-7-(2-chloro-6-isopropylquinolin-3-yl)hepta-4,6-diene-2,3-diol (4c)

According to the procedure **3c**, the sulphone **7** (0.10 g, 0.27 mmol) and aldehyde **5b** (0.07 g, 0.32 mmol), gave compound **4c** (0.07 g, 70% overall yield); mp 121-122 ^{o}C; $[\alpha]_D^{24}$ -24.0 (*c* 0.03, $CHCl_3$); IR (KBr): 3423, 2962, 2924, 1585, 1046, 757 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 8.22 (S, 1H), 7.90 (d, 1H, *J* = 8.9 Hz), 7.60-7.58 (m, 2H), 7.01 (d, 1H, *J* = 15.9 Hz), 6.87 (dd, 1H, *J* = 15.9, 6.9 Hz), 6.59 (dd, 1H, *J* = 15.9, 9.8 Hz), 5.99 (dd, 1H, *J* = 15.9, 6.7 Hz), 4.24 (dd, 1H, *J* = 6.7, 4.5 Hz), 3.98-3.94 (m, 1H), 3.08 (sep, 1H, *J* = 6.9 Hz), 1.34 (d, 6H, *J* = 6.9 Hz), 1.20 (d, 3H, *J* = 6.9 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 149.1, 148.0, 145.6, 133.4, 133.3, 132.6, 132.0, 130.3, 129.6, 128.0, 127.8, 127.4, 123.5, 75.9, 70.3, 34.0, 23.7, 17.7; MS (ESI): *m/z* 332 $(M + H)^+$; HRMS (ESI): *m/z* 332.1409 $(M + H)^+$ (calcd for $C_{19}H_{23}O_2NCl$ 332.1411).

(2R,3S,4E,6E)-7-(2-chloro-7-methylquinolin-3-yl)hepta-4,6-diene-2,3-diol (4d)

According to the procedure **3c**, the sulphone **7** (0.10 g, 0.27 mmol) and aldehyde **5c** (0.06 g, 0.32 mmol) gave compound **4d** (0.07 g, 70% overall yield); mp 134-136 ^{o}C; $[\alpha]_D^{24}$ -75.14 (*c* 0.03, $CHCl_3$); IR (Neat): 3418, 2922, 1622, 1337, 986 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 8.20 (s, 1H), 7.73 (brs, 1H), 7.68 (d, 1H, *J* = 8.3 Hz),7.36 (d, 1H, *J* = 8.3 Hz), 6.99 (d, 1H, *J* = 15.1 Hz), 6.84 (dd, 1H, *J* = 15.1, 10.5 Hz), 6.57 (dd, 1H, *J* = 15.1, 10.5 Hz), 5.98 (dd, 1H, *J* = 15.1, 6.7 Hz), 4.24 (dd, 1H, *J* = 6.7, 3.0 Hz), 4.01-3.92 (m, 1H), 2.54 (s, 3H), 1.20 (d, 3H, *J* = 6.7 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 149.9, 147.0, 141.0, 133.3, 132.6, 131.8, 129.5, 128.8, 127.8, 127.7, 127.2, 127.1, 126.0, 75.9, 70.3, 21.9, 17.7; MS (ESI): *m/z* 304 $(M+H)^+$; HRMS (ESI): *m/z* 304.1097 $(M+H)^+$ (calculated for $C_{17}H_{18}N_3O_6$ 304.1098).

(E)-ethyl 3-((2S,3S)-3-methyloxiran-2-yl)acrylate (19)

To a solution of oxalyl chloride (0.87 mL, 9.96 mmol) in 30 mL of CH_2Cl_2 at -78 ^{o}C, DMSO (1.41 mL, 19.92 mmol) was added dropwise. The solution was stirred for 10 min and a solution of epoxy alcohol **18** (0.73 mg, 8.3 mmol) in 20 mL of CH_2Cl_2 was added. After 20 min at same temperature, triethylamine (3.47 mL, 24.7 mmol) was added. After 5 min, the reaction mixture of was allowed to warm to rt during 30 min. A solution of (carbethoxymethylene) triphenylphosphorane (7.63 g, 20.8 mmol) in 10 mL of CH_2Cl_2 was added and the reaction mixture was stirred for 24 h. Reaction mixture was quenched with aq. NH_4Cl, and extracted with CH_2Cl_2 (2 × 10 mL). The combined organic layer was dried over Na_2SO_4, crude residue was purified by silica gel column chromatography (EtOAc:Hexane, 1:4) to afford epoxide **19** (0.55 g, 56 %); $[\alpha]_D^{24}$ -15.54 (*c* 0.04, $CHCl_3$); IR (Neat): 2927, 1721, 1455, 1272, 1178, 772 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 6.67 (dd, 1H, *J* = 15.6, 7.0 Hz), 6.12 (d, 1H, *J* = 15.6 Hz), 4.20 (q,

2H, *J* = 7.17 Hz), 3.18 (dd, 1H, *J* = 8.7, 7.0 Hz), 2.97 (qd, 1H, *J* = 5.1, 2.07 Hz), 1.39 (d, 3H, *J* = 5.1 Hz), 1.28 (t, 3H, *J* = 7.17 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 165.5, 144.5, 123.5, 60.4, 57.2, 57.1, 17.3, 14.0; Anal. Calcd for $C_8H_{12}O_3$ (156.07): C, 61.52; H, 7.74. Found: C, 61.62; H, 7.82.

(4R,5S,E)-ethyl 4-(benzyloxy)-5-hydroxyhex-2-enoate (20)

To a solution of epoxide ester **19** (3.14 g, 20.15 mmol), benzyl alcohol (4.19 mL, 40.3 mmol) in CH_2Cl_2 (40 ml) at -20°C, $BF_3.OEt_2$ (2.5 mL, 40.3 mmol) was added and whole mixture was stirred at rt for 1 h, then diluted with H_2O and extracted with ether. The ether layer was washed with saturated brine and dried over Na_2SO_4. The organic layer was concentrated under *vacuo*. Crude residue was purified by silica gel column chromatography (EtOAc:Hexane, 3:2) to afford **20** (3.03 g, 60%) as colorless oil; $[\alpha]_D^{25}$ -51.51 (*c* 1.0, $CHCl_3$); IR (Neat): 3508, 2983, 1721, 1264, 836 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.40-7.30 (m, 5H), 6.91 (dd, 1H, *J* = 15.8, 6.8 Hz), 6.08 (d, 1H, *J* = 15.8 Hz), 4.66 (d, 1H, *J* = 11.3 Hz), 4.42 (d, 1H, *J* = 11.3), 4.23 (q, 2H, *J* = 7.5 Hz), 4.01-3.90 (m, 2H), 1.31 (t, 3H, *J* = 7.5 Hz), 1.16 (d, 3H, *J* = 6.0 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 165.7, 143.8, 137.6, 128.4, 127.8, 127.7, 124.7, 81.9, 71.3, 69.2, 60.6, 17.9, 14.2; Anal. Calcd for $C_{15}H_{20}O_4$ (264.13): C, 68.16; H, 7.63. Found: C, 68.22; H, 7.69.

(4R,5S,E)-ethyl 4,5-dihydroxyhex-2-enoate (21)

To a well-stirred solution of $AlCl_3$ (0.99 g, 7.5 mmol) in 10 mL CH_2Cl_2 at 0 °C was added a solution of **20** (0.40 g, 1.5 mmol) in 5 mL m-xylene and the reaction mixture was stirred for 15 min at the same temperature. The reaction mixture was poured in ice cold water and extracted with ether. The ether layer was washed with saturated NaCl solution and dried over Na_2SO_4. The ether layer was concentrated under *vacuo*. Crude residue was purified by silica gel column chromatography (EtOAc:Hexane, 2:1) to give **21** (0.29 g, 76%) as a colorless viscous oil; $[\alpha]_D^{25}$ +9.01 (*c* 0.09, $CHCl_3$); IR (Neat): 3417, 2981, 1706, 1276 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 6.94 (dd, 1H, *J* = 15.6, 5.0 Hz), 6.11 (dd, 1H, *J* = 15.6, 1.5 Hz), 4.31-4.27 (m, 1H), 4.20 (q, 2H, *J* = 7.1 Hz), 3.98-3.90 (m, 1H), 1.29 (t, 3H, *J* = 7.1 Hz), 1.14 (d, 3H, *J* = 6.6 Hz); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 166.5, 145.8, 122.2, 74.5, 69.9, 60.5, 17.2, 14.0; Anal. Calcd for $C_8H_{14}O_4$ (174.19): C, 55.16; H, 8.10. Found: C, 55.12; H, 8.19.

(E)-3-((4R,5R)-2,2,5-(E)-3-((4R,5S)-2,2,5-trimethyl-1,3-dioxolan-4-yl)prop-2-en-1-ol (22)

According to the procedure **10**, Diol **21** (0.26 g, 1.49 mmol)) gave alcohol **22** (0.21 g, 86%) as colourless, viscous liquid; $[\alpha]_D^{24}$ +21.20 (*c* 0.1, $CHCl_3$); IR (Neat): 3422, 2985, 1376, 1217, 772 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 5.92 (td, 1H, *J* = 15.4, 5.1 Hz), 5.70 (tdd, 1H, *J* = 15.4, 7,7, 1.3 Hz), 4.53 (t, 1H, *J* = 7.7 Hz), 4.34 (quin, 1H, *J* = 6.4 Hz), 4.19 (m, 2H), 1.49 (s, 3H), 1.36 (s,

3H), 1.15 (d, 3H, *J* = 6.4 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 133.5, 127.1, 108.0, 79.0, 74.0, 62.8, 28.2, 25.5, 16.1.

1-phenyl-5-(((E)-3-((4R,5S)-2,2,5-trimethyl-1,3-dioxolan-4-yl)allyl)sulfonyl)-1H-tetrazole (17)

According to the procedure **6**, compound **22** (0.1 g, 0.58 mmol) gave sulphone **17** (0.17 g, 81%); $[\alpha]_D^{24}$ +1.12 (*c* 0.1, $CHCl_3$); IR (Neat): 2984, 1497, 1347, 1153, 763 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.69-7.56 (m, 5H), 5.95 (dd, 1H, *J* = 15.1, 6.0 Hz), 5.85-5.75 (m, 1H), 4.52-4.42 (m, 3H), 4.32 (t, 1H, *J* = 6.7 Hz), 1.44 (s, 3H), 1.33 (s, 3H), 1.02 (d, 3H, 6.7 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 152.9, 140.3, 131.4, 129.6, 125.1, 116.1, 108.5, 78.0, 73.8, 59.0, 28.0, 25.3, 15.8; MS (ESI): *m/z* 364 $(M+H)^+$; HRMS (ESI): *m/z* 387.1093 $[M+Na]^+$ (calculated for $C_{16}H_{20}N_4O_4NaS$ 387.1097).

(2S,3R,4E,6E)-7-(3-methylpyridin-2-yl)hepta-4,6-diene-2,3-diol (11*R*, 12*S*)-4a

According to the procedure **3a**, compound **23** (0.05 g, 0.19 mmol) gave **(11*R*, 12*S*)-4a** (0.02 g), and cis isomer **(11*R*, 12*S*)-4b** (0.01 g) as viscous, color less oils, (total yield 82%); $[\alpha]_D^{24}$ -26.9 (*c* 0.04, $CHCl_3$); IR (Neat): 3414, 2924, 2853, 1731, 1617, 995 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 8.42 (d, 1H, *J* = 4.3 Hz), 7.49-7.40 (m, 2H), 7.06 (dd, 1H, *J* = 7.5, 4.3 Hz), 6.80 (d, 1H, *J* = 15.1 Hz), 6.56 (dd, 1H, *J* = 15.1, 11.1 Hz), 6.02 (dd, 1H, *J* = 15.4, 6.2 Hz), 4.23 (dd, 1H, *J* = 6.2, 3.3 Hz), 3.93 (dd, 1H, *J* = 6.2, 3.3 Hz), 2.36 (s, 3H), 1.16 (d, 3H, *J* = 6.2 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 153.2, 146.9, 138.2, 134.3, 133.0, 132.4, 130.8, 128.2, 122.1, 75.9, 70.2, 18.7, 17.6; MS (ESI): *m/z* 220 $(M+H)^+$; HRMS (ESI): *m/z* 220.1327 $[M+H]^+$ (calculated for $C_{13}H_{18}O_2N$ 220.1332).

(2S,3R,4E,6Z)-7-(3-methylpyridin-2-yl)hepta-4,6-diene-2,3-diol (11*R*, 12*S*)-4b

Yield: 0.01 g, as viscous, colour less oil; $[\alpha]_D^{24}$ -59.6 (*c* 0.03, $CHCl_3$); IR (Neat): 3419, 2924, 1628, 1457, 762 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 8.43 (d, 1H, *J* = 4.0 Hz), 7.46 (d, 1H, *J* = 7.1 Hz), 7.30 (dd, 1H, *J* = 15.3, 11.2 Hz), 7.07 (dd, 1H, *J* = 7.1, 4.0 Hz), 6.50 (d, 1H, *J* = 11.2 Hz), 6.43 (t, 1H, *J* = 11.2 Hz), 5.97 (dd, 1H, *J* = 7.1, 15.3 Hz), 4.16 (dd, 1H, *J* = 6.1, 4.0 Hz), 3.89 (dd, 1H, *J* = 4.0, 6.1 Hz), 2.30 (s, 3H), 1.15 (d, 3H, *J* = 6.1 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 154.6, 146.4, 137.8, 135.3, 132.8, 130.2, 128.5, 126.3, 121.9, 76.0, 70.1, 19.0, 17.8; Mass (ESI-MS): *m/z* 220 $(M+H)^+$; HRMS (ESI): *m/z* 220.1327 $(M+H)^+$ (calculated for $C_{13}H_{18}O_2N$ 220.1332).

Molecular Modeling

The calculations of this investigation have been carried out by following the procedure given below. Fuzanin C (**3a**), fuzanin D (**4a**) and **(11*R*, 12*S*)–4a** were considered for computational prediction of optical rotations. Initially, geometry optimization was performed at B3LYP/6-31G (d) basis set and frequency calculations were done for lowest energy conformer in order to ascertain the minima. Optical rotation was calculated at 589.3 nm using B3LYP/Aug-CC-pVDZ method in gas phase for lowest energy conformer of respective molecules.[24] B3LYP/Aug-CC-pVDZ method was opted based on the literature.[22] The solvent effect has been omitted for geometry optimizations and optical rotation calculations, since a methodology does not yet exist which predicts the solvent effects with uniform reliability.[25] All these calculations were performed using Gaussian 09 software.[26]

Biological Activity

Cell culture

HT-29 (Colon cancer) cell line was grown as adherent in RPMI medium, ME-180 (Cervical cancer), MCF-7 (Breast cancer) and MDA-MB-453 (Breast cancer) cell line were grown as adherent in DMEM medium supplemented with 10% fetal bovine serum, 100 µg / ml penicillin, 200 µg/ml streptomycin, 2mM L-glutamine, and culture was maintained in a humidified atmosphere with 5% CO_2. All in vitro experiments were performed during the exponential phase of cell growth.

Preparation of samples for cytotoxicity

20mM stock solution for compounds was prepared in DMSO, from the above stock various dilutions were made with sterile PBS to get required concentration.

Cytotoxicity screening using MTT assay

MTT assay was performed according to the method of Naidu et al.[27] MTT assay is a standard colorimetric assay for measuring cellular proliferation. MTT is a tetrazolium salt, which is yellow in color and is photosensitive. MTT [3-(4, 5- dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide] is taken by the living cells and reduced by a mitochondrial dehydrogenase enzyme to a purple formazan product that is impermeable to the cell membrane. Solubilisation with solvents like DMSO leads to liberation of product and amount of purple formazan product is directly related to the cell viability. $1x10^4$ Cells (counted by Trypan blue exclusion dye method)) in 96- well plates were incubated with series of concentrations of compounds for 48 h at 37 ^oC in DMEM with 10% FBS medium. Then the above media was replaced with 90 µl of fresh serum free media and 10 µl of MTT reagent (5mg/ml) and plates were incubated at 37 ^oC for 4h, there after the above media was replaced with 200 µl of DMSO and incubated at 37 ^oC for 10 min.The absorbance at 570 nm was measured on a spectrophotometer (spectra max, Molecular devices). IC_{50} values were determined from plot: % cell viability (from control) versus concentration.

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Supporting Information

^{1}H NMR spectra and ^{13}C NMR spectra for all final compounds, Molecular Modeling studies, Cartesian Coordinates of fuzanins C, D, (11*R*, 12*S*)-fuzanin D.

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First total synthesis of Fuzanins C, D and their analogues as anticancer agents

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Figure captions

Scheme captions

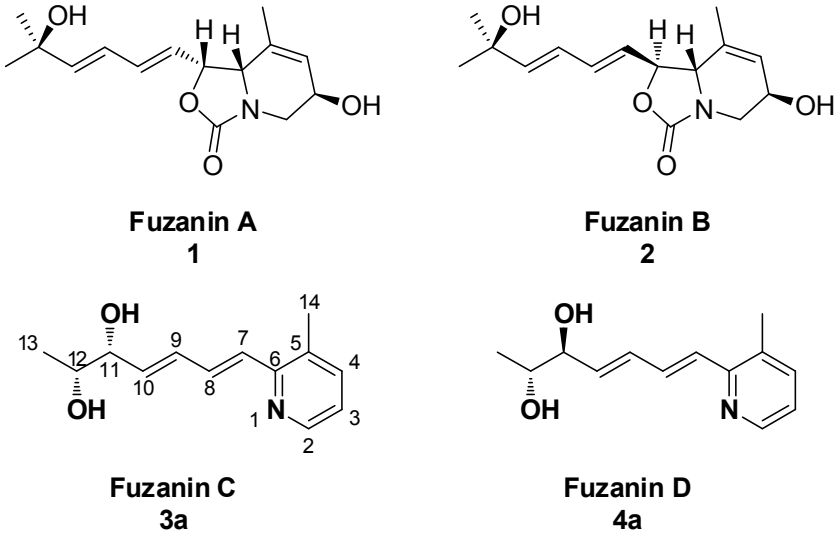


Figure 1. Fuzanins A, B, C, and D

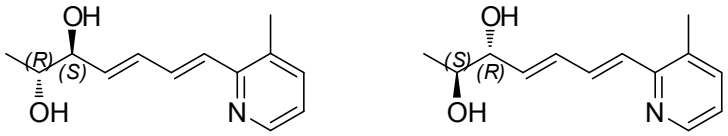


Figure 2. Revised Structure of Fuzanin D

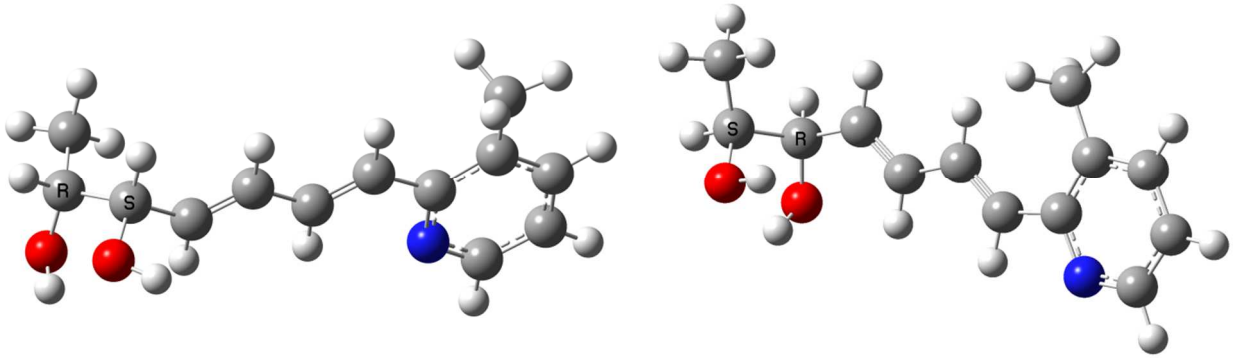


Figure 3. Energy minimized geometries of fuzanin D (**4a**) & (**11*R*, 12*S*)-4a**

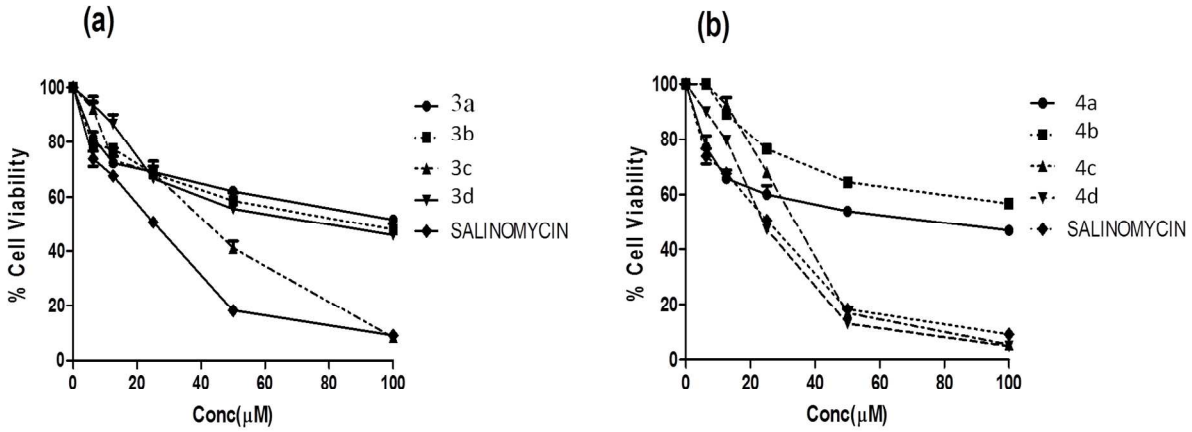
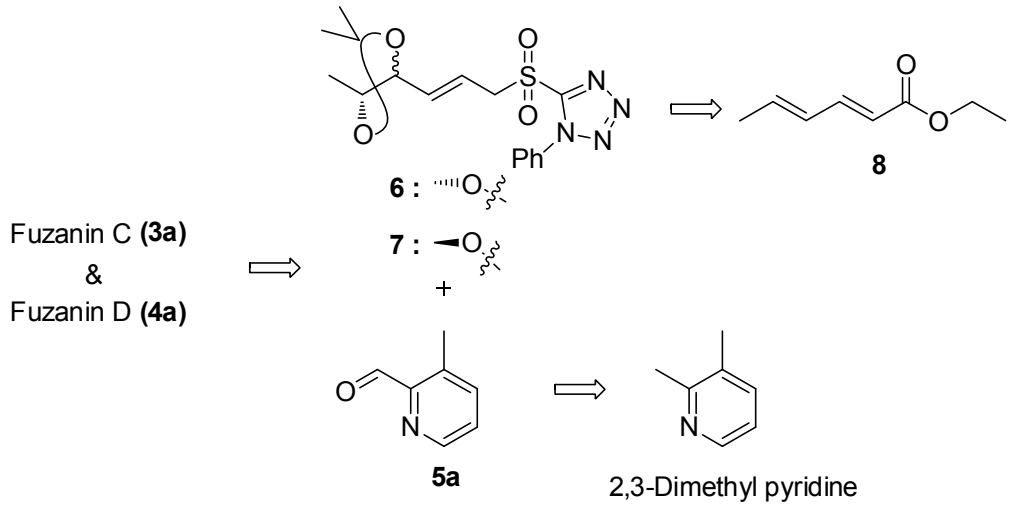
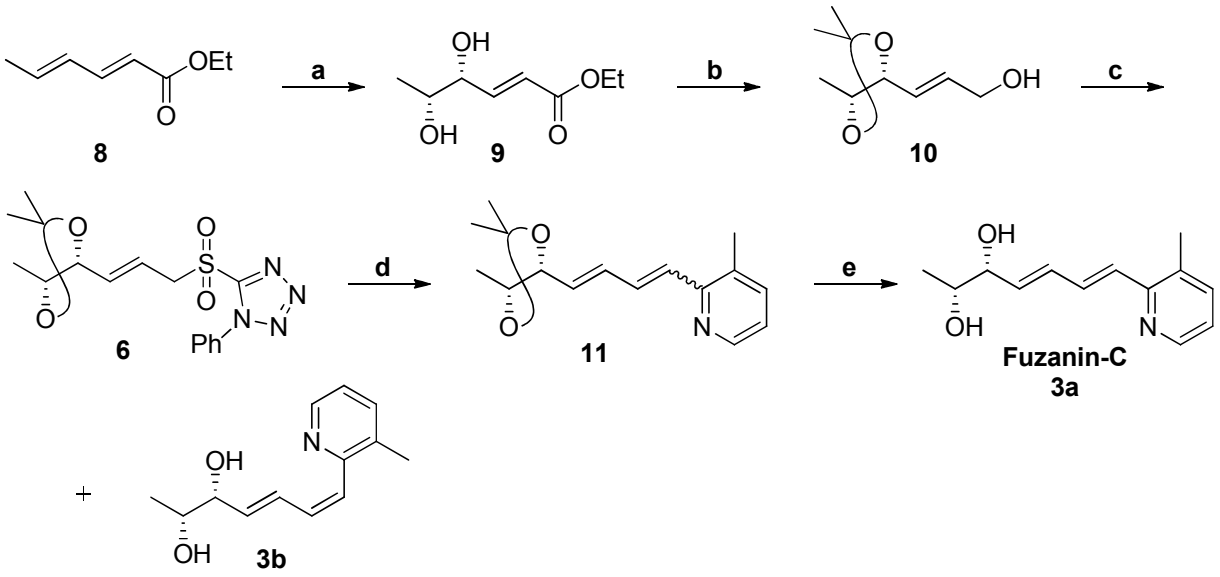


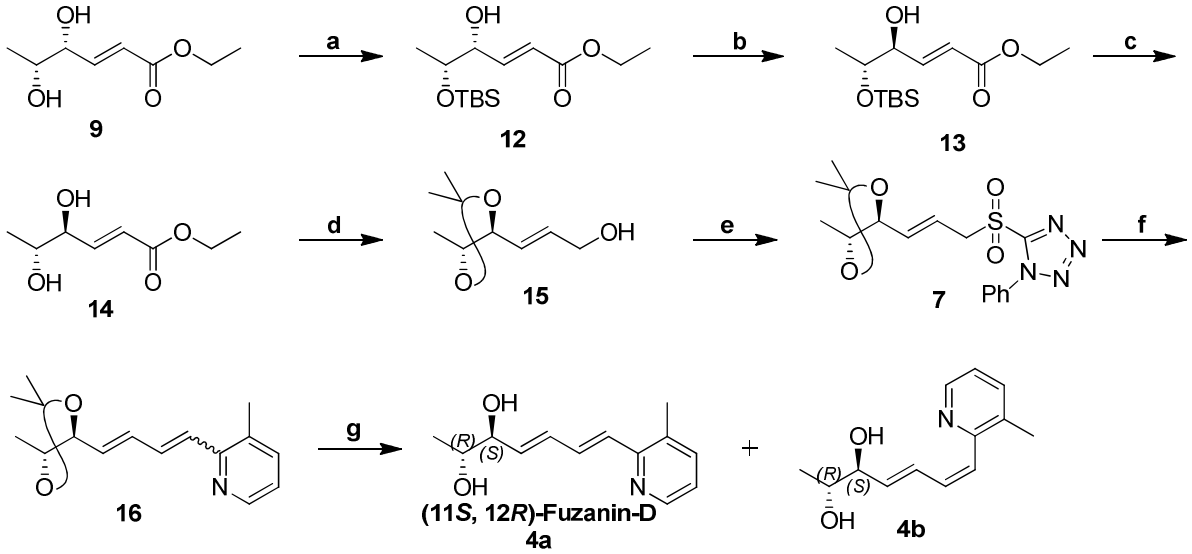
Figure 4. Dose response of fuzanin compounds against HT-29 cancer cell line



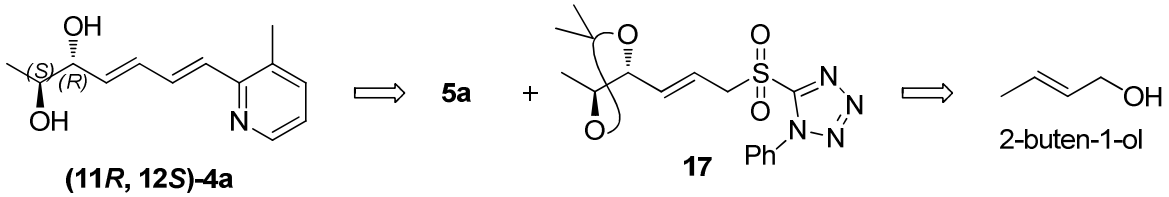
Scheme 1. Retrosynthetic analysis of **3a**, **4a**



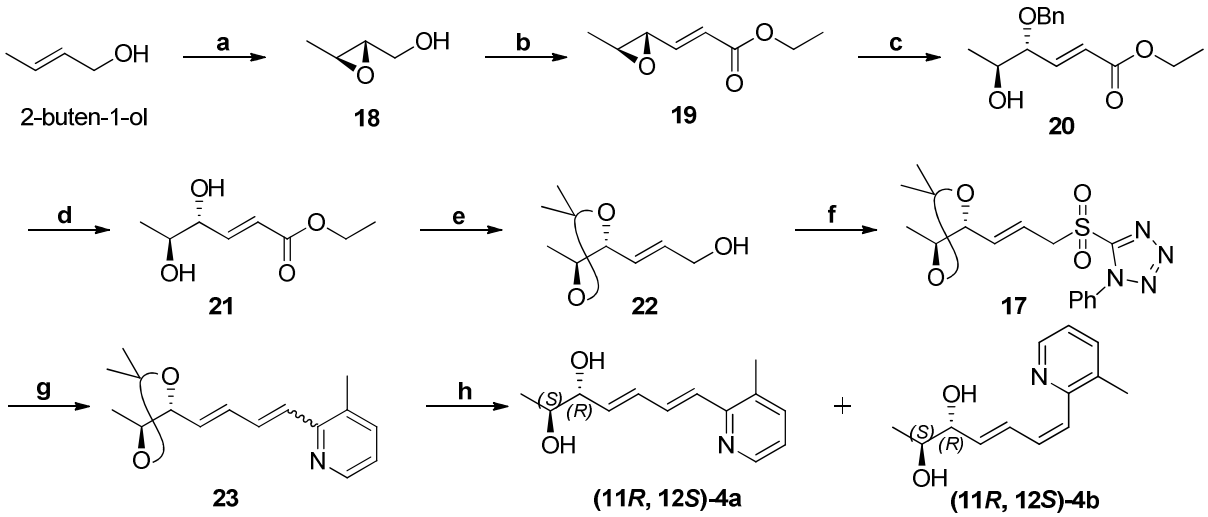
Scheme 2 *Reagents and conditions*: (a) $(DHQD)_2PHAL$, OsO_4, $CH_3SO_2NH_2$, $K_3Fe(CN)_6$, K_2CO_3, *t*-BuOH/H_2O (1:1), 0 °C, 12 h, 84%; (b) (i) 2,2-Dimethoxypropane, *p*-TSA (cat), CH_2Cl_2, rt, 15 min; (ii) DIBAL-H, CH_2Cl_2, 0 °C, 1 h, 81% (for two steps); (c) (i) 1-phenyl-1H-tetrazole-5-thiol, PPh_3, DIAD, THF, 0 °C, 1 h, 90%; (ii) $(NH_4)_6Mo_7O_{24}.4H_2O$, EtOH, 30% H_2O_2, rt, 8h, 92%; (d) **5a**, KHMDS, THF, -78 °C, 2 h, 81%; (e) *p*-TSA, MeOH, rt, 12 h, 82% (total yield).



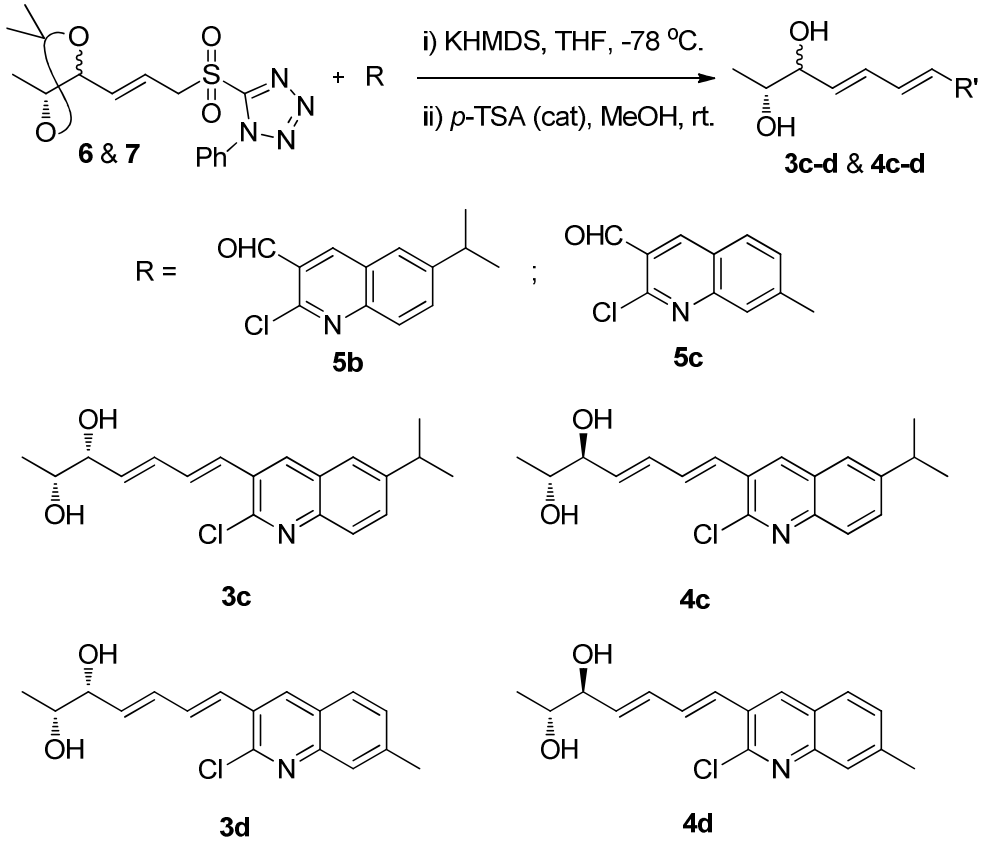
Scheme 3 *Reagents and conditions*: (a) TBS-Cl, Et_3N, DMAP, CH_2Cl_2, rt, 24 h, 65%; (b) (i) PPh_3, DEAD, HCOOH, rt, 2 h; (ii) dil. NH_4OH/MeOH, 3 h, 55% (for two steps); (c) TBAF, CH_2Cl_2, 0 °C, 1 h, 85%; (d) (i) 2,2-Dimethoxypropane, *p*-TSA (cat), CH_2Cl_2, rt, 15 min; (ii) DIBAL-H, CH_2Cl_2, 0 °C, 1 h, 80% (for two steps); (e) (i) 1-phenyl-1H-tetrazole-5-thiol, PPh_3, DIAD, THF, 0 °C, 1 h; (ii) $(NH_4)_6Mo_7O_{24}.4H_2O$, EtOH, 30% H_2O_2, rt, 8 h, 82% (for two steps); (f) **5a,** KHMDS, THF, -78 °C, 2 h, 81%; (g) *p*-TSA, MeOH, rt, 12 h, 82% (total yield).



Scheme 4. Retrosynthetic analysis of **(11*R*,12*S*)-4a**



Scheme 5 *Reagents and conditions*: (a) $Ti(O^iPr)_4$, (+)-DIPT, anhydrous TBHP, CH_2Cl_2, -20 ^oC, 2 h; (b) (i) DMSO, $(COCl)_2$, Et_3N, CH_2Cl_2, -78 ^oC, 30 min. (ii) Ph_3P=CHCOOEt, CH_2Cl_2, rt, 24 h, 56% (for two steps); (c) BnOH, $BF_3.OEt_2$, DCM, -20 ^oC, 1 h, 60%; d) $AlCl_3$, *m*-xylene, 0 ^oC, 15 min, 76%; (e) (i) 2,2-Dimethoxypropane, *p*-TSA, CH_2Cl_2, rt, 15 min. (ii) DIBAL-H, CH_2Cl_2, 0 ^oC, 1 h, 86% (for two steps); (f) (i) 1-phenyl-1H-tetrazole-5-thiol, PPh_3, DIAD, THF, 0 ^oC, 1 h. (ii) $(NH_4)_6Mo_7O_{24}.4H_2O$, EtOH, 30% H_2O_2, rt, 81% (for two steps); g) **5a,** KHMDS, THF, -78 ^oC, 2 h, 80%; h) *p*-TSA, MeOH, rt, 12 h, 82% (total yield).



Scheme 6. Analogues of fuzanins C, D

First total synthesis of Fuzanins C, D and their analogues as anticancer agents

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Table 1. Experimental and calculated specific rotations of selected compounds

Compounds	$[\alpha]_D$ (in ° dm^{-1} g^{-1} cm^3)		
	Isolation paper[a]	Synthesized compounds	Calculated[b]
Fuzanin C (**3a**)	$[\alpha]_D^{15}$ = +34.5	$[\alpha]_D^{24}$ = +39.89	+66.5
Fuzanin D (**4a**)	$[\alpha]_D^{15}$ = -32.9	$[\alpha]_D^{24}$ = +29.47	+22.4
(11*R*, 12*S*)-4a	-	$[\alpha]_D^{24}$ = -26.9	-8.5

[a] reference 11; [b] Optical rotations were computed using B3LYP/aug-cc-pVDZ // B3LYP/6-31G(d) basis set in gas phase.

Table 2. IC_{50} values (μM) of fuzanin compounds and Salinomycin against HT29 human colon cancer cell lines

Compound	IC_{50} /μg mL^{-1}
	HT-29 (Colon cancer)
3a	96.2±2.65
3b	85.3±4.72
3c	48.5±2.56
3d	76.2±2.45
4a	77.9±2.95
4b	98.5±1.88
4c	35.3±0.83
4d	27.4±0.12
(11*R*, 12*S*)-4a	>100
(11*R*, 12*S*)-4b	>100
Salinomycin	20.5±1.26