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Synthesis of (±)-2*R*,4*R*,5*S*-2-methoxy-4-nitro-5-(2,3,4-trimethoxyphenyl)cyclohexanone and (±)-2*R*,4*S*,5*R*-2-methoxy-5-nitro-4-(2,3,4-trimethoxyphenyl)cyclohexanone as colchicine mimetics

Karen Anderson Evans,[†] Kebede Beshah, David H. Young,[‡] Ted T. Fujimoto,[§] Colin M. Tice[¶] and Enrique L. Michelotti*,[§]

Rohm and Haas Company, P.O. Box 904, 727 Norristown Road, Spring House, PA 19477-0904, USA

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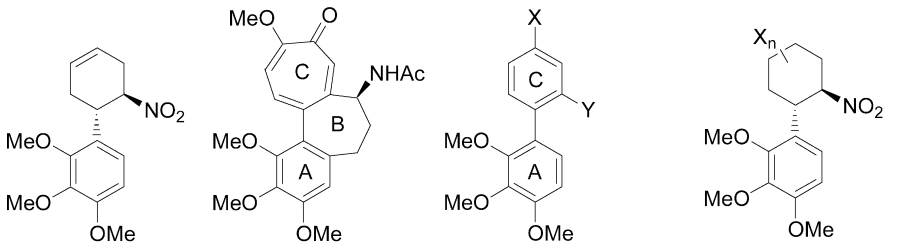
Abstract—Colchicine mimetic (±)-4*S*,5*R*-4-nitro-5-(2,3,4-trimethoxyphenyl)cyclohexene (**1**) was epoxidized to afford a mixture of epoxides. The epoxides were separately converted in two steps, with high stereoselectivity, to two regioisomeric α-methoxyketones. One regioisomer, (±)-2*R*,4*S*,5*R*-2-methoxy-5-nitro-4-(2,3,4-trimethoxyphenyl)cyclohexanone (**17**), proved to be about 12-fold more potent than synthetic precursor **1** against HCT-116 tumor cells while the other regioisomer, (±)-2*R*,4*R*,5*S*-2-methoxy-4-nitro-5-(2,3,4-trimethoxyphenyl)cyclohexanone (**16**), and the synthetic intermediates tested showed no improvement in potency. © 2003 Published by Elsevier Science Ltd.

We recently reported the discovery of a series of phenylcyclohexenes (PCHs) exemplified by **1** (Fig. 1) that bind to the colchicine (**2**) site of tubulin.[1] While **1** is significantly more potent towards plant than animal cells, the reverse is true of **2**.[2] Looking to enhance the potency of **1** we examined the SAR of **2** for clues. A number of SAR studies of **2** have established the importance of the α-methoxyketone moiety for activity.[3] Furthermore the methoxy and carbomethoxy groups at the 4-position of the C-ring of biphenyl colchicine mimetics **3** and **4** increase the potency of these compounds against mammalian tubulin.[3] Thus, PCH analogs in which the alicylic ring is functionalized with an α-methoxyketone moiety or other oxygen containing functionality, as shown in general structure **7**, might be better mimics of **2** than **1** is, and hence possess enhanced potency against mammalian, and possibly plant,



1 **2**

3 X = OMe, Y = Me
4 X = CO_2Me, Y = Me
5 X = H, Y = NO_2
6 X = OMe, Y = NO_2

X = oxygen containing functionality
n = 1,2

7

Figure 1. (±)Phenylcyclohexenes, colchicine and biphenyls. (a) One enantiomer depicted.

Keywords: phenylcyclohexenes; colchicine; epoxides.

* Corresponding author. Tel.: +1-215-358-2026; fax: +1-215-358-2030; e-mail: emichelotti@locuspharma.com
† Present address: GlaxoSmithKline, 1250 South Collegeville Road, Collegeville, PA 19426, USA.
‡ Present address: Dow AgroSciences LLC, 9330 Zionsville Road, Indianapolis, IN 46268, USA.
§ Present address: Locus Discovery Inc., Four Valley Square, 512 Township Line Road, Blue Bell, PA 19422, USA.
¶ Present address: Concurrent Pharmaceuticals, 502 West Office Center Drive, Fort Washington, PA 19034, USA.

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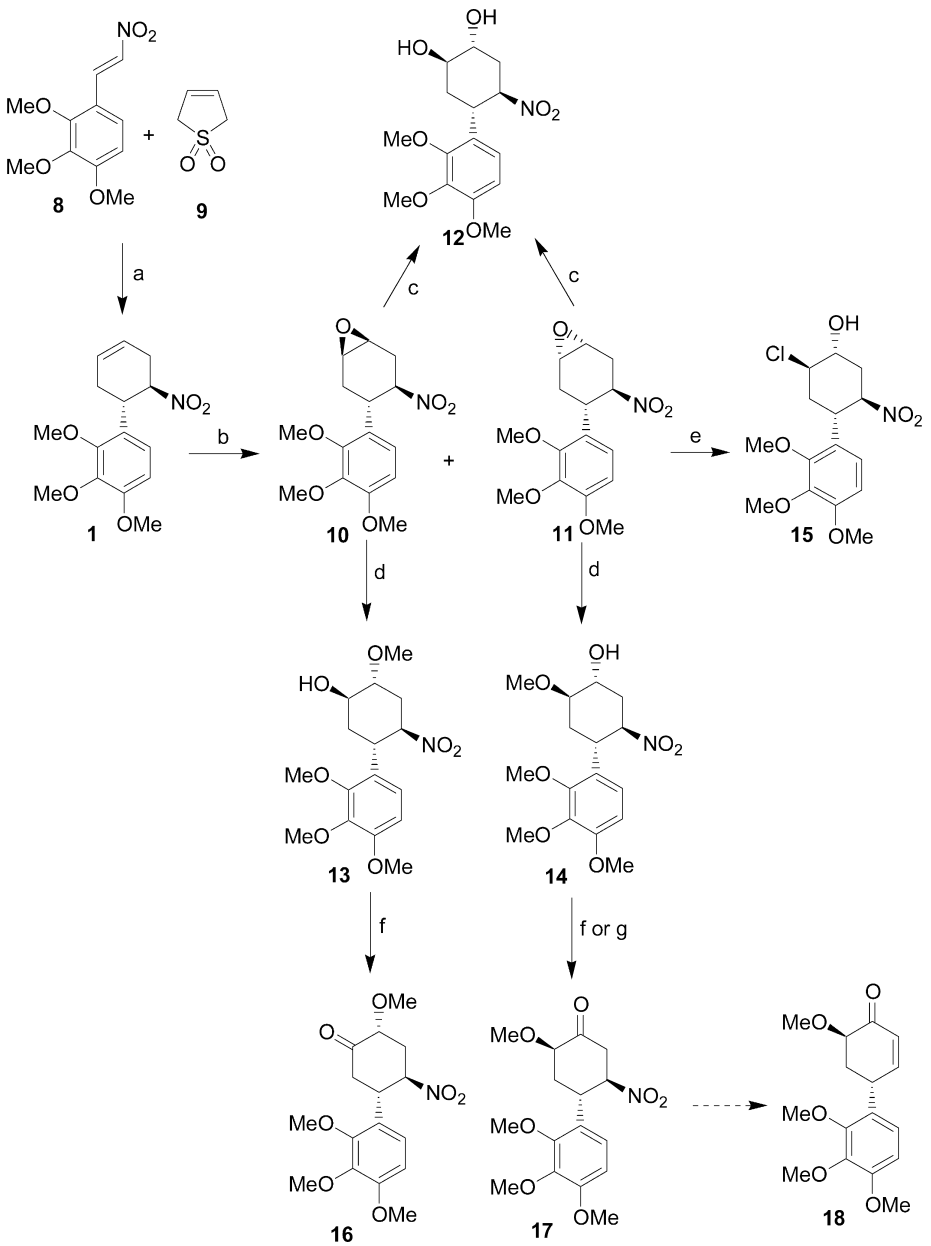
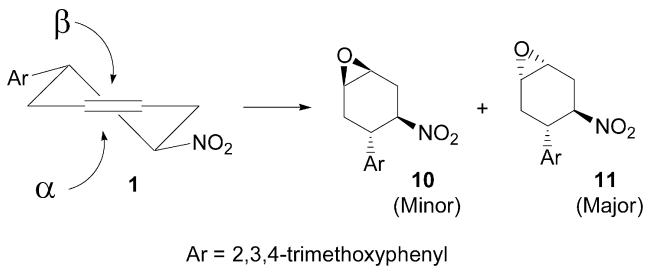


Scheme 1. Synthesis of oxygenated PCHs. All compounds are racemic. One of the two enantiomers has been depicted in a consistent manner across all of the structures including an account of the inversions that take place during the opening of the epoxide ring. (a) dioxane, 120°C, 36 h; (b) *m*-CPBA, $CHCl_3$, rt, 16 h; (c) H_2SO_4, H_2O, THF, rt, 1 h; (d) H_2SO_4, MeOH, rt, 3 h; (e) HCl, MeOH, rt, 1 h; (f) Dess–Martin periodinane, CH_2Cl_2, rt, 1.5 h; g. PCC, CH_2Cl_2, rt, 16 h.

tubulin. Oxygenated 1-nitro-2-arylcyclohexanes related to **7** have previously been prepared by various routes as synthetic intermediates by other workers.[4–11] The synthesis of seven new PCH analogs oxygenated on the alicyclic ring and the results of preliminary biological assays are reported here.

PCH **1** was prepared by Diels–Alder reaction of nitrostyrene **8** with 10 equiv. of sulfolene (**9**) (Scheme 1) in a sealed vessel. Initial experiments using toluene as solvent gave poor yields of **1**.[12,13] When dioxane, which is less often used for Diels–Alder reactions, was employed as solvent the reaction proceeded more quickly (48–72 h, sealed tube, 135°C) to afford cleaner product. The double bond of the cyclohexene ring of **1** was used as a handle to introduce oxygen containing functionality. Thus, **1** was epoxidized with *m*-CPBA to afford two epoxides in a 3:1 ratio. X-Ray crystallography of the major epoxide indicated that it had the relative stereochemistry shown as **11** resulting from delivery of oxygen to the α-face of the alkene (Scheme 2).[14] Huitric et al.[7] reported that the epoxidation of *trans*-4-nitro-5-(4-chlorophenyl)cyclohexene gave a similar ratio of epoxide products, suggesting that the *ortho* methoxy substituent in **1** does not significantly influence the epoxidation reaction. They assigned epoxide stereochemistry based on ^{1}H NMR spectroscopy.

Epoxides rings can readily be opened by water and alcohols to yield diols and alkoxyalcohols, respectively.[15] Ring opening can be carried out under neutral, basic, or acidic conditions,and it is well known that the reaction follows an S_N2 mechanism under neutral or basic conditions. In acidic



Scheme 2. Epoxidation stereochemistry.

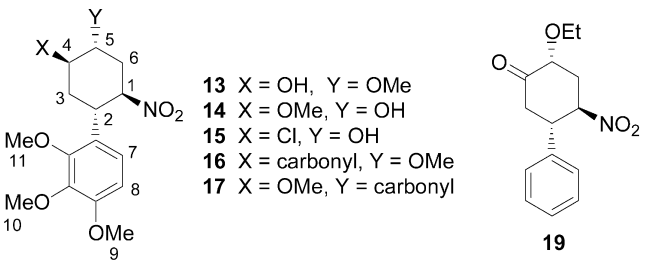


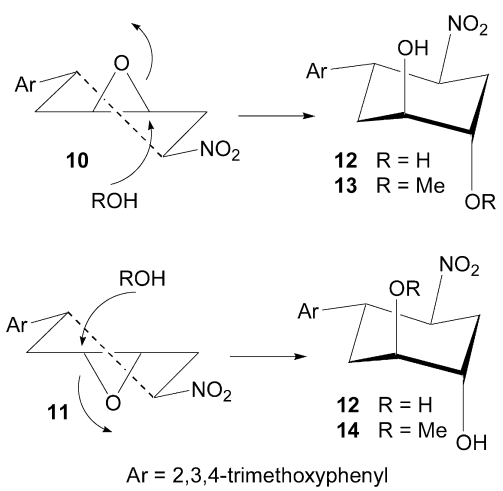
Figure 2. Compound numbering for ^{1}H NMR assignments.

media, protonation of the epoxide precedes nucleophilic attack, but even in this case, backside attack of the nucleophile on an epoxide carbon occurs, resulting in Walden inversion at this center. With unsymmetrical epoxides the regiochemistry of the nucleophilic attack is governed by both the structure of the epoxide and the reaction conditions. In the case of rigid cyclohexene oxides, such as **10** and **11**, ring opening usually occurs to give the *trans* diaxial product (Furst–Plattner rule).[16]

Thus epoxides **10** and **11** were separately treated with aqueous sulfuric acid to afford single diol product **12** in 82 and 84% yields, respectively.[7] The diol products from the two different epoxides were shown to be identical by ^{1}H NMR, ^{13}C NMR and GC co-injection and the relative stereochemistry shown in structure **12**, as was expected from *trans* diaxial ring opening of both **10** and **11** (Scheme 3), was assigned based on high field NMR studies (vide infra).

Treatment of **10** with methanol under acidic conditions afforded the vicinal methoxyalcohol **13**, while similar treatment of **11** afforded the isomeric compound **14** (Scheme 3).[17] The relative stereochemistry of the substituents around the cyclohexane rings in **13** and **14** was again established by a series of high field NMR experiments (vide infra). In addition, major epoxide **11** was treated with HCl to afford a single chlorohydrin **15**, resulting from *trans* diaxial ring opening, in 76% yield.

The final step in the sequence we envisaged was the oxidation of alcohols **13** and **14** to provide **16** and **17** which contain the α-methoxyketone functionality present in the



Scheme 3. Diaxial opening of epoxide rings.

C-ring of colchicine (**2**). We were concerned that **17** would readily undergo β-elimination of nitrite to afford an enone **18**[18] and selected the Dess–Martin periodinane as oxidant since it functions under mild conditions.[19] Thus, alcohols **13** and **14** were smoothly oxidized to afford **16** and **17** in 75 and 67% yields, respectively. In fact only trace amounts of decomposition product **18** were observed in the crude product. Oxidation of **14** to **17** can also be effected with PCC in quantitative yield. No evidence for epimerization of the methoxy group α to the ketone was observed in either case.

The ^{1}H NMR assignments of the protons around the cyclohexane rings of **13**–**17** (Fig. 2) which establish their relative stereochemistry are shown in Table 1. The assignment strategy involved correlating three bond proton coupling correlation using two-dimensional proton–proton correlated spectroscopy. The stereochemistry for the axial and equatorial positions was determined from the coupling constants between the axial neighbors (at greater than 10 Hz) and between the equatorial neighbors at about 3–5 Hz. In addition, from the 2D NOESY cross peak intensities we were able to clearly observe stronger signals between proton pairs close in space such as between adjacent equatorial positions. For example, we observed a weak NOE between protons C^{1}–H_{axial} and C^{6}–H_{axial}, while we saw a much stronger signal between C^{1}–H_{axial} and C^{6}–$H_{equatorial}$ which is consistent with the distances between the pairs: the greater the distance the weaker the signal. The rest of the assignments were confirmed

Table 1. ^{1}H NMR assignments

Position[a]	13	14	15	16	17
1	5.08	5.23	5.28	5.49	5.14
2 ax	3.87	3.75	3.95	3.81	4.08
3 ax	2.04	1.98	2.53	3.13	2.30
3 eq	1.81	1.98	2.02	2.49	2.10
4 ax	(2.57)[b]	(3.39)[c]	–	–	(3.39)[c]
4 eq	4.03	3.37	4.15	–	3.58
5 ax	(3.41)[c]	(2.54)[b]	(2.50)[b]	(3.38)[c]	–
5 eq	3.56	4.17	4.28	3.71	–
6 ax	2.46	2.44	2.70	2.71	3.56
6 eq	2.40	2.26	2.32	2.43	2.86
7	6.85	6.86	6.86	6.85	6.81
8	6.59	6.59	6.61	6.61	6.59
9	3.79	3.79	3.82	3.83	3.83
10	3.81	3.83	3.85	3.84	3.84
11	3.89	3.91	3.94	3.96	3.96

Chemical shifts are in ppm downfield of internal tetramethysilane. See Section 1 for methodology used to make assignments.

[a] See general structure in Figure 2 for numbering scheme, ax=axial, eq=equatorial.

[b] Attached O*H* proton.

[c] Attached O*Me* group.

Table 2. Biological assay results

Compound[a]	HCT116 tumor cell assay[b] EC_{50} (μM)	Tobacco root assay[c] EC_{50} (μM)
1[d]	57.5	0.27
2	0.025	356
5	19.6	52.0
6	2.74	>100
10	>50	>100
11	>50	>100
12	>50	>100
13	>50	>100
14	>50	>100
16	>50	>100
17	4.8	>100

[a] Compounds **1** and **10**–**17** were tested as their racemic mixtures while **2** was tested as its natural levorotatory enantiomer.
[b] See Ref. 21.
[c] See Ref. 22.
[d] Data for **1** represent the mean values from three experiments±standard deviation.

similarly. The signals for the aromatic group and its attachment to the cyclohexyl group were confirmed from a combination of heteronuclear HSQC and HMBC experiments. For example, aromatic C^7 showed correlation to C^2–H_{axial} on the cyclohexane ring. Conversely C^2 showed correlation to aromatic C^7–H. This confirmed where the two rings are bonded to each other. The strong J coupling between C^7–H and C^8–H indicates that the two should be three bonds apart. The remaining assignments for the aryl methoxy groups was established from the NOESY data. For example, the *ortho*-methoxy protons (C^{11}–H_3) showed cross peaks with C^3–$H_{equatorial}$ indicating their proximity to the cyclohexane ring. Similarly *para*-methoxy protons (C^9–H_3) showed an NOE signal with aromatic proton C^8–H. Further examination of the HMBC signals also confirm the same assignments. Our ^{1}H NMR assignments for the cyclohexanone ring of **16** were generally consistent with those for the closely related compound **19** prepared by a different route.[4,20]

New compounds **10**–**14**, **16** and **17** were tested for biological activity against HCT116 tumor cells[21] and in an assay for inhibition of tobacco root growth,[22] as potency in these assays has been shown to correlate with the antimitotic or antitubulin activity of the PCHs.[1] The results for the new

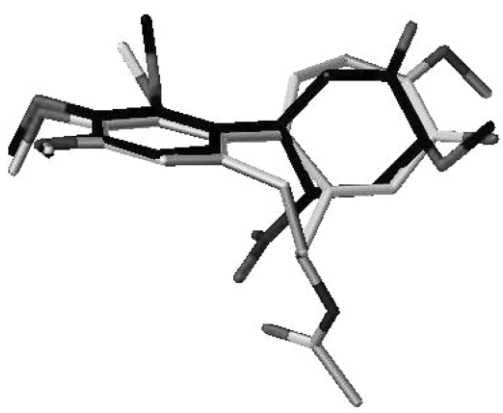


Figure 3. Orthogonal views of α-methoxyketone **16** superimposed on colchicine (**2**). (a) The carbon atoms of **16** are colored black. The carbon atoms of **2** are colored gray.

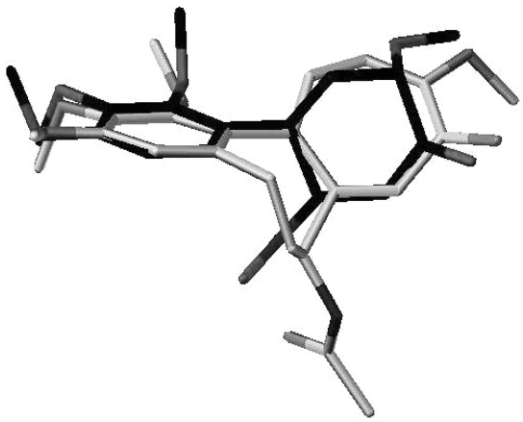


Figure 4. Orthogonal views of α-methoxyketone **17** superimposed on colchicine (**2**). (a) The carbon atoms of **17** are colored black. The carbon atoms of **2** are colored gray.

compounds are shown in Table 2 along with data for the parent PCH **1** and colchicine (**2**). Data are also presented for biphenyls **5** and **6**.[1,23,24] Introduction of oxygen functionality into the PCH scaffold abolished activity in the tobacco root assay (**10**–**14**, **16**, **17** vs **1**) whereas in the HCT116 tumor cell assay α-methoxyketone **17** showed an almost 12-fold improvement in potency over **1**. Similarly, introduction of the methoxy group at the 4-position of the nitrophenyl ring of **5** to give **6** abolished activity in the tobacco root assay while increasing potency in the HCT116 tumor cell assay.

To rationalize this result, molecular modeling was used to compare the structures of inactive α-methoxyketone **16** and its biologically active regioisomer **17** to the X-ray structure of **2**. The results are depicted in Figures 3 and 4. In **16** the orientation of the carbonyl and the methoxy groups are reversed relative to **2** (northeast corner) and the distance between the carbonyl oxygen of **2** and the closest oxygen in **16**, which is a methoxy oxygen, is more than 2 Å. In addition, to allow the lone pair electrons on the methoxy group of **16** to align with the lone pair electrons of the carbonyl of **2**, it is necessary for the methyl group on the methoxy to rotate over the cyclohexane ring, which is an energetically unfavorable conformation. These structural differences possibly account for the lack of potency observed for **16**. On the other hand, overlap of **17** with **2** positioned the α-methoxyketone groups of the two molecules in a more similar orientation. In particular, the carbonyl group of **17** is in proximity to and is oriented parallel to that of **2** which aligns the lone pair electron density of both in the same direction. The methoxy groups of **17** and **2**, while in the same general orientation, are further apart than the carbonyls. It has previously been established that carbonyl group of **2** is more important than the methoxy for efficacy.[25] Thus, we hypothesized that the greater conformational similarity between **17** and **2** accounted for the increased potency of **17** over precursor **1** against mammalian cells.

Introduction of oxygen-containing functionality onto the alicylic ring of the PCHs was generally deleterious to the activity of the molecules in the two assays we ran; however, one compound, α-methoxyketone **17**, showed about a

12-fold improvement over parent PCH **1** in potency against the HCT116 tumor cell line. Molecular modeling was used to rationalize this result.

1. Experimental

1.1. General

The proton and carbon nuclear magnetic resonance spectra (^{1}H, ^{13}C NMR) were recorded on a Bruker DPX300 spectrometer operating at 300.1319 and 75.4773 MHz, respectively. Spectra were taken in the indicated solvent at ambient temperature, and the chemical shifts are reported in ppm relative to the lock of the solvent used. The infrared (IR) spectra were recorded using a thin film (NaCl), unless otherwise specified, on a Mattson Genesis-II FTIR spectrophotometer and are reported in reciprocal centimeters. Gas chromatograph mass spectra (GCMS) were recorded on an HP5890A gas chromatograph (Column is HP-5 (Cross-linked 5% Ph Me Silicone) 15 m×0.25 mm×0.25 mm Film Thickness, HP Part No. 19091J-431) with an HP5970 Series Mass Selective Detector and are reported in *m/z* units for the molecular ion. Gas chromatography (GC) was performed on an HP5890A gas chromatograph with a 10 m capillary column (HP-1, 0.53×0.88 packing). The method normally used started at 100°C, with heating at a rate of 20°C/min to 275°C. The initial time was 2.00 min and the final time was 10 min. Melting points were recorded on a Thomas Hoover Uni-melt capillary apparatus and are uncorrected.

1.2. High-field NMR experiments

We used ^{1}H, ^{13}C, and two-dimensional carbon–proton correlation experiments to make the assignments for one bond carbon–proton correlation (HSQC) as well as multiple bond carbon–proton correlation by heteronuclear multiple bond correlation (HMBC) experiment. For the stereochemistry assignments, we used nuclear Overhauser enhancement experiment in two dimensions (NOESY). All experiments were performed on a Bruker DMX400 spectrometer and inverse detection probe. The samples were all dissolved in deuterated $CDCl_3$.

1.3. Materials

All reactions were carried out under house nitrogen atmosphere with the exclusion of moisture. Reagents and solvents were purchased from Fisher Scientific, VWR, and Aldrich Chemical Company and were used without further purification unless otherwise specified. PCC stands for pyridinium chlorochromate. Dess–Martin periodinane reagent is 1,1,1-tris(acetoxy)-1,1-dihydro-1,2-benziodoxol-3-(1*H*)-one. Concentration or evaporation of solvent refers to removal at reduced pressure using a Büchi rotary evaporator attached to house vacuum. Flash column chromatography was carried out with nitrogen in the indicated solvent system (in the percentage of volume) on 200–400 mesh (60 Å) silica gel (SiO_2) or using an Elution Solutions (Biotage) FlashElute™ apparatus. Prepacked silica cartridges used were: 40S (4.0×7.5 cm^2, 40 g silica), 40M (4.0×15 cm^2, 90 g silica), or 40L (4.0×20 cm^2, 120 g silica). Analytical thin layer chromatography (TLC) was performed on Baker-flex® silica gel IB2-F precoated plastic-backed plates (2.5×7.5 cm^2). TLC visualization was performed using 254 nm wavelength ultraviolet light, or by heating samples stained with vanillin (5 g vanillin, 5 mL sulfuric acid, and 2.5 mL glacial acetic acid in 90 mL absolute ethanol), and/or potassium permanganate (2.5 g sodium carbonate in 50 mL water added to 0.5 g potassium permanganate in 50 mL water).

1.3.1. (±)-1*S*,3*R*,4*S*,6*R*-3-Nitro-4-(2,3,4-trimethoxyphenyl)-7-oxabicyclo[4.1.0]heptane (10) and (±)-1*R*,3*R*,4*S*, 6*S*-3-nitro-4-(2,3,4-trimethoxyphenyl)-7-oxabicyclo-[4.1.0]heptane (11). To a stirred solution of *trans*-4-nitro-5-(2,3,4-trimethoxyphenyl)cyclohexene (**1**) (10.0 g, 34.4 mmol) in chloroform (150 mL) was added portionwise m-chloroperbenzoic acid (13.1 g, 34.8 mmol) at room temperature. The reaction mixture was stirred overnight at room temperature. The resulting suspension was diluted with ethyl acetate (400 mL), washed with saturated aqueous $NaHCO_3$ (2×150 mL) and dried over anhydrous $MgSO_4$. Removal of the solvent under vacuum left a yellow solid (9.82 g) consisting of the two possible epoxides **10** and **11**. The mixture was separated by column chromatography (ethyl acetate/hexane 25:75, 30:70, 40:60 and 50:50) to afford in order of elution compound **11** (6.1 g, 57.4%) and compound **10** (2.0 g, 18.8%). Compound **11** gave the following spectral data: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.78 (1H, d, *J*=8.7 Hz), 6.59 (1H, d, *J*=8.7 Hz), 5.06 (1H, dt, *J*=11.7, 4.5 Hz), 3.94 (3H, s), 3.83 (3H, s), 3.82 (3H, s), 3.44 (2H, m), 3.26 (1H, br t, *J*=4.5 Hz), 2.88 (1H, dt, *J*=12.0, 2.1 Hz), 2.43 (2H, m), 2.12 (1H, br t, *J*=12.0 Hz). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 153.5, 152.2, 142.5, 125.1, 122.3, 107.8, 84.9, 61.6, 61.0, 56.3, 53.4, 51.4, 38.4, 32.2, 31.9. IR (thin film on NaCl): 3004 (m), 2940 (m), 2838 (w), 1601 (w), 1550 (s), 1496 (s), 1467 (s), 1419 (w), 1375 (w), 1291 (m), 1278 (w), 1097 (s) cm^{-1}. Anal. calcd for $C_{15}H_{19}NO_6$: C, 58.25; H, 6.19; N, 4.53. Found: C, 58.12; H, 6.15; N, 4.43. Mp 85–86°C. Compound **10** gave the following spectral data: ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 6.76 (1H, d, *J*=8.6 Hz), 6.57 (1H, d, *J*=8.6 Hz), 4.95 (1H, dt, *J*=11.1, 6.6 Hz), 3.95 (3H, s), 3.83 (3H, s), 3.82 (3H, s), 3.54 (1H, dt, *J*=11.7, 4.5 Hz), 3.30 (2H, m), 2.64 (2H, m), 2.42 (1H, dd, *J*=14.7, 4.5 Hz), 2.20 (1H, br t, *J*=13.2 Hz). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 153.7, 152.6, 142.6, 124.2, 123.6, 107.5, 85.1, 61.5, 61.0, 56.3, 52.7, 49.9, 36.5, 31.0, 30.2. IR (thin film on NaCl): 3017 (m), 2937 (m), 2848 (w), 1601 (w), 1552 (s), 1496 (s), 1468 (s), 1420 (w), 1376 (w), 1286 (m), 1218 (w), 1099 (s), 1015 (w), 755 (s) cm^{-1}. Anal. calcd for $C_{15}H_{19}NO_6$: C, 58.25; H, 6.19; N, 4.53. Found: C, 58.18; H, 6.12; N, 4.43. Mp 86–87°C.

1.3.2. (±)-1*R*,2*R*,4*R*,5*S*-4-Nitro-5-(2,3,4-trimethoxyphenyl)-cyclohexane-1,2-diol (12). To a solution of **10** (0.18 g, 0.58 mmol) dissolved in THF (10 mL), was added water (5 mL) and concentrated sulfuric acid (0.2N, 0.10 mL). After the reaction stirred for 1 h at rt, TLC showed the reaction was complete. The solution was concentrated in vacuo. The oily residue was dissolved in ethyl acetate and washed with saturated sodium bicarbonate solution (3×5 mL), water (1×5 mL), and brine (1×5 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated in vacuo. Flash column chromatography (silica gel, 70% ethyl acetate/hexanes) afforded diol **12**

(0.16 g, 84%) as a white solid. The same procedure was repeated starting with compound **11** and the same diol **12** was isolated. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.88 (1H, d, J=8.7 Hz), 6.62 (1H, d, J=8.7 Hz), 5.25 (1H, dt, J=11.7, 3.9 Hz), 4.16 (1H, m), 3.93 (3H, s), 3.90 (2H, m), 3.84 (3H, s), 3.82 (3H, s), 2.58 (1H, dt, J=12.9, 2.0 Hz), 2.44 (OH, br s), 2.39 (OH, br s), 2.30 (1H, dt, J=12.9, 3.3 Hz), 2.18 (1H, br t, J=14.4 Hz), 1.84 (1H, br d, J=14.4 Hz). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 153.3, 152.2, 142.6, 126.1, 122.5, 107.9, 85.3, 69.7, 69.0, 61.7, 61.1, 56.3, 36.1, 34.7, 33.9. IR (thin film on NaCl): 3454 (br OH), 2940 (s), 2839 (w), 1710 (w), 1602 (w), 1549 (s), 1497 (s), 1467 (s), 1420 (m), 1376 (m), 1288 (m), 1105 (s), 1092 (s), 1043 (s), 911 (m), 800 (w), 733 (m) cm^{-1}. GCMS (*m/z*): 327, calcd for $C_{15}H_{21}NO_7$: 327.34. Mp 61–63°C.

1.3.3. (±)-1*R*,2*R*,4*R*,5*S*-2-Methoxy-4-nitro-5-(2,3,4-trimethoxyphenyl)cyclohexan-1-ol (13). To a solution of **10** (0.26 g, 0.84 mmol) dissolved in methanol (12.5 mL), was added concentrated sulfuric acid (0.2N, 0.08 mL). After the reaction stirred for 3 h at rt, TLC showed the reaction was complete. The solution was concentrated in vacuo. The oily residue was dissolved in ethyl acetate and washed with saturated sodium bicarbonate solution (3×5 mL), water (1×5 mL), and brine (1×5 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated in vacuo. Flash column chromatography (silica gel, 30% ethyl acetate/-hexanes) afforded **13** (0.20 g, 69%) as a clear, colorless oil. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.87 (1H, d, J=8.7 Hz), 6.61 (1H, d, J=8.7 Hz), 5.11 (1H, dt, J=11.1, 5.4 Hz), 4.07 (1H, br s), 3.93 (3H+H, s), 3.84 (3H, s), 3.82 (3H, s), 3.59 (1H, m), 3.44 (3H, s), 2.47 (2H, m), 2.06 (1H, m), 1.84 (2H, m). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 152.9, 152.0, 142.3, 125.7, 122.0, 107.3, 85.1, 78.2, 66.2, 61.2, 60.6, 57.1, 55.9, 35.4, 34.9, 30.2. IR (thin film on NaCl): 3476 (br OH), 2938 (s), 2833 (w), 1601 (w), 1549 (s), 1497 (s), 1466 (s), 1285 (m), 1197 (w), 1104 (s), 1092 (s), 1013 (m) cm^{-1}. High resolution Positive ESI-FTMS (*m/z*): (M+H) 342.1559, calcd for $[C_{16}H_{23}NO_7]$H 342.1553.

1.3.4. (±)-1*R*,2*R*,4*S*,5*R*-2-Methoxy-5-nitro-4-(2,3,4-trimethoxyphenyl)cyclohexanol (14). To a solution of **11** (0.25 g, 0.81 mmol) dissolved in methanol (12.5 mL), was added concentrated sulfuric acid (0.2N, 0.08 mL). After the reaction stirred for 2 h at rt, TLC showed the reaction was complete. The solution was concentrated in vacuo. The oily residue was dissolved in ethyl acetate and washed with saturated sodium bicarbonate solution (3×5 mL), water (1×5 mL), and brine (1×5 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated in vacuo to afford **14** (0.23 g 82%) as a clear colorless oil. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.87 (1H, d, J=8.6 Hz), 6.61 (1H, d, J=8.6 Hz), 5.25 (1H, dt, J=12.3, 4.2 Hz), 4.21 (1H, br s), 3.94 (3H, s), 3.85 (3H, s), 3.82 (3H, s), 3.78 (1H, m), 3.43 (3H, s), 3.40 (1H, m), 2.47 (1H, dt, J=12.6, 2.4 Hz), 2.29 (1H, dt, J=12.9, 3.3 Hz), 2.03 (3H, m). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 153.2, 152.4, 142.6, 126.3, 122.4, 107.7, 85.2, 77.1, 67.7, 61.6, 61.0, 56.8, 56.3, 36.4, 34.5, 30.6. IR (thin film on NaCl): 3449 (br OH), 2936 (s), 2834 (w), 1548 (s), 1497 (s), 1466 (s), 1282 (m), 1214 (w), 1097 (s), 1011 (m) cm^{-1}. High resolution Positive ESI-FTMS (*m/z*): (M+H) 342.1550, calcd for $[C_{16}H_{23}NO_7]$H 342.1553.

1.3.5. (±)-2*R*,4*S*,5*R*-2-Chloro-5-nitro-4-(2,3,4-trimethoxyphenyl)cyclohexanol (15). A solution of compound **11** (0.65 g, mmol), and concentrated hydrochloric acid (0.3 g) in methanol (30 mL) was stirred at room temperature for 1 h. The reaction mixture was diluted with ethyl acetate (100 mL), extracted with saturated aqueous $NaHCO_3$ (2×100 mL), dried over anhydrous $MgSO_4$, and the solvent eliminated under vacuum yielding a light yellow solid. TLC plate analysis showed two compounds that were separated by column chromatography (silica gel, ethyl acetate/hexane 25:75) yielding 0.10 g of methoxyalcohol **14** (0.10 g, 21%) and chlorohydrin **15** (0.48 g, 66%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.87 (1H, d, J=8.6 Hz), 6.60 (1H, d, J=8.6 Hz), 5.29 (1H, dt, J=11.7, 3.9 Hz), 4.33 (1H, m), 4.18 (1H, m), 3.95 (3H+OH, s), 3.84 (3H, s), 3.83 (3H, s), 2.73 (1H, dt, J=14.7, 2.1 Hz), 2.55 (1H, br t, J=12.6 Hz), 2.34 (1H, dt, J=13.5, 3.0 Hz), 2.08 (1H, m), 2.05 (1H, dt, J=13.5, 3.0 Hz). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 153.6, 152.4, 142.7, 125.1, 122.8, 107.7, 84.7, 70.3, 61.6, 61.1, 58.2, 56.3, 36.8, 34.4, 33.2. IR (thin film on NaCl): 3462 (br OH), 2942 (m), 2838 (w), 1601 (w), 1549 (s), 1497 (s), 1467 (s), 1419 (m), 1377 (m), 1282 (m), 1231 (w), 1099 (s), 1000 (m), 910 (w), 800 (w) cm^{-1}. Elemental analysis calcd for $C_{15}H_{20}ClNO_6$: C, 52.10; H, 5.83; N, 4.05. Found: C, 52.02; H, 5.82; N, 3.84. Mp 126–127°C.

1.3.6. (±)-2*R*,4*R*,5*S*-2-Methoxy-4-nitro-5-(2,3,4-trimethoxyphenyl)cyclohexanone (16). To a solution of compound **13** (0.04 g, 0.12 mmol) dissolved in dichloromethane (5 mL) was added commercially available Dess–Martin Periodinane (0.36 g, 15 wt% solution in dichloromethane).[18] The reaction was stirred for 1.5 h at rt. The mixture was quenched with saturated sodium bicarbonate and diluted with ether. Sodium thiosulfate (0.11 g, 0.7 mmol) was added. After 10 min, the ether layer was separated and washed with saturated sodium bicarbonate solution (1×5 mL), water (1×5 mL), and brine (1×5 mL). The organic layer was dried over magnesium sulfate, filtered and concentrated in vacuo. Flash column chromatography (20–50% ethyl acetate/hexanes) afforded 0.30 g product as a clear, colorless oil (75% yield, 100% yield based on recovered starting material). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.85 (1H, d, J=8.6 Hz), 6.61 (1H, d, J=8.6 Hz), 5.49 (1H, dt, J=11.1, 3.9 Hz), 3.97 (3H, s), 3.84 (3H+H, s), 3.83 (3H, s), 3.72 (1H, m), 3.39 (3H, s), 3.13 (1H, br t, J=13.3 Hz), 2.71 (1H, dt, J=13.5, 4.5 Hz), 2.46 (2H, m). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 207.1, 154.1, 152.2, 142.6, 123.7, 122.9, 107.6, 84.1, 81.4, 64.3, 61.5, 61.0, 58.0, 56.3, 42.0, 36.5. IR (thin film on NaCl): 2938 (m), 2833 (w), 1728 (s), 1600 (w), 1553 (s), 1497 (s), 1468 (s), 1421 (m), 1376 (m), 1300 (m), 1280 (m), 1101 (s) cm^{-1}. High resolution ESI-FTMS (*m/z*): (M+Na) 362.1215, calcd for $[C_{16}H_{21}NO_7]$Na 362.1216.

1.3.7. (±)-2*R*,4*S*,5*R*-2-Methoxy-5-nitro-4-(2,3,4-trimethoxyphenyl)cyclohexanone (17). *Procedure A*. To a solution of compound **14** (0.03 g, 0.09 mmol) dissolved in dichloromethane (5 mL) was added commercially available Dess–Martin Periodinane (0.27 g, 15 wt% solution in dichloromethane).[18] The reaction was stirred for 1.5 h at rt. The mixture was quenched with saturated sodium bicarbonate and diluted with ether. Sodium thiosulfate (0.11 g, 0.7 mmol) was added. After 10 min, the ether

layer was separated and washed with saturated sodium bicarbonate solution (1×5 mL), water (1×5 mL), and brine (1×5 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated in vacuo. Flash column chromatography (silica gel, 20–50% ethyl acetate/hexanes) afforded **17** (0.20 g, 67%) as a clear, colorless oil.

Procedure B. To a solution of **14** (0.11 g, 0.32 mmol) dissolved in dichloromethane (5 mL) was added PCC (0.08 g, 0.39 mmol). After the mixture was allowed to stir overnight, TLC showed the reaction was complete. The reaction was diluted with ether. It was then filtered through a small column of silica gel eluted with ether, followed by 50% ethyl acetate/hexanes. The solution was concentrated in vacuo to afford **17** (0.10 g, 100%) as a clear, colorless oil. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.81 (1H, d, J=8.6 Hz), 6.59 (1H, d, J=8.6 Hz), 5.14 (1H, dt, J=12.0, 4.5 Hz), 4.10 (1H, dd, J=11.7, 3.9 Hz), 3.96 (3H, s), 3.84 (6H, s), 3.58 (2H, m), 3.39 (3H, s), 2.86 (1H, dd, J=12.9, 4.5 Hz), 2.31 (1H, dt, J=14.7, 3.6 Hz), 2.11 (1H, dt, J=12.9, 2.4 Hz). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 205.3, 153.6, 152.3, 142.3, 123.3, 122.8, 107.1, 86.9, 81.6, 61.1, 60.6, 57.3, 55.9, 41.8, 38.1, 35.0. IR (thin film on NaCl): 2938 (m), 2832 (w), 1732 (s), 1601 (w), 1556 (s), 1497 (s), 1467 (s), 1419 (m), 1279 (m), 1100 (s) cm^{-1}. High resolution ESI-FTMS (*m*/*z*): (M+Na) 362.1218, calcd for $[C_{16}H_{21}NO_7]$Na 362.1216. Compound **18** was also observed: (M+Na) 315.1209, calcd for $[C_{16}H_{20}O_5]$Na 315.1204.

1.4. Biological assays

Cytotoxicity towards HCT-116 colon cancer cells was measured in 96-well microtiter plates using the sulforhodamine B assay described by Skehan et al.[21] Inhibition of tobacco root growth was determined according to procedures described previously.[22]

1.5. Molecular modeling

The X-ray structure of colchicine (**2**)[26] was imported into Sybyl® 6.6 software.[27] The 3D structure of parent PCH **1** was generated by first setting the torsional angles of the three methoxy groups to be the same as those in the X-ray structure of colchicine. The resulting structure was minimized using the Tripos force field with Gasteiger–Huckel charges. The minimized conformation of the cyclohexene ring of **1** is a diequatorial pseudochair. Two local minima were identified for the aryl-cyclohexyl bond torsional angle: conformer A had an angle of 234.5° and conformer B had an angle of 62.1°. When conformer A of **1** was overlapped with **2** by superimposing the 2,3,4-trimethoxyphenyl rings of the two compounds, the nitro group of **1** remained within the molecular volume occupied by **2**. By contrast, overlap of conformer B of **1** with **2** put the nitro group of **1** outside the colchicine molecular volume. We concluded that conformer B was unlikely to be biologically relevant and focused on the relationship between colchicine and conformer A of PCHs **16** and **17** derived from **1**.

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