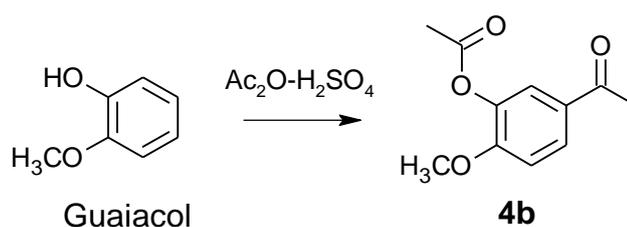


A convenient synthesis of *cis*-restricted combretastatin analogues with pyrazole and isoxazole cores

Dmitry V. Tsyganov, Marina N. Semenova, Leonid D. Konyushkin, Vladimir I. Ushkarov, Mikhail M. Raihstat and Victor V. Semenov

Chemistry. Materials and methods. Melting points were measured using a Boetius melting point apparatus and were uncorrected. ^1H NMR and ^{13}C NMR spectra were recorded on a Bruker DRX-500 instrument [working frequencies of 500.13 MHz (^1H) and 125.76 MHz (^{13}C), respectively]. Chemical shifts were stated in parts per million (ppm) and referenced to the appropriate NMR solvent peaks. Spin-spin coupling constants (J) were reported in Hertz (Hz). Low resolution mass spectra (m/z) were recorded on a Finnigan MAT/INCOS 50 mass spectrometer at 70 eV using direct probe injection. High resolution mass spectra (HRMS) were measured on Bruker maXis and micrOTOF II instruments using electrospray ionization (ESI). Elemental analysis was performed on the automated Perkin-Elmer 2400 CHN micro-analyzer. Flash chromatography was accomplished using silica (Acros, 0.035–0.070 mm, 60 Å). Thin layer chromatography (TLC) analysis was performed using Merck 60 F₂₅₄ plates. Solvents, benzaldehydes **3a,b,d** and acetophenones **4a,c** were purchased from Acros Organics (Belgium) at the highest commercial quality and used as received. Synthesis of aldehydes **3e,f** was described earlier.¹ 3-Acetoxy-4-methoxyacetophenone **4b** was synthesized from guaiacol according to procedure.²

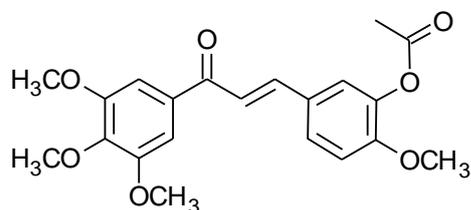


Synthesis of chalcones 5 (general procedure).^{3,4} NaOH (60 mmol) was added to a vigorously stirred solution of benzaldehyde **3** (20 mmol) and arylacetophenone **4** (20 mmol) in EtOH (150 ml) at +5 °C (ice bath). The reaction mixture was stirred at room temperature for 24 h, concentrated *in vacuo*, the residue was treated with distilled water (130 ml), neutralized with 15% HCl and extracted with CH_2Cl_2 (3 \times 80 ml). Organic extracts were washed with brine (2 \times

80 ml), dried over anhydrous Na₂SO₄ and concentrated *in vacuo*. The resulting residue was crystallized from EtOAc–petroleum ether (1:1) and dried to afford chalcones **5**.

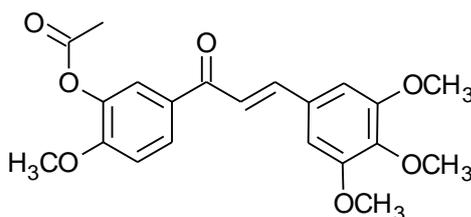
Synthesis of chalcone acetates 5-Ac (general procedure). A mixture of AcCl (1.18 g, 15 mmol) and pyridine (1.19 g, 15 mmol) in abs. CH₂Cl₂ (15 ml) was added by small portions to a stirred solution of chalcone **5** (10 mmol) and pyridine (0.79 g, 10 mmol) in abs. CH₂Cl₂ (50 ml) at 20 °C. The reaction mixture was stirred at room temperature for 3 h, diluted with water (50 ml), and the organic layer was separated. Water layer was extracted by CH₂Cl₂ (30 ml). Combined organic extracts were washed with water (3 × 50 ml), dried with Na₂SO₄ and evaporated. The residue was crystallized from EtOAc–petroleum ether (1:1) and dried to afford chalcone acetates **5a-Ac**.

2-Methoxy-5-[(1E)-3-oxo-3-(3,4,5-trimethoxyphenyl)-1-propenyl]phenyl acetate (5a-Ac).



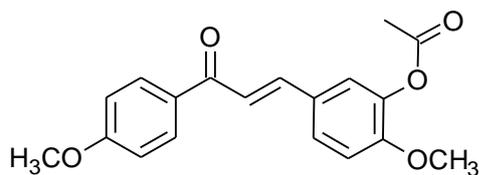
Yellow crystals; 2.1 g (72%); mp 114–116 °C; ¹H NMR (DMSO-*d*₆) δ: 7.84 (1H, d, *J* 15.5 Hz, H-2), 7.82 (1H, d, *J* 2.2 Hz, H-6'), 7.74 (1H, dd, *J* 8.6 Hz, *J* 2.2 Hz, H-4'), 7.71 (1H, d, *J* 15.5 Hz, H-1), 7.43 (2H, s, H-2'',6''), 7.21 (1H, d, *J* 8.6 Hz, H-3'), 3.91 (6H, s, OMe-3'',5''), 3.85 (3H, s, OMe), 3.77 (3H, s, OMe), 2.30 (3H, s, MeC(O)); EIMS *m/z*: 386 (M⁺, 15%), 344 (28), 329 (21), 313 (9), 301 (7), 195 (18), 177 (15), 156 (13), 89 (12), 77 (16), 43 (100). Anal. Calc. for C₂₁H₂₂O₇: C, 65.28; H, 5.74. Found: C, 65.37; H, 5.78.

2-Methoxy-5-[(2E)-3-(3,4,5-trimethoxyphenyl)-2-propenoyl]phenyl acetate (5b-Ac).



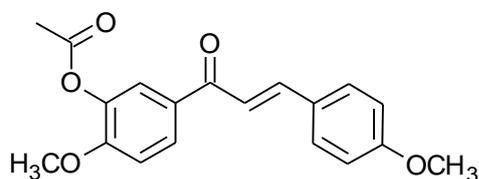
Yellow crystals; 7.7 g (86%); mp 135–137 °C; ¹H NMR (DMSO-*d*₆) δ: 8.16 (1H, dd, *J* 8.6, 1.9 Hz, H-4'), 7.93 (1H, d, *J* 1.9 Hz, H-6'), 7.88 (1H, d, *J* 15.5 Hz, H-2), 7.69 (1H, d, *J* 15.5 Hz, H-3), 7.30 (1H, d, *J* 8.7 Hz, H-3'), 7.24 (2H, s, H-2'',6''), 3.90 (3H, s, OMe), 3.87 (6H, s, 2 OMe), 3.71 (3H, s, OMe), 2.30 (3H, s, MeC(O)). Anal. Calc. for C₂₁H₂₂O₇: C, 65.28; H, 5.74. Found: C, 65.00; H, 5.79.

2-Methoxy-5-[(1E)-3-(4-methoxyphenyl)-3-oxo-1-propenyl]phenyl acetate (5c-Ac).



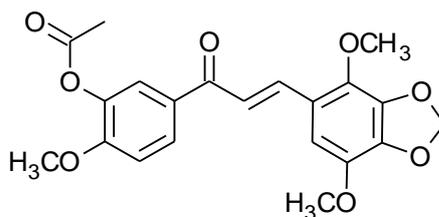
Yellow crystals; 0.46 g (81%); mp 136–138 °C (EtOAc–petroleum ether, 1:1); $^1\text{H NMR}$ (DMSO- d_6) δ : 8.17 (2H, d, J 8.9 Hz, H-2'',6''), 7.85 (1H, d, J 15.5 Hz, H-2), 7.79 (1H, d, J 2.1 Hz, H-6'), 7.72 (1H, dd, J 8.6, 2.1 Hz, H-4'), 7.66 (1H, d, J 15.5 Hz, H-1), 7.20 (1H, d, J 8.6 Hz, H-3'), 7.08 (2H, d, J 8.9 Hz, H-3'',5''), 3.87 (3H, s, OMe), 3.84 (3H, s, OMe), 2.30 (3H, s, MeC(O)); EIMS m/z : 326 (M^+ , 6%), 284 (14), 269 (9), 135 (22), 92 (13), 77 (25), 43 (100). Anal. Calc. for $\text{C}_{19}\text{H}_{18}\text{O}_5$: C, 69.93; H, 5.56. Found: C, 69.99; H, 5.59.

2-Methoxy-5-[(2E)-3-(4-methoxyphenyl)-2-propenoyl]phenyl acetate (5d-Ac).



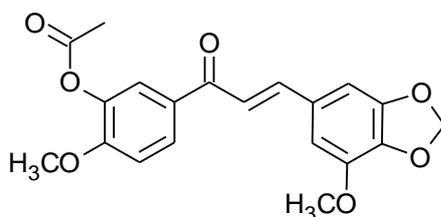
Yellow crystals; 2.9 g (89%); mp 126–128 °C; $^1\text{H NMR}$ (DMSO- d_6) δ : 8.13 (1H, dd, J 8.7, 2.0 Hz, H-4'), 7.93 (1H, d, J 2.0 Hz, H-6'), 7.86 (2H, d, J 8.7 Hz, H-2'',6''), 7.81 (1H, d, J 15.5 Hz, H-2), 7.71 (1H, d, J 15.5 Hz, H-3), 7.30 (1H, d, J 8.7 Hz, H-3'), 7.02 (2H, d, J 8.7 Hz, H-3'',5''), 3.89 (3H, s, OMe), 3.83 (3H, s, OMe), 2.31 (3H, s, MeC(O)); EIMS m/z : 327 ($[\text{M}+1]^+$, 16%), 326 (M^+ , 78), 285 (20), 284 (100), 283 (30), 269 (43), 253 (16), 161 (41), 151 (21), 133 (14), 89 (7), 77 (8). Anal. Calc. for $\text{C}_{19}\text{H}_{18}\text{O}_5$: C, 69.93; H, 5.56. Found: C, 69.99; H, 5.59.

5-[(2E)-3-(4,7-Dimethoxy-2H-1,3-benzodioxol-5-yl) 2-propenoyl]phenyl acetate (5e-Ac).



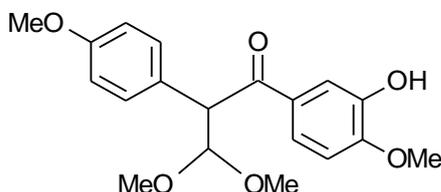
Yellow crystals; 2.97 g (88 %); mp 186–187 °C; $^1\text{H NMR}$ (DMSO- d_6) δ : 8.14 (1H, dd, J 8.7, 2.2 Hz, H-4'), 7.93 (1H, d, J 15.6 Hz, H-2), 7.89 (1H, d, J 2.2 Hz, H-6'), 7.80 (1H, d, J 15.6 Hz, H-3), 7.33 (1H, s, H-5''), 7.29 (1H, d, J 8.7 Hz, H-3'), 6.11 (2H, s, OCH₂O), 3.92 (3H, s, OMe), 3.90 (3H, s, OMe), 3.89 (3H, s, OMe), 2.30 (3H, s, MeC(O)). EIMS m/z : 400 (M^+ , 18%), 370 (21), 369 (100), 328 (20), 327 (96), 151 (26), 43 (54). Anal. Calc. for $\text{C}_{21}\text{H}_{20}\text{O}_8$: C, 63.00; H, 5.03. Found: C, 63.23; H, 4.86.

2-Methoxy-5-[(2E)-3-(7-methoxy-2H-1,3-benzodioxol-5-yl) 2-propenoyl]phenyl acetate (5f-Ac).



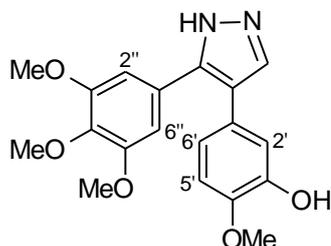
Yellow crystals; 7.6 g (87 %); mp 176–178 °C (EtOH); $^1\text{H NMR}$ ($\text{DMSO-}d_6$) δ : 8.14 (1H, dd, J 8.7, 2.2 Hz, H-4'), 7.93 (1H, d, J 2.2 Hz, H-6'), 7.83 (1H, d, J 15.4 Hz, H-2), 7.67 (1H, d, J 15.4 Hz, H-3), 7.33 (1H, d, J 2.4 Hz, H-4''), 7.29 (1H, d, J 8.7 Hz, H-3'), 7.20 (1H, d, J 2.4 Hz, H-6''), 6.09 (2H, s, OCH_2O), 3.90 (6H, s, 2OMe), 2.31 (3H, s, MeC(O)). Anal. Calc. for $\text{C}_{20}\text{H}_{18}\text{O}_7$: C, 64.86; H, 4.90. Found: C, 64.65; H, 5.09.

1-(3-Hydroxy-4-methoxyphenyl)-3,3-dimethoxy-2-(4-methoxyphenyl)-1-propanone (6d).



White solid; 5.82 g (84%); mp 104–107 °C (EtOAc–petroleum ether, 1:1); $^1\text{H NMR}$ (CDCl_3): δ : 7.56 (1H, dd, J 8.2, 2.1 Hz, H-6'), 7.55 (1H, d, J 2.1 Hz, H-2'), 7.32 (2H, d, J 8.7 Hz, H-2'',6''), 6.84 (1H, d, J 8.2 Hz, H-5'), 6.83 (2H, d, J 8.7 Hz, H-3'',5''), 5.61 (1H, s, OH), 5.07 (1H, d, J 8.5 Hz, H-2), 4.77 (1H, d, J 8.5 Hz, H-3), 3.91 (3H, s, OMe), 3.75 (3H, s, OMe), 3.43 (3H, s, OMe), 3.21 (3H, s, OMe); EIMS m/z : 347 $[\text{M}+1]^+$, 346 $[\text{M}]^+$. Anal. Calc. for $\text{C}_{19}\text{H}_{22}\text{O}_6$: C, 65.88; H, 6.40. Found: C, 66.02; H, 6.46.

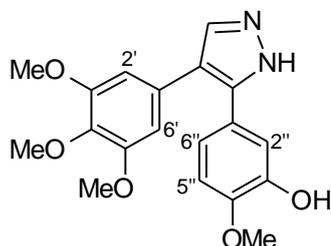
4-(3-Hydroxy-4-methoxyphenyl)-5-(3,4,5-trimethoxyphenyl)-1H-pyrazole (1a) (Scheme 1).



Light brown solid; 140 mg (72%); mp 171–173 °C (lit.⁵ 198–200 °C); $^1\text{H NMR}$ ($\text{DMSO-}d_6$) δ : 13.1 (0.5H, s, NH), 12.9 (0.5H, s, NH), 8.95 (1H, s, OH), 7.80 (0.5H, s, H-3), 7.56 (0.5H, s, H-3), 6.90 (1H, d, J 8.3 Hz, H-5'), 6.76 (2H, s, H-2'',6''), 6.75–6.66 (2H, m, H-2',6'), 3.75 (3H, s, OCH_3 -4'), 3.67 (6H, s, OMe-3'',5''), 3.63 (3H, s, OMe-4''); $^{13}\text{C NMR}$ ($\text{DMSO-}d_6$) δ : 152.6, 146.4, 146.3, 139.4, 137.1, 128.5, 126.2, 119.6, 119.3, 118.9, 115.8, 112.3, 104.9, 60.0, 56.0, 55.6; EIMS m/z : 357 ($[\text{M}+1]^+$, 21%), 356 (M^+ , 100), 341 (28), 162 (13), 155 (12), 140 (10), 133 (10),

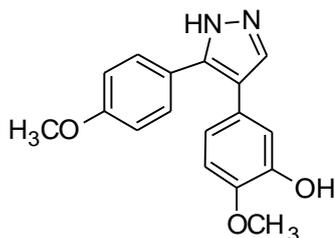
127 (10), 119 (49), 105 (14), 91 (21), 77 (15). Anal. Calc. for C₁₉H₂₀N₂O₅: C 64.04; H 5.66; N 7.86. Found: C 64.12; H 5.70; N 7.78.

5-(3-Hydroxy-4-methoxyphenyl)-4-(3,4,5-trimethoxyphenyl)-1H-pyrazole (**1b**) (Scheme 2).



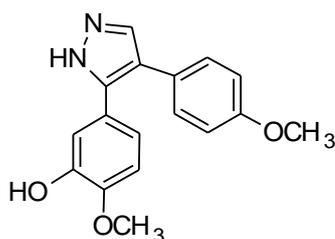
Gray solid; 32 mg (12%); mp 232–234 °C (lit.⁵ 90–93 °C); ¹H NMR (DMSO-*d*₆) δ: 13.08 (0.5H, s, NH), 13.00 (0.5H, s, NH), 9.04 (0.5H, br, OH), 8.99 (0.5H, br, OH), 7.30 (1H, m, H-6''), 7.21 (0.5H, s, H-3), 7.13 (2H, s, H-2',6'), 7.04 (0.5H, s, H-3), 7.02 (1H, s, H-2''), 6.98 (1H, m, H-5''), 3.86 (6H, s, OMe-3',5'), 3.81 (3H, s, OMe), 3.69 (3H, s, OMe); EIMS *m/z*: 357 ([M+1]⁺, 20%), 356 (M⁺, 100), 342 (6), 341 (40), 327 (1), 313 (7), 118 (11), 91 (16). Anal. Calc. for C₁₉H₂₀N₂O₅: C 64.04; H 5.66; N 7.86. Found: C 64.15; H 5.70; N 7.78.

4-(3-Hydroxy-4-methoxyphenyl)-5-(4-methoxyphenyl)-1H-pyrazole (**1c**) (Scheme 1).



White solid; 38 mg (32%); mp 151–153 °C; ¹H NMR (DMSO-*d*₆) δ: 12.97 (0.5H, s, NH), 12.83 (0.5H, s, NH), 8.88 (1H, s, OH), 7.77 (0.5H, s, H-3), 7.54 (0.5H, s, H-3), 7.34 (2H, d, *J* 7.6 Hz, H-2'',6''), 6.97 (1H, d, *J* 7.9 Hz, H-3'',5''), 6.88 (1H, d, *J* 7.9 Hz, H-3'',5''), 6.85 (1H, d, *J* 8.3 Hz, H-5'), 6.70–6.64 (2H, m, H-2',6'), 3.77 (3H, s, OMe), 3.75 (3H, s, OMe); ¹³C NMR (DMSO-*d*₆) δ: 158.4, 146.2, 139.2, 128.6, 126.4, 119.4, 118.6, 117.9, 115.7, 115.2, 114.4, 113.5, 112.3, 55.5, 55.0; EIMS *m/z*: 297 ([M+1]⁺, 21%), 296 (M⁺, 100), 281 (22), 263 (12), 253 (12), 235 (16), 118 (10). Anal. Calc. for C₁₇H₁₆N₂O₃: C 68.91; H 5.44; N 9.45. Found: C 69.02; H 5.47; N 9.42.

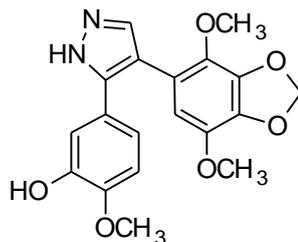
5-(3-Hydroxy-4-methoxyphenyl)-4-(4-methoxyphenyl)-1H-pyrazole (**1d**) (Scheme 2).



White solid; 65 mg (48%); mp 166–168 °C; ¹H NMR (DMSO-*d*₆, 500 MHz) δ: 12.93 and 12.85 (1H, br, NH), 9.08 and 8.92 (1H, br, OH), 7.76 and 7.60 (1H, br, H-3), 7.19 (2H, d, *J* 8.4 Hz, H-

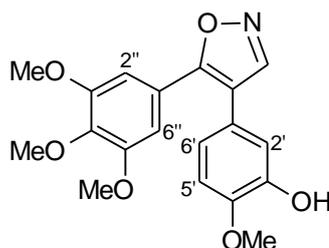
2',6'), 6.88 (2H, m, H-2'',6''), 6.88 (2H, d, *J* 8.4 Hz, H-3',5'), 6.79 (1H, d, *J* 8.0 Hz, H-5''), 3.77 (3H, s, OMe), 3.74 (3H, s, OMe); ¹³C NMR (DMSO-*d*₆) δ: 157.6, 147.3, 146.2, 139.1, 128.9, 128.4, 125.8, 122.5, 118.8, 117.7, 115.1, 113.8, 112.0, 55.5, 55.0; EIMS *m/z*: 297 ([M+1]⁺, 18%), 296 (M⁺, 100), 281 (7), 263 (8), 253 (6), 235 (12), 221 (11), 127 (12), 118 (12), 91 (12), 77 (30). Anal. Calc. for C₁₇H₁₆N₂O₃: C 68.91; H 5.44; N 9.45. Found: C 68.98; H 5.46; N 9.39.

4-(4,7-Dimethoxy-1,3-benzodioxol-5-yl)-5-(3-hydroxy-4-methoxyphenyl)-1H-pyrazole (**1e**)
(Scheme 2).



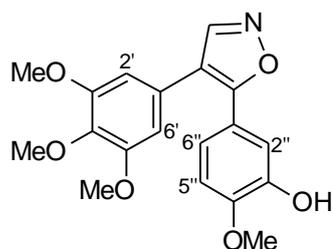
Dark gray solid; 25 mg (18%); mp 128–132 °C; ¹H NMR (DMSO-*d*₆) δ: 12.95 (0.5H, br, NH), 12.83 (0.5H, br, NH), 8.98 (1H, br, OH), 7.70 (0.5H, br, H-3), 7.48 (0.5H, br, H-3), 6.88 (2H, m, H-2'',6''), 6.77 (1H, d, *J* 7.6 Hz, H-5''), 6.02 (2H, s, OCH₂O), 3.74 (3H, s, OMe), 3.66 (3H, s, OMe), 3.48 (3H, s, OMe); ¹³C NMR (DMSO-*d*₆) δ: 146.1, 138.8, 138.5, 135.6, 135.2, 119.9, 117.9, 114.4, 113.6, 111.9, 109.6, 101.4, 59.2, 56.3, 55.4; EIMS *m/z*: 371 ([M+1]⁺, 20%), 370 (M⁺, 100), 325 (5), 171 (13), 170 (11), 169 (33), 140 (12), 127 (11). Calc. for C₁₉H₁₈N₂O₆: C 61.62; H 4.90; N 7.56. Found: C 61.74; H 4.93; N 7.47.

4-(3-Hydroxy-4-methoxyphenyl)-5-(3,4,5-trimethoxyphenyl)isoxazole (**2a**) (Scheme 1).



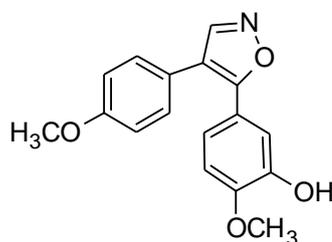
White crystals; 710 mg (55%); mp 158–160 °C (lit.⁶ mp 167–168 °C); ¹H NMR (DMSO-*d*₆) δ: 9.17 (1H, s, OH), 8.78 (1H, s, H-3), 7.0 (1H, d, *J* 8.0 Hz, H-5'), 6.92 (2H, s, H-2'',6''), 6.90 (1H, d, *J* 8.0 Hz, H-6'), 6.88 (1H, s, H-2'), 3.79 (3H, s, OMe-4'), 3.72 (3H, s, OMe-4''), 3.69 (6H, s, 2OMe-3',5'); ¹³C NMR (DMSO-*d*₆) δ: 162.2 (C-5), 153.1 (2C, C-3'',5''), 152.3 (C-3), 147.7 (C-4'), 146.8 (C-3'), 139.0 (C-4''), 122.5 (C-1'), 121.9 (C-1''), 119.7 (C-6'), 115.7 (C-4), 115.6 (C-2'), 112.6 (C-5'), 104.5 (2C, C-2'',6''), 60.2 (OMe), 55.9 (2OMe), 55.7 (OMe); EIMS *m/z*: 357 (M⁺, 100%), 358 (21), 342 (11), 314 (10), 195 (56). Anal. Calc. for C₁₉H₁₉NO₆: C 63.86; H 5.36; N 3.92. Found: C 63.74; H 5.29; N 3.97.

5-(3-Hydroxy-4-methoxyphenyl)-4-(3,4,5-trimethoxyphenyl)isoxazole (**2b**) (Scheme 2).



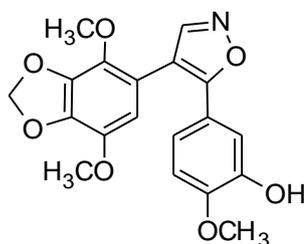
White crystals; 335 mg (26%); mp 171–173 °C (lit.⁷ no mp value and NMR spectra); ¹H NMR (DMSO-*d*₆) δ: 9.40 (1H, s, OH), 8.83 (1H, s, H-3), 7.11 (1H, d, *J* 2.1 Hz, H-2''), 7.10 (1H, dd, *J* 6.6, 2.1 Hz, H-6''), 7.03 (1H, d, *J* 6.6 Hz, H-5''), 6.74 (2H, s, H-2',6'), 3.81 (3H, s, OMe-4''), 3.71 (6H, s, 2OMe-3',5'), 3.70 (3H, s, OMe-4'); EIMS *m/z*: 357 (M⁺, 100%), 358 (20), 342 (14), 329 (12), 283 (17), 151 (44). Anal. Calc. for C₁₉H₁₉NO₆: C 63.86; H 5.36; N 3.92. Found: C 63.74; H 5.28; N 4.12.

5-(3-Hydroxy-4-methoxyphenyl)-4-(4-methoxyphenyl)isoxazole (**2d**) (Scheme 2).



White crystals; 560 mg (52%); mp 147–149 °C (lit.⁸ no mp value); ¹H NMR (DMSO-*d*₆) δ: 9.37 (1H, s, OH), 8.73 (1H, s, H-3), 7.35 (2H, d, *J* 8.7 Hz, H-2',6'), 7.01 (3H, br.s, H-2'',5'',6''), 6.99 (2H, d, *J* 8.7 Hz, H-3'',5''), 3.80 (3H, s, OMe), 3.79 (3H, s, OMe); ¹³C NMR (DMSO-*d*₆) δ: 162.8 (C-5), 158.9 (C-4'), 152.3 (C-3), 149.4 (C-4''), 146.7 (C-3''), 129.7 (2C, C-2',6'), 121.9 (C-1''), 119.8 (C-1'), 118.6 (C-6''), 114.4 (2C, C-3',5'), 113.8 (C-4, C-2''), 112.3 (C-5''), 55.6 (OMe), 55.2 (OMe); EIMS *m/z*: 297 (M⁺, 100%), 298 (19), 254 (39), 227 (13), 151 (65). Anal. Calc. for C₁₇H₁₅NO₄: C 68.68; H 5.09; N 4.71. Found: C 68.83; H 5.19; N 4.47.

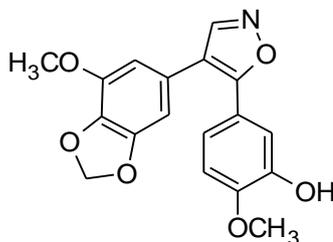
4-(4,7-Dimethoxy-1,3-benzodioxol-5-yl)-5-(3-hydroxy-4-methoxyphenyl)-isoxazole (**2e**) (Scheme 2).



White crystals; 307 mg (23%); mp 138–140 °C; ¹H NMR (DMSO-*d*₆) δ: 9.43 (1H, s, OH), 8.56 (1H, s, H-3), 7.01–6.99 (3H, m, H-2',5',6'), 6.53 (1H, s, H-6'), 6.08 (2H, s, OCH₂O), 3.79 (3H, s, OMe), 3.72 (3H, s, OMe), 3.62 (3H, s, OMe); ¹³C NMR (DMSO-*d*₆) δ: 163.6 (C-5), 153.1 (C-3), 149.3 (C-4''), 146.6 (C-3''), 139.1 (C-3a'), 138.8 (C-7'), 136.8 (C-7a'), 135.9 (C-4'), 119.9 (C-1''),

118.1 (C-5'), 115.7 (C-5'), 113.3 (C-2''), 112.2 (C-5''), 110.3 (C-4), 109.6 (C-6'), 102.0 (C-2'), 59.7 (OMe), 56.6 (OMe), 55.6 (OMe). EIMS m/z : 371 (M^+ , 18%), 232 (10), 152 (11), 151 (100), 123 (13), 108 (10). Anal. Calc. for $C_{19}H_{17}NO_7$: C 61.45; H 4.61; N 3.77. Found: C 61.54; H 4.64; N 3.64.

4-(7-methoxy-1,3-benzodioxol-5-yl)-5-(3-hydroxy-4-methoxyphenyl)-isoxazole (**2f**)
(Scheme 2).



Light yellow crystals; 220 mg (18%); mp 166 °C; 1H NMR (DMSO- d_6) δ : 9.4 (1H, s, OH), 8.77 (1H, s, H-3), 7.04 (3H, m, H-2'',5'',6''), 6.72 (1H, d, J 1.5 Hz, H-4'), 6.64 (1H, d, J 1.5 Hz, H-6'), 6.03 (2H, s, OCH₂O), 3.81 (3H, s, OMe), 3.78 (3H, s, OMe); ^{13}C NMR (DMSO- d_6) δ : 163.1 (C-5), 152.3 (C-3), 149.5 (C-4''), 148.8 (C-3''), 146.7 (C-3a'), 143.5 (C-7'), 134.6 (C-7a'), 123.8 (C-5'), 119.6 (C-1''), 118.8 (C-6''), 114.6 (C-4), 113.9 (C-2''), 112.4 (C-5''), 108.4 (C-6'), 102.4 (C-4'), 101.5 (C-2'), 56.3 (OMe), 55.6 (OMe); EIMS m/z : 341 (M^+ , 24%), 342 (5), 152 (11), 151 (100), 123 (12). Anal. Calc. for $C_{18}H_{15}NO_6$: C 63.34; H 4.43; N 4.10. Found: C 63.42; H 4.47; N 4.03.

Biology. *Phenotypic sea urchin embryo assay.*⁹ Adult sea urchins, *Paracentrotus lividus* Lamarck (Echinidae), were collected from the Mediterranean Sea on the Cyprus coast and kept in an aerated seawater tank. Gametes were obtained by intracoelomic injection of 0.5 M KCl. Eggs were washed with filtered seawater and fertilized by adding drops of diluted sperm. Embryos were cultured at room temperature under gentle agitation with a motor-driven plastic paddle (60 rpm) in filtered seawater. The embryos were observed with a Biolam light microscope (LOMO, St. Petersburg, Russia). For treatment with the test compounds, 5 ml aliquots of embryo suspension were transferred to six-well plates and incubated as a monolayer at a concentration up to 2000 embryos/ml. Stock solutions of test compounds were prepared in DMSO at 10 mmol dm⁻³ concentration followed by a 10-fold dilution with 96% EtOH. This procedure enhanced the solubility of the test compounds in the salt-containing medium (seawater), as evidenced by microscopic examination of the samples. The maximal tolerated concentrations of DMSO and EtOH in the *in vivo* assay were determined to be 0.2% and 0.5%, respectively. Higher concentrations of either DMSO ($\geq 0.5\%$) or EtOH ($\geq 1\%$) caused nonspecific alteration and retardation of the sea urchin embryo development, independently of the treatment

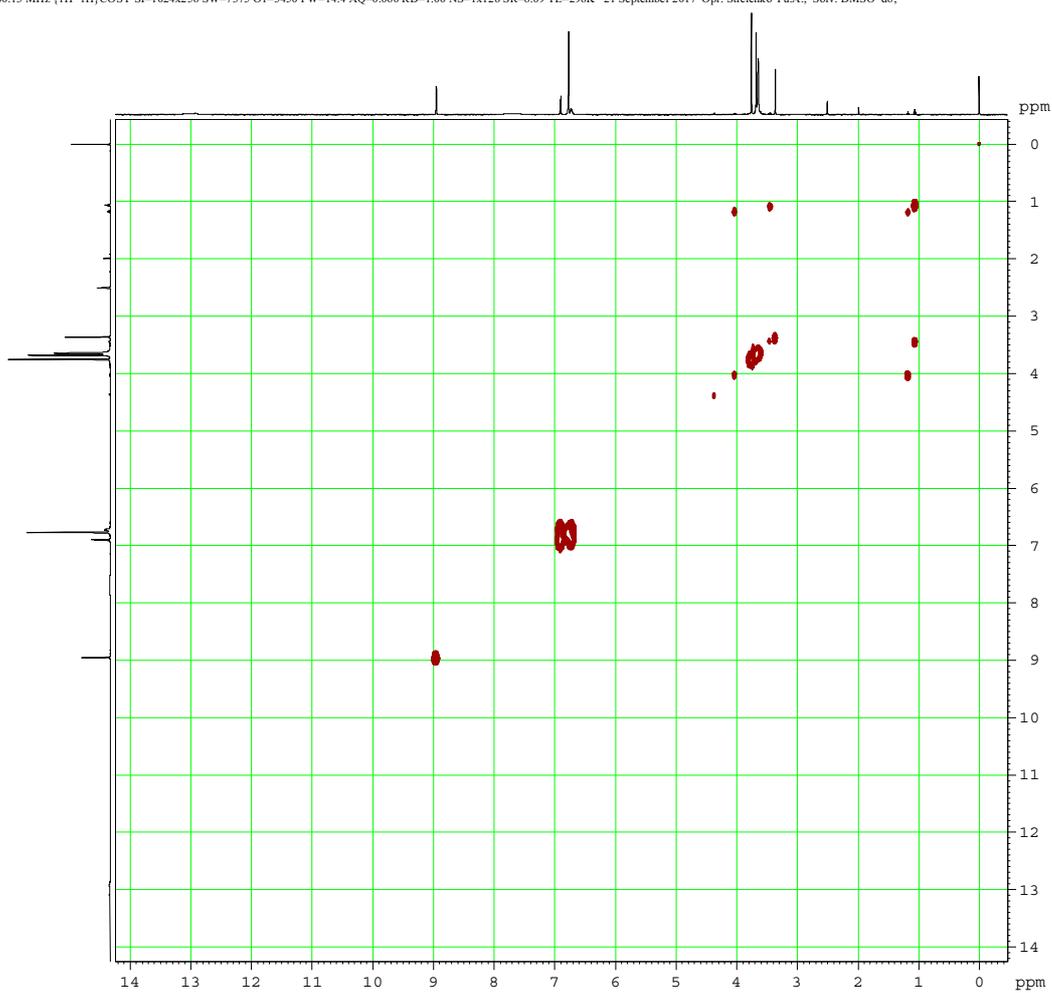
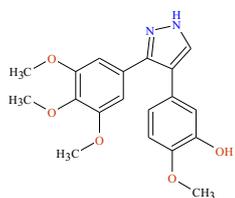
stage. Combretastatins A-4 and A-2 (synthesized as reported previously^{1,10} served as reference compounds. The antiproliferative activity was assessed by exposing fertilized eggs (8–15 min after fertilization, 45–55 min before the first mitotic cycle completion) to two-fold decreasing concentrations of the compound. Cleavage alteration and arrest were clearly detected at 2.5 h and 5.5 h after fertilization, when control embryos reached eight-cell and early blastula stages, respectively. The effects were estimated quantitatively as an effective threshold concentration (lowest observed effect concentration, LOEC) resulting in cleavage alteration and embryo death before hatching or full mitotic arrest. At these concentrations all tested microtubule destabilizers caused 100% cleavage alteration and embryo death before hatching, whereas at two-fold lower concentrations the compounds failed to produce any effect. For microtubule-destabilizing activity, the compounds were tested on free-swimming blastulae just after hatching (8–10 h after fertilization), which originated from the same embryo culture. Embryo spinning was observed after 15 min to 20 h of treatment, depending on the structure and concentration of the compound. Both spinning and lack of forward movement were interpreted to be the result of the microtubule-destabilizing activity of a molecule. Video illustrations are available at <http://www.chemblock.com>. Sea urchin embryo assay data are available at <http://www.zelinsky.ru>. Experiments with the sea urchin embryos fulfill the requirements of biological ethics. The artificial spawning does not cause animal death, embryos develop outside the female organism, and both post spawned adult sea urchins and the excess of intact embryos are returned to the sea, their natural habitat.

References

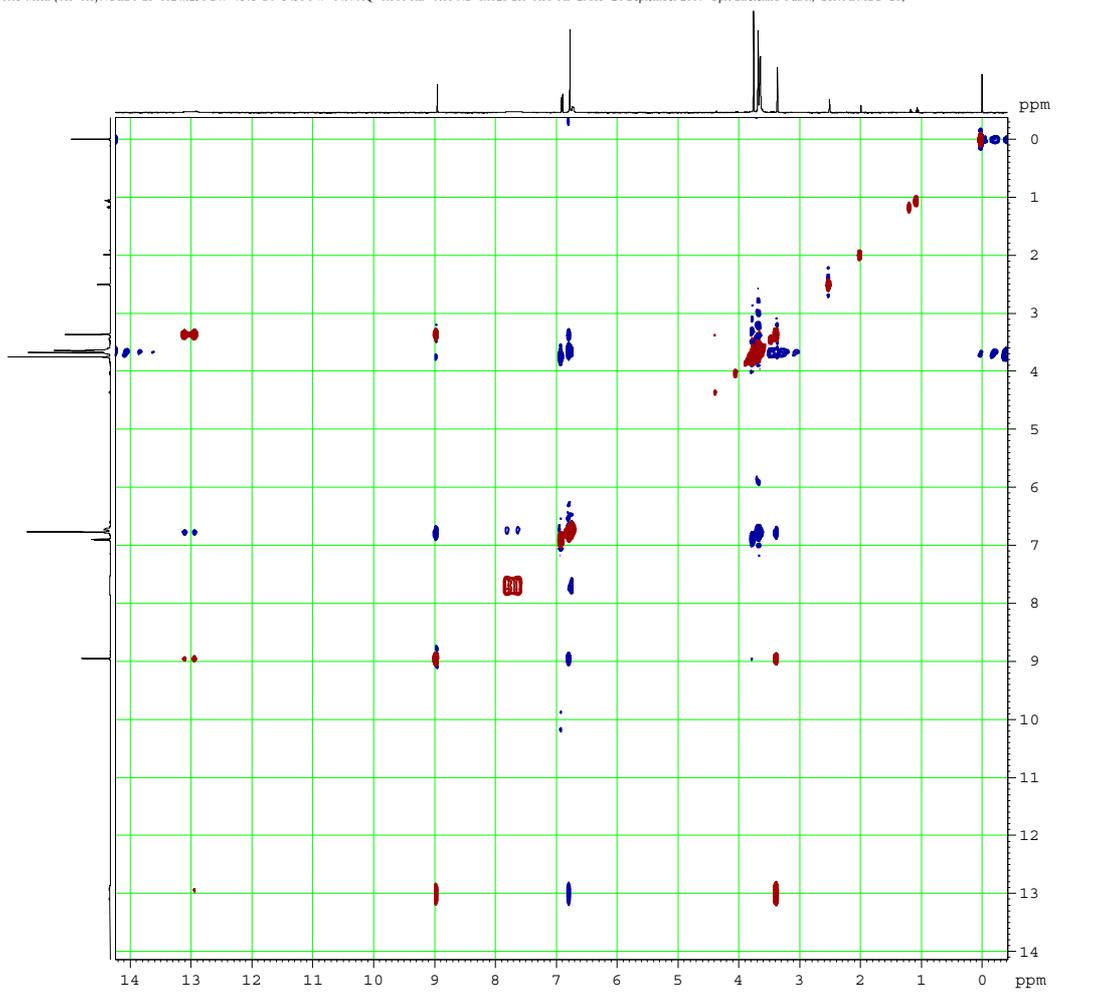
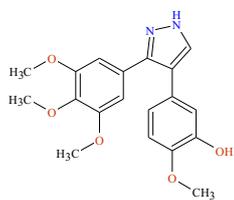
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1a (NMR/24267672)
COSY

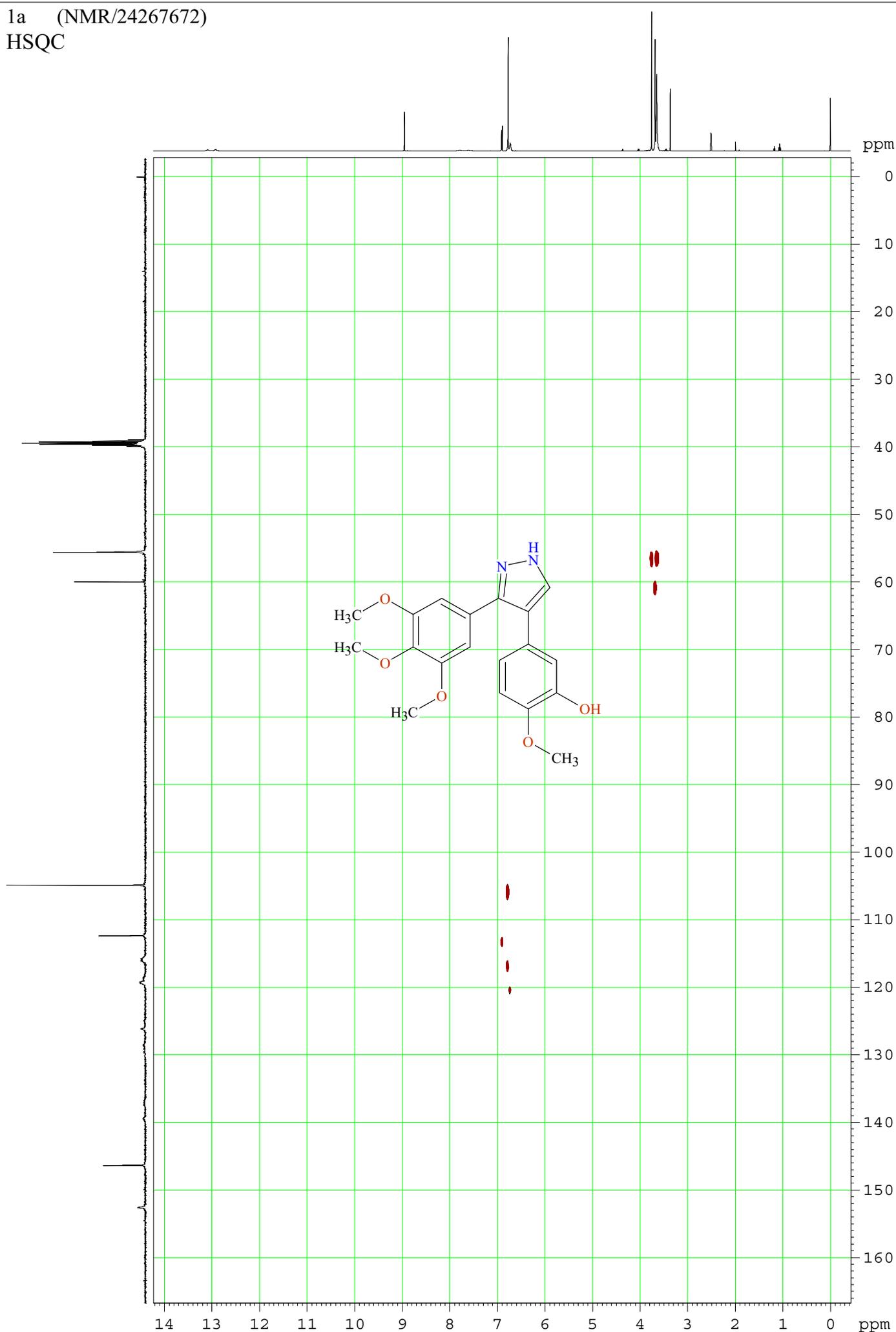


1a (NMR/24267672)
NOESY



1a (NMR/24267672)
HSQC

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1a (NMR/24267672)

HMBC

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