Supplementary data

7-Deacetyl-10-alkylthiocolchicine derivatives – new compounds with potent anticancer and fungicidal activity

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1. Chemistry

1.1. Materials

Colchicine 1 is commercially available on ApplyChem. For all reactions a natural isomer (-)-(\(aR,7S\)) was used. 10-Alkylthiocolchicine 2-6 were obtained according literature procedure from colchicine and respective sodium alkylthiolate RSNa\(^1\). Sodium alkylthiolates are commercially available of Fluka.


1.2. Experimental Measurements

The NMR spectra of 7-dacetyl-10-alkilthiocolchicines 7-11 (0.07 mol L\(^{-1}\)) were recorded in DMSO-\(d_6\) and CDCl\(_3\) solutions using a Varian Gemini 300 MHz spectrometer. All spectra were locked to deuterium resonance of DMSO. The \(^1\)H NMR measurements in DMSO-\(d_6\) and CDCl\(_3\) were carried out at the operating frequency 300.075 MHz; flip angle, \(\text{pw} = 45^\circ\); spectral width 4500 Hz; acquisition time 2.0 s; relaxation delay, \(d_1=1.0\) s; \(T = 293.0\) K and using TMS as the internal standard. No window function or zero filling was used. Digital resolution was 0.2 Hz per point. The error of chemical shift value was 0.01 ppm. \(^{13}\)C NMR spectra were recorded at the operating frequency 75.454 MHz; \(\text{pw} = 60^\circ\); \(\text{sw} = 19000\) Hz; \(at = 1.8\) s; \(d_1=1.0\) s; \(T = 293.0\) K and TMS as the internal standard. Line broadening parameters were 0.5 or 1 Hz. The error of chemical shift value was 0.01 ppm. The \(^1\)H and \(^{13}\)C NMR signals were assigned for each species using one or two-dimensional (COSY, HETCOR, HMBC) spectra. The FT IR spectra (0.07 mol dm\(^{-3}\)) were recorded in the mid infrared region in KBr pellets. The spectra were taken with an IFS 113v FT IR spectrophotometer (Bruker,
Karlsruhe) equipped with a DTGS detector; resolution 2 cm⁻¹, NSS = 125. A cell with Si windows and wedge-shaped layers was used to avoid interferences (mean layer thickness 170 μm). The Happ-Genzel apodization function was used. All manipulations with the substances were performed in a carefully dried and CO₂-free glove box. The EI mass spectra were recorded on a Waters/Micromass (Manchester, UK) ZQ mass spectrometer equipped with a Harvard Apparatus syringe pump. Elemental analysis (% C, N, S, H) was carried out by means of a Elementar Analyser Vario EL III. Melting point was determined on BUCHI SMP-20. Melt-Temp II apparatus (Laboratory Devices Inc.).

The EI mass spectra were recorded on an AMD-402 two-sector mass spectrometer (AMD Intectra GmbH Co. Harpstedt, Germany) with an acceleration voltage of 8 kV, electron energy 70 eV, mass resolution 6000, and an ion source temperature of ∼150 °C. The samples were introduced using a direct insertion probe. The UV-Vis spectra were recorded in methanol by JASCO V-550 spectrophotometer at 200-600 nm range.

1.3. Synthesis of 7-deacetyl-10-alkylthiocolchicines 7-11

100 mg of 2, 3, 4, 5 and 6 was dissolved in 5 mL of methanol. To this solution 100-150 mL of 1M hydrochloric acid in MeOH was added, then reaction mixture was refluxed for 6-16h (temperature ~70°C). The reaction progress was checked by TCL analysis (chloroform : aceton, 3:2, v/v). New derivatives 7-11 were obtained with very good yields. Carbon atoms numbering of colchicine 1 and derivatives 2-11 are given below in Figure 1.

![Figure S1. Carbon atom numbering of Colchicine 1 and derivatives 2-11.](image)
7-deacetyl-10-methylthiocolchicine (7)

The title compound was prepared from 10-methylthiocolchicine 2 and methanolic solution of hydrochloric acid. M.p. 183-186°C, yield 86%, $^1$H NMR (300 Hz, DMSO-$d_6$, TMS, ppm): 6.84 (HC-4, s), 2.21, 2.69 (HC-5, m), 1.98, 2.51 (HC-6, m), 2.32 (HC-7, m), 7.07 (HC-8, s), 7.35 (HC-11, d), 7.20 (HC-12, d), 3.62 (H$_3$C-13, s), 3.85 (H$_3$C-14, s), 3.79 (H$_3$C-15, s), 8.79 (NH), 2.57 (H$_3$C-16, s); $^{13}$C NMR (75 MHz, DMSO-$d_6$, TMS, ppm): 144.88 (C-1), 124.52 (C-1a) 140.84 (C-2), 153.51 (C-3), 107.82 (C-4), 134.90 (C-4a), 28.58 (C-5), 34.75 (C-6), 52.57 (C-7), 150.38 (C-7a), 128.20 (C-8), 180.78 (C-9), 157.93 (C-10), 126.98 (C-11), 133.64 (C-12), 136.48 (C-12a), 61.02 (C-13) 60.60 (C-14) 55.4 (C-15) 14.42 (C-16),

$^{13}$C NMR (75 MHz, CDCl$_3$, TMS, ppm): 145.84 (C-1), 124.49 (C-1a), 141.48 (C-2), 154.08 (C-3), 107.63 (C-4), 137.98 (C-4a), 29.51 (C-5), 35.59 (C-6), 54.02 (C-7), 150.90 (C-7a), 129.24 (C-8), 181.71 (C-9), 159.05 (C-10), 127.39 (C-11), 135.82 (C-12), 133.69 (C-12a), 61.67 (C-13), 61.17 (C-14) 56.02 (C-15) 15.05 (C-16), $^1$H NMR (300 Hz, CDCl$_3$, TMS, ppm): 6.56 (HC-4, s), 2.36, 2.56 (HC-5, m), 1.96, 2.45 (HC-6, m), 4.70 (HC-7, m), 7.63 (HC-8, s), 7.08 (HC-11, d), 7.34 (HC-12, d), 3.71 (H$_3$C-13, s), 3.93 (H$_3$C-14, s), 3.87 (H$_3$C-15, s), 9.19 (NH), 2.36 (H$_3$C-16, s); Anal. elem. calc. for C$_{20}$H$_{23}$NO$_4$S·3.5H$_2$O C 55.04, H 6.88, N 3.21, S 7.33 %; found: C 54.88, H 6.84, N 2.89, S 5.53 %. UV (CH$_3$OH) [nm]: $\lambda_{max1}$ 360, $\lambda_{max2}$ 245; FT IR (KBr): 3377 (NH), 2933, 2858, 1598 (C=O), 1535, 1487, 1232, 1138, 1092, 844 (C-S).

7-deacetyl-10-ethylthiocolchicine (8)

The title compound was prepared from 10-ethylthiocolchicine 3 and methanolic solution of hydrochloric acid. M.p. 174-176°C, yield 80%, $^1$H NMR (300 Hz, DMSO- $d_6$, TMS, ppm): 6.85 (HC-4, s), 2.23, 2.70 (HC-5, m), 1.97, 2.51 (HC-6, m), 2.32 (HC-7, m), 7.08 (HC-8, s), 7.41 (HC-11, d), 7.18 (HC-12, d), 3.69 (H$_3$C-13, s), 3.86 (H$_3$C-14, s), 3.79 (H$_3$C-15, s), 8.89
(NH), 2.57 (H$_3$C-16, s); $^{13}$C NMR (75 MHz, DMSO-$d_6$, TMS, ppm): 144.79 (C-1), 124.51 (C-1a), 140.82 (C-2), 153.48 (C-3), 107.81 (C-4), 134.87 (C-4a), 28.59 (C-5) 34.70 (C-6) 52.53 (C-7) 150.37 (C-7a), 128.48 (C-8), 180.79 (C-9), 156.66 (C-10), 127.21 (C-11), 133.64 (C-12), 136.60 (C-12a), 61.00 (C-15) 60.58 (C-15) 55.92 (C-16), 24.48 (C-17), 12.51 (C-16),

$^{13}$C NMR (75 MHz, CDCl$_3$, TMS, ppm): 145.62 (C-1), 124.52 (C-1a), 141.49 (C-2), 154.01 (C-3), 107.60 (C-4), 138.10 (C-4a), 29.51 (C-5), 35.44 (C-6), 53.98 (C-7), 150.92 (C-7a), 129.41 (C-8), 181.62 (C-9), 158.16 (C-10), 127.67 (C-11), 135.74 (C-12), 133.70 (C-12a), 61.74 (C-13) 61.17 (C-14), 56.00 (C-15), 25.43 (C-17), 12.35 (C-16), $^1$H NMR (300 Hz, CDCl$_3$, TMS, ppm): 6.55 (HC-4, s), 2.47, 2.69 (HC-5, m), 1.99, 2.55 (HC-6, m), 4.27 (HC-7, m), 7.62 (HC-8, s), 7.29 (HC-11, d), 7.14 (HC-12, d), 3.74 (H$_3$C-13, s), 3.94 (H$_3$C-14, s), 3.92 (H$_3$C-15, s), 9.52 (NH), 1.40 (H$_2$C-16, s), 2.83 (H$_2$C-17);

Anal. elem. calc. for C$_{21}$H$_{25}$NO$_4$S·3H$_2$O C 57.14, H 7.02, N 3.17, S 7.25 %, found C 57.60, H 7.58, N 2.90, S 6.53 %; UV (CH$_3$OH) [nm]: $\lambda_{\text{max}1}$ 375, $\lambda_{\text{max}2}$ 245; FT IR (KBr): 3375 (NH), 2931, 1596 (C=O), 1538, 1487, 1234, 1136, 1092, 844 (C-S).

**7-deacetyl-10-\(n\)-propylthiocolchicine (9)**

The title compound was prepared from 10-\(n\)-propylthiocolchicine and methanolic solution of hydrochloric acid. m.p. 133-135°C, yield 73%, $^1$H NMR (300 Hz, DMSO- $d_6$, TMS, ppm): 6.84 (HC-4, s), 2.23, 2.68 (HC-5, m), 1.99, 2.51 (HC-6, m), 2.34 (HC-7, m), 7.08 (HC-8, s), 7.41 (HC-11, d), 7.18 (HC-12, d), 3.62 (H$_3$C-13, s), 3.86 (H$_3$C-14, s), 3.79 (H$_3$C-15, s), 8.88 (NH), 2.92 (H$_2$C-18),1.69 (H$_2$C-17), 1.04 (H$_3$C-16); $^{13}$C NMR (75 MHz, DMSO-$d_6$, TMS, ppm): 144.77 (C-1), 124.53 (C-1a), 140.83 (C-2), 153.49 (C-3), 107.82 (C-4), 134.87 (C-4a), 28.59 (C-5), 34.71 (C-6), 52.53 (C-7), 150.38 (C-7a), 128.41 (C-8), 180.81 (C-9), 156.83 (C-10), 127.25 (C-11), 133.65 (C-12), 136.58 (C-12a), 61.01 (C-13), 60.59 (C-14), 55.92 (C-15), 32.28 (C-18), 20.65 (C-17), 13.55 (C-16),

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$^{13}$C NMR (75 MHz, CDCl$_3$, TMS, ppm): 145.71 (C-1), 124.56 (C-1a), 141.46 (C-2), 153.95 (C-3), 107.59 (C-4), 137.94 (C-4a), 29.56 (C-5), 35.47 (C-6), 54.00 (C-7), 150.93 (C-7a), 129.36 (C-8), 181.61 (C-9), 158.29 (C-10), 127.60 (C-11), 135.62 (C-12), 133.76 (C-12a), 61.77 (C-13), 61.19 (C-14), 55.99 (C-15), 13.85 (C-16), 20.88 (C-17), 33.43 (C-18), $^1$H NMR (300 Hz, CDCl$_3$, TMS, ppm): 6.56 (HC-4, s), 2.21, 2.55 (HC-5, m), 1.98, 2.49 (HC-6, m), 4.26 (HC-7, m), 7.64 (HC-8, s), 7.27 (HC-11, d), 7.12 (HC-12, d), 3.94 (H$_3$C-13, s), 3.92 (H$_3$C-14, s), 9.62 (NH), 2.80 (H$_3$C-16, s), 1.76 (H$_2$C-17, m), 1.10 (H$_2$C-18, t); Anal. elem. calc. for C$_{22}$H$_{27}$NO$_4$S$\cdot$3H$_2$O C 58.02, H 7.25, N 3.07, S 7.03 %, found C 58.53, H 7.64, N 2.69, S 5.89 %. UV (CH$_3$OH) [nm]: $\lambda_{\text{max}}$1 370, $\lambda_{\text{max}}$2 250; IR (KBr): 3386 (NH), 2959, 2869, 1597 (C=O), 1538, 1487, 1233, 1137, 1093, 841 (S-C).

7-deacetyl-10-$i$-propylthiocolchicine (10)

The title compound was prepared from 10-$i$-propylthiocolchicine and methanolic solution of hydrochloric acid. m.p. 165-167°C, yield 75%, $^1$H NMR (300 Hz, DMSO- $d_6$, TMS, ppm): 6.84 (HC-4, s), 2.23, 2.68 (HC-5, m), 1.98, 2.51 (HC-6, m), 2.92 (HC-7, m), 7.06 (HC-8, s), 7.47 (HC-11, d), 7.19 (HC-12, d), 3.62 (H$_3$C-13, s), 3.85 (H$_3$C-14, s), 3.78 (H$_3$C-15, s), 8.79 (NH), 2.32 (HC-17), 1.30, 1.36 (H$_3$C-16, s); $^{13}$C NMR (75 MHz, DMSO-$d_6$, TMS, ppm): 144.72 (C-1), 124.48 (C-1a), 140.83 (C-2), 153.50 (C-3), 107.82 (C-4), 134.90 (C-4a), 28.60 (C-5), 34.73 (C-6), 52.51 (C-7), 150.40 (C-7a), 128.66 (C-8), 180.87 (C-9), 155.89 (C-10), 127.93 (C-11), 133.67 (C-12), 136.68 (C-12a), 61.01 (C-13), 60.60 (C-14), 55.93 (C-15), 33.70 (C-17), 21.93, 21.93 (C-16). $^{13}$C NMR (75 MHz, CDCl$_3$, TMS, ppm): 145.47 (C-1), 124.51 (C-1a), 141.48 (C-2), 154.03 (C-3), 107.61 (C-4), 138.08 (C-4a), 29.57 (C-5), 35.47 (C-6), 54.00 (C-7), 150.96 (C-7a), 129.70 (C-8), 181.77 (C-9), 157.44 (C-10), 128.38 (C-11), 135.67 (C-12), 133.75 (C-12a), 61.85 (C-13) 61.22 (C-14), 56.03 (C-15), 34.48 (C-17), 22.02 (C-16$^x$2), $^1$H NMR (300 Hz, CDCl$_3$, TMS, ppm): 6.56 (HC-4, s), 2.21, 2.58 (HC-5, m), 1.98,
2.55 (HC-6, m), 4.25 (HC-7, m), 7.63 (HC-8, s), 7.27 (HC-11, d), 7.18 (HC-12, d), 3.94 (H$_3$C-13, s), 3.92 (H$_3$C-14, s), 3.71 (H$_3$C-15, s), 9.52 (NH), 1.24 (H$_3$C-16, s); 3.36 (H$_3$C-17, m), 3.94 (H$_3$C-13, s), 3.92 (H$_3$C-14, s), 3.71 (H$_3$C-15, s), 9.52 (NH), 1.24 (H$_3$C-16, s); 3.36 (H$_3$C-17, m), 3.94 (H$_3$C-13, s), 3.92 (H$_3$C-14, s), 3.71 (H$_3$C-15, s), 9.52 (NH), 1.24 (H$_3$C-16, s); 3.36 (H$_3$C-17, m), Anal. elem. calc. for C$_{22}$H$_{27}$NO$_4$S·4H$_2$O C 55.81, H 8.0, N 2.95, S 6.76 %, found C 55.64, H 7.41, N 2.66, S 6.55 %; UV (CH$_3$OH) [nm]: $\lambda_{\text{max}}$1 375, $\lambda_{\text{max}}$2 250; FT IR (KBr): 3381 (NH), 2963, 2930, 2866, 1597  (C=O), 1536, 1488, 1237, 1137, 1093, 844 (C-S).

7-deacetyl-10-\(n\)-buthylthiocolchicine (11)

The title compound was prepared from 10-\(n\)-buthylthiocolchicine and methanolic solution of hydrochloric acid. M.p. 140-142°C, yield 85%, $^1$H NMR (300 Hz, DMSO- $d_6$, TMS, ppm): 6.84 (HC-4, s), 2.25, 2.68 (HC-5, m), 1.98, 2.51 (HC-6, m), 2.32 (HC-7, m), 7.07 (HC-8, s), 7.41 (HC-11, d), 7.18 (HC-12, d), 3.62 (H$_3$C-13, s), 3.85 (H$_3$C-14, s), 3.79 (H$_3$C-15, s), 8.81 (NH), 2.94 (H$_2$C-19, t), 1.66 (H$_2$C-18, m), 1.47 (H$_2$C-17, m), 0.93 (H$_2$C-16, t). $^{13}$C NMR (75 MHz, DMSO-$d_6$, TMS, ppm): 144.77 (C-1), 124.52 (C-1a), 140.84 (C-2), 153.50 (C-3), 107.82 (C-4), 134.91 (C-4a), 28.59 (C-5), 34.74 (C-6), 52.54 (C-7), 150.39 (C-7a), 128.37 (C-8), 180.81 (C-9), 156.90 (C-10), 127.30 (C-11), 133.65 (C-12), 136.57 (C-12a), 61.02 (C-13), 60.60 (C-14), 55.93 (C-15), 30.11 (C-19), 29.17 (C-18), 21.70 (C-17), 13.54 (C-16), $^{13}$C NMR (75 MHz, CDCl$_3$, TMS, ppm): 145.57 (C-1), 124.64 (C-1a), 141.53 (C-2), 154.09 (C-3), 107.65 (C-4), 137.94 (C-4a), 29.44 (C-5), 35.40 (C-6), 54.06 (C-7), 151.06 (C-7a), 129.48 (C-8), 181.61 (C-9), 158.83 (C-10), 127.61 (C-11), 135.65 (C-12), 133.78 (C-12a), 61.87 (C-13), 61.24 (C-14), 56.04 (C-15), 31.21 (C-19), 29.59 (C-18), 22.38 (C-17), 13.64 (C-16), $^1$H NMR (300 Hz, CDCl$_3$, TMS, ppm): 6.56 (HC-4, s), 2.22, 2.71 (HC-5, m), 1.98, 2.56 (HC-6, m), 4.28 (HC-7, m), 7.65 (HC-8, s), 7.27 (HC-11, d), 7.10 (HC-12, d), 3.95 (H$_3$C-13, s), 3.92
(H_{3}C-14, s), 3.71 (H_{3}C-15, s), 9.60 (NH), 0.98 (H_{3}C-16, t), 1.51 (H_{2}C-17, m), 1.69 (H_{2}C-18, m), 2.66 (H_{2}C-19, t); Anal. elem. calc. for C_{23}H_{29}NO_{4}S \cdot 4.5H_{2}O C 55.6, H 7.66, 2.82 N, 6.45 S\%, found C 55.32, H 7.23, N 2.73, S 6.03 \%. UV (CH_{3}OH) [nm]: \lambda_{\text{max1}} 375, \lambda_{\text{max2}} 250; FT IR (KBr): 3387 (NH), 2953, 2930, 2866, 1596 (C=O), 1538, 1487, 1232, 1136, 1092, 844 (C-S).

1.4. EI MS mass spectra for 7-deacetyl-10-alkylthiocolchicines 7-11

Figure S2. EI MS mass spectra of 7-deacetyl-10-methylthiocolchicine 7
**Figure S3.** EI MS mass spectra of 7-deacetyl-10-ethylthiocolchicine 8

**Figure S4.** EI MS mass spectra of 7-deacetyl-10-\(n\)-propylthiocolchicine 9

**Fig S5.** EI MS mass spectra of 7-deacetyl-10-\(n\)-butylthiocolchicine 11
1.5. FT IR spectra of 7-deacetyl-10-alkylthiocolchicines 7-11

Fig. S6. FT IR spectra for 2 and 7 in the region of carbonyl group

Fig. S7. FT IR spectra for 3 and 8 in the region of carbonyl group
Fig. S8. FT IR spectra for 4 and 9 in the region of carbonyl group

Fig. S9. FT IR spectra for 5 and 10 in the region of carbonyl group
Fig. S10. FT IR spectra for 6 and 11 in the region of carbonyl group
$^{13}$C NMR

![C NMR spectrum]
$^1$H NMR

[H NMR spectrum]

[Chemical structure diagram]

[H$_3$CO] 15
[H$_3$CO] 14
[H$_3$CO] 13

[SCH$_2$CH$_2$CH$_2$CH$_3$]
$^1$H NMR

SCH$_2$CH$_2$CH$_3$

H$_2$CO

H$_2$CO

H$_2$CO

H$_2$CO

H$_2$CO

H$_2$CO

H$_2$CO

H$_2$CO
$^13$C NMR

[Diagram of a molecular structure with chemical shifts and peaks labeled]

- Chemical shifts and peaks are indicated on the right side of the diagram.

- The molecular structure includes carbon atoms labeled from C1 to C19.

- The peaks correspond to different chemical shifts in ppm (parts per million).

- The diagram shows the complexity of the molecular structure and its resonance behavior.
2. Lipophilicity of the molecules

Fig S11. Hydrophobic (blue color) and hydrophilic (orange and red colors) parts of molecules of derivatives 1, 2, 6, 7 and 11 (space-filling CPK models).
Fig S12. Comparison of hydrophobic (blue color) and hydrophilic (orange and red colors) parts of molecules of derivatives with propylthio chain 4, 5, 9 and 10 (space-filling CPK models).

Differences between 4, 9 and 5, 10 derivatives with unbranched CH$_3$CH$_2$CH$_2$S- and branched (CH$_3$)$_2$CHS- propylthio chain were visualized in Fig. 5.

3. DFT calculation

Information on geometry of the new compounds was obtained using quantum-chemical calculations. The calculations were carried out by the density functional theory method (DFT) at the B3LYP/6-311G level implemented in the Gaussian 03 program package.$^2$

4. Molecular docking

The structures of all synthesized molecules were prepared using LigPrep v3.6 \(^3\), and the appropriate ionization states at pH = 7.4 were assigned using Epik v3.4.\(^4\) The crystal structures of human tubulin in complex with colchicine (PDB ID: 1SA0)\(^5\) and the mitochondrial cytochrome bcl enzyme complex (CYTBC1) with azoxystrobin (PDB ID: 1SQB)\(^6\) were retrieved from Brookhaven Protein Data Bank.\(^7\) The Protein Preparation Wizard\(^8\) was used to assign the bond orders, check the steric clashes, and assign appropriate amino acid ionization states. The receptor grids were generated (the OPLS_2005 force field) by set up the grid box on the center of co-crystalized ligand. Automated docking of all synthetized compounds was performed by using Glide v6.9 \(^9\) at SP level with the flexible docking option turned on. The ligand-receptor complexes were visualized by means of the PyMOL Molecular Graphics System.

4.2. Sequence alignment and construction of fungal tubulin models

The sequences of fungal β-tubulins were obtained from the UniProtKB/Swiss-Prot database:

- *Aspergillus niger* van Tiegen ID: **A2QQP0**,  
- *Aspergillus versicolor* ID: **A0A1L9P7L4**,  
- *Paecilomyces variotii* ID: **V5G9H5**,  
- *Penicillium funiculosum* ID: **not available**,  
- *Chaetomium globosum* ID: **Q2GSL5**,  
- *Aureobasidium pullulans* ID: **A0A074XVP4**,  
- *Penicillium cyclopium* ID: **G5CIU9**,  
- *Trichoderma viride* Pers ID: **P31863**.

A crystal structure of human β-tubulin (PDB ID: 1SA0, co-crystalized with colchicine) was used as the 3-dimensional template for the homology modeling. The multiple sequence alignment (Figure S11) and identity matrix (Table S1) were obtained using Schrödinger Suit. The homology models for all fungal β-tubulin were generated using Prime module in
Schrödinger. Next, the models were energetically optimized using the steepest descent algorithm and OPLS3 force field using MacroModel. The minimization was completed when the RMS gradient convergence reached a 0.05 kJ/(Å·mol).

**Figure S13.** Multiple sequence alignment between human and fungal β-tubulin units. The key amino acids in the colchicine binding site are indicated by a red frame.

**Table S1.** Identity matrix calculated between sequences of human and fungal β-tubulins.

<table>
<thead>
<tr>
<th></th>
<th>Human sapiens</th>
<th>Chaetomium globosum</th>
<th>Penicillium cyclopium</th>
<th>Aureobasidium pullulans</th>
<th>Paecilomyces variotii</th>
<th>Aspergillus versicolor</th>
<th>Aspergillus niger</th>
<th>Trichoderma viride Pers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human sapiens</td>
<td>100.00</td>
<td>84.41</td>
<td>83.13</td>
<td>40.91</td>
<td>80.73</td>
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**Figure S14.** Comparison of the colchicine binding site shapes between human wt and several fungal β-tubulin homology models. The human wild-type tubulin was visualized with co-crystalized ligand (colchicine, cyan).
5. Cytotoxicity assay

In *in vitro* experiments one cancer cell line was used. The cytotoxicity of the synthesized colchicine analogues was tested against SKOV-3 ovarian cell line human colon cancer cell line LoVo, two human breast cancer cell lines: MFC-7, MDA-MB-231 and lung fibroblasts CCD39Lu obtained from the *European Collection of Cell Cultures* (ECACC) Salisbury UK via Sigma-Aldrich Poland. For experiments two types (for SKOV-3 cell line) of cell culture were used: proliferating and growth arrested cells. The cells were seeded in 96-well plates at density 15000 cells/well and 25000 cells/well for experiments with proliferating and growth arrested cells respectively. Cells were incubated at 37°C with a 5% CO₂ atmosphere in DMEM supplemented with 2 mM glutamine, 100 μg/mL streptomycin 100
U/mL penicillin and 10% foetal bovine serum (FBS). For proliferating cells regular FBS while for growth arrested cells charcoal treated FBS was used. The cells were allowed to attach and after 24 h, the compounds (0.1–100 µM) dissolved in DMSO were added to each well and incubated for 72 h. Control cells were treated with DMSO alone. The final DMSO concentration in both treated and control samples was 0.1%. The growth of tumour cells was quantified by the ability of the living cells to reduce the yellow dye 3-(4,5- dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) to a purple formazan product.\(^{10}\) The formazan product is formed and accumulates only in healthy cells, therefore colorimetric signal generated from the assay is proportional to the number of living cells in the sample.\(^{10}\)

At the end of the incubation, the plates were centrifuged and the medium was replaced by fresh medium (200 µL) containing 0.5 mg/mL MTT. Three hours later, the MTT formazan product was dissolved in 150 µL DMSO, and the absorbance was measured using a multiplate reader (BioTek Elx-800, BioTek Instruments, Inc. Winooski, Vermont, USA). The drug effect was quantified as the percentage of the absorbance of reduced dye at 550 nm in relation to control wells. Statistical analyses were carried out using one-way ANOVA with Dunnett's multiple comparison tests. The results presented as the mean ± SD from three independent experiments. The values indicated cytotoxicity concentration (EC50) were calculated according to the Hill’s equation (sigmoidal model of concentration-response curve) and expressed as a mean ± SEM (standard error of mean) using GraphPad Prism version 5.00 for Windows, GraphPad Software, San Diego California USA.

6. Fungicidal activity


**Antimicrobial assay**

The cultures were prepared by single-spore isolation technique on PDA (potato dextrose agar) slants and maintained by periodic transfer on the same medium for further experiments. The fungal spores suspensions were obtained from two-week agar slants. The species were provided by the BAM Federal Institute for Materials Research and Testing collection or by the Institute of Chemical Wood Technology (Poznan University of Life Science).

**96-Well fungal bioassay.** The 96-well microtiter assay was used to determine the sensitivity of eight strains of fungi *A. niger*, *A. versicolor*, *P. variotii*, *P. funiculosum*, *Ch. globosum*, *A. pullulans*, *P. cyclopium* and *T. viride* to the new obtained derivatives of colchicine. Tested compounds (10 mg) were dissolved in 200 µL methanol to obtain a high concentration of the solution. After complete dissolving 10 µL volumes of tested solutions were added using micropipette to 100 µL PDA as a culture medium into the wells. Before that, PDA powder was dissolved in distilled water to a final concentration of 39 g/L a water bath to lower the temperature. To each wells was added 10 µL of freshly made fungal spores suspension (10^{-5} to 10^{-6} CFU/mL). The plates were incubated aerobically for 7 days in a moist chamber with relative humidity (RH) above 95% at 28±1°C in the dark. Differences in mycelial growth in each of the wells in the 96-well plates demonstrate sensitivity of pure compounds and indicated fungistatic or fungicidal effects. Fungal growth was evaluated macroscopically according to the three point scale of intensity mycelium growth: „+“ - no visible growth under the microscope; „±“ - growth visible with the naked eye, growth of hyphae without spores; „-“ - growth visible with the naked eye, sporulation mycelium. For reproducibility and accuracy evaluation of the microtiter plate screening method experiments
were done in triplicates and the results for each compounds were compared to the control wells (without any additives, with 10 µL (5 mg/L) of commercial fungicide such as 3-iodo-2-propynylbutylcarbamate (IPBC) as Preventol® MP100 from Lanxess. IPBC (Iodopropynyl butylcarbamate) is a water-soluble preservative used globally in the paints & coatings, wood preservatives, personal care, and cosmetics industries. It is used as an active substance for formulation of antimicrobial products. It is effective against a wide range of fungal species, such as *Aspergillus niger* and *Trichoderma virens*. The results of bioassay tests against microfungi for compounds 7-11 are given in Table 1S below. Concentration of IPBC was chosen based on previous studies. 11


**Table S2.** The results of bioassay tests against microfungi for compounds 7-11

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<thead>
<tr>
<th>Compound</th>
<th>A. niger</th>
<th>A. versicolor</th>
<th>P. variotti</th>
<th>P. funiculosum</th>
<th>T. viride</th>
<th>P. cyclopium</th>
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*IPBC* (3-Iodo-2-propynyl butyl carbamate) Antimicrobial Preventol® MP100 from Lanxess

,,+“ - no visible growth under the microscope;
,,±“ - growth of hyphae without spores;
,,−“ - sporulation mycelium.
### Table S3. Antifungal activity of compounds 1 and 8-11 the results of MFC [µg/mL] and [mMol/mL]

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<th>Compounds</th>
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<th>A. versicolor</th>
<th>P. varioti</th>
<th>P. funiculosum</th>
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