Electronic Supplementary Material (ESI) for MedChemComm.

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 (-)-(a*R*,7*S*) was used. 10-Alkylthiocolchicine **2-6** were obtained according literature procedure from colchicine and respective sodium alkylthiolate RSNa[1]. **S**odium alkylthiolates are commercially available of Fluka.

(1) Kurek, J.; Boczoń, Wł.; Murias, M.; Myszkowski, K.; Borowiak, T.; Wolska, I., Synthesis of sulfur containing colchicine derivatives and their biological evaluation as cytotoxic agents, *Let. Drug Des. Disc*. **2014,** 11, 279–289.

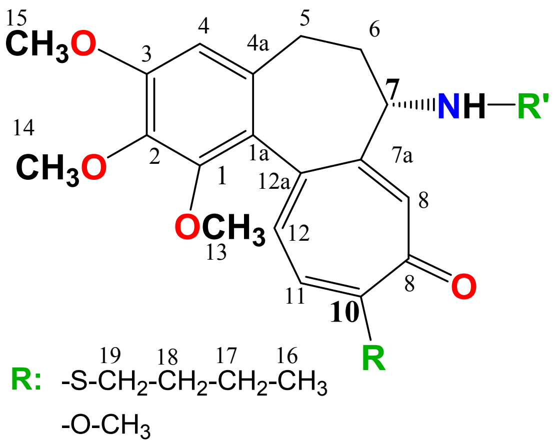
1.2. Experimental Measurements

The NMR spectra of 7-dacetyl-10-alkilthiocolchicines **7-11** (0.07 mol L^{-1}) were recorded in DMSO-*d6* 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-*d6* and $CDCl_3$ were carried out at the operating frequency 300.075 MHz; flip angle, pw = 45^0; 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; pw = 60^0; 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 CO_2-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**.



	R	R'
1	OCH_3	$COCH_3$
2	SCH_3	$COCH_3$
3	SCH_2CH_3	$COCH_3$
4	$SCH_2CH_2CH_3$	$COCH_3$
5	$SCH(CH_3)_2$	$COCH_3$
6	$SCH_2CH_2CH_2CH_3$	$COCH_3$
7	SCH_3	H
8	SCH_2CH_3	H
9	$SCH_2CH_2CH_3$	H
10	$SCH(CH_3)_2$	H
11	$SCH_2CH_2CH_2CH_3$	H

Figure S1. Carbon atom numbering of Colchicine **1** and derivatives **2-11**.

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_3C-13, s), 3.85 (H_3C-14, s), 3.79 (H_3C-15, s), 8.79 (NH), 2.57 (H_3C-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_3C-13, s), 3.93 (H_3C-14, s), 3.87 (H_3C-15, s), 9.19 (NH), 2.36 (H_3C-16, s); Anal. elem. calc. for $C_{20}H_{23}NO_4S{\cdot}3.5H_2O$ 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_3OH) [nm]: λ_{max1} 360, λ_{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_3C-13, s), 3.86 (H_3C-14, s), 3.79 (H_3C-15, s), 8.89

(NH), 2.57 (H_3C-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_3C-13, s), 3.94 (H_3C-14, s), 3.92 (H_3C-15, s), 9.52 (NH), 1.40 (H_3C-16, s), 2.83 (H_2C-17);
Anal. elem. calc. for $C_{21}H_{25}NO_4S{\cdot}3H_2O$ 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_3OH) [nm]: λ_{max1} 375, λ_{max2} 245; FT IR (KBr): 3375 (NH), 2931, 2869, 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_3C-13, s), 3.86 (H_3C-14, s), 3.79 (H_3C-15, s), 8.88 (NH), 2.92 (H_2C-18),1.69 (H_2C-17), 1.04 (H_3C-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),

^{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_3C-13, s), 3.92 (H_3C-14, s), 3.71 (H_3C-15, s), 9.62 (NH), 2.80 (H_3C-16, s), 1.76 (H_2C-17, m), 1.10 (H_2C-18, t); Anal. elem. calc. for $C_{22}H_{27}NO_4S{\cdot}3H_2O$ 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_3OH) [nm]: λ_{max1} 370, λ_{max2} 250; IR (KBr): 3386 (NH), 2959, 2929, 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_3C-13, s), 3.85 (H_3C-14, s), 3.78 (H_3C-15, s), 8.79 (NH), 2.32 (HC-17), 1.30, 1.36 (H_3C-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-16x2), ^{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_3C-13, s), 3.92 (H_3C-14, s), 3.71 (H_3C-15, s), 9.52 (NH), 1.24 (H_3C-16, s); 3.36(H_2C-17, m), Anal. elem. calc. for $C_{22}H_{27}NO_4S{\cdot}4H_2O$ 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_3OH) [nm]: λ_{max1} 375, λ_{max2} 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^{o}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_3C-13, s), 3.85 (H_3C-14, s), 3.79 (H_3C-15, s), 8.81 (NH), 2.94 (H_2C-19, t), 1.66 (H_2C-18, m), 1.47 (H_2C-17, m), 0.93 (H_3C-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), 2.23, 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_3C-13, s), 3.92

(H_3C-14, s), 3.71 (H_3C-15, s), 9.60 (NH), 0.98 (H_3C-16, t), 1.51 (H_2C-17, m), 1.69 (H_2C-18, m), 2.66 (H_2C-19, t); Anal. elem. calc. for $C_{23}H_{29}NO_4S \cdot 4.5H_2O$ 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_3OH) [nm]: λ_{max1} 375, λ_{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

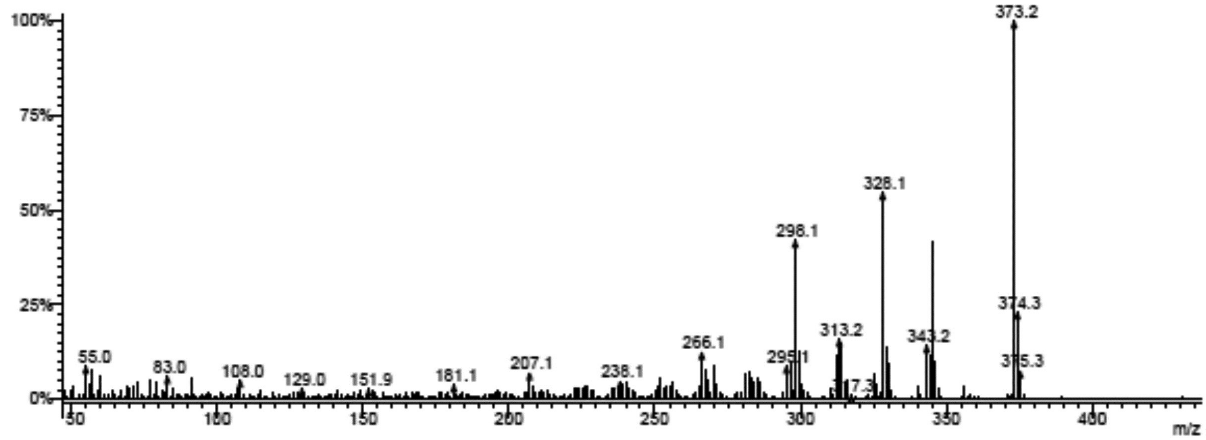


Figure S2. EI MS mass spectra of 7-deacetyl-10-methylthiocolchicine **7**

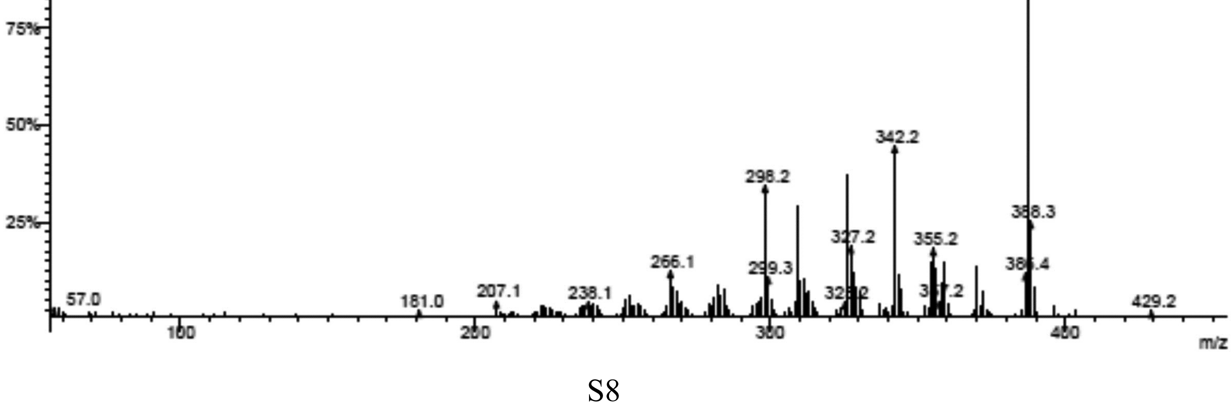


Figure S3. EI MS mass spectra of 7-deacetyl-10-etylthiocolchicine **8**

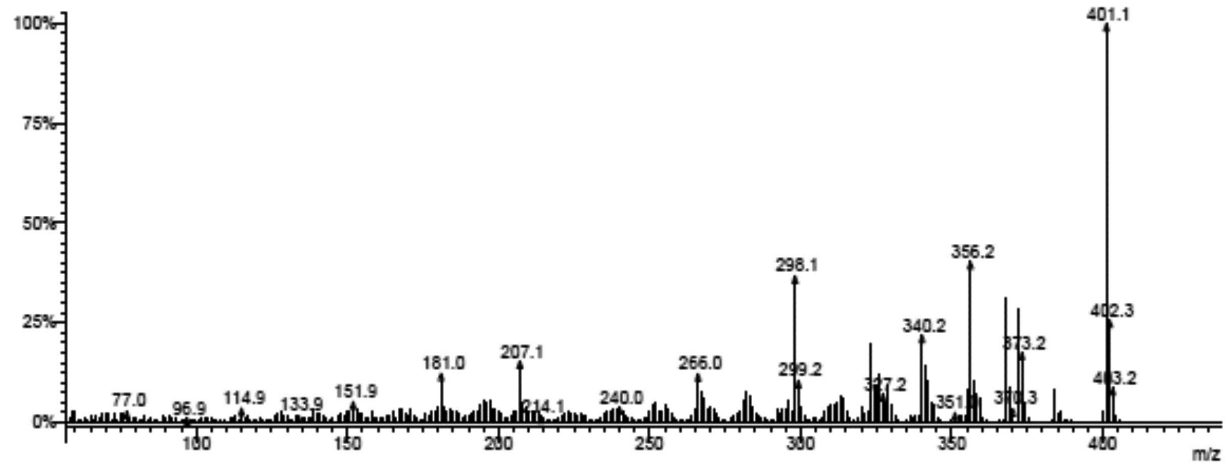


Figure S4. EI MS mass spectra of 7-deacetyl-10-*n*-propylthiocolchicine **9**

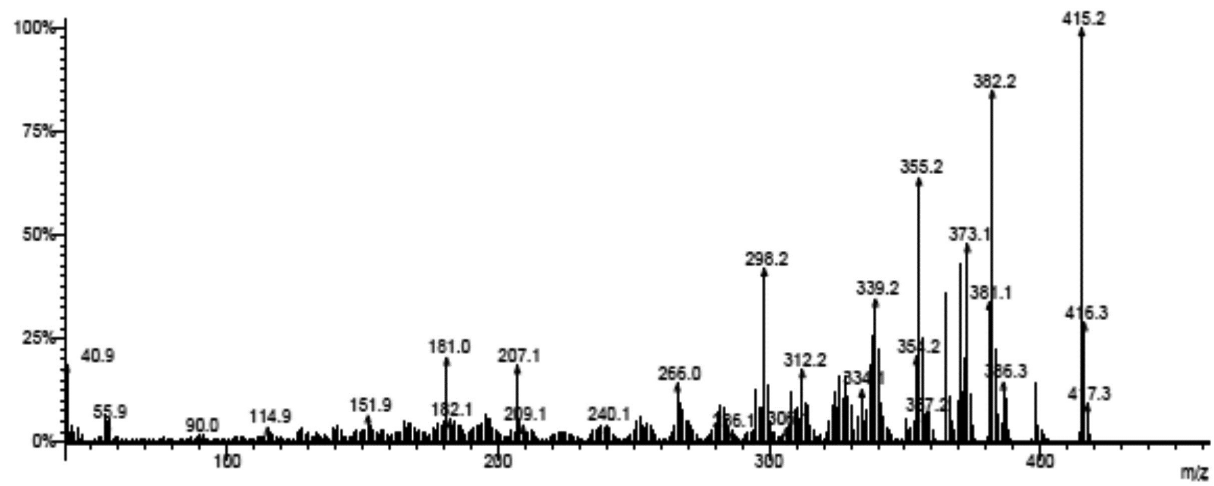


Fig S5. EI MS mass spectra of 7-deacetyl-10-*n*-butylthiocolchicine **11**

1.5. FT IR spectra of 7-deacetyl-10-alkylthiocolchicines **7-11**

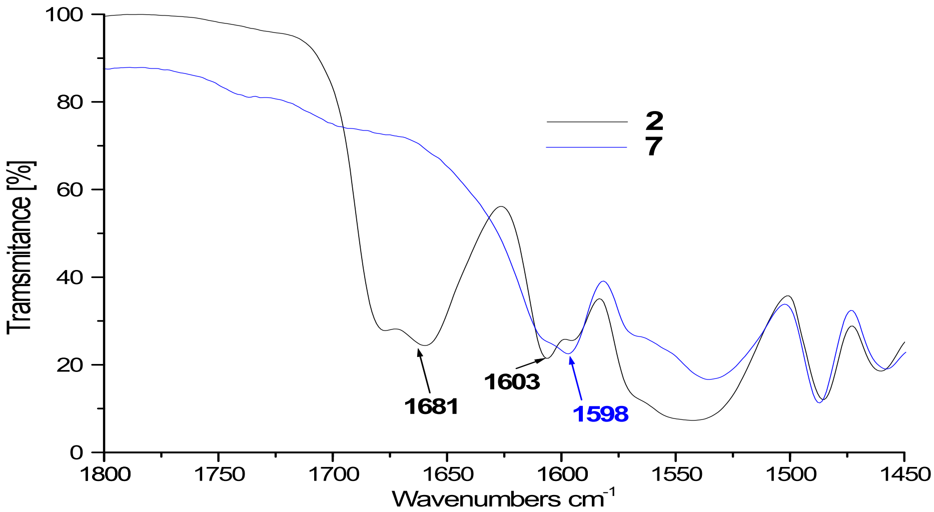


Fig. S6. FT IR spectra for **2** and **7** in the region of carbonyl group

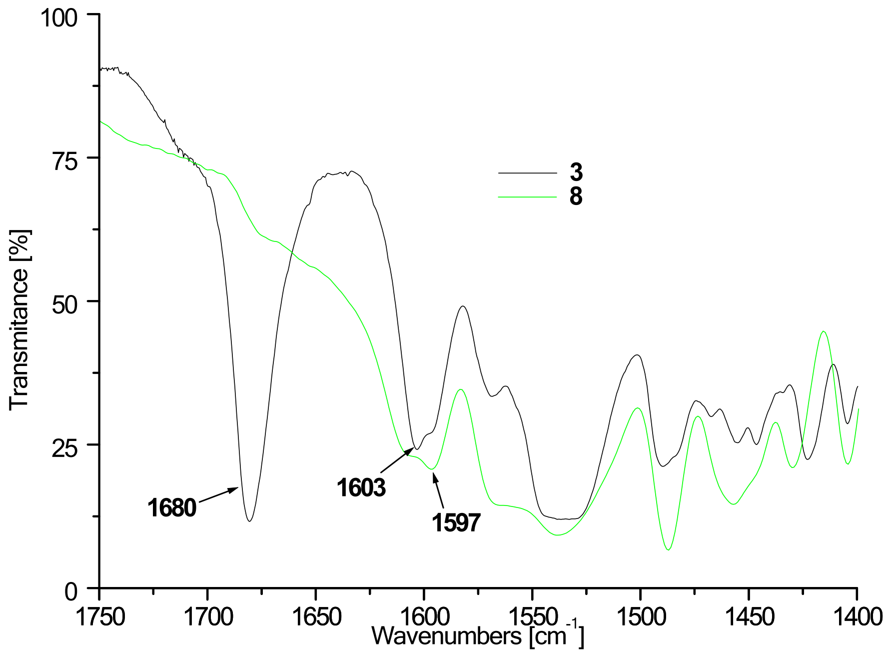


Fig. S7. FT IR spectra for **3** and **8** in the region of carbonyl group

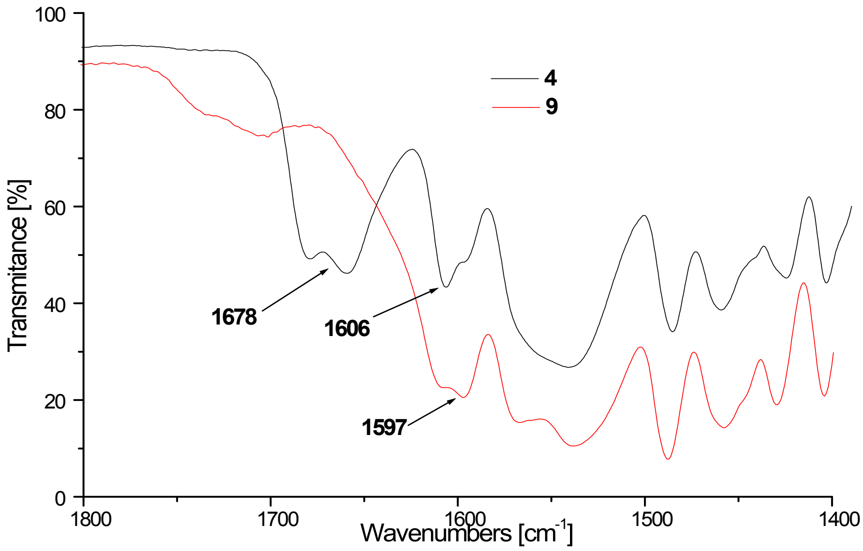


Fig. S8. FT IR spectra for **4** and **9** in the region of carbonyl group

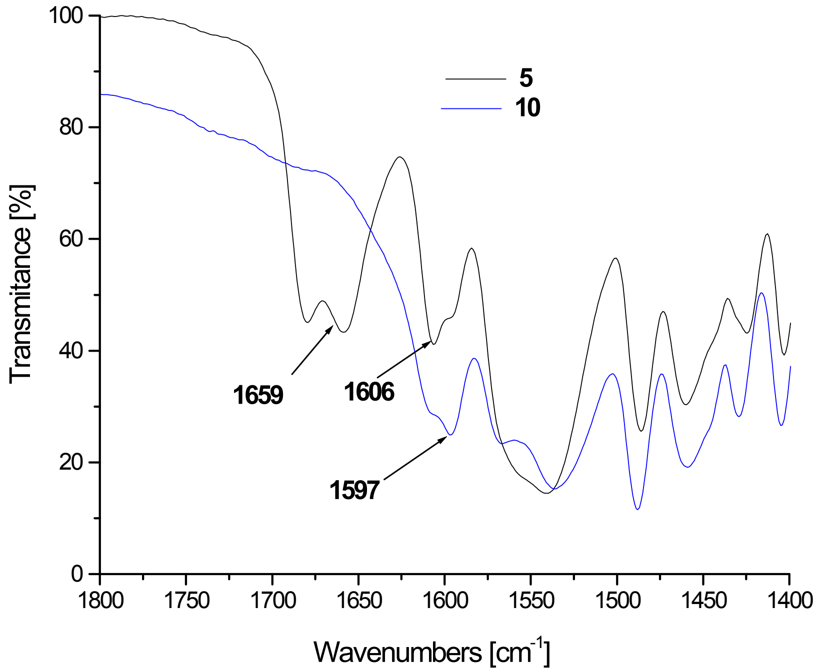


Fig. S9. FT IR spectra for **5** and **10** in the region of carbonyl group

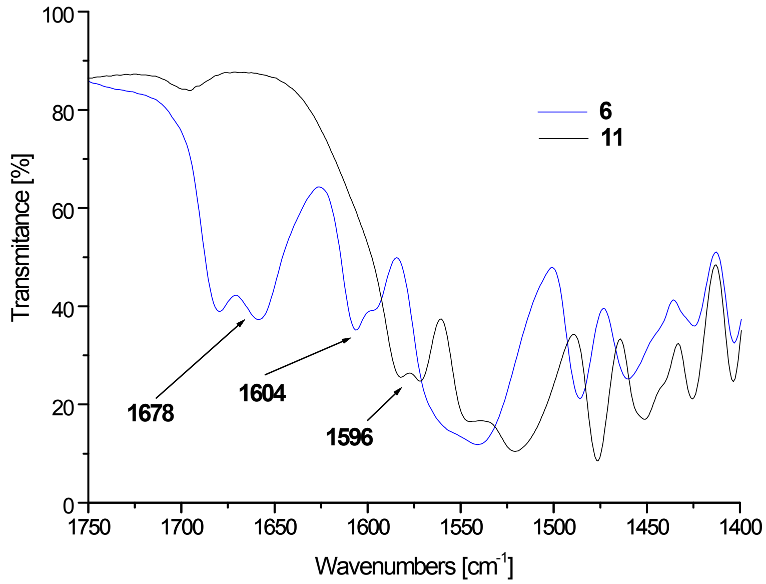
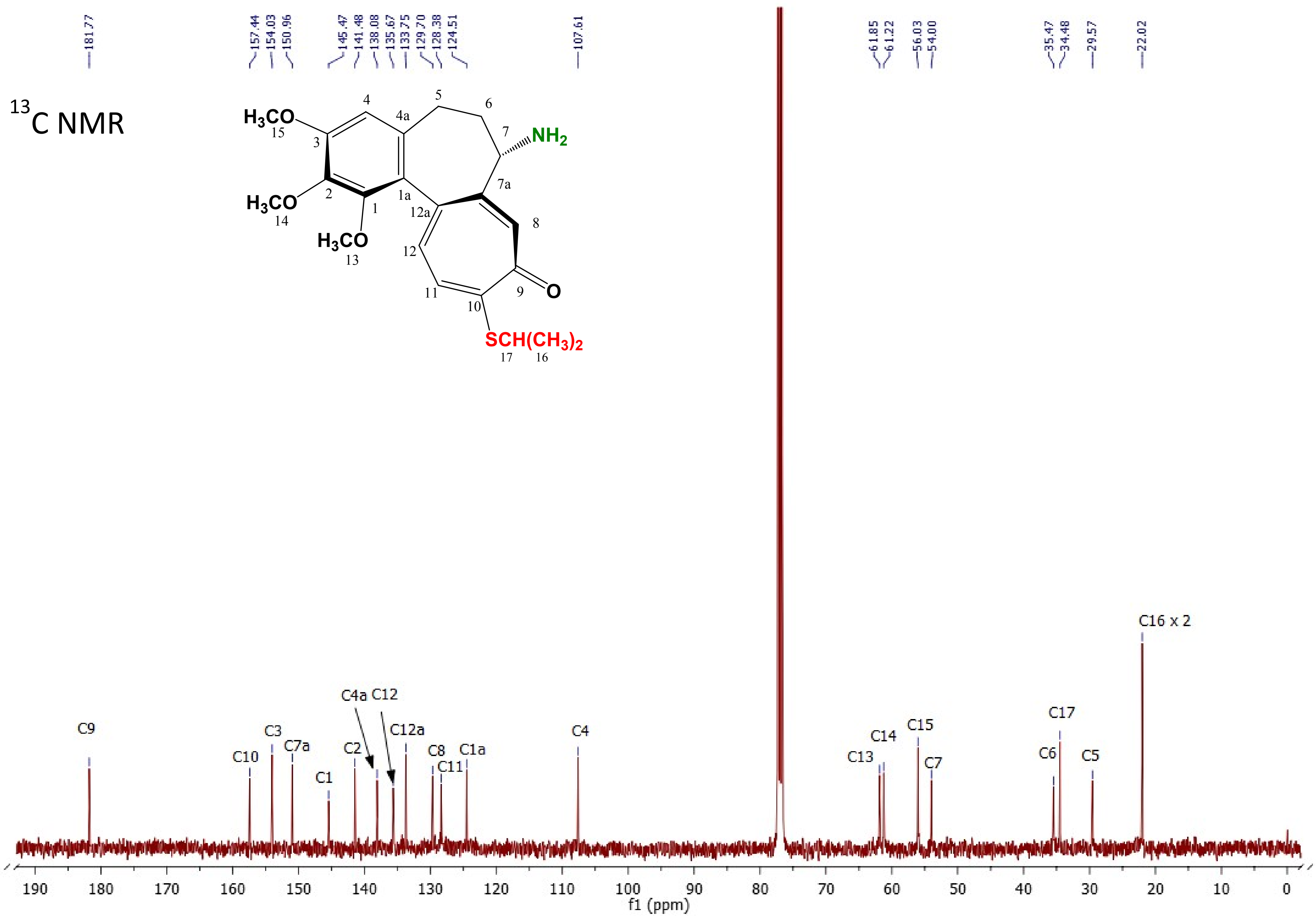
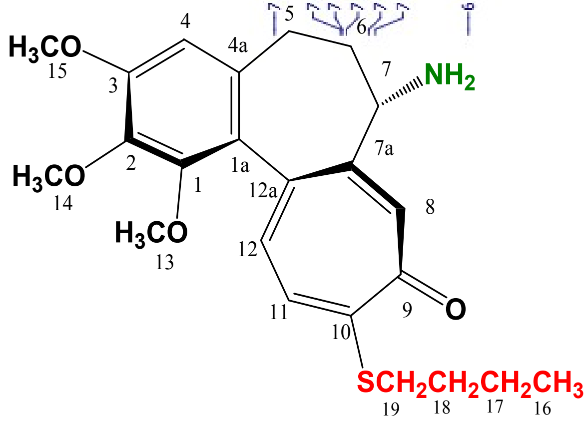


Fig. S10. FT IR spectra for 6 and 11 in the region of carbonyl group

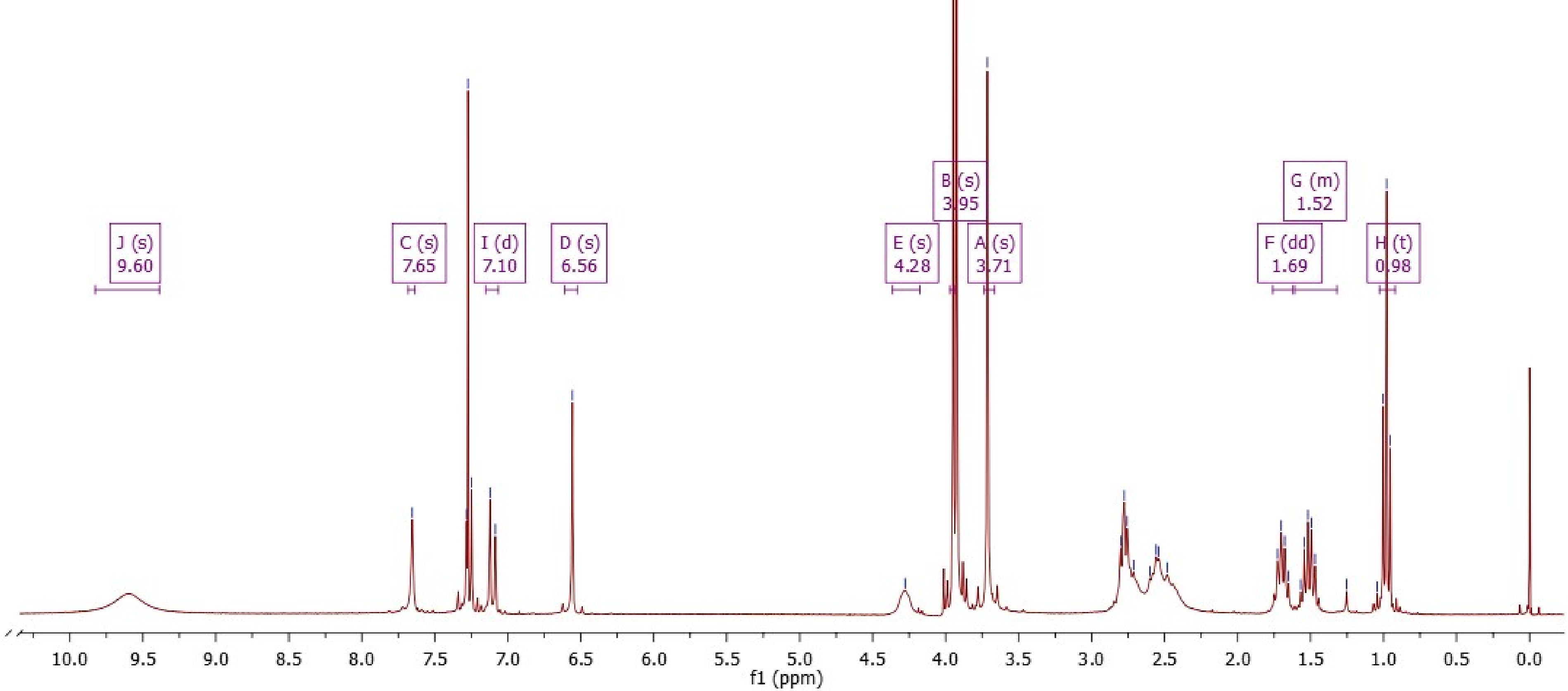


13C NMR
181.77
157.44
154.03
150.96
145.47
141.48
138.08
135.67
133.75
129.70
128.38
124.51
107.61
61.85
61.22
56.03
54.00
35.47
34.48
29.57
22.02
H3CO
15
H3CO
14
H3CO
13
NH2
O
SCH(CH3)2
17
16
C9
C10
C3
C7a
C1
C2
C4a
C12
C12a
C8
C11
C1a
C4
C13
C14
C15
C7
C6
C17
C5
C16 x 2
f1 (ppm)

^1H NMR



H3CO
15
3
4
4a
5
6
7
NH2
7a
2
H3CO
14
1a
1
12a
8
H3CO
13
12
11
10
9
O
SCH2CH2CH2CH3
19
18
17
16



7.65
7.28
7.27
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7.09
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2.76
2.71
2.60
2.56
2.54
2.48
1.73
1.70
1.68
1.65
1.57
1.54
1.52
1.49
1.47
1.25
1.04
1.00
0.98
0.95
J (s)
9.60
C (s)
7.65
I (d)
7.10
D (s)
6.56
E (s)
4.28
B (s)
3.95
A (s)
3.71
F (dd)
1.69
G (m)
1.52
H (t)
0.98
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
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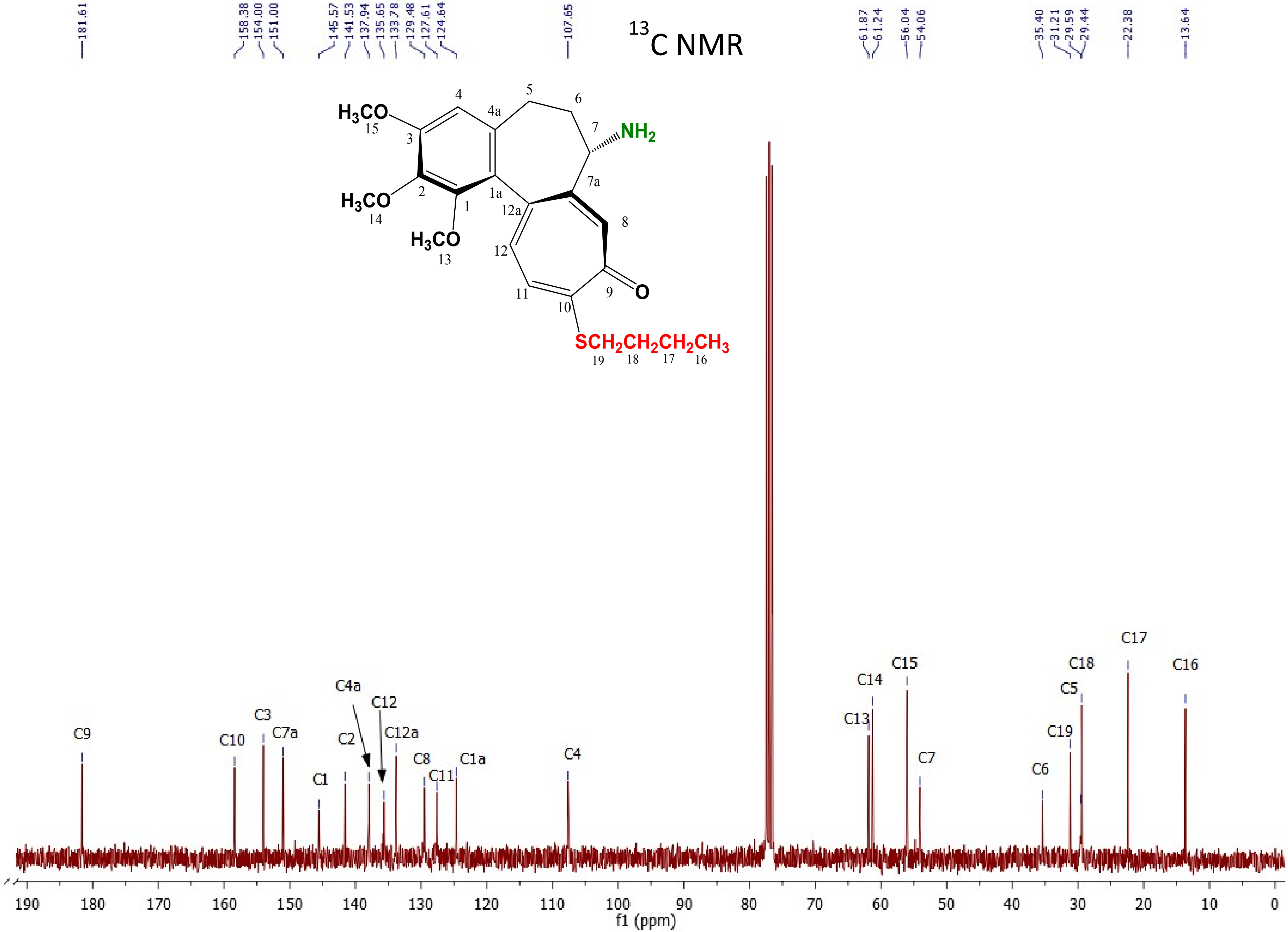
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)



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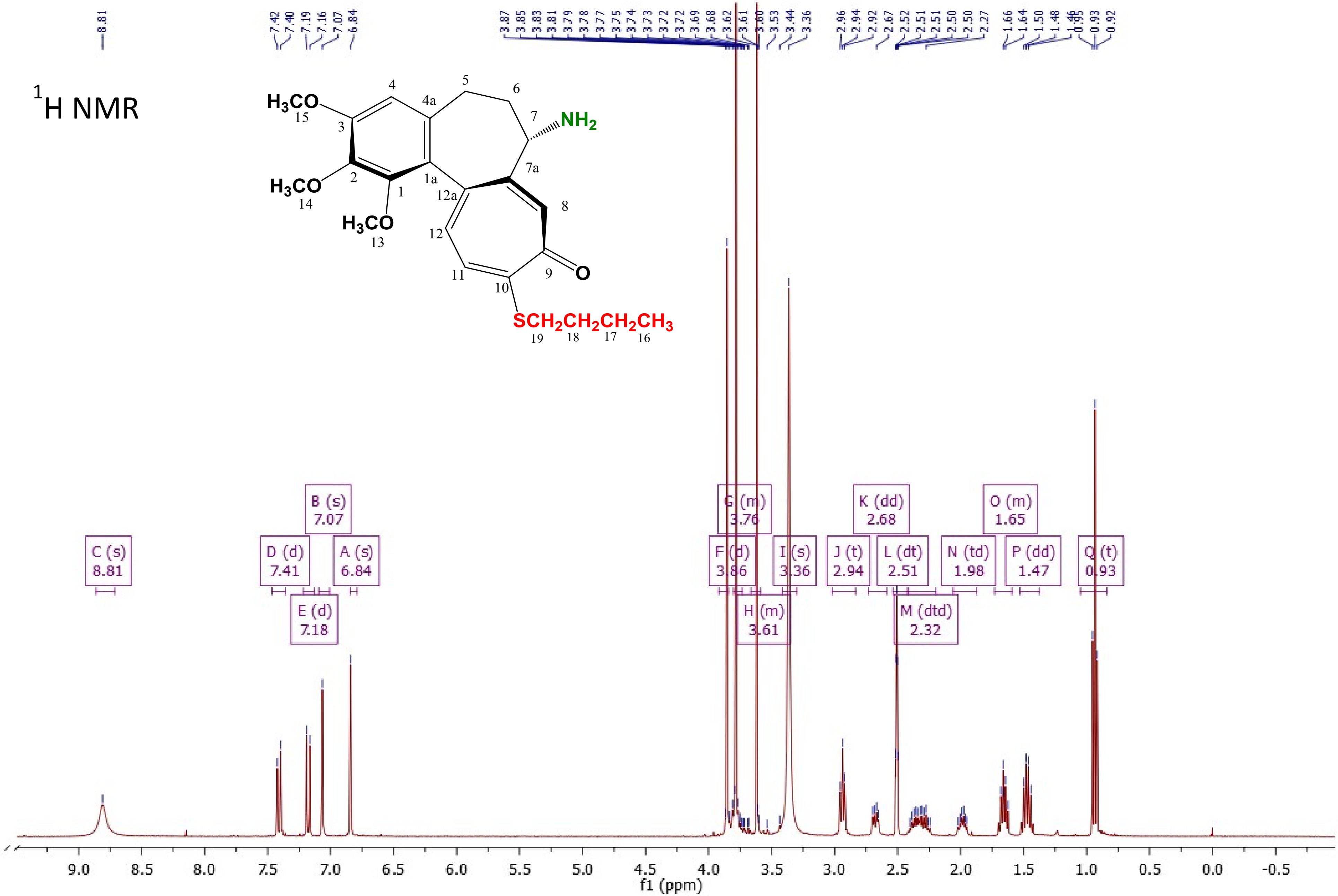
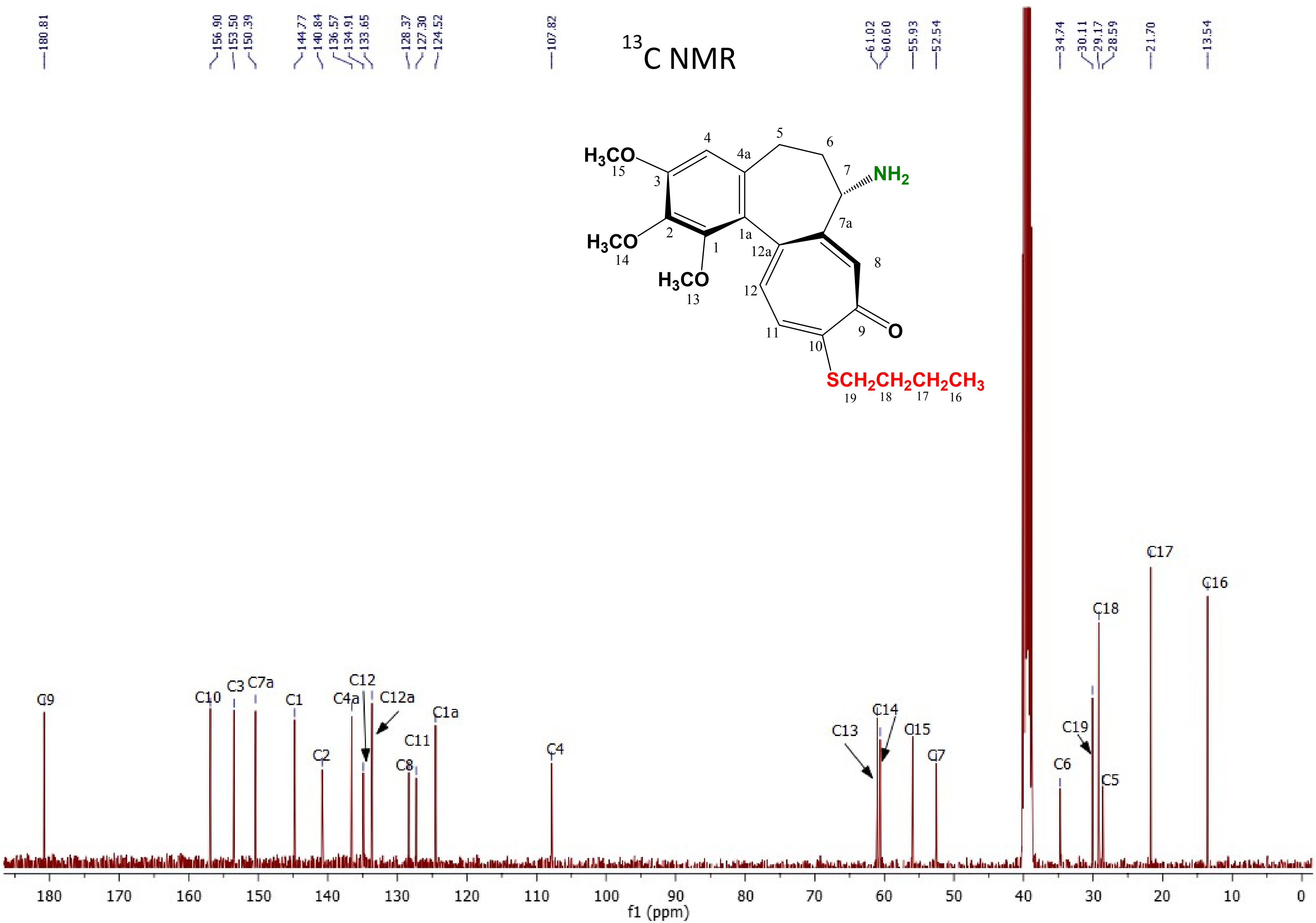
158.38
154.00
151.00
145.57
141.53
137.94
135.65
133.78
129.48
127.61
124.64
107.65
61.87
61.24
56.04
54.06
35.40
31.21
29.59
29.44
22.38
13.64
H3CO 15
H3CO 14
H3CO 13
NH2
O
SCH2CH2CH2CH3
19 18 17 16
C9
C10
C3
C7a
C1
C2
C4a
C12
C12a
C8
C11
C1a
C4
C13
C14
C15
C7
C6
C19
C5
C18
C17
C16
f1 (ppm)

^1H NMR



H3CO
H3CO
H3CO
NH2
O
SCH2CH2CH2CH3
C (s) 8.81
D (d) 7.41
B (s) 7.07
E (d) 7.18
A (s) 6.84
F (d) 3.86
G (m) 3.76
H (m) 3.61
I (s) 3.36
J (t) 2.94
K (dd) 2.68
L (dt) 2.51
M (dtd) 2.32
N (td) 1.98
O (m) 1.65
P (dd) 1.47
Q (t) 0.93
f1 (ppm)



^{13}C NMR
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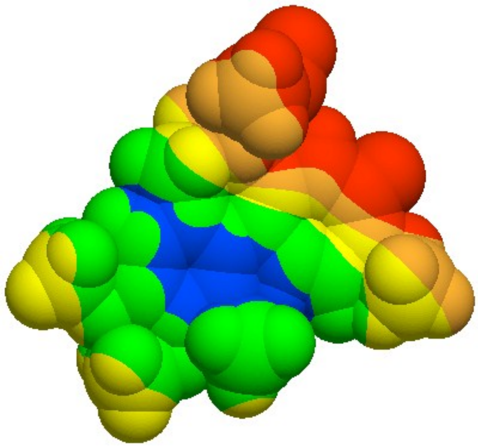
153.50
150.39
144.77
140.84
136.57
134.91
133.65
128.37
127.30
124.52
107.82
61.02
60.60
55.93
52.54
34.74
30.11
29.17
28.59
21.70
13.54
H_3CO
H_3CO
H_3CO
NH_2
O
$SCH_2CH_2CH_2CH_3$
C9
C10
C3
C7a
C1
C2
C4a
C12
C12a
C8
C11
C1a
C4
C13
C14
C15
C7
C6
C19
C18
C5
C17
C16
f1 (ppm)

2. Lipophilicity of the molecules



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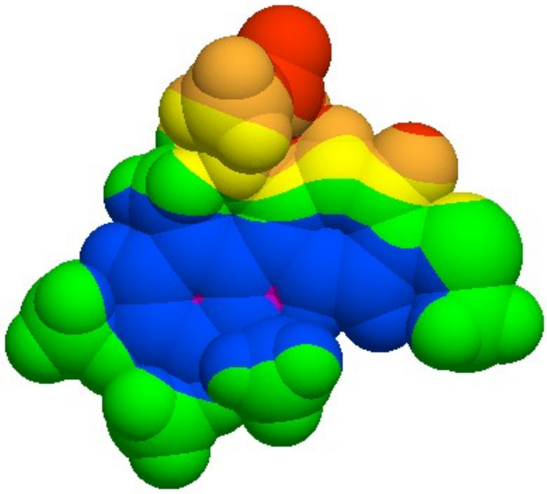

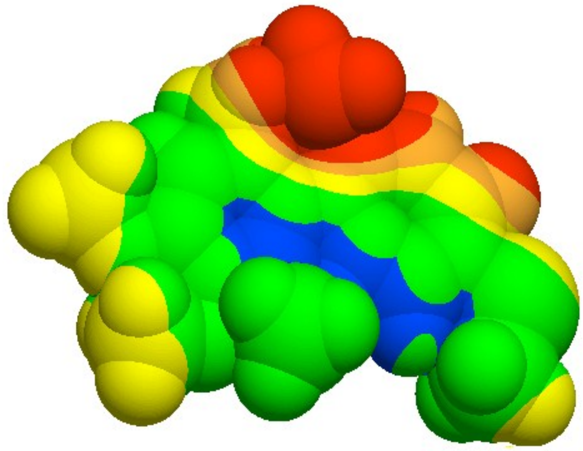

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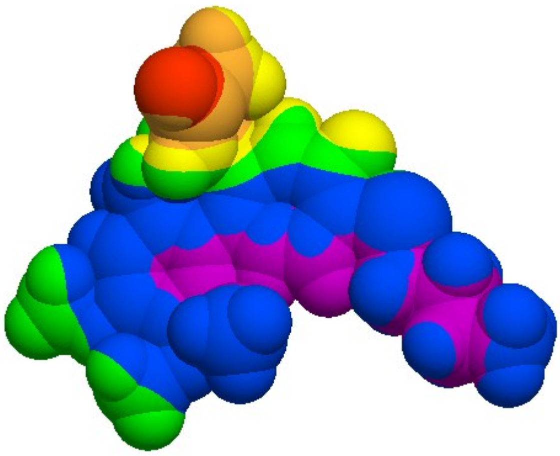
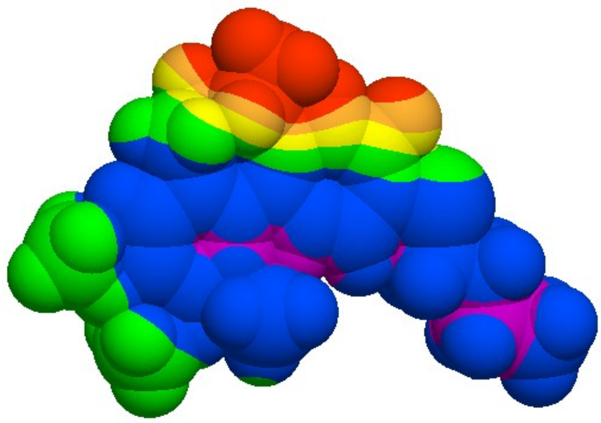
2

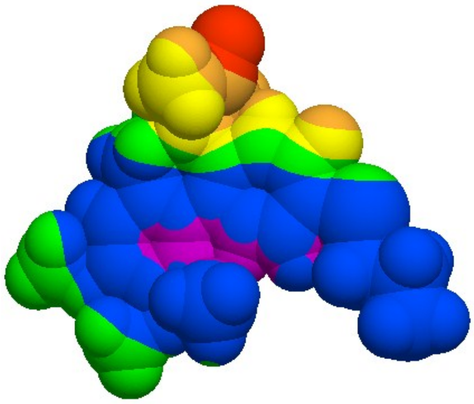




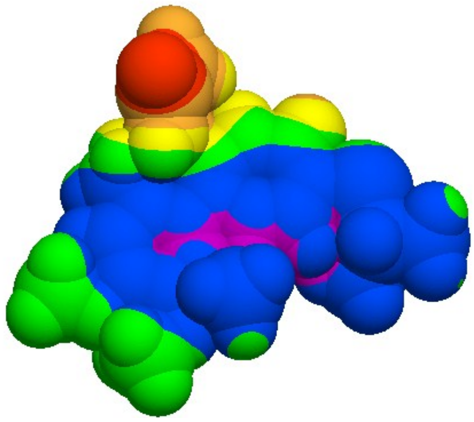
11

7

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).



4



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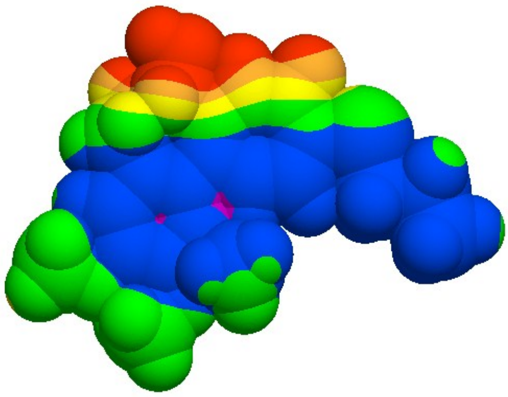
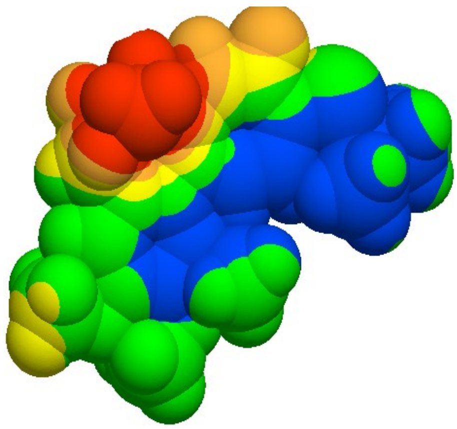


9



10

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 $CH_3CH_2CH_2S$- and branched $(CH_3)_2CHS$- 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]

(2) Frisch, M.J.; Trucks, G.W.; Schlegel, H.B.; Scuseria, G.E.; Robb, M.A.; Cheeseman, J.R.; Montgomery, J.A.; Vreven, Jr. T.; Kudin, K.N.; Burant, J.C.; Millam, J.M.; Iyengar, S.S.; Tomasi, J.; Barone, V.; Mennucci, B.; Cossi, M.; Scalmani, G.; Rega, N.; Petersson, G.A.; Nakatsuji, H.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Klene, M.; Li, X.; Knox, J.E.; Hratchian, H.P.; Cross, J.B.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R.E.; Yazyev, O.; Austin, A.J.; Cammi, R..; Pomelli, C.; Ochterski, J.W.; Ayala, P.Y.; K. Morokuma, G.A.; Voth, P.; Salvador, J.J.; Dannenberg, V.G.; Zakrzewski, S.; Dapprich, A.D.; Daniels, M.C.; Strain, O.; Farkas, D.K.; Malick, A.D.; Rabuck, K.; Raghavachari, J.B.; Foresman, J.V.; Ortiz, Q;. Cui, A.G.; Baboul, S.; Clifford, J.; Cioslowski, B.B.; Stefanov, G.; Liu, A.; Liashenko, P.; Piskorz, I.; Komaromi, R.L.; Martin, D.J;. Fox, T.; Keith, M.A.; Al-Laham, C.Y.; Peng, A.; Nanayakkara, M.; Challacombe, P.M.; Gill, W.; Johnson, B. Chen, W.; Wong, M.W., Gonzalez, C.; Pople. J.A.; Gaussian 03, Revision B.04, Gaussian, Inc., Pittsburgh PA, **2003**.

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 bc1 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.

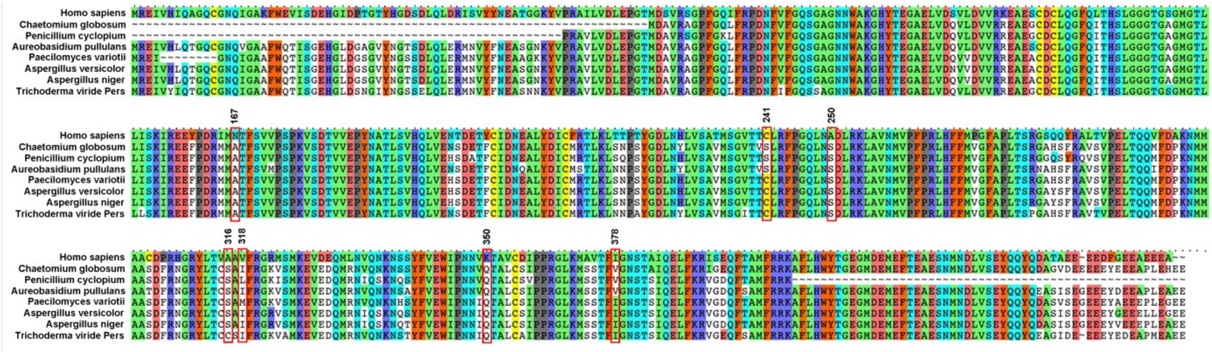
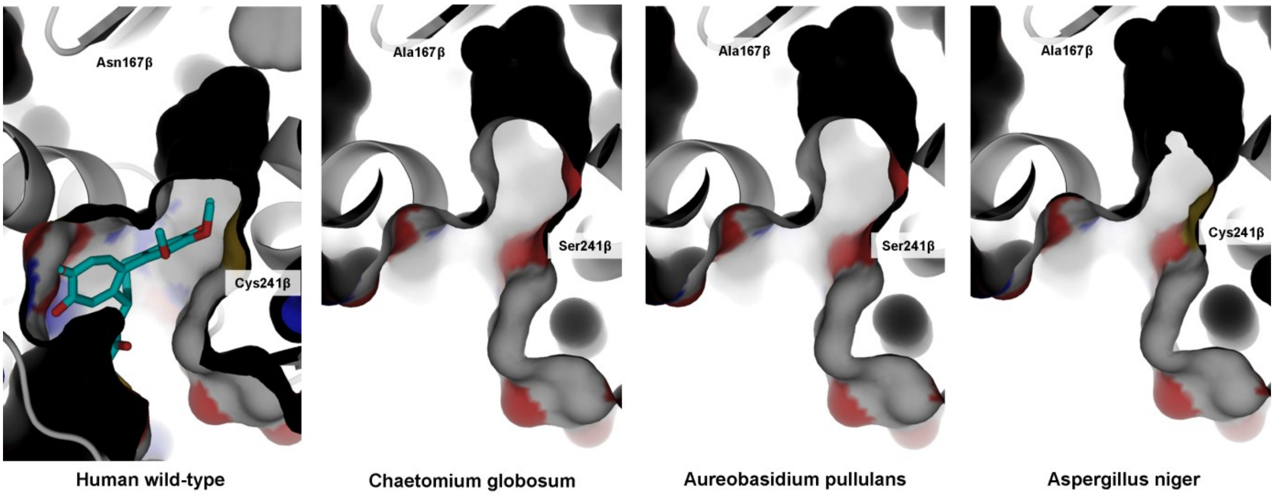


Table S1. Identity matrix calculated between sequences of human and fungal β-tubulins.

	Human sapiens	*Chaetomium globosum*	*Penicillium cyclopium*	*Aureobasidium pullulans*	*Paecilomyces variotii*	*Aspergillus versicolor*	*Aspergillus niger*	*Trichoderma viride Pers*
Human sapiens	100.00	84.41	83.13	40.91	80.73	81.53	81.31	80.36
Chaetomium globosum	84.41	100.00	93.75	41.85	94.93	93.33	93.60	93.05
Penicillium cyclopium	83.13	93.75	100.00	41.57	94.28	94.58	94.58	89.46
Aureobasidium pullulans	40.91	41.85	41.57	100.00	39.35	39.55	39.77	40.55
Paecilomyces variotii	80.73	94.93	94.28	39.35	100.00	96.13	96.13	90.41
Aspergillus versicolor	81.53	93.33	94.58	39.55	96.13	100.00	98.88	90.36
Aspergillus niger	81.31	93.60	94.58	39.77	96.13	98.88	100.00	91.26
Trichoderma viride Pers	80.36	93.05	89.46	40.55	90.41	90.36	91.26	100.00

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).



(3) Schrödinger Release 2016-3: LigPrep, Schrödinger, LLC, New York, NY, **2016**.
(4) Schrödinger Release 2016-3: Epik, Schrödinger, LLC, New York, NY, **2016**.
(5) Ravelli, R. B.G.; Gigant, B.; Curmi, P. A.; Jourdain, I.; Lachkar, S.; Sobel, A.; Knossow, M.; Insight into tubulin regulation from a complex with colchicine and a stathmin-like domain, Nature, 2004, 428, 198-202.
(6) Zhou, Y.; Chen, L.; Hu, J.; Duan, H.; Lin, D.; Liu, P.; Meng, Q.; Li, B.; Si, N.; Liu, C.; Liu, X.; Resistance Mechanisms and Molecular Docking Studies of Four Novel QoI Fungicides in *Peronophythora litchi*, *Nature*, *Sci. Rep.* **2015**, 5, 17466
(7) Berman, H. M. The Protein Data Bank. *Nucleic Acids Res*. **2000**, 28, 1, 235–242.
(8) Madhavi Sastry, G.; Adzhigirey, M.; Day, T.; Annabhimoju, R.; Sherman, W. Protein and Ligand Preparation: Parameters, Protocols, and Influence on Virtual Screening Enrichments. J. *Comput. Aided. Mol. Des.* **2013**, 27 (3), 221–234.
(9) Schrödinger Release 2016-3: Glide, Schrödinger, LLC, New York, NY, **2016**.

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% CO_2 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-2*H*-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.

(10) Berridge, M.V.; Herst, P.M.; Tan, A.S. *Biotechnol Annu Rev*. **2005,** 11, 127-133.

6. Fungicidal activity

Fungi strains. The antifungal activity of tested compounds was evaluated against microfungi commonly known as mold: *Aspergillus niger* van Tiegen BAM 4 (ATCC 6275), *Aspergillus versicolor* BAM 8 (ATCC 11730), *Paecilomyces variotii* BAM 19 (ATCC 18502), *Penicillium funiculosum* BAM 22 (ATCC 11797) *Chaetomium globosum* BAM 12 (ATCC 6205), *Aureobasidium pullulans* BAM 10 (ATCC 9348), *Penicillium cyclopium* Westling, *Trichoderma viride* Pers.

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]

(11) Viitanen, H.; Ritschkoff, A.C.; Coating and surface treatment of wood. In: Adan and Samson (Ed.) Fundamentals of mold growth in indoor environments and strategies for healthy living, Wageningen Academic Publishers, **2011**, 463-488

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

Compound	Fungal species							
	A. niger	*A. versicolor*	*P. variotti*	*P. funiculosum*	*T. viride*	*P. cyclopium*	*A. pullulans*	*Ch. globosum*
7	-	-	-	-	-	-	±	+
8	-	-	-	-	-	-	±	+
9	-	-	+	+	+	+	±	±
10	-	-	+	+	±	±	±	+
11	-	-	+	+	+	+	+	+
chalkone	+	+	+	+	+	+	+	+
IPBC	+	+	+	+	+	+	+	+
control	-	-	-	-	-	-	-	-

IPBC (3-Iodo-2-propynyl butyl carbamate) Antimicrobial Preventol® MP100 from Lanxes

,,+“ - 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]

Compounds	**Fungi**							
	A. niger	*A. versicolor*	*P. variotti*	*P. funiculosum*	*T. viride*	*P. cyclopium*	*A. pullulans*	*Ch. globosum*
1	>4000	>4000	>4000	>4000	>4000	>4000	$1\cdot10^{-3}$ [$2.5\cdot10^{-7}$]	>4000
2	>4000	>4000	>4000	>4000	>4000	>4000	>4000	>4000
3	>4000	>4000	>4000	>4000	>4000	>4000	>4000	>4000
4	>4000	>4000	>4000	>4000	>4000	>4000	>4000	>4000
5	>4000	>4000	>4000	>4000	>4000	>4000	>4000	>4000
6	>4000	>4000	>4000	>4000	>4000	>4000	>4000	>4000
7	>4000	>4000	>4000	>4000	>4000	>4000	>4000	>4000
8	>4000	>4000	>4000	>4000	>4000	>4000	>4000	>4000
9	>4000	>4000	1000±0.0 [$2.5\cdot10^{-10}$]	2000±600 [$4.9\cdot10^{-10}$]	2000±0.0 [$4.9\cdot10^{-10}$]	2000±0.0 [$4.9\cdot10^{-10}$]	>4000	>4000
10	>4000	>4000	1000±0.0 [$2.5\cdot10^{-10}$]	1000±0.0 [$2.5\cdot10^{-10}$]	2000±0.0 [$4.9\cdot10^{-10}$]	500±0.0 [$1.2\cdot10^{-10}$]	260±0.0 [$6.5\cdot10^{-11}$]	130±0.0 [$3.2\cdot10^{-11}$]
11	>4000	>4000	1000±0.0 [$2.4\cdot10^{-10}$]	1000±0.0 [$2.4\cdot10^{-10}$]	2000±0.0 [$4.8\cdot10^{-10}$]	260±0.0 [$6.3\cdot10^{-11}$]	260±0.0 [$6.3\cdot10^{-11}$]	130±0.0 [$3.1\cdot10^{-11}$]
chalcone (fungicide)*	65±0.0 [$3.1\cdot10^{-11}$]	2000±0.0 [$9.6\cdot10^{-10}$]	1000±0.0 [$4.8\cdot10^{-10}$]	130±0.0 [$6.2\cdot10^{-11}$]	500±0.0 [$2.4\cdot10^{-10}$]	260±0.0 [$1.2\cdot10^{-10}$]	500±0.0 [$2.4\cdot10^{-10}$]	130±0.0 [$6.2\cdot10^{-11}$]
IPBC (fungicide)*	2±0.0 [$7\cdot10^{-12}$]	2±0.0 [$7\cdot10^{-12}$]	2±0.0 [$7\cdot10^{-11}$]	2±0.0 [$7\cdot10^{-12}$]	100±0.0 [$3.5\cdot10^{-8}$]	2±0.0 [$7\cdot10^{-12}$]	1±0.0 [$3.5\cdot10^{-12}$]	5±0.0 [$1.7\cdot10^{-11}$]