Synthesis and spectroscopic characterisation of a combinatorial library based on the fungal natural product 3-chloro-4-hydroxyphenylacetamide

Rohan A. Davis,[1,*] Gregory K. Pierens[1] and Peter G. Parsons.[2]

[1] Natural Product Discovery, Eskitis Institute for Cell and Molecular Therapies, Griffith University, Brisbane, QLD 4111, Australia

[2] Melanoma Genomics Group, Queensland Institute of Medical Research, Herston, Brisbane, QLD 4006, Australia

* Correspondence to: Rohan A. Davis, Natural Product Discovery, Eskitis Institute for Cell and Molecular Therapies, Griffith University, Brisbane, QLD 4111, Australia. E-mail: r.davis@griffith.edu.au

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ABSTRACT

Parallel solution-phase chemistry has yielded a series of secondary amide analogues of the fungal natural product 3-chloro-4-hydroxyphenylacetamide. 3-Chloro-4-hydroxyphenylacetic acid was coupled to a variety of primary amines using 1-ethyl-3-(3'-dimethylaminopropyl)-carbodiimide hydrochloride. The desired products were obtained in good yield and high-purity following rapid silica purification. All analogues were spectroscopically characterised by NMR, UV, IR and MS data. One compound displayed moderate cytotoxicity against the human melanoma and prostate cell lines, MM96L and DU145.

INTRODUCTION

Natural products continue to play an important role in the drug discovery process as evidenced by the fact that 15 new natural product-derived drugs were launched by the pharmaceutical industry between 2000 and 2003.[1] Natural products are renowned for their structural diversity, and several studies have noted that the chemical space they occupy is different from that of synthetic libraries.[2-4] Hence, their use as templates in combinatorial chemistry has been identified as a good method for obtaining unique and chemically diverse libraries. A number of libraries incorporating a natural product motif have been published,[5-13] and several examples exist where the specific and desirable biological activity of a natural product has been improved with rather small libraries that have integrated only simple functional group modifications.[14]

We have recently reported the isolation and structure elucidation of the fungal metabolites, 3-chloro-4-hydroxyphenylacetamide (**1**) and 3-chloro-4-hydroxyphenylacetic acid (**2**) (Figure 1).[15] With our interest in the use of natural products as scaffolds for combinatorial chemistry we decided to generate a small library based on the common core structure of **1** and **2**. Using 1-ethyl-3-(3'-dimethylaminopropyl)-carbodiimide hydrochloride (EDCI) coupling chemistry we generated a high-purity secondary amide library in good yield. This library (**3-13**) (Figure 2) along with the fungal natural products (**1** and **2**) have been added to the Eskitis Institute's chemical repository and made accessible for agrochemical or pharmaceutical high-throughput screening. During separate studies on this library one member (compound **11**) has recently been identified as a potent bovine carbonic anhydrase II inhibitor.[16] In this paper we report the solution-phase parallel synthesis and spectroscopic data of a series of secondary amides that are all analogues of the fungal natural product 3-chloro-4-hydroxyphenylacetamide.

RESULTS AND DISCUSSION

The secondary amide library (**3-13)** was synthesised by coupling the commercial reagent 3-chloro-4-hydroxyphenylacetic acid (**2**) with 11 different primary amines using EDCI and 4-dimethylaminopyridine (DMAP) in CH_3CN at room temperature. The work-up involved partitioning the reaction mixture between CH_2Cl_2 and acidified H_2O; the CH_2Cl_2-soluble material was chromatographed using silica solid-phase extraction (SPE) cartridges and a

hexanes/EtOAc stepwise gradient. ^{1}H NMR spectroscopy was used to determine which silica SPE fractions to combine; only high-purity compounds were added to the library. This protocol afforded 11 secondary amides (Figure 2) in yields ranging from 8 to 99%. Purity analysis of compounds **1-13** was performed using C_{18} analytical HPLC and showed purities ranging from 91 to 99%. Synthetics **3-13** were all spectroscopically characterised using 1D and 2D NMR (^{1}H, ^{13}C, DEPT, gCOSY, gHSQC, gHMBC), IR, UV and MS data. The amide stereochemistry for **3-13** was assigned as *trans* since acyclic secondary amides typically prefer this conformation about the amide C-N bond.[17] Support for the *trans* stereochemical assignment was provided by ROESY data for compounds **6** and **7**, which both showed strong ROESY correlations between 8-NH and H-7.

Compounds **1-13** were tested for cytotoxicity against the human melanoma cell line MM96L using the colorimetric sulphorhodamine B assay.[18] Compounds **7** and **13** were the only analogues that displayed any significant cytotoxicity with IC_{50} values of 72 and 350 μM, respectively. In order to determine if **7** or **13** showed any selective anticancer activity, both compounds were tested against the human prostate cell line DU145 and showed IC_{50} values of 51 and 335 μM, respectively.

CONCLUSIONS

Eleven secondary amide analogues of the fungal natural product 3-chloro-4-hydroxyphenylacetamide have been prepared using solution-phase parallel synthesis. All analogues were fully characterised by spectroscopic methods. Screening of the high-purity combinatorial library against the human melanoma cell line MM96L and human prostate cell line DU145 identified one library member, compound **7**, which displayed moderate cytotoxicity.

EXPERIMENTAL

General

Low-resolution electrospray ionization mass spectra (LRESIMS) were recorded on a Waters ZQ mass spectrometer. High-resolution electrospray ionization mass spectra (HRESIMS) were recorded on a Bruker Daltonics Apex III 4.7e Fourier transform mass spectrometer, fitted with an

Apollo API source, funded by ARC LIEF (2002). IR and UV spectra were recorded on a Bruker Tensor 27 spectrometer and a Camspec M501 spectrophotometer, respectively. Melting points were determined on a Gallenkamp melting point apparatus and are uncorrected. Optical rotations were recorded on a Jasco P-1020 polarimeter. Phenomenex Strata Si-1 (1g, 50 μm, 70 Å) SPE cartridges were used for reaction purifications. A Waters 600 pump equipped with a Waters 996 PDA detector and a Waters 717 autosampler were used for analytical HPLC. A Thermo Electron Hypersil C_{18} BDS 5 μm 143 Å analytical column (4.6 mm × 150 mm) was used for HPLC purity analyses. All solvents used for chromatography, UV and MS were Lab-Scan HPLC grade, and the H_2O used was Millipore Milli-Q PF filtered. All synthetic reagents were purchased from Sigma-Aldrich.

General synthetic procedure.

3-Chloro-4-hydroxyphenylacetic acid (**2**, 186 mg, 1 mmol), EDCI (288 mg, 1.5 mmol), and DMAP (12 mg, 0.1 mmol) were stirred in CH_3CN (3 mL) at room temperature for 1 h. The primary amine (3 mmol) was then added, and the solution was stirred for a further 16 h at room temperature. The reaction mixture was poured into CH_2Cl_2 (30 mL) and extracted with 2N HCl (1 × 30 mL). The CH_2Cl_2-soluble material was pre-absorbed to silica then loaded onto a silica SPE cartridge which was subsequently flushed using a 20% stepwise gradient from 100% hexanes to 100% EtOAc. All resulting fractions were dried under N_2, then analysed by ^{1}H NMR spectroscopy with only high purity fractions combined and added to the library.

Compound 3: Yellow gum (238 mg, 99%); UV (CH_3OH) λ_{max} (log ε) 211 (3.51), 230 (3.52), 281 (3.35) nm; IR (NaCl) ν_{max} 1647, 1559, 1509, 1430 cm^{-1}; ^{1}H and ^{13}C NMR data see Tables 1 and 2; (-)-LRESIMS *m/z* (rel. int.) 240 (100), 242 (33); (-)-HRESIMS *m/z* 240.07921 ($C_{12}H_{15}{}^{35}ClNO_2$ $[M-H]^-$ requires 240.07968).

Compound 4: Pale yellow solid (20 mg, 8%); UV (CH_3OH) λ_{max} (log ε) 211 (3.52), 229 (3.50), 281 (3.21) nm; IR (NaCl) ν_{max} 1651, 1557, 1505 cm^{-1}; ^{1}H and ^{13}C NMR data see Tables 1 and 2; (-)-LRESIMS *m/z* (rel. int.) 240 (100), 242 (33); (-)-HRESIMS *m/z* 240.07993 ($C_{12}H_{15}{}^{35}ClNO_2$ $[M-H]^-$ requires 240.07968).

Compound 5: Yellow gum (150 mg, 62%); UV (CH_3OH) λ_{max} (log ε) 212 (3.53), 231 (3.53), 282 (3.30) nm; IR (NaCl) ν_{max} 1636, 1557, 1537 cm^{-1}; ^{1}H and ^{13}C NMR data see Tables 1 and 2; (-)-LRESIMS *m/z* (rel. int.) 242 (100), 244 (33); (-)-HRESIMS *m/z* 242.05895 ($C_{11}H_{13}{}^{35}ClNO_3$ $[M-H]^-$ requires 242.05895).

Compound 6: Brown gum (278 mg, 99%); $[\alpha]^{23}{}_D$ 0° (*c* 1.240, CH_3OH); UV (CH_3OH) λ_{max} (log ε) 212 (3.58), 229 (3.56), 281 (3.27) nm; IR ν_{max} (NaCl) 1644, 1556, 1510 cm^{-1}; ^{1}H and ^{13}C NMR data see Tables 1 and 2; (-)-LRESIMS *m/z* (rel. int.) 278 (100), 280 (33); (-)-HRESIMS *m/z* 278.09464 ($C_{15}H_{17}{}^{35}ClNO_2$ $[M-H]^-$ requires 278.09533).

Compound 7: White needles (189 mg, 69%); mp 165-167 °C; UV (CH_3OH) λ_{max} (log ε) 209 (3.58), 230 (3.58), 281 (3.31) nm; IR ν_{max} (NaCl) 1653, 1561, 1541 cm^{-1}; ^{1}H and ^{13}C NMR data see Tables 1 and 2; (-)-LRESIMS *m/z* (rel. int.) 274 (100), 276 (33); (-)-HRESIMS *m/z* 274.06469 ($C_{15}H_{13}{}^{35}ClNO_2$ $[M-H]^-$ requires 274.06403).

Compound 8: Yellow gum (298 mg, 96%); UV (CH_3OH) λ_{max} (log ε) 207 (3.62), 225 (3.62), 281 (3.27) nm; IR (NaCl) ν_{max} 1651, 1552, 1510 cm^{-1}; ^{1}H and ^{13}C NMR data see Tables 1 and 2; (-)-HRESIMS *m/z* 308.02387 ($C_{15}H_{12}{}^{35}Cl_2NO_2$ $[M-H]^-$ requires 308.02506).

Compound 9: Yellow gum (284 mg, 98%); UV (CH_3OH) λ_{max} (log ε) 211 (3.60), 229 (3.59), 283 (3.29) nm; IR (NaCl) ν_{max} 1645, 1557, 1506 cm^{-1}; ^{1}H and ^{13}C NMR data see Tables 1 and 2; (-)-LRESIMS *m/z* (rel. int.) 288 (100), 290 (33); (-)-HRESIMS *m/z* 288.07946 ($C_{16}H_{15}{}^{35}ClNO_2$ $[M-H]^-$ requires 288.07968).

Compound 10: Yellow gum (222 mg, 70%); UV (CH_3OH) λ_{max} (log ε) 210 (3.65), 229 (3.65), 273 sh (3.42), 280 (3.48), 288 sh (3.29) nm; IR (NaCl) ν_{max} 1647, 1610, 1584 cm^{-1}; ^1H and ^{13}C NMR data see Tables 1 and 2; (-)-LRESIMS *m/z* (rel. int.) 318 (100), 320 (33); (-)-HRESIMS *m/z* 318.09121 ($C_{17}H_{17}{}^{35}ClNO_3$ $[M-H]^-$ requires 318.09025).

Compound 11: Off-white amorphous solid (99 mg, 27%); UV (CH_3OH) λ_{max} (log ε) 210 (3.71), 233 (3.72), 281 (3.32) nm; IR (NaCl) ν_{max} 1647, 1558, 1537 cm^{-1}; ^1H and ^{13}C NMR data see Tables 1 and 2; (-)-LRESIMS *m/z* (rel. int.) 367 (100), 369 (33); (-)-HRESIMS *m/z* 367.05241 ($C_{16}H_{16}{}^{35}ClN_2O_4S$ $[M-H]^-$ requires 367.05248).

Compound 12: Yellow needles (337 mg, 97%); mp 148-150 °C; UV (CH_3OH) λ_{max} (log ε) 214 (3.67), 235 (3.69), 280 (3.64) nm; IR (NaCl) ν_{max} 1651, 1515 cm^{-1}; ^1H and ^{13}C NMR data see Tables 1 and 2; (-)-LRESIMS *m/z* (rel. int.) 348 (100), 350 (33); (-)-HRESIMS *m/z* 348.09862 ($C_{18}H_{19}{}^{35}ClNO_4$ $[M-H]^-$ requires 348.10081).

Compound 13: Dark green gum (281 mg, 86%); UV (CH_3OH) λ_{max} (log ε) 212 (3.66), 230 (3.66), 279 (3.67) nm; IR (NaCl) ν_{max} 1645, 1558, 1509 cm^{-1}; ^1H and ^{13}C NMR data see Tables 1 and 2; (-)-LRESIMS *m/z* (rel. int.) 327 (100), 329 (33); (-)-HRESIMS *m/z* 327.08940 ($C_{18}H_{16}{}^{35}ClN_2O_2$ $[M-H]^-$ requires 327.09058).

NMR data

NMR spectra (^1H, ^{13}C, DEPT, gCOSY, gHSQC, gHMBC and ROESY) were recorded at 30 ^oC on a Varian Inova 500 MHz spectrometer equipped with a switchable probe. The ^1H and ^{13}C chemical shifts were referenced to the residual solvent peak of DMSO-d_6 at δ 2.49 and 39.5 ppm, respectively. The ^1H spectral width was set at 12 ppm for all experiments with a 90° pulse for ^1H of 10.8 μs and a ^{13}C 90° pulse of 7.6 μs. The ^{13}C and DEPT experiments were acquired with

776 and 256 transients, respectively. All 2D experiments were acquired with 1024 complex points. The gradient COSY was acquired with 128 F1 increments with 4 transients per increment. Sinebell weighting was applied to each dimension and zero filled to 2K points. The HSQC was acquired with a ^{13}C spectral width of 160 ppm and 200 t1 increments. Each increment was acquired with 4 transients. Gaussian weighting was applied to both ^{1}H and ^{13}C dimensions, which were then zero filled to 2K and 1K, respectively. A one-bond coupling constant delay was set using 140 Hz, and WURST decoupling was applied during acquisition. The gradient HMBC was acquired using 8 transients per increment with 400 F1 increments. A spectral width of 220 ppm was used for the ^{13}C dimension. A one-bond coupling constant of 140 Hz and long-range coupling constant of 8 Hz were used to set the delays in the pulse sequence. Sinebell weighting was applied to both ^{1}H and ^{13}C dimensions, which were then zero filled to 4K and 1K, respectively. The ROESY was acquired with 256 F1 increments with 16 transients per increment. A mixing time of 500 ms was used. Gaussian weighting was applied to both dimensions, which were then zero filled to 2K.

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SUPPLEMENTARY INFORMATION

^{1}H and ^{13}C NMR spectra for compounds **3-13** and the full details of the HPLC library purity analysis and results are available from the author upon request.

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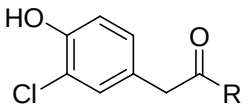
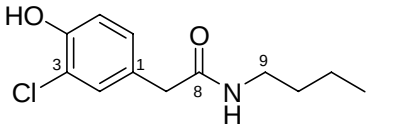
Figure 1. Fungal natural products **1** and **2**.



1 R = NH_2
2 R = OH

Figure 2. Chemical structures of the library members **3-13**.



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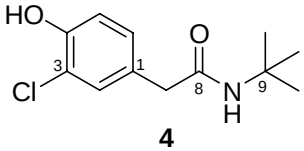

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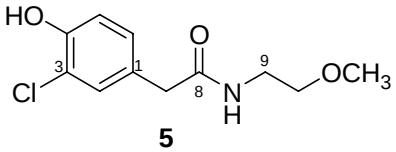

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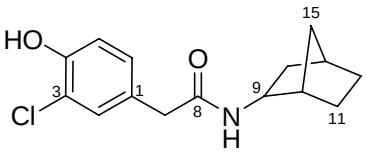

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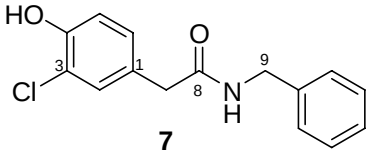

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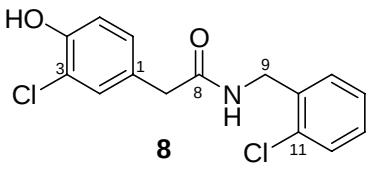

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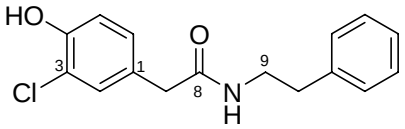

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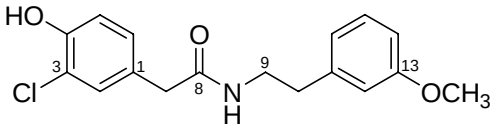

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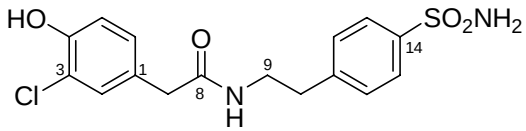

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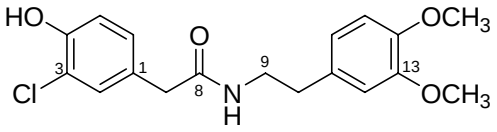

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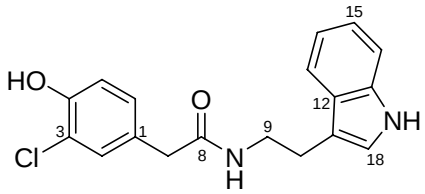

13

Table 1. ^{1}H NMR (500 MHz) data for compounds **3-13**.[a]

Position	3	4	5	6	7	8	9	10	11	12	13
2	7.19, s	7.17, d, 1.5	7.20, s	7.18, d, 1.5	7.22, d, 2.0	7.25, d, 1.5	7.19, s	7.17, s	7.19, s	7.17, s	7.21, s
5	6.86, d, 8.5	6.86, d, 8.5	6.86, d, 8.5	6.85, d, 8.5	6.89, d, 8.5	6.89, d, 8.0	6.87, d, 8.0	6.86, d, 8.5	6.87, d, 8.5	6.86, d, 8.5	6.88, d, 8.5
6	6.98, d, 8.5	6.97, dd, 8.5, 1.5	6.98, d, 8.5	6.98, dd, 8.5, 1.5	7.02, dd, 8.5, 2.0	7.03, dd, 8.0, 1.5	6.97, d, 8.0	6.96, d, 8.5	6.96, d, 8.5	6.96, d, 8.5	6.97, d, 8.5
7	3.25, s	3.22, s	3.28, s	3.23, s	3.36, s	3.40, s	3.27, s	3.26, s	3.25, s	3.26	3.29, s
9	3.01, dt, 5.5, 7.0	-	3.19, dt, 5.5, 5.5	3.45, m	4.26, d, 6.0	4.32, d, 5.5	3.26, dt, 5.5, 7.0	3.26, dt, 5.5, 7.5	3.28, dt, 5.5, 7.0	3.23, dt, 5.5, 7.0	3.33, dt, 5.5, 7.0
10	1.35, tt, 7.0, 7.0	1.22, s	3.31, t, 5.5	2.00, br s	-	-	2.69, t, 7.0	2.66, t, 7.5	2.76, t, 7.0	2.62, t, 7.0	2.81, t, 7.0
11	1.24, tq, 7.0, 7.5	-	-	1.07, m 1.39, m	7.21, d, 7.5	-	-	-	-	-	-
12	0.84, t, 7.5	-	-	1.07, m 1.39, m	7.29, dd, 7.5, 7.5	7.42, m[b]	7.14, d, 7.5	6.74, s	7.33, d, 8.0	6.76, s	-
13	-	-	-	2.18, br s	7.22, t, 7.5	7.27, m[b]	7.25, dd, 7.5, 7.0	-	7.72, d, 8.0	-	7.51, d, 8.0
14	-	-	-	1.21, br d, 13.0 1.55, dd, 13.0, 9.5	-	7.27, m[b]	7.17, t, 7.0	6.75, d, 7.5	-	-	6.96, dd, 7.5, 8.0
15	-	-	-	1.07, m 1.39, m	-	7.27, m[b]	-	7.15, dd, 7.5, 7.5	-	6.81, d, 8.0	7.05, dd, 8.0, 7.5
16	-	-	-	-	-	-	-	6.72, d, 7.5	-	6.64, d, 8.0	7.33, d, 8.0
18	-	-	-	-	-	-	-	-	-	-	7.11, s
4-OH	9.91, s	9.89, s	9.92, s	9.91, s	9.95, s	9.95, s	9.94, s	9.92, s	9.93, s	9.93, s	9.93, s
8-NH	7.88, br t, 5.5	7.56, br s	8.02, br t, 5.5	7.82, d, 7.0	8.45, br t, 6.0	8.46, t, 5.5	7.99, br t, 5.5	7.97, t, 5.5	8.03, t, 5.5	7.94, t, 5.5	8.04, br t, 5.5
10-OCH_3	-	-	3.22, s	-	-	-	-	-	-		-
13-OCH_3	-	-		-	-	-	-	3.71, s	-	3.70, s	-
14-SO_2NH_2	-	-		-	-	-	-	-	7.26, s		-
14-OCH_3	-	-		-	-	-	-		-	3.70, s	-
17-NH	-	-		-	-	-	-		-		10.77, s

[a] Spectra were recorded in DMSO-d_6 at 30 °C. [b] Interchangeable signals.

Table 2. ^{13}C NMR (125 MHz) data for compounds **3-13**.[a]

Position	3	4	5	6	7	8	9	10	11	12	13
1	128.3	128.6	128.1	128.4	128.0	127.9	128.1	128.1	128.0	128.1	128.2
2	130.0	130.0	130.0	130.0	130.1	130.2	130.1	130.1	130.1	130.1	130.1
3	119.1	119.1	119.2	119.1	119.2	119.2	119.2	119.2	119.2	119.2	119.2
4	151.5	151.3	151.5	151.4	151.5	151.6	151.5	151.5	151.5	151.5	151.5
5	116.3	116.3	116.3	116.3	116.4	116.4	116.3	116.3	116.3	116.3	116.3
6	128.4	128.4	128.5	128.4	128.5	128.6	128.5	128.5	128.5	128.5	128.5
7	41.0	41.6	40.9	40.9	41.0	40.8	41.1	41.0	41.0	41.1	41.1
8	169.9	169.6	170.2	169.2	170.1	170.3	170.0	170.0	170.1	170.0	170.0
9	38.2	50.0	38.5	52.1	42.2	40.1	40.3	40.1	39.9	40.4	39.7
10	31.1	28.4	70.6	41.9	139.4	136.2	35.0	35.0	34.7	35.6	25.2
11	19.5	-	-	26.1	127.2	132.1	139.4	141.1	143.7	131.9	111.8
12	13.6	-	-	28.0	128.2	129.1[b]	128.6	114.2	129.1	112.5	127.2
13	-	-	-	35.0	126.7	128.6[b]	128.2	159.2	125.6	148.6	118.2
14	-	-	-	38.6	-	127.0[b]	126.0	111.5	142.0	147.2	118.2
15	-	-	-	34.9	-	128.9[b]	-	129.2	-	111.9	120.9
16	-	-	-	-	-	-	-	120.9	-	120.5	111.3
17	-	-	-	-	-	-	-	-	-	-	136.2
18	-	-	-	-	-	-	-	-	-	-	122.6
10-OCH_3	-	-	57.8	-	-	-	-	-	-	-	-
13-OCH_3	-	-	-	-	-	-	-	54.8	-	55.3[c]	-
14-OCH_3	-	-	-	-	-	-	-	-	-	55.5[c]	-

[a] Spectra were recorded in DMSO-d_6 at 30 °C. [b, c] Interchangeable signals.