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Supporting Information

Clostrindolin is an Antimycobacterial Pyrone Alkaloid from the Anaerobic Bacterium *Clostridium beijerinckii*

Sebastian Schieferdecker, [a,†] Gulimila Shabuer [a,†] , Uwe Knuepfer [b] and Christian Hertweck* [a,c]

[a] Department of Biomolecular Chemistry

Leibniz Institute for Natural Product Research and Infection Biology (HKI)

Beutenbergstrasse 11, 07745 Jena, Germany.

[b] Biopilot Plant

Leibniz Institute for Natural Product Research and Infection Biology (HKI)

Beutenbergstrasse 11, 07745 Jena, Germany.

[c] Faculty of Biological Sciences, Friedrich Schiller University Jena, 07743 Jena, Germany

[†] Both authors have contributed equally to this work

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General experimental procedures

NMR data were recorded on AVANCE III 500 MHz and 600 MHz instruments. LC-HRMS measurements were performed using an Exactive Q Orbitrap high performance benchtop device with an electrospray ion source and an Accela HPLC system (Thermo Fisher Scientific, Bremen, Germany) consisting of an Autosampler equipped with a column oven, a 1250 Pump and a PDA Detector.

Fermentation, extraction and isolation of compound 1

C. beijerinckii HKI805 was grown in P2 medium in bioreactors (50 L, BIOSTAT UD, U20K, Braun Biotech International) at 37 °C. After 3 days of fermentation, the fermentation broth was extracted twice with 50 L ethyl acetate. The organic layers were combined, residual water dried with Na_2SO_4 and the solvent removed under reduced pressure. The crude extract was chromatographed on a Shimadzu Prominence preparative HPLC system equipped with a diode array detector and a C18 Htec 21 x 250 mm, 5 µm column (Macherey Nagel). Chromatographic conditions were as followed: 10% MeOH in H_2O with 0.1% TFA for 5 min followed by a linear gradient to 100% MeOH in 30 min and an additional isocratic step at 100% MeOH for 10 min. Final purification of compound **1** was achieved by the same HPLC system employing a PFP 10 x 250 mm, 5 µm column (Macherey Nagel) and the same solvent gradient.

Preparation of 3-bromo-4-methoxy-6-methyl-2*H*-pyran-2-one (6)

To a solution of 1.4 mmol of 4-methoxy-6-methyl-2H-pyran-2-one dissolved in 40 mL of carbon tetrachloride 1.4 mmol of NBS was added. The mixture was stirred at 80 °C for one hour, afterwards the solvent was removed under reduced pressure and the crude product purified by flash column chromatography (dichloromethane : ethyl acetate 4:1).

White solid, yield: 89%, ^{1}H NMR ($CDCl_3$, 500 MHz): 6.09 (1H, s), 3.99 (3H, s), 2.29 (3H, s) ppm; ^{13}C NMR ($CDCl_3$, 125 MHz): 166.8, 162.8, 160.8, 95.1, 88.3, 57.3, 20.2 ppm.

Preparation of 4-hydroxy-3-(1*H*-indol-3-yl)-6-methyl-2*H*-pyran-2-one (8)

To a solution of 0.02 mmol of 3-(1*H*-indol-3-yl)-4-methoxy-6-methyl-2*H*-pyran-2-one dissolved in 200 µL of dimethyl formamide 0.08 mmol of sodium thiomethylate was added and the solution was stirred at 60 °C for six hours. Afterwards, the solvent was removed under reduced pressure and the product purified by reverse phase HPLC.

Yellow solid, yield: 43%, ^{1}H NMR (MeCN-d_3, 600 MHz): 9.52 (1H, s), 7.97 (1H, s), 7.47 (1H, dd, *J* = 8.3, 1.1 Hz), 7.38 (1H, dd, *J* = 8.5, 1.3 Hz), 7.35 (1H, d, *J* = 2.5 Hz), 7.19 (1H, ddd, *J* = 8.3, 6.9, 1.3 Hz), 7.09 (1H, ddd, *J* = 8.5, 6.9, 1.1 Hz), 6.10 (1H, d, *J* = 1.1 Hz), 2.28 (3H, d, *J* = 1.1 Hz) ppm; ^{13}C NMR (MeCN-d_3 150 MHz): 165.3, 164.1, 161.5, 136.4, 127.1, 126.0, 121.7, 120.0, 111.4, 104.7, 99.7, 96.6, 19.0 ppm.

Preparation of 3-(1*H*-indol-3-yl)-4-methoxy-6-methyl-2*H*-pyran-2-one (9)

To a solution of 1.0 mmol of 3-bromo-4-methoxy-6-methyl-2*H*-pyran-2-one dissolved in 3 mL of 1,4-dioxane and 1 mL of water, 1 mmol of 3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-indole, 3 mmol of potassium carbonate and a catalytic amount of tetrakis(triphenylphosphine)palladium(0) were added and the solution was refluxed at 100 °C for 24h. Afterwards, the solvent was removed under reduced pressure and the crude product purified by flash column chromatography employing a solvent gradient from dichloromethane : ethyl acetate 3:1 to ethyl acetate.

Yellow solid, yield: 42%: ^{1}H NMR (MeOH-d_4, 600 MHz): 7.34 (2H, m), 7.09 (1H, dd, *J* = 7.7, 7.6 Hz), 6.99 (1H, dd, J = 7.6, 7.2 Hz), 6.50 (1H, s), 3.82 (3H, s), 2.32 (3H, s) ppm; ^{13}C NMR (MeOH-d_4, 150 MHz): 168.6, 167.4, 163.4, 137.5, 128.1, 127.1, 122.1, 121.6, 119.9, 112.3, 106.2, 100.6, 97.4, 57.2, 20.1 ppm.

Preparation of 3-bromo-4,6-dimethyl-2*H*-pyran-2-one (5)

A mixture of 1 mmol of 3,5-dimethyl-2H-pyran-2-one and 1 mmol of NBS in 30 mL of carbon tetrachloride was heated for one hour at 80 °C. Afterwards, the solvent was removed under reduced pressure and the crude product purified by flash column chromatography employing a solvent mixture gradient of 4:1 dichloromethane to ethyl acetate.

White solid, yield: 72%, ^{1}H NMR ($CDCl_3$, 300 MHz): 5.93 (1H, s), 2.23 (3H, s), 2.18 (3H, s) ppm; ^{13}C NMR ($CDCl_3$, 75 MHz): 159.5, 159.3, 154.8, 1085.3, 107.2, 23.2, 19.4 ppm.

Preparation of 3-(1*H*-indol-3-yl)-4,6-dimethyl-2*H*-pyran-2-one (10)

A solution of 0.5 mmol of 3-bromo-4,6-dimethyl-2*H*-pyran-2-one, 0.5 mmol of 2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-indole, 1.5 mmol of potassion carbonate and a catalytic amount of tetrakis(triphenylphosphine)palladium(0) were dissolved in 1800 µL of 1.4-dioxane and 600 µL water and refluxed at 100 °C for 24 h. Subsequently the solvent was removed under reduced pressure and the crude product purified by flash column chromatography employing a solvent mixture gradient of 4:1 dichloromethane to ethyl acetate.

Yellow solid, yield 38%, ^{1}H NMR (MeOH-d_4, 600 MHz): 7.43 (1H, dd, *J* = 8.1, 0.9 Hz), 7.28 (1H, s), 7.27 (1H, dd, *J* = 8.1, 0.9 Hz), 7.15 (1H, ddd. *J* = 8.1, 7.2, 1.4 Hz), 7.05 (1H, ddd, *J* = 8.0, 7.2, 0.9 Hz), 6.27 (1H, d, *J* = 0.9 Hz), 2.32 (3H, d, *J* = 0.9 Hz), 2.10 (3H, s) ppm; ^{13}C NMR (MeOH-d_4, 150 MHz): 166.1, 160.2, 155.3, 137.7, 128.1, 126.6, 122.6, 120.4, 120.3, 117.8, 112.6, 109.2, 108.9, 21.0, 19.4 ppm.

Preparation of 3-(1*H*-indol-2-yl)-4-methoxy-6-methyl-2*H*-pyran-2-one (11)

A mixture of 0.5 mmol of 3-bromo-4-methoxy-6-methyl-2*H*-pyran-2-one, 0.5 mmol of 3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-indole, 1.5 mmol of potassion carbonate and a catalytic amount of tetrakis(triphenylphosphine)palladium(0) were dissolved in 1800 µL of 1.4-dioxane and 600 µL water and refluxed at 100 °C for 24 h. Afterwards, the solvent was removed under reduced pressure and the crude product purified by flash column chromatography employing a solvent gradient from dichloromethane : ethyl acetate 3:1 to ethyl acetate.

Yellow solid, yield: 41%, ^{1}H NMR (MeOH-d_4, 600 MHz): 7.49 (1H, d, *J* = 7.8 Hz), 7.40 (1H, dd, *J* = 8.1, 0.7 Hz), 7.09 (1H, d, *J* = 0.7 Hz), 7.07 (1H, m), 6.97 (1H, m), 5.48 (1H, s), 4.13 (3H, s), 2.35 (3H, d, *J* = 0.7 Hz) ppm; ^{13}C NMR (MeOH-d_4, 150 MHz): 168.4, 165.9, 163.5, 136.6, 129.5, 125.9, 122.6, 120.9, 120.2, 111.9, 104.9, 98.4, 97.4, 57.8, 20.0 ppm.

Preparation of 4-hydroxy-3-(1*H*-indol-2-yl)-6-methyl-2*H*-pyran-2-one (12)

To a solution of 0.02 mmol of 3-(1*H*-indol-2-yl)-4-methoxy-6-methyl-2*H*-pyran-2-one dissolved in 200 µL of dimethyl formamide 0.08 mmol of sodium thiomethylate was added and the solution was stirred at 60 °C for six hours. Afterwards, the solvent was removed under reduced pressure and the product purified by reverse phase HPLC.

Yellow solid, yield: 37%, ^{1}H NMR (MeCN-d_3, 600 MHz): 2.13 (3H, s)*, 6.36 (1H, s), 7.19 (1H, s), 7.29 (1H, dd, *J* = 7.7, 7.4 Hz), 7.42 (1H, dd, *J* = 7.7, 7.4 Hz), 7.49 (d, *J* = 7.7 Hz), 7.62 (1H, d, *J* = 7.7 Hz) ppm; ^{13}C NMR (MeCN-d_3, 150 MHz): 19.2, 102.6, 106.9, 111.1, 113.1, 121.2, 124.6, 124.8, 128.0, 129.9, 131.4, 160.9, 162.8, 164.2 ppm. *signal below HDO signal

Preparation of 4-methoxy-6-methyl-3-(quinolin-3-yl)-2*H*-pyran-2-one (13)

To a solution of 0.5 mmol of 3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)quinoline dissolved in 1800 µL of 1.4-dioxane and 600 µL water 0.5 mmol of 3-bromo-4-methoxy-6-methyl-2*H*-pyran-2-one, 1.5 mmol of potassium carbonate and a catalytic amount of tetrakis(triphenylphosphine)palladium(0) was added and the mixture was subsequently refluxed at 100 °C for 24 h. Afterwards, the solvent was removed under reduced pressure

and the crude product purified by flash column chromatography employing a gradient of dichloromethane : ethyl acetate 4:1 to ethylacetate.

Yellow solid, yield 42%, ^{1}H NMR ($CDCl_3$, 600 MHz): 9.37 (1H, d, *J* = 2.0 Hz), 9.16 (1H, d, *J* = 2.0 Hz), 8.25 (1H, dd, *J* = 8.5, 0.9 Hz), 7.98 (1H, dd, *J* = 8.5, 1.3 Hz), 7.96 (1H, m), 7.94 (1H, dd, *J* = 8.4, 1.3 Hz), 7.79 (1H; ddd, *J* = 8.5, 7.1, 1.0 Hz), 6.36 (1H, d, *J* = 1.0 Hz), 3.82 (3H, s,), 2.32 (3H, d, *J* = 1.0 Hz) ppm; ^{13}C NMR ($CDCl_3$, 150 MHz): 169.0, 164.1, 163.8, 145.6, 145.2, 135.6, 133.7, 129.7, 128.9, 128.3, 128.0, 120.7, 100.9, 95.1, 61.3, 20.2 ppm.

Preparation of 4-hydroxy-6-methyl-3-(quinolin-3-yl)-2*H*-pyran-2-one (14)

To a solution of 0.02 mmol of 4-methoxy-6-methyl-3-(quinolin-3-yl)-2*H*-pyran-2-one dissolved in 200 µL of dimethyl formamide 0.08 mmol of sodium thiomethylate was added and the solution was stirred at 60 °C for six hours. Afterwards, the solvent was removed under reduced pressure and the product purified by reverse phase HPLC.

Yellow solid, yield: 39%, ^{1}H NMR (MeOH-d_4, 600 MHz): 9.07 (1H, d, *J* = 1.9 Hz), 8.51 (1H, d, *J* = 1.6 Hz), 7.95 (1H, d, *J* = 8.7 Hz), 7.87 (1H, d, *J* = 8.1 Hz), 7.67 (1H, ddd, *J* = 8.4, 7.0, 1.2 Hz), 7.54 (1H, ddd, *J* = 8.4, 7.0, 1.2 Hz), 5.93 (1H, s), 2.20 (3H, s) ppm; ^{13}C NMR (MeOH-d_4, 150 MHz): 169.2, 161.5, 154.3, 148.3, 146.0, 138.7, 131.0, 129.9, 129.1, 128.2, 127.5, 108.3, 98.6, 19.6 ppm.

Calculation of proton NMR data

Proton NMR data of compound **1** were calculated by DFT employing jaguar 9.5, release 12 from the small-drug discovery suite (Schrodinger Inc.) using the following parameters. SFC spin treatment was set to automaticand B3LYP hybrid function and nonrelativistic hamiltonians were used. The SFC accuracy level was set to automatic and atomic overlap was used as initial guess. As convergence criteria 48 maximum iterations, an energy change of $5 \cdot 10^{-5}$ hartree and a RMS density matrix charge of $5 \cdot 10^{-6}$ were used. Geometry optimization was used in maximum 100 steps with default convergence criteria and a Schlegel guess as initial hessian. Coordinates were set to redundant internal. The standard Poisson-Boltzmann continuum solvation model was used employing solvent data of acetonitrile (dielectric constant 37.5, probe radius 21.9 nm, density 0.777 g/mL, molecular weight 41.05 g/mol). Gas-phase reference energy was optimized for gas-phase structure.

Evaluation of antiproliferative and cytotoxic effects

The test substances were dissolved in DMSO before being diluted in the respective medium. The adherent cells were harvested at the logarithmic growth phase after soft trypsinization using 0.25% trypsin in phosphate buffered saline (PBS) containing 0.02% ethylenediaminetetraacetic acid (EDTA). For each experiment, approximately 10,000 cells were seeded with 0.1 mL culture medium per well of the 96-well microplates. HeLa cells were pre-incubated for 48 h prior to the addition of the test compounds, which were carefully diluted on the subconfluent monolayers. Incubation was then conducted in a humidified atmosphere at 37 °C and 5% CO_2. In case of K-562 cells, the number of viable cells in every well was determined using the CellTiter-Blue1 assay. The adherent HUVEC and HeLa cells were fixed by glutaraldehyde and stained with a 0.05% solution of methylene blue for 10 min. After gently washing, the stain was eluted with 0.2 mL of 0.33 N HCl in the wells. The optical densities were measured at 660 nm in a SUNRISE microplate reader (TECAN).

Determination of antimicrobial activity

Antimicrobial activities of both compounds were determined in a primary screen against *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* SG511, *Mycobacterium vaccae* IMET 10670, *Pseudomonas aeruginosa* K799/61, *Escherichia coli* SG458, *Sporobolomyces salmonicolor* SBUG 549, *Candida albicans* ATCC14053 and *Penicillium notatum* JP 36. To this end, holes with 7 mm diameter were aseptically punched in the respective agar medium. Subsequently, the agar plates were inoculated with the test organisms. 0.8 mg of test compound was dissolved in 1 mL of methanol, and 50 µL of this solution was transferred to a single hole. Ciprofloxacin and amphotericin B served as positive controls. After evaporation of the solvent, the agar plates were incubated depending on the growth conditions of the test organisms. MIC data were obtained by broth microdilution in 96 well plates. Bacterial growth was determined by measuring of the optical density at 600 nm.

Table S1. NMR data of compound **1** in MeCN-d_3.

#	^{13}C [ppm]	^{1}H [ppm, multiplicity, *J* in Hz]	COSY	HMBC
1	106.8	7.06 s		3, 9, 10, 13
2	109.7			
3	128.0			
4	120.0	7.94 (1H, dd, *J* =1.0, 8.0)	5	2, 6, 8
5	122.2	7.21 (1H, ddd, *J* = 1.0, 7.2, 8.0)	4, 6	3, 7
6	124.0	7.26 (1H, ddd, *J* = 1.0, 7.2, 8.1)	5, 7	4, 8
7	113.1	7.51(1H, dt, *J* = 1.0, 8.1)	6	3, 5
8	137.0			
9	143.8			
10	132.3	8.01 (1H, d, *J* = 2.8)		2, 3, 12
11	160.6			
12	137.0			
13	181.1			
14	12.5	2.32 s		11, 12

Table S2. Antimicrobial activity of compound **1** (agar duffusion assay).

Teststrain	**diameter of inhibition zone [mm]**
Bacillus subtilis ATCC	0
Staphylococcus aureus SG511	0
Escherichia coli SG458	15
Pseudomonas aeruginosa K799/61	0
Mycobacterium vaccae IMET 10670	42
Sporobolomyces salmonicolor SBUG 549	0
Candida albicans ATCC14053	0
Penicillium notatum JP 36	13

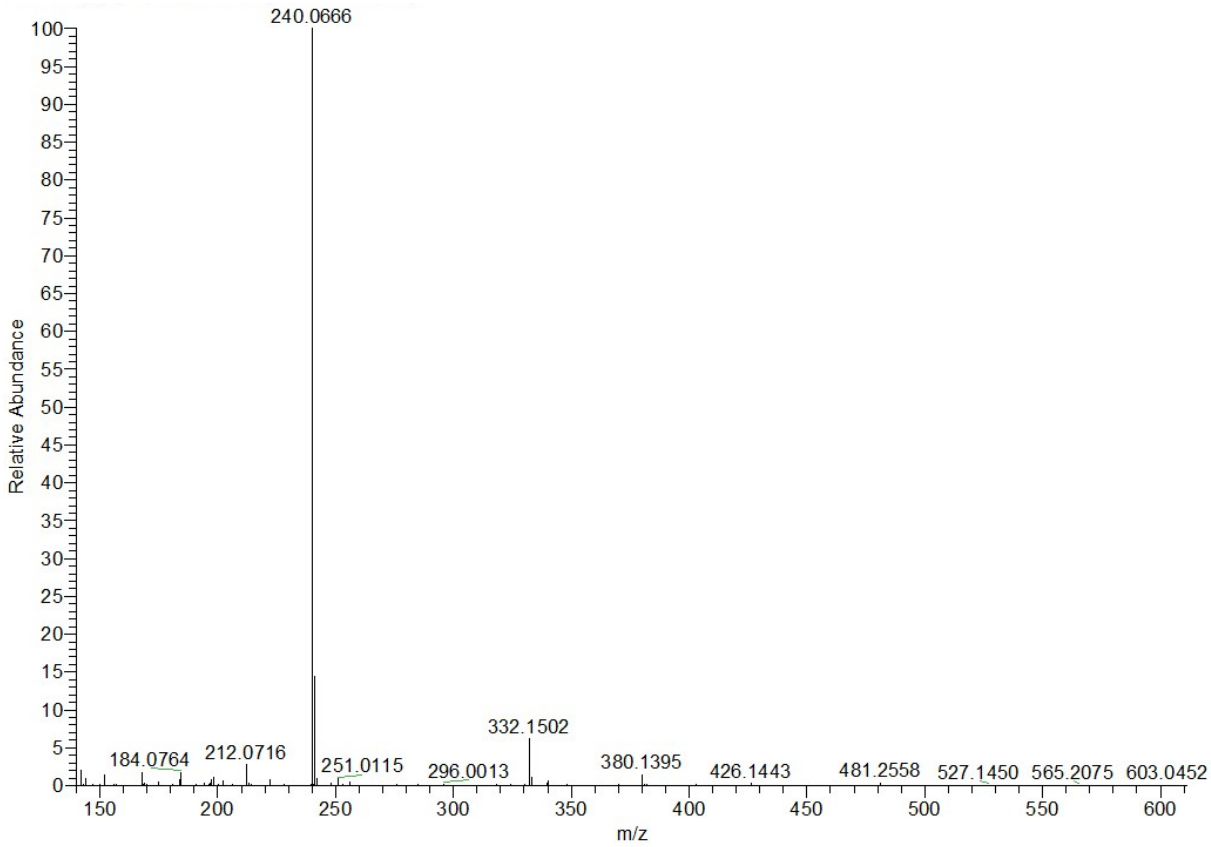

Figure S1. HR-MS spectrum of clostroindolin (**1**) (negative mode).

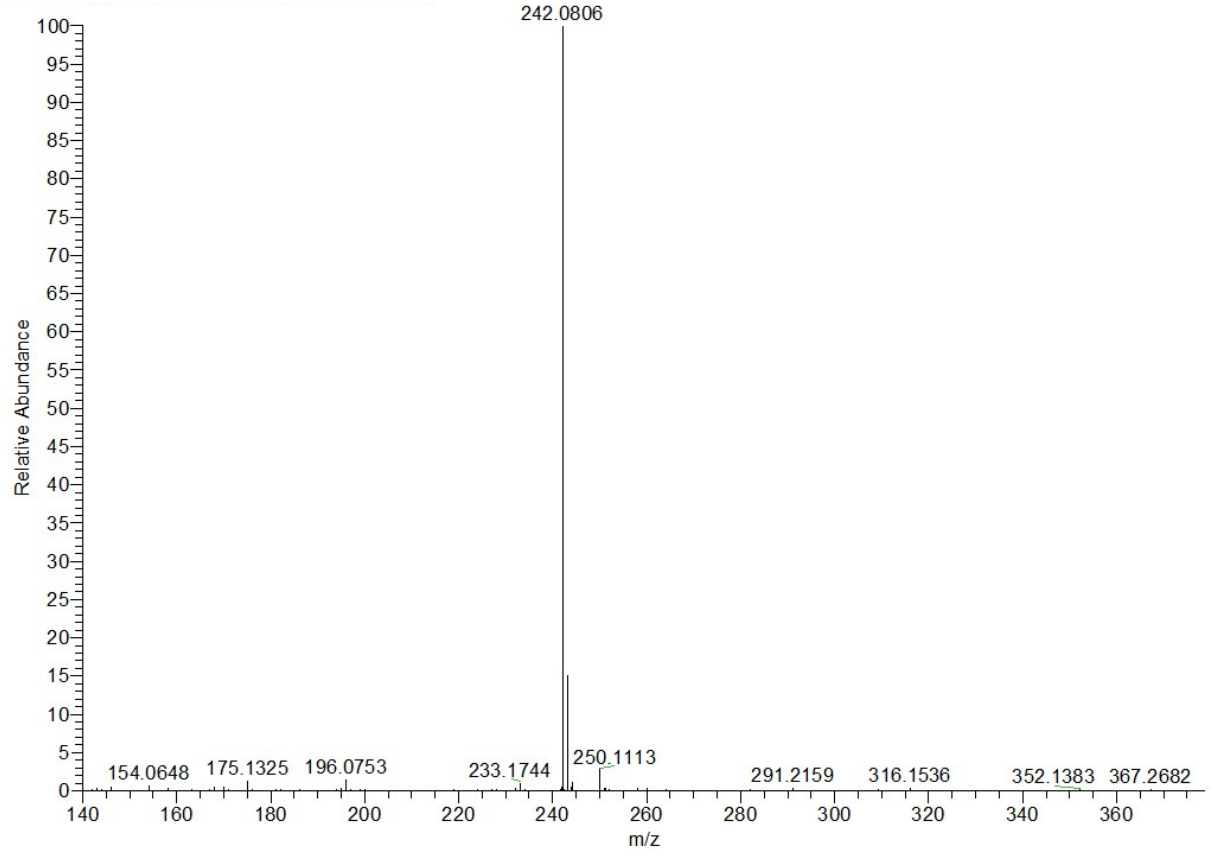

Figure S2. HR-MS spectrum of clostroindolin (**1**) (positive mode).

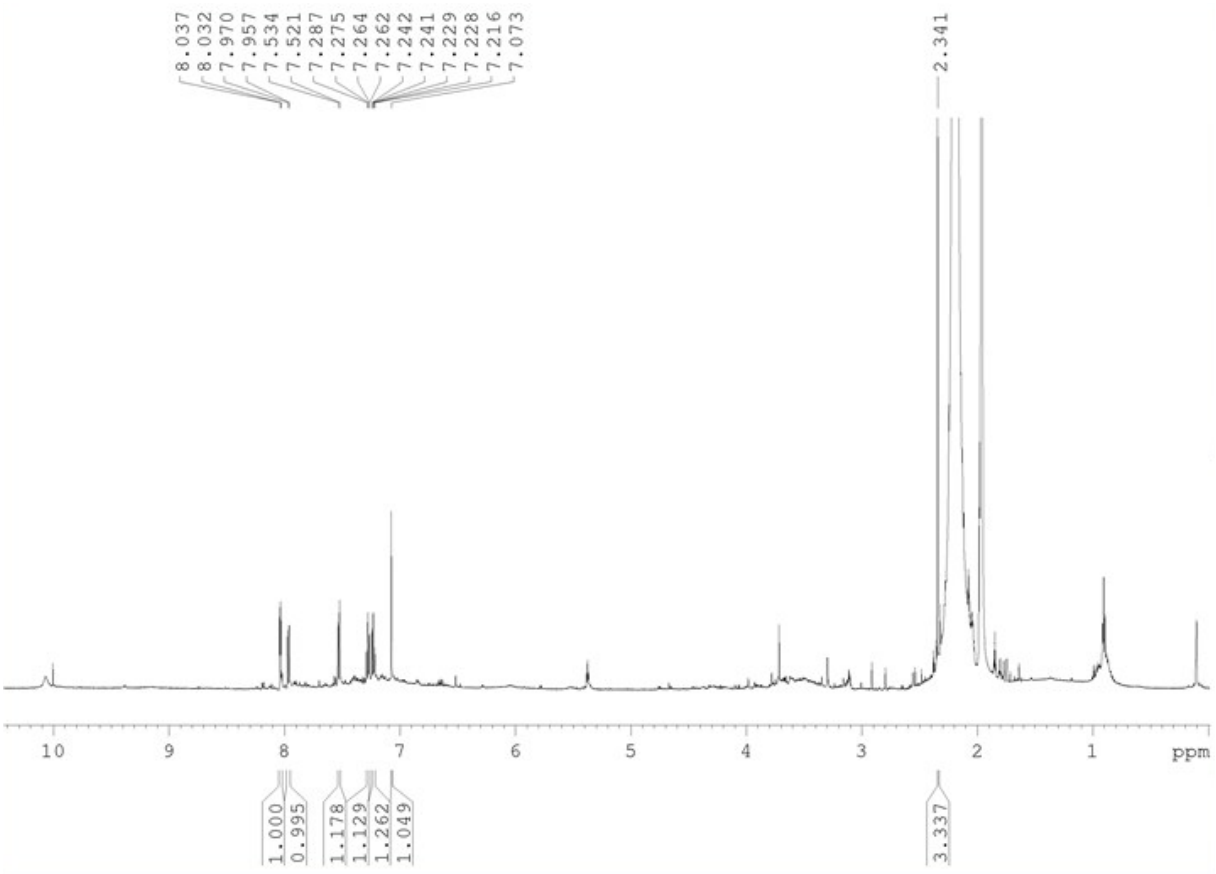

Figure S3. ^{1}H NMR spectrum of clostroindolin (**1**) in MeCN-d_3 measured at 600 MHz.

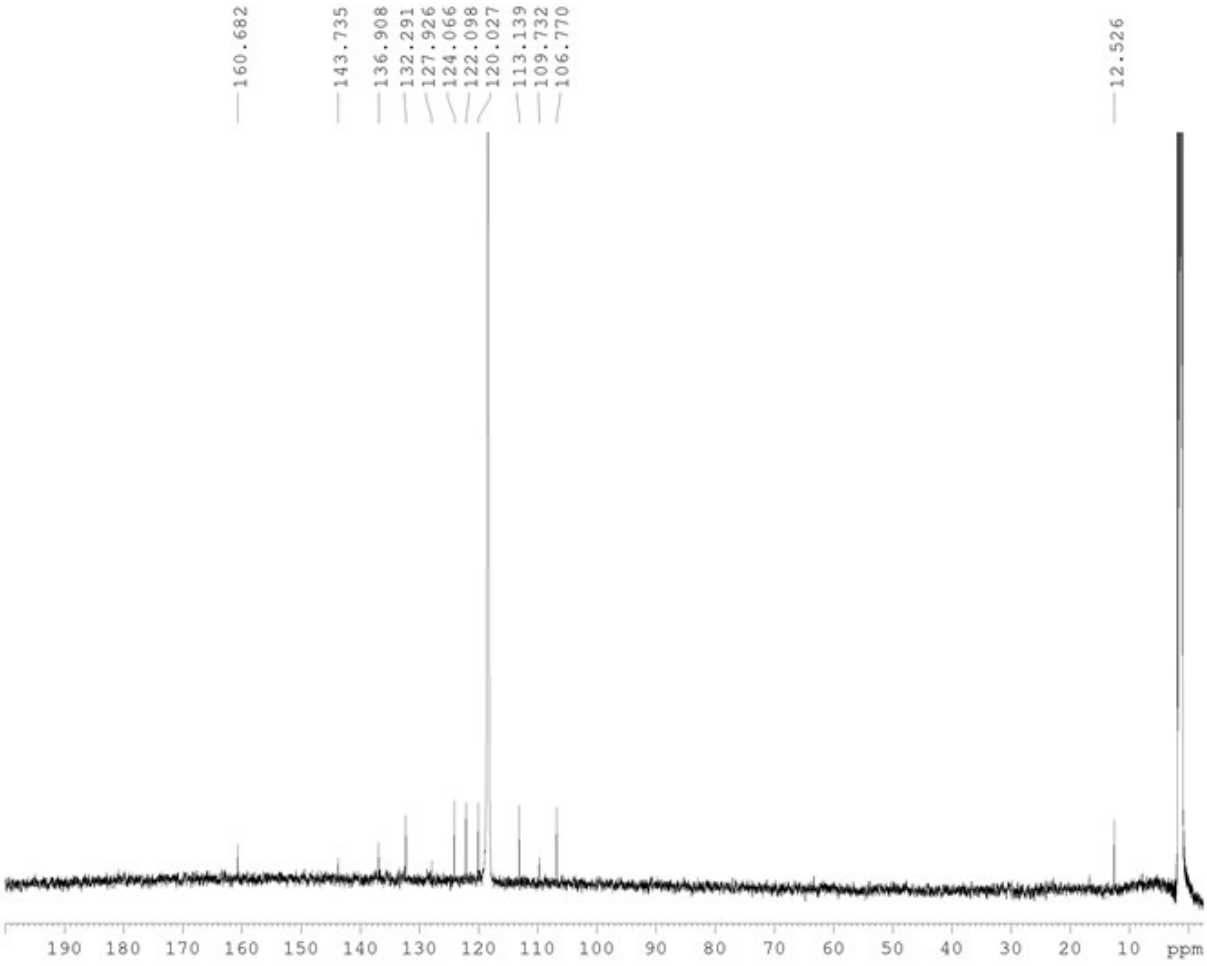

Figure S4. ^{13}C NMR spectrum of clostroindolin (**1**) in MeCN-d_3 measured at 150 MHz.

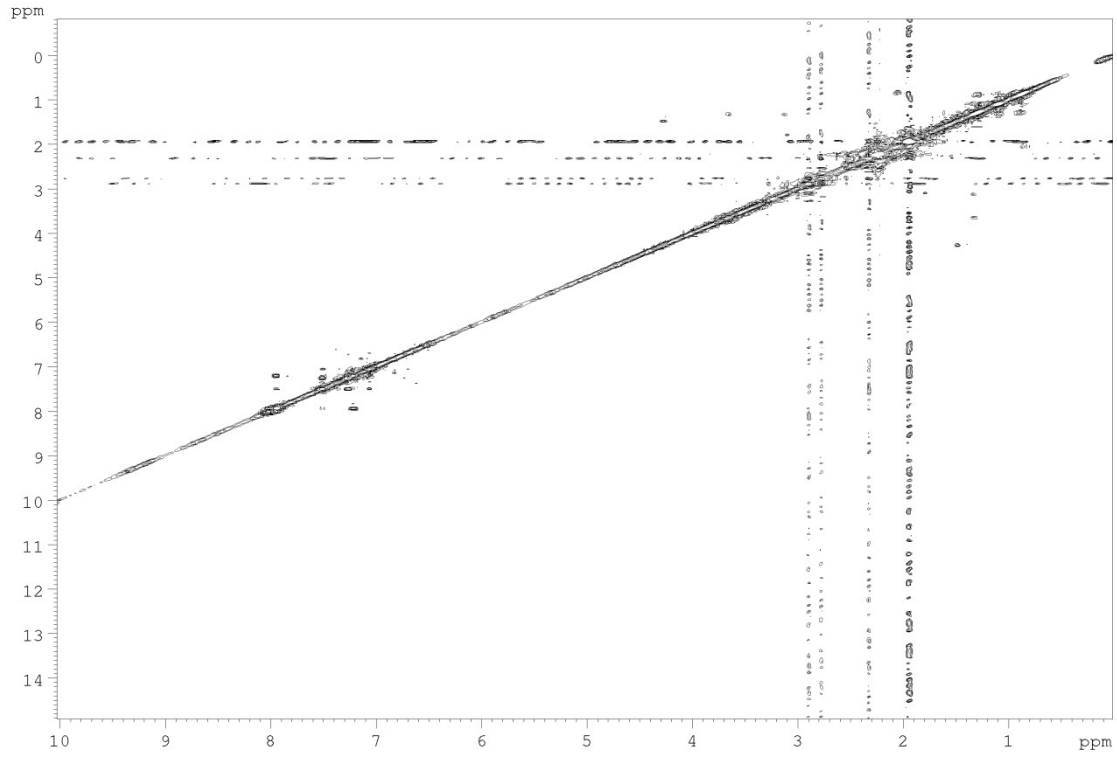

Figure S5. COSY spectrum of clostroindolin (**1**) in MeCN-d_3.

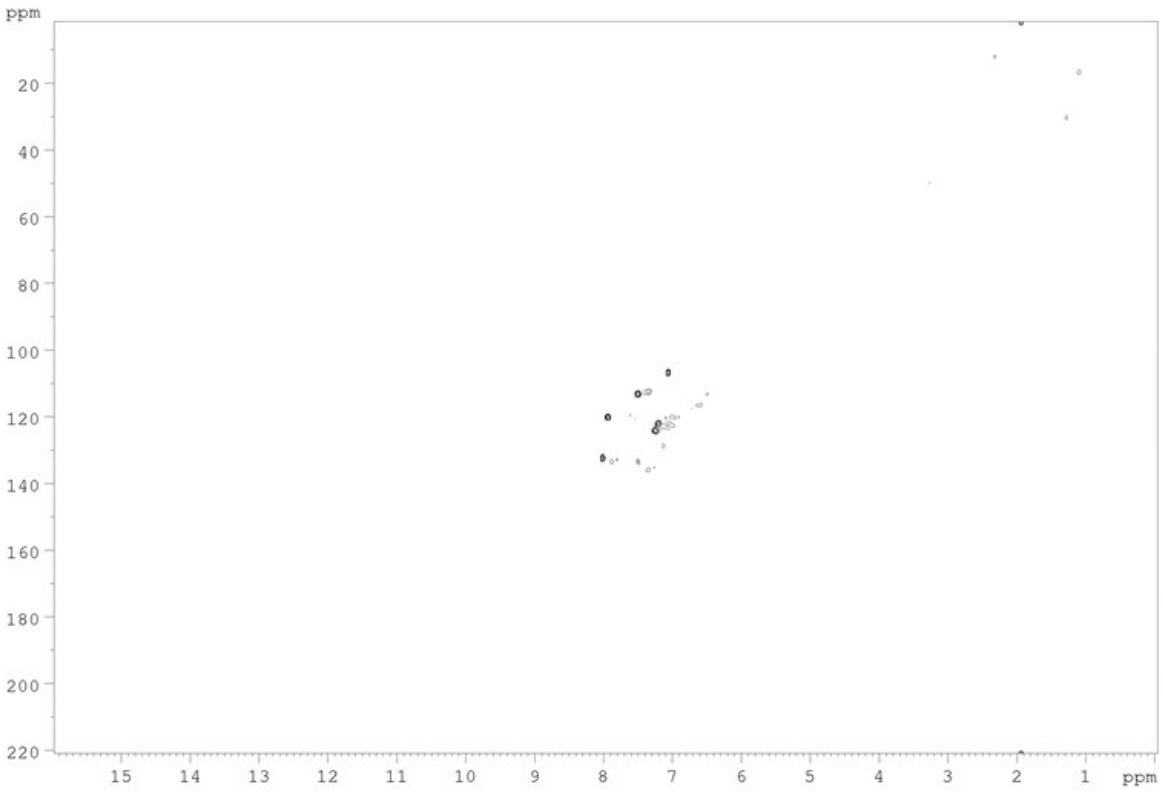

Figure S6. HSQC spectrum of clostroindolin (**1**) in MeCN-d_3.

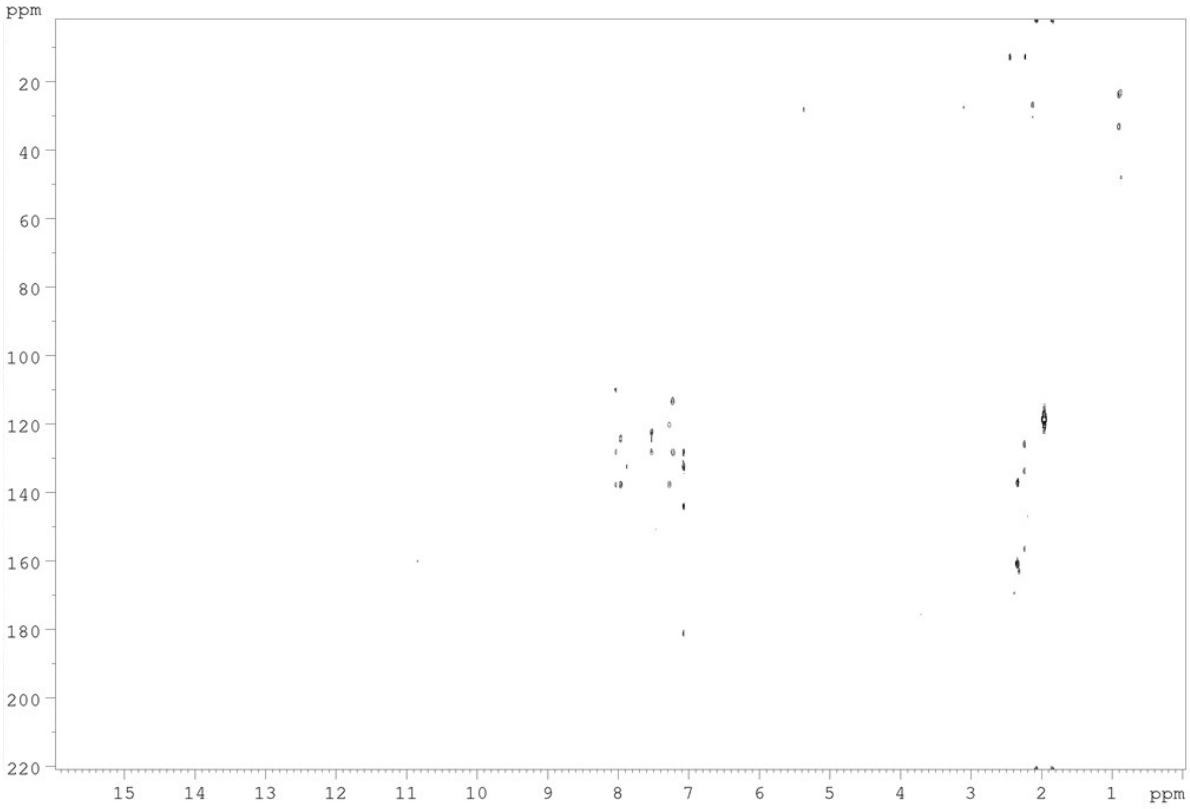

Figure S7. HMBC spectrum of clostroindolin (**1**) in MeCN-d_3.

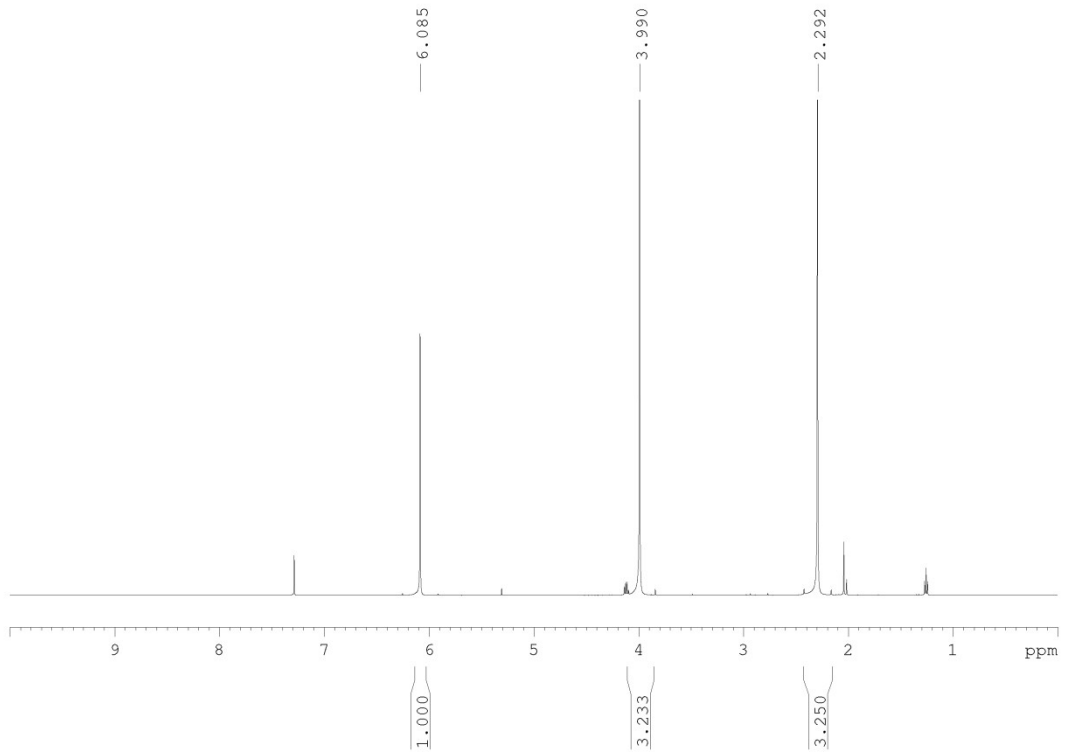

Figure S8. ^{1}H NMR spectrum of 3-bromo-4-methoxy-6-methyl-2H-pyran-2-one (**12**) in $CDCl_3$.

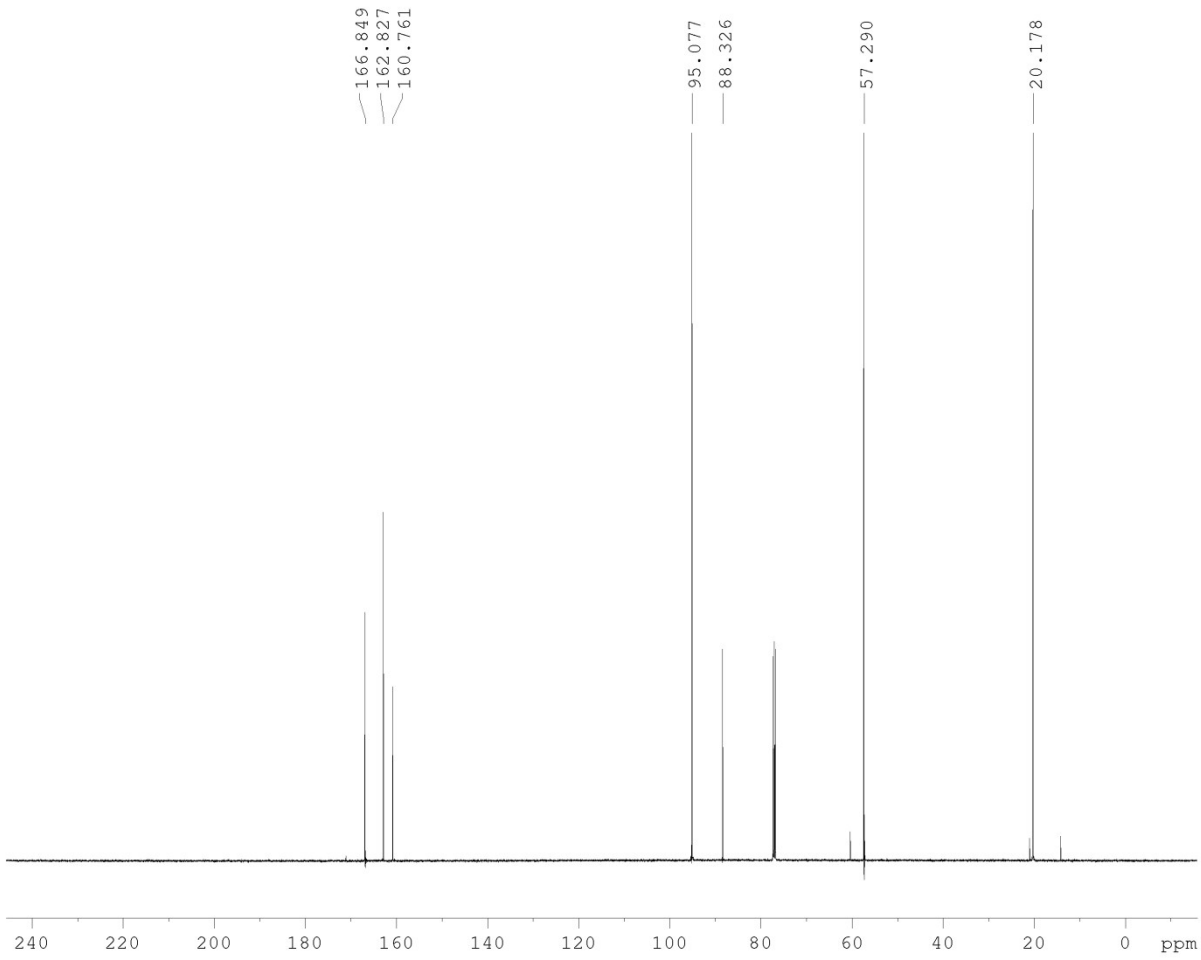

Figure S9. ^{13}C NMR spectrum of 3-bromo-4-methoxy-6-methyl-2H-pyran-2-one (**12**) in $CDCl_3$.

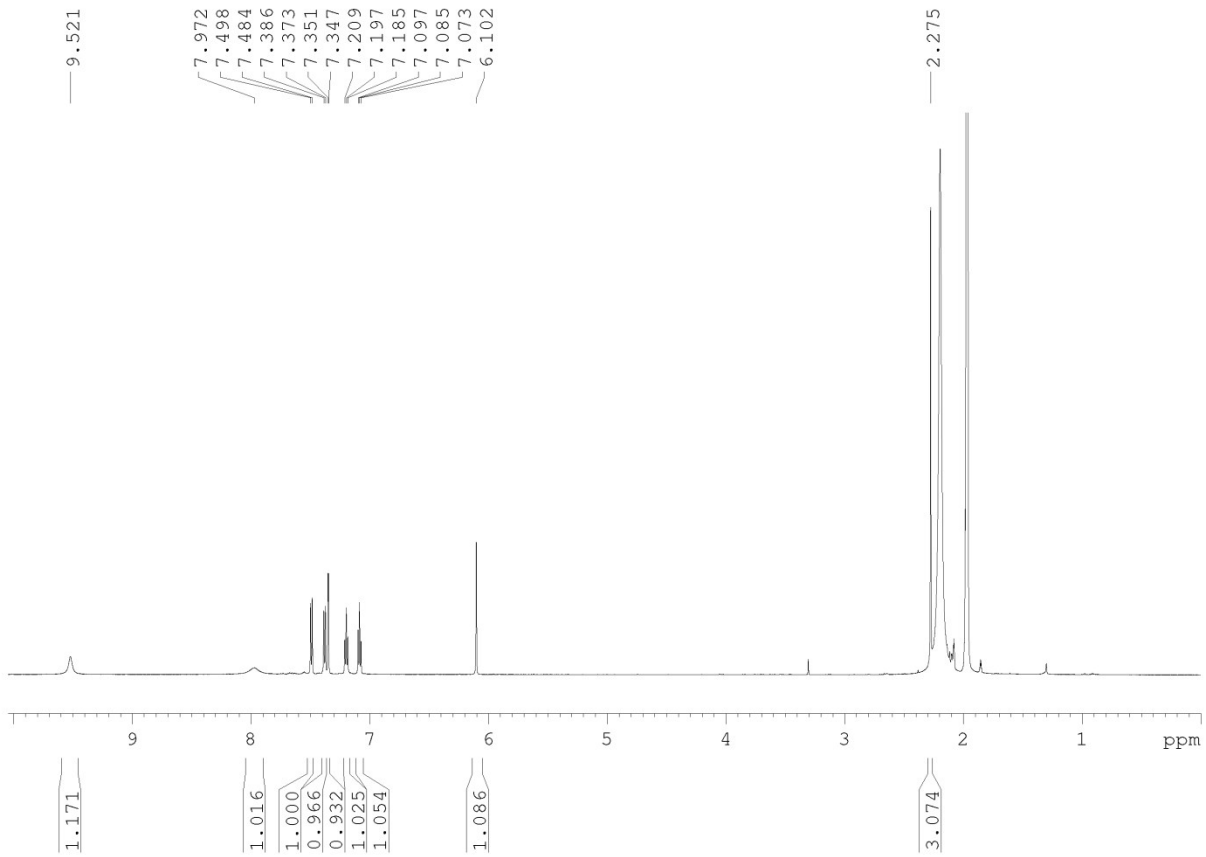

Figure S10. ^{1}H NMR spectrum of 4-hydroxy-3-(1H-indol-3-yl)-6-methyl-2H-pyran-2-one (**3**) in $CDCl_3$.

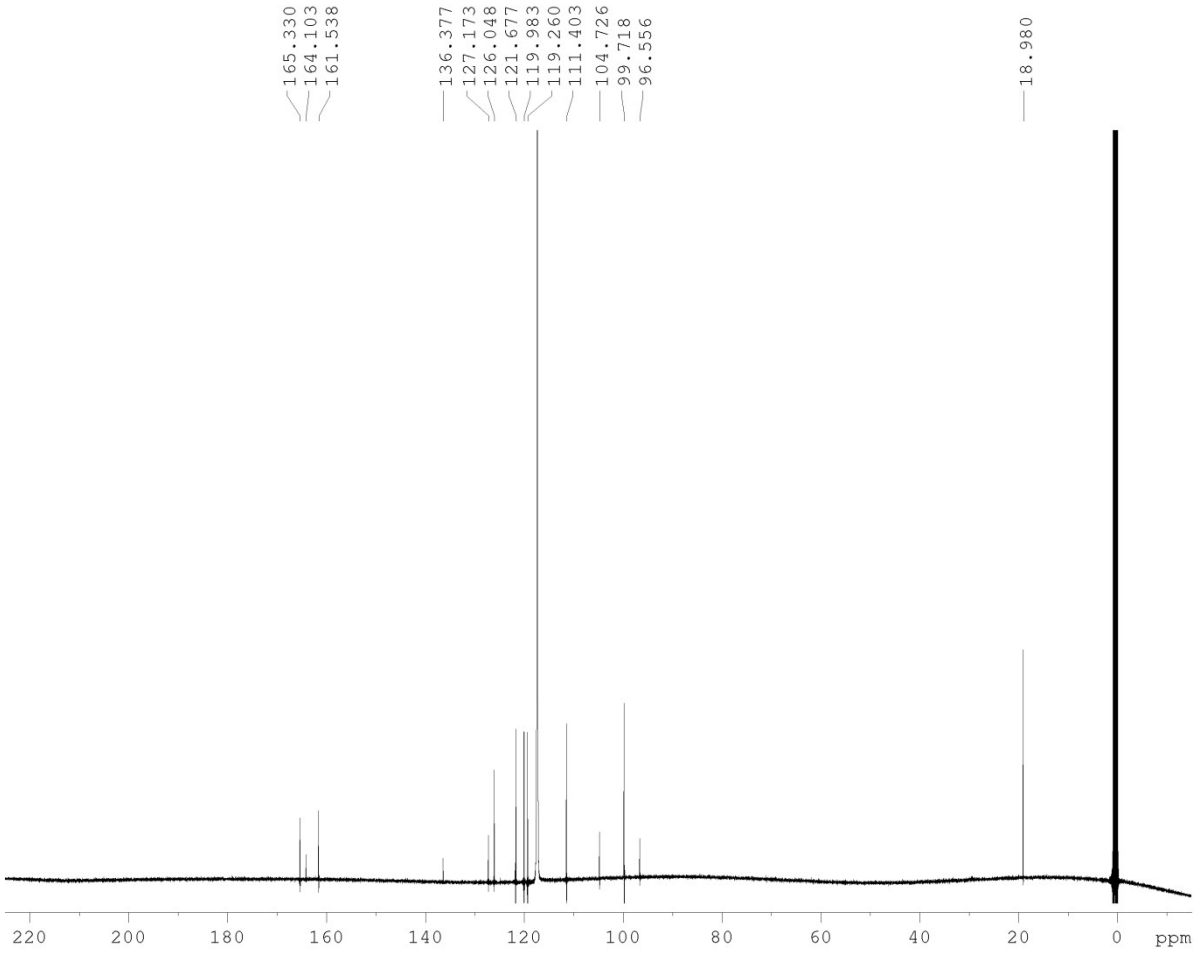

Figure S11. ^{13}C NMR spectrum of 4-hydroxy-3-(1H-indol-3-yl)-6-methyl-2H-pyran-2-one (**3**) in $CDCl_3$.

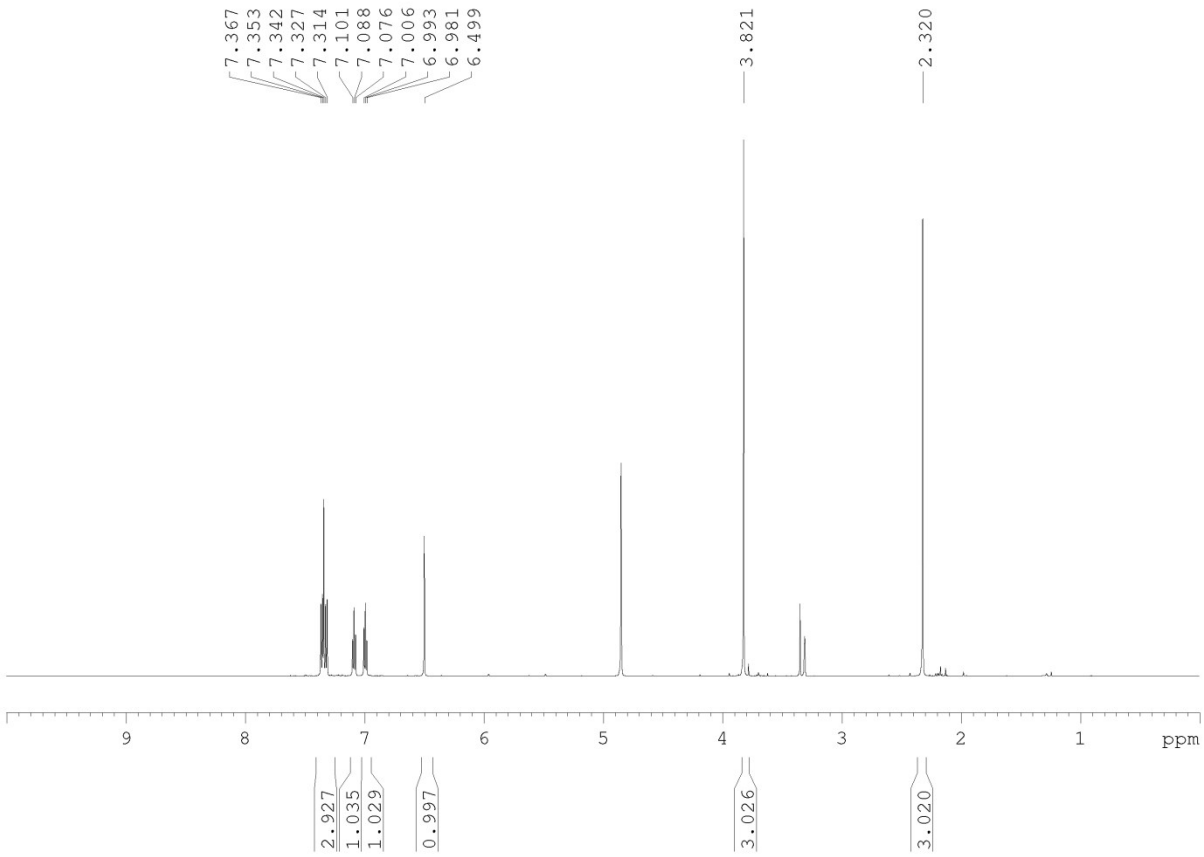

Figure S12. ^{1}H NMR spectrum of 3-(1H-indol-3-yl)-4-methoxy-6-methyl-2H-pyran-2-one (**4**) in $CDCl_3$.

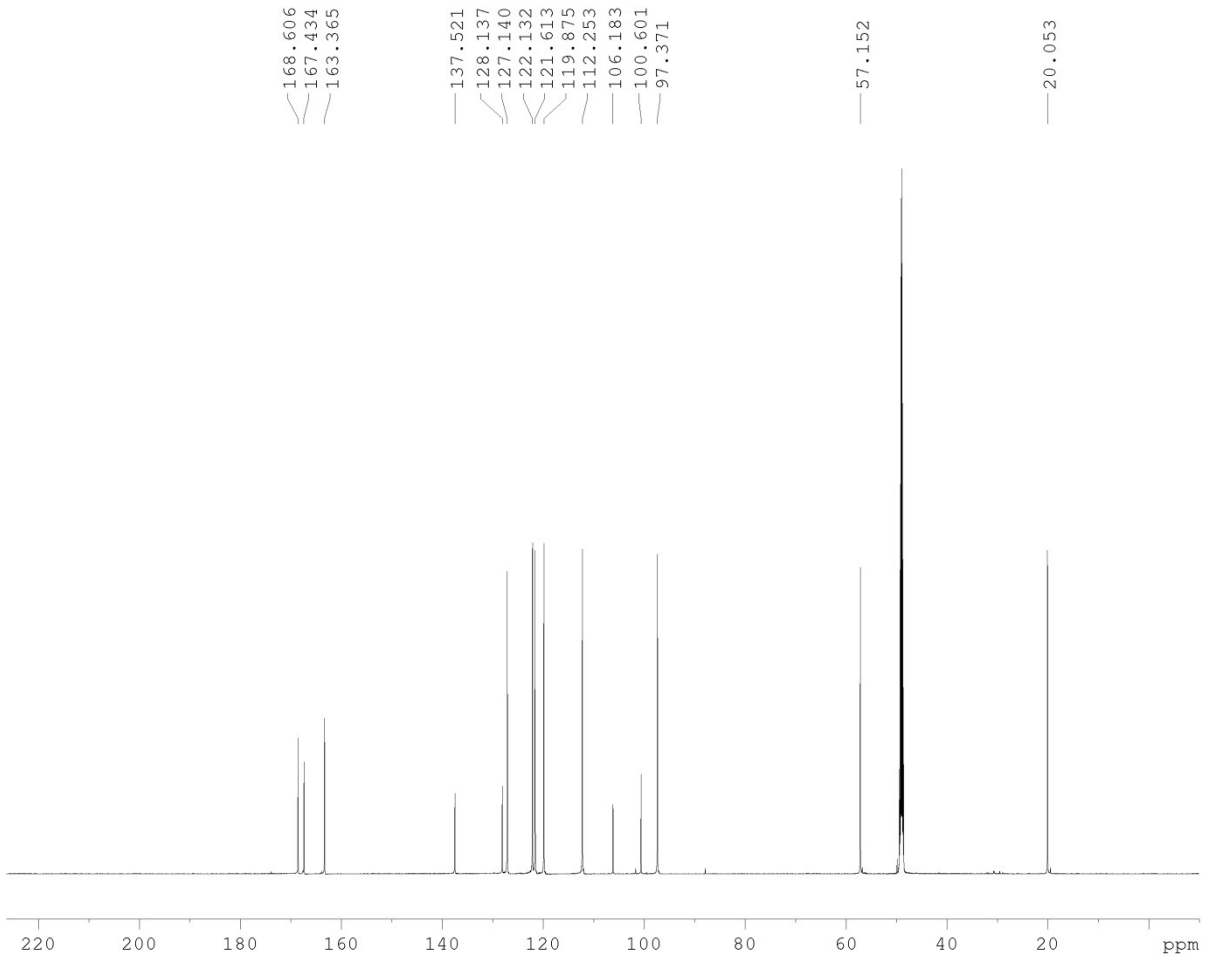

Figure S13. ^{13}C NMR spectrum of 3-(1H-indol-3-yl)-4-methoxy-6-methyl-2H-pyran-2-one (**4**) in $CDCl_3$.

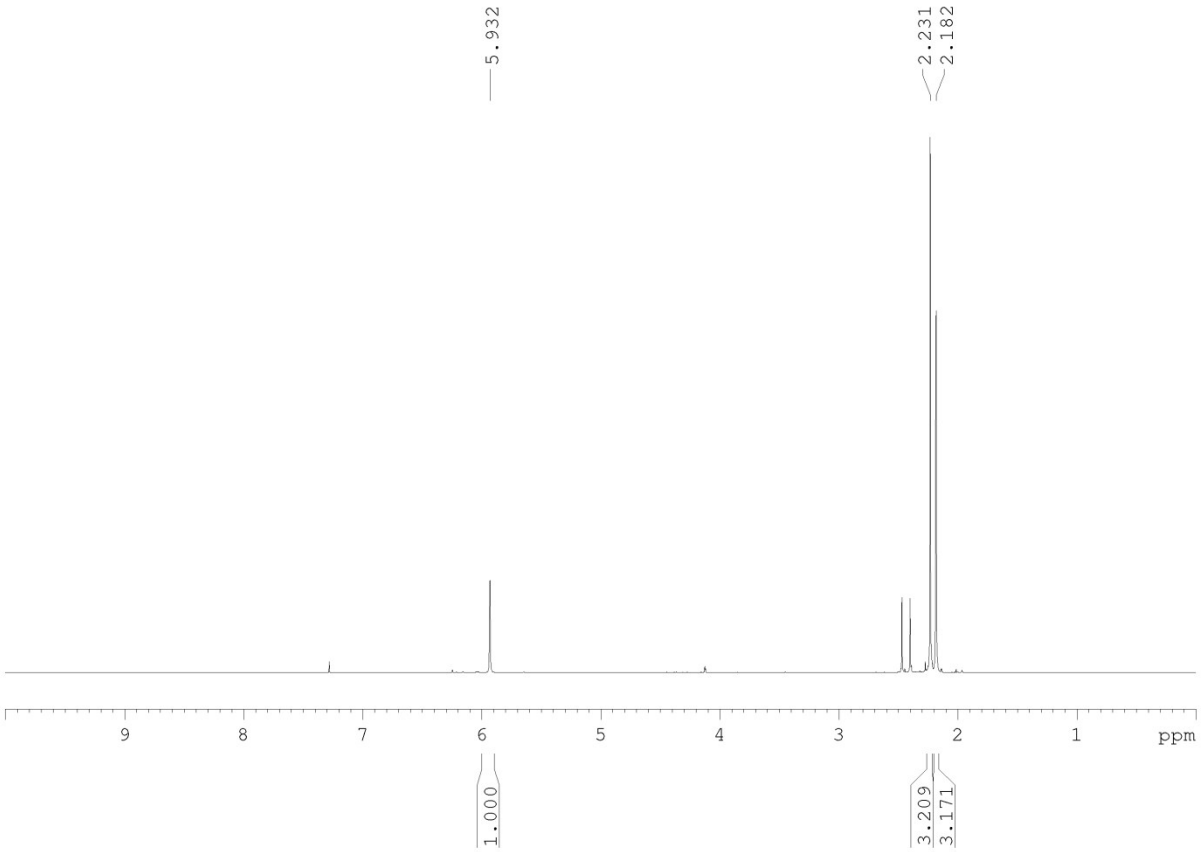

Figure S14. ^{1}H NMR spectrum of 3-bromo-4,6-dimethyl-2H-pyran-2-one (**13**) in $CDCl_3$.

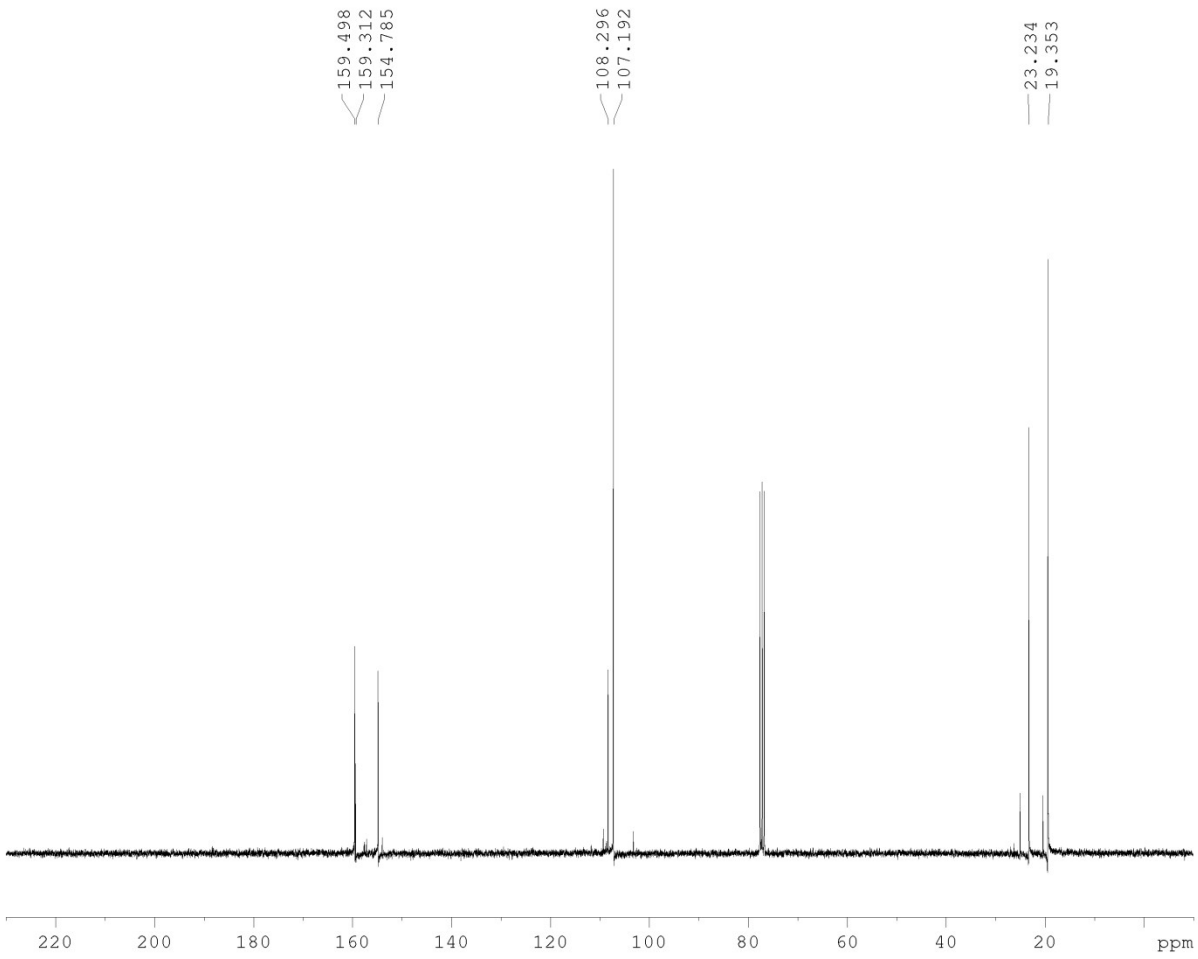

Figure S15. ^{13}C NMR spectrum of 3-bromo-4,6-dimethyl-2H-pyran-2-one (**13**) in $CDCl_3$.

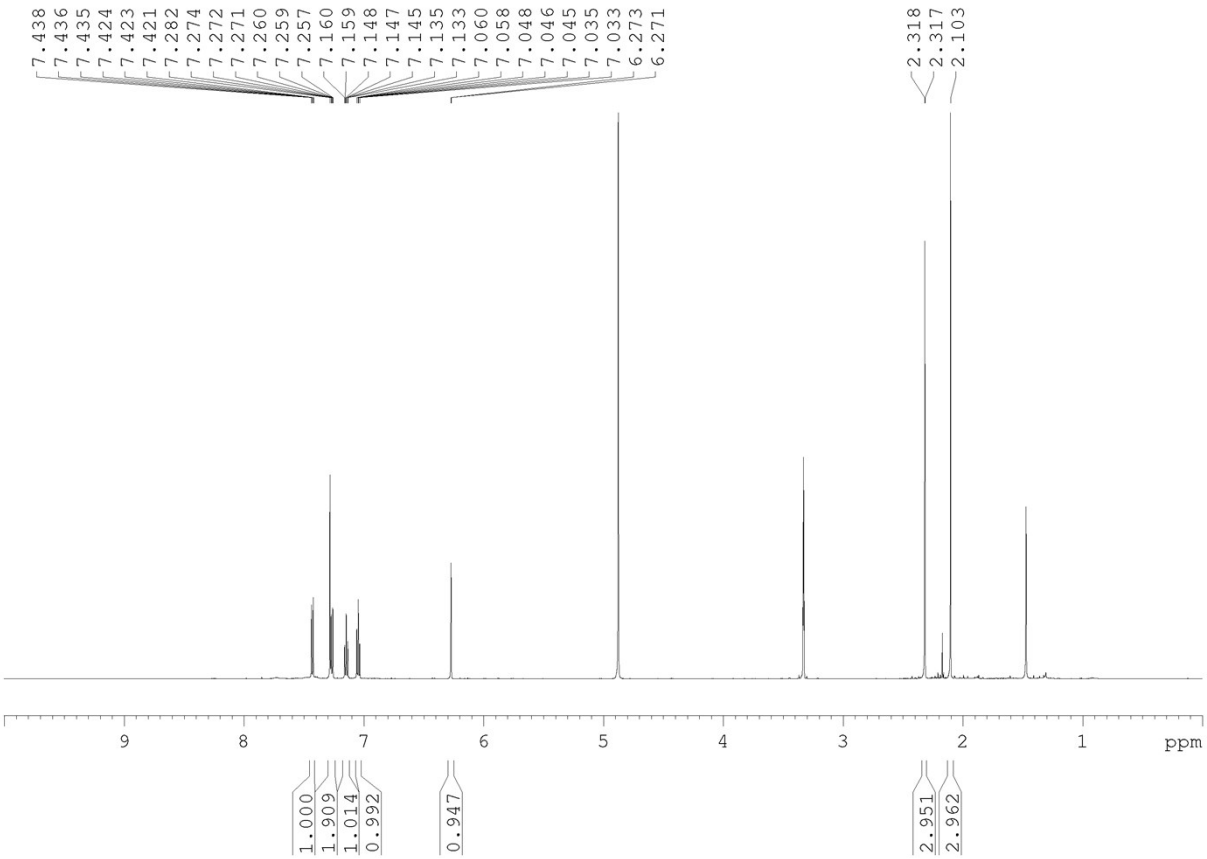

Figure S16. ^{1}H NMR spectrum of 3-(1H-indol-3-yl)-4,6-dimethyl-2H-pyran-2-one (**5**) in MeOH-d_4.

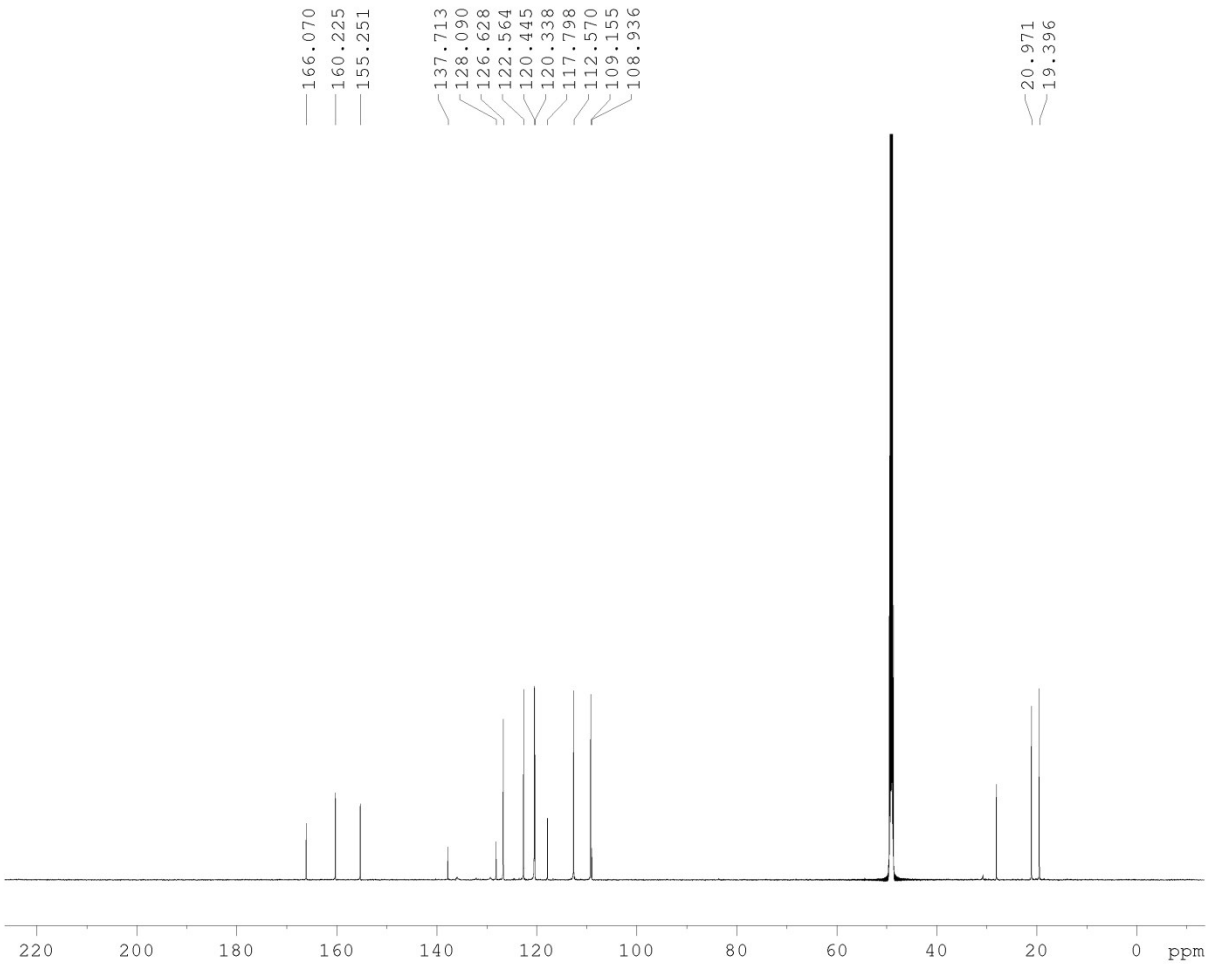

Figure S17. ^{13}C NMR spectrum of 3-(1H-indol-3-yl)-4,6-dimethyl-2H-pyran-2-one (**5**) in MeOH-d_4.

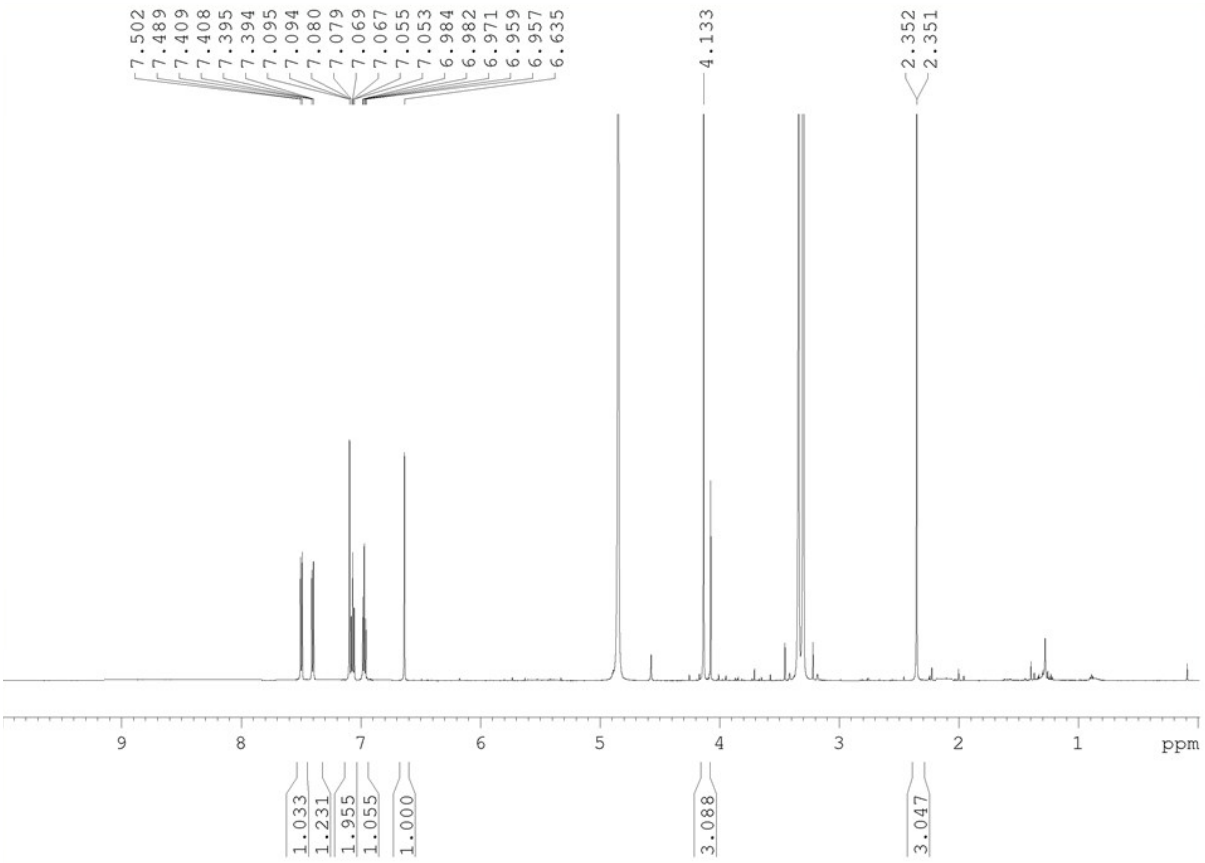

Figure S18. ^{1}H NMR spectrum of 3-(1H-indol-2-yl)-4-methoxy-6-methyl-2H-pyran-2-one (**6**) in MeOH-d_4.

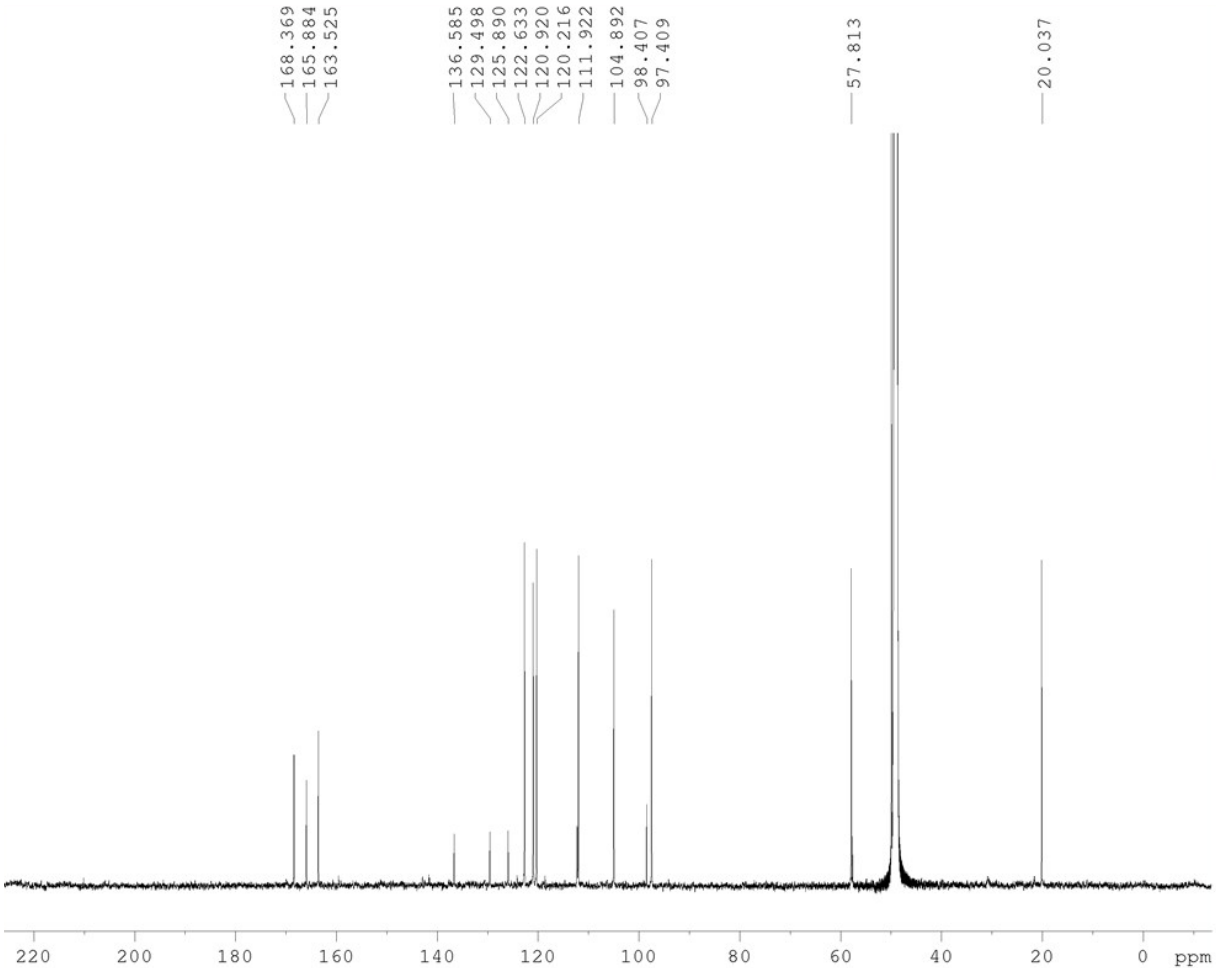

Figure S19. ^{13}C NMR spectrum of 3-(1H-indol-2-yl)-4-methoxy-6-methyl-2H-pyran-2-one (**6**) in MeOH-d_4.

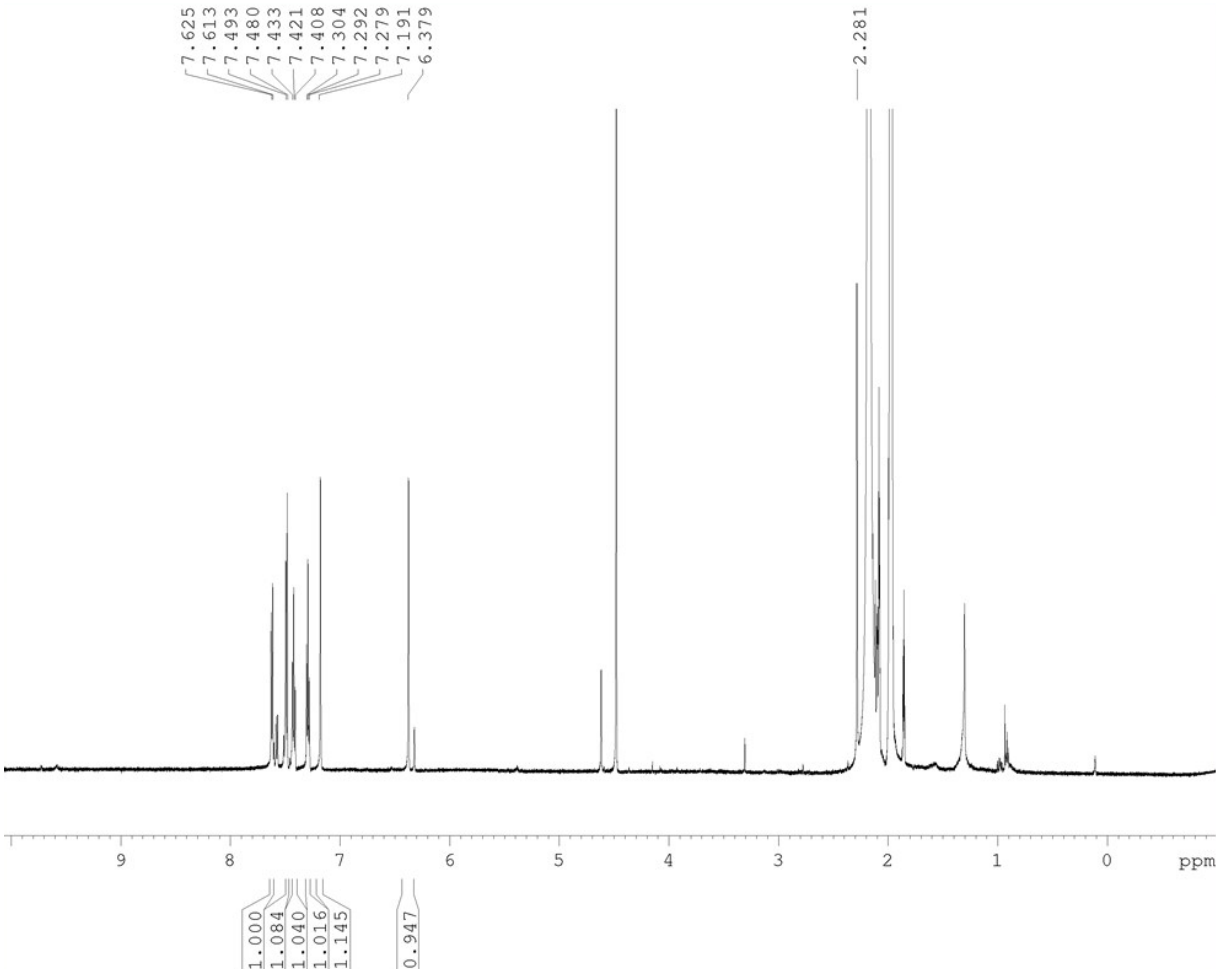

Figure S20. ^{1}H NMR spectrum of 4-hydroxy-3-(1H-indol-2-yl)-6-methyl-2H-pyran-2-one (**7**) in MeCN-d_3.

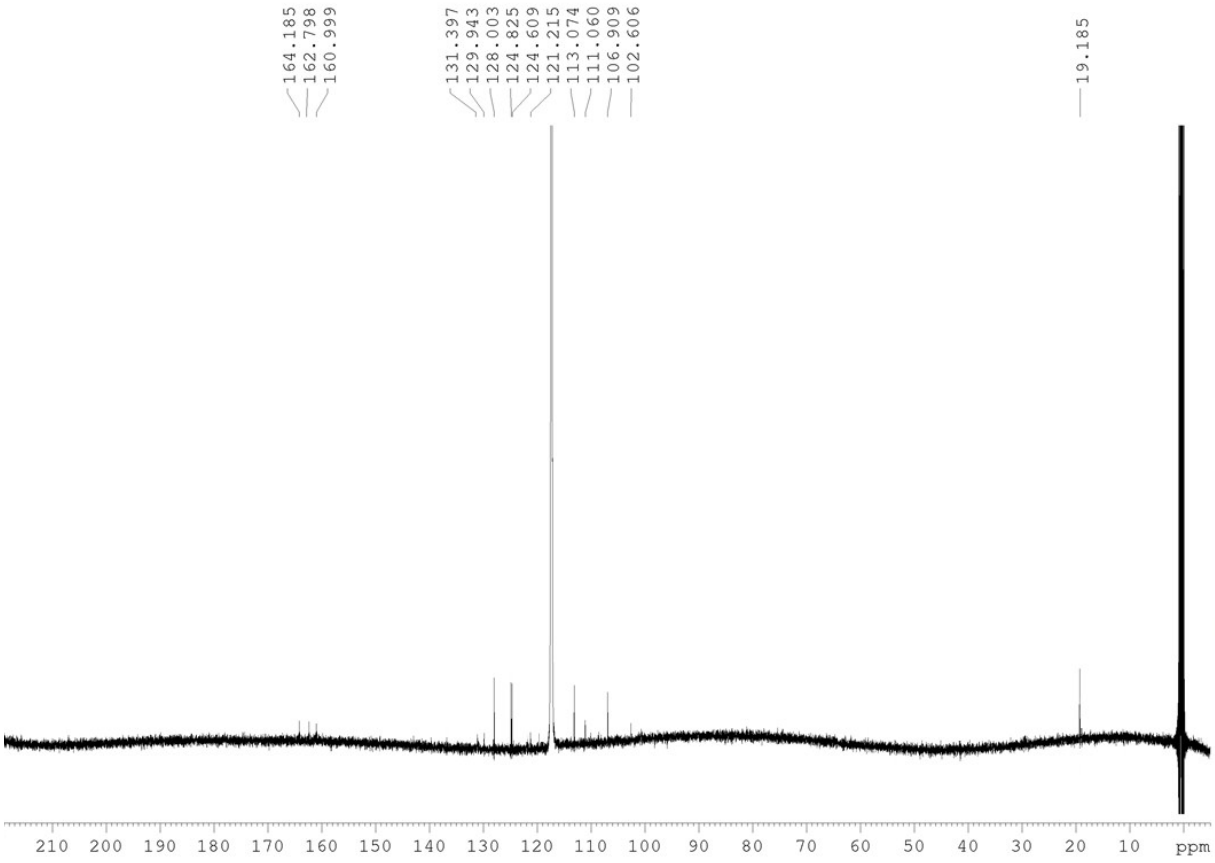

Figure S21. ^{13}C NMR spectrum of 4-hydroxy-3-(1H-indol-2-yl)-6-methyl-2H-pyran-2-one (**7**) in MeCN-d_3.

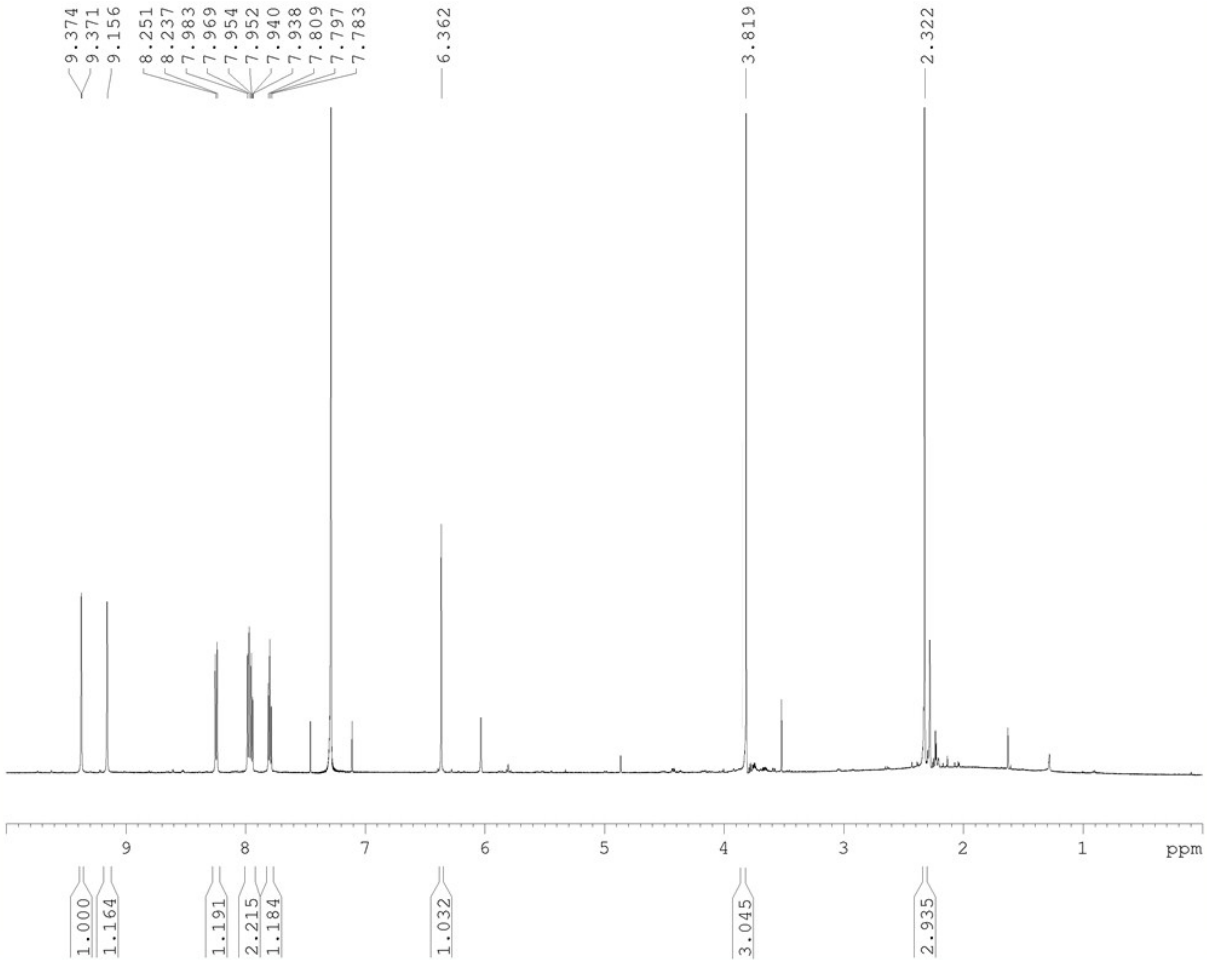

Figure S22. ^{1}H NMR spectrum of 4-methoxy-6-methyl-3-(quinolin-3-yl)-2H-pyran-2-one (**8**) in $CDCl_3$.

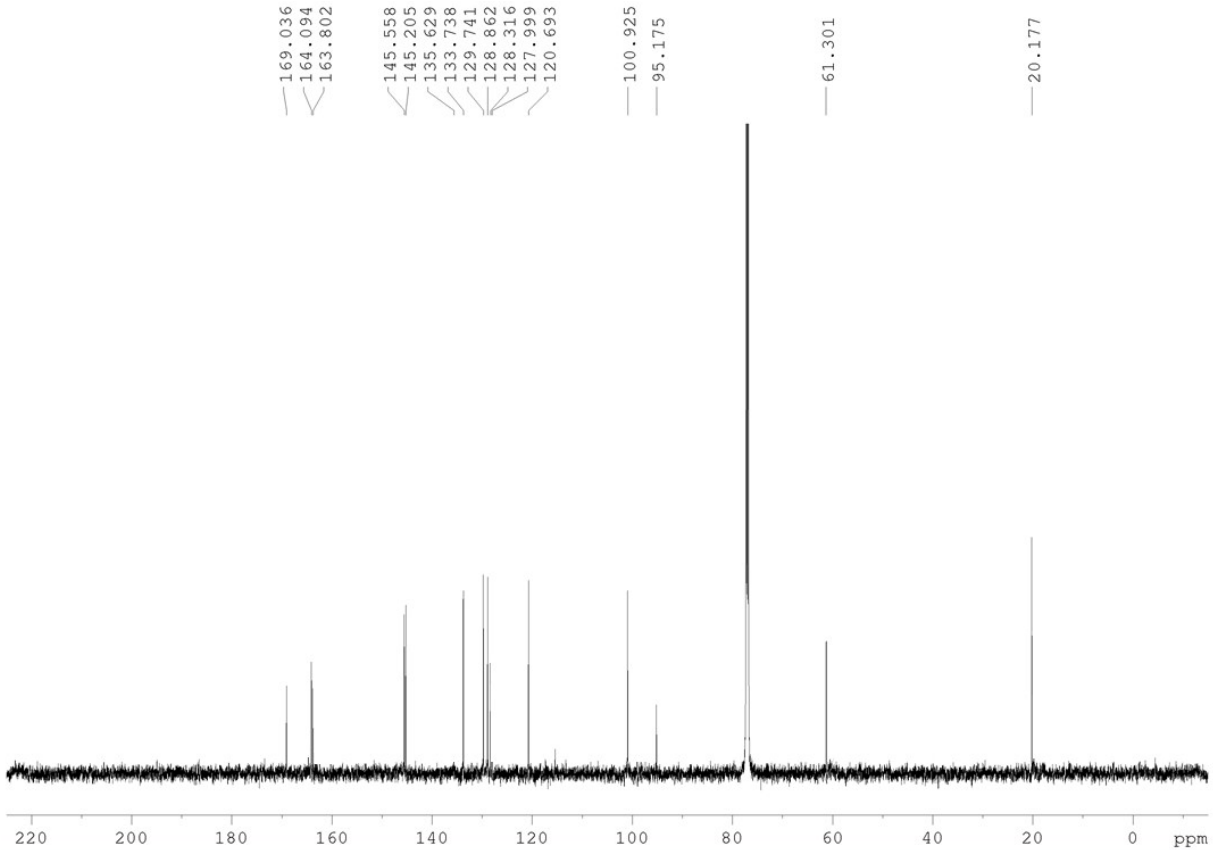

Figure S23. ^{13}C NMR spectrum of 4-methoxy-6-methyl-3-(quinolin-3-yl)-2H-pyran-2-one (**8**) in $CDCl_3$.

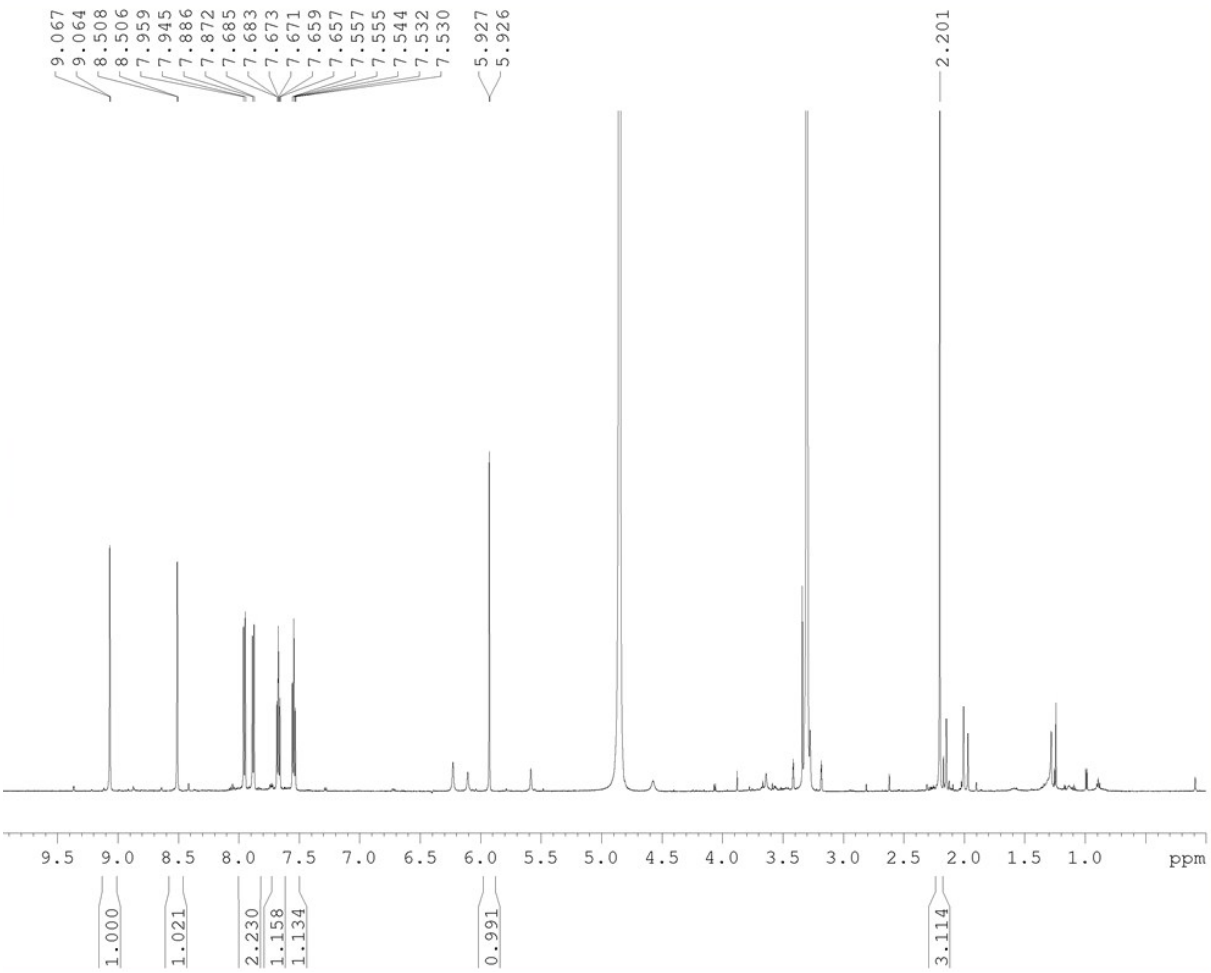

Figure S24. ^{1}H NMR spectrum of 4-hydroxy-6-methyl-3-(quinolin-3-yl)-2H-pyran-2-one (**9**) in MeOH-d_4.

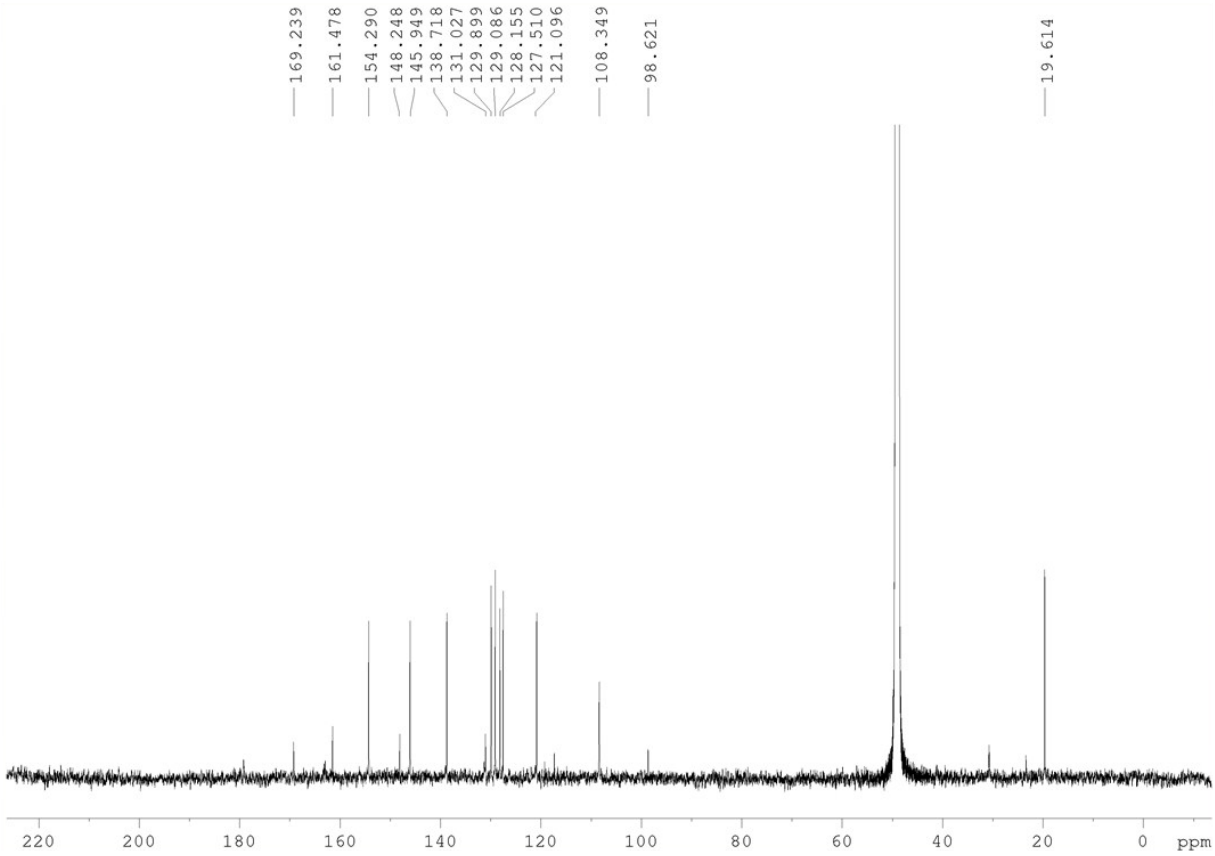

Figure S25. ^{13}C NMR spectrum of 4-hydroxy-6-methyl-3-(quinolin-3-yl)-2H-pyran-2-one (**9**) in MeOH-d_4.