

**A new pyrano[2,3,4-*cd*]indole as a scaffold for selective
non-basic 5-HT₆R ligands**

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Table 1. Parent structures used for the bioisostere generation in the Pipeline Pilot program. The binding affinity data are taken from the literature.

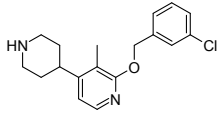
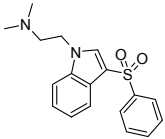
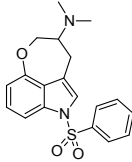
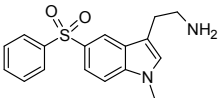
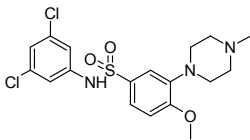
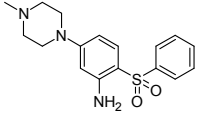
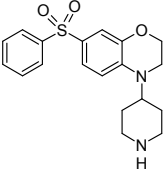
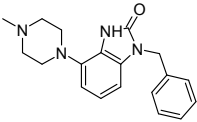
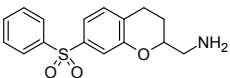
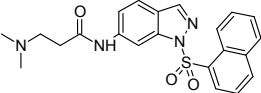
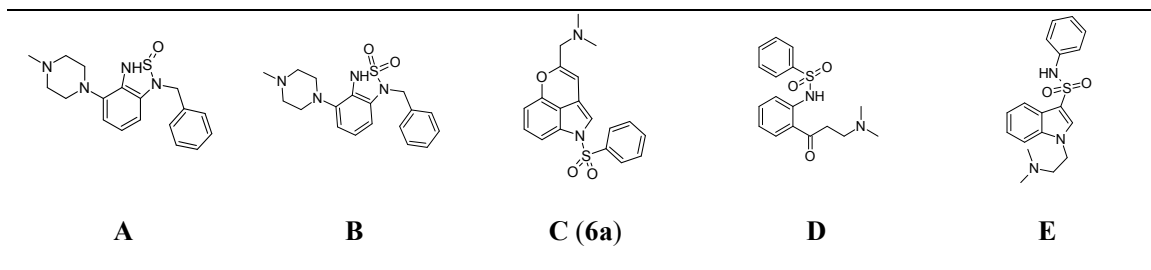
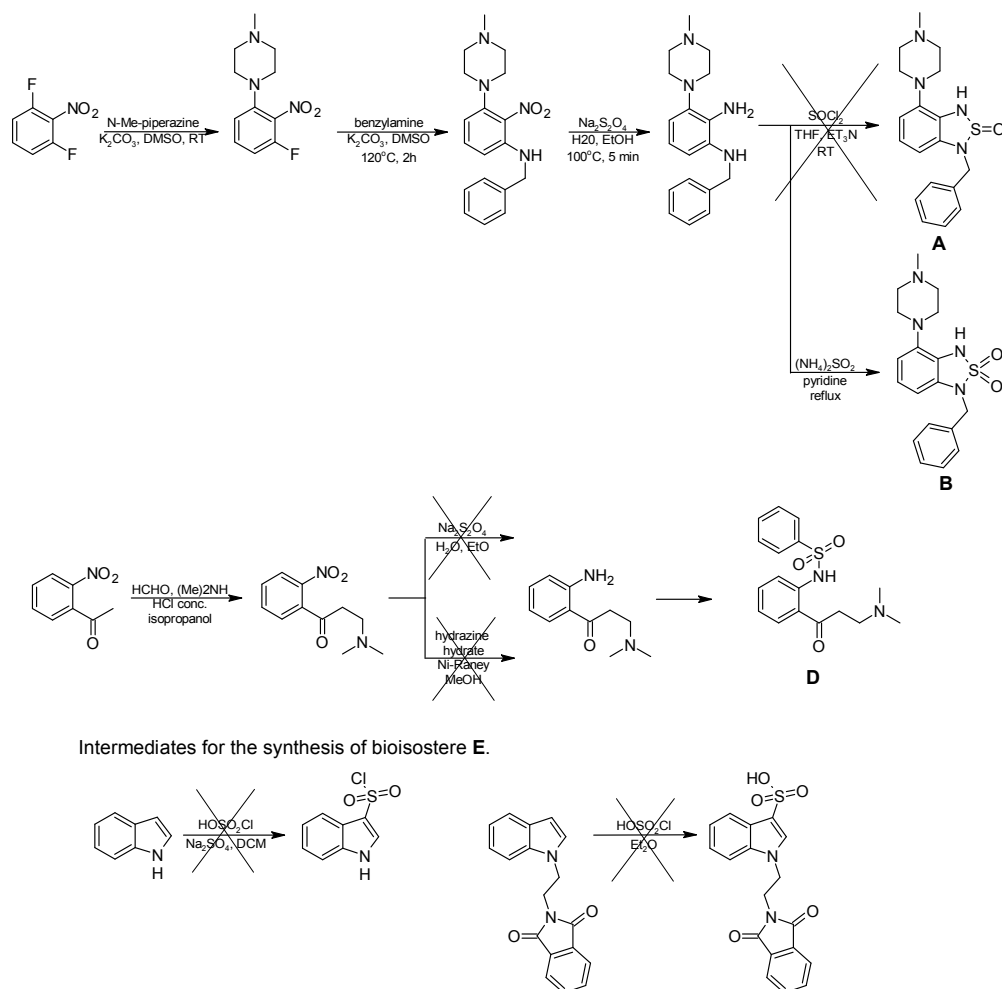
Structure	5-HT ₆ K_i [nM] reference	Reference
	8.1	1
	18	2
	1.7	3
	1.5	4
	0.6	5
	3.6	6
	0.1	7
	0.6	8
	0.1	9
	1.8	10

Table 2. Daughter structures that resulted in an automatic bioisostere generation and were selected for further evaluation.



Synthesis of bioisostere **A** was unsuccessful (Scheme 1), no further attempts were made. Bioisostere **B** (Scheme 1) was obtained in a scale of a few mg, however it turned out to be totally insoluble in any solvent (even DMSO). As in the case of bioisostere **D**, the intermediate 3-(dimethylamino)-1-(2-nitrophenyl)propan-1-one turned out to be unstable in a free base form, thus any attempt to reduce the nitro group was unsuccessful. Protection of carbonyl with acetals also did not allow to obtain the desired product. Synthesis of bioisostere **E** was also unsuccessful, as any attempt to introduce the sulfonyl group to an indole ring resulted in a black oily mass with no product.

Scheme 1. Synthesis of bioisosteres **A**, **B**, **D** and **E**.



Radioligand binding assays

Cell pellets were thawed and homogenized in 20 volumes of assay buffer using an Ultra Turrax tissue homogenizer and centrifuged twice at 35 000 g for 20 min at 4°C, with incubation for 15 min at 37°C in between the rounds of centrifugation. The composition of the assay buffers was as follows: for 5-HT_{1A}R: 50 mM Tris-HCl, 0.1 mM EDTA, 4 mM MgCl₂, 10 µM pargyline and 0.1% ascorbate; for 5-HT₆R: 50 mM Tris-HCl, 0.5 mM EDTA and 4 mM MgCl₂, for 5-HT_{7b}R: 50 mM Tris-HCl, 4 mM MgCl₂, 10 µM pargyline and 0.1% ascorbate; for dopamine D_{2L}R: 50 mM Tris-HCl, 1 mM EDTA, 4 mM MgCl₂, 120 mM NaCl, 5 mM KCl, 1.5 mM CaCl₂ and 0.1% ascorbate.

All assays were incubated in a total volume of 200 µl in 96-well microtiter plates for 1 h at 37°C, excluding 5-HT_{1A}R, which was incubated at room temperature for 1 h. The equilibration process was terminated by rapid filtration through Unifilter plates with a 96-well cell harvester, and the radioactivity retained on the filters was quantified using a Microbeta plate reader.

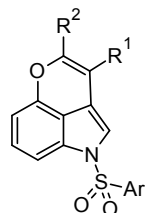
For the displacement studies, the assay samples contained the following as radioligands: 1.5 nM [³H]-8-OH-DPAT (187 Ci/mmol) for 5-HT_{1A}R, 2 nM [³H]-LSD (85.2 Ci/mmol) for 5-HT₆R, 0.6 nM [³H]-5-CT (39.2 Ci/mmol) for 5-HT_{7b}R or [³H]-Raclopride (74.4 Ci/mmol) for D₂R.

Non-specific binding was defined using 10 µM of 5-HT in the 5-HT_{1A}R and 5-HT_{7b}R binding experiments, whereas 10 µM methiothepine or 1 µM (+)butaclamol was used in the 5-HT₆R and D_{2L} assays, respectively. Each compound was tested in triplicate at 7–8 concentrations (10⁻¹¹–10⁻⁴ M). The inhibition constants (*K_i*) were calculated using the Cheng-Prusoff equation,¹¹ and the results are expressed as the means of at least two independent experiments.

5-HT₆R functional activity assay

HEK293 cells overexpressing 5-HT₆ receptors (prepared using Lipofectamine 2000) were maintained at 37°C in a humidified atmosphere with 5% CO₂ and grown in Dulbecco's Modified Eagle's Medium containing 10% dialyzed foetal bovine serum and 500 mg/ml G418 sulfate. For the functional experiments, the cells were subcultured in 25-cm² diameter dishes, grown to 90% confluence, washed twice with phosphate-buffered saline (PBS) prewarmed to 37°C and centrifuged for 5 min (160 × g). The supernatant was aspirated, and the cell pellet was resuspended in stimulation buffer (1 × HBSS, 5 mM HEPES, 0.5 mM IBMX, 0.1% BSA). The functional properties of compounds were evaluated using their ability to inhibit cAMP production induced by agonist the 5-CT – 100 nM. Total cAMP was measured using the LANCE cAMP detection kit (PerkinElmer) according to the manufacturer's directions. To quantify the levels of cAMP, the cells (5 µl) were incubated with the test compounds and 5-CT (5 µl) for 30 min at room temperature in 384-well white opaque microtiter plate. After incubation, the reaction was stopped, and the cells were lysed by addition of 10 µl of a working solution (5 µl of Eu-cAMP and 5 µl of ULIGHT-anti-cAMP). The assay plate was incubated for 1 h at room temperature. Time-resolved fluorescence resonance energy transfer (TR-FRET) was detected using an Infinite M1000 Pro (Tecan) with instrument settings according to the LANCE cAMP detection kit manual. K_b values were calculated using the Cheng-Prusoff equation¹¹ specific for the analysis of functional inhibition curves: $K_b = IC_{50}/(1 + A/EC_{50})$, where A is the agonist concentration, IC_{50} is the concentration of antagonist that produces a 50% reduction in the response to agonist, and EC_{50} is the agonist concentration that causes a half maximal response.

Table 1. Standard deviation parameters for binding affinities of synthesized compounds.



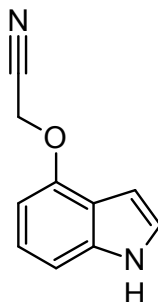
Cmpd.	Ar	R1	R2	K_i [nM] \pm SD ^a				
				5-HT ₆	5-HT _{1A}	5-HT _{2A}	5-HT ₇	D ₂
4b	1-naphthyl	H	CN	755 \pm 126	> 10 000	n.d.	> 10 000	> 10 000
5b	1-naphthyl	H	CHO	266 \pm 65	> 10 000	n.d.	> 10 000	5 162 \pm 874
6a	Ph	H	CH ₂ N(Me) ₂	5 \pm 0.4	> 10 000	65 \pm 6	3 692 \pm 628	6 698 \pm 402
6b	1-naphthyl	H	CH ₂ N(Me) ₂	1 \pm 0.2	5 967 \pm 895	489 \pm 59	75 \pm 19	1 489 \pm 283
6c	Ph	H	CH ₂ N(Et) ₂	28 \pm 5	> 10 000	1 703 \pm 255	> 10 000	82 \pm 13
7a	Ph	H	CH ₂ OH	36 \pm 5	> 10 000	> 10 000	> 10 000	> 10 000
7b	1-naphthyl	H	CH ₂ OH	4 \pm 1	n.d.	> 10 000	> 10 000	4 287 \pm 900
7c	Ph	Me	CH ₂ OH	52 \pm 10	> 10 000	> 10 000	> 10 000	> 10 000
7d	1-naphthyl	Me	CH ₂ OH	20 \pm 4	> 10 000	> 10 000	> 10 000	> 10 000

^a Affinity of the reference drugs: 5-HT₆R and 5-HT_{2A}R, Olanzapine – K_i = 10.7 \pm 2.1 nM and K_i = 6.2 \pm 0.9 nM, respectively; 5-HT_{1A}R, Buspirone – K_i = 34.3 \pm 4.2 nM; 5-HT₇R, Clozapine – K_i = 45.5 \pm 5.1 nM; D₂R, Ziprasidone – K_i = 2.1 \pm 0.3 nM. n.d. – not determined.

Synthetic procedures

The ^1H NMR spectra were recorded using a Bruker Avance III HD 400 NMR spectrometer. Mass spectra were recorded using a TQD Waters LC/MS spectrometer with electrospray ionization. LC analysis was performed on a Waters H-Class UPLC using ACQUITY UPLC BEH C18 $1.7\ \mu\text{m}$ $2.1 \times 50\text{ mm}$ column with solvent gradient beginning at 80% water:20% ACN up to 100% ACN for 3 min, then 100% ACN for 1 min and back to 80% water:20% ACN through 2 min, solvent flow was set on 0.3 ml/min. HRMS analysis was performed using QTOF impact II Bruker mass spectrometer with ESI ionization. Substrates and solvents were purchased from Avantor, Sigma-Aldrich, Combi-Blocks or Apollo Scientific and used without further purification.

2-(1H-Indol-4-yloxy)acetonitrile (**1**)

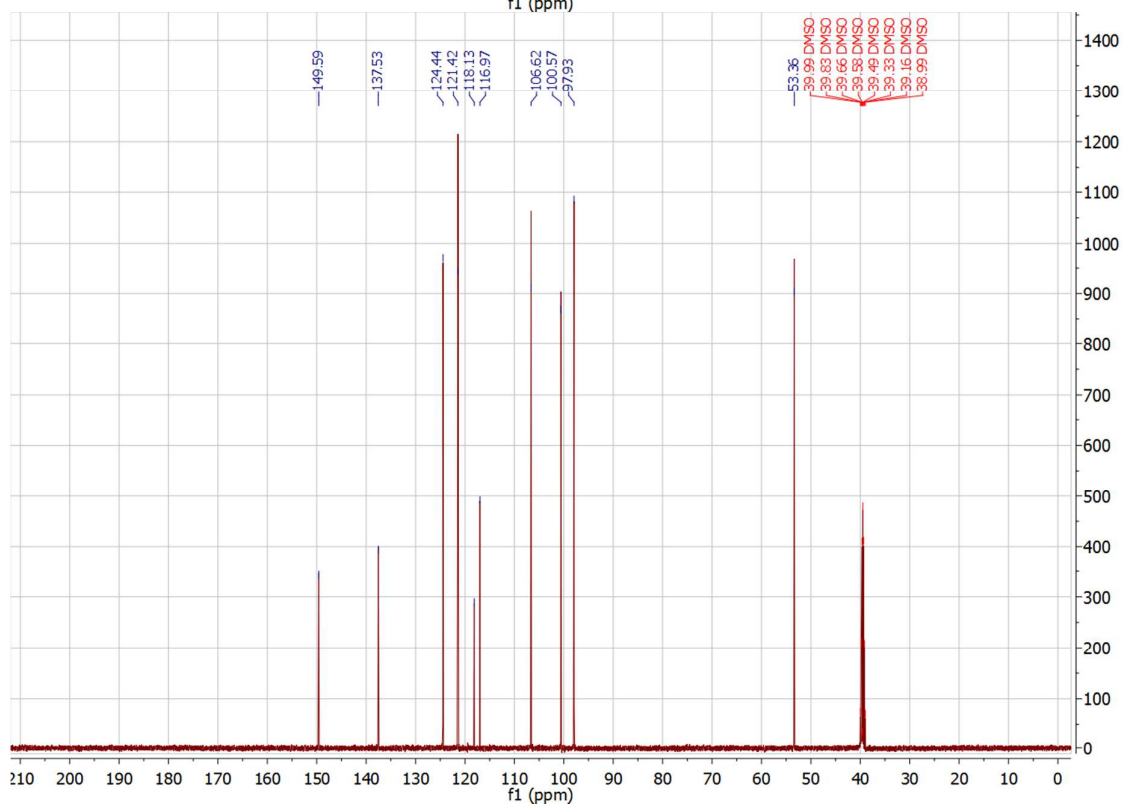
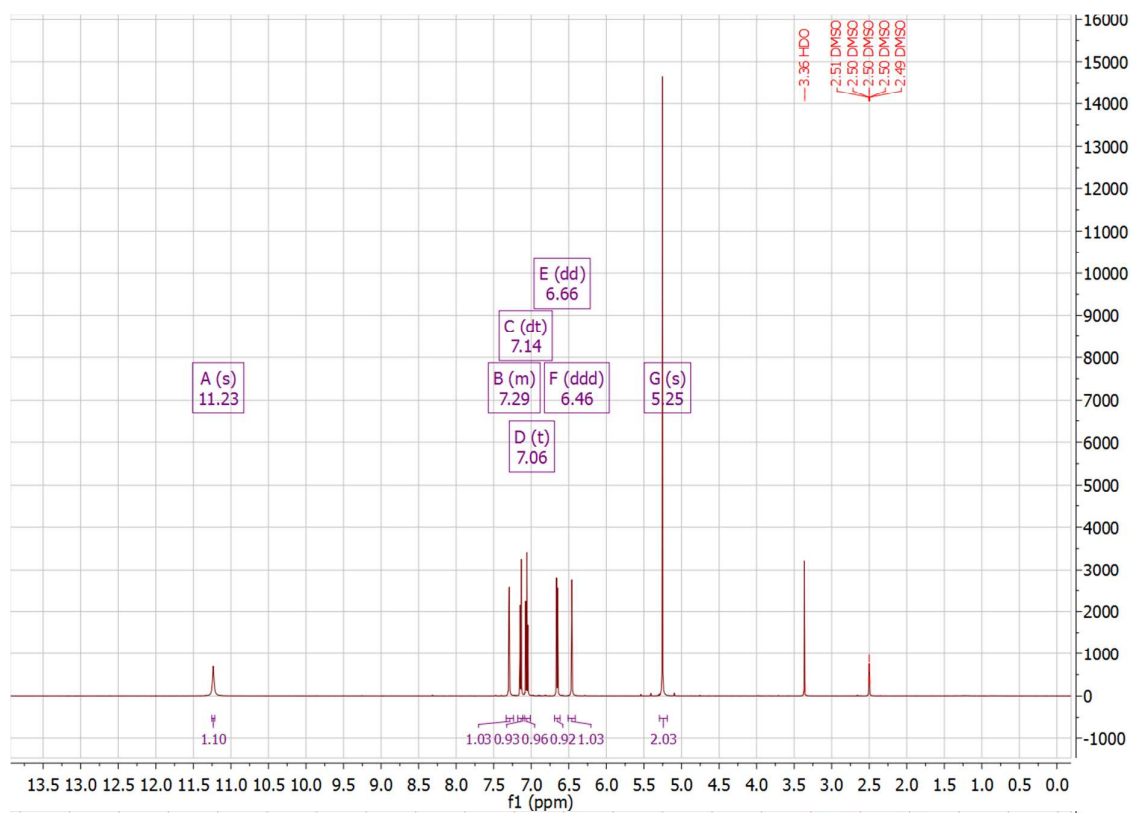


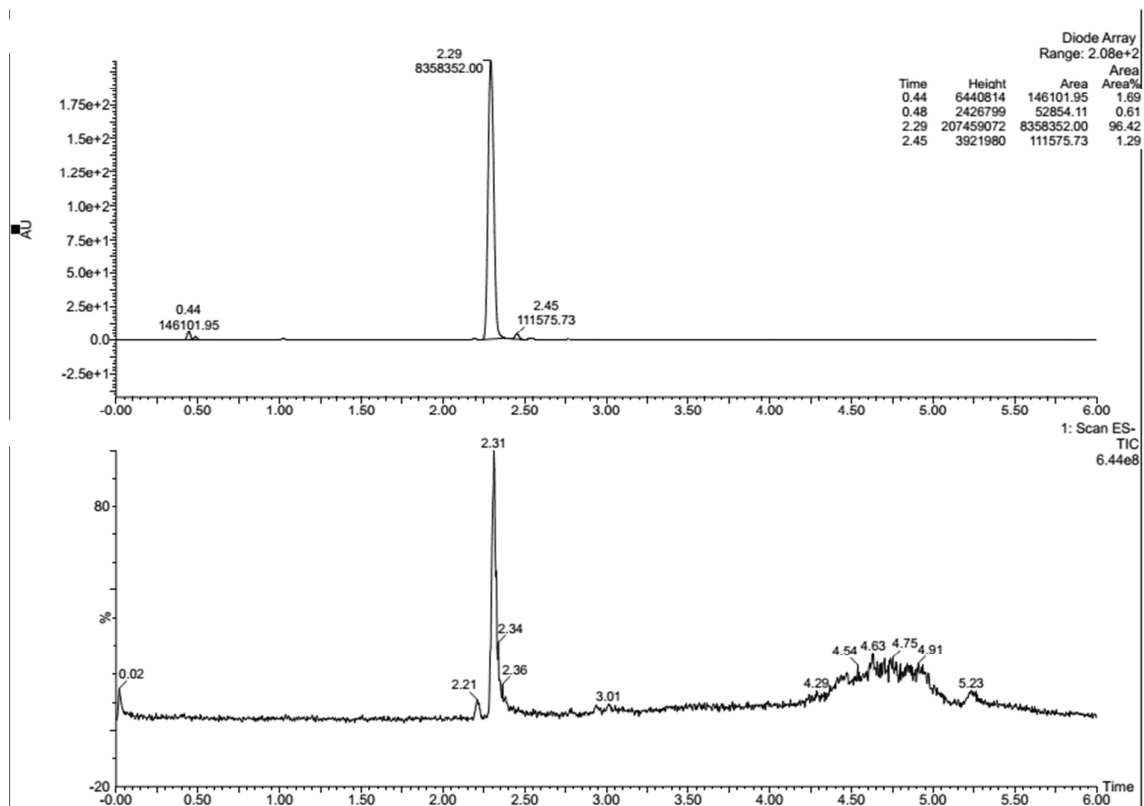
Chloroacetonitrile (1 eq., 2.37 ml) was added to a solution of 4-hydroxyindole (5g, 0.037 mol) in acetone (125 ml) with Cs_2CO_3 (1.6 eq., 20 g) and the reaction mixture stirred at room temperature for 72 hours. After completion, precipitate was filtered, washed with acetone (100 ml) and evaporated under reduced pressure. Product was purified using column chromatography (SiO_2 , CHCl_3). A white solid of **1** was obtained (3.35 g, 53%).

LCMS $[\text{M}-1] = 171.06\text{ m/z}$ (172.19 calc.).

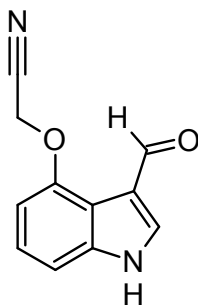
^1H NMR (500 MHz, $\text{DMSO}-d_6$) δ 11.23 (s, 1H), 7.33 – 7.24 (m, 1H), 7.14 (dt, $J = 8.2, 0.8\text{ Hz}$, 1H), 7.06 (t, $J = 7.9\text{ Hz}$, 1H), 6.66 (dd, $J = 7.7, 0.7\text{ Hz}$, 1H), 6.46 (ddd, $J = 3.0, 2.1, 0.9\text{ Hz}$, 1H), 5.25 (s, 2H).

^{13}C NMR (126 MHz, $\text{DMSO}-d_6$) δ 53.36, 97.93, 100.57, 106.62, 116.97, 118.13, 121.42, 124.44, 137.53, 149.59.





2-[(3-Formyl-1H-indol-4-yl)oxy]acetonitrile (2a)

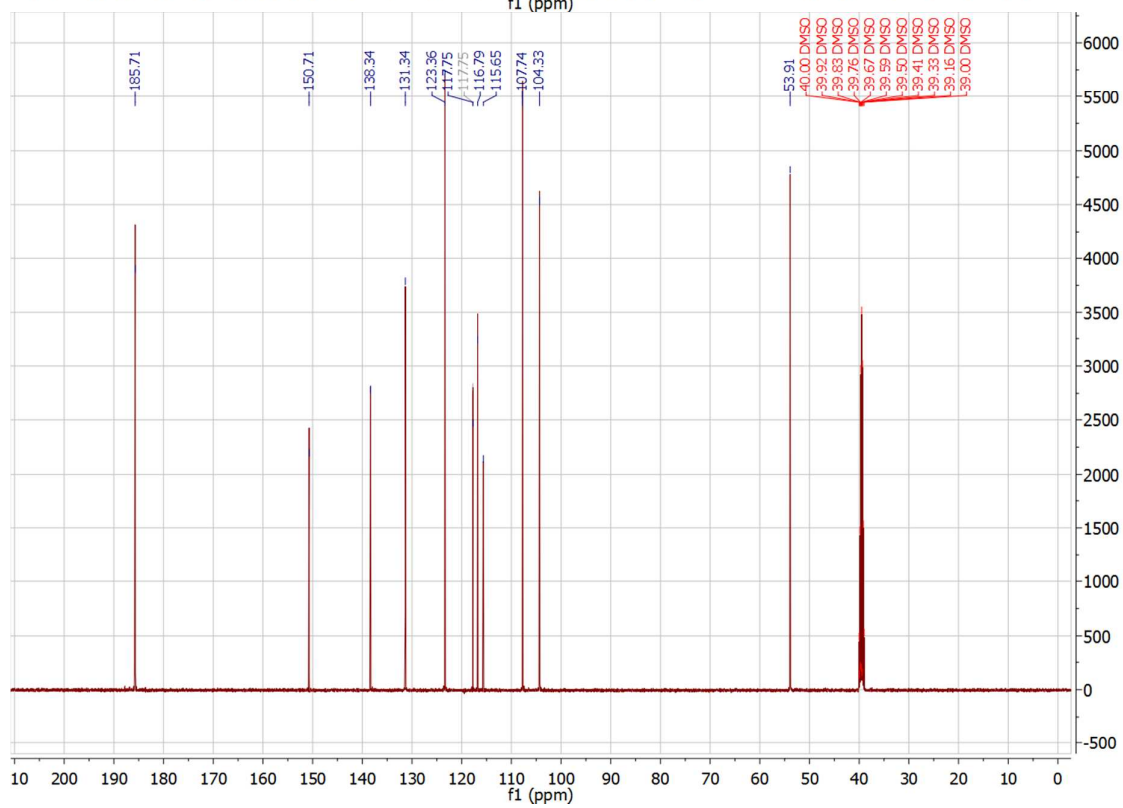
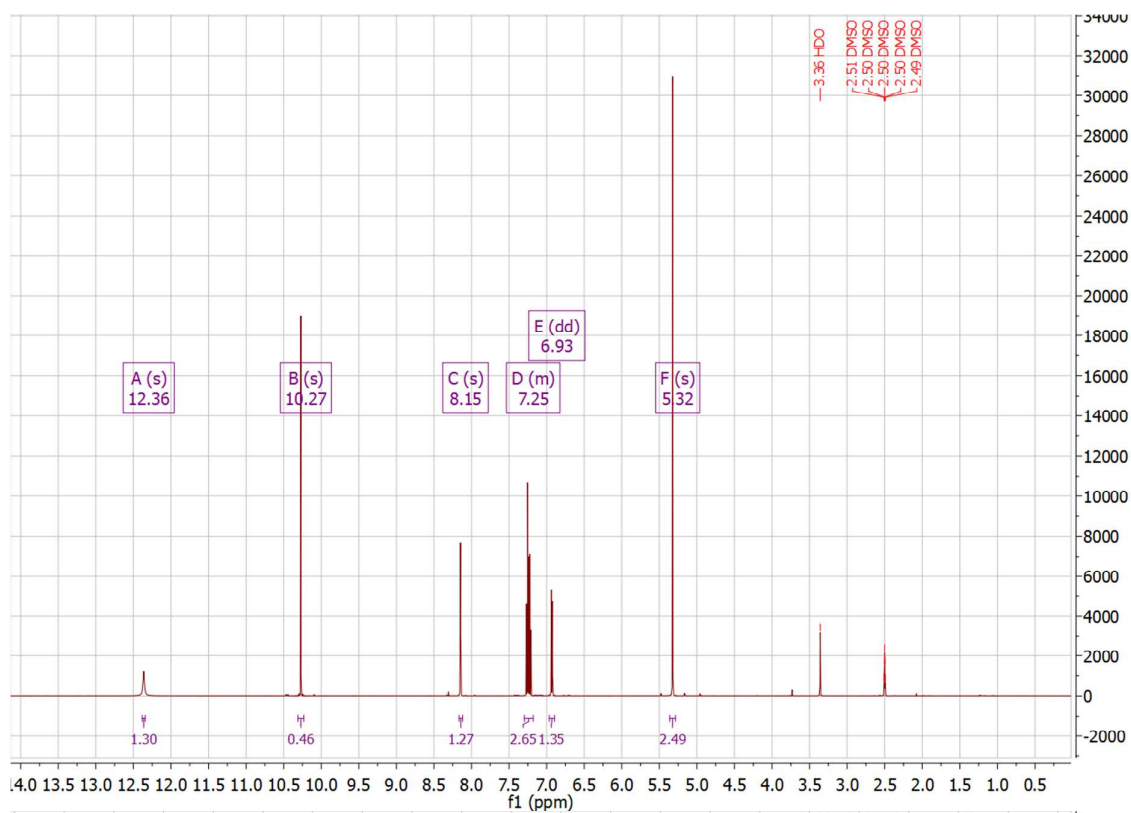


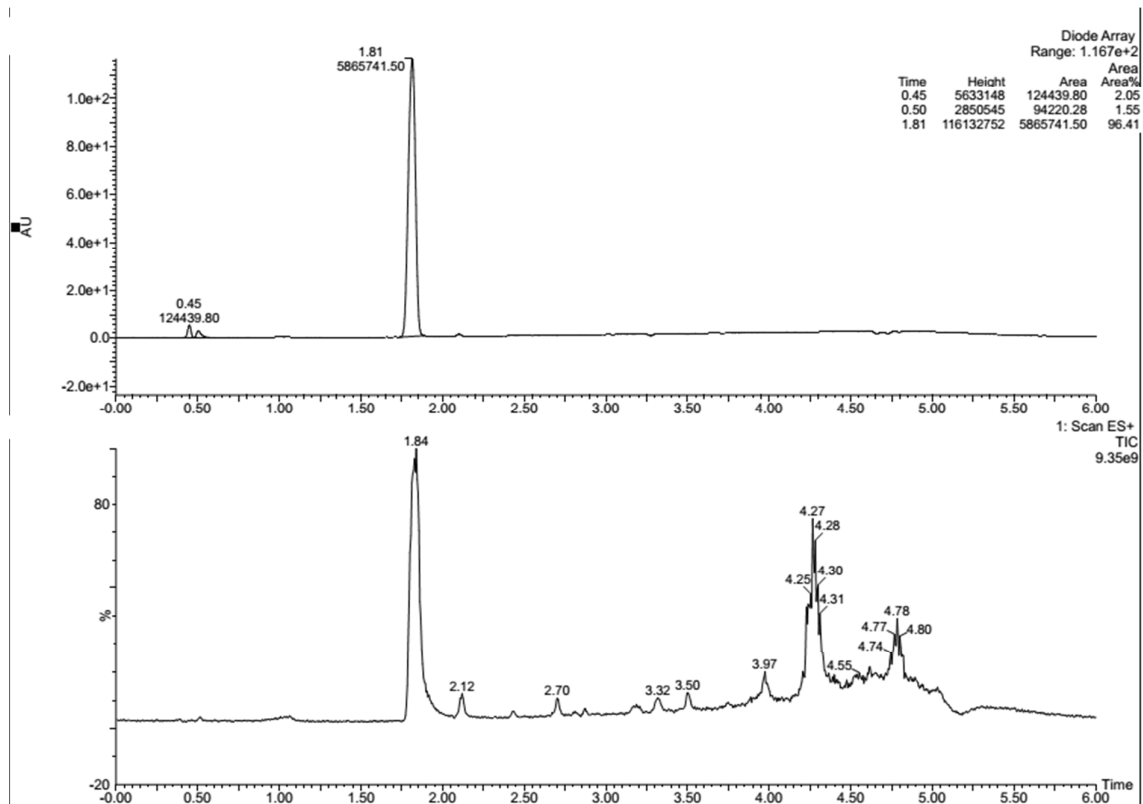
N-Chlorosuccinimide (3 eq., 1.2 g) was added portionwise to a solution of PPh₃ (3 eq., 2.36 g) in THF (44 ml) and stirred at room temperature for 30 min. DMF (6 eq., 1.39 ml) was added and reaction mixture was heated to reflux for 1 hour. 2-(1H-indol-4-yloxy)acetonitrile (**1**) (0.5 g, 0.003 mol) was added and reaction mixture was heated to reflux for 1 hour. After cooling to room temperature solvent was evaporated under reduced pressure and distilled water (10 ml) was added. Reaction mixture was heated to reflux for another 1 hour. After cooling to room temperature reaction mixture was basified with 10% NaOH and extracted with ethyl acetate (3x 50ml). Combined organic extracts were washed with brine (20 ml), dried over anhydrous magnesium sulfate and evaporated under reduced pressure. Product was purified using column chromatography (SiO₂, CHCl₃). A light tan solid was obtained (0.44 g, 76%).

LCMS [M+1] = 200.91 m/z (200.20 calc.).

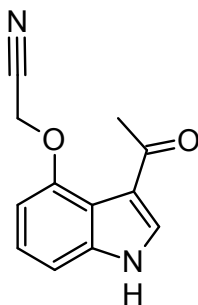
¹H NMR (500 MHz, DMSO-d₆) δ 12.36 (s, 1H), 10.27 (s, 1H), 8.15 (s, 1H), 7.30 - 7.18 (m, 2H), 6.93 (dd, *J* = 7.5, 1.0 Hz, 1H), 5.32 (s, 2H).

¹³C NMR (126 MHz, DMSO-d₆) δ 53.91, 104.33, 107.74, 115.65, 116.79, 117.75, 123.36, 131.34, 138.34, 150.71, 185.71.





2-[(3-Acetylo-1H-indol-4-yl)oxy]acetonitrile (2b)

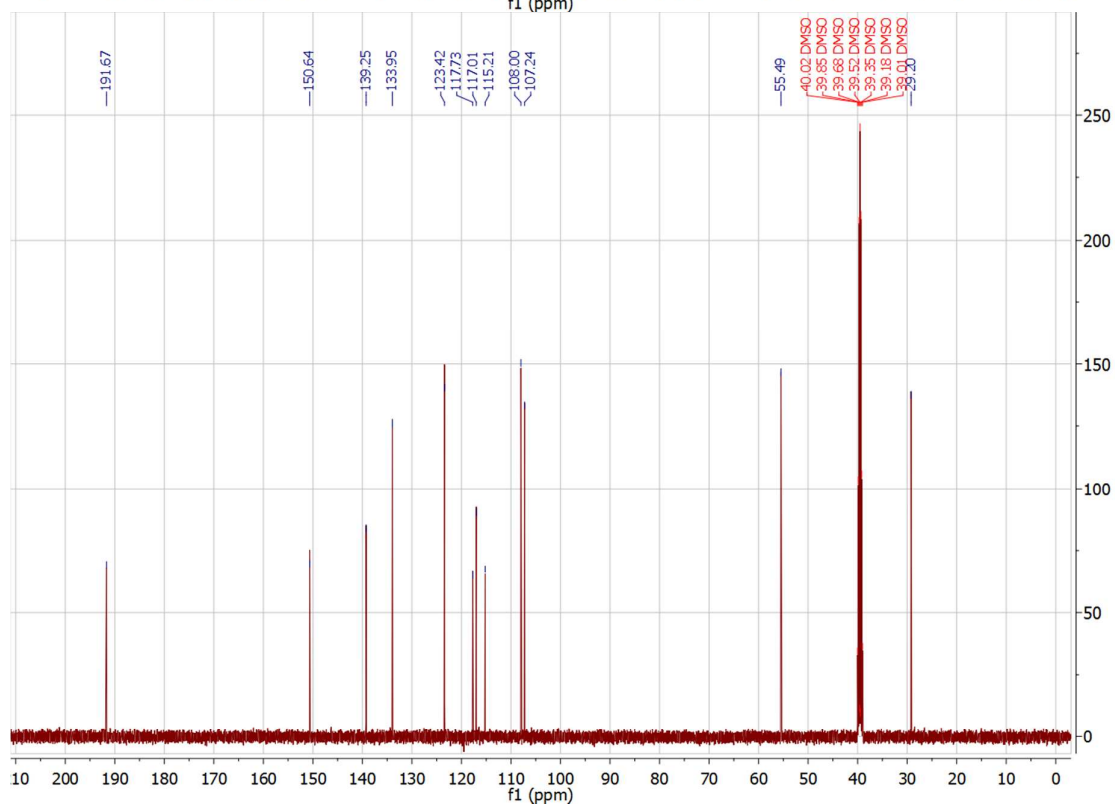
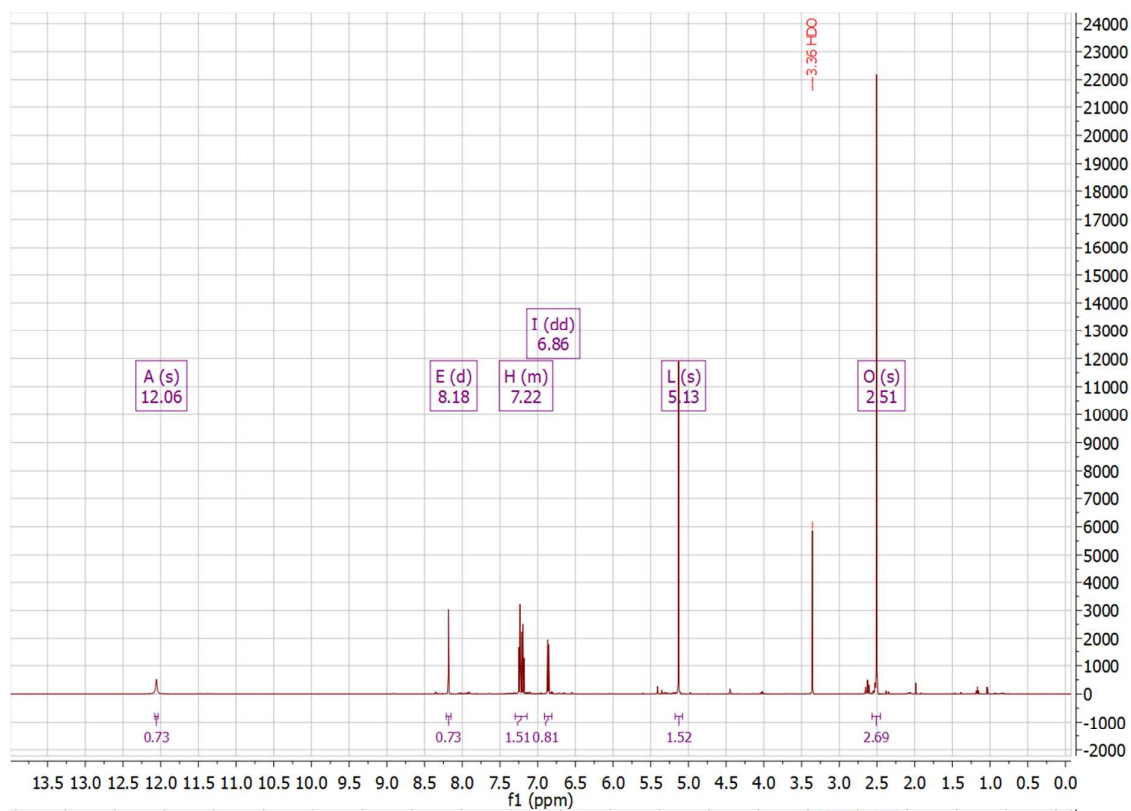


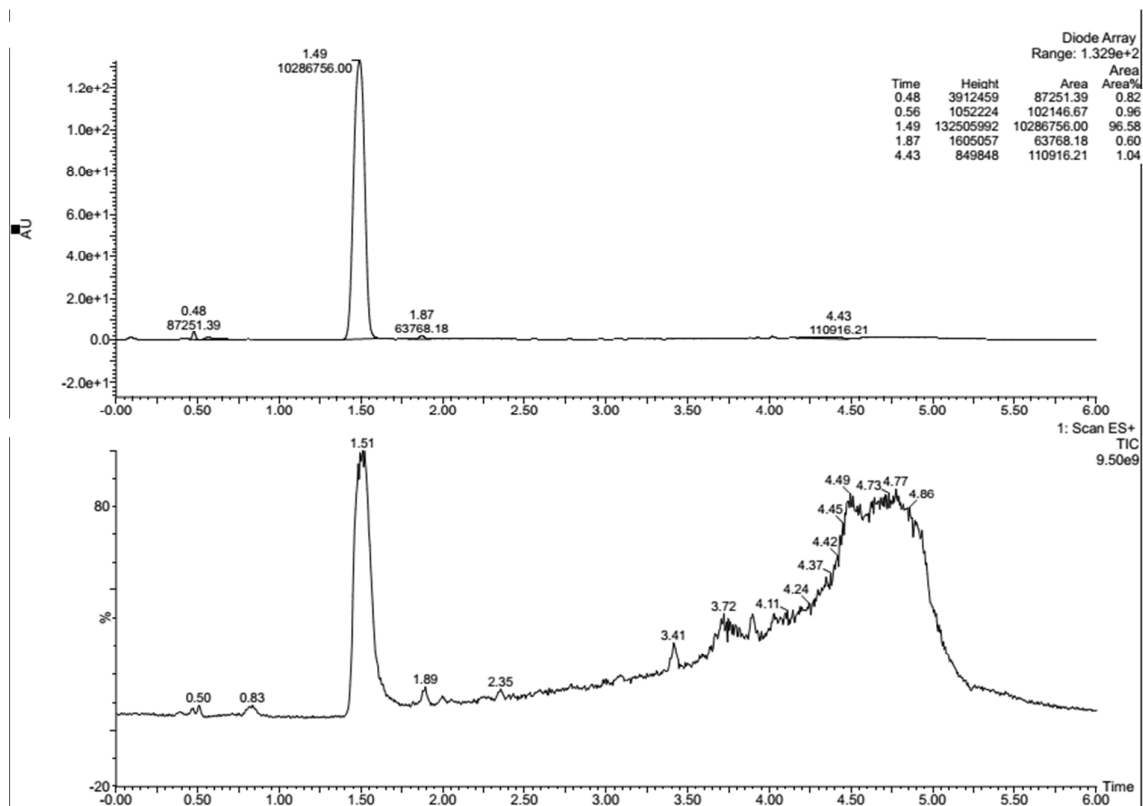
Diethylaluminium chloride (1M solution in hexane, 1.5 eq., 2.61 ml) was added dropwise to a solution of 2-(1H-indol-4-yloxy)acetonitrile (**1**) (0.3 g, 0.00174 mol) in dichloromethane (7 ml) at 0° C, and the reaction mixture was stirred at 0° C for 30 min. A solution of acetyl chloride (2.1 eq., 0.26 ml) in dichloromethane (3.5 ml) was added dropwise and the reaction mixture was again stirred at 0° C for 2 hours. After completion distilled water was added (20 ml) and the product was extracted with dichloromethane (2x 10 ml). Combined organic extracts were washed with brine, dried over anhydrous magnesium sulfate and evaporated under reduced pressure. A white solid was obtained (0.3 g, 80%) pure enough for the next step.

LCMS [M+1] = 215.10 m/z (214.22 calc.).

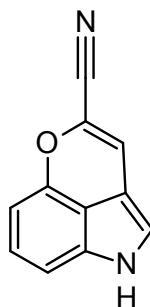
¹H NMR (500 MHz, DMSO-d₆) δ 12.06 (s, 1H), 8.18 (d, *J* = 3.1 Hz, 1H), 7.30 – 7.14 (m, 2H), 6.86 (dd, *J* = 7.6, 1.0 Hz, 1H), 5.13 (s, 2H), 2.51 (s, 3H).

¹³C NMR (126 MHz, DMSO-d₆) δ 29.20, 55.49, 107.24, 108.00, 115.21, 117.01, 117.73, 123.42, 133.95, 139.25, 150.64, 191.67.





Pyrano[2,3,4-*cd*]indole-4-carbonitrile (3a)



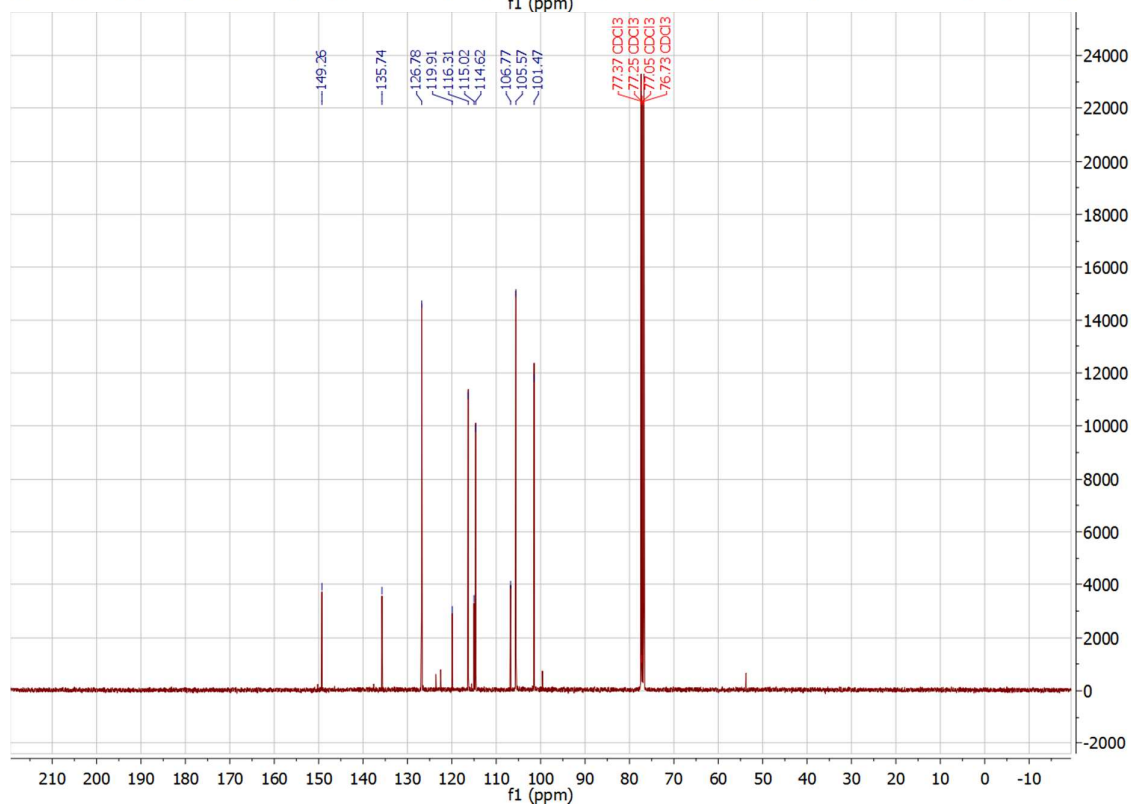
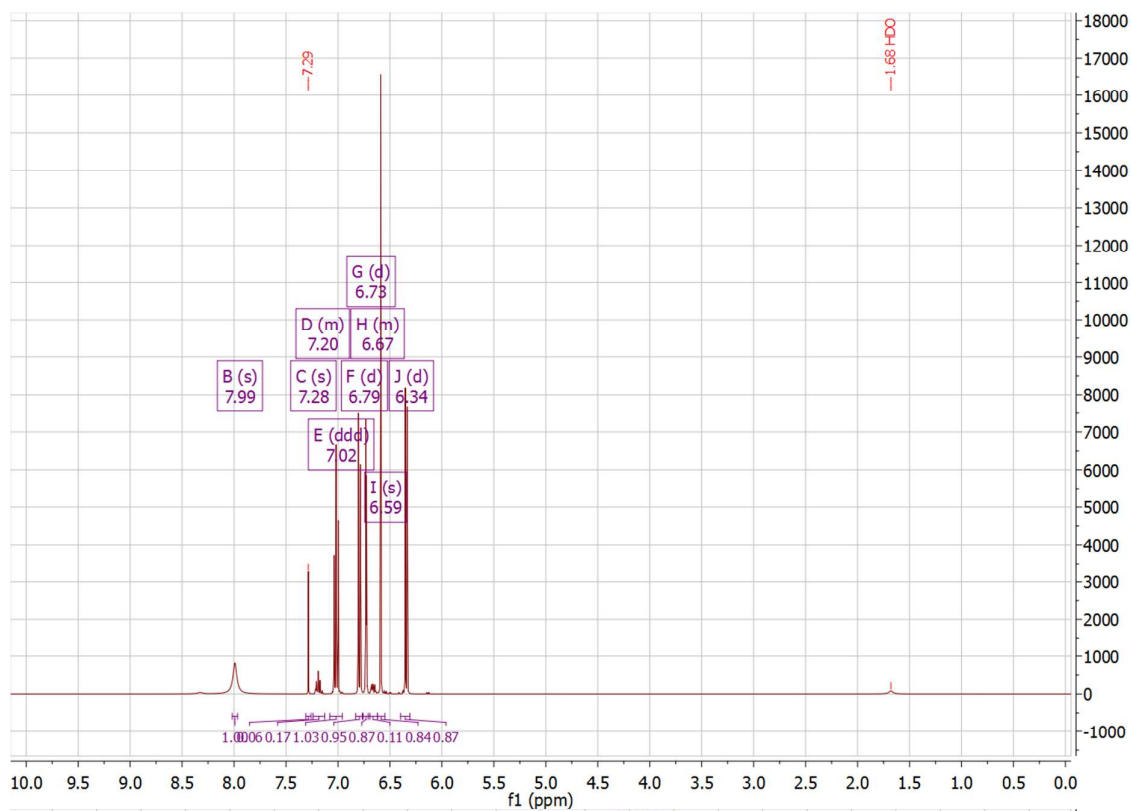
2-[(3-Formyl-1H-indol-4-yl)oxy]acetonitrile (**1b**) (1.1 g, 0.0056 mol) was dissolved in anhydrous dimethylacetamide (11 ml), anhydrous K₂CO₃ (2 eq., 1.5 g) was added and the reaction mixture was sealed under argon and heated to 160° C for 20 min in a microwave oven (150W). After cooling to room temperature distilled water (100 ml) was added and the mixture extracted with ethyl acetate (3x 50 ml). Combined organic extracts were washed with brine (20 ml), dried over anhydrous magnesium sulfate and evaporated under reduced pressure. Product was purified using column chromatography (SiO₂, CHCl₃). A light green solid was obtained (0.36 g, 35%).

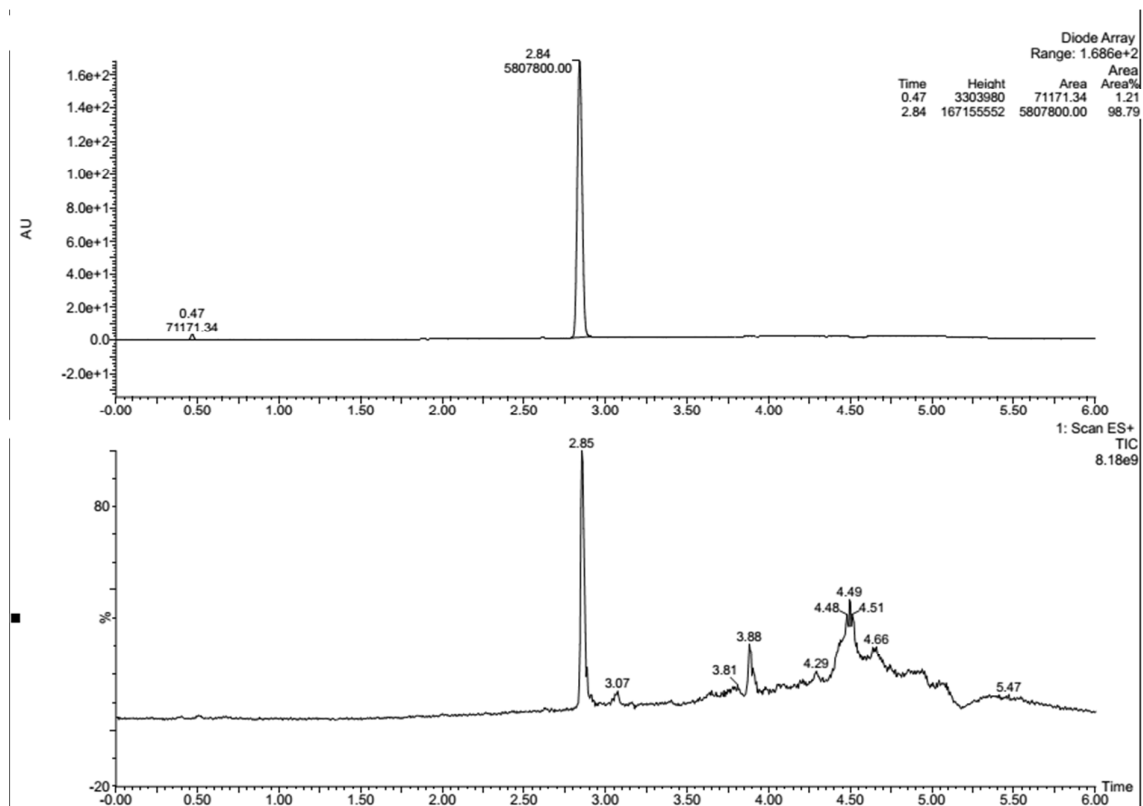
LCMS [M+1] = 182.84 m/z (182.18 calc.).

HRMS [M-1] = 181.0376 (181.0407 calc.)

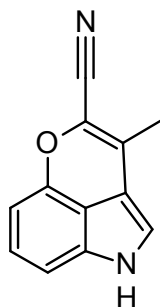
¹H NMR (400 MHz, CDCl₃) δ 7.99 (s, 1H), 7.02 (ddd, *J* = 8.2, 7.7, 0.4 Hz, 1H), 6.79 (d, *J* = 8.2 Hz, 1H), 6.73 (d, *J* = 2.1 Hz, 1H), 6.59 (s, 1H), 6.34 (d, *J* = 7.7 Hz, 1H).

¹³C NMR (101 MHz, CDCl₃) δ 101.47, 105.57, 106.77, 114.62, 115.02, 116.31, 119.91, 126.78, 135.74, 149.26.





3-Methyl-pyrano[2,3,4-*cd*]indole-4-carbonitrile (3b)

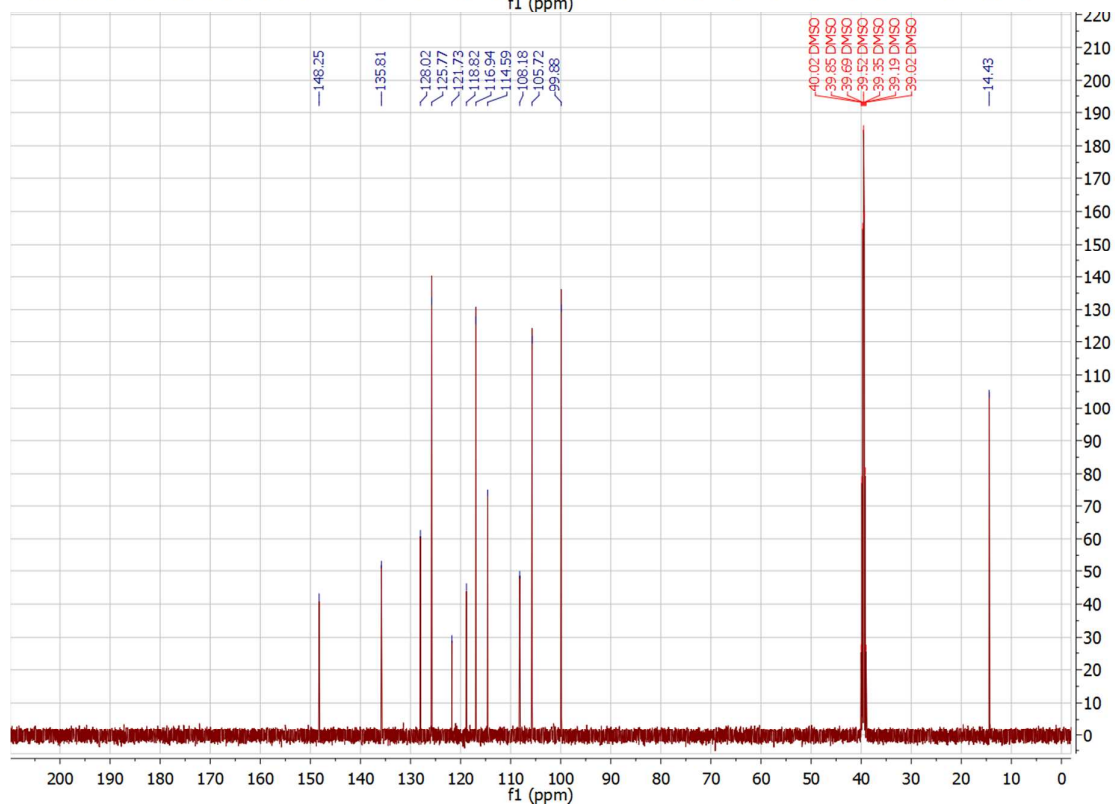
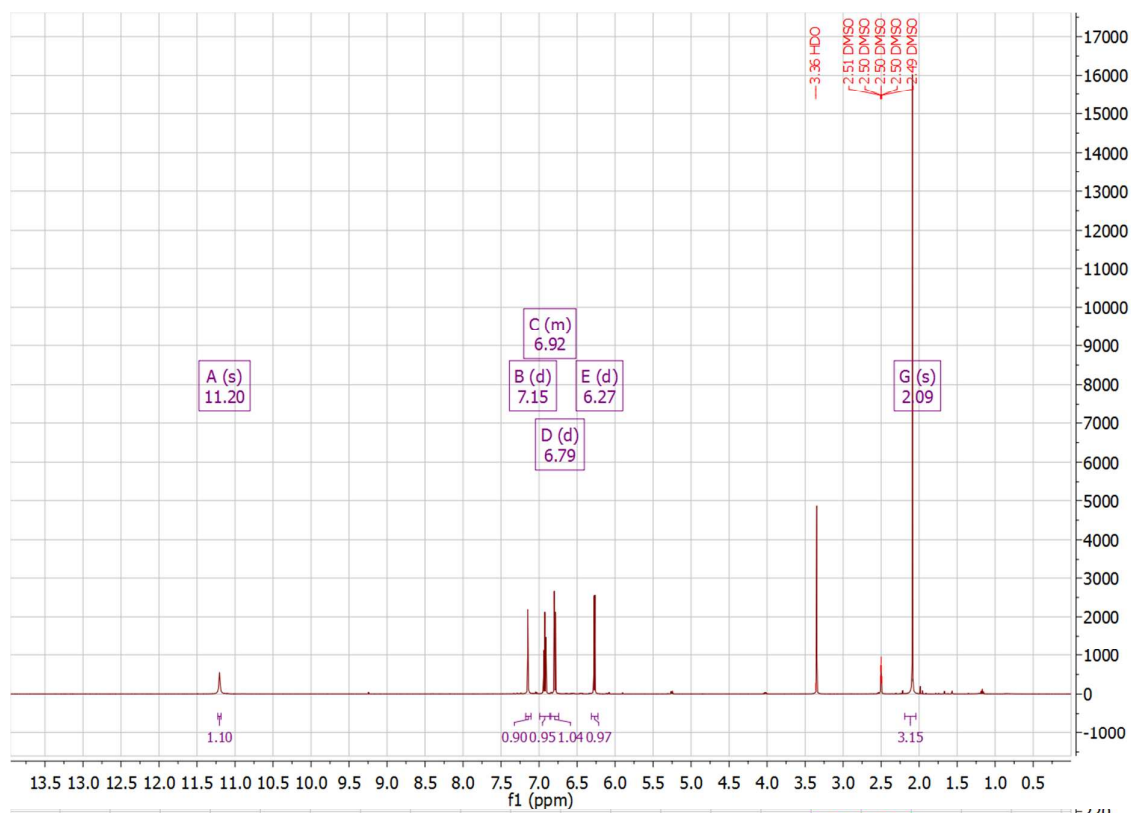


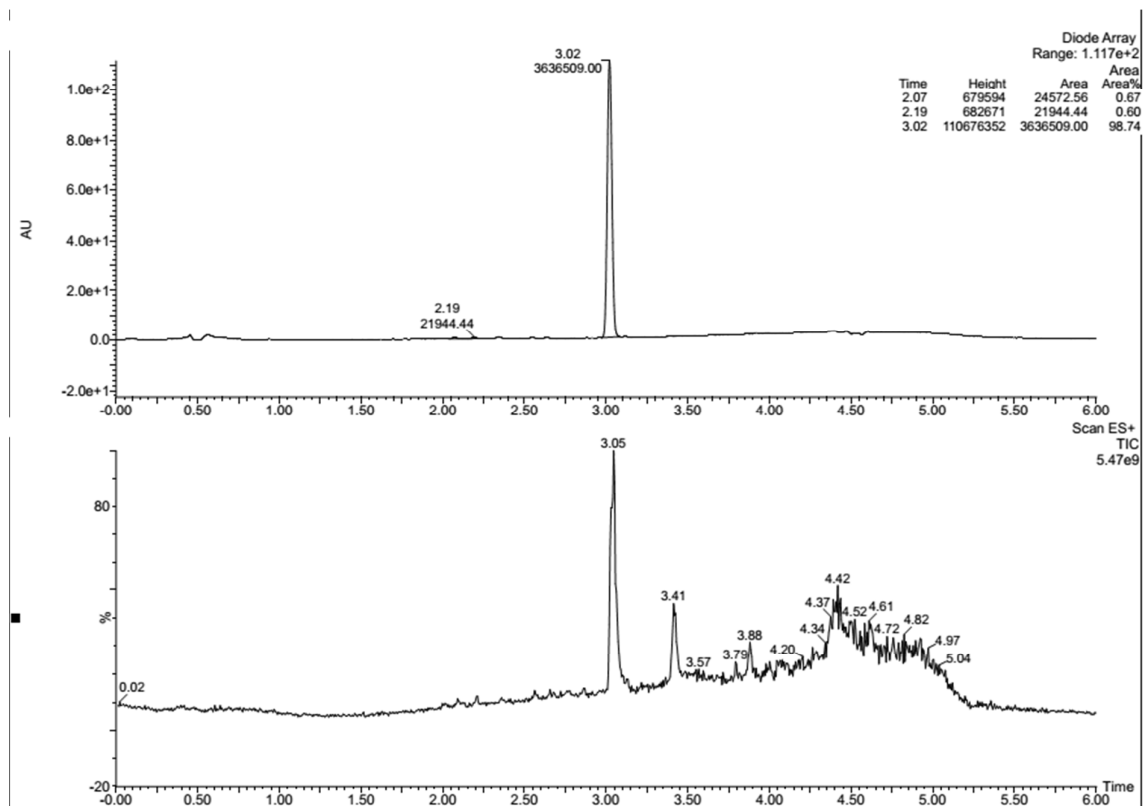
2-{[3-(Prop-1-en-2-yl)-1H-indol-4-yl]oxy}acetonitrile (**2b**) (2 g, 9.3 mmol) was dissolved in anhydrous dimethylformamide (25 ml), anhydrous K₂CO₃ (2 eq., 2.55 g) was added and the reaction mixture was sealed under argon and heated to 160° C for 4 hours in a microwave oven (150W). After cooling to room temperature distilled water (200 ml) was added and the mixture extracted with ethyl acetate (3x 50 ml). Combined organic extracts were washed with brine (20 ml), dried over anhydrous magnesium sulfate and evaporated under reduced pressure. Product was purified using column chromatography (SiO₂, CHCl₃). A brown solid was obtained (0.65 g, 35%).

LCMS [M+1] = 197.11 m/z (196.21 calc.).

¹H NMR (500 MHz, DMSO-d₆) δ 11.20 (s, 1H), 7.15 (d, *J* = 2.2 Hz, 1H), 6.99 – 6.86 (m, 1H), 6.79 (d, *J* = 8.2 Hz, 1H), 6.27 (d, *J* = 7.6 Hz, 1H), 2.09 (s, 3H).

¹³C NMR (126 MHz, DMSO-d₆) δ 14.43, 99.88, 105.72, 108.18, 114.59, 116.94, 118.82, 121.73, 125.77, 128.02, 135.81, 148.25.

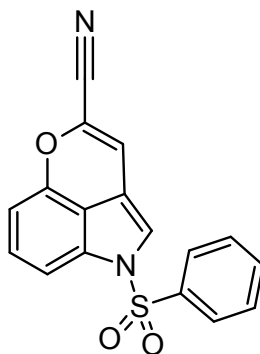




General procedure for the synthesis of 4a - 4d.

7-Oxa-2-azatricyclo[6.3.1.0^{4,1,2}]dodeca-1(12),3,5,8,10-pentaene-6-carbonitrile (**3a**) (0.3 g, 1.6 mmol) was dissolved in anhydrous THF (10 ml). Sodium hydride (60% in oil, 1.2 eq., 0.079 g) was added portionwise and reaction mixture was stirred at room temperature for 30 min. Benzenesulfonyl chloride (1.2 eq., 0.34 g) was added and reaction mixture was stirred for another 30 min. After completion distilled water was added (100 ml) and mixture was extracted with ethyl acetate (3x 50 ml). Combined organic extracts were washed with brine (20 ml), dried over anhydrous magnesium sulfate and evaporated under reduced pressure. Product was purified using column chromatography (SiO₂, CHCl₃).

1-(Benzenesulfonyl)-pyrano[2,3,4-*cd*]indole-4-carbonitrile (4a)

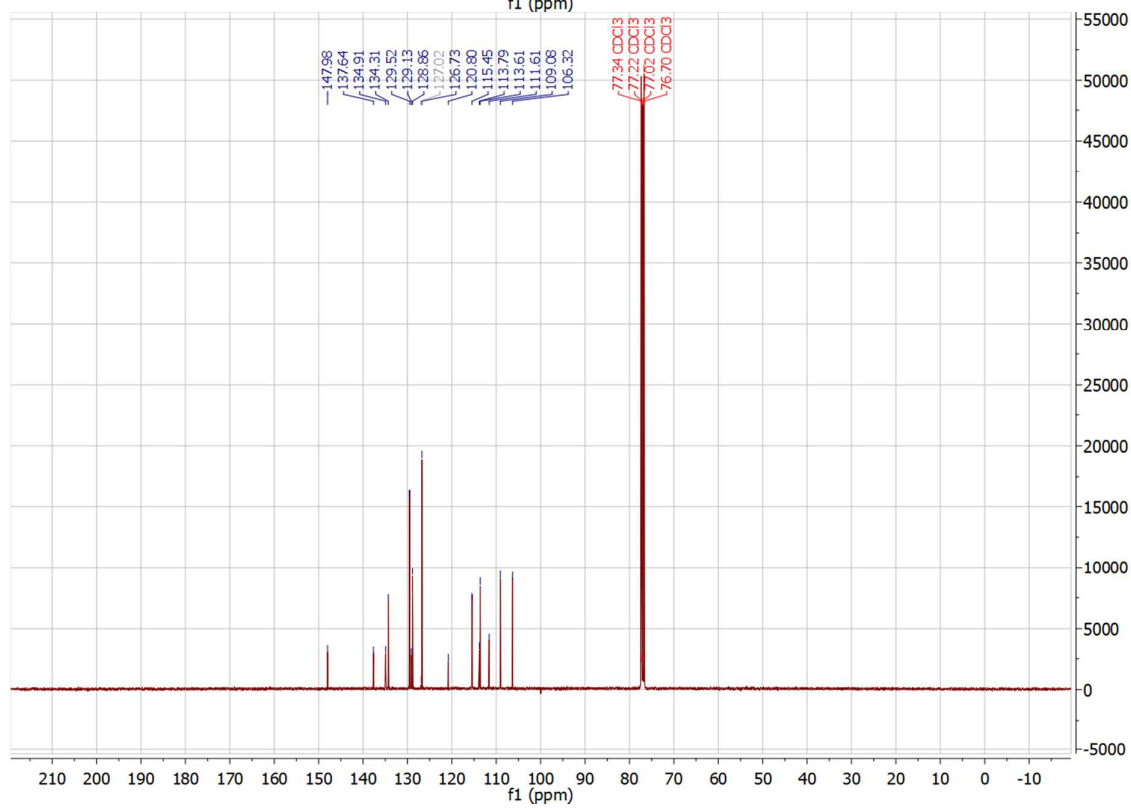
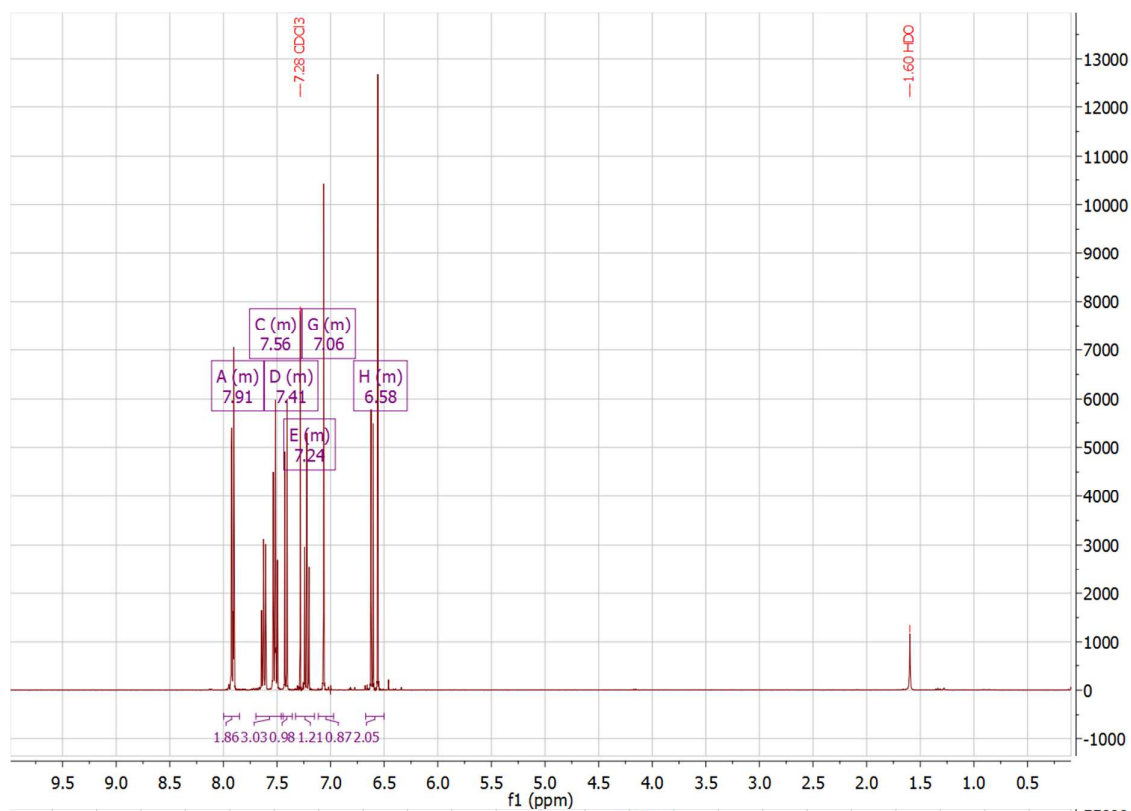


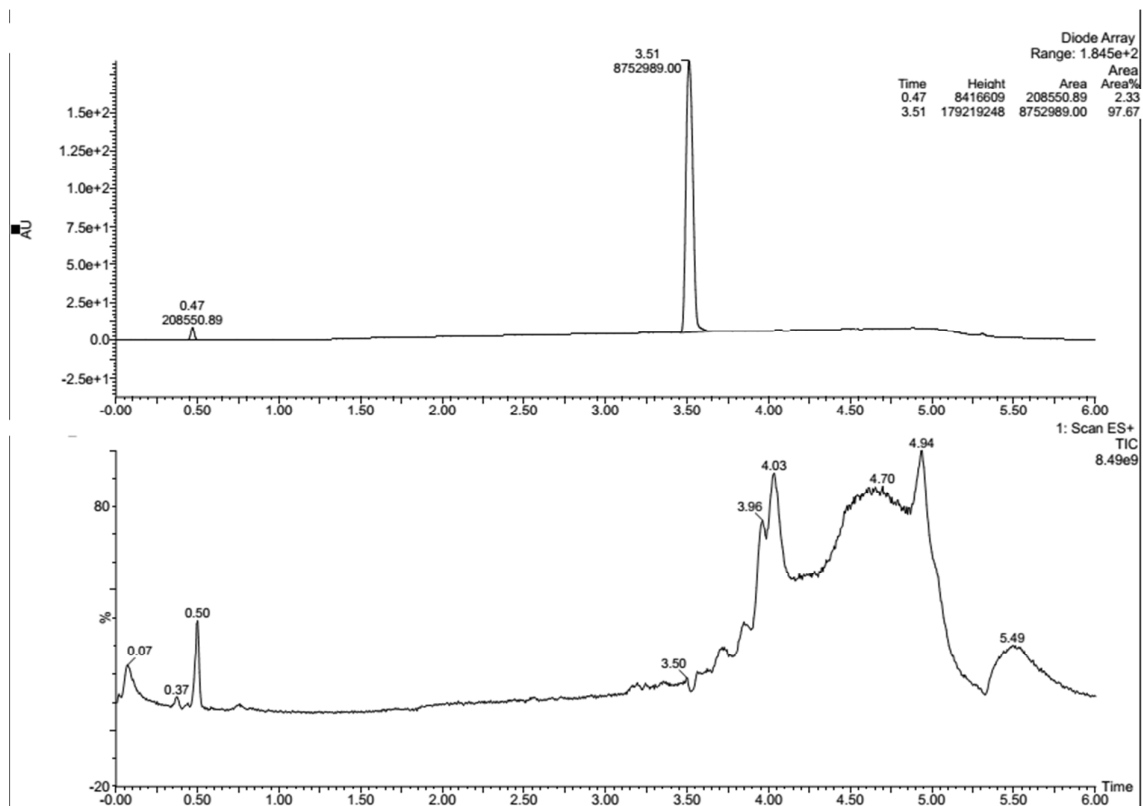
3a (0.3 g, 1.6 mmol), THF (10 ml), NaH (60% in oil, 1.2 eq., 0.079 g), benzenesulfonyl chloride (1.2 eq., 0.34 g). A white solid was obtained (0.36 g, 70%).

LCMS [M+1] = 322.94 m/z (322.34 calc.).

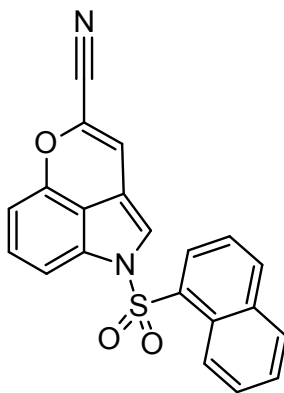
¹H NMR (400 MHz, CDCl₃) δ 8.00 - 7.85 (m, 2H), 7.70 - 7.44 (m, 3H), 7.46 - 7.36 (m, 1H), 7.33 - 7.15 (m, 1H), 7.11 - 6.97 (m, 1H), 6.67 - 6.50 (m, 2H).

¹³C NMR (101 MHz, CDCl₃) δ 106.32, 109.08, 111.61, 113.61, 113.79, 115.45, 120.80, 126.73, 128.86, 129.13, 129.52, 134.31, 134.91, 137.64, 147.98.





1-(Naphthalene-1-sulfonyl)-pyrano[2,3-*cd*]indole-4-carbonitrile (4b)



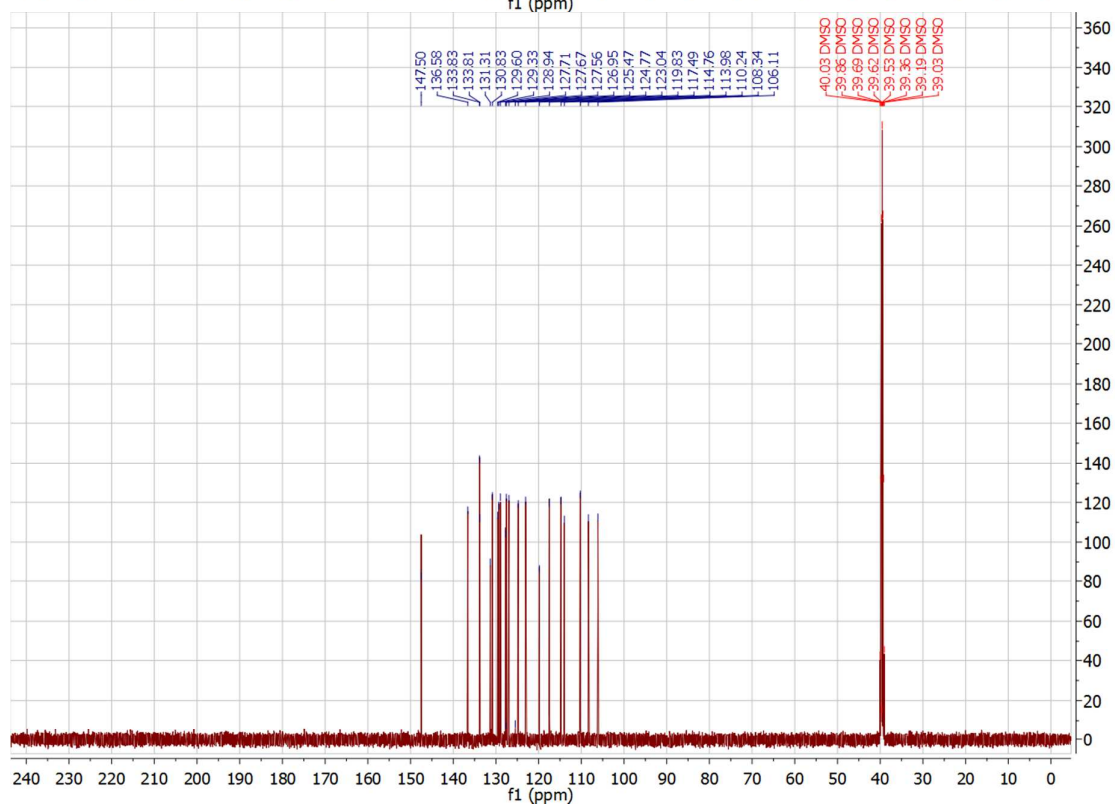
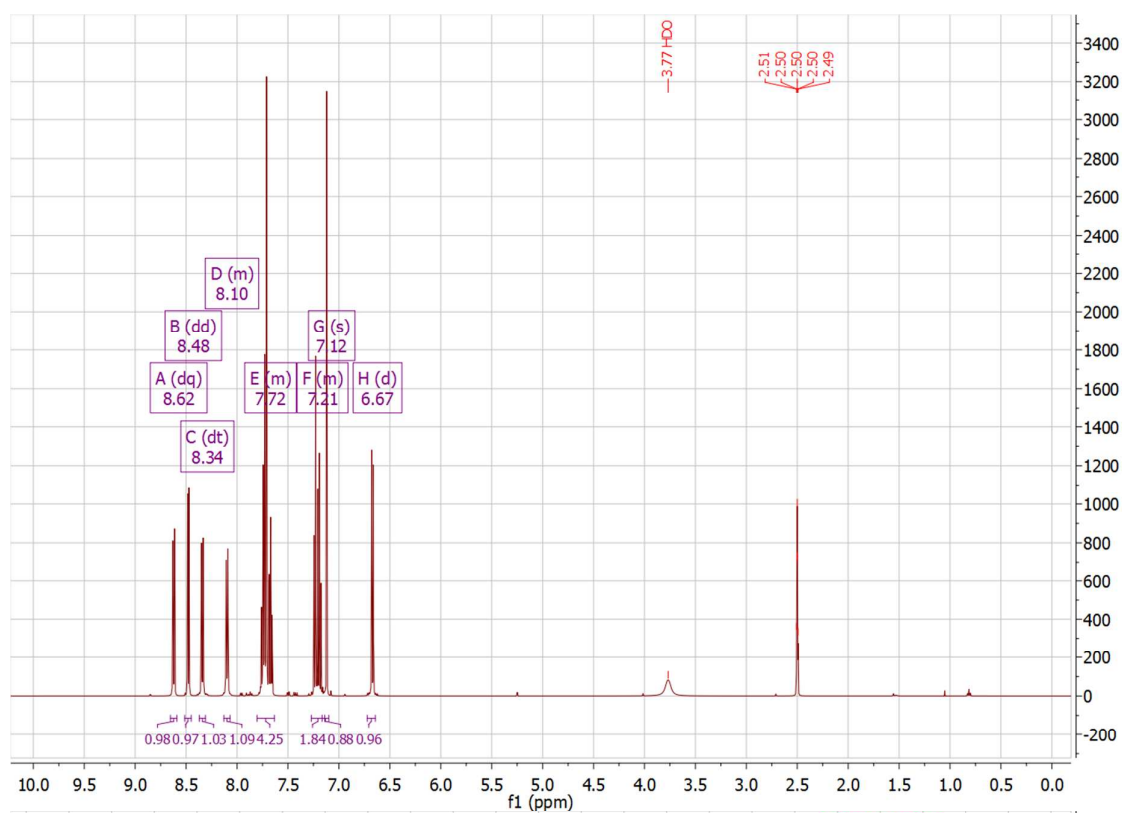
3a (0.3 g, 1.6 mmol), THF (10 ml), NaH (60% in oil, 1.2 eq., 0.079 g), 1-naphthylsulfonyl chloride (1.2 eq., 0.45 g). A white solid was obtained (0.46 g, 78%).

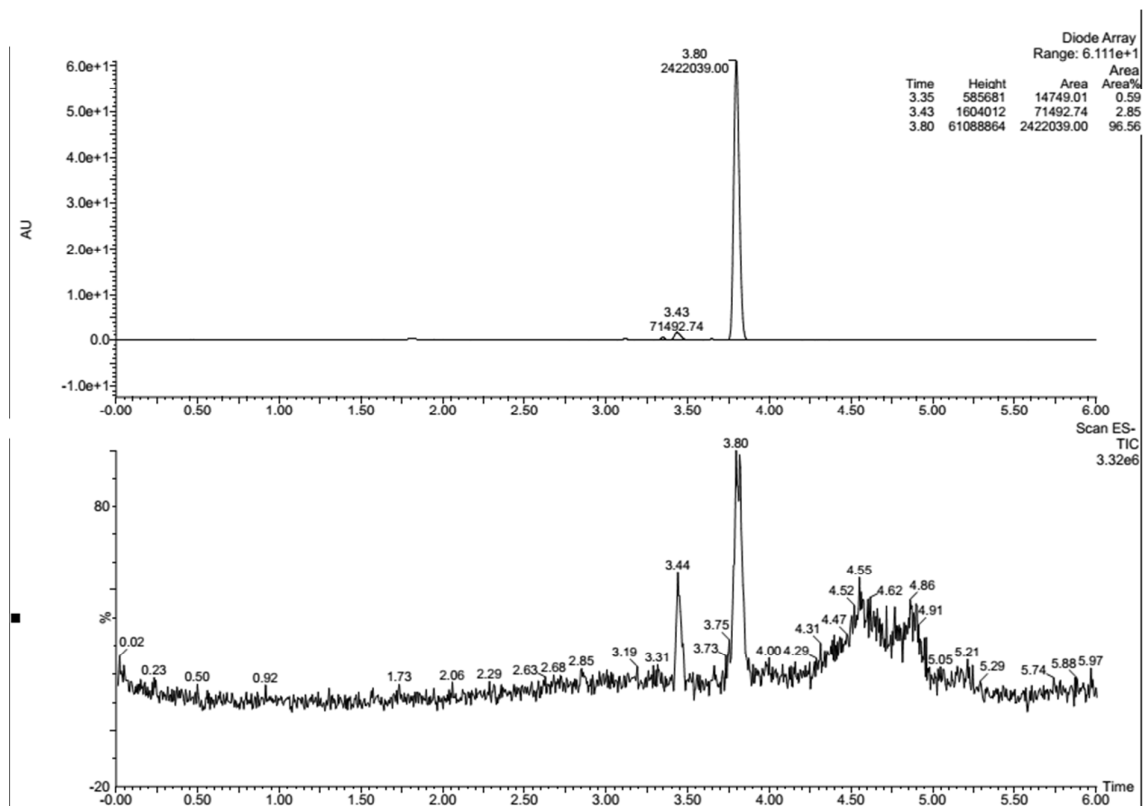
LCMS [M-1] = 371.08 m/z (372.40 calc.).

HRMS [M-1] = 371.0528 (371.0495 calc.)

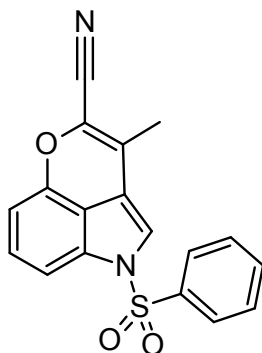
¹H NMR (500 MHz, DMSO-*d*₆) δ 8.62 (dq, *J* = 8.7, 0.9 Hz, 1H), 8.48 (dd, *J* = 7.5, 1.2 Hz, 1H), 8.34 (dt, *J* = 8.4, 1.0 Hz, 1H), 8.13 - 8.06 (m, 1H), 7.81 - 7.62 (m, 4H), 7.28 - 7.14 (m, 2H), 7.12 (s, 1H), 6.67 (d, *J* = 7.7 Hz, 1H).

¹³C NMR (126 MHz, DMSO-*d*₆) δ 106.11, 108.34, 110.24, 113.98, 114.76, 117.49, 119.83, 123.04, 124.77, 125.47, 126.95, 127.56, 127.67, 127.71, 128.94, 129.33, 129.60, 130.83, 131.31, 133.81, 133.83, 136.58, 147.50.





1-(Benzenesulfonyl)-3-methyl-pyrano[2,3,4-*cd*]indole-4-carbonitrile (4c)

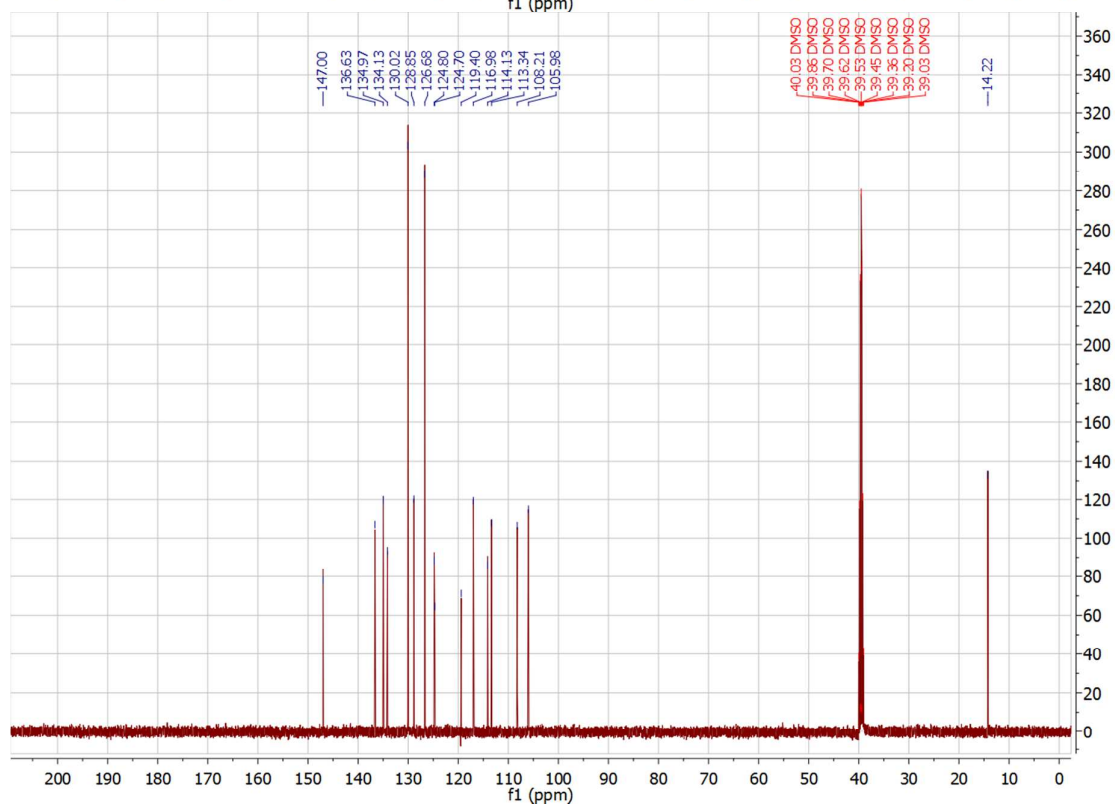
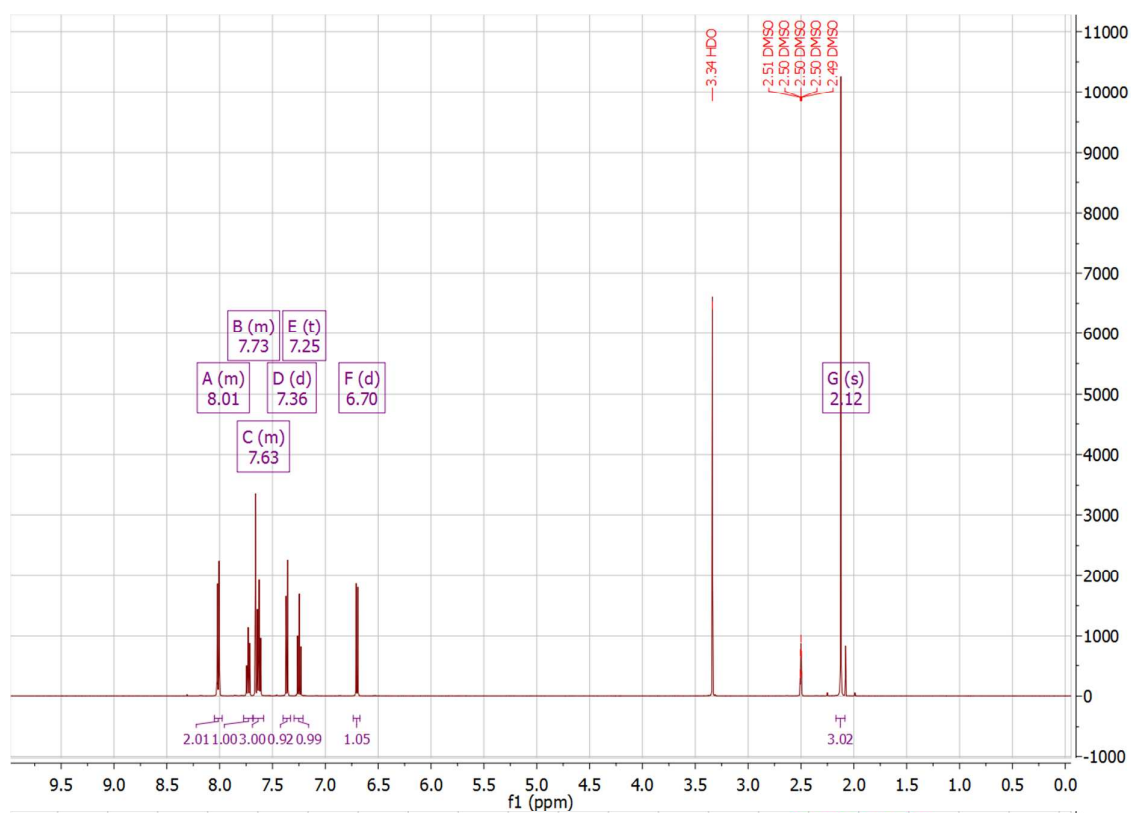


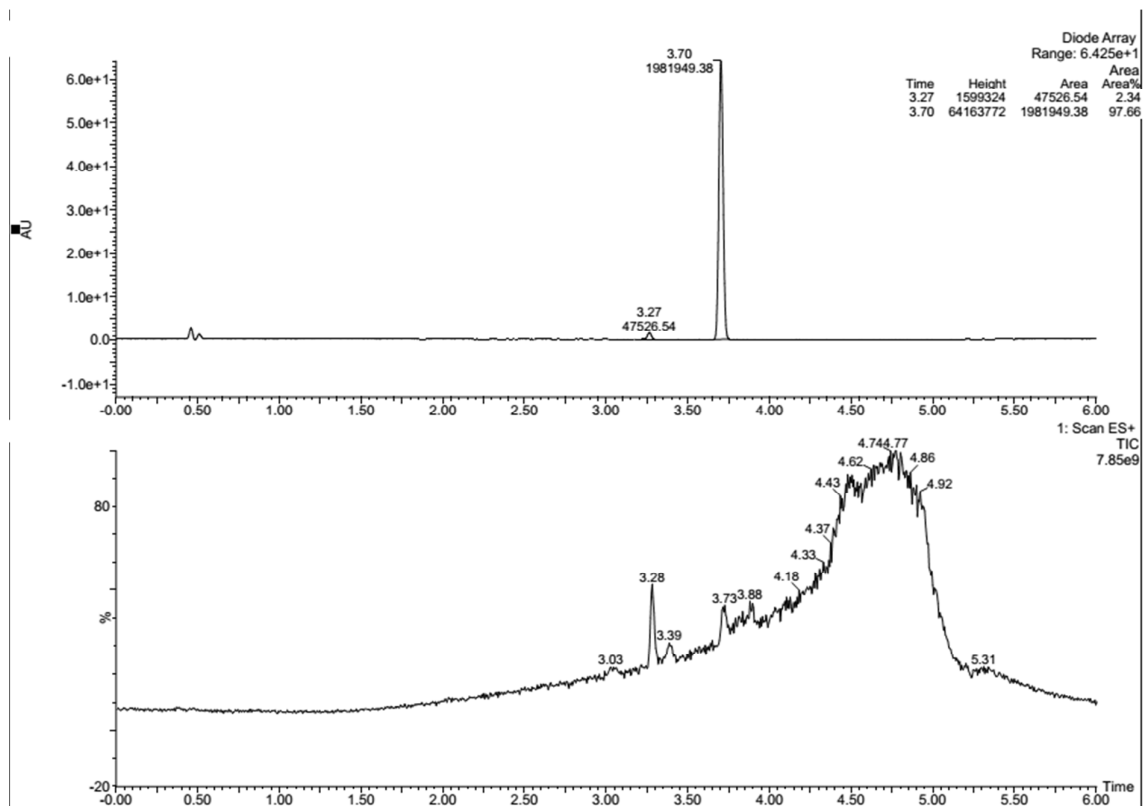
3b (0.22 g, 1.1 mmol), THF (10 ml), NaH (60% in oil, 1.2 eq., 0.054 g), benzenesulfonyl chloride (1.2 eq., 0.168 ml). A white solid was obtained (0.28 g, 76%).

LCMS [M+1] = 336.94 m/z (336.37 calc.).

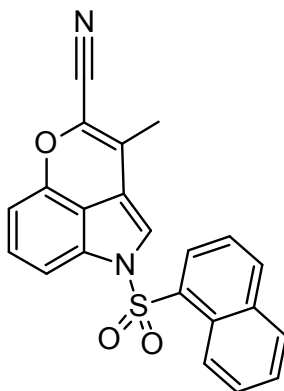
¹H NMR (500 MHz, DMSO-*d*₆) δ 8.05 – 7.98 (m, 2H), 7.77 – 7.68 (m, 1H), 7.69 – 7.58 (m, 3H), 7.36 (d, *J* = 8.3 Hz, 1H), 7.25 (t, *J* = 8.1 Hz, 1H), 6.70 (d, *J* = 8.0 Hz, 1H), 2.12 (s, 3H).

¹³C NMR (126 MHz, DMSO-*d*₆) δ 14.22, 105.98, 108.21, 113.34, 114.13, 116.98, 119.40, 124.70, 124.80, 126.68, 128.85, 130.02, 134.13, 134.97, 136.63, 147.00.





1-(Naphthalene-1-sulfonyl)-3-methyl-pyrano[2,3,4-*cd*]indole-4-carbonitrile (4d)

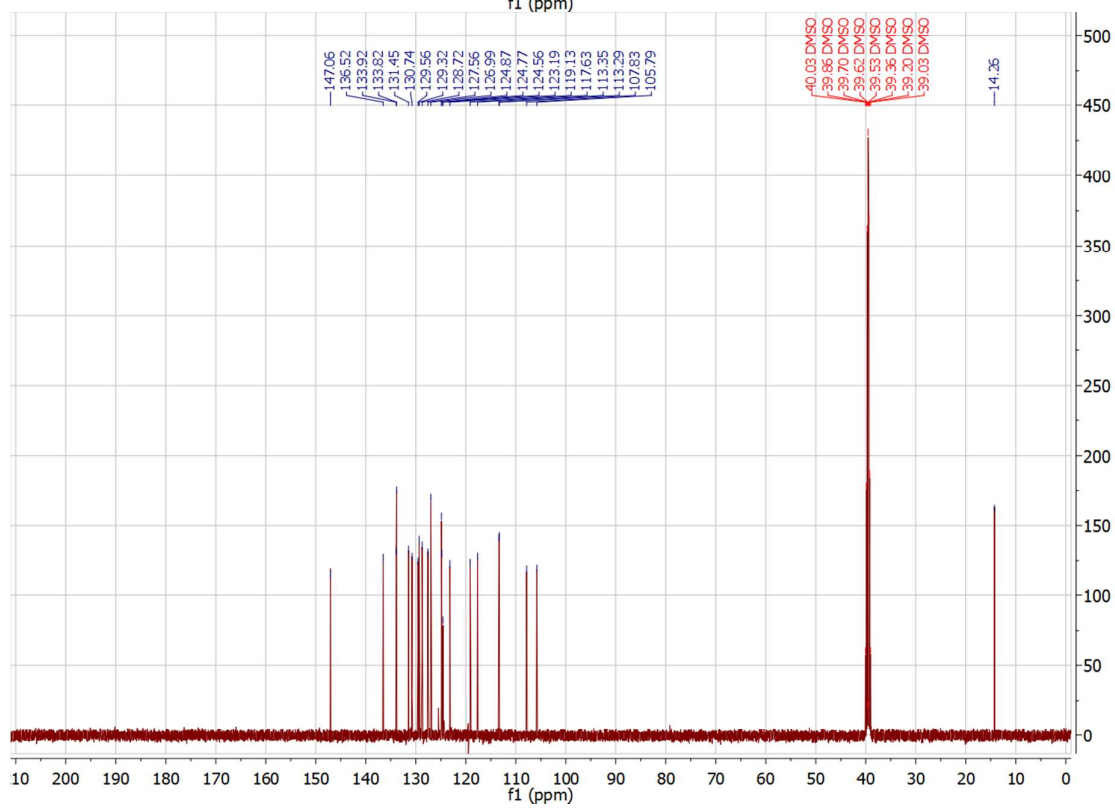
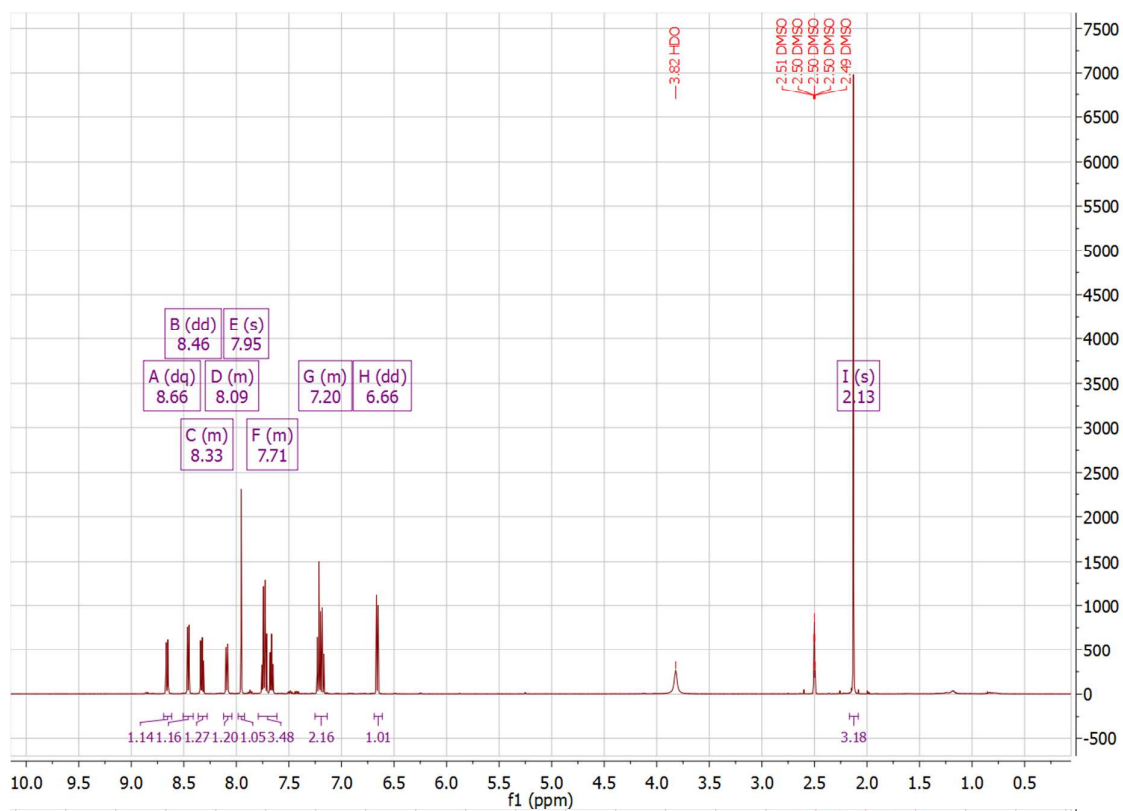


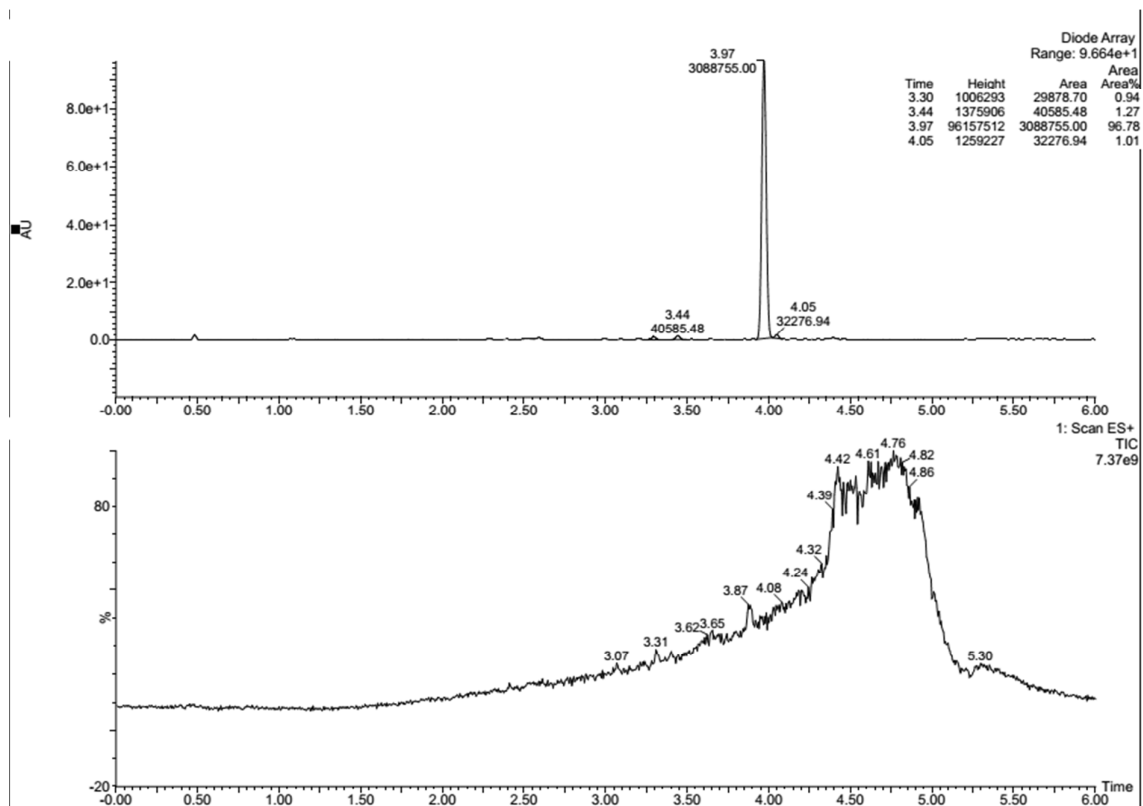
3b (0.22 g, 1.1 mmol), THF (10 ml), NaH (60% in oil, 1.2 eq., 0.054 g),), 1-naphthylsulfonyl chloride (1.2 eq., 0.30 g). A white solid was obtained (0.32 g, 73%).

LCMS [M+1] = 387.06 m/z (386.43 calc.).

¹H NMR (500 MHz, DMSO-*d*₆) δ 8.66 (dq, *J* = 8.7, 0.9 Hz, 1H), 8.46 (dd, *J* = 7.5, 1.2 Hz, 1H), 8.36 – 8.28 (m, 1H), 8.12 – 8.04 (m, 1H), 7.95 (s, 1H), 7.79 – 7.61 (m, 3H), 7.25 – 7.14 (m, 2H), 6.66 (dd, *J* = 7.7, 0.7 Hz, 1H), 2.13 (s, 3H).

¹³C NMR (126 MHz, DMSO-*d*₆) δ 14.26, 105.79, 107.83, 113.29, 113.35, 117.63, 119.13, 123.19, 124.56, 124.77, 124.87, 126.99, 127.56, 128.72, 129.32, 129.56, 130.74, 131.45, 133.82, 133.92, 136.52, 147.06.

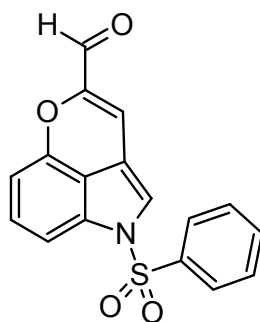




General procedure for the synthesis of 5a - 5d.

4a (0.3 g, 0.93 mmol) was dissolved in dichloromethane (3 ml) and cooled to -78°C . A 1M solution of DIBAL-H in THF (2 eq., 2 ml) was added dropwise and the mixture was allowed to warm to room temperature. Methanol (35 ml) was added followed by 1M HCl (2 ml). Solvent was evaporated under reduced pressure. Product was purified using column chromatography (SiO_2 , CHCl_3). A white solid was obtained (0.24g, 79%).

1-(Benzenesulfonyl)-pyrano[2,3,4-*cd*]indole-4-carbaldehyde (5a)

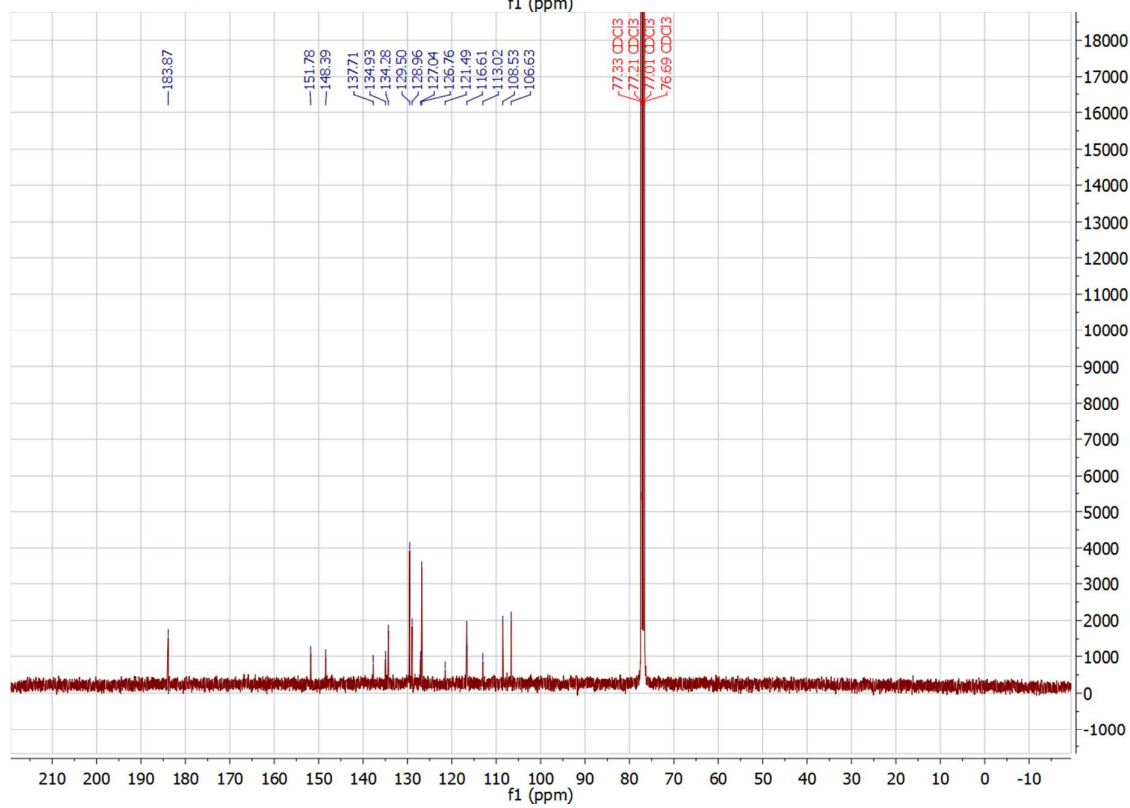
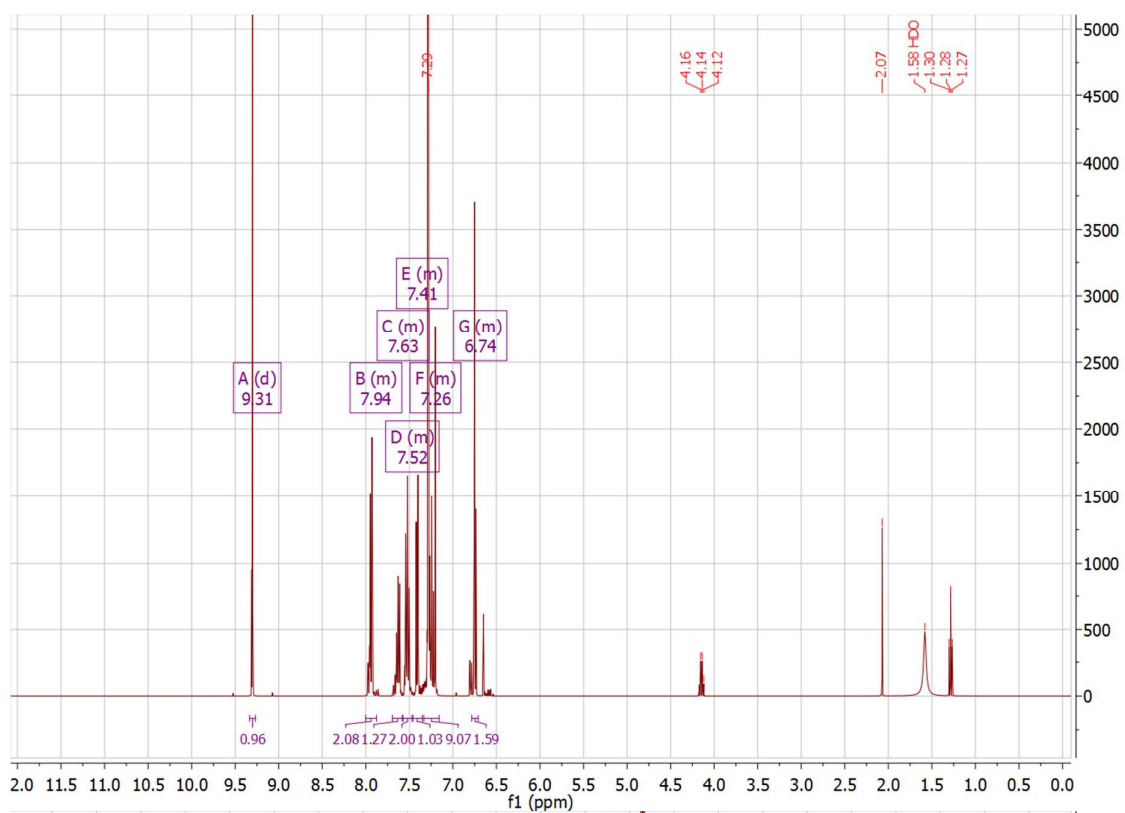


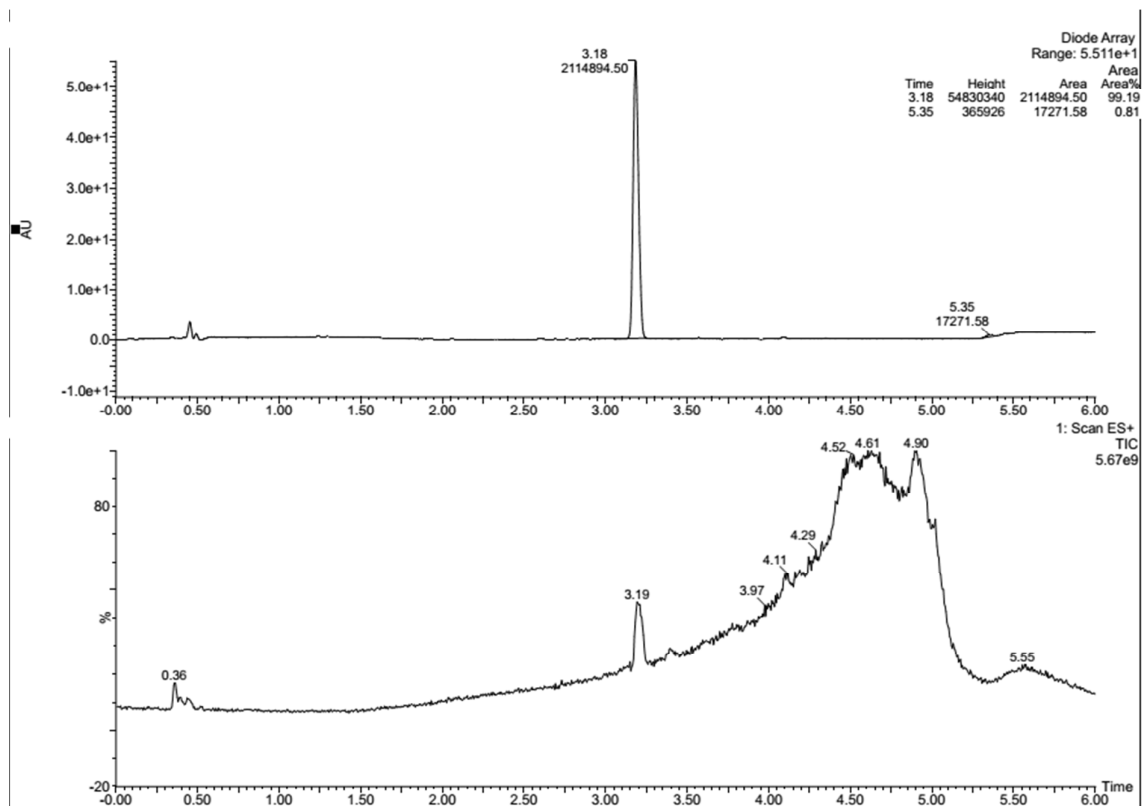
4a (0.3 g, 0.93 mmol), dichloromethane (3 ml) DIBAL-H (1M in THF, 2 eq., 2 ml), methanol (35 ml), 1M HCl (2 ml). A white solid was obtained (0.24 g, 79%).

LCMS $[M+1] = 325.14\text{ m/z}$ (325.34 calc.).

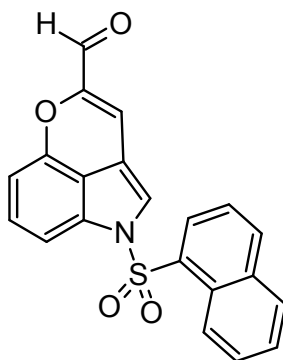
^1H NMR (400 MHz, Chloroform- d) δ 9.31 (d, $J = 3.7\text{ Hz}$, 1H), 8.01 - 7.83 (m, 1H), 7.71 - 7.44 (m, 3H), 7.44 - 7.16 (m, 5H), 6.83 - 6.71 (m, 1H).

^{13}C NMR (101 MHz, CDCl_3) δ 106.63, 108.53, 113.02, 116.61, 121.49, 126.76, 127.04, 128.96, 129.50, 134.28, 134.93, 137.71, 148.39, 151.78, 183.87.





1-(Naphthalene-1-sulfonyl)-pyrano[2,3,4-*cd*]indole-4-carbaldehyde (5b)



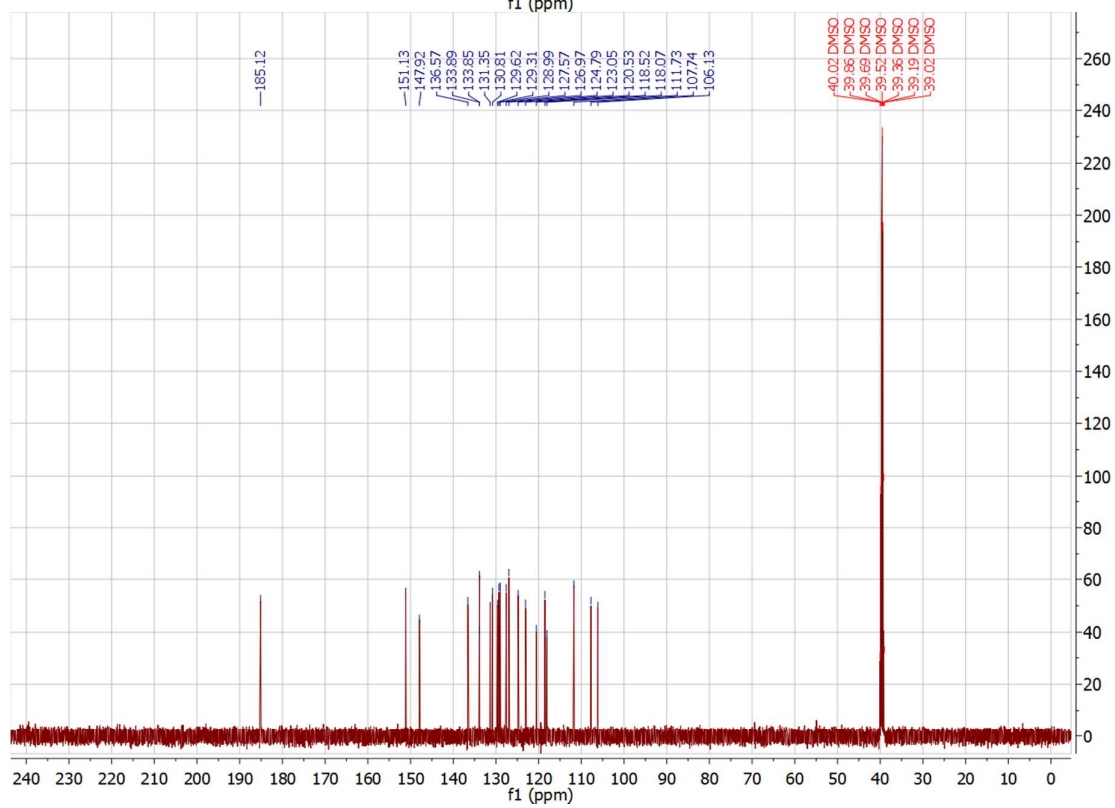
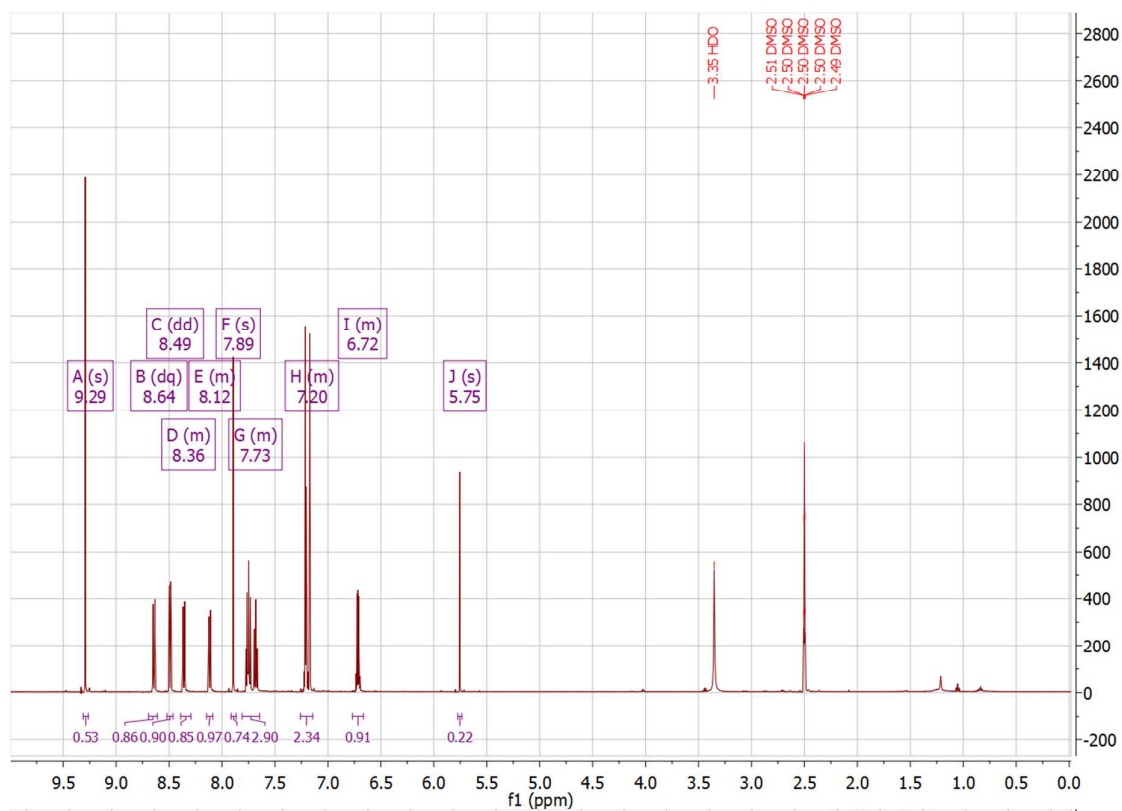
4b (0.3 g, 0.81 mmol), dichloromethane (3 ml) DIBAL-H (1M in THF, 2 eq., 2 ml), methanol (35 ml), 1M HCl (2 ml). A white solid was obtained (0.11 g, 36%).

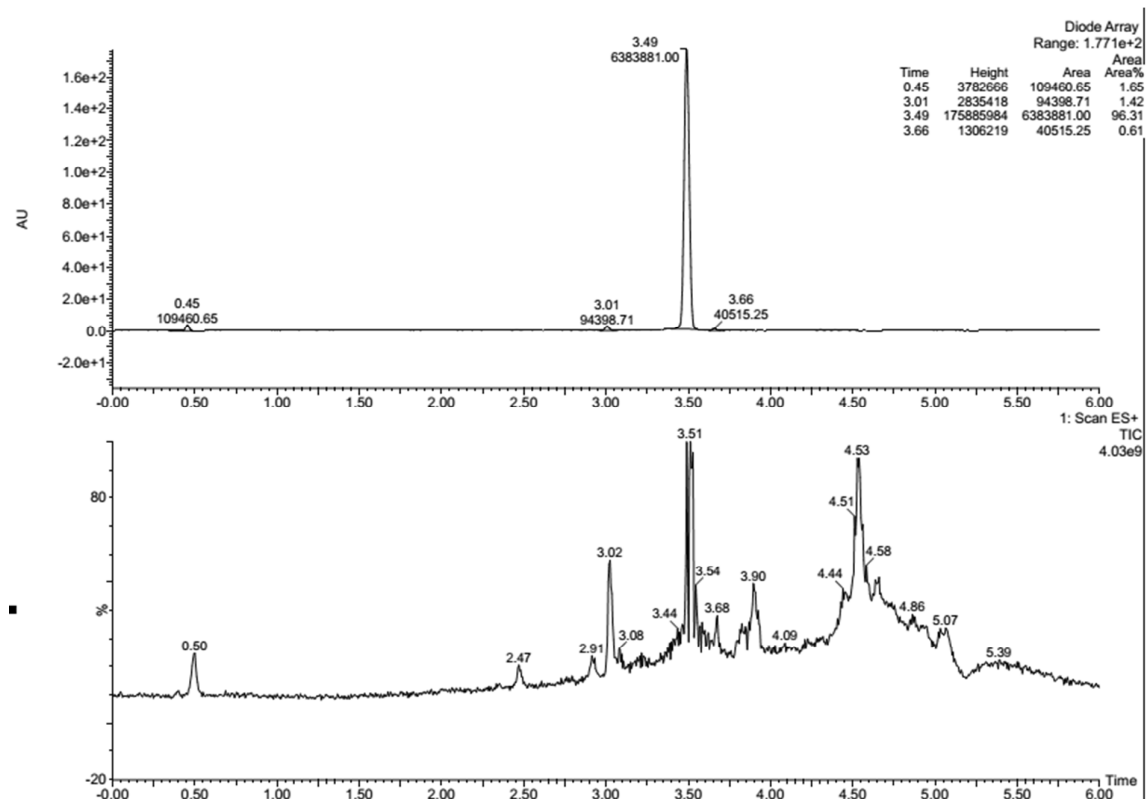
LCMS [M+1] = 375.93 m/z (375.40 calc.).

HRMS [M-1] = 374.0492 (374.0521 calc.)

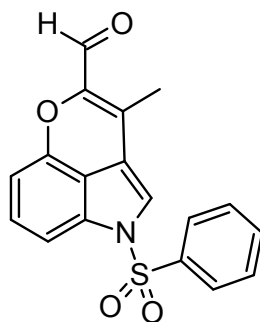
¹H NMR (500 MHz, DMSO-*d*₆) δ 9.29 (s, 1H), 8.64 (dq, *J* = 8.7, 0.9 Hz, 1H), 8.49 (dd, *J* = 7.5, 1.2 Hz, 1H), 8.39 - 8.29 (m, 1H), 8.15 - 8.09 (m, 1H), 7.89 (s, 1H), 7.81 - 7.64 (m, 3H), 7.26 - 7.14 (m, 3H), 6.77 - 6.66 (m, 1H), 5.75 (s, 0H).

¹³C NMR (126 MHz, DMSO-*d*₆) δ 106.13, 107.74, 111.73, 118.07, 118.52, 120.53, 123.05, 124.79, 126.97, 127.57, 128.99, 129.31, 129.62, 130.81, 131.35, 133.85, 133.89, 136.57, 147.92, 151.13, 185.12.





1-(Benzenesulfonyl)-3-methyl-pyrano[2,3,4-*cd*]indole-4-carbaldehyde (5c)

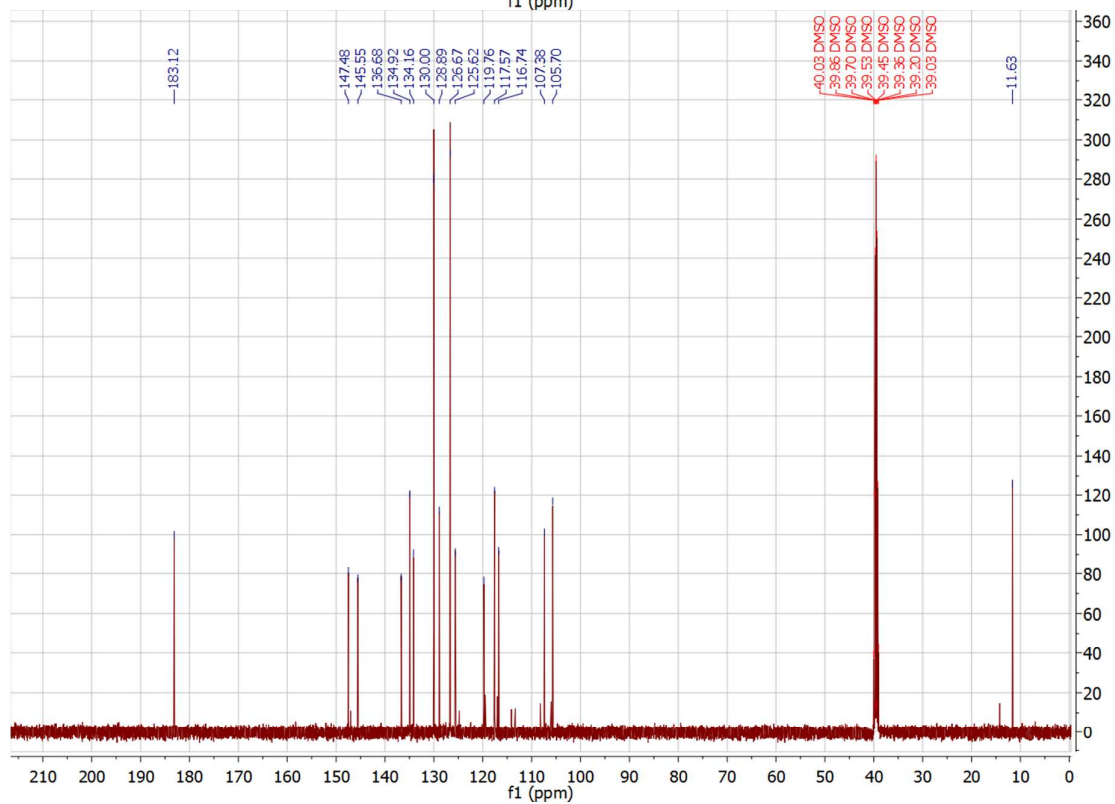
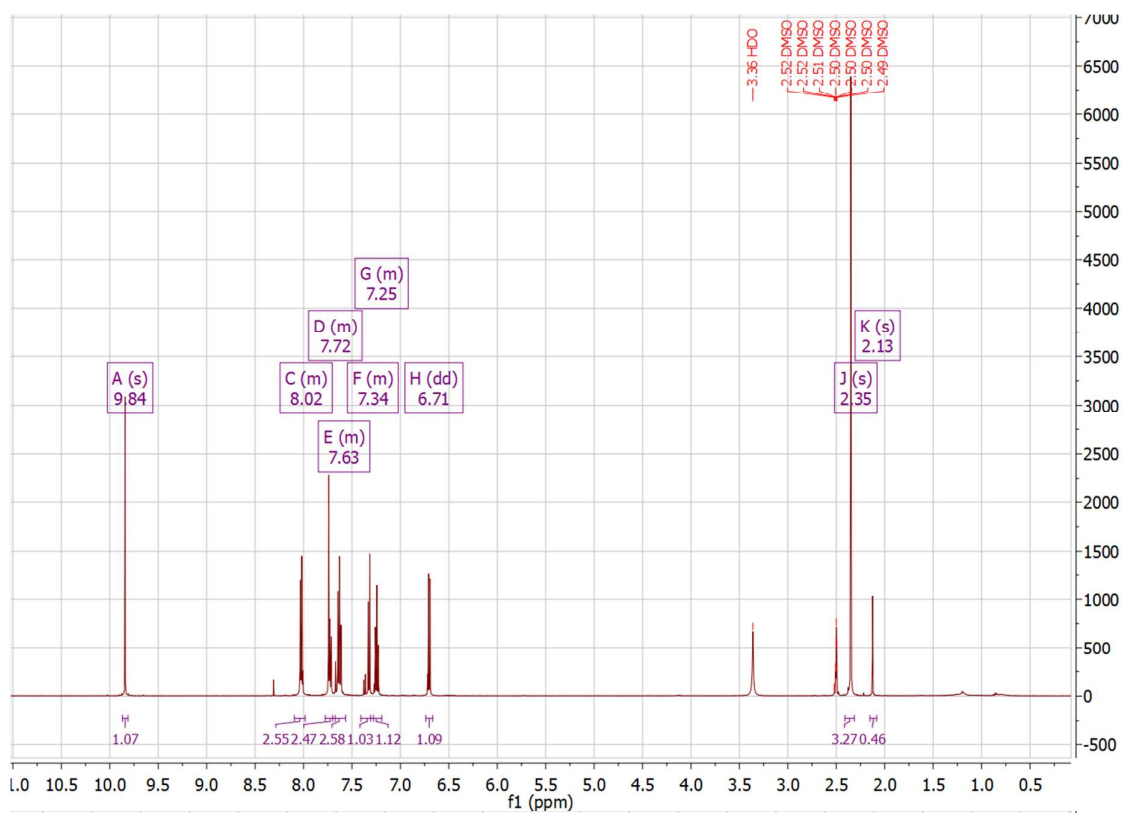


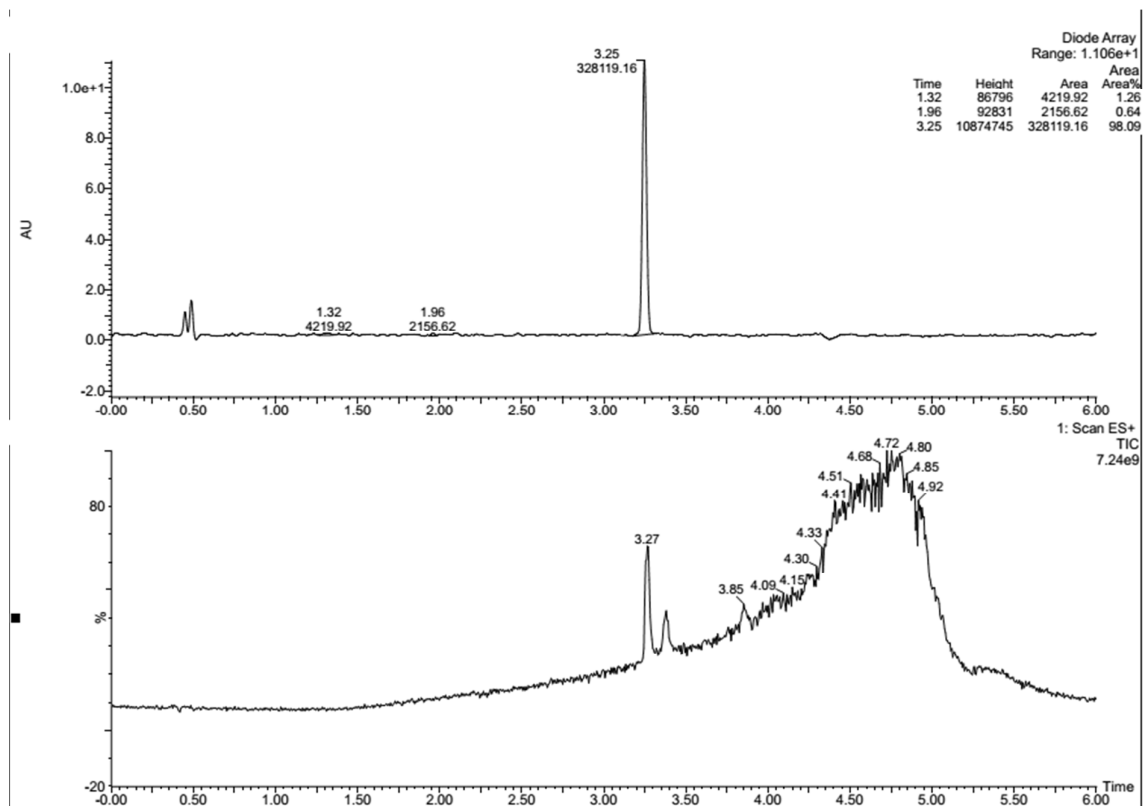
4c (0.15 g, 0.44 mmol), dichloromethane (3 ml) DIBAL-H (1M in THF, 2 eq., 0.9 ml), methanol (10 ml), 1M HCl (1 ml). A white solid was obtained (0.10 g, 67%).

LCMS [M+1] = 339.94 m/z (339.37 calc.).

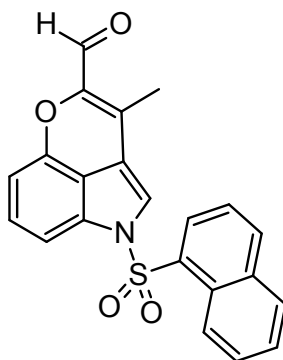
¹H NMR (500 MHz, DMSO-*d*₆) δ 9.84 (s, 1H), 8.09 – 7.98 (m, 2H), 7.78 – 7.67 (m, 2H), 7.70 – 7.57 (m, 2H), 7.41 – 7.28 (m, 1H), 7.31 – 7.19 (m, 1H), 6.71 (dd, *J* = 7.9, 4.5 Hz, 1H), 2.35 (s, 3H), 2.13 (s, 0H).

¹³C NMR (126 MHz, DMSO-*d*₆) δ 11.63, 105.70, 107.38, 116.74, 117.57, 119.76, 125.62, 126.67, 128.89, 130.00, 134.16, 134.92, 136.68, 145.55, 147.48, 183.12.





1-(Naphthalene-1-sulfonyl)-3-methyl-pyrano[2,3,4-*cd*]indole-4-carbaldehyde (5d)

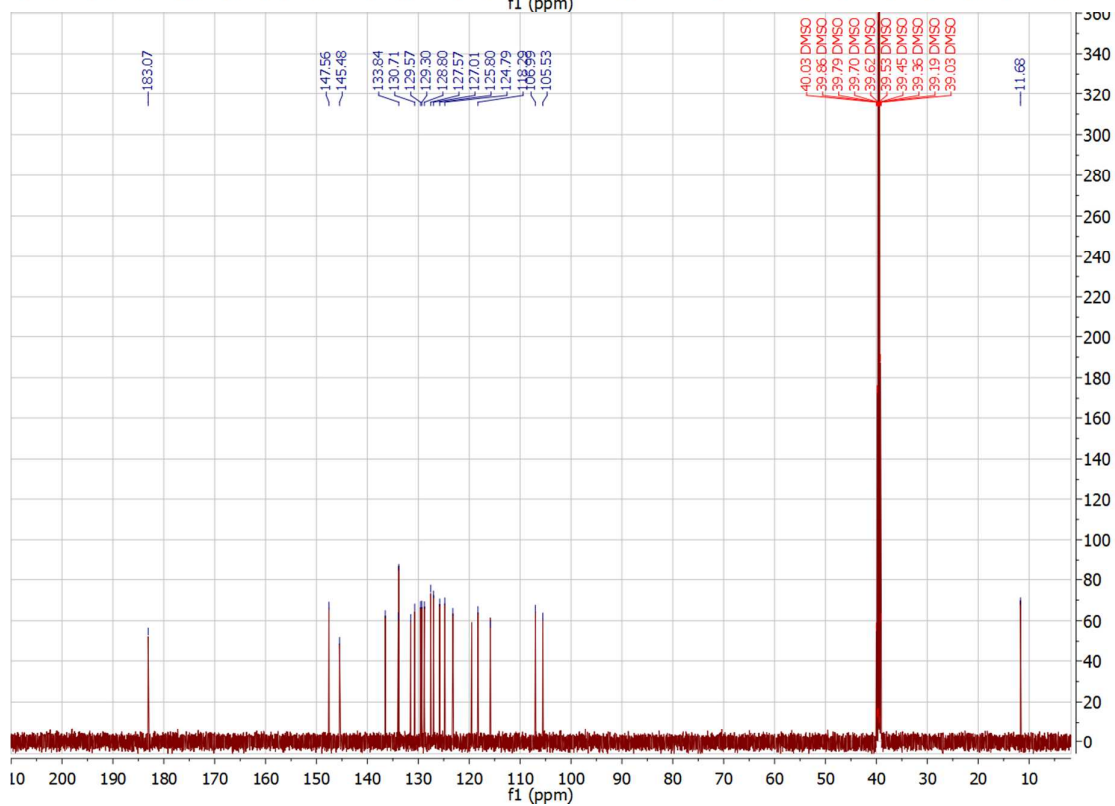
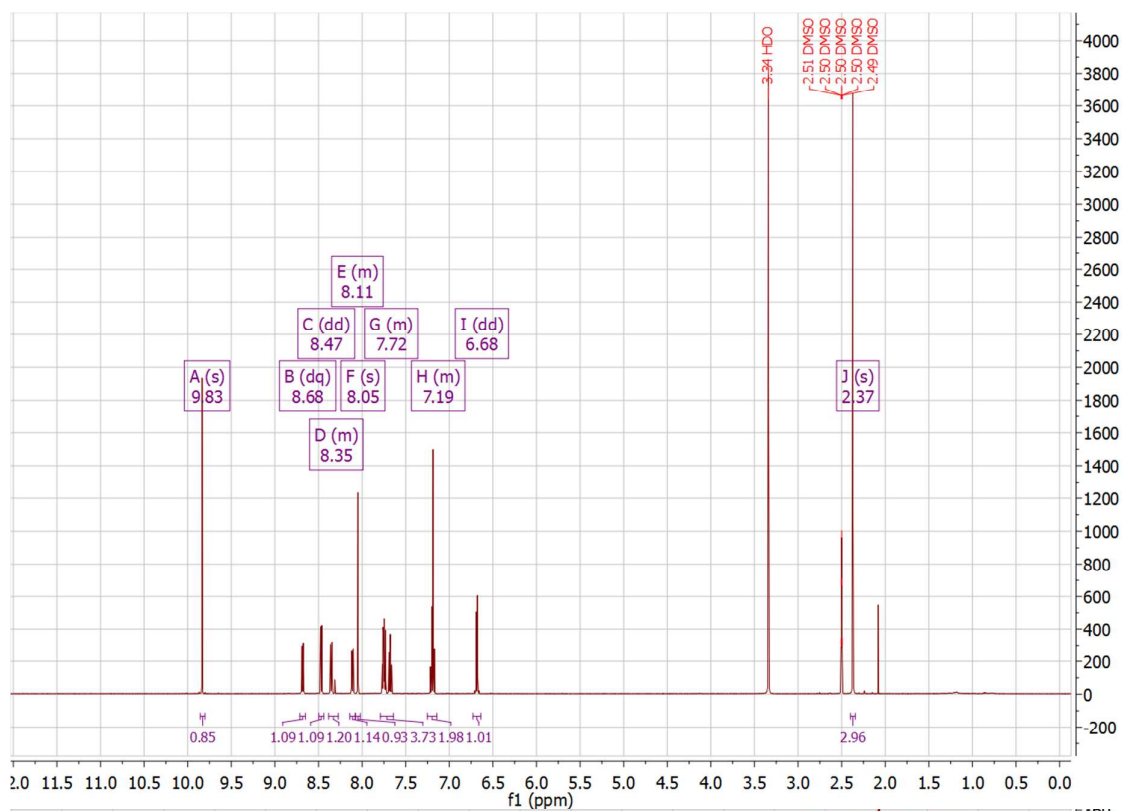


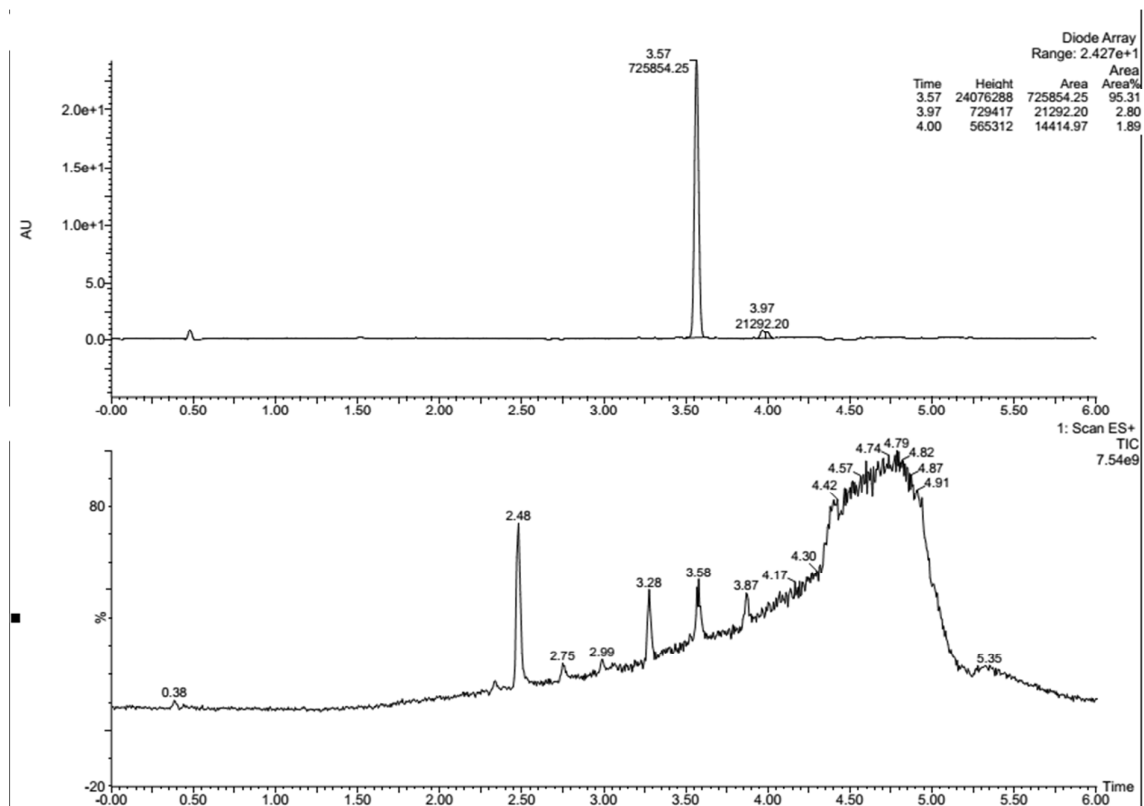
4d (0.33 g, 0.87 mmol), dichloromethane (10 ml) DIBAL-H (1M in THF, 2 eq., 1.74 ml), methanol (10 ml), 1M HCl (2 ml). A white solid was obtained (0.23 g, 68%).

LCMS [M+1] = 390.00 m/z (389.43 calc.).

¹H NMR (500 MHz, DMSO-*d*₆) δ 9.83 (s, 1H), 8.68 (dq, *J* = 8.7, 0.9 Hz, 1H), 8.47 (dd, *J* = 7.5, 1.2 Hz, 1H), 8.38 – 8.27 (m, 1H), 8.14 – 8.08 (m, 1H), 8.05 (s, 1H), 7.79 – 7.64 (m, 3H), 7.25 – 7.14 (m, 2H), 6.68 (dd, *J* = 6.9, 1.4 Hz, 1H), 2.37 (s, 3H).

¹³C NMR (126 MHz, DMSO-*d*₆) δ 11.68, 105.53, 106.99, 115.85, 118.29, 123.21, 124.79, 125.80, 127.01, 127.57, 128.80, 129.30, 129.57, 130.71, 131.49, 133.84, 133.94, 136.49, 145.48, 147.56, 183.07.

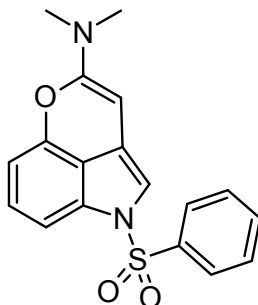




General procedure for the synthesis of 6a - 6e.

4a (0.1 g, 0.3 mmol) was dissolved in dichloroethane (5 ml) with NaBH(OAc)₃ (3 eq., 0.195 g) and dimethylamine hydrochloride (2 eq., 0.050 g). Reaction mixture was stirred at room temperature for 25 hours. After completion distilled water was added (50 ml) and the obtained mixture was extracted with ethyl acetate (3x 50ml). Combined organic extracts were washed with brine (20 ml), dried over anhydrous magnesium sulfate and evaporated under reduced pressure. Product was purified using column chromatography (SiO₂, CHCl₃:MeOH 9:1).

1-(Benzenesulfonyl)-pyrano[2,3,4-*cd*]indole-4-methyldimethylamine (6a)



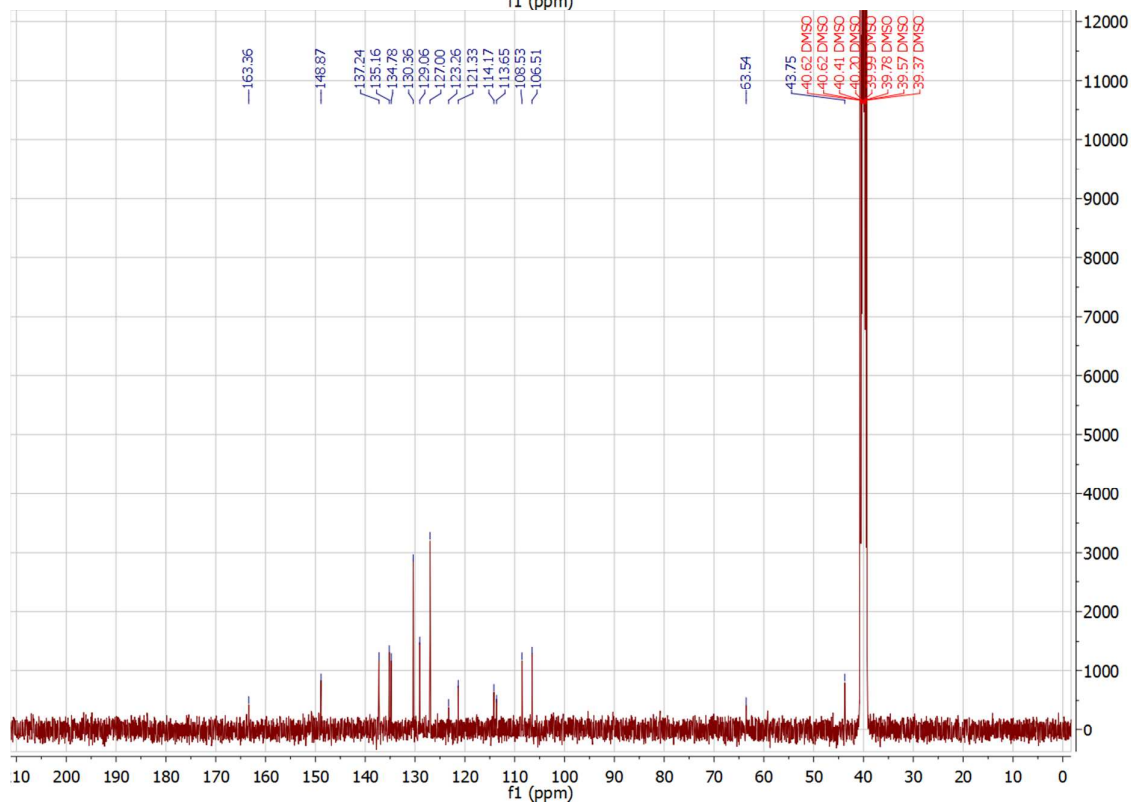
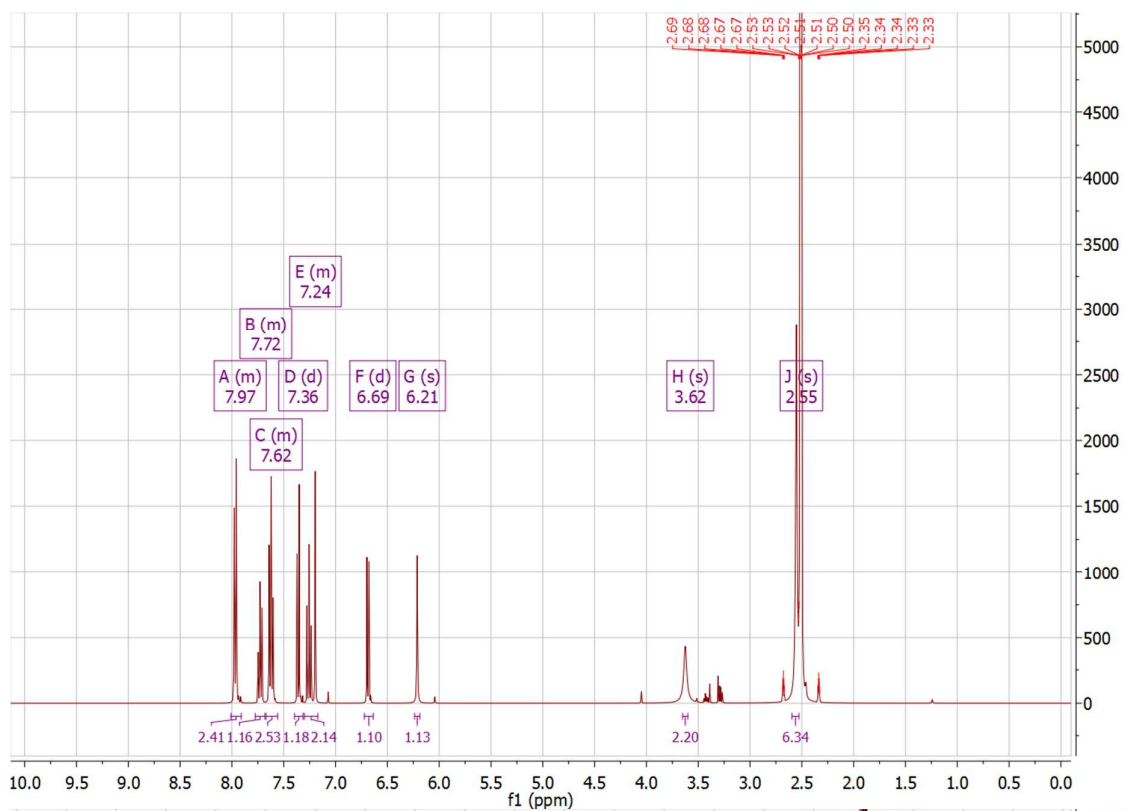
4a (0.1 g, 0.3 mmol), dichloroethane (5 ml), NaBH(OAc)₃ (3 eq., 0.195 g), dimethylamine hydrochloride (2 eq., 0.050 g). A light brown oil was obtained (0.050 g, 47%).

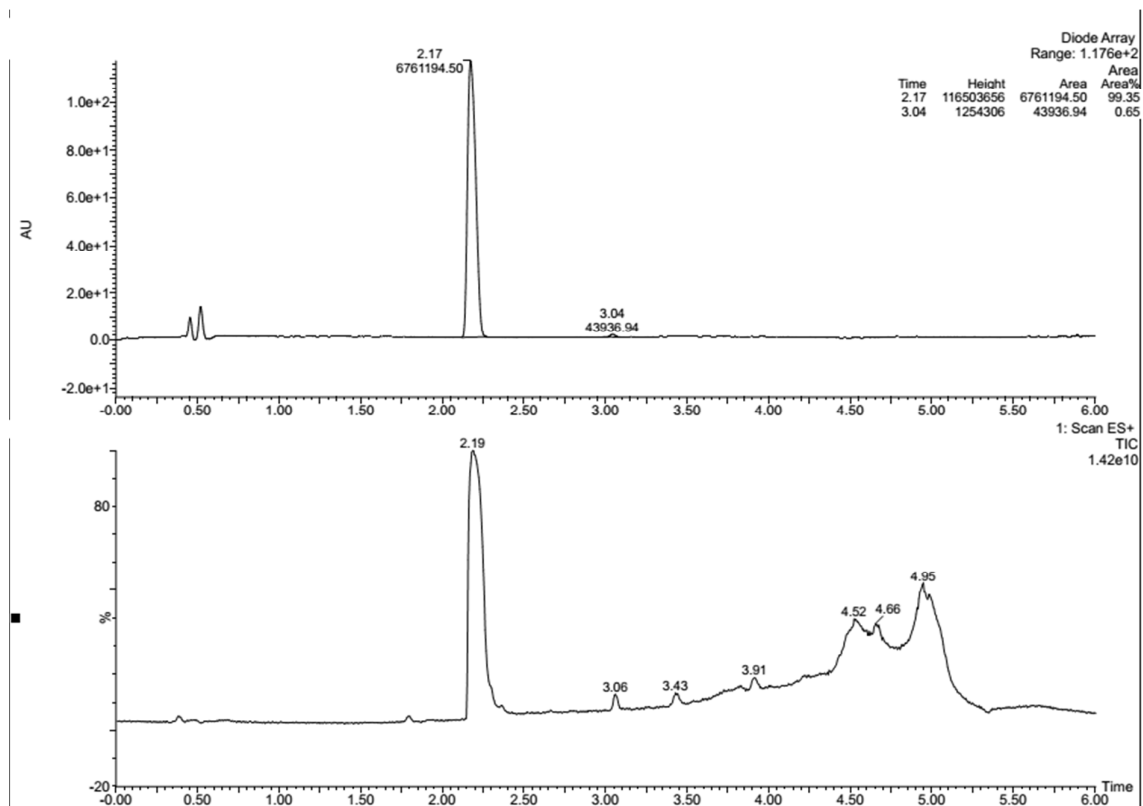
LCMS [M+1] = 355.20 m/z (354.42 calc.).

HRMS [M+1] = 355.1101 (355.1116 calc.)

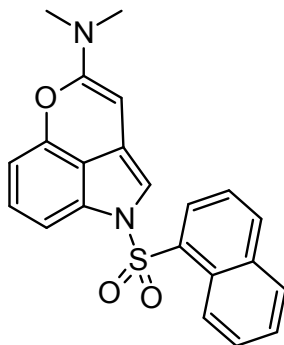
¹H NMR (400 MHz, DMSO-d₆) δ 8.01 - 7.91 (m, 2H), 7.78 - 7.67 (m, 1H), 7.67 - 7.56 (m, 2H), 7.36 (d, *J* = 8.2 Hz, 1H), 7.30 - 7.17 (m, 2H), 6.69 (d, *J* = 7.9 Hz, 1H), 6.21 (s, 1H), 3.17 (s, 2H), 2.55 (s, 6H).

¹³C NMR (126 MHz, DMSO-d₆) δ 40.62, 43.75, 63.54, 106.51, 108.53, 113.65, 114.17, 121.33, 127.00, 129.06, 130.36, 134.78, 135.16, 137.24, 148.87, 163.36.





1-(Naphthalene-1-sulfonyl)-pyrano[2,3,4-*cd*]indole-4-methyldimethylamine (6b)



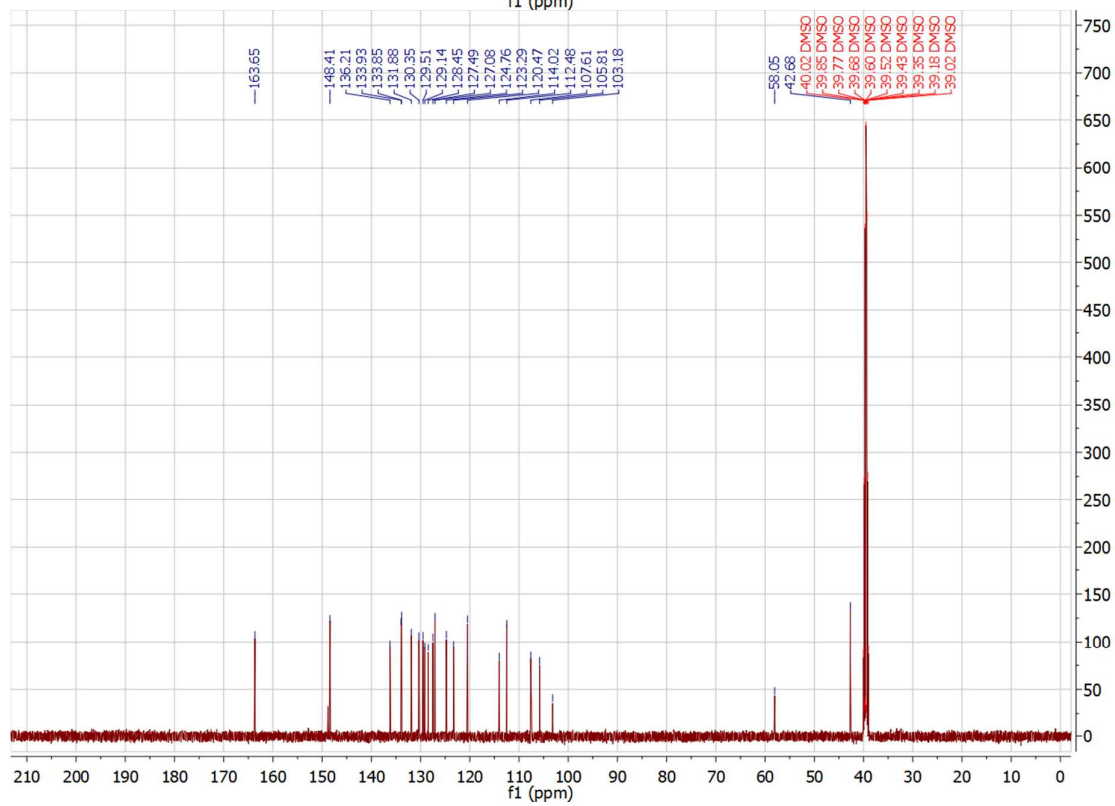
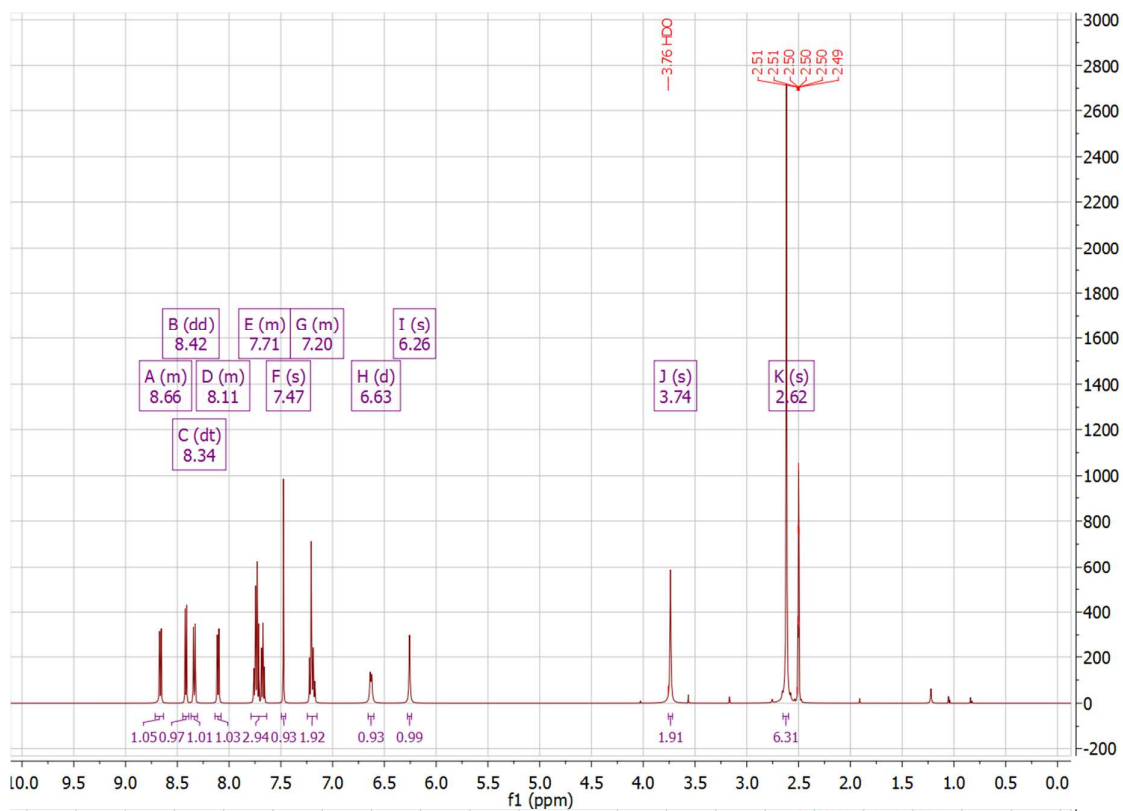
4b (0.1 g, 0.3 mmol), dichloroethane (5 ml), NaBH(OAc)₃ (3 eq., 0.240 g), dimethylamine hydrochloride (2 eq., 0.060 g). A light brown oil was obtained (0.093 g, 86%).

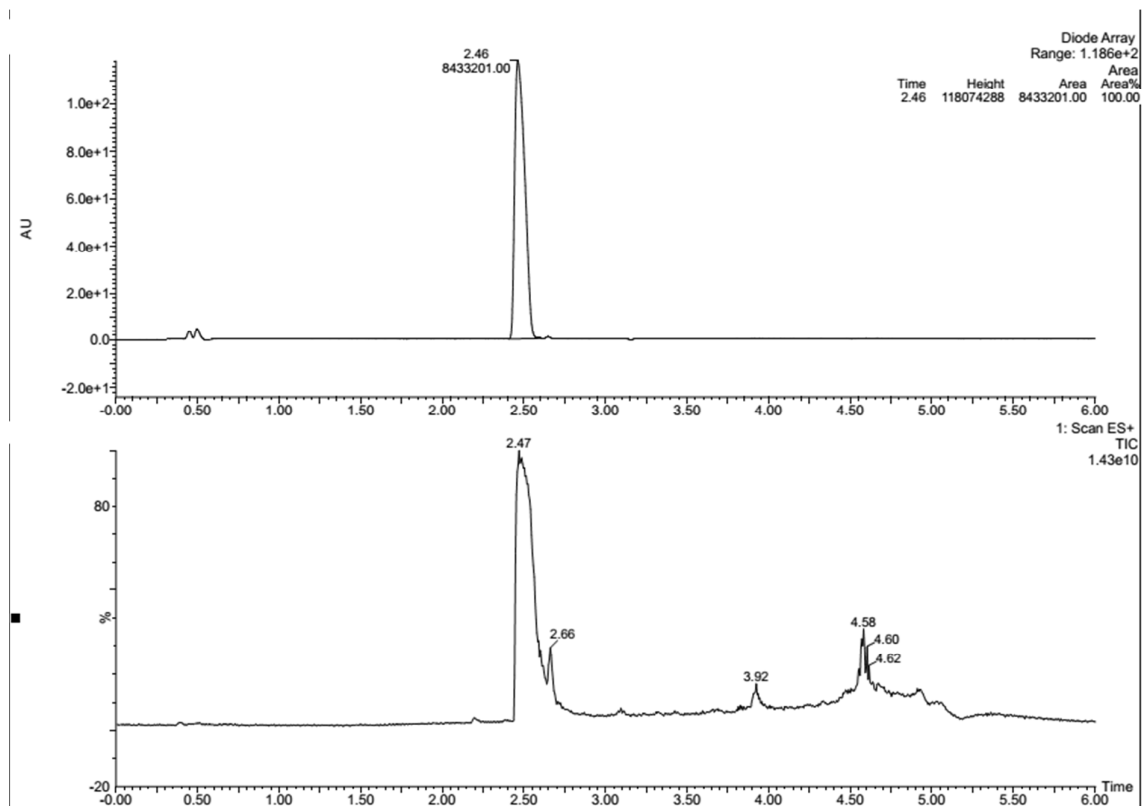
LCMS [M+1] = 405.26 m/z (404.48 calc.).

HRMS [M+1] = 405.1257 (405.1272 calc.)

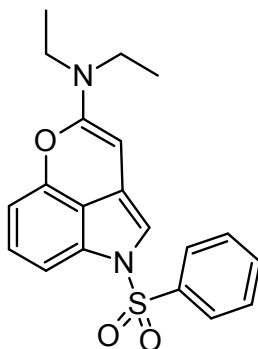
¹H NMR (500 MHz, DMSO-*d*₆) δ 8.71 - 8.63 (m, 1H), 8.42 (dd, *J* = 7.5, 1.2 Hz, 1H), 8.34 (dt, *J* = 8.4, 1.0 Hz, 1H), 8.14 - 8.08 (m, 1H), 7.79 - 7.64 (m, 3H), 7.47 (s, 1H), 7.24 - 7.15 (m, 2H), 6.63 (d, *J* = 7.2 Hz, 1H), 6.26 (s, 1H), 3.74 (s, 2H), 2.62 (s, 6H).

¹³C NMR (126 MHz, DMSO-*d*₆) δ 42.68, 58.05, 103.18, 105.81, 107.61, 112.48, 114.02, 120.47, 123.29, 124.76, 127.08, 127.49, 128.45, 129.14, 129.51, 130.35, 131.88, 133.85, 133.93, 136.21, 148.41, 163.65.





1-(Benzenesulfonyl)-3-methyl-pyrano[2,3,4-*cd*]indole-4-methyldiethylamine (6c)



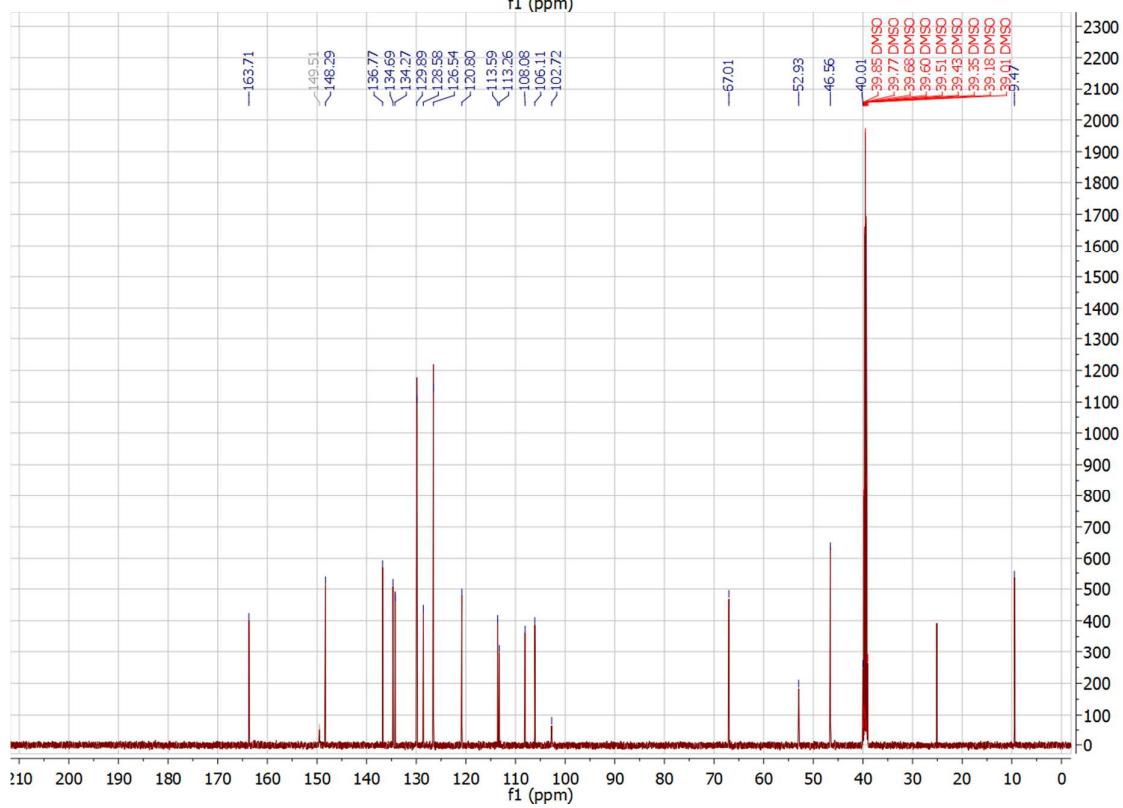
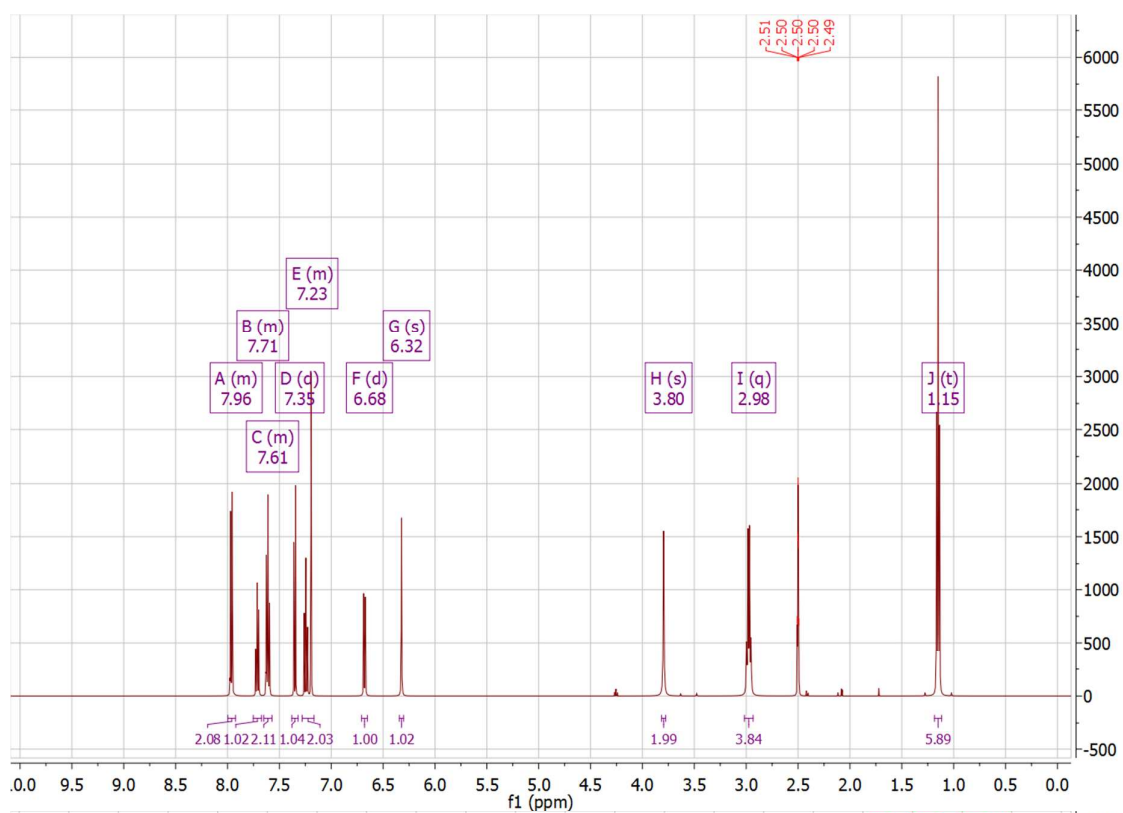
4a (0.2 g, 0.61 mmol), dichloroethane (10 ml), NaBH(OAc)₃ (3 eq., 0.4 g), diethylamine (2 eq., 0.050 g). A light brown oil was obtained (0.15 g, 64%).

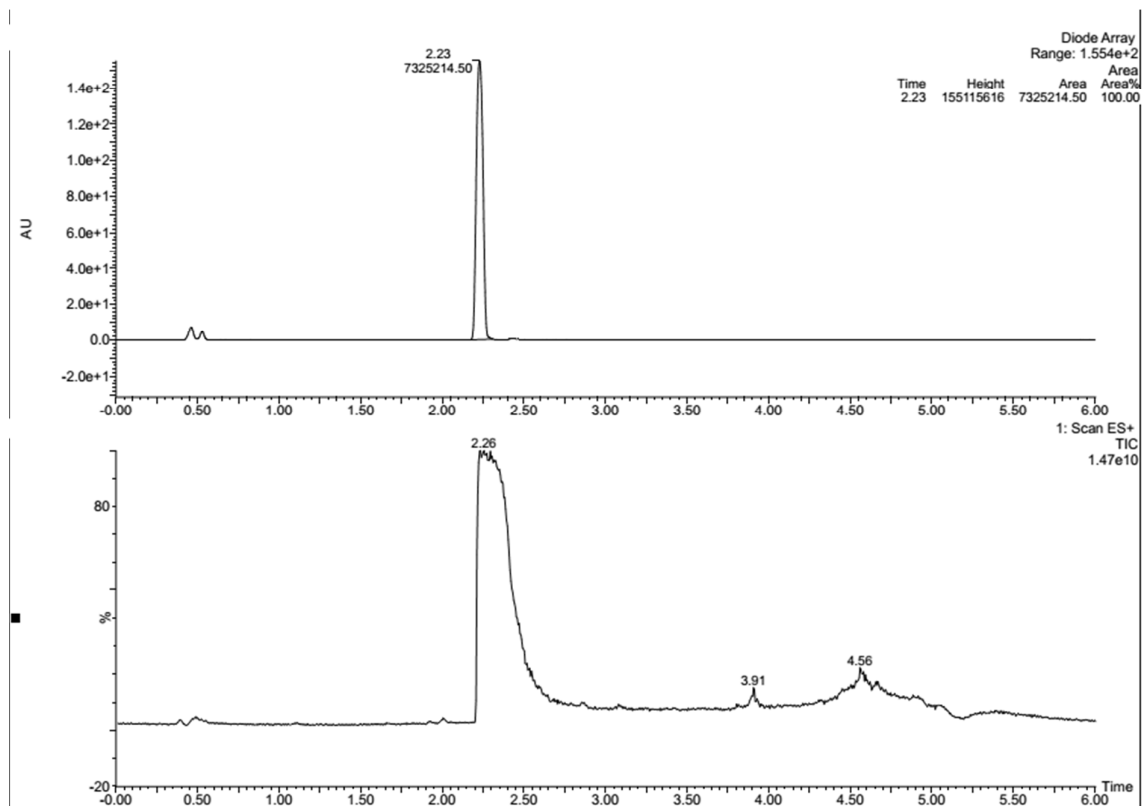
LCMS [M+1] = 383.06 m/z (382.48 calc.).

HRMS [M+1] = 383.1429 (383.1411 calc.)

¹H NMR (500 MHz, DMSO-*d*₆) δ 8.00 – 7.92 (m, 2H), 7.75 – 7.67 (m, 1H), 7.65 – 7.57 (m, 2H), 7.35 (d, *J* = 8.2 Hz, 1H), 7.28 – 7.17 (m, 2H), 6.68 (d, *J* = 7.9 Hz, 1H), 6.32 (s, 1H), 3.80 (s, 2H), 2.98 (q, *J* = 7.2 Hz, 4H), 1.15 (t, *J* = 7.2 Hz, 6H).

¹³C NMR (126 MHz, DMSO-*d*₆) δ 9.47, 40.01, 46.56, 52.93, 67.01, 102.72, 106.11, 108.08, 113.26, 113.59, 120.80, 126.54, 128.58, 129.89, 134.27, 134.69, 136.77, 148.29, 163.71.

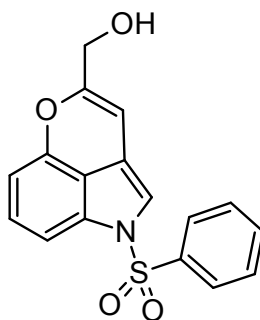




General procedure for the synthesis of 7a - 7d.

4a (0.1 g, 0.3 mmol) was dissolved in methanol (5 ml) and NaBH₄ (2 eq., 20 mg) was added. Reaction mixture was stirred at room temperature for 1 hour, then distilled water was added (50 ml). Obtained mixture was extracted with ethyl acetate (3x 50ml), combined organic extracts were washed with brine (20 ml), dried over anhydrous magnesium sulfate and evaporated under reduced pressure. Product was purified using column chromatography (SiO₂, CHCl₃).

[1-(Benzenesulfonyl)-pyrano[2,3,4-*cd*]indol-4-yl]methanol (7a)



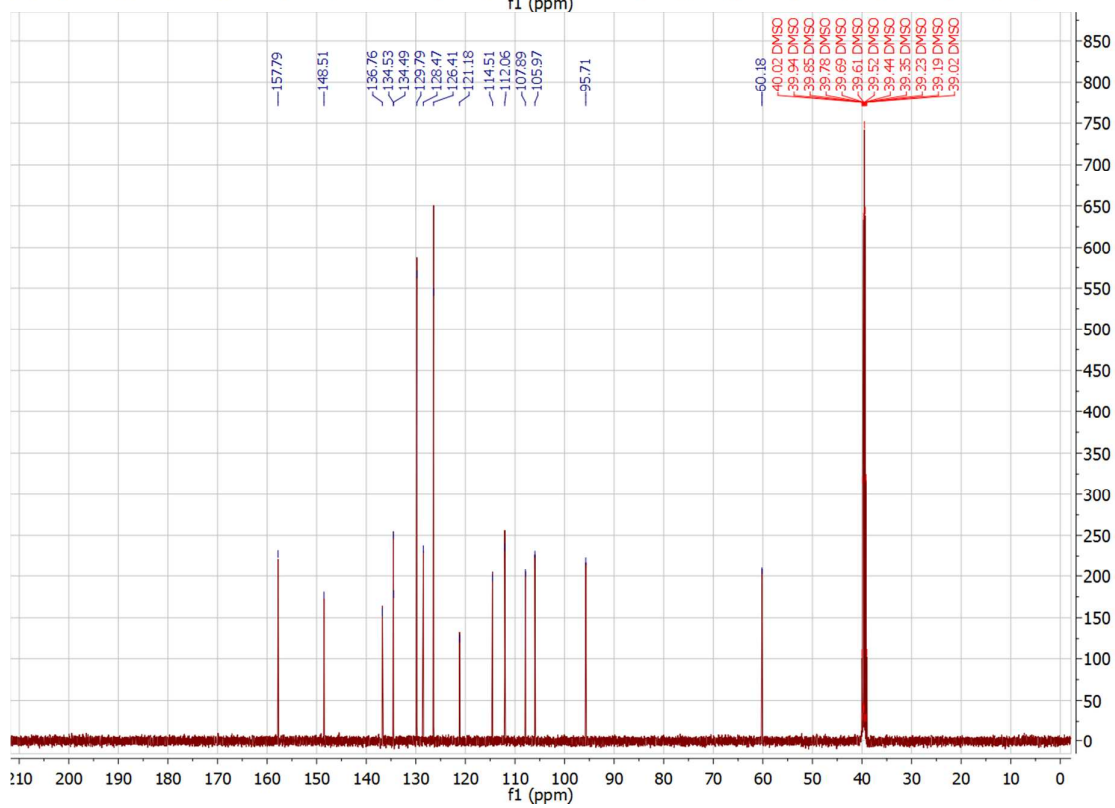
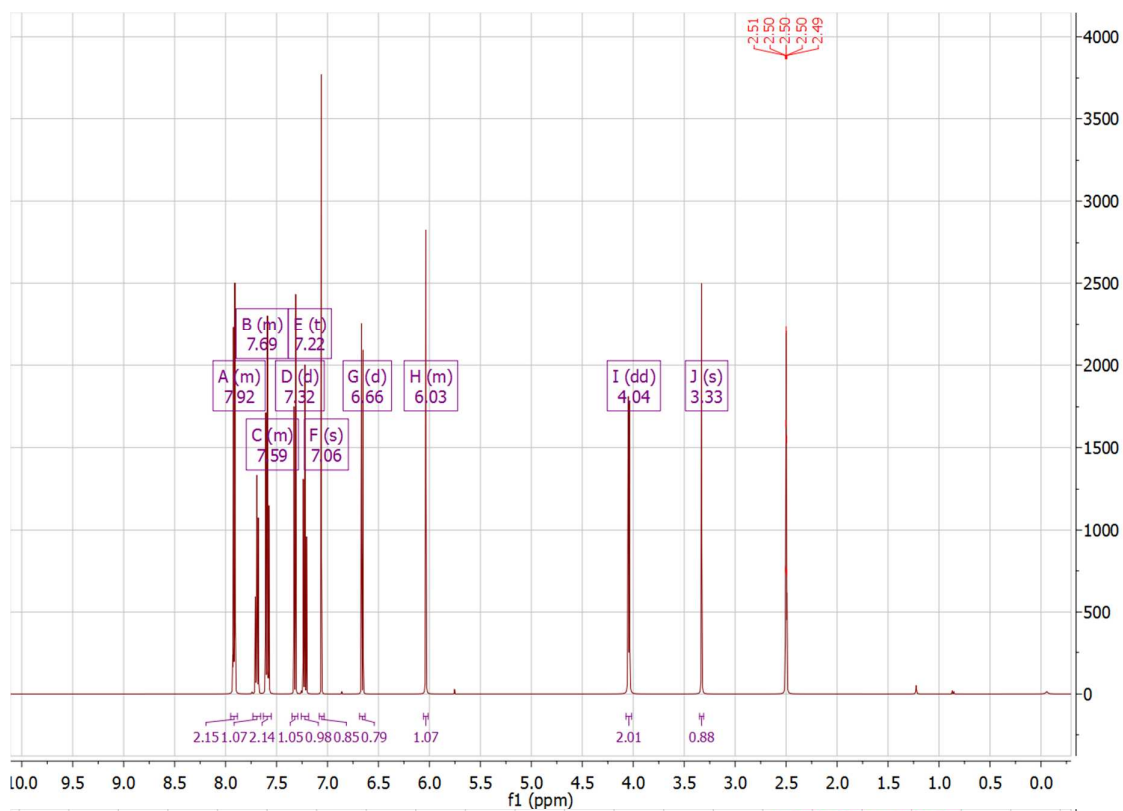
4a (0.1 g, 0.3 mmol), methanol (5 ml), NaBH₄ (2 eq., 20 mg). A light tan solid was obtained (0.083 g, 83%).

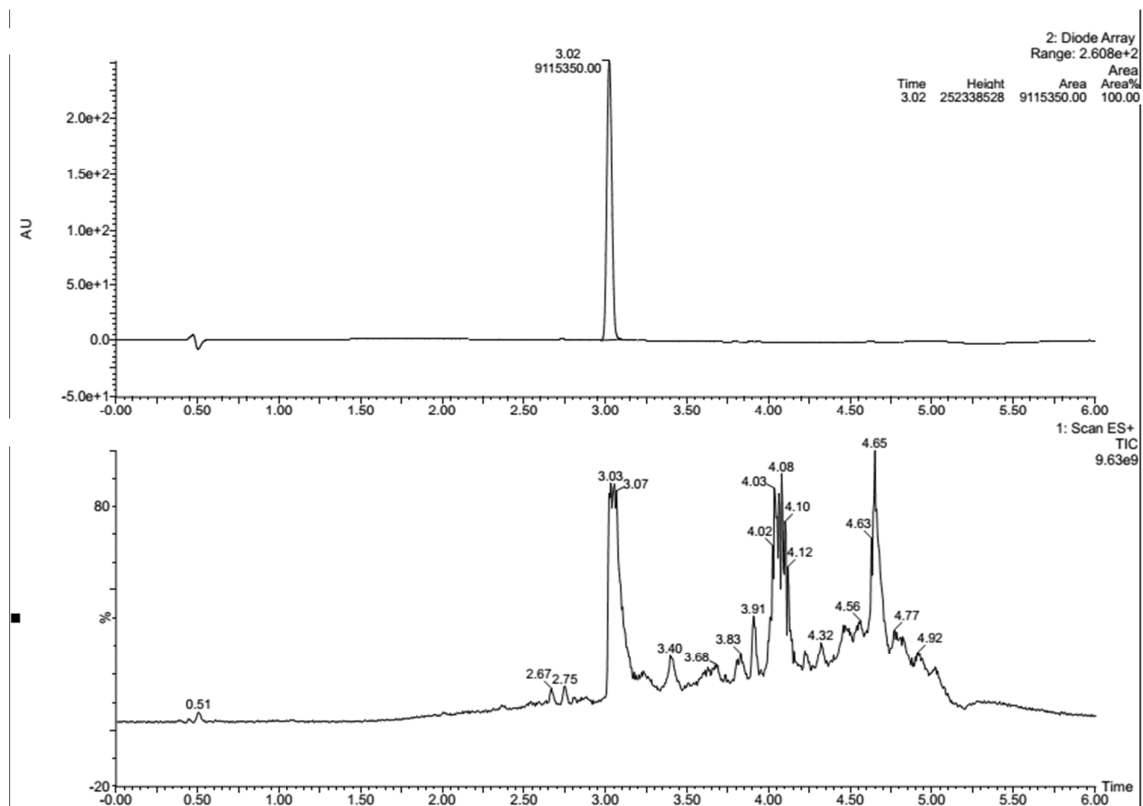
LCMS [M+1] = 328.01 m/z (327.35 calc.).

HRMS [M+1] = 328.0630 (328.0643 calc.)

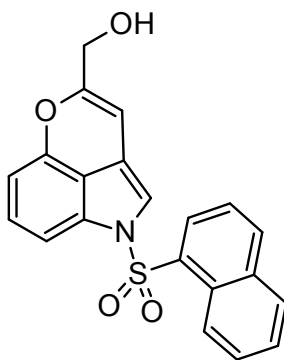
¹H NMR (500 MHz, DMSO-d₆) δ 7.97 - 7.88 (m, 2H), 7.74 - 7.65 (m, 1H), 7.64 - 7.55 (m, 2H), 7.32 (d, *J* = 8.2 Hz, 1H), 7.22 (t, *J* = 8.1 Hz, 1H), 7.06 (s, 1H), 6.66 (d, *J* = 7.7 Hz, 1H), 6.06 - 6.01 (m, 1H), 5.39 (t, *J* = 6.0 Hz, 1H), 4.04 (dd, *J* = 6.0, 1.1 Hz, 2H), 3.33 (s, 1H).

¹³C NMR (126 MHz, DMSO-d₆) δ 60.18, 95.71, 105.97, 107.89, 112.06, 114.51, 121.18, 126.41, 128.47, 129.79, 134.49, 134.53, 136.76, 148.51, 157.79.





[1-(Naphthalene-1-sulfonyl)-pyrano[2,3,4-*cd*]indol-4-yl]methanol (7b)



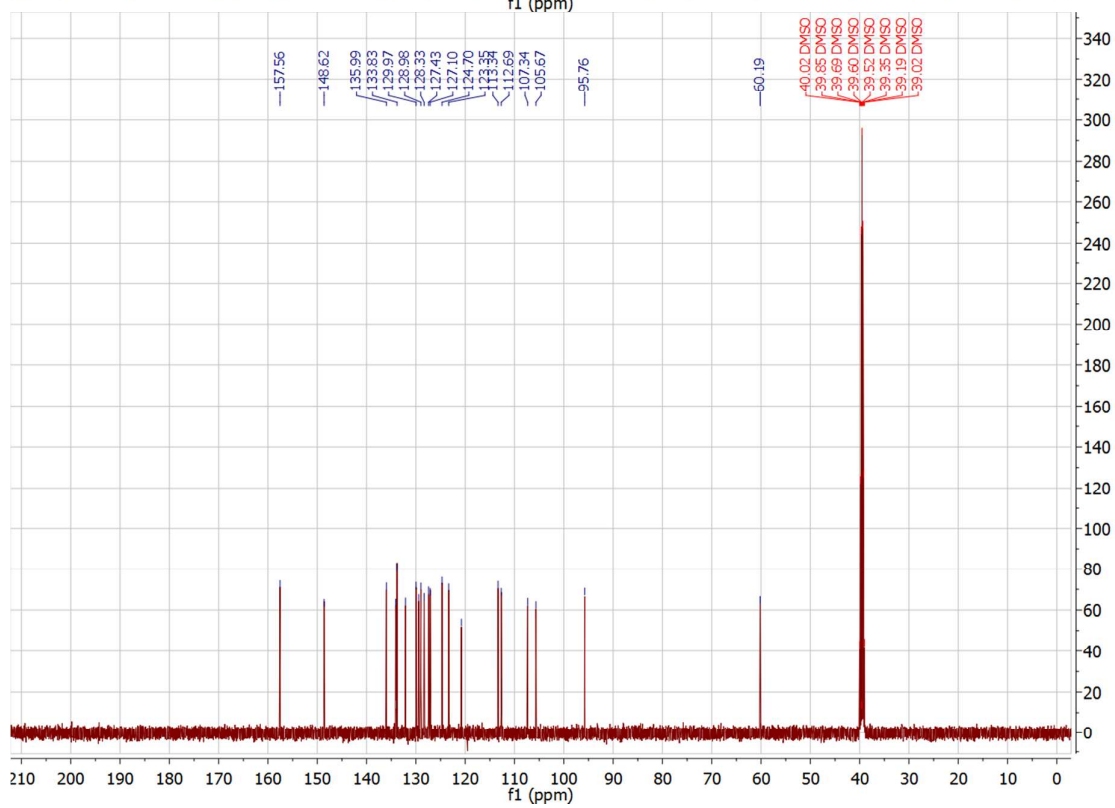
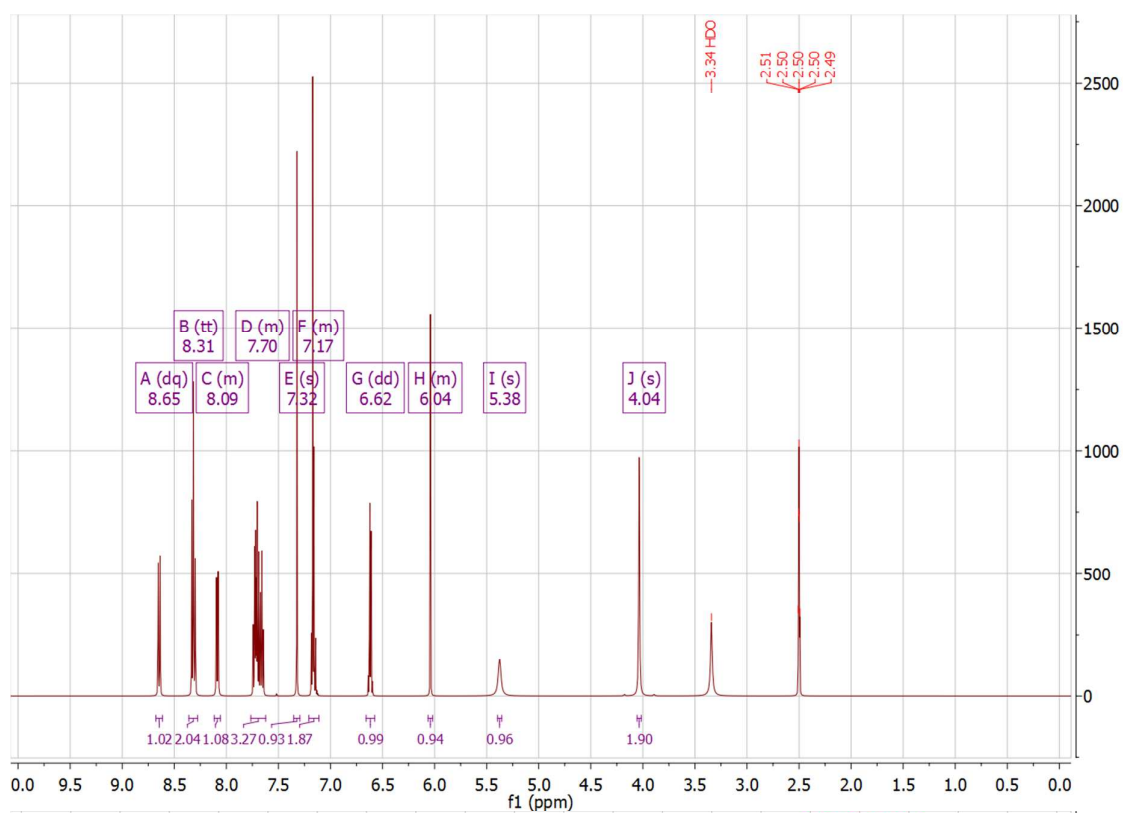
4b (0.1 g, 0.26 mmol), methanol (5 ml), NaBH₄ (2 eq., 20 mg). A light tan solid was obtained (0.085 g, 85%).

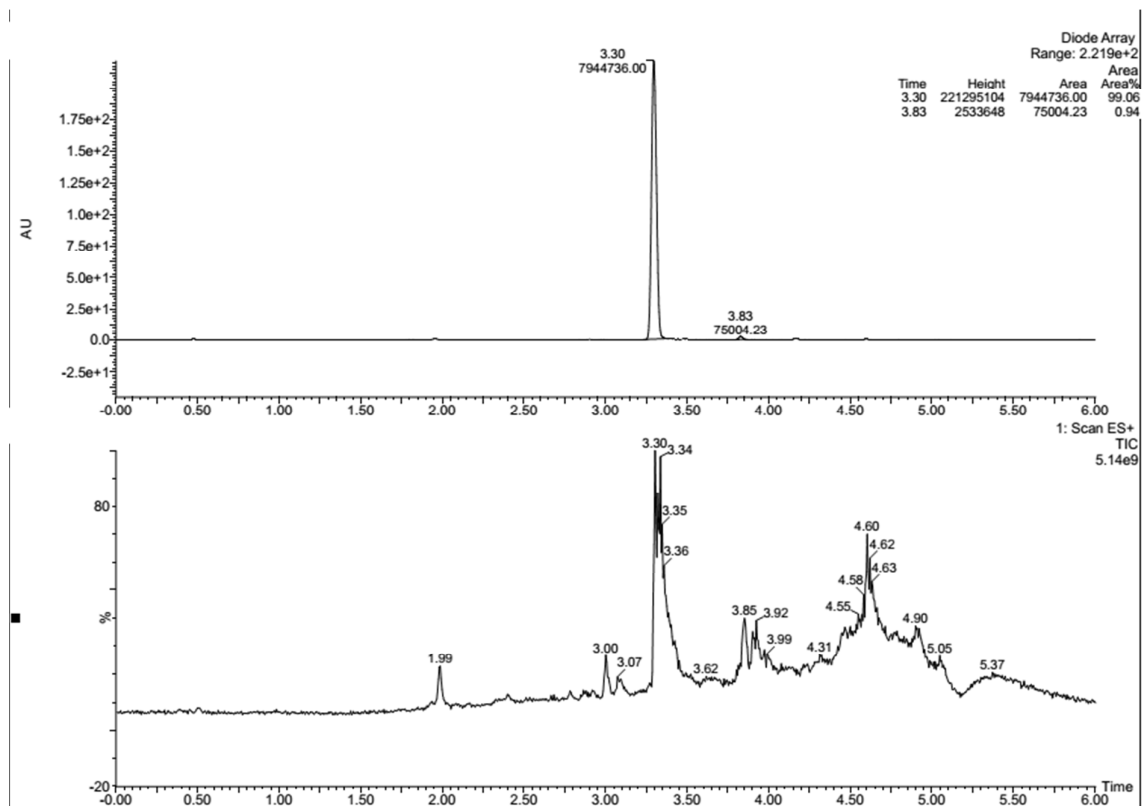
LCMS [M+1] = 377.86 m/z (377.41 calc.).

HRMS [M-1] = 376.0679 (376.0649 calc.)

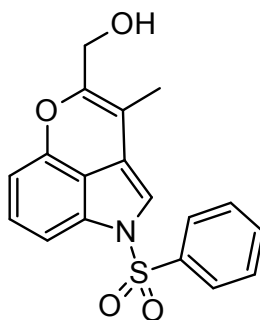
¹H NMR (500 MHz, DMSO-*d*₆) δ 8.65 (dq, *J* = 8.7, 0.9 Hz, 1H), 8.31 (tt, *J* = 8.5, 1.1 Hz, 2H), 8.12 - 8.06 (m, 1H), 7.78 - 7.61 (m, 3H), 7.32 (s, 1H), 7.22 - 7.11 (m, 2H), 6.67 - 6.56 (m, 1H), 6.04 (d, *J* = 1.1 Hz, 1H), 5.38 (s, 1H), 4.04 (s, 2H).

¹³C NMR (126 MHz, DMSO-*d*₆) δ 60.19, 95.76, 105.67, 107.34, 112.69, 113.34, 120.78, 123.35, 124.70, 127.10, 127.43, 128.33, 128.98, 129.43, 129.97, 132.12, 133.83, 134.11, 135.99, 148.62, 157.56.





[1-(Benzenesulfonyl)-3-methyl-pyrano[2,3,4-*cd*]indol-4-yl]methanol (7c)



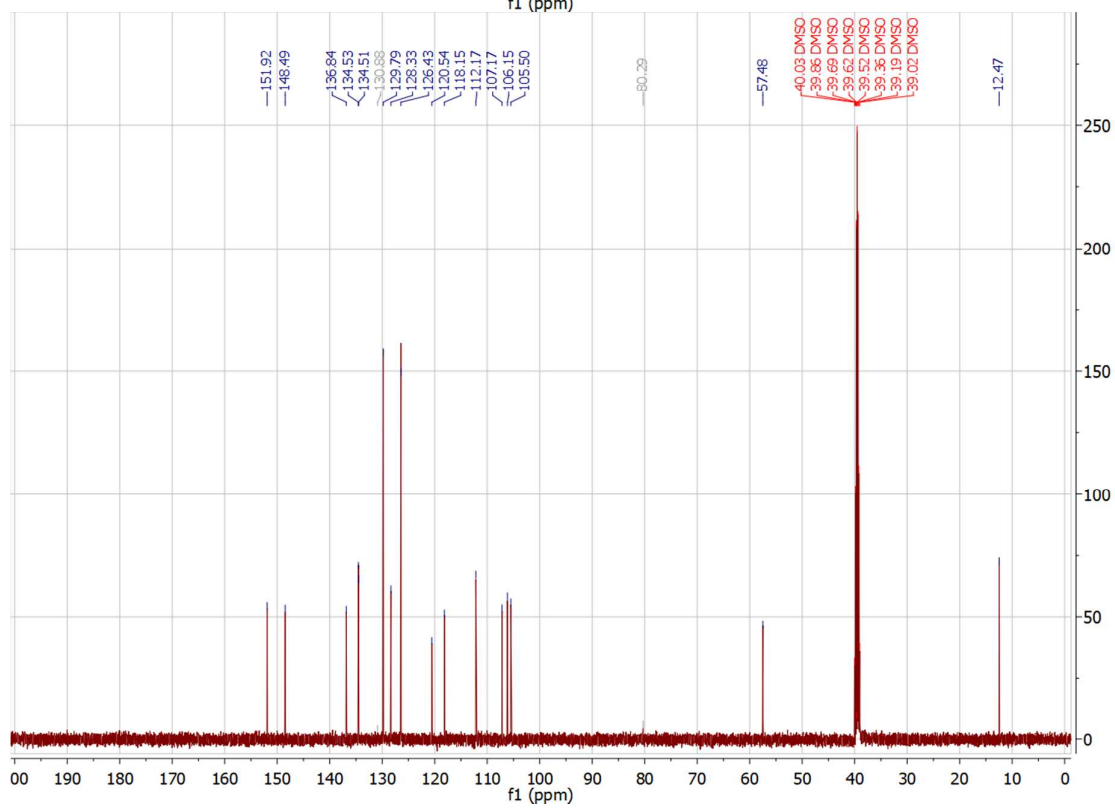
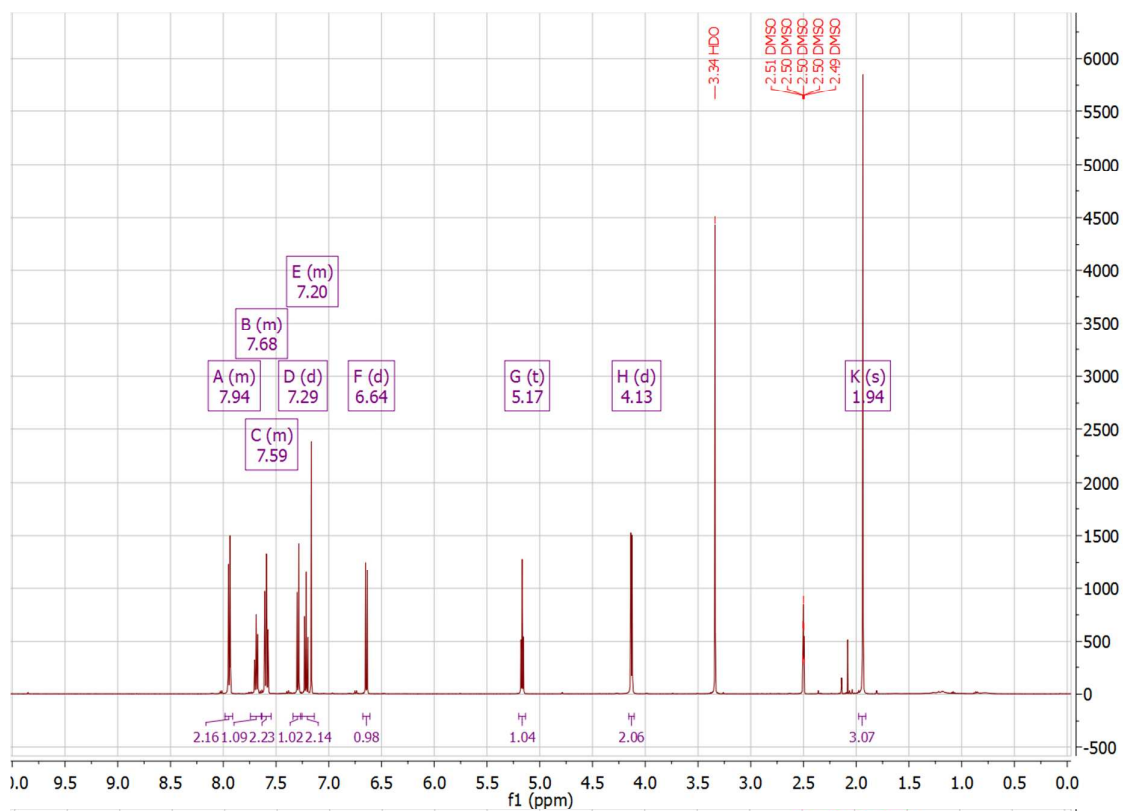
4c (0.050 g, 0.15 mmol), methanol (2 ml), NaBH₄ (2 eq., 11 mg). A light tan solid was obtained (0.022 g, 43%).

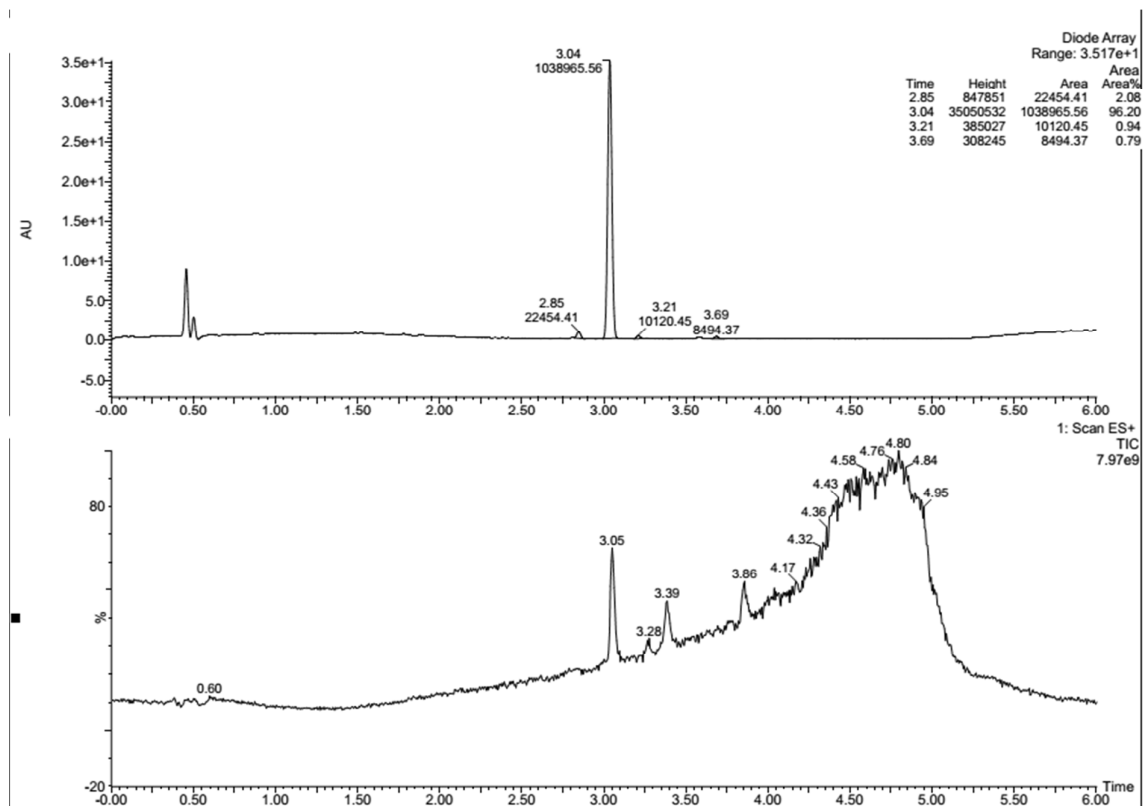
LCMS [M+1] = 342.20 m/z (341.38 calc.).

HRMS [M+1] = 342.0779 (342.0800 calc.)

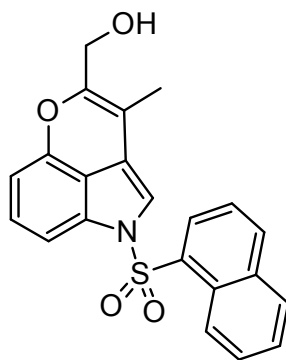
¹H NMR (500 MHz, DMSO-*d*₆) δ 7.98 – 7.91 (m, 2H), 7.74 – 7.63 (m, 1H), 7.64 – 7.55 (m, 2H), 7.29 (d, *J* = 8.1 Hz, 1H), 7.27 – 7.14 (m, 2H), 6.64 (d, *J* = 7.8 Hz, 1H), 5.17 (t, *J* = 5.9 Hz, 1H), 4.13 (d, *J* = 5.9 Hz, 2H), 1.94 (s, 3H).

¹³C NMR (126 MHz, DMSO-*d*₆) δ 12.47, 57.48, 105.50, 106.15, 107.17, 112.17, 118.15, 120.54, 126.43, 128.33, 129.79, 134.51, 134.53, 136.84, 148.49, 151.92.





[1-(Naphthalene-1-sulfonyl)-3-methyl-pyrano[2,3,4-*cd*]indol-4-yl]methanol (7d)



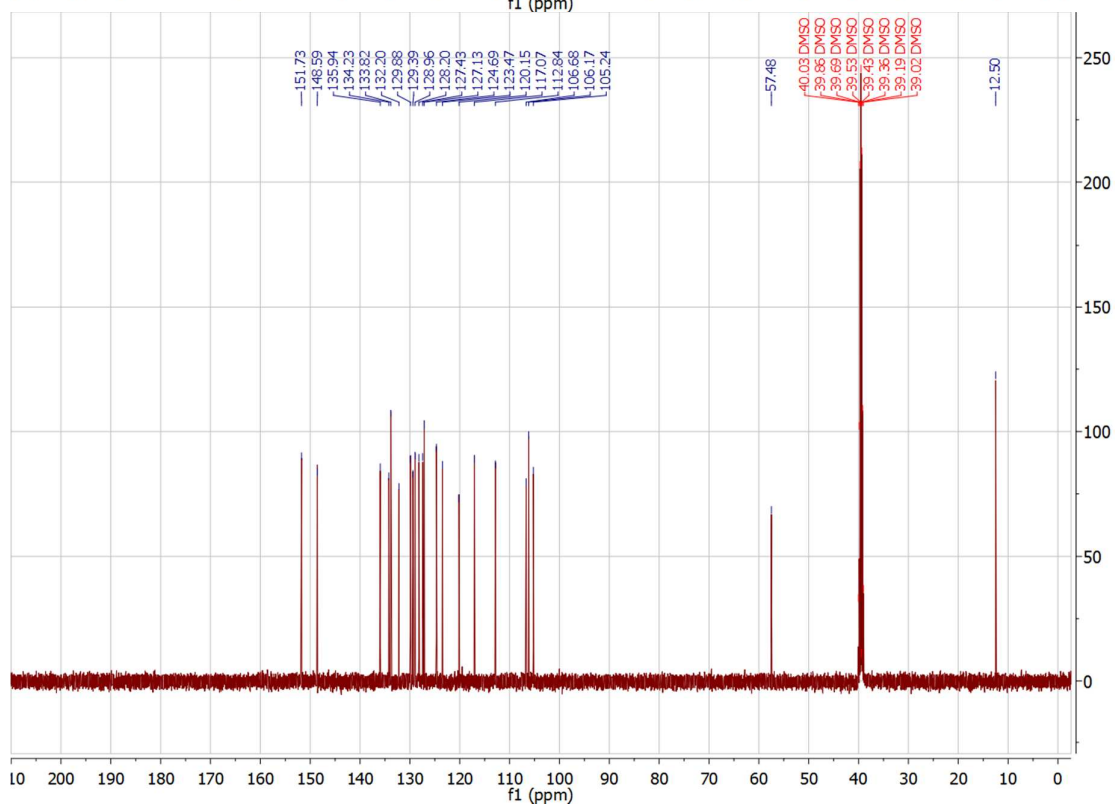
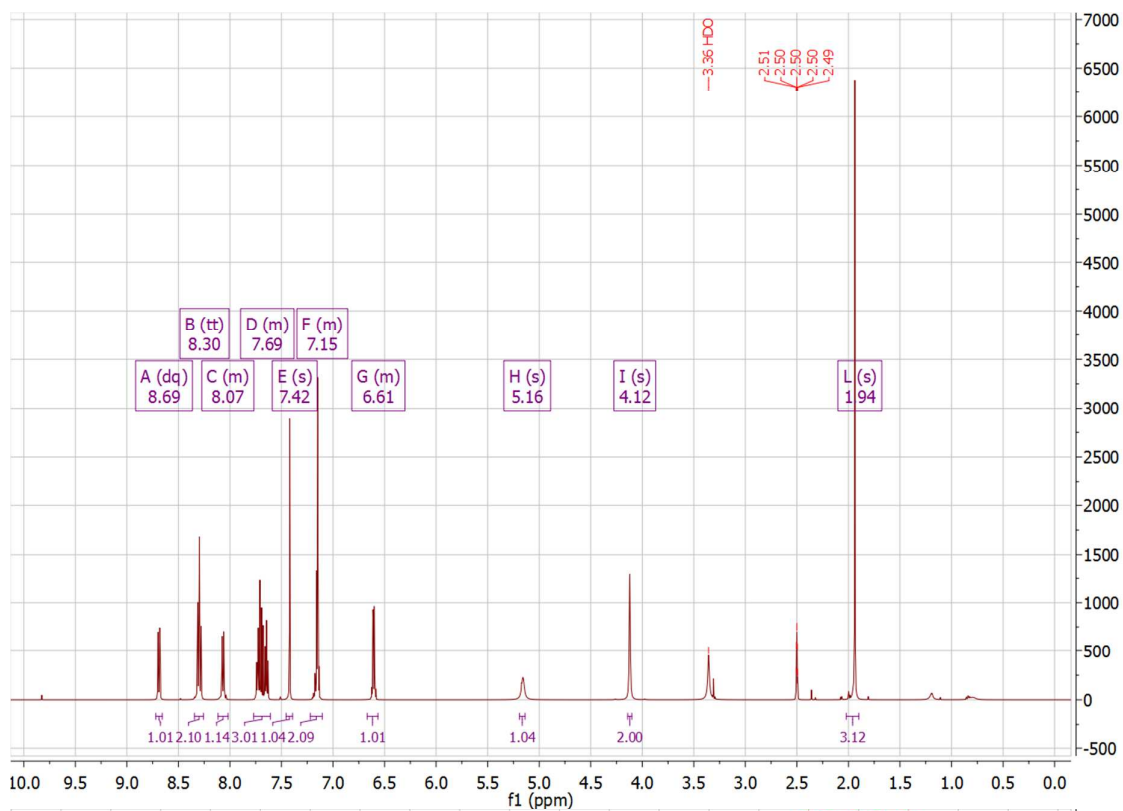
4d (0.1 g, 0.26 mmol), methanol (5 ml), NaBH₄ (2 eq., 20 mg). A light tan solid was obtained (0.038 g, 37%).

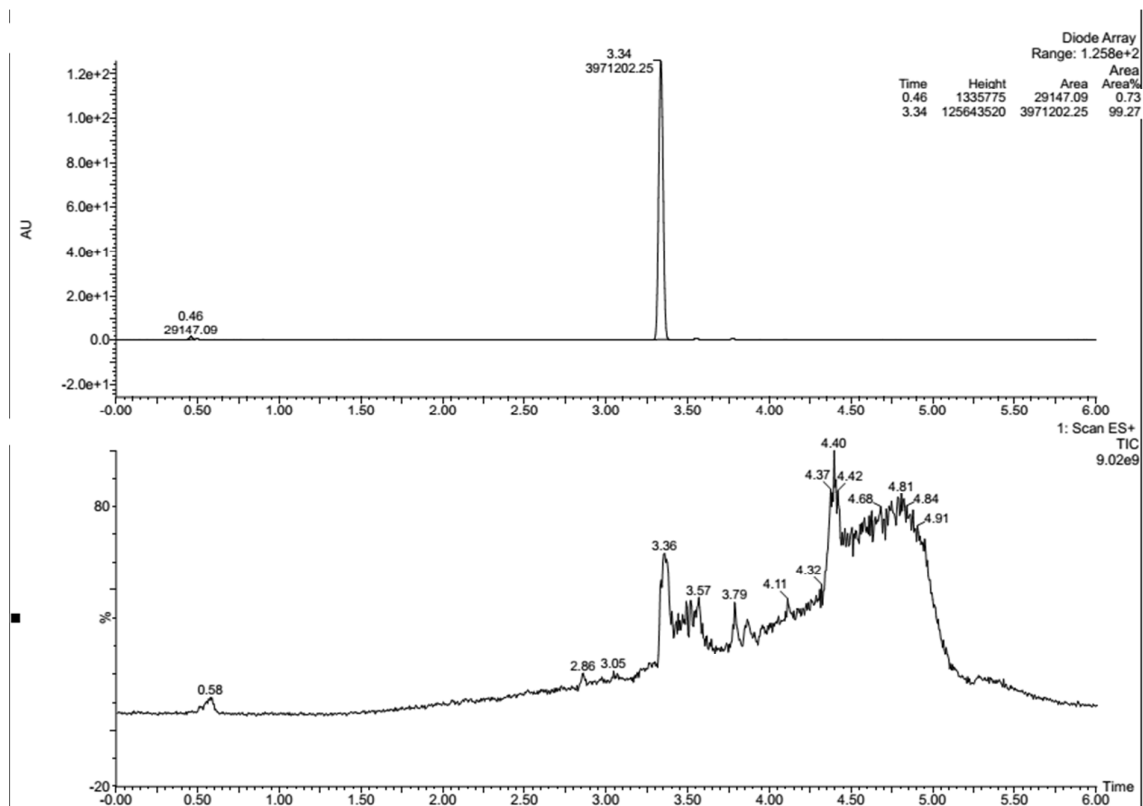
LCMS [M+1] = 392.00 m/z (391.44 calc.).

HRMS [M-1] = 390.0834 (390.0805 calc.)

¹H NMR (500 MHz, Common NMR Solvents) δ 8.69 (dq, *J* = 8.7, 0.9 Hz, 1H), 8.30 (tt, *J* = 8.4, 1.1 Hz, 2H), 8.12 – 8.02 (m, 1H), 7.77 – 7.61 (m, 3H), 7.42 (s, 1H), 7.22 – 7.10 (m, 2H), 6.67 – 6.56 (m, 1H), 5.16 (s, 1H), 4.12 (s, 2H), 1.94 (s, 3H).

¹³C NMR (126 MHz, DMSO-*d*₆) δ 12.50, 57.48, 105.24, 106.17, 106.68, 112.84, 117.07, 120.15, 123.47, 124.69, 127.13, 127.43, 128.20, 128.96, 129.39, 129.88, 132.20, 133.82, 134.23, 135.94, 148.59, 151.73.





Fluorescence spectra

Fluorescence spectra of **3a** were recorded using spectrofluorimeter Max-P (Horiba Jobin Yvon, Kyoto, Japan) in methanol diluted to $A_{300\text{ nm}} = 0,1\text{ cm}^{-1}$ in a quartz 1 cm cuvette at room temperature.

GENERAL PARAMETERS:

Number of Data Points: 326

Integration Time: 0.100000s

EX1: Excitation 1 (Mono3)

Park: 300.00nm or 360.00 nm

Front Entrance Slit: 2.00 nmBandpass

Front Exit Slit: 2.00 nmBandpass

EM1: Emission 1 (Mono4)

Start: 250.00nm

End: 900.00nm

Increment: 2.00nm

Front Entrance Slit: 2.00 nmBandpass

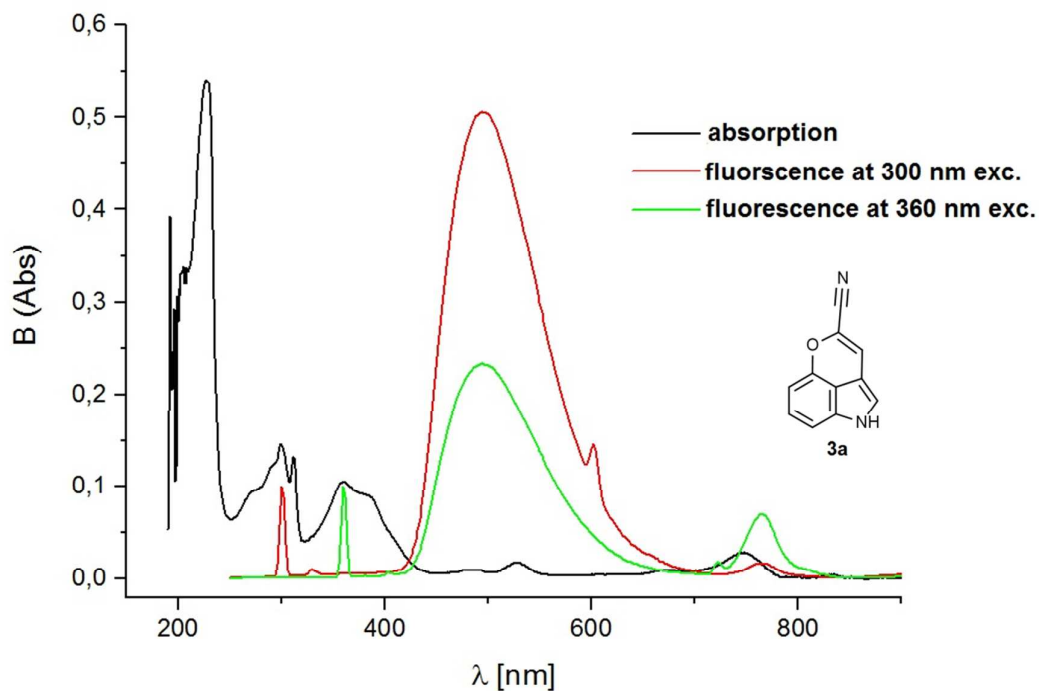
Front Exit Slit: 2.00 nmBandpass

Grating: Density 1200 (Blaze: 500)

Detector Accumulations: 1

Accumulations Mode: None

Detector Cycles: 1



Molecular modeling

The homology model of the 5-HT₆R was generated with Schrödinger Prime based on the 5-HT_{1B}R template (PDB code 4IAR¹²) and the sequence alignment retrieved from GPCRdb.org [ref]. As the crystal template was in the agonist-bound conformation, a 250 ns MD simulation was conducted with a ligand ChEMBL1642416 (an antagonist with nanomolar affinity towards 5-HT₆R). to obtain an inactive (antagonist-bound) form of the target. MD was run with Desmond program, with NPT ensemble at 310K. Explicit POPC membrane was used along with TIP3P water model. A set of 250 frames was extracted from the trajectory and the best 5-HT₆R conformation was extracted based on docking of the set of 42 known basic and non-basic ligands. The receptor model with the most consistent binding mode and the best average Glide score was selected for the docking experiments performed on the tricyclic compounds introduced in this paper

ABBREVIATIONS

5-HT, 5-hydroxytryptamine; 5-HT₆R, 5-hydroxytryptamine receptor 6; DMSO, dimethyl sulfoxide; EDTA, ethylenediaminetetraacetic acid; tris(hydroxymethyl) aminomethane; 8-OH-DPAT, 8-Hydroxy-2-(dipropylamino)tetralin hydrobromide; LSD, lysergic acid diethylamide; PBS, phosphate-buffered saline; HBSS, Hank's Buffered Salt Solution; HEPES, (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid); IBMX, (3-isobutyl-1-methylxanthine); BSA, Bovine Serum Albumin; cAMP, Cyclic adenosine monophosphate; 5-CT, 5-Carboxamidotryptamine; NMR, nuclear magnetic resonance; TQD, triple quadrupole; LC, liquid chromatography; MS, mass spectrometry; UPLC, ultra performance liquid chromatography; ACN, acetonitrile; HRMS, high resolution mass spectrometry; THF, tetrahydrofuran; DMF, dimethylformamide; NCS, N-chlorosuccimide; PPh₃, triphenylphosphine; DIBAL-H, diisobutylaluminium hydride; PDB, protein data bank; MD, molecular dynamics.

References

- (1) Singer, J. M.; Wilson, M. W.; Johnson, P. D.; Graham, S. R.; Cooke, L. W.; Roof, R. L.; Boxer, P. A.; Gold, L. H.; Meltzer, L. T.; Janssen, A.; Roush, N.; Campbell, J. E.; Su, T.-Z.; Hurst, S. I.; Stoner, C. L.; Schwarz, J. B. Synthesis and SAR of tolylamine 5-HT₆ antagonists *Bioorg. Med. Chem. Lett.* **2009**, *19* (9), 2409–2412.
- (2) Bernotas, R.; Lenicek, S.; Antane, S.; Zhang, G. M.; Smith, D.; Coupet, J.; Harrison, B.; Schechter, L. E. 1-(2-Aminoethyl)-3-(arylsulfonyl)-1H-indoles as novel 5-HT₆ receptor ligands. *Bioorg. Med. Chem. Lett.* **2004**, *14* (22), 5499–5502.
- (3) Fukui, Y.; Adachi, M.; Sasatani, T. Tricyclic indole compounds having affinity for serotonin receptor. WO2002024641, 2002.
- (4) Fu, J.-M. 5-Arylsulfonyl indoles useful for treating disease. US2003060498, 2003.
- (5) Haupt, A.; Pohlki, F.; Drescher, K.; Wicke, K. N-phenyl-(piperazinyl or homopiperazinyl)-benzenesulfonamide compounds for treating disorders. WO2010125134, 2010.
- (6) Jacobsen, E. J.; King, S. J. Bis-arylsulfones. US2002115660, 2002.
- (7) Bonhaus, D. W.; Martin, R. S. Compositions and methods for treating cognitive disorders. US2006069094, 2006.
- (8) Owens, T.; Sethofer, S.; Walker, K. A.; Zhao, S. H. Benzimidazolone and dihydroindolone derivatives and uses thereof. US2007015744, 2007.
- (9) Berger, J.; Caroon, J. M.; Javier Lopez-Tapia, F.; Lowrie JR, L. E.; Nitzan, D.; Zhao, S.-H. Arylsulfonyl chromans as 5-HT₆ inhibitors. US2007099908, 2007.
- (10) Liu, K.; Lo, J. R.; Robichaud, A. J.; Elokda, H. M. 1-sulfonylindazolylamine and -amide derivatives as 5-hydroxytryptamine-6 ligands. US2007281922, 2007.
- (11) Cheng, Y.-C.; Prusoff, W. H. Relationship between the inhibition constant (K_i) and the concentration of inhibitor which causes 50 per cent inhibition (I₅₀) of an enzymatic reaction *Biochem. Pharmacol.* **1973**, *22* (23), 3099–3108.
- (12) Wang, C.; Jiang, Y.; Ma, J.; Wu, H.; Wacker, D.; Katritch, V.; Han, G. W.; Liu, W.; Huang, X.-P.; Vardy, E.; McCorvy, J. D.; Gao, X.; Zhou, E. X.; Melcher, K.; Zhang, C.; Bai, F.; Yang, H.; Yang, L.; Jiang, H.; Roth, B. L.; Cherezov, V.; Stevens, R. C.; Xu, H. E. Structural Basis for Molecular Recognition at Serotonin Receptors *Science*. **2013**, *340* (6132), 610–614.