Small molecule inhibitors of Gli transcriptional factors of the Hedgehog Signalling Pathway

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Electronic Supporting Information

Contents

Biology

Dual Luciferase Reporter Assay results for Tryptophan derivatives (Table 1)

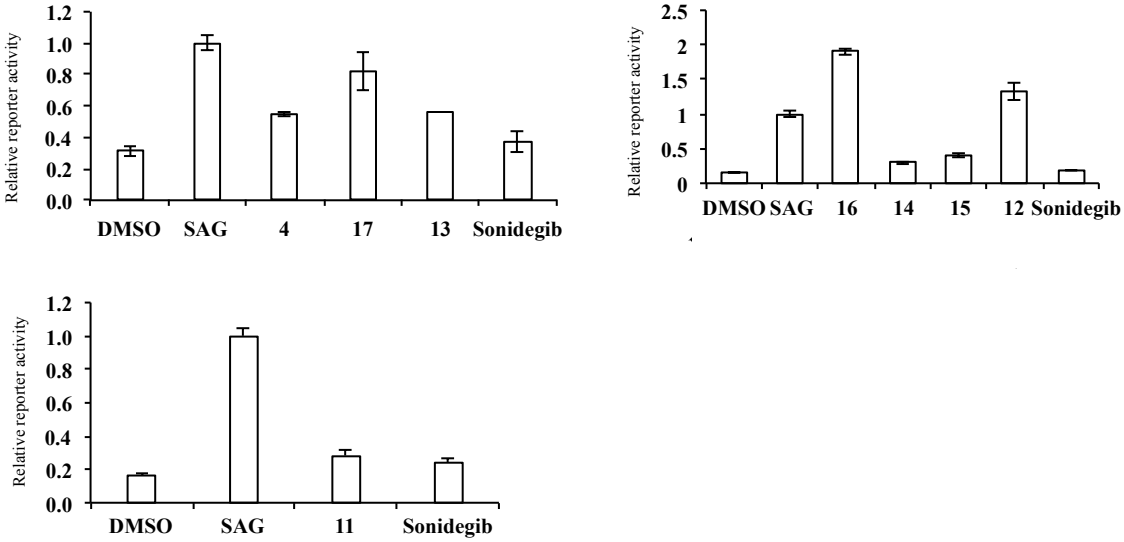


Figure S1. The relative total Gli protein expression in Shh LIGHT 2 cells after treatment with 100 nM SAG and subsequent treatment with 10 μM **4**, **11-17**. Sonidegib at 100 nM was used as the positive control. * $P < 0.05$, ** $P < 0.001$, *** $P < 0.0001$ compared to SAG treatment. All experiments were performed in triplicate.

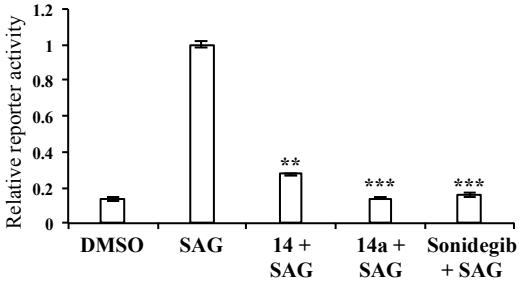


Figure S2. The suppression of Gli protein expression in 100nM SAG-activated Shh LIGHT2 cells by L- and D-Tryptophan analogues **14** and **14a** at 10 μM, respectively. Sonidegib (100 nM) was used as the positive control. All experiments were performed in triplicate. **$P < 0.001$, ***$P < 0.0001$ compared to SAG treatment.

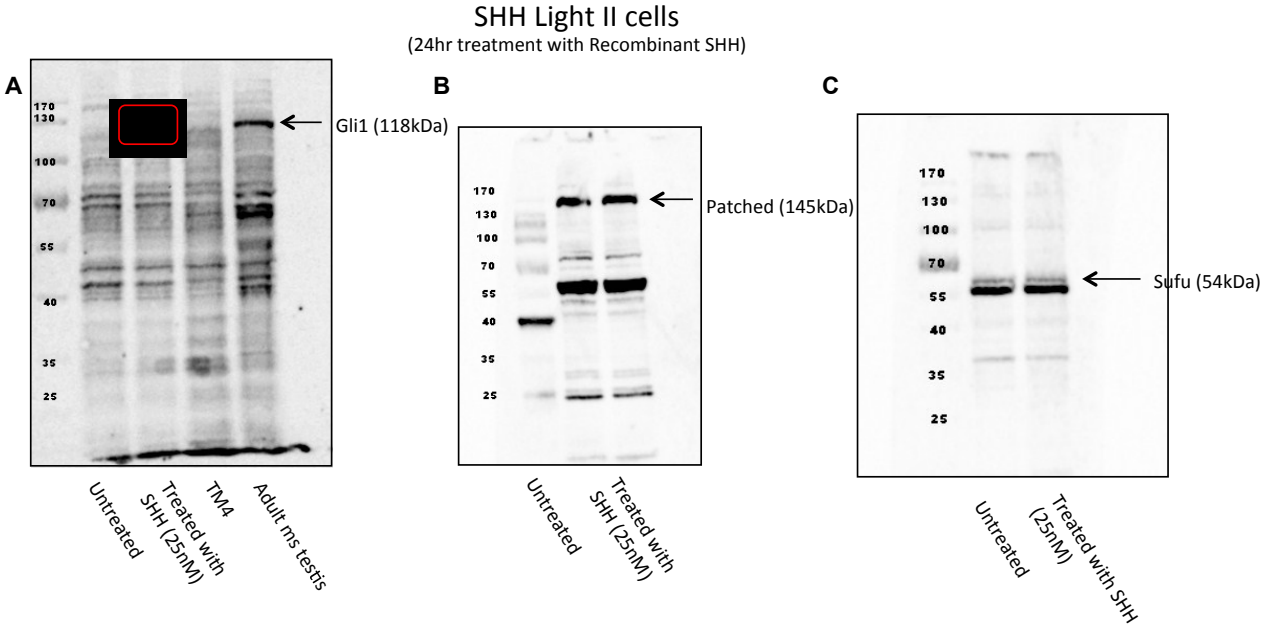


Figure S3. Western blotting analysis identifying the presence of Gli1 in adult mouse testes, but its absence on treatment (red box); (B) presence of Ptch1 (treated and untreated); and (C) presence of Sufu (treated and untreated).

Reverse phase and chiral separation of L− and D−Tryptophan derivatives

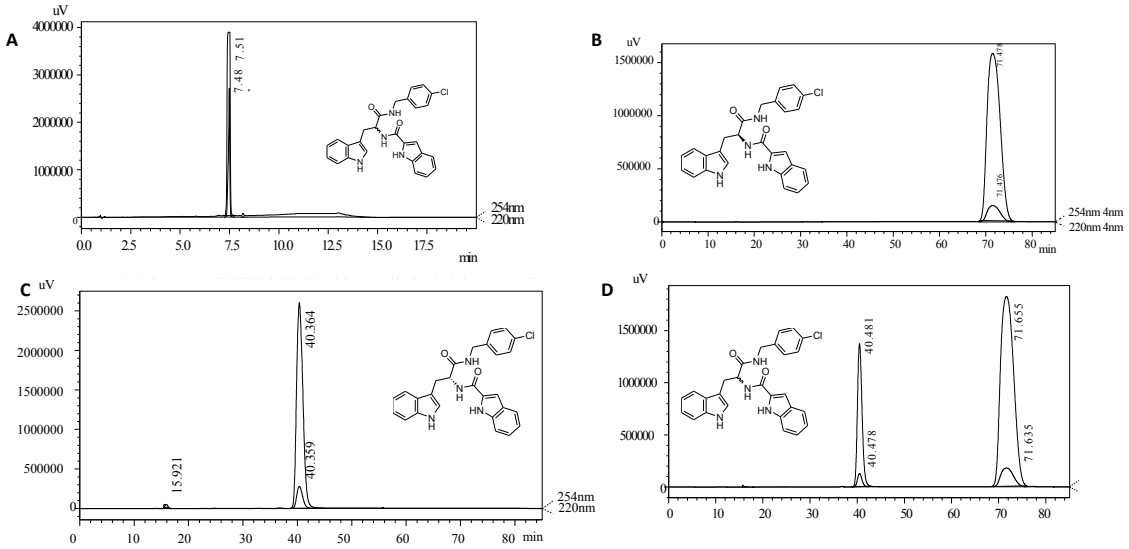


Figure S4. The HPLC chromatograms (A) reverse phase HPLC analysis of a mixture of **14** and **14a**; (B) chiral column HPLC analysis of **14** prepared from L-Tryptophan; (C) chiral column HPLC analysis of **14a** prepared from D-Tryptophan; (D) chiral column HPLC analysis of a mixture of **14** and **14a**.

Amide rotamer of benzo[1,3]dioxol-5-ylmethyl-[2-(1H -indol-3-yl)-ethyl]-amine derivatives

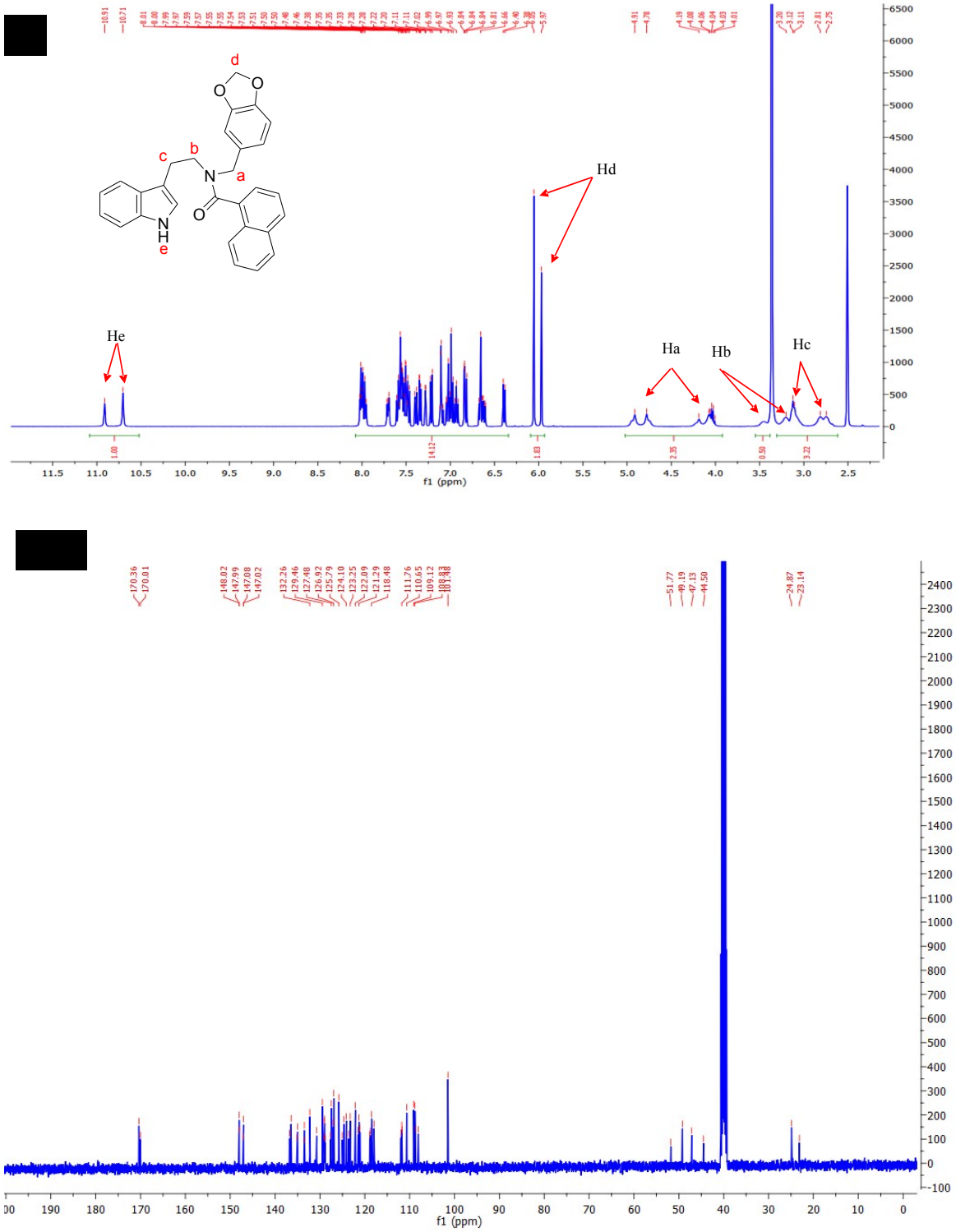


Figure S5. A) ^{1}H NMR (**A**) and (**B**) ^{13}C NMR spectra of analogue **5** highlighting the presence of duplicative peaks.

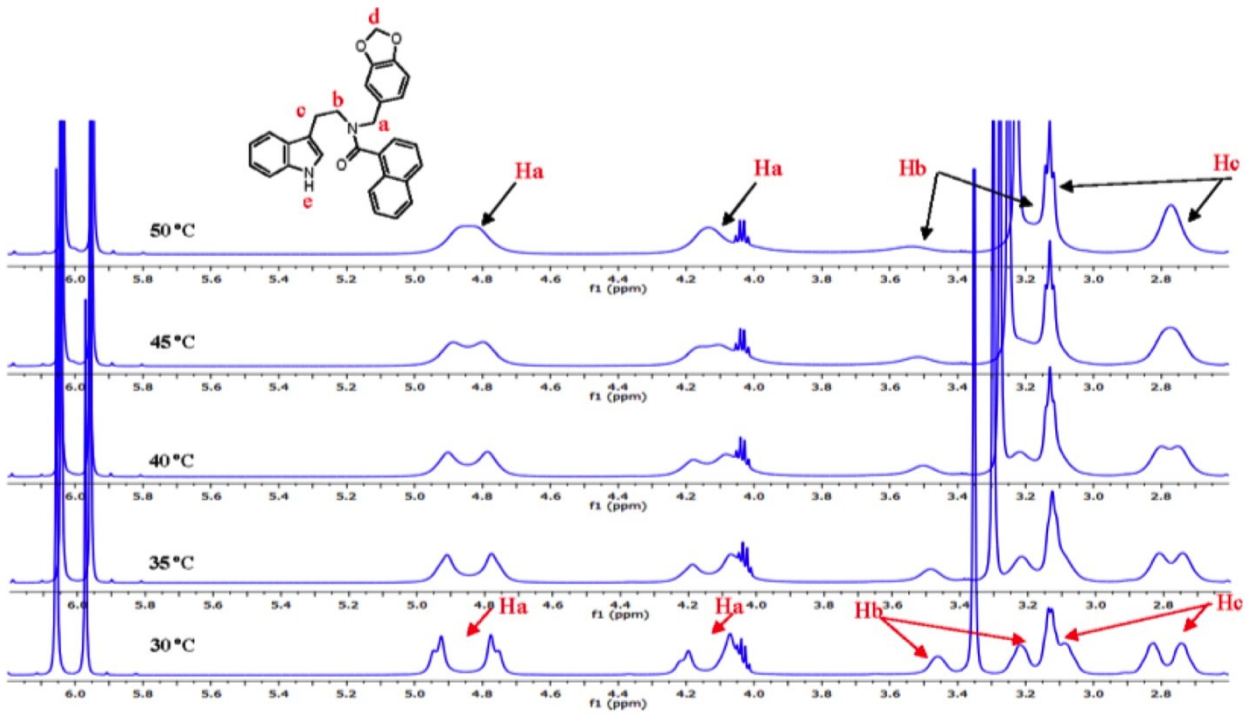


Figure S6. Variable-temperature NMR experiment with analogue **5** at 30, 35, 40, 45, and 50 ºC. Arrows indicated the splitting (red arrows) merging into individual peaks (black arrows) when increasing the temperatures from 30 to 50 ºC. Displayed is the aliphatic region of the ^{1}H NMR in DMSO-d_6.

1D selective NOSEY NMR Experiment

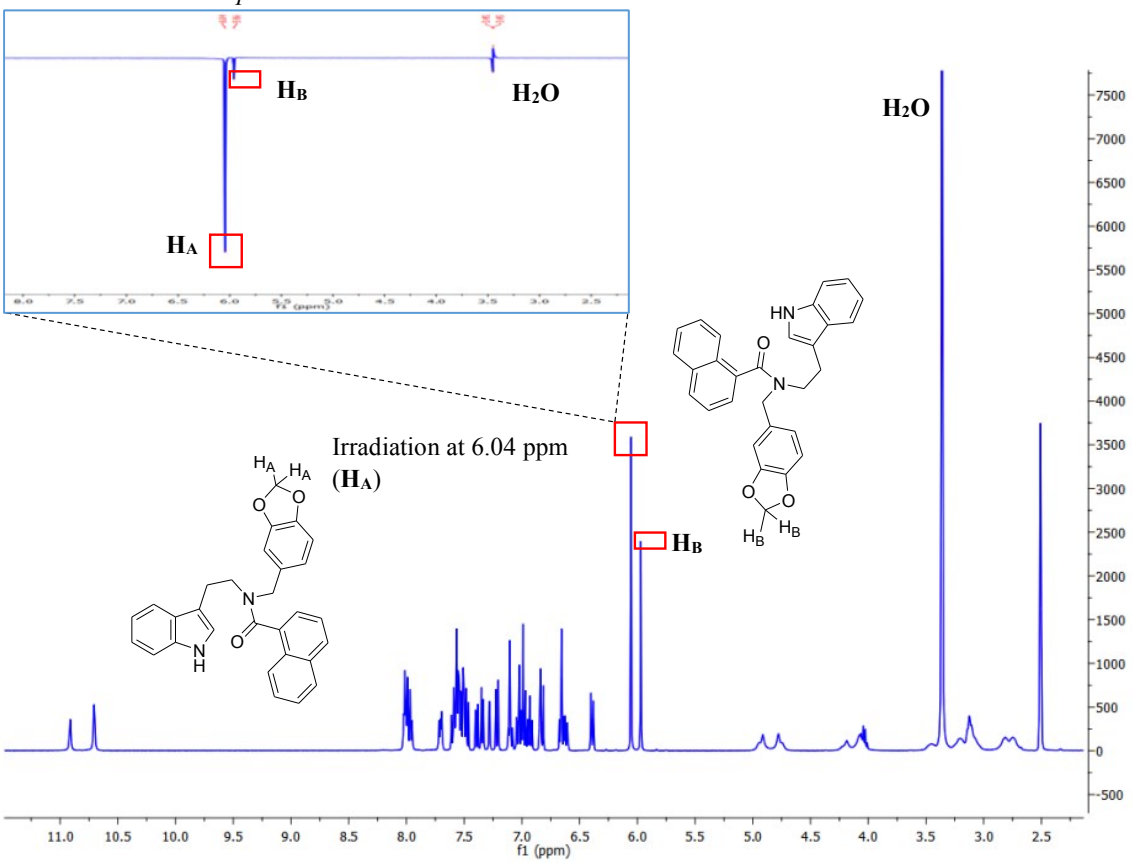


Figure S7. 1D selective NOSEY and ^{1}H NMR spectra of analogue **5**. The irradiation at the peak at 6.04 ppm results in two peaks at the same phase at 6.04 and 5.96 ppm which correspond to H_A and H_B, respectively. Other peaks are not visible.

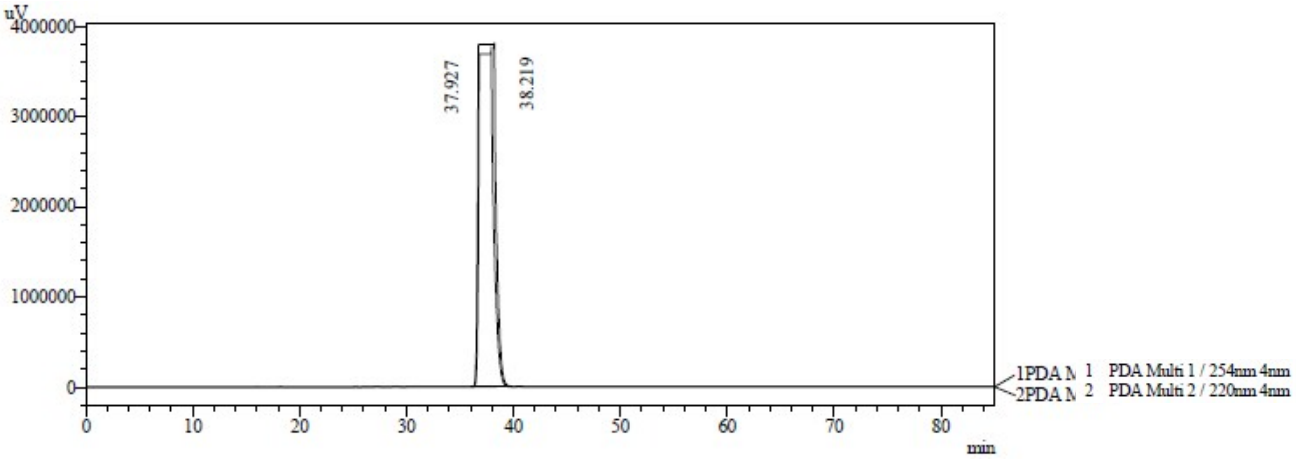


Figure S8. Chiral HPLC analysis of amide rotamer analogue **5**.

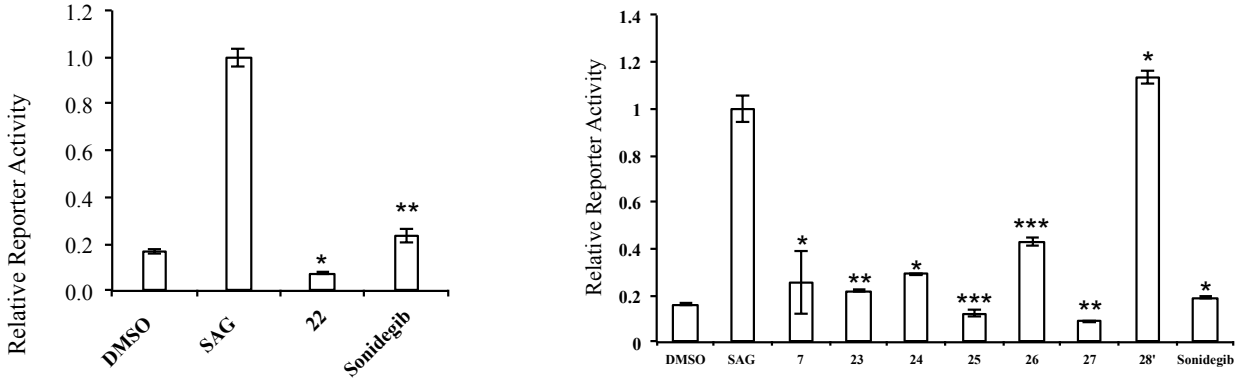


Figure S9. The relative total Gli protein expression in Shh LIGHT 2 cells after treatment with 100 nM SAG and subsequent treatment with 10 μM **7 (A)** and **23-28 (B)**. Sonidegib at 100 nM was used as the positive control. * $P < .05$, ** $P < .001$, *** $P < .0001$ compared to SAG treatment. All treatments were performed in triplicate.

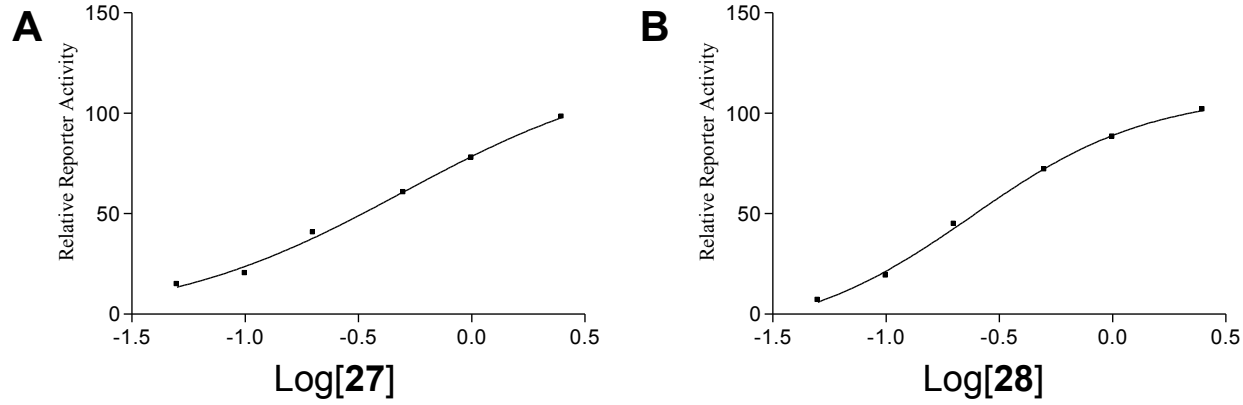


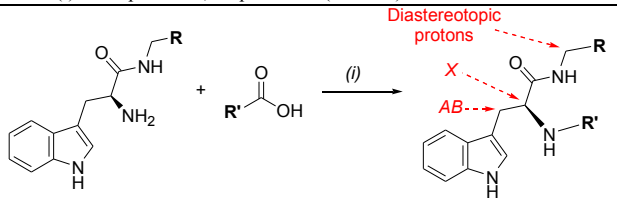
Figure S9. IC_{50} curves for analogues **27** and **28**.

Compound Characterization

Chemistry

*Synthesis of L-tryptophan derivatives (**4, 11**−**17**)*

Table S1. Isolated yields, ^{1}H NMR featuring the ABX systems and the diastereotopic protons, and base cations in HRMS $[M+H]^{+}$ of L-Tryptophan derivatives (**4**, **11**–**17**). *Reagents and conditions:* (*i*) 1.5 eq. HATU, 3eq. DIPEA (Table 1)



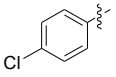
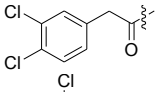
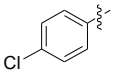
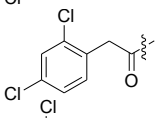
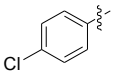
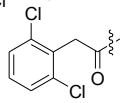
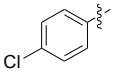
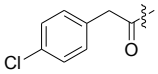
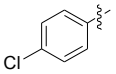
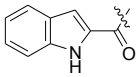
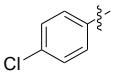
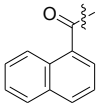
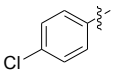
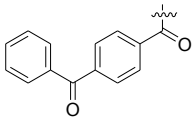
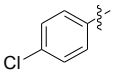
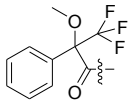
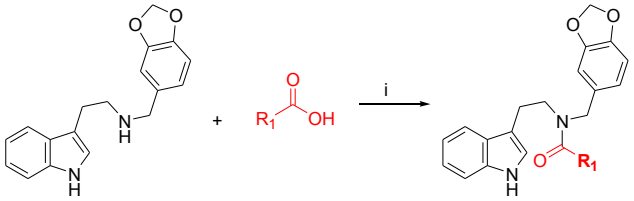
Compounds	R	R'	Yield (%)	^{1}H NMR AB protons (ppm)	Proton-X (ppm)	Diastereotopic protons (ppm)	HRMS $[M+H]^{+}$ (m/z)
4			18	3.05 (ddd*, J_{AX} = 22.8, J_{BX}=14.4, J_{AB}=7.2 Hz, 2H)	4.59 (dd, J = 14.4, 8.1 Hz, 1H)	4.24 (qd, J = 15.6, 5.9 Hz, 2H)	514.0850
11			13	3.07 (ddd, J_{AX} = 22.9, J_{BX}= 14.4, J_{AB}= 7.2 Hz, 2H)	4.63 (dd, J = 14.3, 8.2 Hz, 1H)	4.25 (qd, J = 15.5, 5.9 Hz, 2H)	514.0850
12			22	3.07 (ddd, J_{AX} = 22.3, J_{BX}=14.4, J_{AB}=7.1 Hz, 2H)	4.60 (dd, J = 14.4, 7.9 Hz, 1H)	4.24 (qd, J = 15.5, 5.9 Hz, 2H)	514.0850
13			18	3.05 (ddd, J_{AX} = 22.8, J_{BX} = 14.4, J_{AB} = 7.3 Hz, 2H)	4.59 (dd, J = 14.4, 8.2 Hz, 1H)	4.23 (qd, J = 15.5, 5.9 Hz, 2H)	480.1240
14			28	3.24 (ddd, J_{AX} = 28.6, J_{BX} = 14.6, J_{AB} = 5.2 Hz, 2H)	4.80 (dd, J = 9.2, 8.4, 5.2 Hz, 1H)	4.31 (qd, J = 16.0, 6.0 Hz, 2H)	471.1582
15			23	3.23 (ddd, J_{AX} = 36.4, J_{BX}= 14.4, J_{AB} = 5.2 Hz, 2H)	4.93 (dd, J = 9.3, 5.2 Hz, 1H)	4.37 (qd, J = 16.0, 6.0 Hz, 2H)	482.1630
16			25	3.25 (ddd, J_{AX} = 45, J_{BX} = 14.9, J_{AB} = 4.8 Hz, 2H)	4.81 (dd, J = 9.3, 4.8 Hz, 1H)	4.37 – 4.23 (m, 2H) overlapping	536.1735
17			39	3.22 – 3.07 (m, 2H) overlapping	4.81 (dd, J = 8.6, 6.0 Hz, 1H)	4.32 (qd, J = 15.4, 5.8 Hz, 2H)	544.1610

Table S2. Isolated yields, and base cations in HRMS [M+H]+ of benzo[1,3]dioxol-5-ylmethyl-[2-(1*H*-indol-3-yl)-ethyl]-amine derivatives **5**, **20**-**29**.



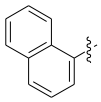
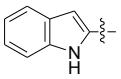
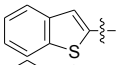
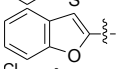
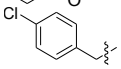
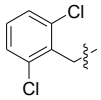
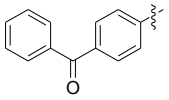
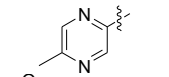
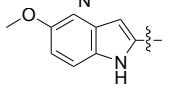
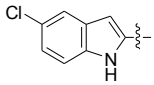
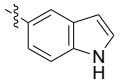
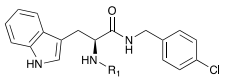
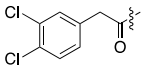
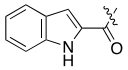
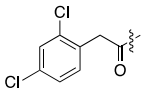
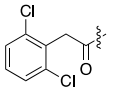
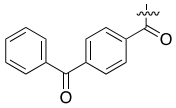
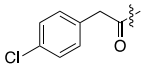
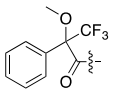
Compounds	R	Isolated yields (%)	HRMS (ES^+) m/z $[M+H]^+$
5		67	449.1859
20		67	439.1811
21		53	455.1423
22		63	439.1652
23		58	447.1647
24		56	447.1467
25		40	503.1964
26		22	415.1761
27		62	468.1919
28		64	472.1422
29		67	438.1812

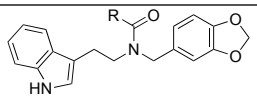
Table S3. Percentage inhibition of Gli protein expression in 100 nM SAG-activated Shh LIGHT2 cells by L-Tryptophan analogues **4** and **11**-**17** at 10 μM compound concentration and cLogP values.



R_1	Inhibition (%)[a]	cLogP	R_1	Inhibition (%)[a]	cLogP
 **4**	66	4.84	 **14** [**14a**; D-isomer]	86 [99]	2.72
 **11**	83	4.84	 **15**	71	4.77
 **12**	-38	4.84	 **16**	-105	4.99
 **13**	64	4.28	 **17**	26	4.46

[a] Inhibition calculated as Gli signal (SAG) – Gli Signal (SAG+Compound).

Table S4. Percentage inhibition of Gli expression in 100 nM SAG-activated Shh LIGHT2 cells by benzo[1,3]dioxol-5-ylmethyl-[2-(1*H*-indol-3-yl)-ethyl]-amine derivatives analogues **5** and **20**-**26** at 10 μM compound concentration and cLogP values.



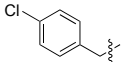
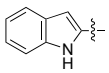
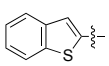
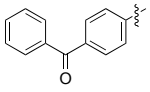
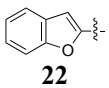
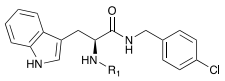
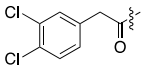
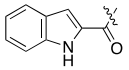
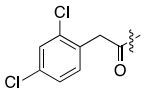
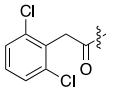
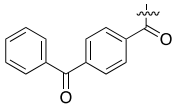
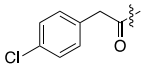
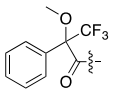
R_1	Inhibition (%)[a]	cLogP	R_1	Inhibition (%)[a]	cLogP
 **5**	89	5.23	 **23**	102	4.73
 **20**	111	3.18	 **24**	67	5.29
 **21**	92	4.12	 **25**	108	5.44
 **22**	84	3.55	 **26**	-16	2.68

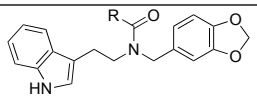
Table S3. Percentage inhibition of Gli protein expression in 100 nM SAG-activated Shh LIGHT2 cells by L-Tryptophan analogues **4** and **11**-**17** at 10 μM compound concentration.



R_1	Inhibition (%)[a]	cLogP	R_1	Inhibition (%)[a]	cLogP
 **4**	66	4.84	 **14** [**14a**; D-isomer]	86 [99]	2.72
 **11**	83	4.84	 **15**	71	4.77
 **12**	-38	4.84	 **16**	-105	4.99
 **13**	64	4.28	 **17**	26	4.46

[a] Inhibition calculated as Gli signal (SAG) – Gli Signal (SAG+Compound).

Table S4. Percentage inhibition of Gli expression in 100 nM SAG-activated Shh LIGHT2 cells by benzo[1,3]dioxol-5-ylmethyl-[2-(1*H*-indol-3-yl)-ethyl]-amine derivatives analogues **5** and **20**-**26** at 10 μM compound concentration.



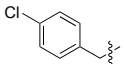
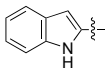
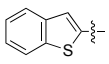
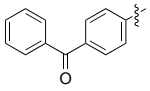
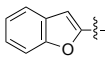
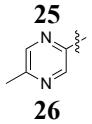
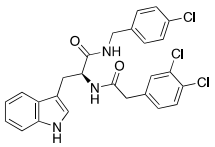
R_1	Inhibition (%)[a]	cLogP	R_1	Inhibition (%)[a]	cLogP
 **5**	89	5.23	 **23**	102	4.73
 **20**	111	3.18	 **24**	67	5.29
 **21**	92	4.12	 **25**	108	5.44
 **22**	84	3.55	 **26**	-16	2.68

Table S5. Primer sequences used in qPCR assay.

Human gene			
	Forward Sequence (5'-3')	**Reverse Sequence (5'-3')**	**Annealing Temp (ºC)**
Gli_2	ATCTCTTGCCACCATTCCAT	GGACAGAATGAGGCTCGTAA	60
Smo	CTGCCACTTCTACGACTTCT	GGCCTGACATAGCACATAGT	56
SuFu	GACCCCTTGGACTATGTTAG	CTGATGTAGTGCCAGTGCTC	55
$Ptch_1$	CCCTCACGTCCATCAGCAAT	AACACCACTACTACCGCTGC	58
Mouse gene			
Gli_2	TCCAGTCAATGGTTCTGTCC	TGGCTCAGCATCGTCACTTC	60
Gli_3	GGCCGTTACCATTATGATCC	CTGAGGCTGCAGTGGGATTA	60
Shh	TGCTTTGTAACCGCCACTTT	CGCTGCTAGGTGCACTTTTA	61
Smo	GAACTCCAATCGCTACCCTG	ATCTGCTCGGCAAACAATCT	60
SuFu	GACCCCTTGGACTATGTTAG	CTGATGTAGTGCCAGTGCTC	55
$Ptch_1$	CATAGCTGCCCAGTTCAAGT	GGTCGTAAAGTAGGTGCTGG	55

N-(4-Chlorobenzyl)-2-[2-(3,4-dichlorophenyl)-acetylamino]-3-(1H-indol-3-yl)-propionamide (***4***)



Yield: 182 mg, 35%. MP 208 – 209 °C;

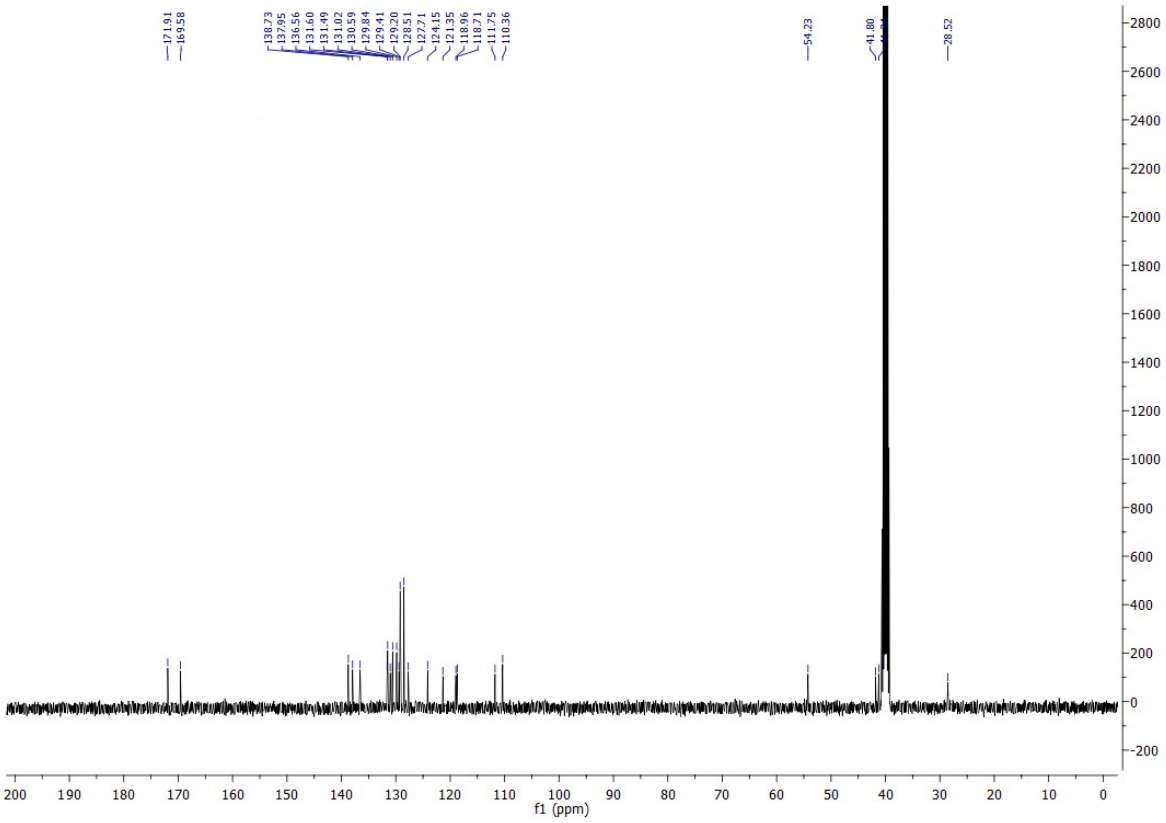
IR: ν_{max}/cm^{-1} 3410 (NH), 3277 (NH), 3068 (CH), 1636 (CON);

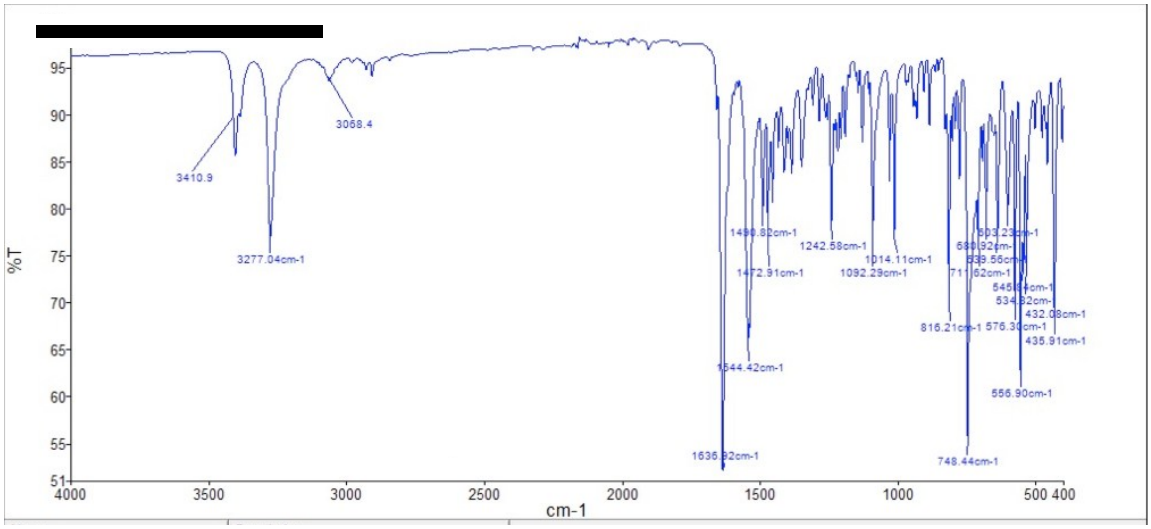
^{1}H NMR (400 MHz, DMSO-d_6) δ 10.84 (s, 1H), 8.56 (t, J = 5.9 Hz, 1H), 8.43 (d, J = 8.1 Hz, 1H), 7.60 (d, J = 7.9 Hz, 1H), 7.47 (d, J = 8.2 Hz, 1H), 7.42 (d, J = 1.8 Hz, 1H), 7.35 (d, J = 8.1 Hz, 1H), 7.28 (d, J = 8.4 Hz, 2H), 7.16 – 7.02 (m, 5H), 6.97 (t, J = 7.1 Hz, 1H), 4.59 (dd, J = 14.4, 8.1 Hz, 1H), 4.24 (qd, J = 15.6, 5.9 Hz, 2H), 3.47 (s, 2H), 3.05 (ddd, J_{AX} = 22.8, J_{BX} = 14.4, J_{AB} = 7.2 Hz, 2H);

^{13}C NMR (101 MHz, DMSO-d_6) δ 171.9, 169.6, 138.7, 138.0, 136. 6, 131.6, 131.5, 131.0, 130.6, 129.8, 129.4, 129.2 (Cx2), 128.5 (Cx2), 127.7, 124.2, 121.4, 119.0, 118.7, 111.8, 110.4, 54.2, 41.8, 41.2, 28.5;

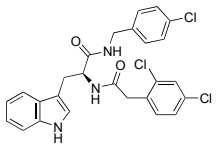
RP-HPLC Alltima™ C18 5 μm 150 μm x 4.6 mm, 10–100% B in 15 min, R_t =14.31 min, 100%;

LRMS (ESI$^+$) m/z: 513, 514 $[M+H]^+$, 95%. HRMS (ES$^+$) for $C_{26}H_{22}Cl_3N_3O_2$, calculated 514.0850, found 514.08498.





*N-(4-Chlorobenzyl)-2-[2-(2,4-dichlorophenyl)-acetylamino]-3-(1H-indol-3-yl)-propionamide (**11**)*



Yield: 55 mg, 24%. MP 207-208 °C;

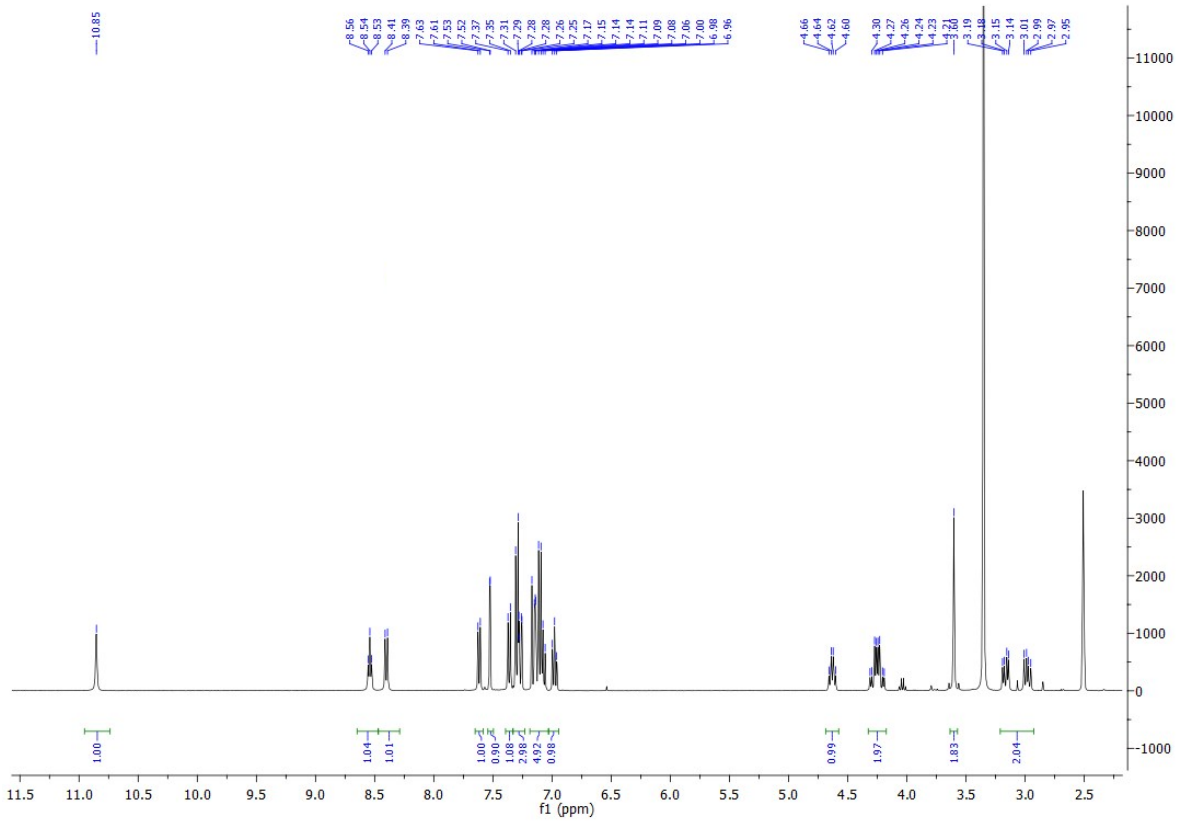
IR: ν_{max}/cm^{-1} 3410 (NH), 3280 (NH), 3065 (CH), 1642 (CON);

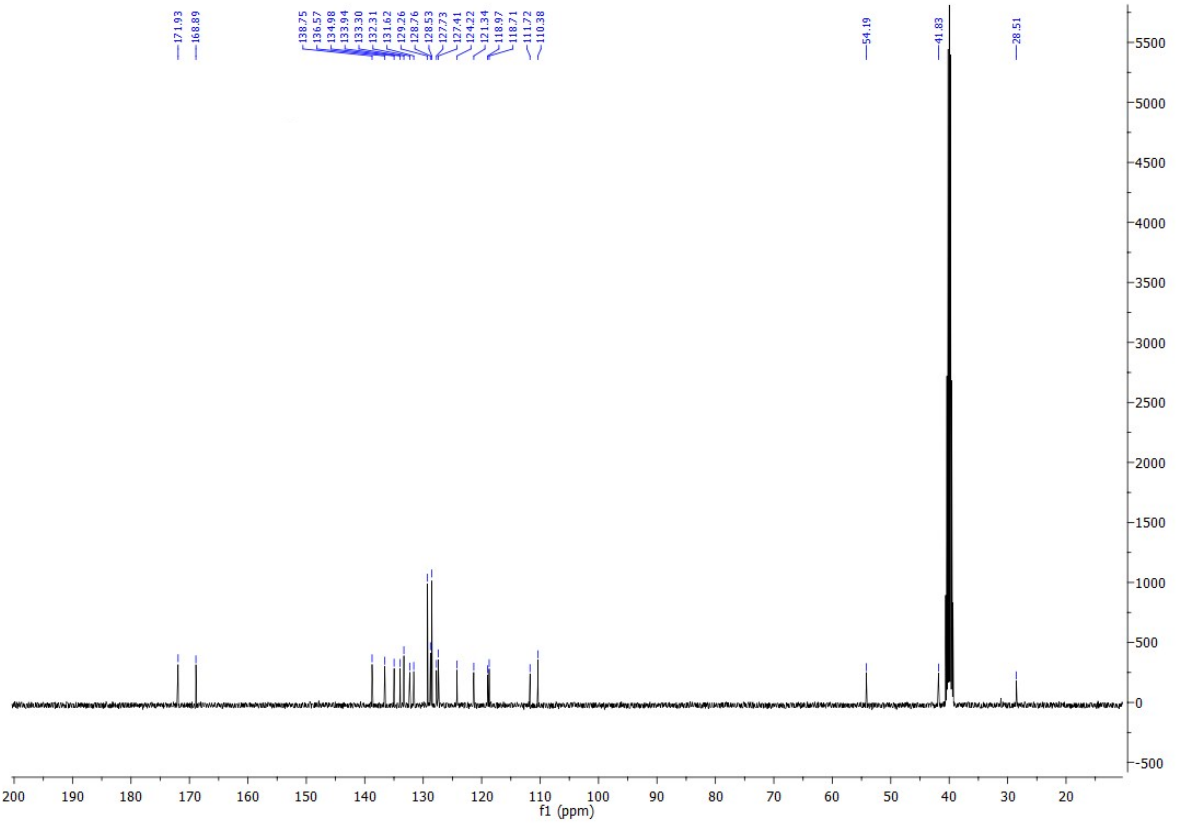
^{1}H NMR (400 MHz, DMSO-d_6) δ 10.85 (s, 1H), 8.54 (t, J = 5.9 Hz, 1H), 8.40 (d, J = 8 1H), 7.62 (d, J = 7.8 Hz, 1H), 7.53 (d, J = 2.1 Hz, 1H), 7.36 (d, J = 8.1 Hz, 1H), 7.33 – 7.2 3H), 7.12 (ddd, J = 22.2, 12.5, 7.8 Hz, 5H), 6.98 (t, J = 7.4 Hz, 1H), 4.63 (dd, J = 14.3, 8. 1H), 4.25 (qd, J = 15.5, 5.9 Hz, 2H), 3.60 (s, 2H), 3.07 (ddd, J_{AX} = 22.9, J_{BX}= 14.4, J_{AB}= 7.2 Hz, 2H);

^{13}C NMR (101 MHz, DMSO-d_6) δ 171.9, 168.9, 138.8, 136.6, 135.0, 133.9, 133.3, 132.3, 131.6, 129.3 (Cx2), 128.5 (Cx2) 127.7, 127.4, 124.2, 121.3, 119.0, 118.7, 111.7, 110.4, 54.2, 41.8, 31.0, 28.5;

RP-HPLC Alltima™ C18 5 µm 150 mm x 4.6 mm, 10–100% B in 15 min, R_t =14.38 min, 99.2%;

LRMS (APCI$^+$) m/z 513, 514 $[M+1H]^+$ 50%. HRMS (ES$^+$) for $C_{26}H_{22}Cl_3N_3O_2$, calculated 514.0850, 514.0850.


10.85
8.56
8.54
8.53
8.41
8.39
7.63
7.61
7.53
7.52
7.37
7.35
7.31
7.29
7.28
7.28
7.26
7.25
7.17
7.15
7.14
7.14
7.11
7.09
7.08
7.06
7.00
6.98
6.96
4.66
4.64
4.62
4.60
4.30
4.27
4.26
4.24
4.23
3.64
3.19
3.18
3.15
3.14
3.01
2.99
2.97
2.95
1.00
1.04
1.01
1.00
0.90
1.08
2.98
4.92
0.98
0.99
1.97
1.83
2.04
f1 (ppm)


171.93
168.89
138.75
136.57
134.98
133.94
133.30
132.31
131.62
129.26
128.76
128.53
127.73
127.41
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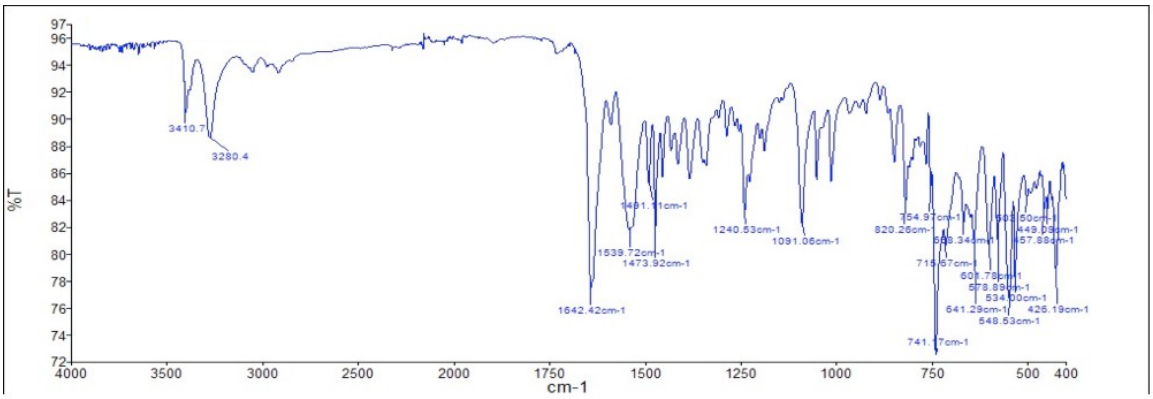
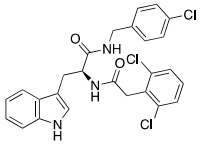
110.38
54.19
41.83
28.51
f1 (ppm)



*N-(4-Chlorobenzyl)-2-[2-(2,6-dichlorophenyl)-acetylamino]-3-(1H-indol-3-yl)-propionamide (**14**)*



Yield: 80 mg, 40%. MP 265-256 °C;

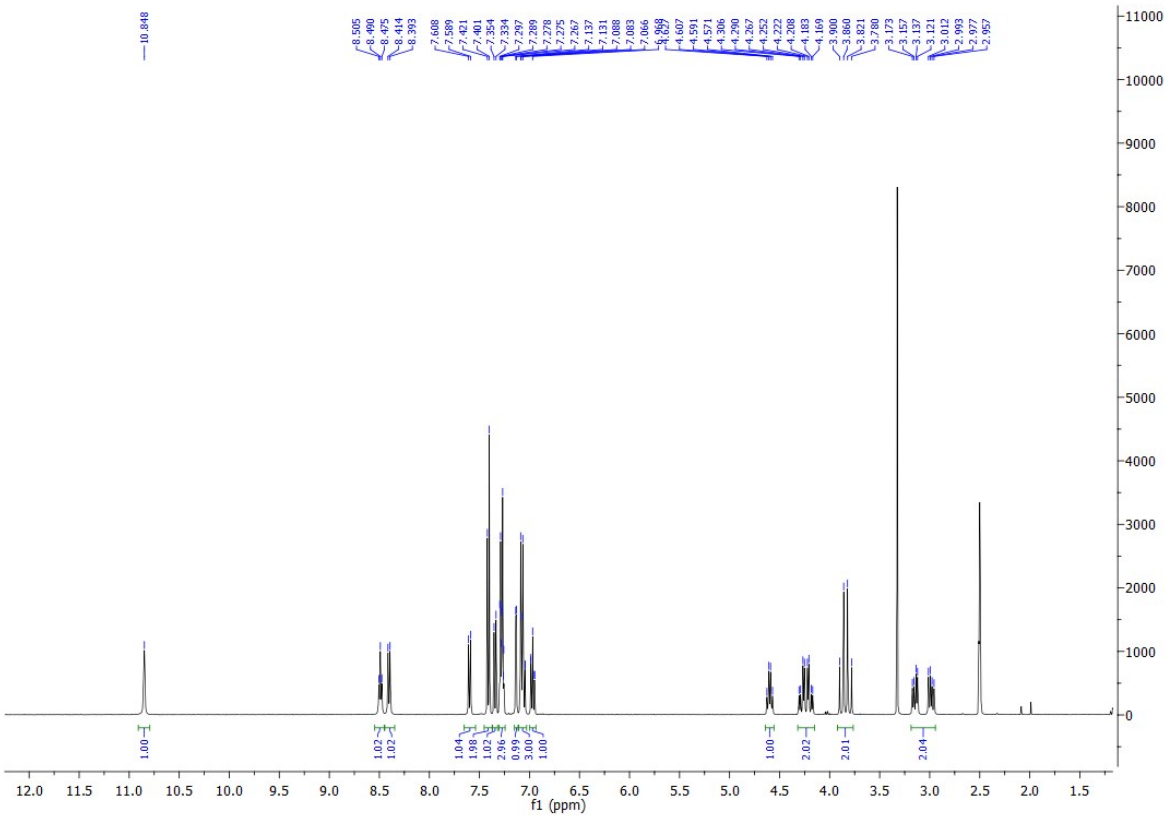
IR: ν_{max}/cm^{-1} 3410 (NH), 3292 (NH), 3252 (NH), 1641 (CON);

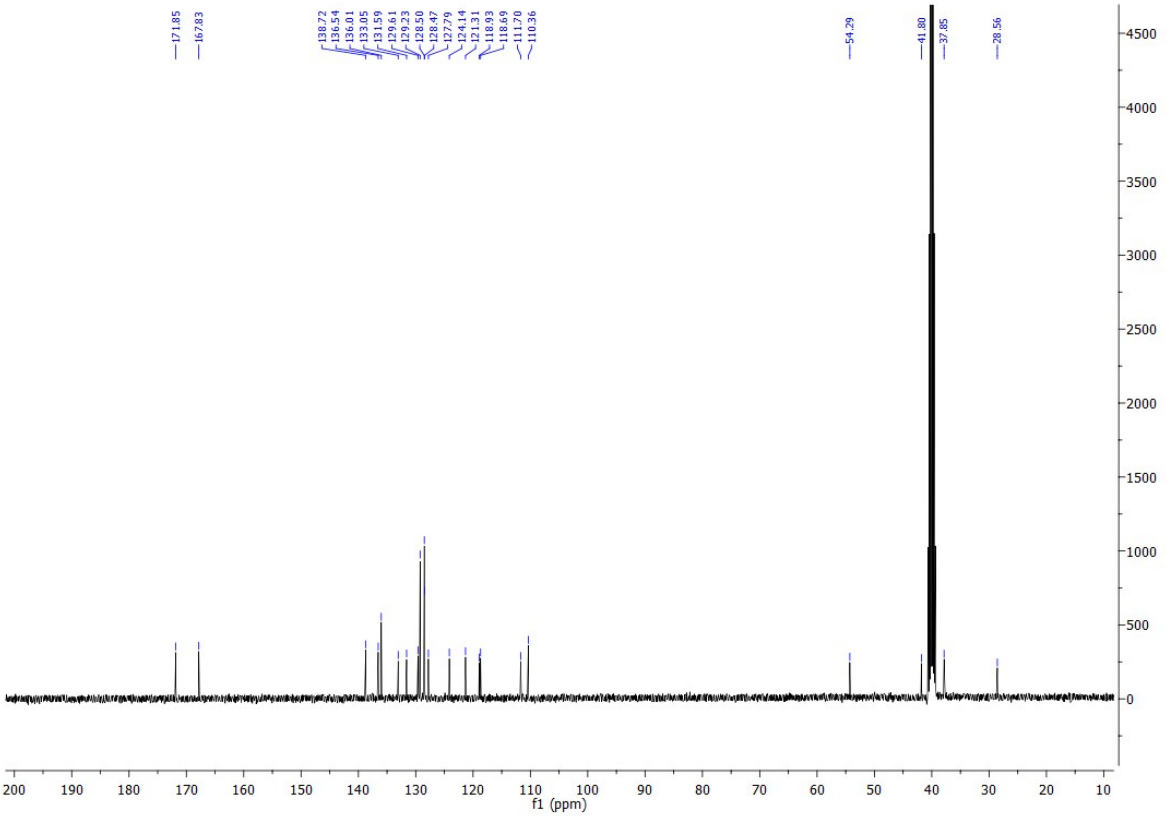
^{1}H NMR (400 MHz, DMSO-d_6) δ 10.85 (s, 1H), 8.49 (t, J = 6.0 Hz, 1H), 8.40 (d, J = 8.2 Hz, 1H), 7.60 (d, J = 7.9 Hz, 1H), 7.41 (d, J = 7.9 Hz, 2H), 7.34 (d, J = 8.1 Hz, 1H), 7.28 (ddd, J = 8.6, 4.7, 2.5 Hz, 3H), 7.13 (d, J = 2.2 Hz, 1H), 7.11 – 7.03 (m, 3H), 7.00 – 6.94 (m, 1H), 4.60 (dd, J = 14.4, 7.9 Hz, 1H), 4.24 (qd, J = 15.5, 5.9 Hz, 2H), 3.84 (q, J = 16.3 Hz, 2H), 3.07 (ddd, J_{AX} = 22.3, J_{BX} = 14.4, J_A = 7.1 Hz, 2H);

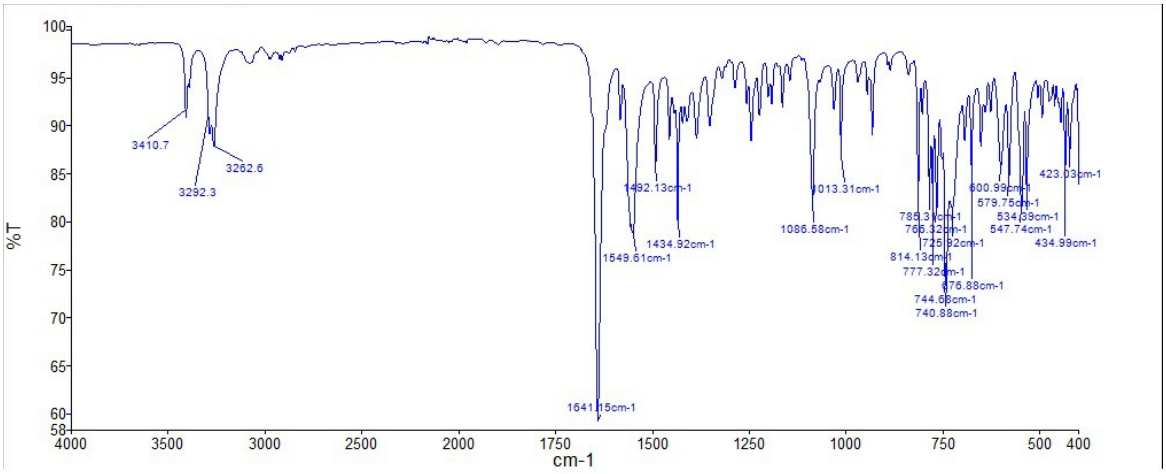
^{13}C NMR (101 MHz, DMSO-d_6) δ 171.9, 167.8, 138.7, 136.5, 136.0 (Cx2), 133.1, 131.6, 129.6, 129.2 (Cx2), 128.5 (Cx2), 128.5 (Cx2), 127.8, 124.1, 121.3, 118.9, 118.7, 111.7, 110.4, 54.3, 41.8, 37.9, 28.6;

RP-HPLC Alltima™ C18 5 μm 150 mm x 4.6 mm, 10–100% B in 15 min, Rt = 6.54 min, 100%;

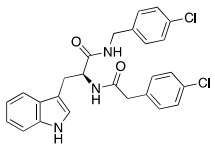
LRMS (ESI+) m/z 513, 514 $[M+H]^+$ 95%. HRMS (ES^+) for $C_{26}H_{22}Cl_3N_3O_2$, calculated 514.0850, found 514.08496.







*N-(4-Chlorobenzyl)-2-[2-(4-chlorophenyl)-acetylamino]-3-(1H-indol-3-yl)-propionamide (**13**)*



Yield: 60 mg, 32%. MP 205.2-206.3 ºC;

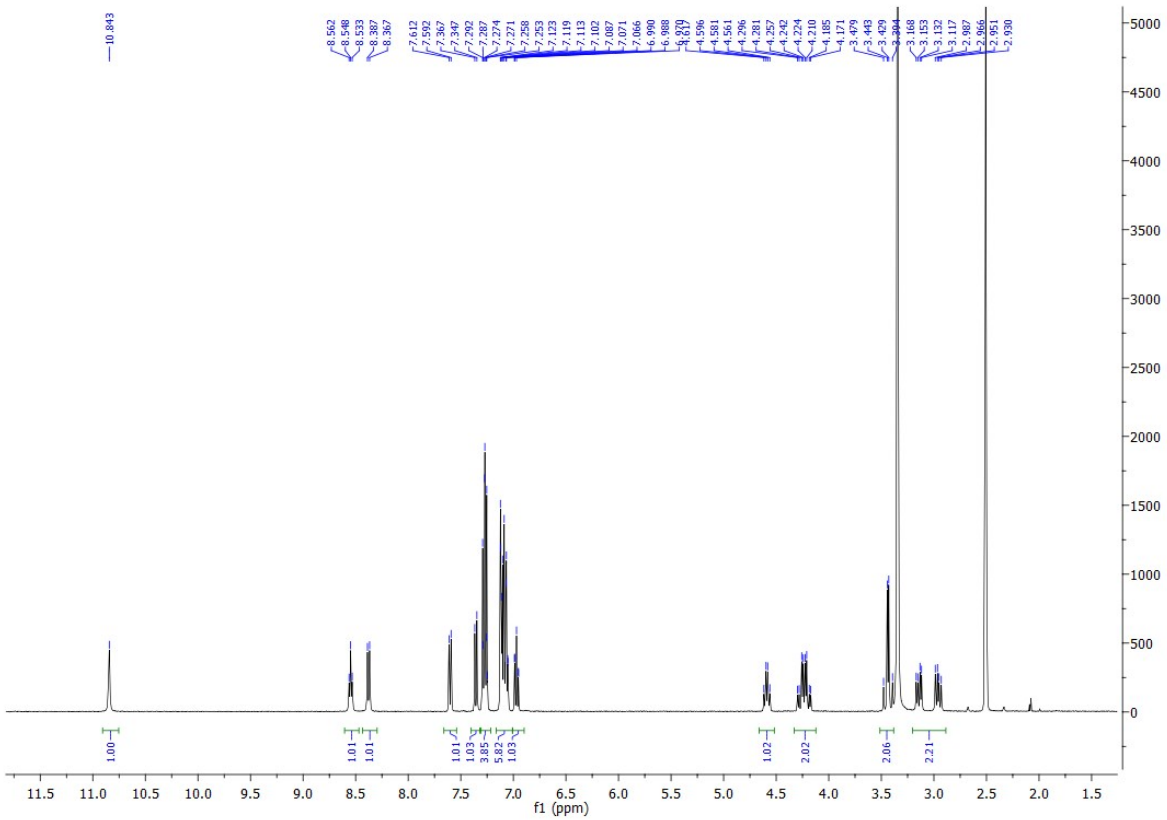
IR: ν_{max}/cm^{-1} 3410 (NH), 3292 (NH), 3061 (CH), 1635 (CON);

^{1}H NMR (400 MHz, DMSO-d_6) δ 10.84 (s, 1H), 8.55 (t, J = 5.9 Hz, 1H), 8.38 (d, J = 8.2 1H), 7.60 (d, J = 7.9 Hz, 1H), 7.36 (d, J = 8.1 Hz, 1H), 7.32 – 7.21 (m, 4H), 7.16 – 7.01 (m, 7.01 – 6.90 (m, 1H), 4.59 (td, J = 14.4, 8.2 Hz, 1H), 4.23 (qd, J = 15.5, 5.9 Hz, 2H), 3.44 (dd, 19.8, 14.4 Hz, 2H), 3.05 (ddd, J_{AX} = 22.8, J_{BX} = 14.4, J_{AB} = 7.3 Hz, 2H);

^{13}C NMR (101 MHz, DMSO-d_6) δ 172.0, 170.0, 138.7, 136.6, 135.8, 131.6, 131.4, 131.3 (Cx2), 129.2 (Cx2), 1 (Cx2), 128.4 (Cx2), 127.7, 124.2, 121.4, 119.0, 118.7, 111.7, 110.4, 54.1, 41.8, 41.7, 28.5;

RP-HPLC Alltima™ C18 5µm 150 mm x 4.6 mm, 10–100% B in 15 min, Rt =13.69 min, 100%;

LRMS (ESI^+) m/z 479, 480 $[M+1H]^+$, 100%; HRMS (ES^+) for $C_{26}H_{23}Cl_2N_3O_2$, calculated 480.1240, fc 480.12396.


10.843
8.562
8.548
8.533
8.387
8.367
7.612
7.592
7.367
7.347
7.292
7.287
7.274
7.271
7.258
7.253
7.123
7.119
7.113
7.102
7.087
7.071
7.066
6.990
6.988
6.819
6.817
4.596
4.581
4.561
4.296
4.281
4.257
4.242
4.224
4.210
4.185
4.171
3.479
3.443
3.429
3.304
3.168
3.153
3.132
3.117
2.987
2.966
2.951
2.930
1.00
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1.01
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1.03
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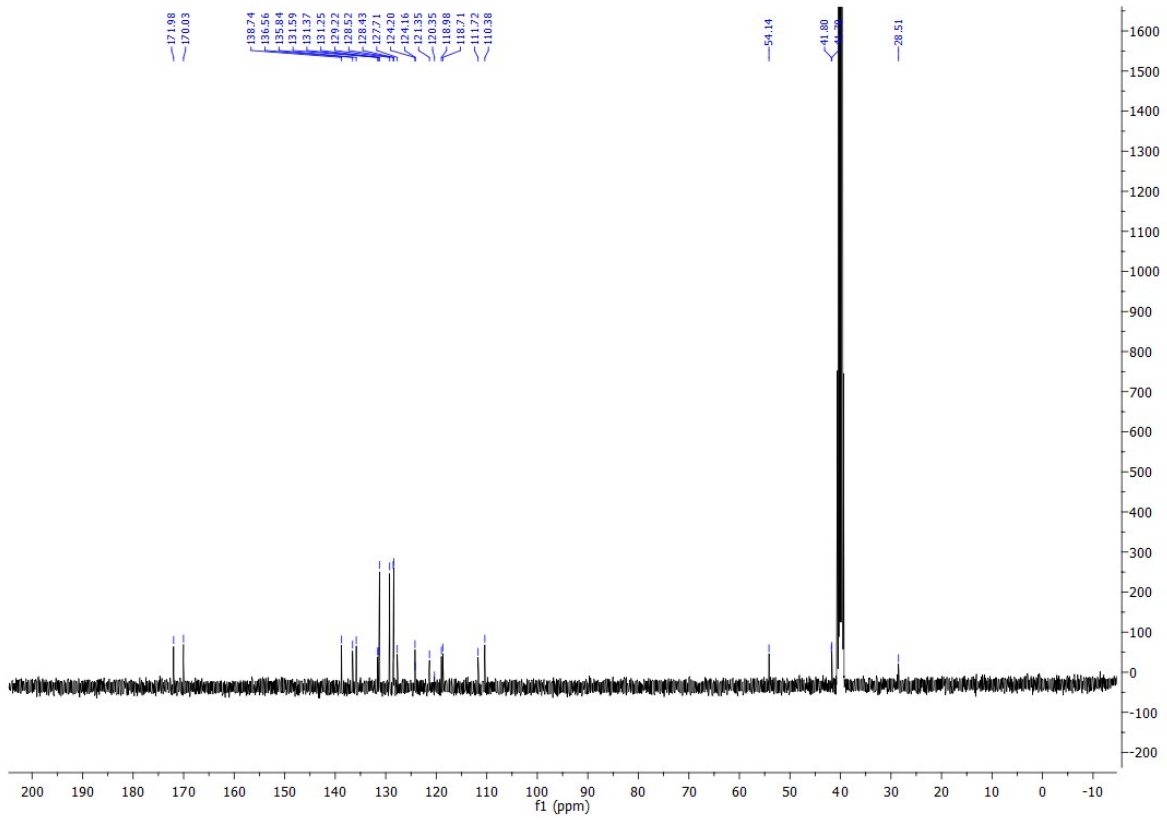
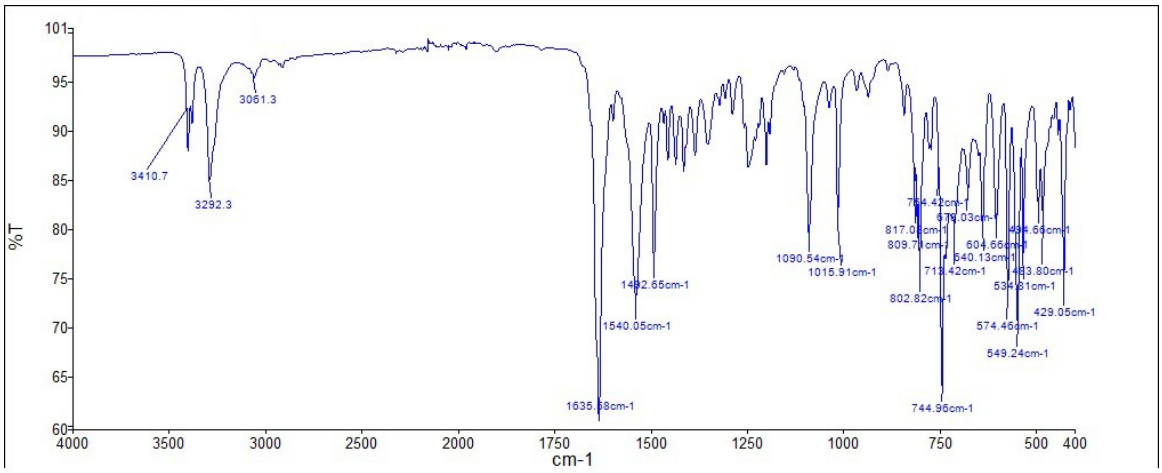
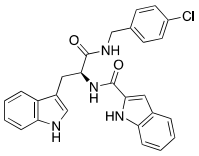
5.82
1.03
1.02
2.02
2.06
2.21
f1 (ppm)


171.98
170.03
138.74
136.56
135.84
131.59
131.37
131.25
129.22
128.52
128.43
127.71
124.20
124.16
121.35
120.35
118.98
118.71
111.72
110.38
54.14
41.80
28.51
f1 (ppm)



*1H-Indole-2-carboxylic acid [1-(4-chlorobenzylcarbamoyl)-2-(1H-indol-3-yl)-ethyl]-amide (**14**, L-isomer)*



Yield: 97 mg, 50%. MP 229.5-230.7 ºC;

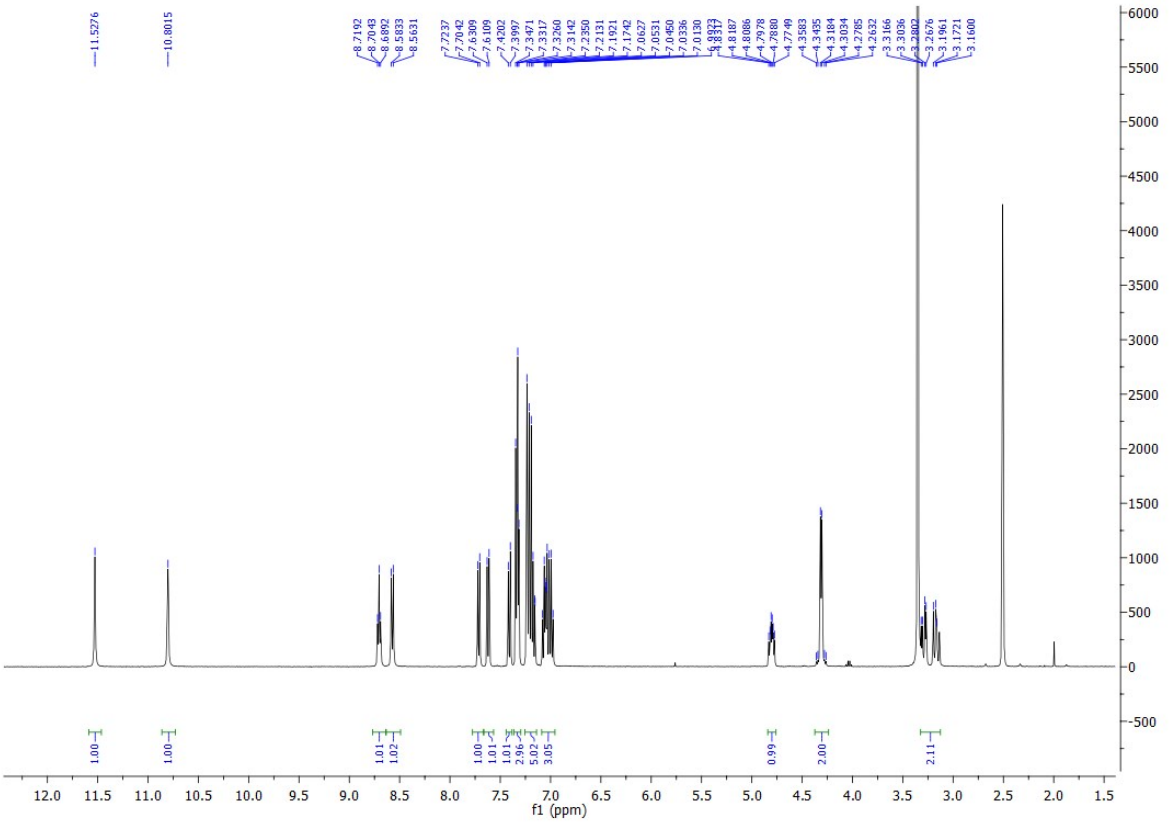
IR: ν_{max}/cm^{-1} 3422 (NH), 3381 (NH), 3316 (NH), 1630 (CON);

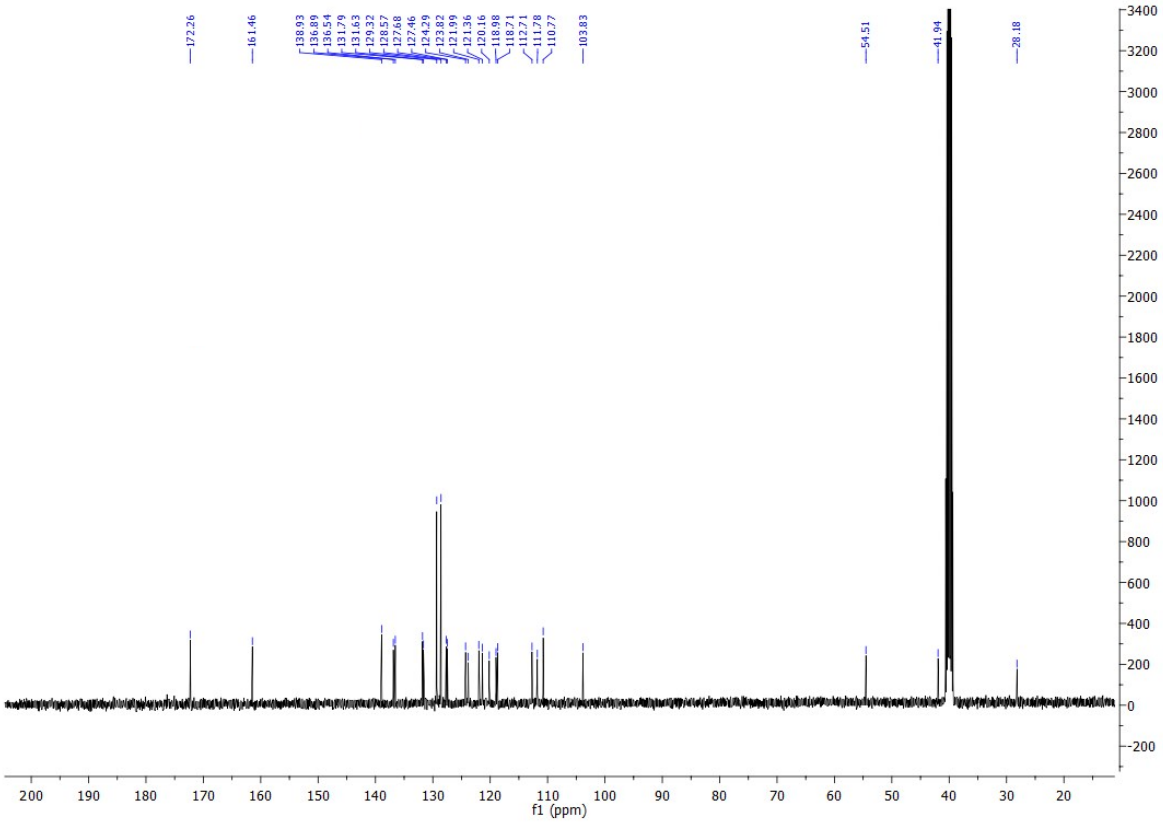
^{1}H NMR (400 MHz, DMSO-*d6*) δ 11.53 (s, 1H), 10.80 (s, 1H), 8.70 (t, *J* = 6.0 Hz, 1H), 8.57 (d, *J* = 8.1 Hz, 1H), 7.71 (d, *J* = 7.8 Hz, 1H), 7.62 (d, *J* = 8.0 Hz, 1H), 7.41 (d, *J* = 8.2 Hz, 1H), 7.33 (dd, *J* = 7.7, 5.5 Hz, 3H), 7.25 – 7.14 (m, 5H), 7.09 – 6.96 (m, 3H), 4.80 (ddd, *J* = 9.2, 8.4, 5.2 Hz, 1H), 4.31 (dd, *J* = 15.96, 6.0 Hz, 2H), 3.24 (ddd, J_{AX} = 28.6 Hz, J_{BX} = 14.56 Hz, J_{AB} = 5.2 Hz, 2H);

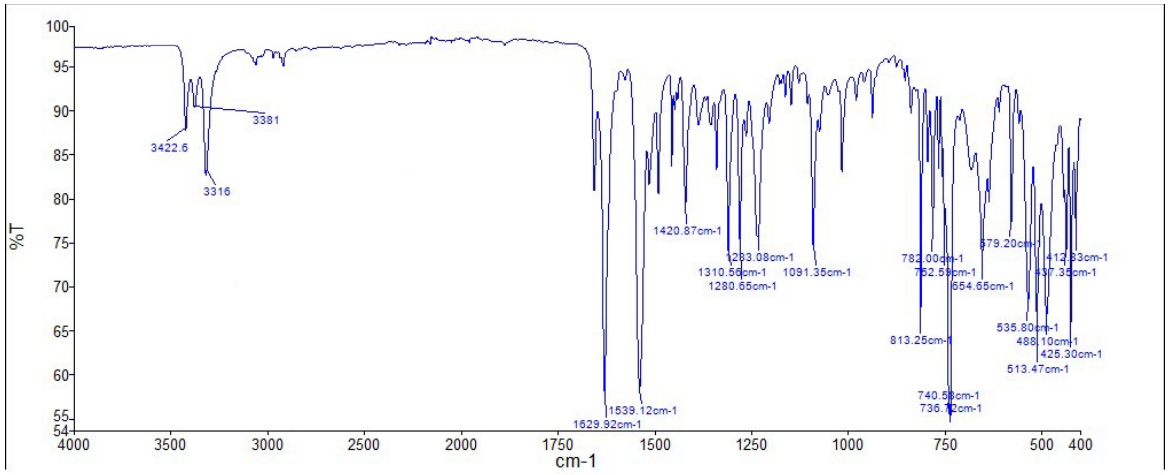
^{13}C NMR (101 MHz, DMSO-*d6*) δ 172.3, 161.5, 138.9, 136.9, 136.5, 131.7, 129.3 (Cx2), 128.6 (Cx2), 127.7, 127.5, 124.3, 123.8, 122.0, 121.4, 120.2, 119.0, 118.7, 112.7, 111.8, 110.8, 103.8, 54.5, 41.9, 28.2;

RP-HPLC Alltima™ C18 5 μm 150 mm x 4.6 mm, 10–100% B in 15 min, R_t = 13.35 min, 100%;

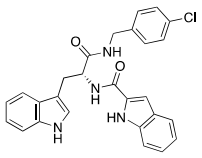
LRMS ($APCI^+$) m/z 470, 471 $[M+1H]^+$, 90%; HRMS (ES^+) for $C_{27}H_{23}ClN_4O_2$ calculated 471.1582, found 471.1582.







*1H-Indole-2-carboxylic acid [1-(4-chlorobenzylcarbamoyl)-2-(1H-indol-3-yl)-ethyl]-amide (**14a**, D-isomer)*



Yield: 156 mg, 48%. MP 227-227.5 ºC;

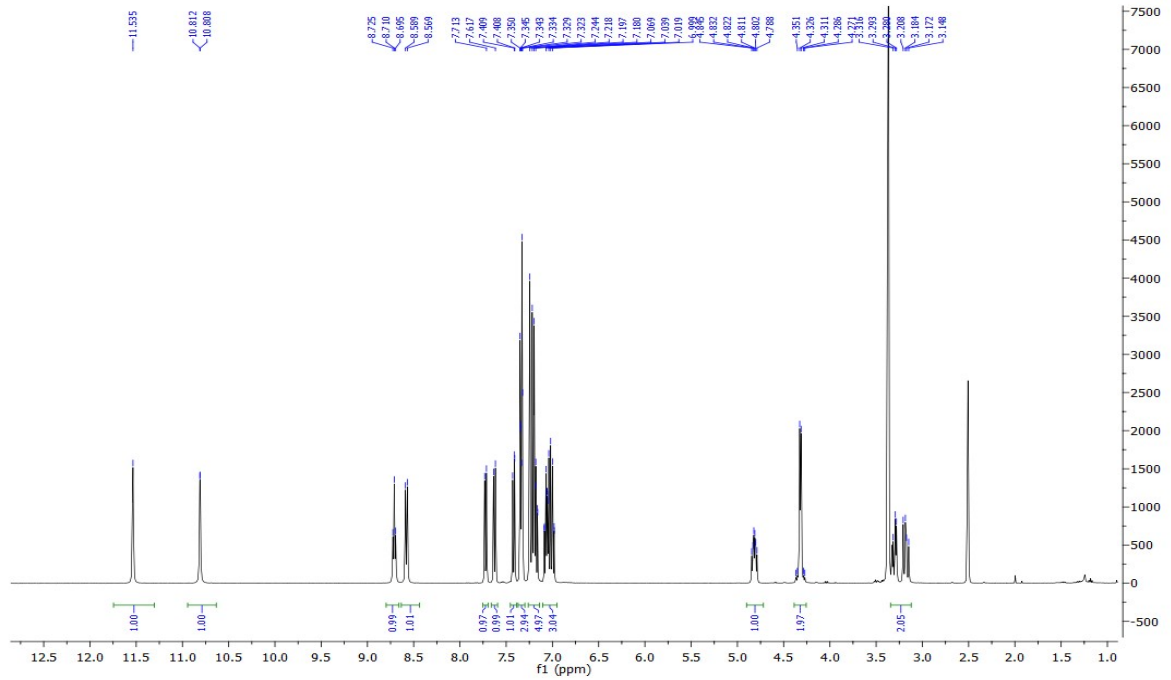
IR: ν_{max}/cm^{-1} 3420 (NH), 3382 (NH), 3325 (NH), 1630 (CONH), 1656 (CON), 737 (CH-aromatics);

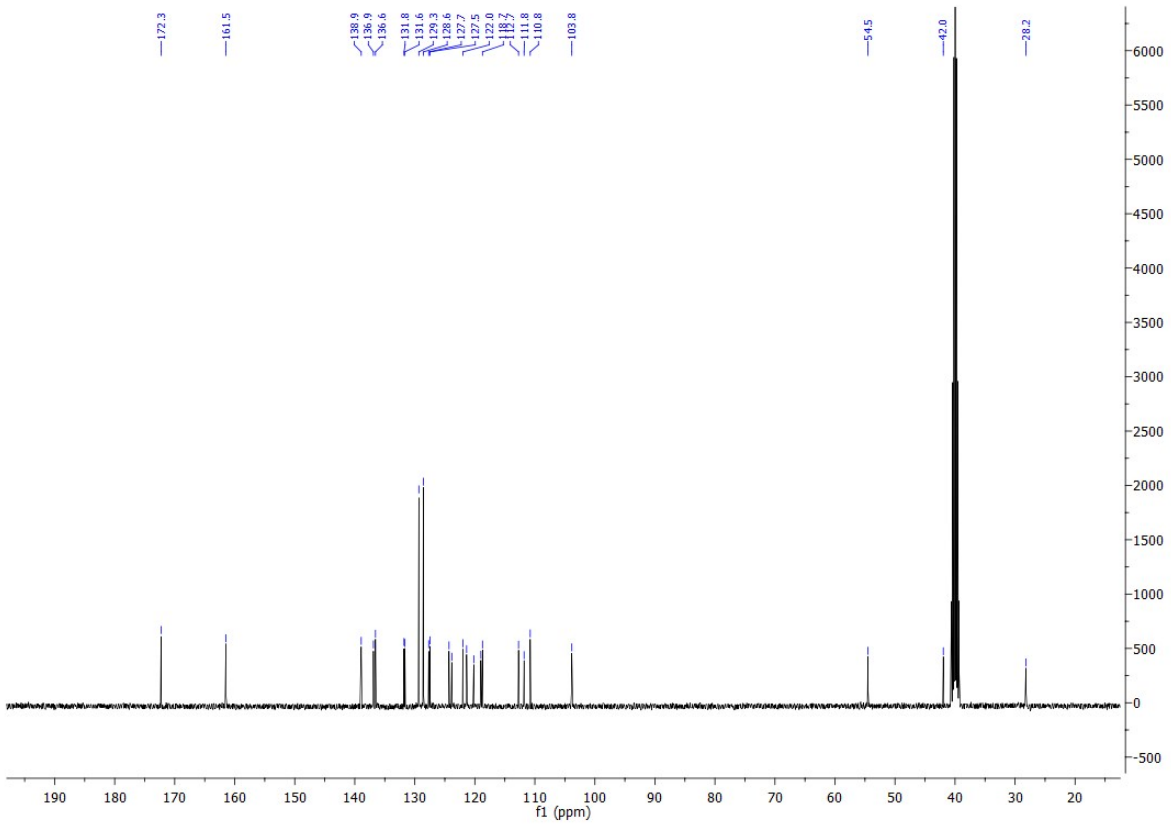
^{1}H NMR (400 MHz, DMSO-d_6) δ 11.53 (s, 1H), 10.81 (d, J = 1.6 Hz, 1H), 8.71 (t, J = 6.0 Hz, 1H), 8.58 (d, J = 8.1 Hz, 1H), 7.72 (d, J = 7.8 Hz, 1H), 7.63 (d, J = 8.0 Hz, 1H), 7.45 – 7.39 (m, 1H), 7.37 – 7.30 (m, 3H), 7.26 – 7.14 (m, 5H), 7.11 – 6.95 (m, 3H), 4.82 (ddd, J = 9.2, 8.4, 5.2 Hz, 1H), 4.32 (dd, J = 16.4, 6.0 Hz, 2H), 3.23 (ddd, J_{AX} = 28.8 Hz , J_{BX} = 14.4 Hz, J_{AB} = 9.2 Hz, 2H);

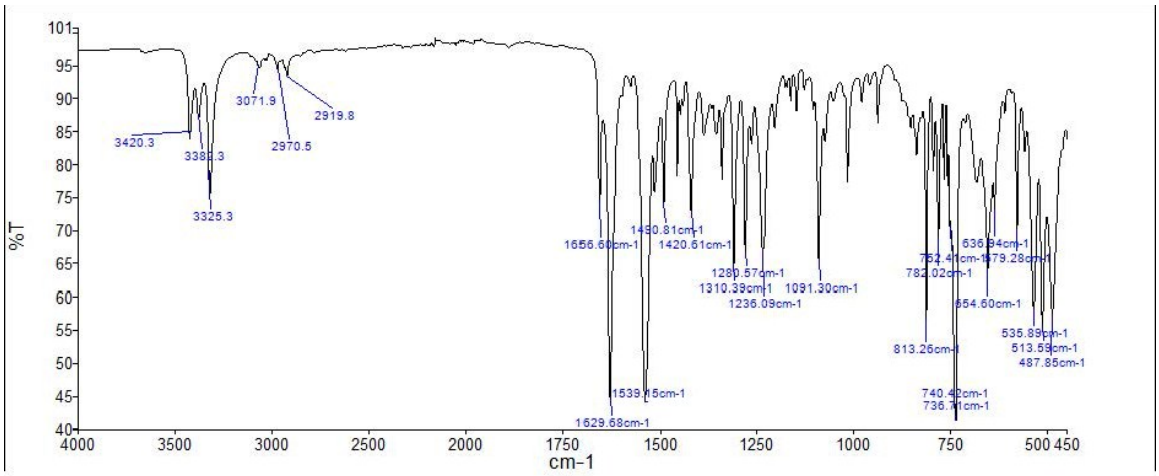
^{13}C NMR (101 MHz, DMSO-d_6) δ 172.3, 161.5, 138.9, 136.9, 136.6, 131.8, 131.7, 129.3 (2C), 128.6 (2C), 127.7, 127.5, 124.3, 123.8, 122.0, 121.4, 120.2, 119.0, 118.7, 112.7, 111.8, 110.8, 103.9, 54.5, 42.0, 28.2;

RP-HPLC Alltima™ C18 5 µm 150 mm x 4.6 mm, 10–100% B in 15 min, R_t = 6.44 min, 100%;

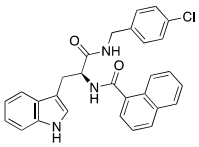
LRMS (ESI$^+$) m/z 470, 470 $[M+H]^+$, 80%. HRMS (ES+) for $C_{27}H_{23}ClN_4O_2$ calculated 471.1582, found 471.1584.


11.535
10.812
10.808
8.725
8.710
8.695
8.589
8.569
7.713
7.617
7.409
7.408
7.350
7.345
7.343
7.334
7.329
7.323
7.244
7.238
7.197
7.180
7.069
7.059
7.019
6.999
4.832
4.822
4.811
4.802
4.788
4.351
4.326
4.311
4.286
3.293
3.280
3.208
3.184
3.172
3.148
1.00
1.00
0.99
1.01
0.87
0.99
1.01
2.94
4.97
3.04
1.00
1.97
2.05
f1 (ppm)


172.3
161.5
138.9
136.9
136.6
131.8
131.6
129.3
128.6
127.7
127.5
122.0
111.8
110.8
103.8
54.5
42.0
28.2
f1 (ppm)



Naphthalene-1-carboxylic acid [1-(4-chlorobenzylcarbamoyl)-2-(1H-indol-3-yl)-ethyl]-amide ***(15)***



Yield: 57 mg, 41%. MP 165.7-166.5 ºC;

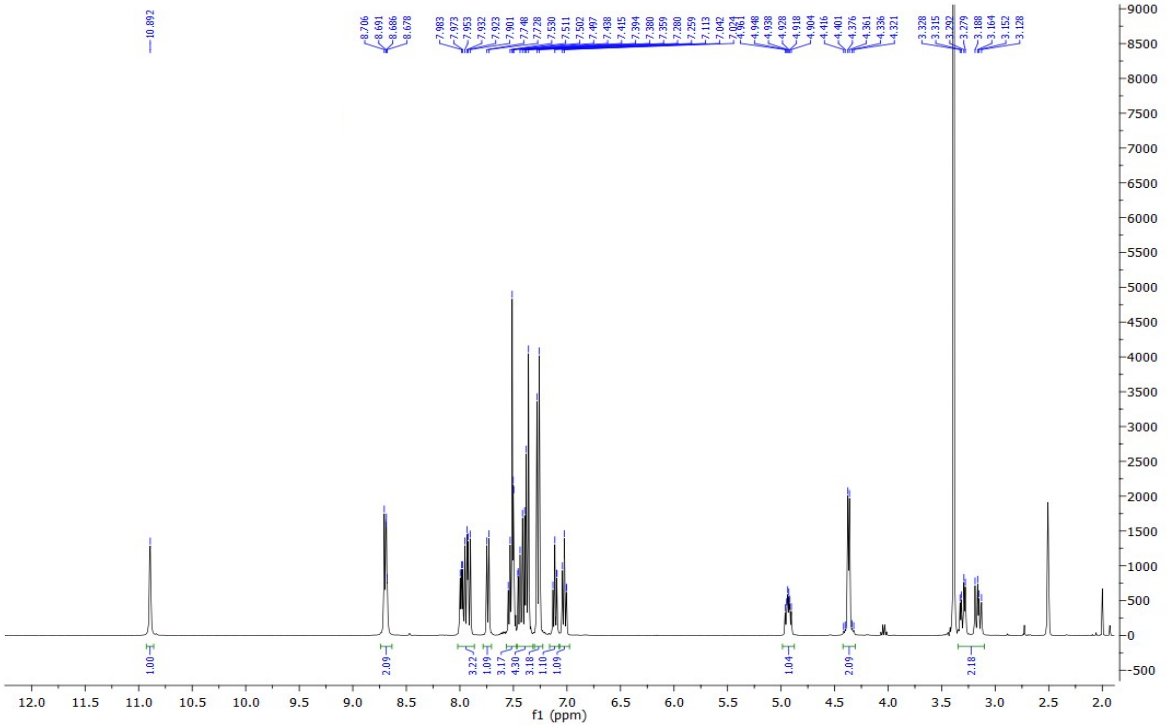
IR: ν_{max}/cm^{-1} 3398 (NH), 3268 (bp NH), 3049 (CH), 1627 (CON), 739 (CH-aromatics);

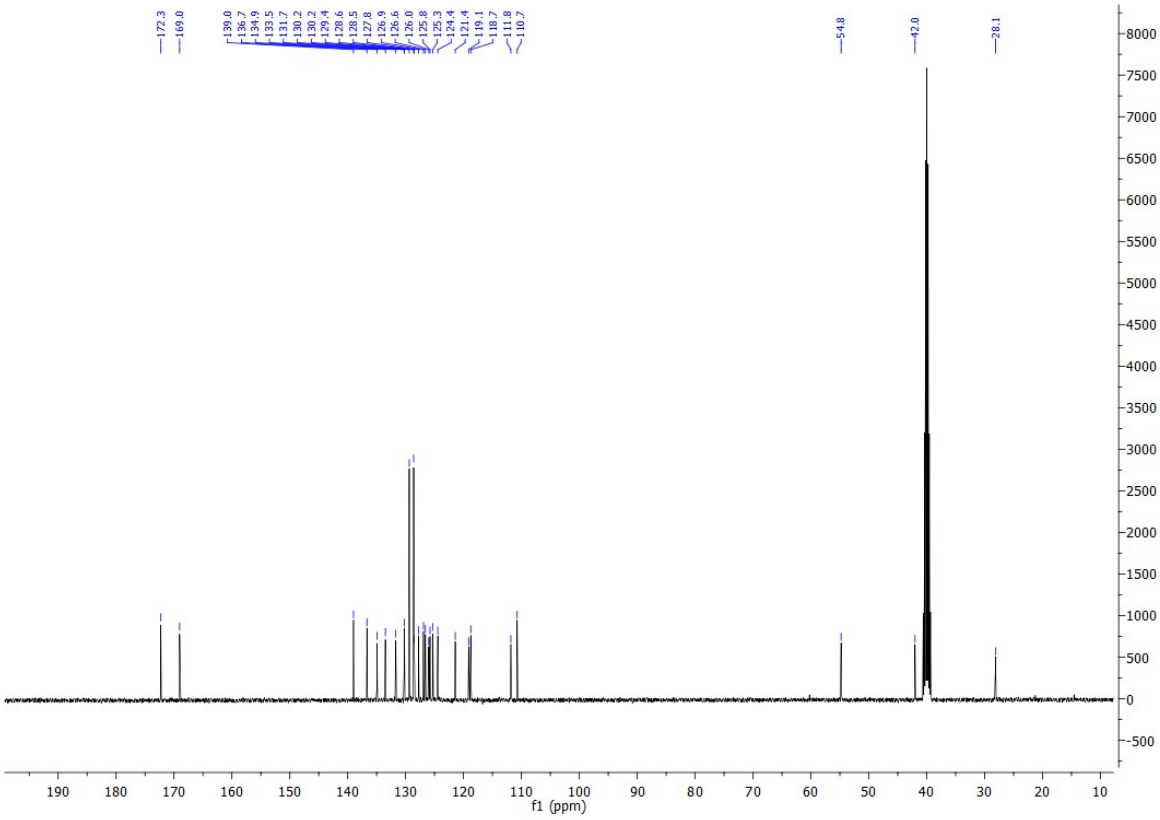
^{1}H NMR (400 MHz, DMSO-d_6) δ 10.89 (s, 1H), 8.73−8.67 (m, 2H) (overlapping two NH amides), 8.02 – 7.86 (m, 3H), 7.74 (d, J = 7.8 Hz, 1H), 7.57 – 7.47 (m, 3H), 7.47 – 7.32 (m, 4H), 7.27 (d, J = 8.5 Hz, 3H), 7.11 (dd, J = 11.1, 3.9 Hz, 1H), 7.02 (dd, J = 11.0, 3.9 Hz, 1H), 4.93 (td, J = 9.3, 5.2 Hz, 1H), 4.37 (ddd, J = 16.0, 6.0 Hz, 2H), 3.23 (ddd, J_{AX} = 36.4, J_{BX} = 14.4, J_{AB} = 5.2 Hz, 2H);

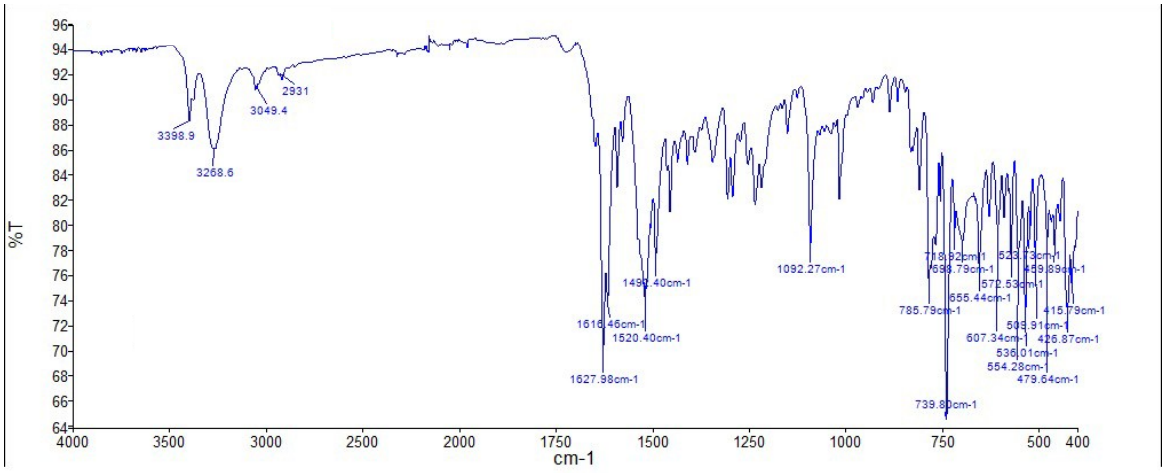
^{13}C NMR (101 MHz, DMSO-d_6) δ 172.3, 169.0, 139.0, 136.7, 135.0, 133.5, 131.7, 130.2 (Cx2), 129.4(Cx2), 128.6 (Cx3), 127.8, 126.9, 126.6, 126.0, 125.8, 125.3, 124.4, 121.4, 119.1, 118.7, 111.8, 110.7, 54.8, 42.0, 28.1;

RP-HPLC Alltima™ C18 5 µm 150 mm x 4.6 mm, 10–100% B in 15 min, Rt = 13.62 min, 99.1%;

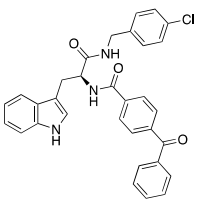
LRMS (APCI^{+}) m/z 481, 482 $[M+1H]^{+}$, 90%. HRMS (ES^{+}) for $C_{29}H_{24}ClN_3O_2$, calculated 482.1630, found 482.1630.







4-Benzoyl-N-[1-(4-chlorobenzylcarbamoyl)-2-(1H-indol-3-yl)-ethyl]-benzamide ***(16)***



Yield: 120 mg, 45%. MP 202 − 202.5 ºC;

IR: ν_{max}/cm^{-1} 3440 (NH), 3304 (NH), 1662(CO), 1632 (CON), 743 (CH-aromatics);

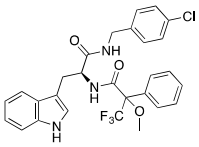
^{1}H NMR (400 MHz, DMSO-d_6) δ 10.80 (s, 1H), 8.80 (d, J = 8.0 Hz, 1H), 8.68 (t, J = Hz, 1H), 8.00 (d, J = 8.4 Hz, 2H), 7.81 – 7.66 (m, 6H), 7.58 (t, J = 7.6 Hz, 2H), 7.33 (dd 8.2, 3.5 Hz, 3H), 7.21 (dd, J = 9.2, 5.3 Hz, 3H), 7.03 (dt, J = 30.0, 7.0 Hz, 2H), 4.81 (td, J = 4.4 Hz, 1H), 4.37 – 4.23 (m, 2H), 3.25 (ddd, J_{AX} = 45 Hz, J_{BX} = 14.9 Hz, J_{AB} = 4.8 Hz, 2H).

^{13}C NMR (101 MHz, DMSO-d_6) δ 195.9, 172.1, 166.0, 139.7, 138.9, 137.8, 137.1, 136.6, 133.5, 131.7, 130.2 ((129.8 (Cx2), 129.3 (Cx2), 129.1 (Cx2), 128.6 (Cx2), 128.2 (Cx2), 127.7, 124.2, 121.4, 119.0, 118.7, 111.8, 110.8, 42.0, 28.0;

RP-HPLC Alltima™ C18 5 µm 150 mm x 4.6 mm, 10–100% B in 15 min, Rt = 14.14 min, 100%;

LRMS (APCI$^+$) m/z 535, 536 $[M+1H]^+$, 20%. HRMS (ES$^+$), for $C_{32}H_{26}ClN_3O_3$, calculated 536.1735, fou 536.1735.

*N-[1-(4-Chlorobenzylcarbamoyl)-2-(1H-indol-3-yl)-ethyl]-3,3,3-trifluoro-2-methoxy-2-phenyl-propionamide (**17**)*



Yield: 171 mg, 71%. MP 171 – 172 ºC;

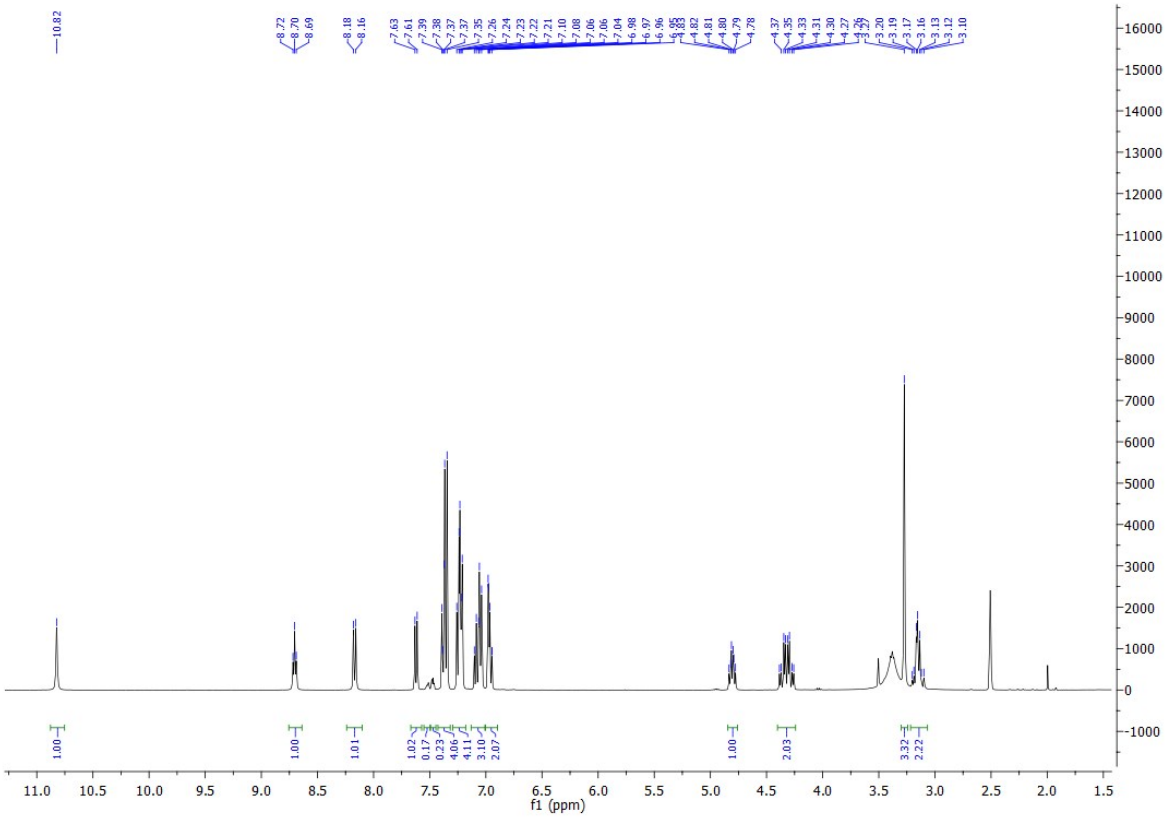
IR: ν_{max}/cm^{-1} 3310 (bp, NH), 2925 (CH), 1657(CON), 741 (CH-aromatics);

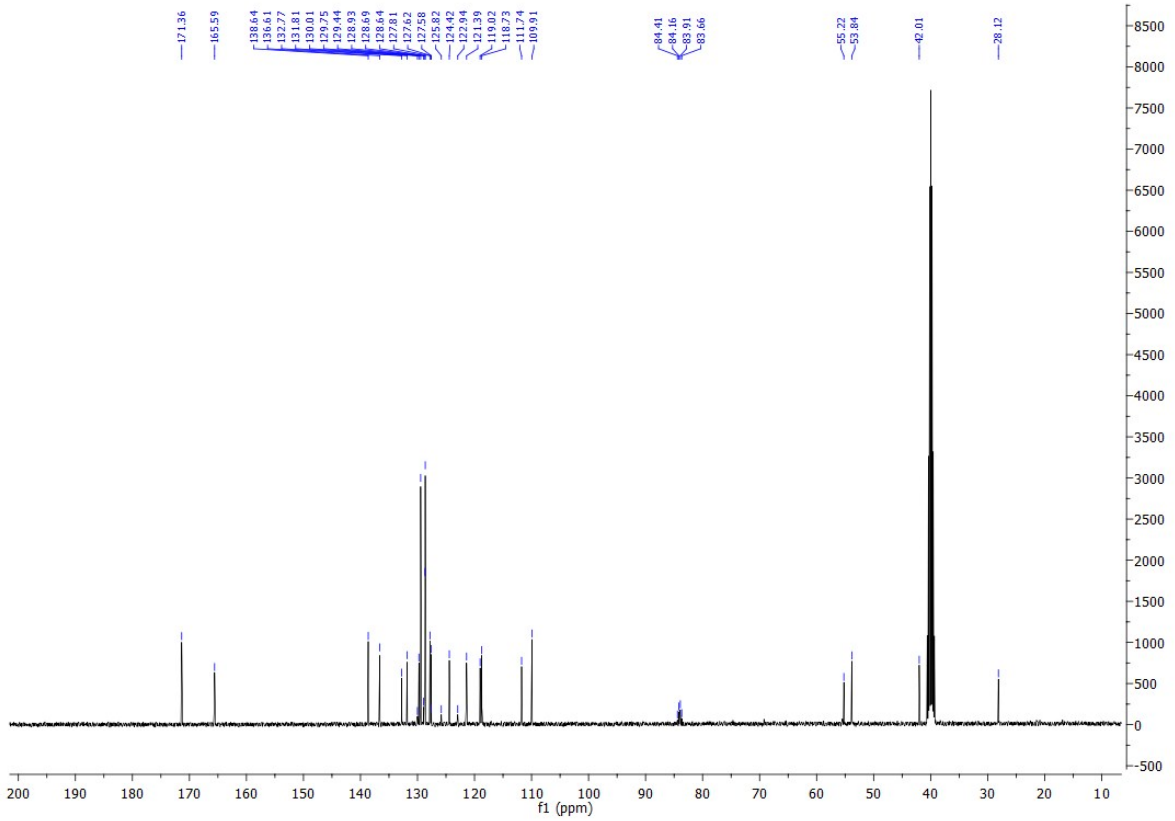
^{1}H NMR (400 MHz, DMSO-d_6) δ 10.82 (s, 1H), 8.70 (t, J = 5.9 Hz, 1H), 8.17 (d, J = 8.5 Hz, 1H), 7.62 (d, J = 7.9 Hz, 1H), 7.43 – 7.32 (m, 4H), 7.30 – 7.18 (m, 4H), 7.13 – 7.01 (m, 3H), 7.01 – 6.90 (m, 2H), 4.81 (td, J = 8.6, 6.0 Hz, 1H), 4.32 (qd, J = 15.4, 5.8 Hz, 2H), 3.27 (s, 3H), 3.22 – 3.07 (m, 2H);

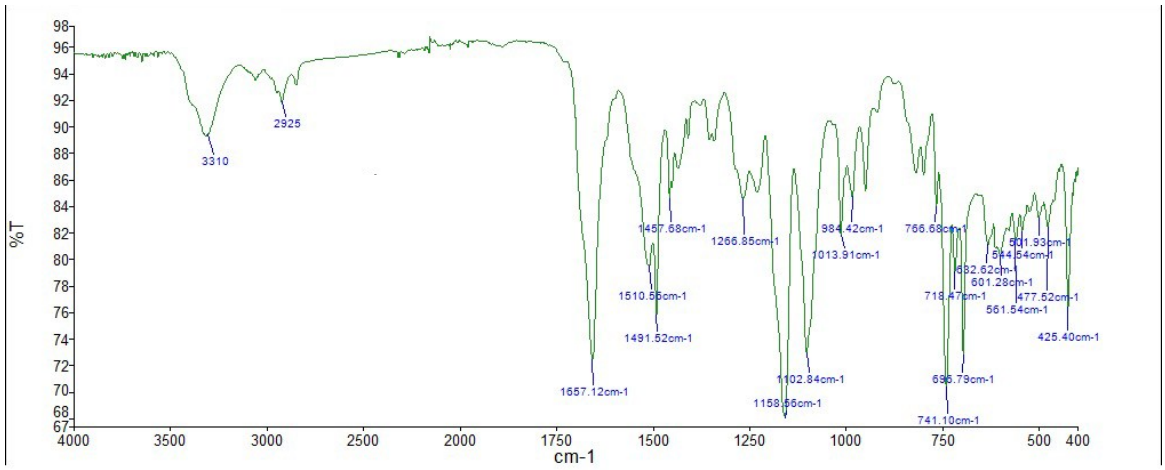
^{13}C NMR (101 MHz, DMSO-d_6) δ 171.4, 165.6, 138.6, 136.6, 132.8, 131.8, 129.8, 129.4 (Cx4), 128.7 (Cx2), 128.6 (Cx4), 127.8, 124.4, 121.4, 119.0, 118.7, 111.7, 109.9, *84.4, 84.2, 83.9, 83.7*, 55.2, 53.8, 42.0, 28.1. *Note: CF_3 splitting at 84.0 (q, J = 25.2 Hz) and presented in italics;*

RP-HPLC Alltima™ C18 5 µm 150 mm x 4.6 mm, 10–100% B in 15 min, R_t = 14.53 min, 100%

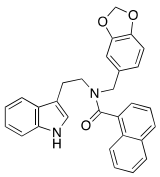
LRMS (APCI$^+$) m/z 543, 544 [M+ H]$^+$, 50%. HRMS (ES$^+$) for $C_{28}H_{25}ClF_3N_3O_3$, calculated 543.1646, found 544.16105







*N-(1,3-Benzodioxol-4-ylmethyl)-N-[2-(1H-indol-3-yl)ethyl]-napthalene carboxamide (**18**)*



Yield: 110 mg, 67%. MP 199 − 200 ºC;

IR: ν_{max}/cm^{-1} 3215 (NH), 1608 (CON), 743 (CH-aromatics);

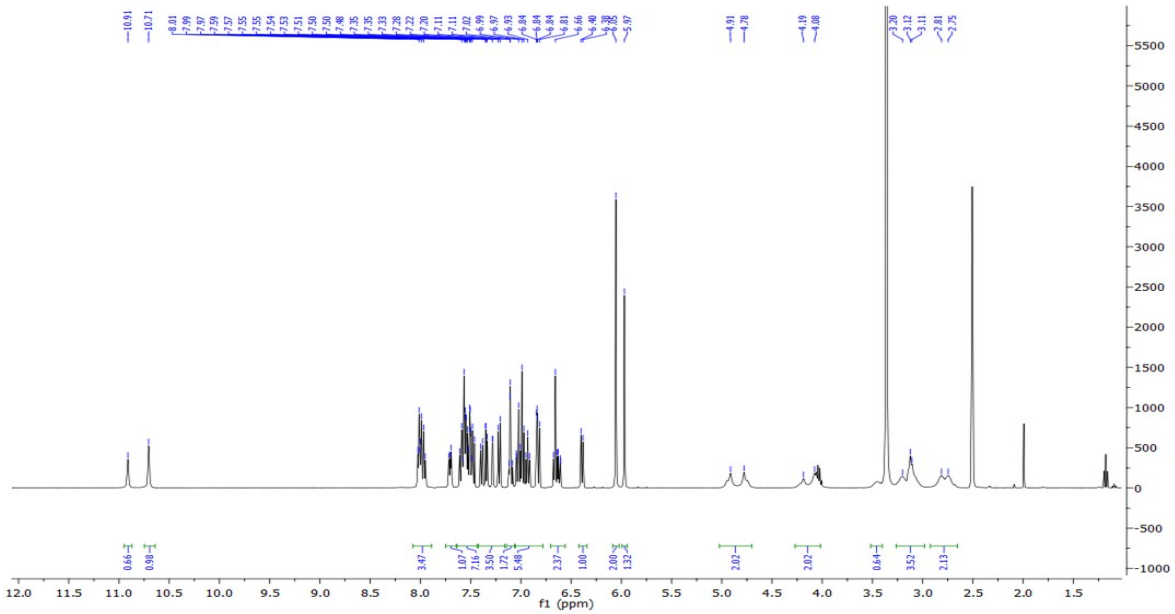
Proton and carbon spectra displays an atropoisomeric property of compound **5, with the approximate ratio 1:0.66 calculated based on the proton benzodioxole $C\underline{H}_2$ peaks at 6.05 and 5.97 ppm, respectively.*

^{1}H NMR (400 MHz, DMSO-d_6) δ 10.91 (s, 0.67H), 10.71 (s, 1H), 8.07 – 7.89 (m, 3.33H), 7.75 – 7.64 (m, 1H), 7.64 – 7.44 (m, 7H), 7.42 – 7.16 (m, 3.3H), 7.14 – 7.06 (m, 1.67H), 7.06 – 6.78 (m, 5.33H), 6.71 – 6.56 (m, 2.33H), 6.39 (d, *J* = 7.9 Hz, 1H), 6.05 (s, 2H), 5.97 (s, 1.33H), 5.01 – 4.70 (m, 2H), 4.26 −4.02 (m, 2H), 3.43 (d, *J* = 20.9 Hz, 0.67H), 3.26 – 2.98 (m, 3.33H), 2.91 −2.66 (m, 2H);

^{13}C NMR (101 MHz, DMSO-d_6) δ 170.4, 170.0, 148.0, 148.0, 147.1, 147.0, 136.8, 136.4, 135.1, 135.0, 133.5 133.4, 132.3, 130.7, 129.5, 129.2, 129.0, 128.9, 128.9, 127.7, 127.5, 127.1, 126.9, 125.8, 125.0, 124.7, 124.1, 123.6, 123.3, 122.1, 121.5, 121.3, 121.1, 118.9, 118.8, 118.5, 118.0, 111.9, 111.8, 111.7, 110.7, 109.1, 108.8, 108.7, 108.1, 101.5, 51.8, 49.2, 47.1, 44.5, 24.9, 23.1;

RP-HPLC Alltima™ C18 5 µm 150 mm x 4.6 mm, 10–100% B in 15 min, Rt = 18.06 min, 100%

LRMS (APCI$^+$) m/z 448, 449 $[M+1H]^+$, 100%. HRMS (ES+) for $C_{29}H_{24}N_2O_3$, calculated 449.1860, found 449.1859.


10.91
10.71
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7.99
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7.59
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7.55
7.54
7.53
7.51
7.50
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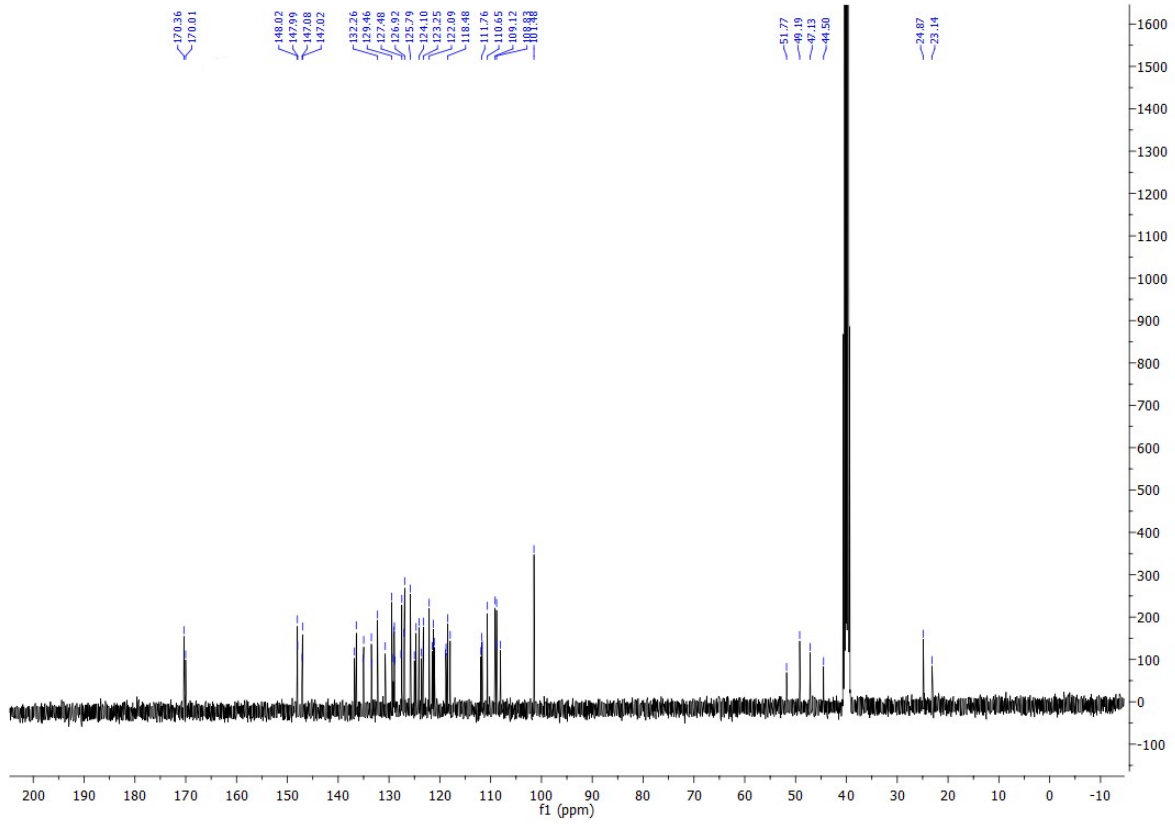
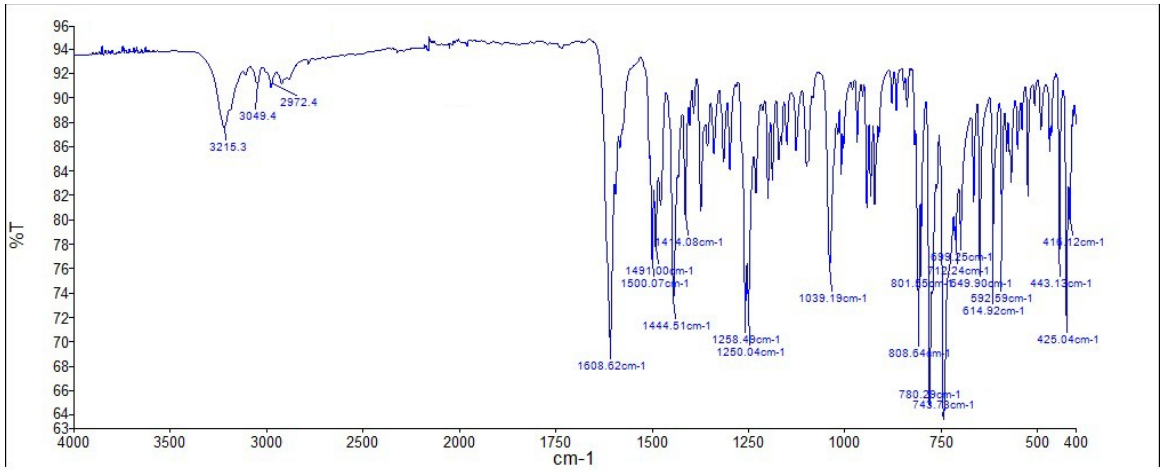
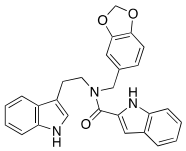
7.48
7.35
7.35
7.33
7.28
7.22
7.20
7.11
7.11
7.02
6.99
6.97
6.93
6.84
6.84
6.84
6.81
6.66
6.40
6.38
6.35
5.97
4.91
4.78
4.19
4.08
3.20
3.12
3.11
2.81
2.75
0.68
0.98
3.47
1.07
7.16
3.50
1.72
5.48
2.37
1.00
2.00
1.32
2.02
2.02
0.64
3.52
2.13
f1 (ppm)


170.36
170.01
148.02
147.99
147.08
147.02
132.26
129.46
127.48
126.92
125.79
124.10
123.25
122.09
118.48
111.76
110.65
109.12
101.38
51.77
49.19
47.13
44.50
24.87
23.14
f1 (ppm)



N-(2-(1H-Indol-3-yl)ethyl)-N-(benzo[d][1,3]dioxol-5-ylmethyl)-1H-indole-2-carboxamide (**20**)



Yield: 140 mg, 67%. MP 198-199 ºC;

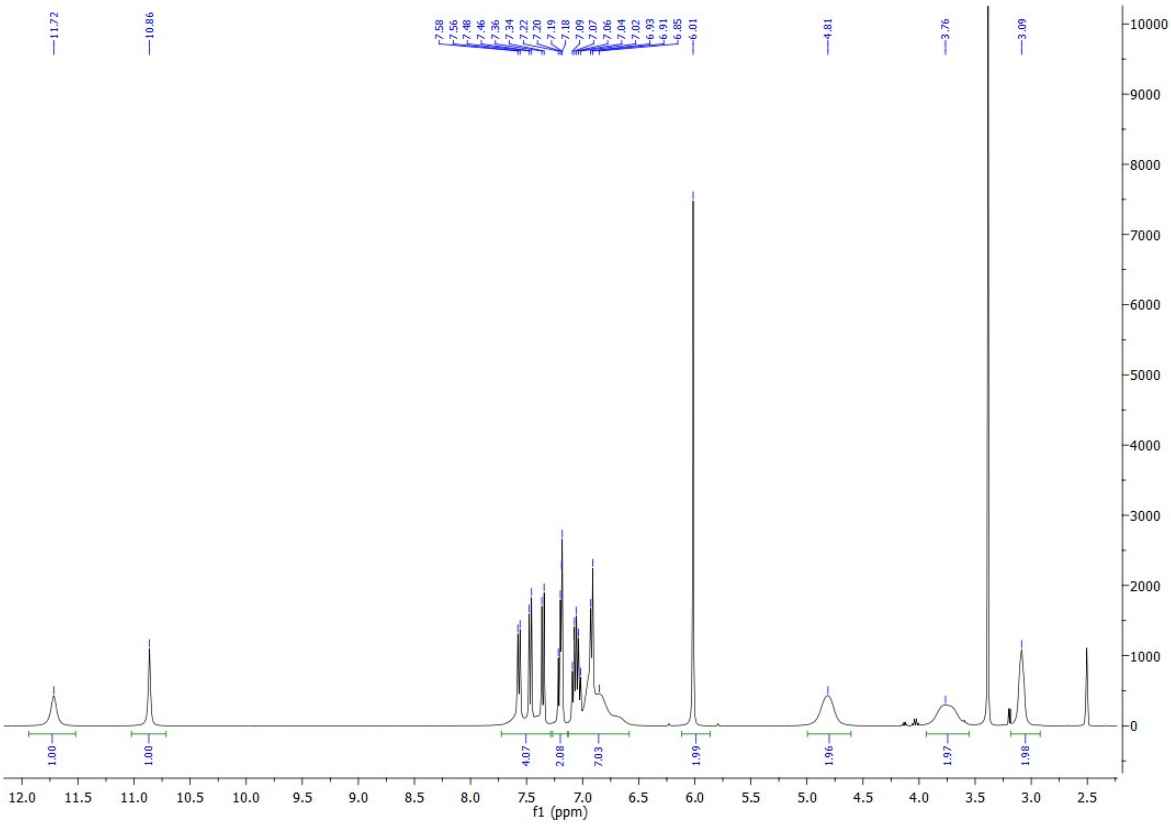
IR: ν_{max}/cm^{-1} 3440 (NH), 3274 (NH), 1620 (CON);

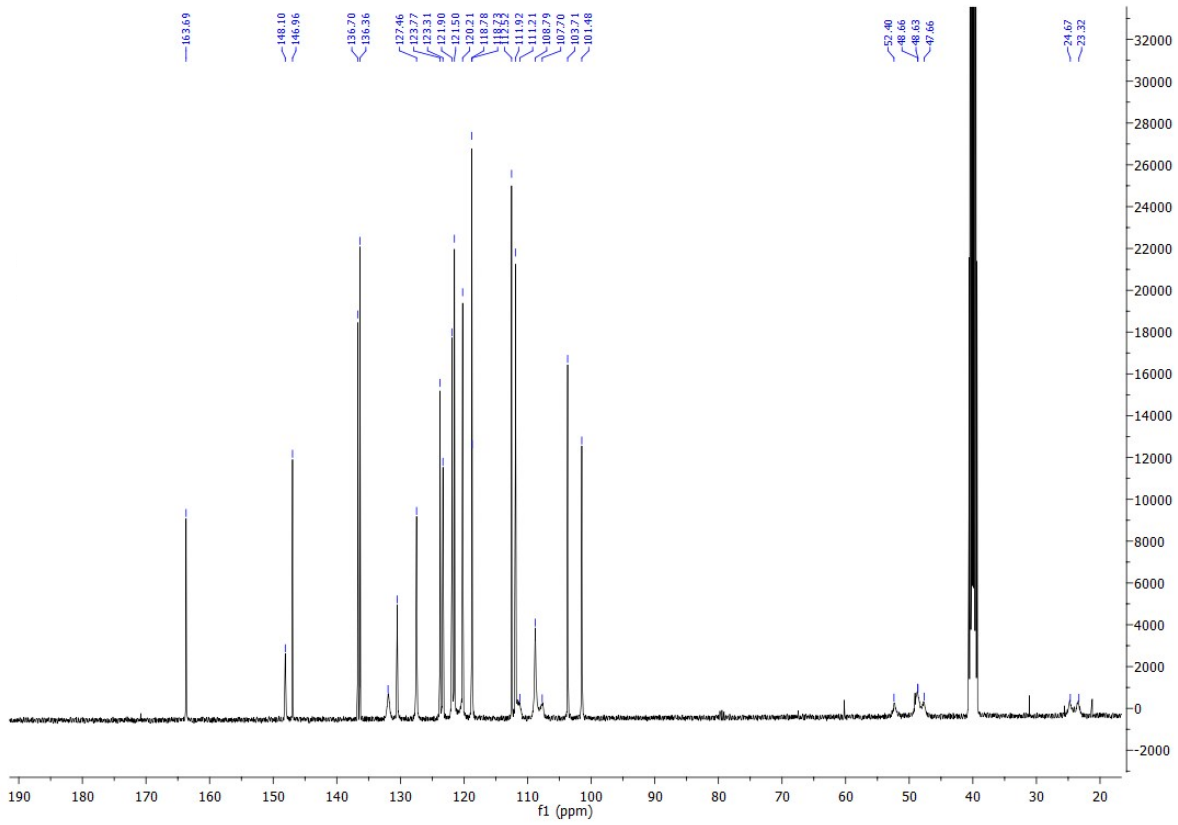
^{1}H NMR (400 MHz, DMSO-d_6) δ 11.72 (s, 1H), 10.86 (s, 1H), 7.72 – 7.29 (m, 4H), 7.20 (dd, *J* = 9.2, 4.8 Hz, 2H), 7.13 – 6.58 (m, 7H), 6.01 (s, 2H), 4.81 (bs, 2H), 3.76 (bs, 2H), 3.09 (s, 2H);

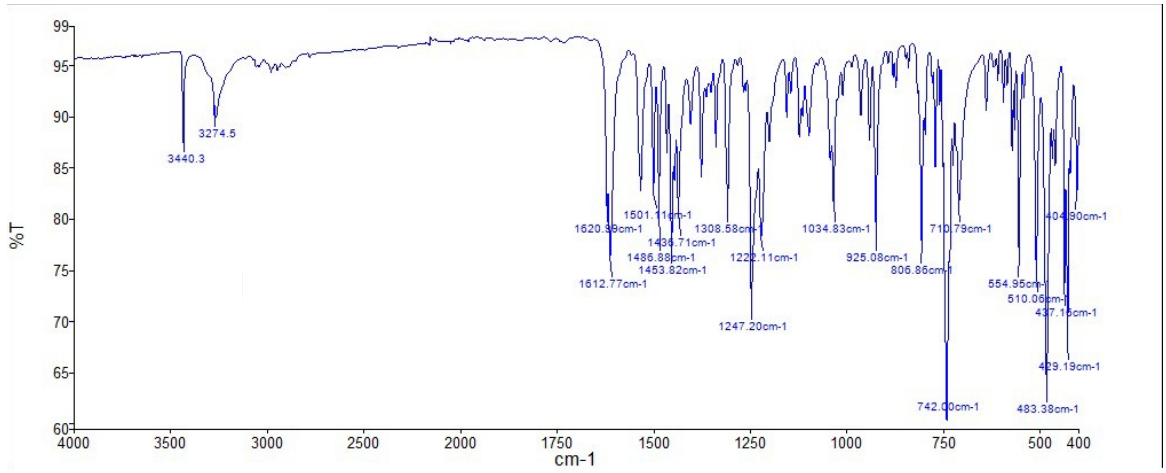
^{13}C NMR (101 MHz, DMSO-d_6) δ 163.7, 148.1, 147.0, 136.7, 136.4, 131.9, 130.5, 127.5, 123.8, 123.3, 121.9, 121.5, 120.2, 118.8, 118.7, 112.5, 111.9, 111.2, 108.8, 107.7, 103.7, 101.5, 52.4, 48.6, 47.7, 24.7, 23.3;

RP-HPLC Alltima™ C18 5 µm 150 mm x 4.6 mm, 10–100% B in 15 min, Rt = 14.68 min, 100%;

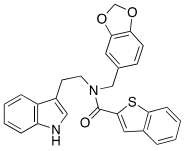
LRMS (APCI^{+}) m/z 437, 438 [M+1], 70%. HRMS (ES^{+}) for $C_{27}H_{23}N_3O_3$, calculated 438.18122, found 439.18110.







*Benzo[b]thiophene-2-carboxylic acid benzo[1,3]dioxol-5-ylmethyl-[2-(1H-indol-3-yl)-ethyl]-amide (**21**)*



Yield: 118 mg, 53%. MP 166 – 167 ºC;

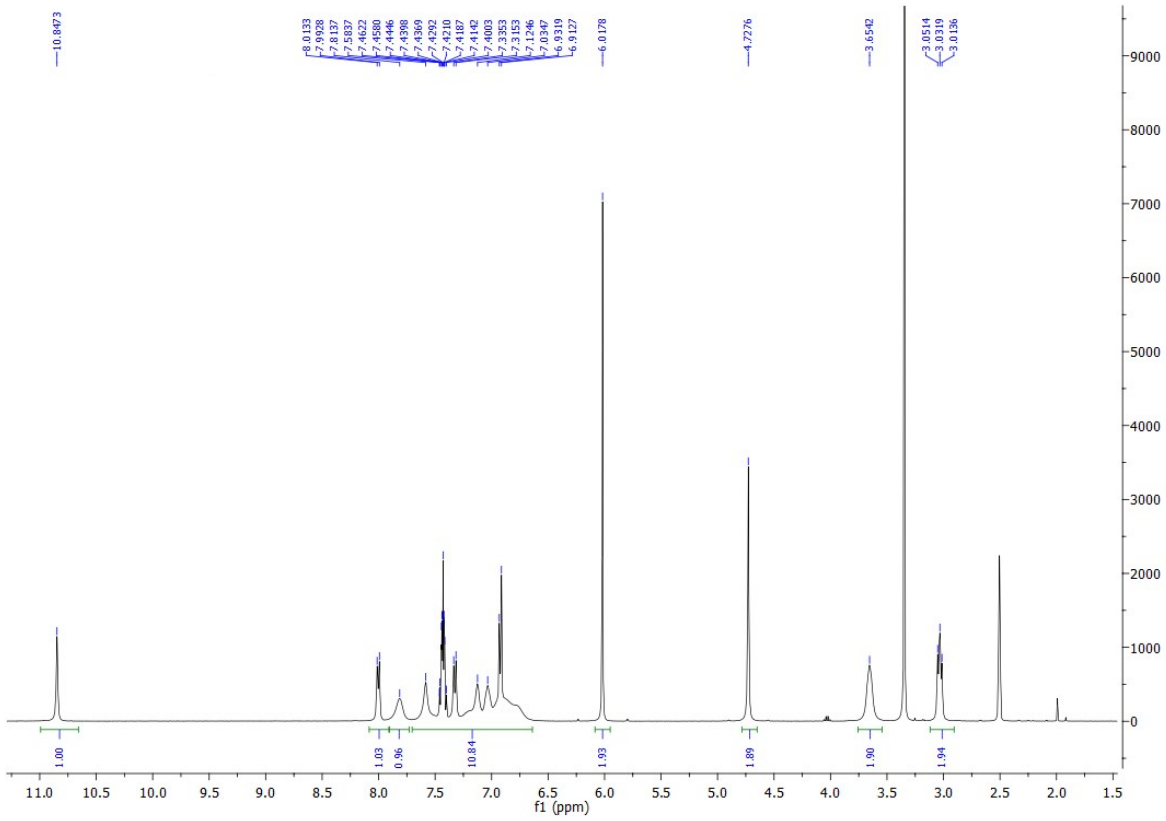
IR: νmax/cm^{-1} 3331 (NH), 1627 (CON);

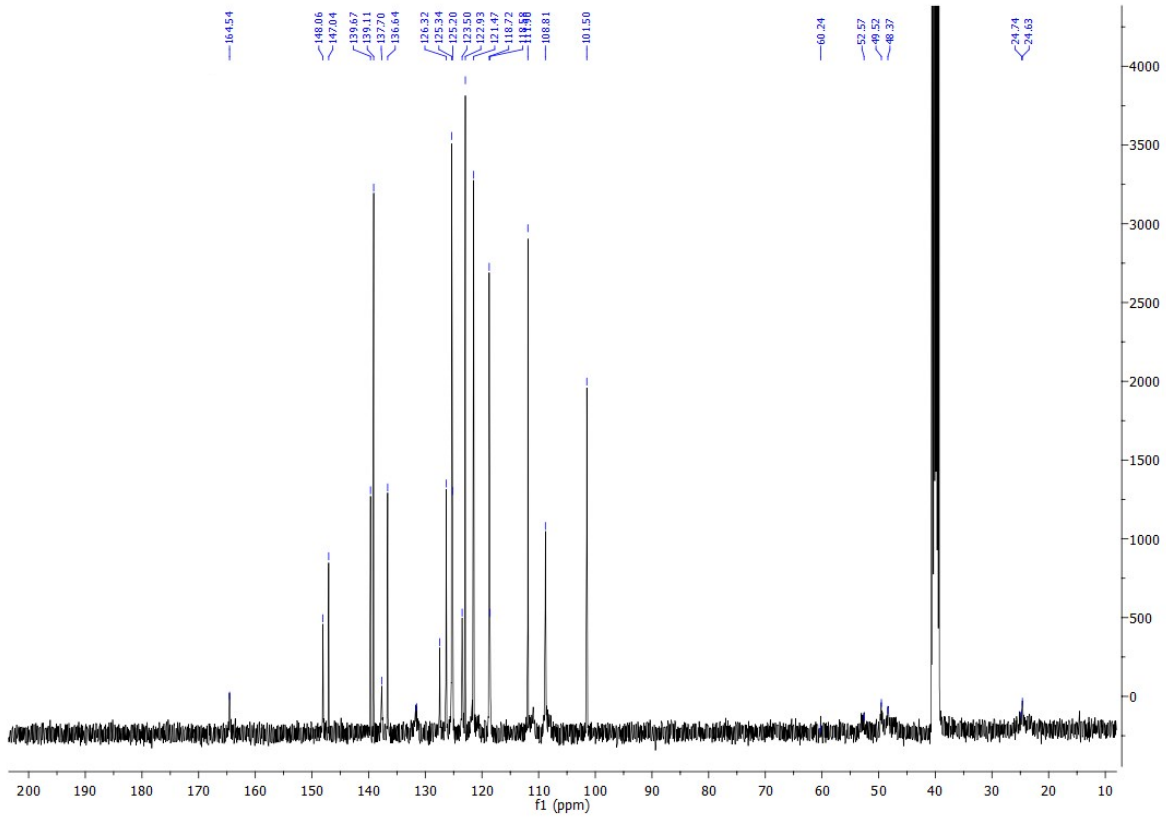
^{1}H NMR (400 MHz, DMSO-d_6) δ 10.85 (s, 1H), 8.00 (d, J = 8.2 Hz, 1H), 7.81 (s, 1H), 7.70 – 6.64 (m, 11H), 6.02 (s, 2H), 4.73 (s, 2H), 3.65 (s, 2H), 3.12 – 2.91 (m, 2H);

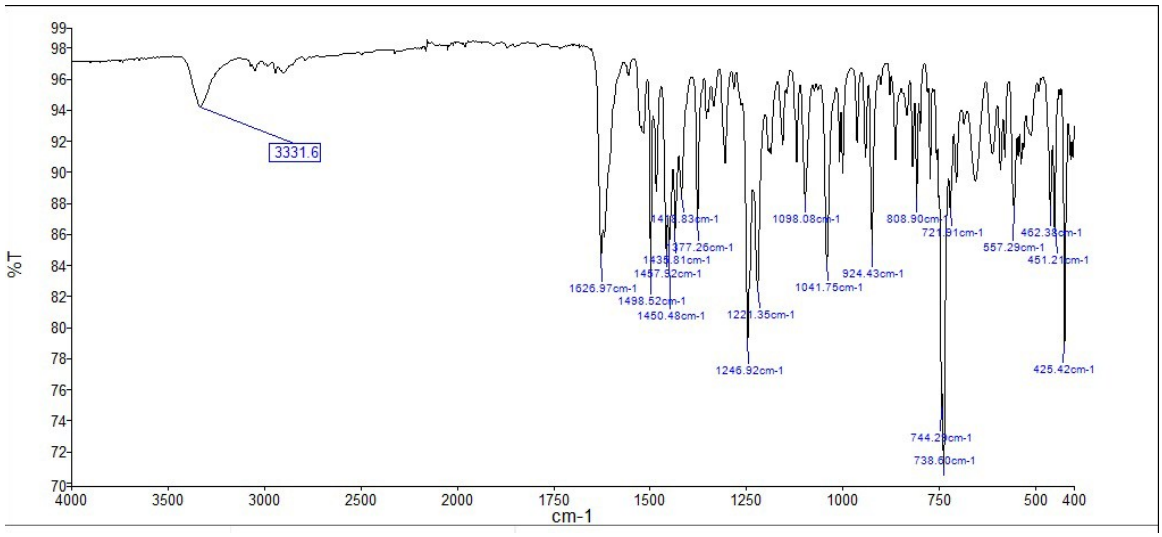
^{13}C NMR (101 MHz, DMSO-d_6) δ 164.5, 148.1, 147.0, 139.7, 139.1, 137.7, 136.6, 131.5, 127.5, 126.32, 125.3 (Cx2), 125.2, 123.5, 122.9, 121.5 (Cx2), 118.7 (Cx2), 118.6, 111.9, 110.9, 108.8, 101.5, 49.6, 48.4, 24.7;

RP-HPLC Alltima™ C18 5 µm 150 mm x 4.6 mm, 10–100% B in 15 min, Rt = 14.97 min, 100%;

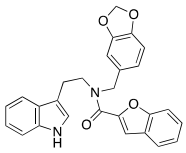
LRMS (APCI^{+}) m/z 454, 455 [M+H]$^{+}$, 100%. HRMS (ES^{+}) for $C_{27}H_{22}N_2O_3S$, calculated 455.14239, found 455.14231.


10.8473
8.0133
7.9928
7.8137
7.5637
7.4622
7.4580
7.4446
7.4398
7.4369
7.4292
7.4210
7.4187
7.4142
7.4003
7.3353
7.3153
7.1296
7.0347
6.9319
6.9127
6.0178
4.7276
3.6542
3.0514
3.0319
3.0136
1.00
1.03
0.96
10.84
1.93
1.89
1.90
1.94
f1 (ppm)


164.54
148.06
147.04
139.67
139.11
137.70
136.64
126.32
125.34
125.20
123.50
122.93
121.47
118.72
108.81
101.50
60.24
52.57
49.52
48.37
24.74
24.63
f1 (ppm)



*Benzofuran-2-carboxylic acid benzo[1,3]dioxol-5-ylmethyl-[2-(1H-indol-3-yl)-ethyl]-amide (**22**)*



Yield: 156 mg, 63%. MP 173 − 173.6 ºC;

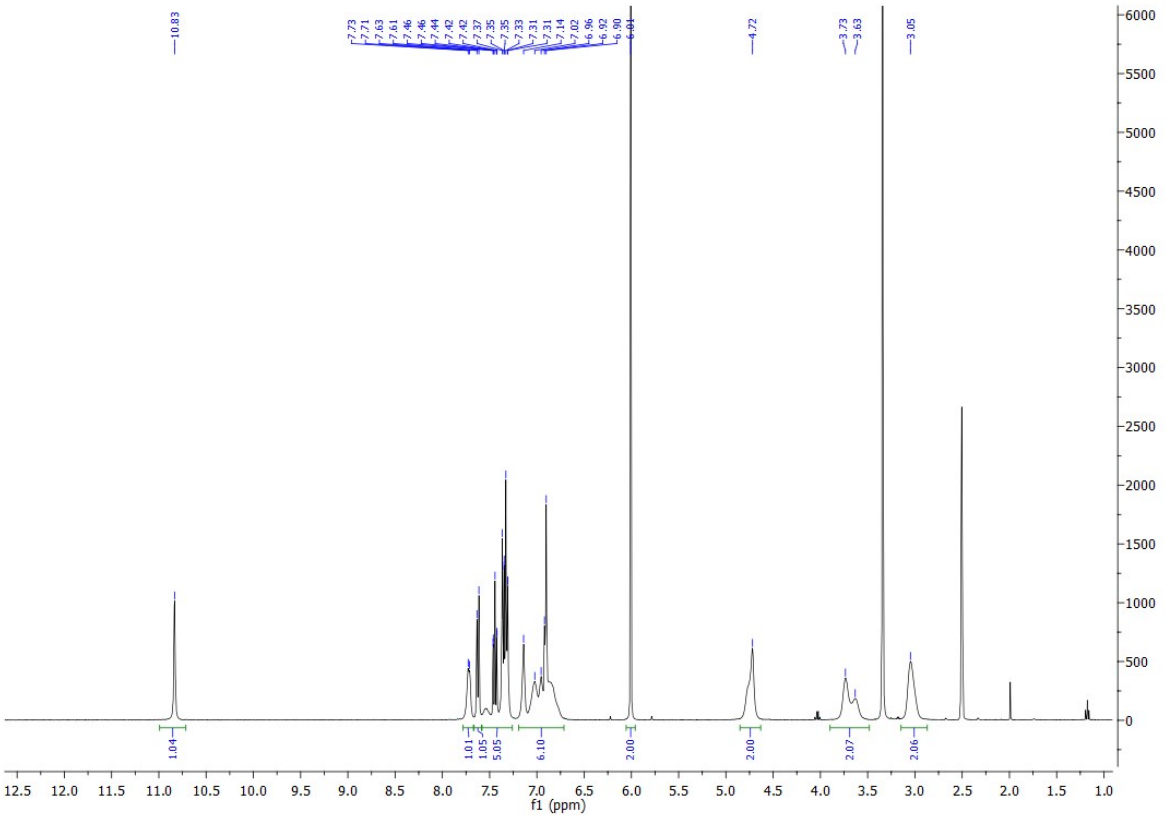
IR: ν_{max}/cm^{-1} 3316 (NH), 1627 (CON), 737 (CH-aromatic);

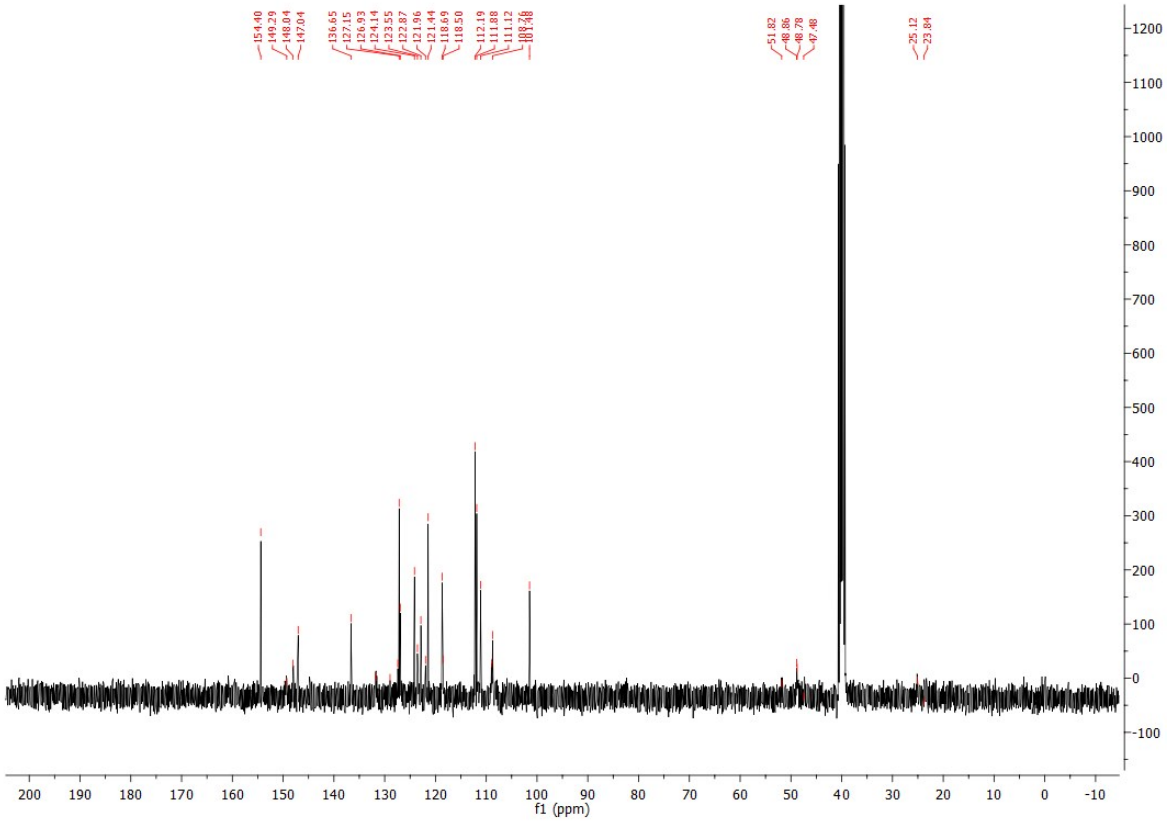
^{1}H NMR (400 MHz, DMSO-d_6) δ 10.83 (s, 1H), 7.72 (d, J = 5.8 Hz, 1H), 7.62 (d, J = 8.3 Hz, 1H), 7.58 – 7.26 (m, 5H), 7.19 – 6.71 (m, 6H), 6.01 (s, 2H), 4.72 (s, 2H), 3.91 −3.51 (m, 2H), 3.05 (s, 2H);

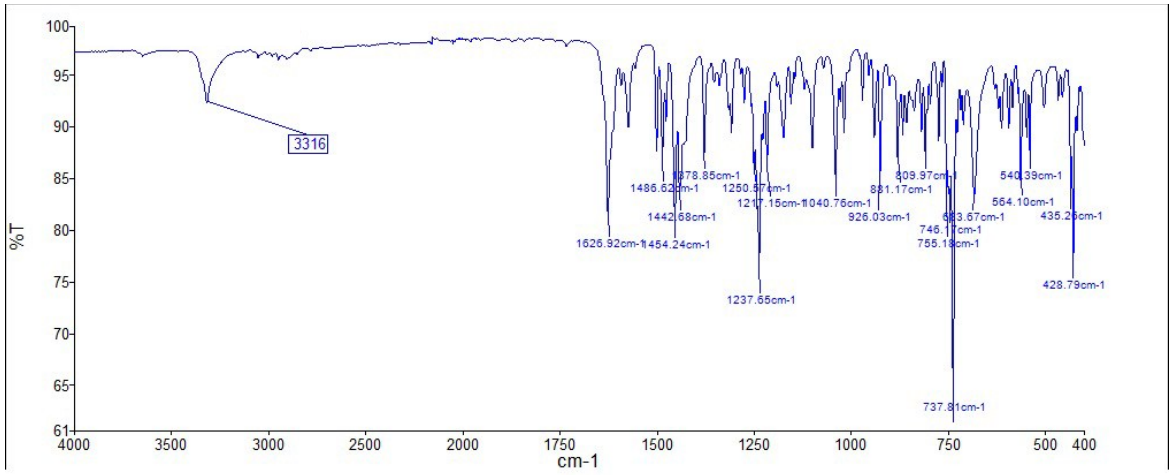
^{13}C NMR (101 MHz, DMSO-d_6) δ 154.4, 149.3, 148.0, 147.0, 136.7, 131.7, 129.0, 127.4, 127.2, 126.9, 124.1, 123.6, 122.9, 122.0, 121.4, 118.7, 118.5, 112.2, 111.9, 111.1, 109.0, 108.8, 101.5, 48.7, 48.8, 25.1;

RP-HPLC Alltima™ C18 5 µm 150 mm x 4.6 mm, 10–100% B in 15 min, Rt = 7.12 min, 100%;

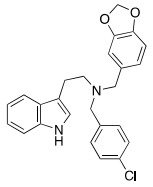
LRMS (APCI+/-) m/z 438, 439 $[M+H]^+$, 100%. HRMS (ES+) for $C_{27}H_{22}N_2O_4$, calculated 439.16523, found 439.16523.







N-Benzo[1,3]dioxol-5-ylmethyl-4-chloro-N-[2-(1H-indol-3-yl)-ethyl]-benzamide ***(23)***



Yield: 90 mg, 58%. MP 132-133 °C;

IR: ν_{max}/cm^{-1} 3203 (NH), 1626 (CON), 1500 (C=C aromatic), 1251 (C-N), 747 (C-H aromatic);

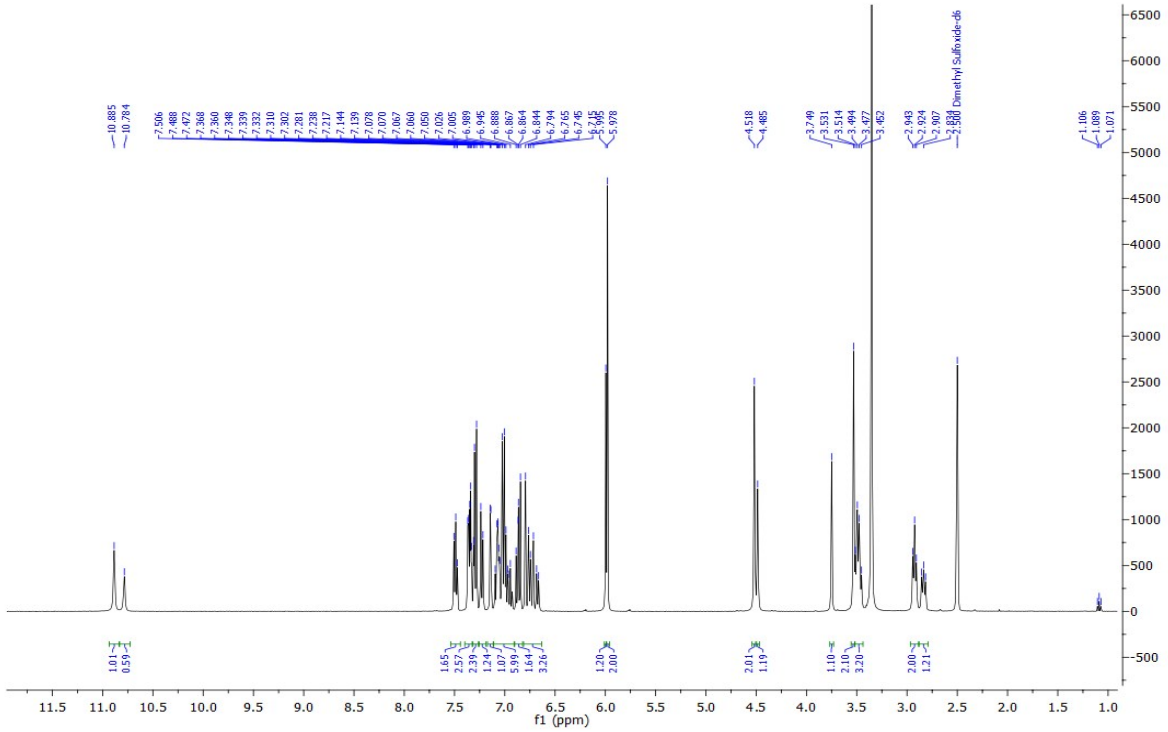
This is a mixture of atropoisomers of compound ***23*** *with the ratio approximately 2.0 : 1.2 calculated on the* CH_2 *splitting peaks at 2.93 and 2.84 ppm of the proton NMR.*

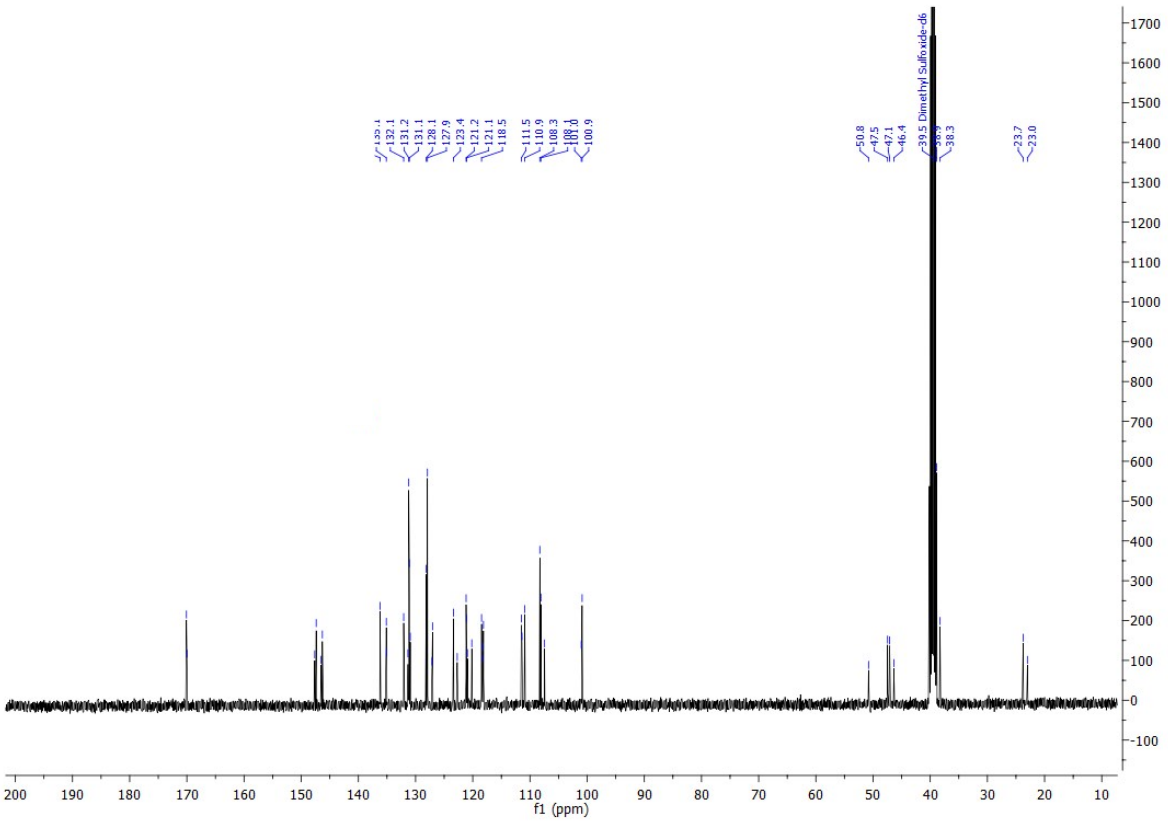
^{1}H NMR calculated separately for splitting peaks (400 MHz, DMSO-d_6) δ 10.89 (s, 2H), 10.79 (s, 1H), 7.50 (t, J = 6.8 Hz, 3H), 7.40-7.26 (m, 9H), 7.23 (d, J = 8.4 Hz, 2H), 7.15 (d, J = 1.9 Hz, 2H), 7.12-6.91 (m, 11H), 6.90-6.82 (m, 3H), 6.82 – 6.62 (m, 6H), 6.03-5.94 (m, 6H), 4.57-4.46 (m, 6H), 3.59 – 3.43 (m, 10H), 2.93 (t, J = 7.2 Hz, 4H), 2.89 – 2.78 (m, 2H);

^{13}C NMR calculated separately for splitting peaks (101 MHz, DMSO-d_6) δ 170.1 and 170.0 (1C), 147.6 and 147.4 (1C), 146.5 and 146.3 (1C), 136.2 (1C), 135.1 and 135.0 (1C), 132.1, 131.3 and 131.2 (2C), 131.1 and 130.9 (1C), 128.1 and 127.9 (2C), 127.1 and 127.0 (1C), 123.4, 122.7, 121.2 and 121.1 (1C), 120.9 and 120.2 (1C), 118.5 and 118.3 (1C), 118.2 and 118.1 (1C), 111.5 and 111.4 (1C), 110.9, 108.3 and 108.1 (1C), 107.4, 101.0 and 100.9 (1C), 50.8 and 47.1 (1C), 47.5 and 46.4 (1C), 38.9 and 38.3(1C), 23.8 and 23.0 (1C);

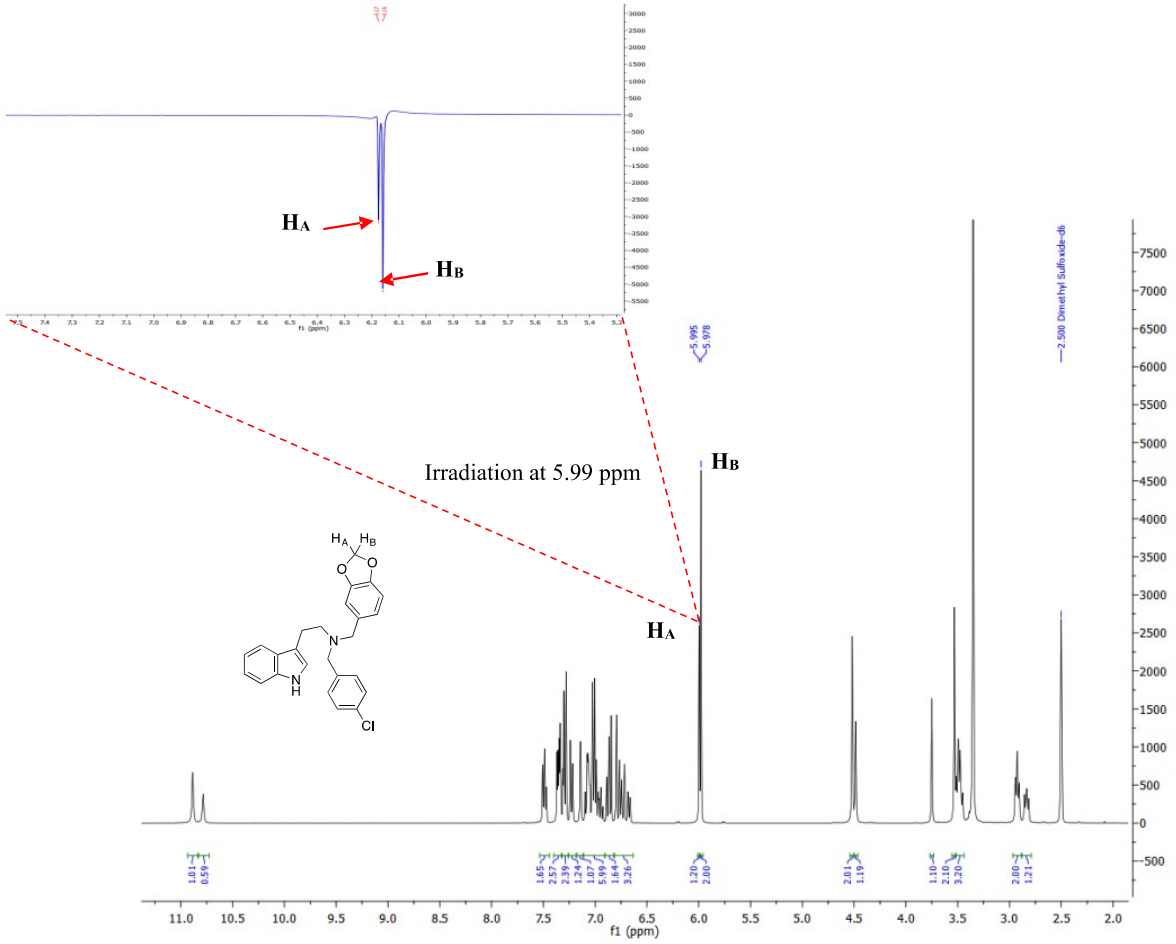
RP-HPLC Alltima™ C18 5 μm 150 mm x 4.6 mm, 10–100% B in 15 min, Rt = 14.76 min, 100%;

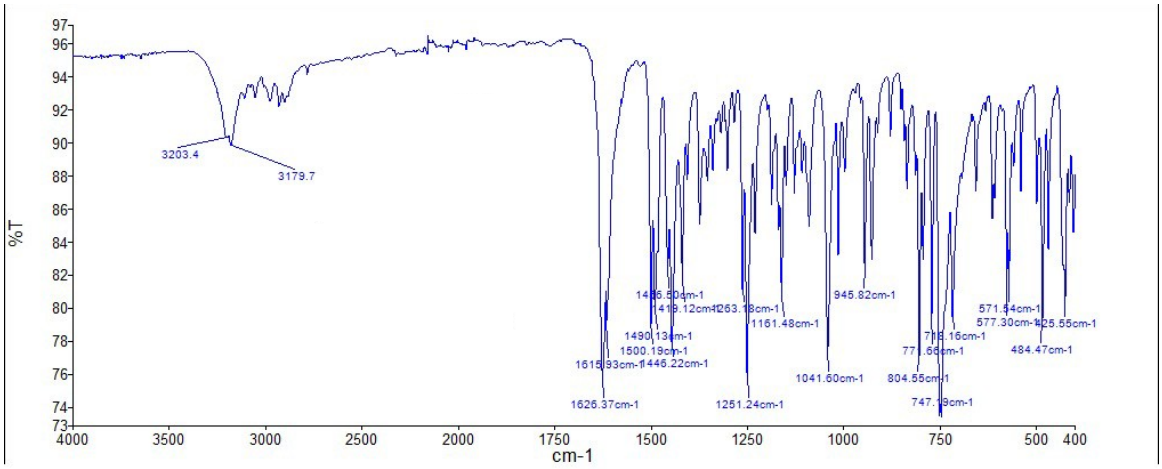
LRMS m/z APCI (+) 446, 447 $[M+H]^+$ 100%; HRMS (ES^+) calculated for $C_{26}H_{23}ClN_2O_3$ 446.1397, fo 447.1467.



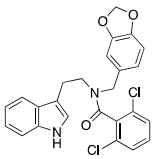


Selective NOSEY at 5.99 ppm





N-Benzo[1,3]dioxol-5-ylmethyl-2,6-dichloro-N-[2-(1H-indol-3-yl)-ethyl]-benzamide ***(24)***



Yield: 100mg, 65%. MP 157-158 ºC;

IR: ν_{max}/cm^{-1} 3280 (NH), 2937 (CH), 1626 (CON), 739 (CH- aromatics);

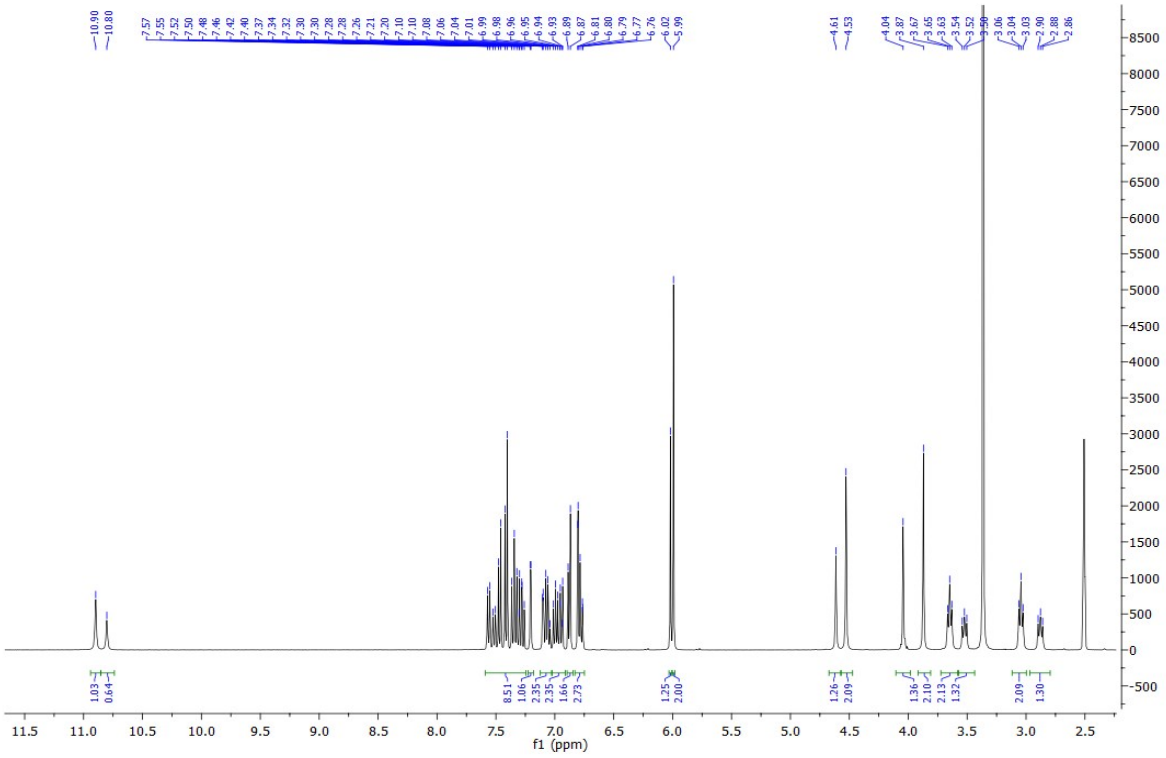
This is a mixture of atropoisomers of compound ***24*** *with the ratio approximately 2.0 : calculated on the CH_2 splitting peaks at 5.99 and 6.02 ppm of the proton NMR.*

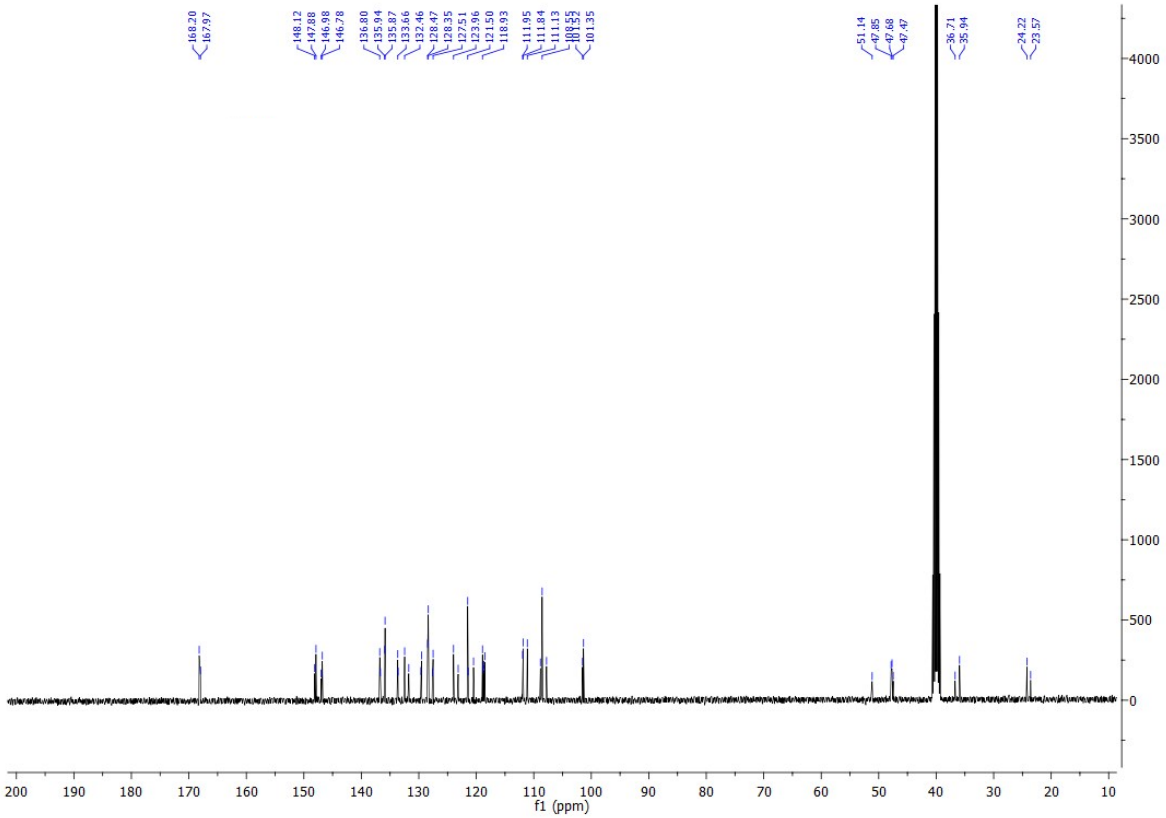
^{1}H NMR (400 MHz, DMSO-d_6) δ 10.90 (s, 1H), 10.80 (s, 0.7H), 7.59 – 7.25 (m, 8.3H), 7.21 (d, 2.2 Hz, 1H), 7.10 (s, 0.3H), 7.07 (dd, J = 15.0, 8.0 Hz, 2H), 7.03 – 6.91 (m, 2.3H), 6.88 (d, J = 7.7 Hz, 1.7H), 6.8 6.75 (m, 2.7H), 6.02 (s, 1.3H), 5.99 (s, 2H), 4.61 (s, 1.3H), 4.53 (s, 2H), 4.04 (s, 1.3H), 3.87 (s, 2H), 3.65 (t, J = 7.2 H 2H), 3.58 – 3.44 (m, 1.3H), 3.04 (t, J = 7.1 Hz, 2H), 2.97 – 2.80 (m, 1.3H);

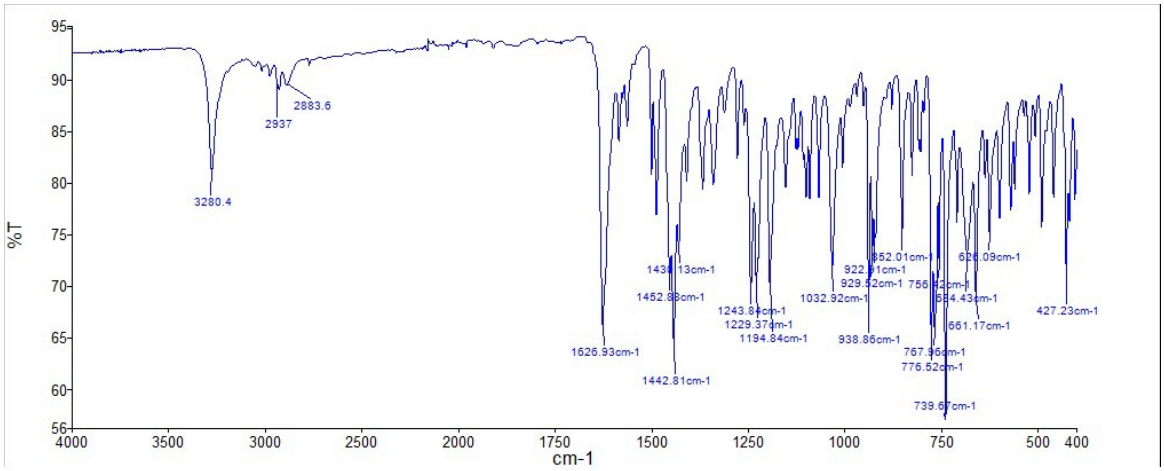
^{13}C NMR (101 MHz, DMSO-d_6) δ 168.2, 168.0, 148.1, 147.9, 147.0, 146.8, 136.8, 136.7, 135.9, 135.9, 13
133.6, 132.5, 131.8, 129.6, 129.5, 128.5, 128.4, 127.6, 127.5, 124.0, 123.2, 121.5, 121.4, 120.5, 118.9, 118.7, 11
118.5, 112.0, 111.8, 111.1, 108.9, 108.6, 107.8, 101.5, 101.4, 51.1, 47.9, 47.7, 47.5, 36.7, 35.9, 24.2, 23.6;

RP-HPLC Alltima™ C18 5 µm 150 mm x 4.6 mm, 10–100% B in 15 min, Rt = 18.81 min, 100%;

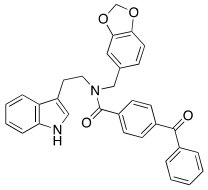
LRMS (ESI$^+$) m/z 481, 481[M]$^+$, 100%. HRMS (ES$^+$) for $C_{26}H_{22}Cl_2N_2O_3$, calculated 480.1007, found 481.1080.







*N-Benzo[1,3]dioxol-5-ylmethyl-4-benzoyl-N-[2-(1H-indol-3-yl)-ethyl]-benzamide (**25**)*



Yield: 77 mg, 40%. MP 181.2-181.7 ºC;

IR: ν_{max}/cm^{-1} 3191 (NH), 2990 (CH), 1643 (CON), 742 (CH-aromatic);

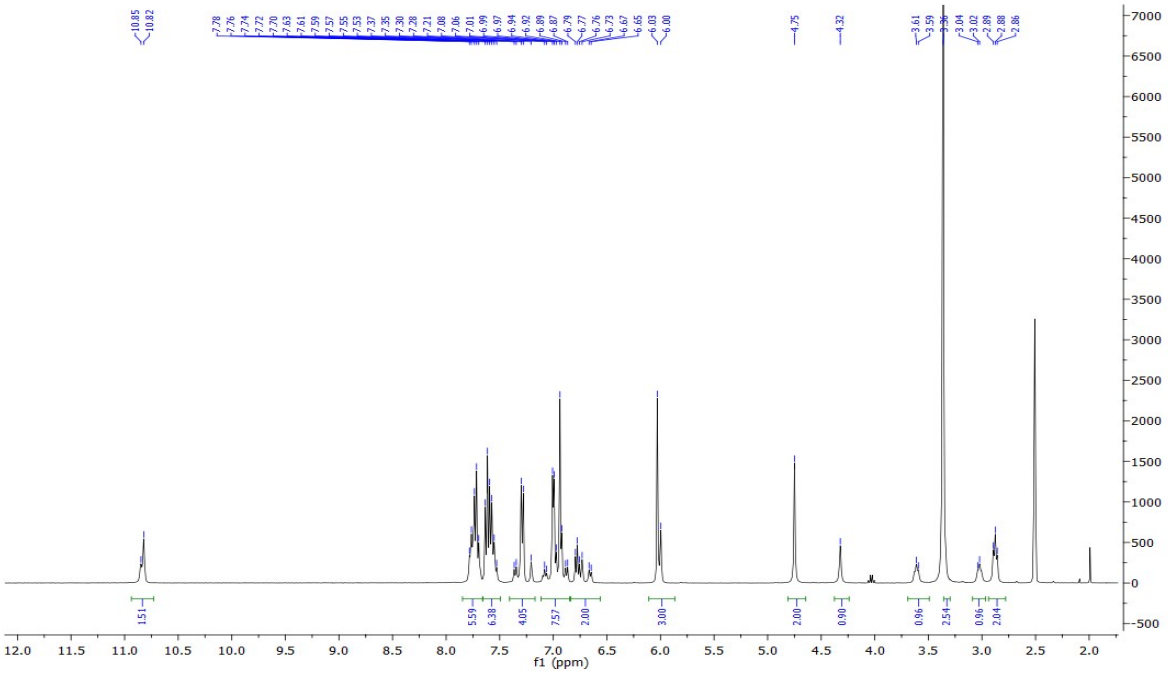
*This is a mixture of atropoisomers of compound **25** with the ratio approximately 2.0 : 0.9 calculated on the CH_2 splitting peaks at 4.75 and 6.02 ppm of the proton NMR.*

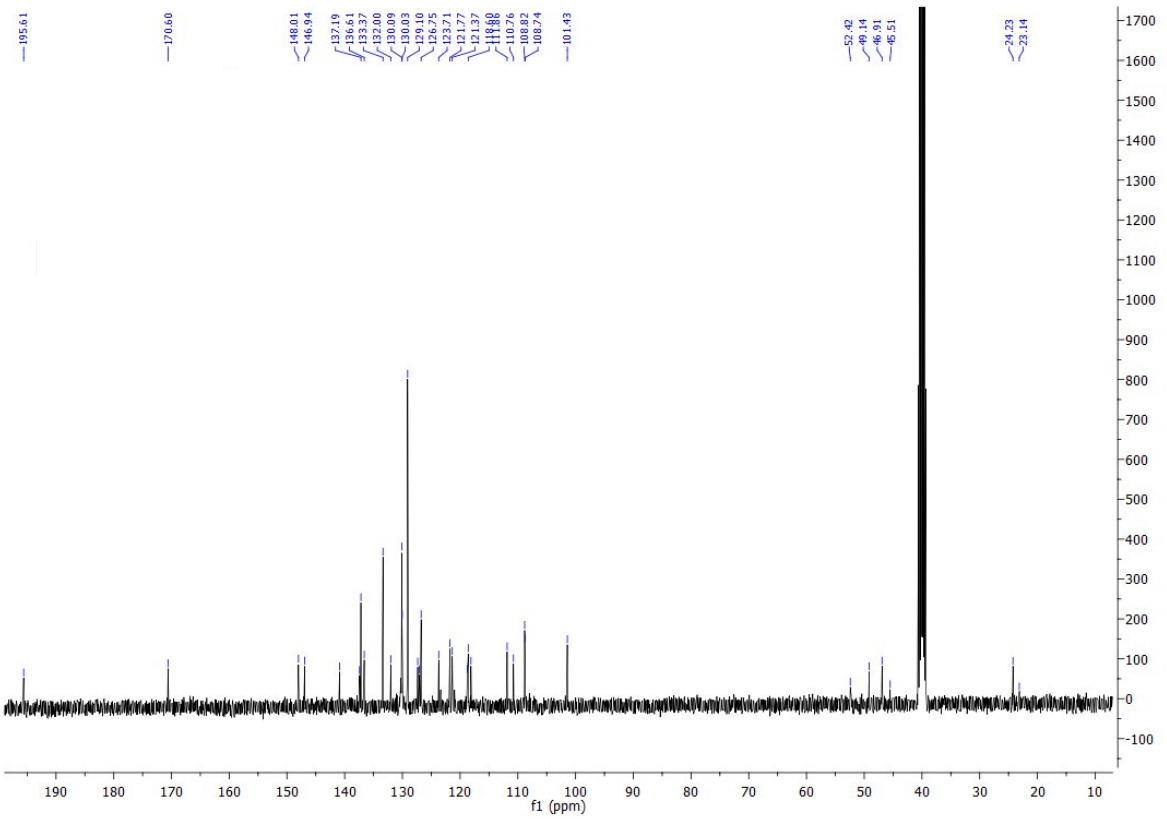
^{1}H NMR (400 MHz, DMSO-d_6) δ 10.83 (d, J = 10.1 Hz, 1.5H), 7.85 – 7.66 (m, 5.5H), 7.58 (dt, J = 18.2, 8.8 Hz, 6.5H), 7.41 – 7.17 (m, 4H), 7.11 – 6.85 (m, 7.5H), 6.84 – 6.56 (m, 2H), 6.01 (d, J = 13.2 Hz, 3H), 4.75 (s, 2H), 4.32 (s, 1H), 3.60 (d, J = 7.1 Hz, 1H), 3.36 – 3.30 (overlapped by water) (m, 2.5H), 3.03 (d, J = 7.1 Hz, 1H), 2.88 (t, J = 7.1 Hz, 2H);

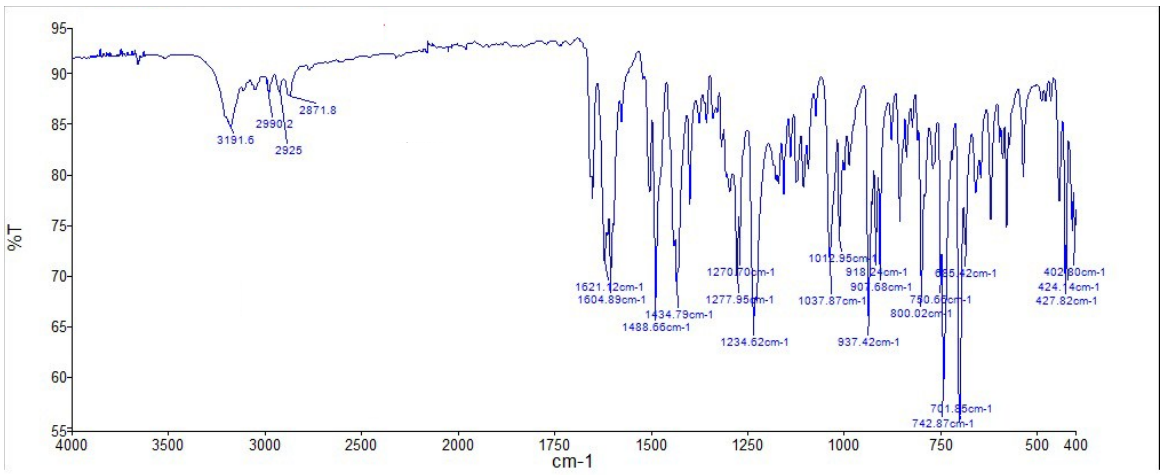
^{13}C NMR (101 MHz, DMSO-d_6) δ 195.6, 170.6, 148.0, 146.9, 140.9, 137.4, 137.2, 136.6, 133.4, 132.0, 130.1, 130.0, 129.1, 127.4, 127.1, 126.8, 123.7, 121.8, 121.4, 118.7, 118.6, 118.1, 111.9, 110.8, 108.8, 108.7, 101.4, 52.4, 49.1, 46.9, 45.5, 24.2, 23.1;

RP-HPLC Alltima™ C18 5 µm 150 mm x 4.6 mm, 10–100% B in 15 min, Rt =17.71 min, 100 %

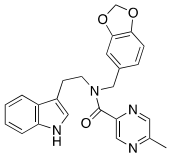
LRMS (APCI+/-) m/z 502, 503 [M+1], 100%. HRMS (ES$^+$) for $C_{32}H_{26}N_2O_4$, calculated 503.19653, found 503.19636.







*5-Methylpyrazine-2-carboxylic acid benzo[1,3]dioxol-5-ylmethyl-[2-(1H-indol-3-yl)-ethyl]-amide (**26**)*



Yield: 47 mg, 22%. MP 132 −133 ºC;

IR: ν_{max}/cm^{-1} 3316 (NH), 1632 (CON), 1632 (CON), 739 (CH-aromatic);

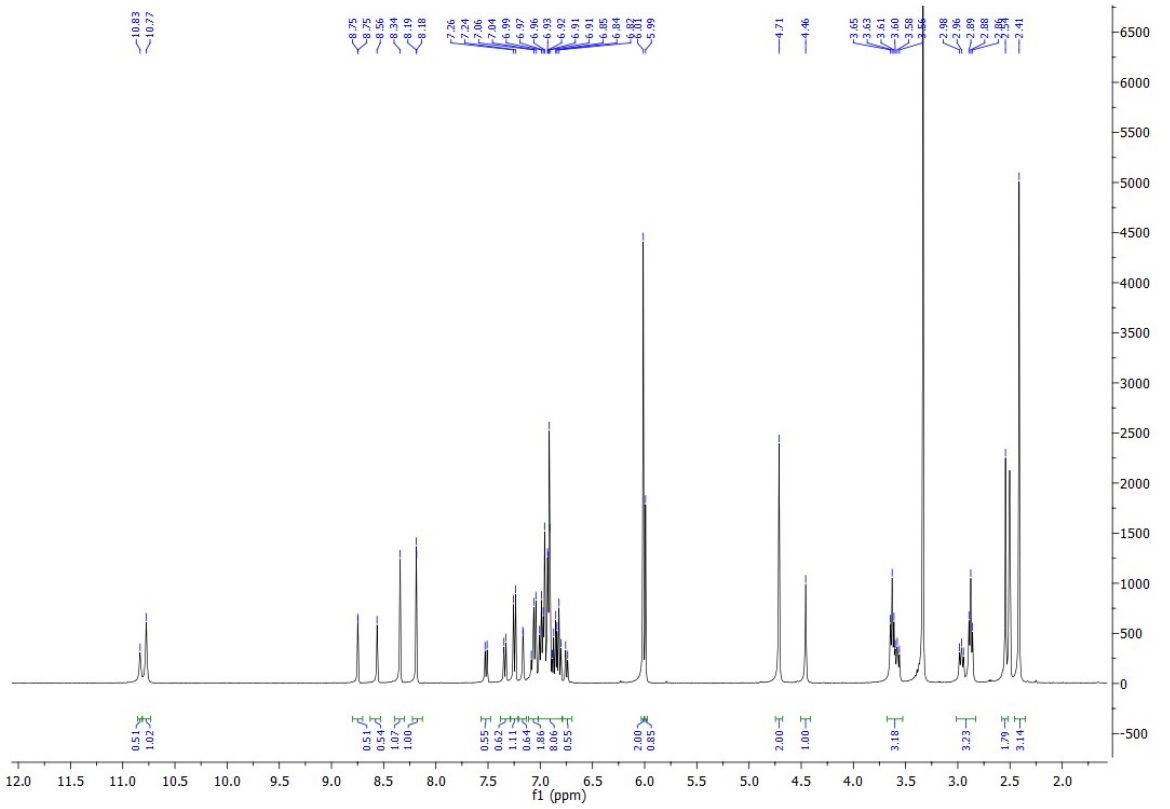
*This is a mixture of atropoisomers of compound **26** with the ratio approximately 2 : 1 calculated on the CH_2 splitting peaks at 4.71 and 4.46 ppm of the proton NMR. ^{1}H NMR is reported as displayed on spectra. All peaks in ^{13}C NMR are reported.*

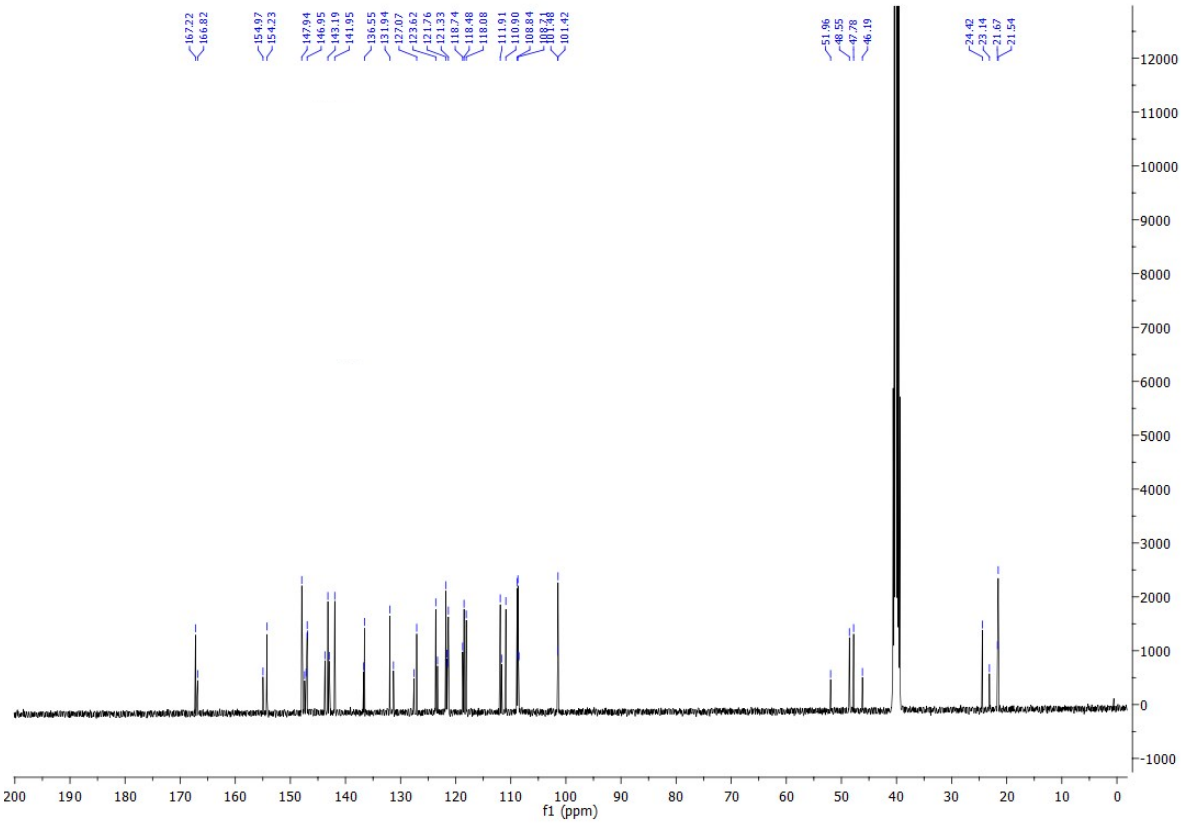
^{1}H NMR (400 MHz, DMSO-d_6) δ 10.83 (s, 0.5H), 10.77 (s, 1H), 8.75 (d, J = 1.2 Hz, 0.5H), 8.56 (s, 0.5H), 8.34 (s, 1H), 8.19 (d, J = 1.3 Hz, 1H), 7.52 (d, J = 7.8 Hz, 0.5H), 7.34 (d, J = 8.1 Hz, 0.7H), 7.25 (d, J = 8.1 Hz, 1.2H), 7.16 (d, J = 2.0 Hz, 0.7H), 7.11 – 7.02 (m, 1.8H), 7.02 – 6.79 (m, 8H), 6.75 (d, J = 7.9 Hz, 0.5H), 6.01 (s, 2H), 5.99 (s, 0.8H), 4.71 (s, 2H), 4.46 (s, 1H), 3.60 (dt, J = 15.8, 7.5 Hz, 3.2H), 2.92 (dt, J = 13.8, 7.5 Hz, 3.2H), 2.54 (s, 1.8H), 2.41 (s, 3.2);

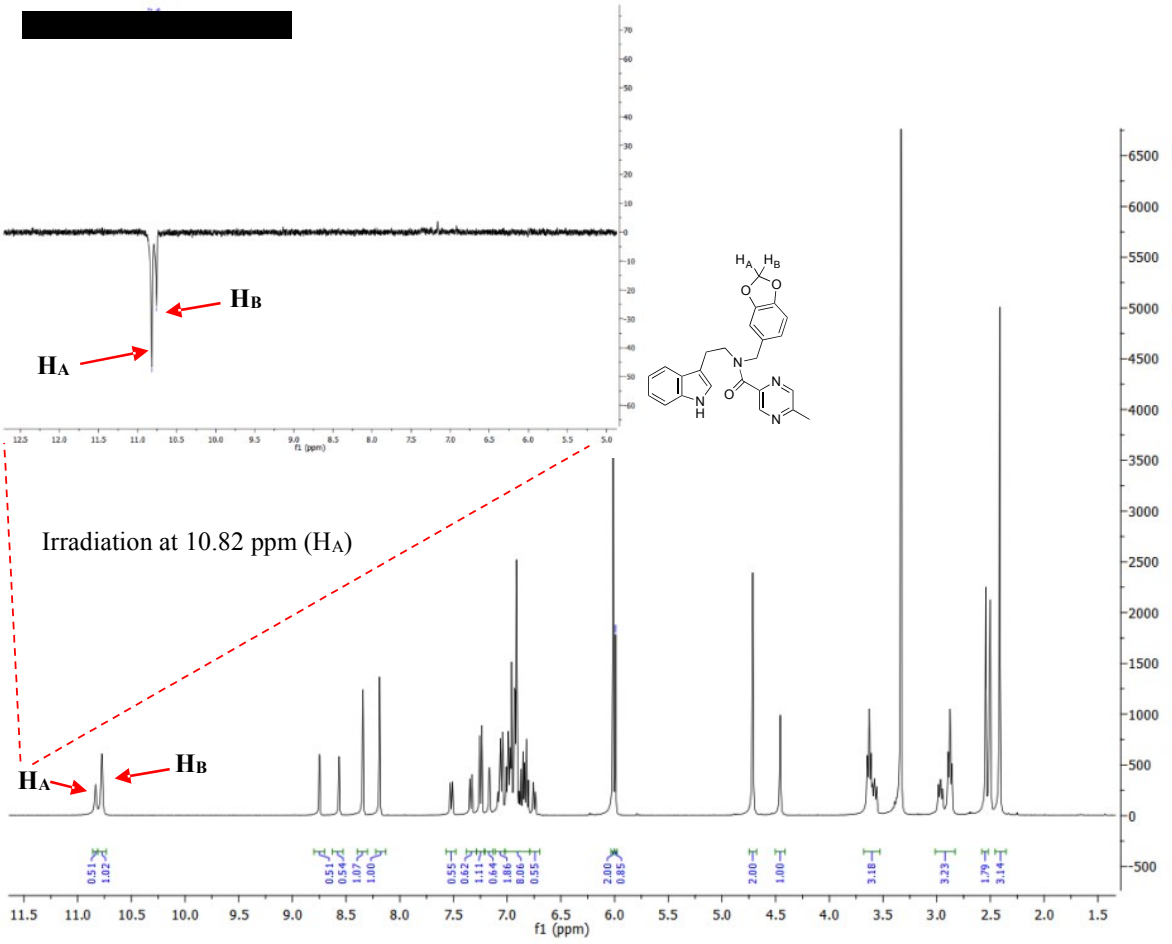
^{13}C NMR (101 MHz, DMSO-d_6) δ 167.2, 166.8, 155.0, 154.2, 147.9, 147.4, 147.1, 147.0, 146.9, 143.7, 143.2, 143.0, 142.0, 136.7, 136.6, 131.9, 131.3, 127.6, 127.1, 123.6, 123.3, 121.8, 121.6, 121.5, 121.3, 118.7, 118.5, 118.1, 111.9, 111.7, 110.9, 108.8, 108.7, 108.6, 108.5, 101.5, 101.4, 52.0, 48.6, 47.8, 46.192, 24.4, 23.1, 21.7, 21.5;

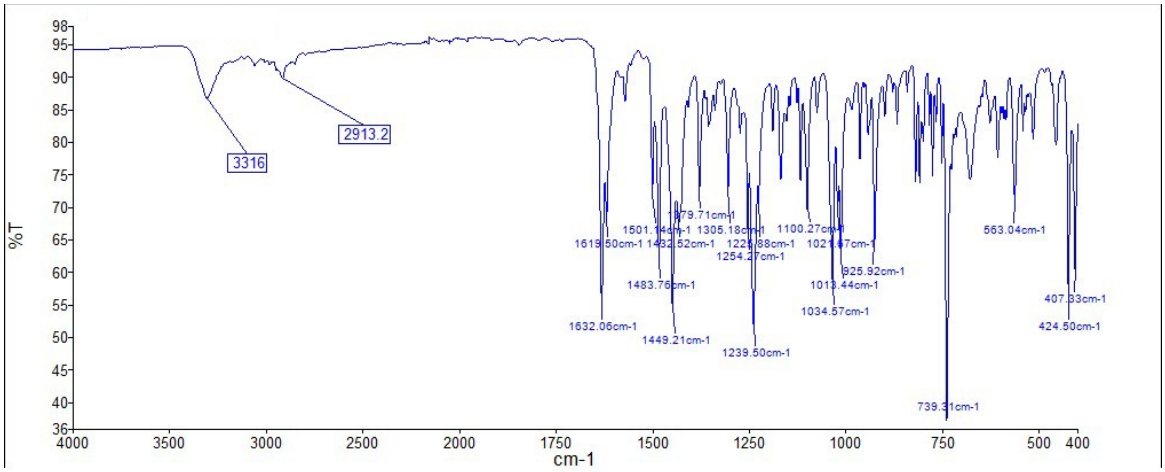
RP-HPLC Alltima™ C18 5 µm 150 mm x 4.6 mm, 10–100% B in 15 min, Rt = 12.40 min, 100%;

LRMS (APCI±) m/z 414, 415 $[M+H]^+$, 100%. HRMS (ES^+) for $C_{24}H_{22}N_4O_3$, calculated 415.17647, fo
415.17611.

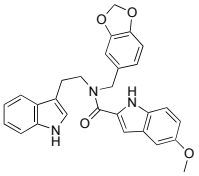








*5-Methoxy-1H-indole-2-carboxylic acid benzo[1,3]dioxol-5-ylmethyl-[2-(1H-indol-3-yl)-ethyl]-amide (**27**)*



Yield: 149 mg, 62%. MP 202-202.5 ºC;

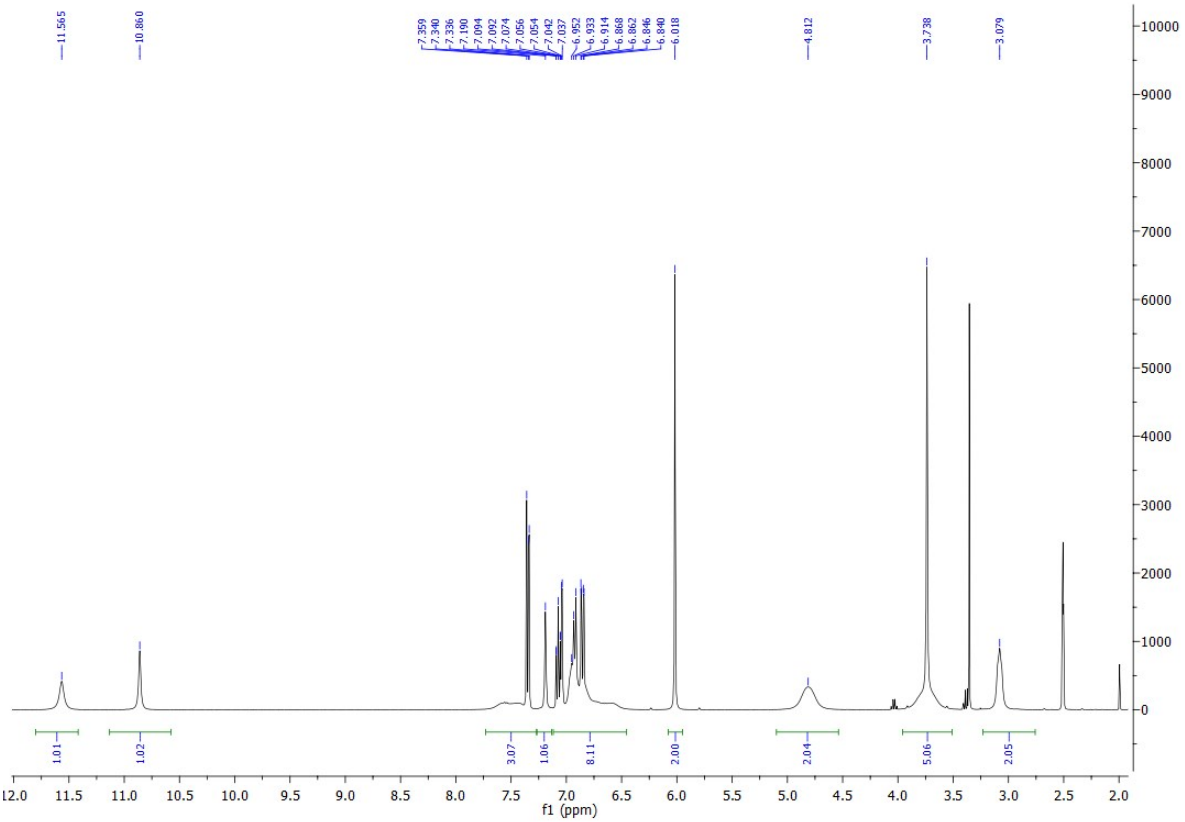
IR: ν_{max}/cm^{-1} 3439 (NH), 3258 (NH), 1612 (CON), 1450 (C-C ring), 738 (C-H ring);

^{1}H NMR (400 MHz, DMSO-d_6) δ 11.57 (s, 1H), 10.86 (s, 1H), 7.68-7.26 (m, 3H), 7.19 (s, 1
7.13 – 6.48 (m, 8H), 6.01 (s, 2H), 4.81 (s, 2H), 3.73 (s, 5H), 3.08 (s, 2H);

^{13}C NMR (101 MHz, DMSO-d_6) δ 163.6, 154.2, 148.1, 146.9, 136.7, 132.0, 131.6, 13(
127.8, 127.6, 123.3, 121.5 (Cx2), 118.8 (Cx2), 115.0, 113.4, 111.9, 108.8, 103.5 (Cx2), 102
101.5, 65.4, 55.7, 48.5, 48.1, 23.6;

RP-HPLC Alltima™ C18 5 µm 150 mm x 4.6 mm, 10–100% B in 15 min, R_t = 6.92 min, 100%;

LRMS (ESI^+) m/z 467, 467 $[M]^+$, 100%; HRMS (ES^+) for $C_{28}H_{25}N_3O_4$, calculated 468.19178, found 468.19186.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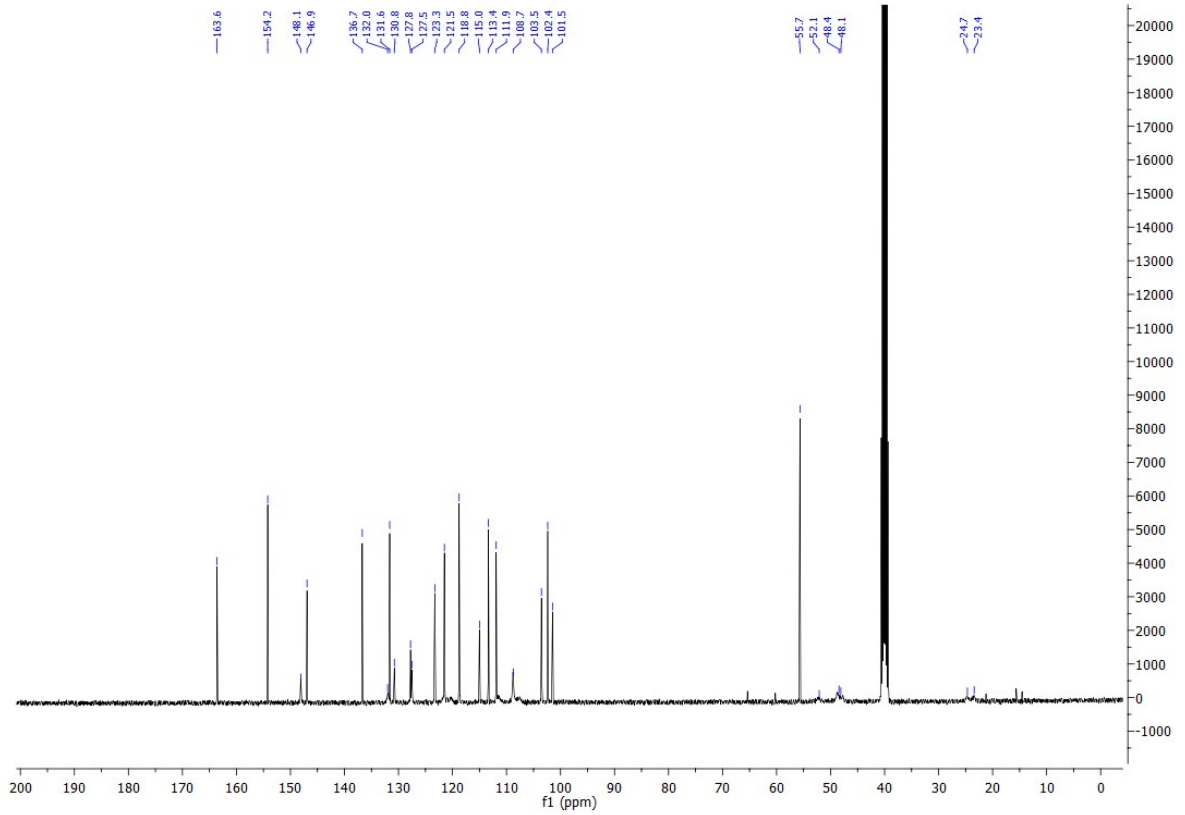
10.860
7.359
7.340
7.336
7.190
7.094
7.092
7.074
7.056
7.054
7.044
7.042
7.037
6.952
6.933
6.914
6.868
6.862
6.846
6.840
6.018
4.812
3.738
3.079
1.01
1.02
3.07
1.06
8.11
2.00
2.04
5.06
2.05
f1 (ppm)


163.6
154.2
148.1
146.9
136.7
132.0
131.6
130.8
127.8
123.5
123.3
121.5
118.8
115.0
113.4
111.9
108.7
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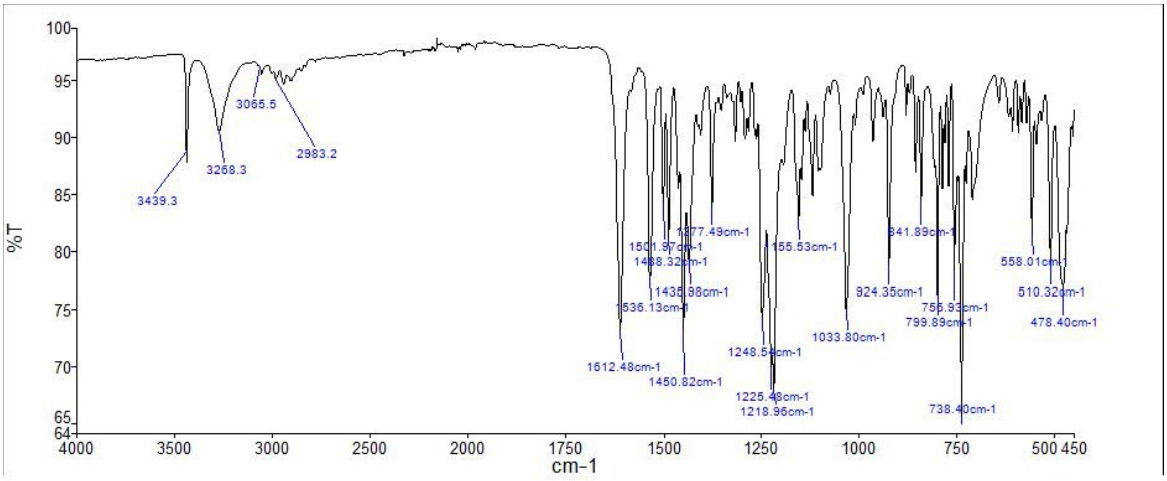
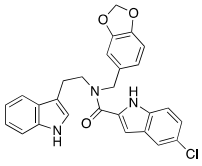
102.4
101.5
55.7
52.1
48.4
48.1
24.7
23.4
f1 (ppm)



5-Chloro-1H-indole-2-carboxylic acid benzo[1,3]dioxol-5-ylmethyl-[2-(1H-indol-3-yl)-ethyl]-amide (***28***)



Yield: 170 mg, 64%. MP 194 −194.5 ºC;

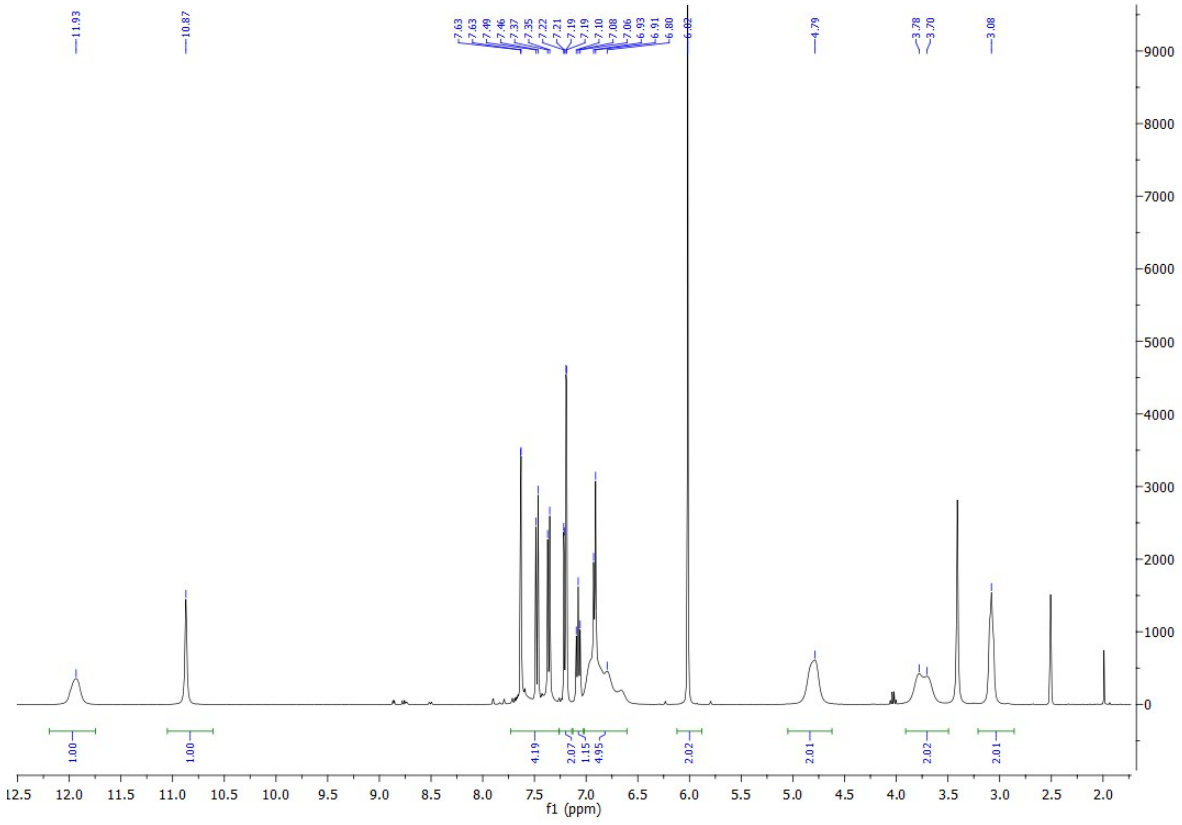
IR: ν_{max}/cm^{-1} 3433 (NH), 3265(NH), 1612 (CON), 739 (CH-aromatics);

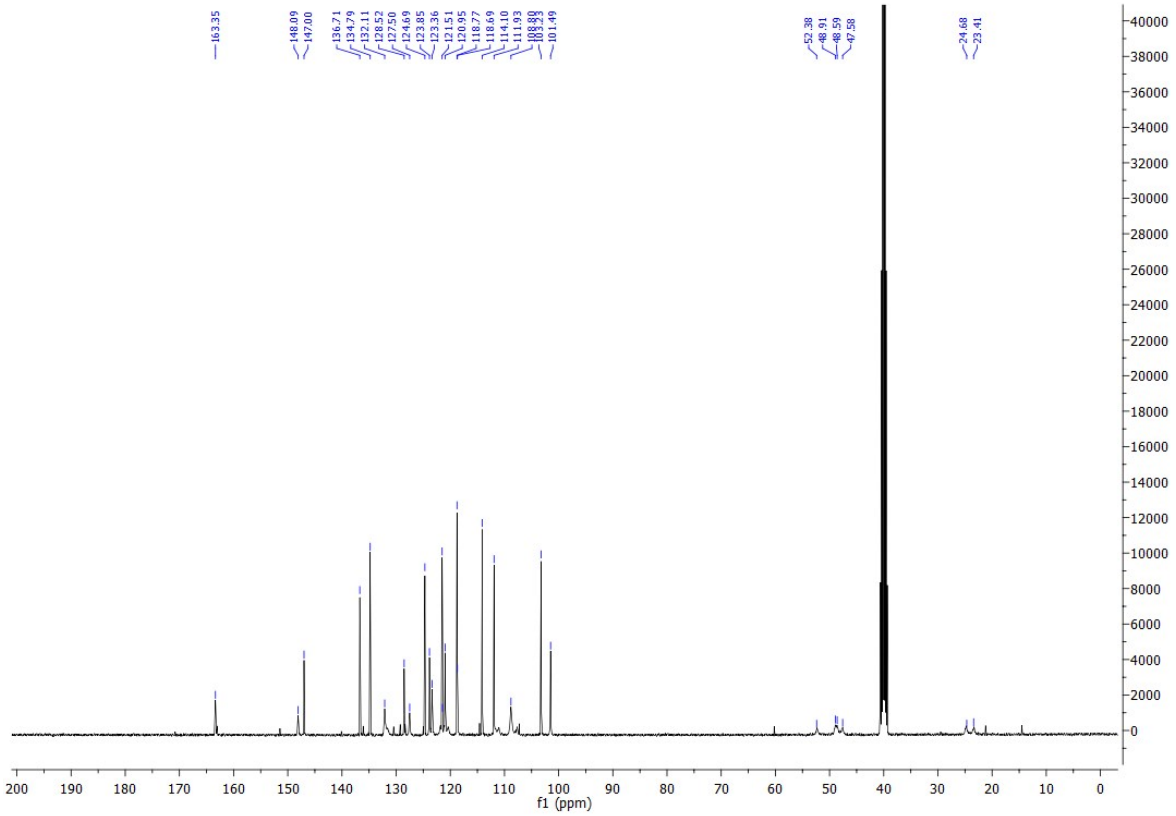
^{1}H NMR (400 MHz, DMSO-d_6) δ 11.93 (s, 1H), 10.87 (s, 1H), 7.73 – 7.26 (m, 4H), 7.25 – 7.13 (m, 2H), 7.08 (t, J = 7.5 Hz, 1H), 7.02 – 6.60 (m, 5H), 6.02 (s, 2H), 4.79 (br.s, 2H), 3.74 (br.s, 2H), 3.08 (s, 2H);

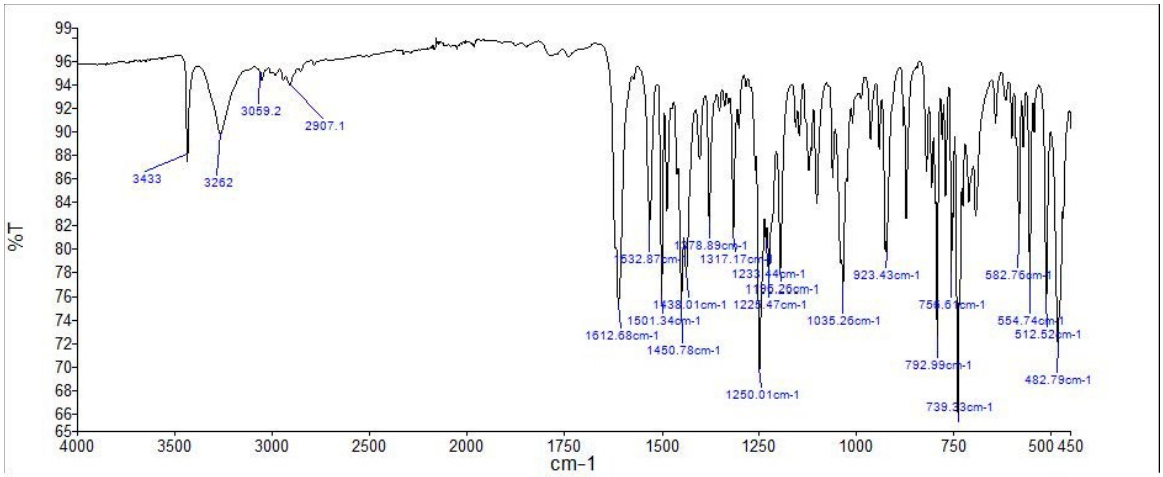
^{13}C NMR (101 MHz, DMSO-d_6) δ 163.4, 148.1, 147.0, 136.7, 134.8, 132.1, 128.5, 127.5, 124.7, 123.9 (Cx2), 123.4, 121.5, 121.4, 121.0, 118.8 (Cx2), 118.7, 114.1, 111.9, 108.8, 103.2 (Cx2), 101.5, 52.4, 48.9, 48.6, 47.6, 24.7, 23.4; **Note: 52.38 and 48.59 are the splitting of 1 C (Ar-CH$_2$-N-); 48.91 and 47.58 are the splitting of (CH$_2$-CH$_2$-N-), and 24.68, 23.41 are the splitting of (CH$_2$-CH$_2$-N-).*

UPLC: Mobile phase A= 100% H_2O with 0.1% formic acid; Mobile phase B = 90% ACN : 10% H_2O and 0.1% formic acid. RP-HPLC Agilent Zorbax SB-C18 1.8 µm, 50 mm x 2.1 mm, isocratic 80% mobile phase B at 0.6 mL/min in 8 minutes, Rt = 5.05 min, 100%;

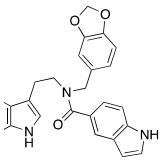
LRMS (ESI$^-$) m/z 471, 470 $[M-H]^+$, 100%; m/z 471, 472 $[M+H]^+$, 100%. HRMS (ES$^+$) for $C_{27}H_{22}ClN_3O_3$, calculated 472.1423, found 472.1422.







*(2-(1H-indol-3-yl)ethyl)-N-(benzo[d][1,3]dioxol-5-ylmethyl)-1H-indole-5-carboxamide (**29**)*



Yield: 158 mg, 67%. MP 162.5-163 ºC;

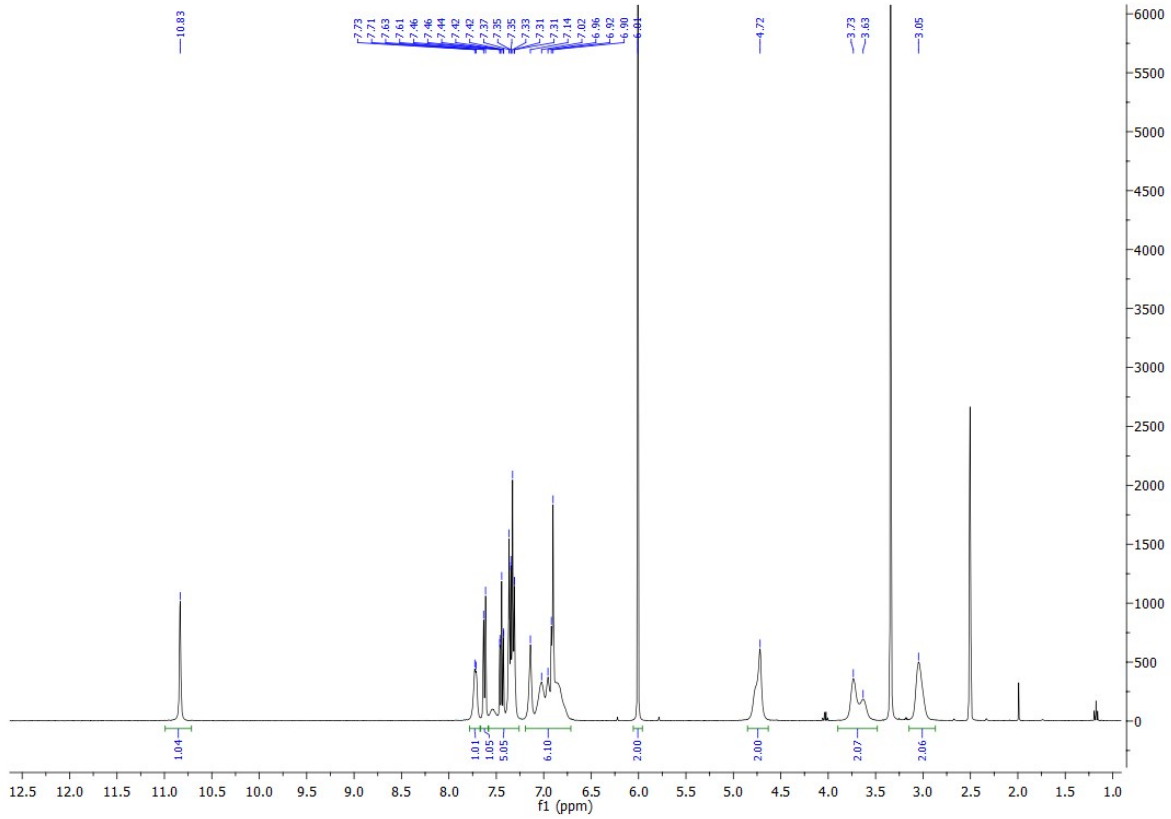
IR: ν_{max}/cm^{-1} 3638 (NH), 3227 (NH), 1614(CON), 740 (CH-aromatic);

^{1}H NMR (400 MHz, DMSO-d_6) δ 11.29 (s, 1H), 10.78 (s, 1H), 7.61 (s, 1H), 7.51 – 7.39 2H), 7.56 – 6.38 (m, 9H), 6.47 (s, 1H), 6.01 (s, 2H), 4.87 – 4.29 (m, 2H), 3.65 – 3.45 (m, 2H), 3 – 2.70 (m, 2H);

^{13}C NMR (101 MHz, DMSO-d_6) δ 186.0, 172.8, 148.0, 146.9, 136.6, 136.5, 127.8, 127.5, 127.0, 123.3, 12 0.4, 119.1, 118.6, 111.8, 111.7, 108.7, 102.2, 101.4, 49.1, 47.3, 45.6, 24.6, 23.4; **Note* : Signs of atropoisomers phatic CH_2, in which *47.3 is the splitting of 1 C (Ar-$\underline{C}H_2$-N-); 49.1 and 45.6 are the splitting of (CH_2-$\underline{C}H_2$-N-); 2 d 23.4 are the splitting of ($\underline{C}H_2$-CH_2-N-);*

RP-HPLC Alltima™ C18 5 µm 150 mm x 4.6 mm, 10–100% B in 15 min, Rt = 6.48 min, 96%

LRMS (ESI+) m/z 437, 437 $[M]^+$, 70%. HRMS (ES+) for $C_{27}H_{23}N_3O_3$, calculated 438.18122, found 438.18122.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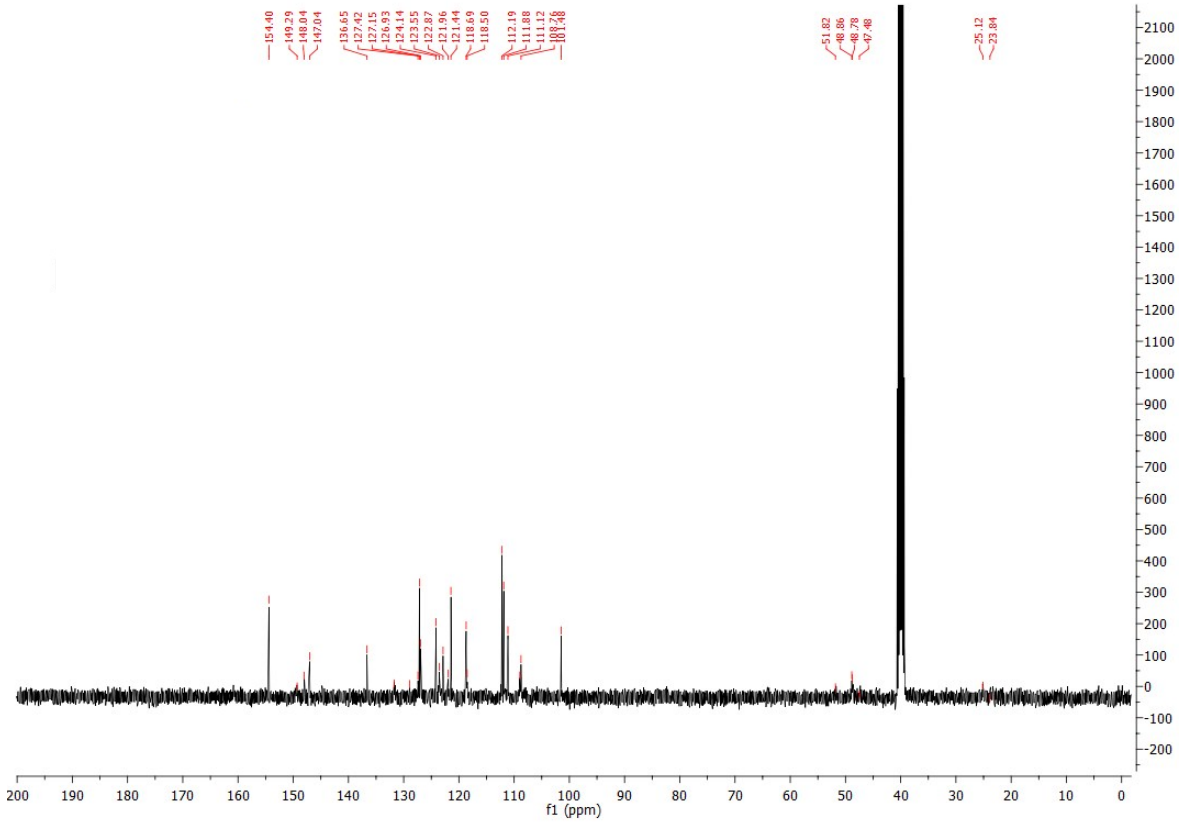
7.46
7.44
7.42
7.42
7.37
7.35
7.35
7.33
7.31
7.31
7.14
7.02
6.96
6.92
6.90
6.84
4.72
3.73
3.63
3.05
1.04
1.01
1.05
5.05
6.10
2.00
2.00
2.07
2.06
f1 (ppm)


154.40
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147.04
136.65
127.42
127.15
126.93
124.14
123.55
122.87
121.96
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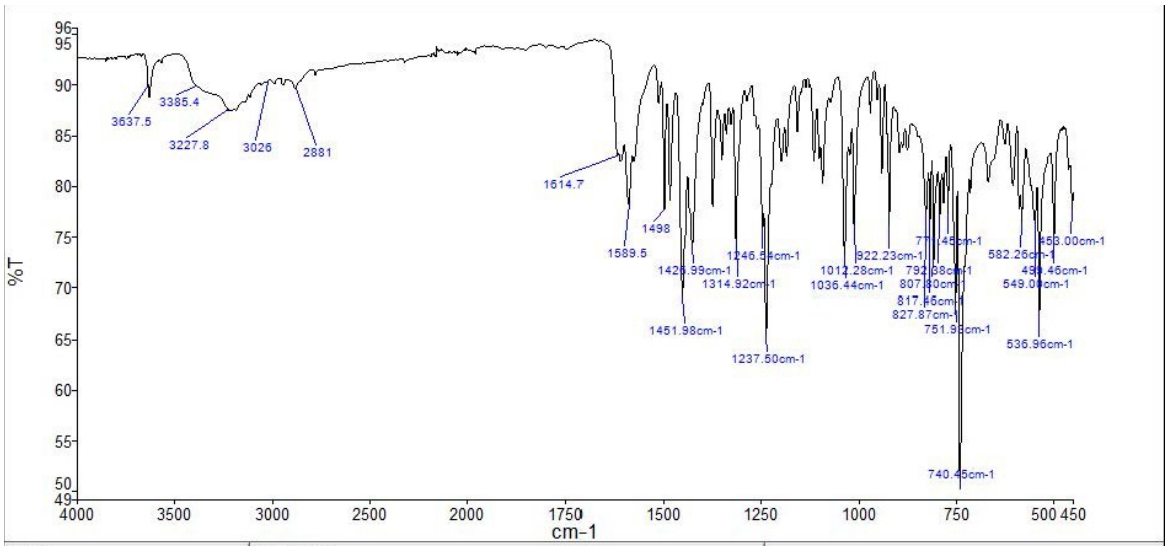
48.78
47.48
25.12
23.84
f1 (ppm)


%T
cm-1
3637.5
3385.4
3227.8
3026
2881
1614.7
1589.5
1498
1451.98cm-1
1428.99cm-1
1314.92cm-1
1246.54cm-1
1237.50cm-1
1036.44cm-1
1012.28cm-1
922.23cm-1
827.87cm-1
817.16cm-1
807.30cm-1
792.38cm-1
771.45cm-1
751.98cm-1
740.45cm-1
582.26cm-1
549.09cm-1
536.96cm-1
453.00cm-1