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Electronic Supplementary Information

Pharmacophore-based tailoring of biphenyl amide derivatives as selective 5-hydroxytryptamine 2B receptor antagonists

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Contents

Biological Screening

Antagonist Activity Assay

CHO-K1/5-HT_{2B} cell line was obtained from GenScript and used for cellular screening of the compounds. CHO-K1 cells expressing 5-HT_{2B} were seeded in a 384-well black-wall, clear-bottom plate at a density of 20,000 cells per well in 20 µL of growth medium (10% FBS + 90% F12), 18 h prior to the experiment and maintained at 37 ℃/5% CO_2. 20 µL of dye-loading solution and 10 µL of tested compound solution (at concentrations five times to the final assay concentrations) were added into the well. Then the plate was placed into a 37 °C incubator for 60 min, followed by 15 min at room temperature. At last, 12.5 µL of control agonist (at concentrations five times to the EC80 concentrations) was added. The control agonist was added to reading plate at 20 s and the fluorescence signal was monitored for an additional 100 s. The cells stimulated with assay buffer (HBSS-HEPES) containing 0.1% DMSO were chosen as background; cells stimulated with 12 nM (EC80 of the cell line) of 5-HT were chosen as the agonist control; cell treated with SB206553 were chosen as positive control of the screening.

The baseline reading was specified as the average fluorescent intensity value during 1s to 20 s. The relative fluorescent units (ΔRFU) intensity values were calculated with the maximal fluorescent units (21 s to 120 s) subtracting the average value of baseline reading. The % inhibition of the tested compound was calculated from the following equation:

%inhibition = [1- (ΔRFUcompound – ΔRFUbackground) / (ΔRFUagonist control – ΔRFUbackground)]*100

Binding Assay

The experimental binding assays were performed following the standard protocol.[1] The radiolabeled reference compounds ([^{3}H]8-OH-DPAT for 5-HT_{1A}; [^{3}H]GR127543 for 5-HT_{1B}; [^{3}H]5-HT for 5-HT_{1E}; [^{3}H]ketanserin for 5-HT_{2A}; [^{3}H]LSD for 5-HT_{2B} and 5-HT_{2C}; [^{3}H]LY278584 for 5-HT_3) were used in the K_i determination assays. The radio-labeled reference compounds are diluted to 5X final assay concentration (50 µM for a final assay concentration of 10 µM) in the standard binding buffer. Subsequently, 50 µL aliquots of buffer (negative control), test compound, and reference compound are added in quadruplicate to the wells of a 96-well plate, each of which contains 50 µL of 5X radioligand and 100 µL of buffer. Finally, receptor-containing, crude membrane fractions are resuspended in an appropriate volume of buffer and dispensed (50 µL per well) into the 96-well plate. Radioligand binding is allowed to equilibrate (typically for 1.5 hours at room temperature), and then bound radioactivity is isolated by filtration onto 0.3% polyethyleneimine-treated, 96-well filter mats using a 96-well Filtermate harverster. The radioactivity retained on the filters is counted in a Microbeta scintillation counter. Total bound radioactivity is estimated from quadruplicate wells without containing test or reference compound and adjusted to 100%; non-specifically bound radioactivity is assessed from quadruplicate wells containing 10 µM of a suitable reference compound and adjusted to 0%. The average bound radioactivity in the presence of the test compound (10 µM final assay concentration, quadruplicate determinations) is expressed on the percent scale. The percent inhibition of radioligand binding is calculated as follows:

%inhibition = 100% - % radioactivity-bound.

Pharmacokinetic Profiling

- Stability in simulated fluids ($t_{1/2}$, min) was evaluated using erythromycin as a reference compound.[2]

  Stability in simulated fluids ($t_{1/2}$, min)

	Gastric (pH 1.6)	Intestinal (pH 6.5)
Compound **7**	259	>500
Erythromycin	89.6	491

- Human plasma stability was evaluated using propantheline as a reference compound.[2]

  Plasma stability (% remaining at 120 min)

	% remaining
Compound **7**	97.4
Propantheline	2.75

- Stability in rat liver microsomes was evaluated using verapamil as a reference compound.[2]

	$t_{1/2}$, min
Compound **7**	179
Verapamil	4.1

- CACO cell permeability was evaluated using propranolol as a reference compound.[2]

	P_{app}, A to B	P_{app}, B to A	Efflux ratio	Classification
Compound **7**	16.3	32.8	2.01	High
Propranolol	33.1	26.9	0.81	High

- Cytotoxicity in HepG2 cells was evaluated by MTT assay using puromycin as a reference compound.[2]

	IC_{50}
Compound **7**	>50
Puromycin	1.54

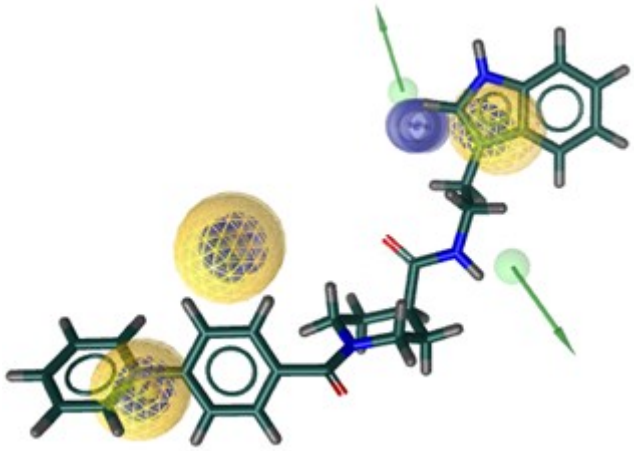

Fig. S1. The 3D overlay of compound **7** to the 5-HT_{2B} receptor-based pharmacophore. The pharmacophore color coding is yellow for hydrophobic regions and green for hydrogen donors.

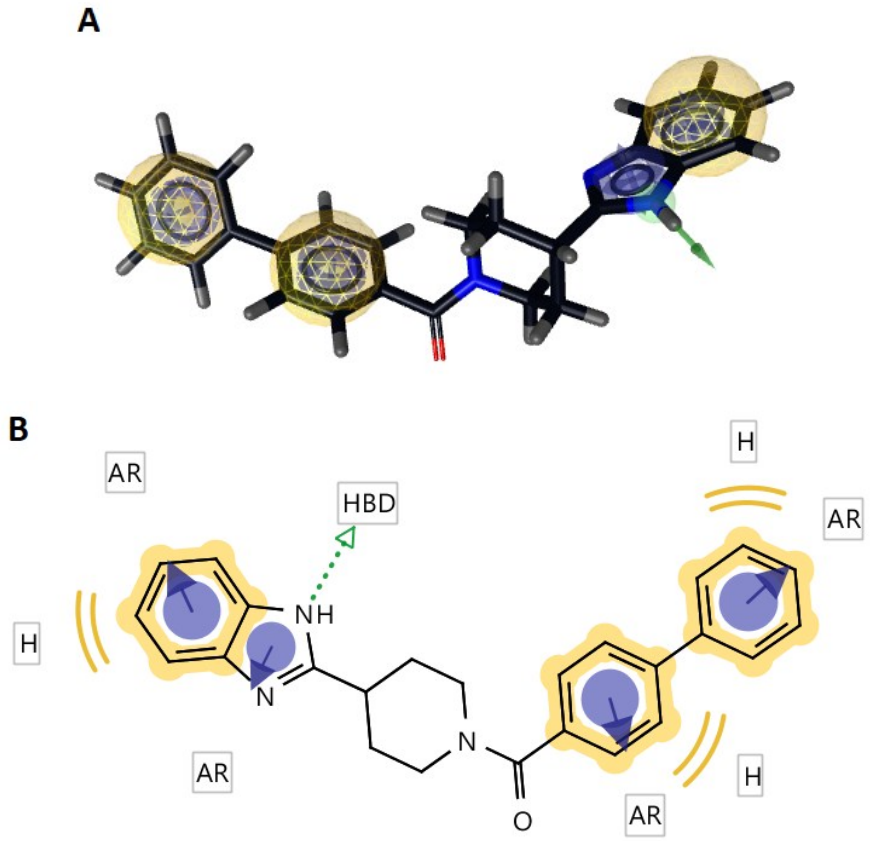

Fig. S2. The 3D and 2D pharmacophoric maps of lead compound **1**; (A) The 3D pharmacophoric map of compound **1**. The pharmacophore color coding is yellow for hydrophobic regions and green for hydrogen donors, (B) The 2D pharmacophoric map of compound **1**. H, hydrophobic center; HBD, hydrogen bond donor; AR, aryl.

References:

1. J. Besnard, G. F. Ruda, V. Setola, K. Abecassis, R. M. Rodriguiz, X. P. Huang, S. Norval, M. F. Sassano, A. I. Shin, L. A. Webster, F. R. C. Simeons, L. Stojanovski, A. Prat, N. G. Seidah, D. B. Constam, G. R. Bickerton, K. D. Read, W. C. Wetsel, I. H. Gilbert, B. L. Roth, A. L. Hopkins *Nature* 2012, **492**, 215−220.
2. D. K. Tosh, A. Ciancetta, E. Warnick, S. Crane, Z.-G. Gao, K. A. Jacobson, *J. Med. Chem.* 2016, **59**, 11006-11026.

Absolute Intensity
Chemical Shift (ppm)
9.89
7.70
7.67
7.54
7.52
7.50
7.49
7.46
7.43
7.41
7.41
4.61
4.58
4.55
3.97
3.87
3.84
3.22
3.18
3.15
2.71
2.70
2.69
2.66
2.11
0.47
4.11
5.04
1.00
1.01
2.01
1.04
2
9
N
O
OH

28.05
40.66
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140.16
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170.53
179.09
Chemical Shift (ppm)
Absolute Intensity
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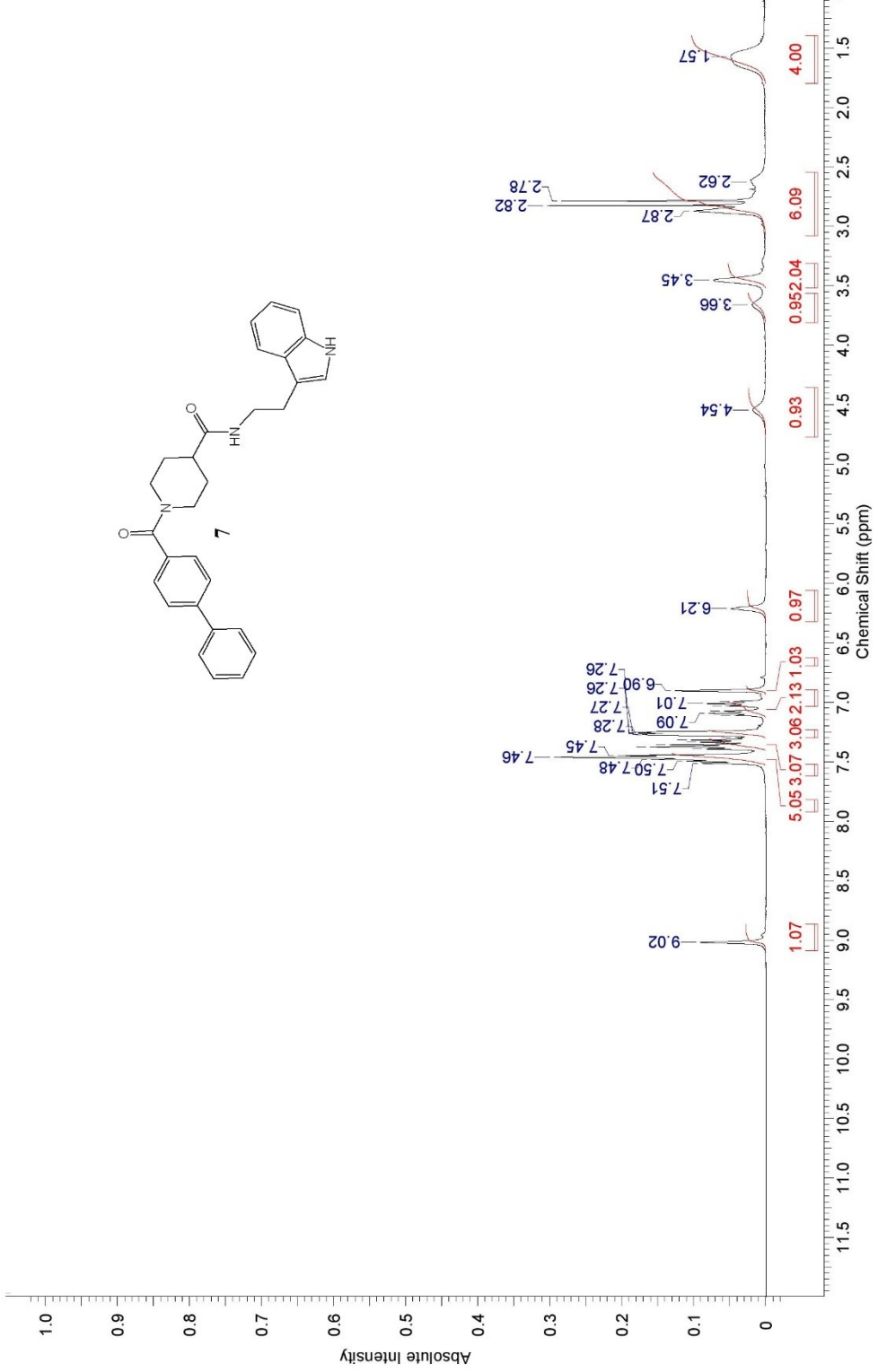
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2.82
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1.07
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2.13
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4.00

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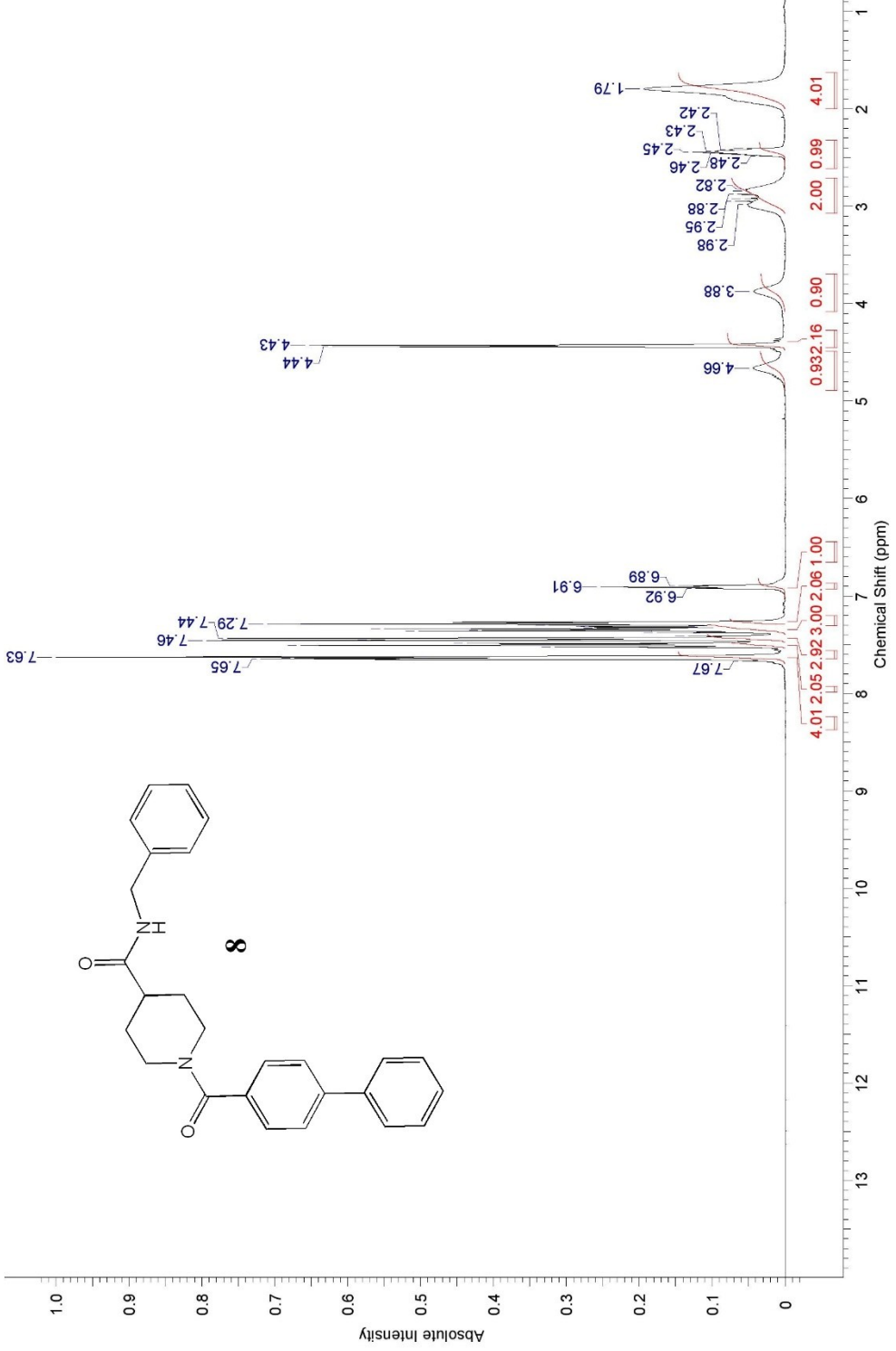
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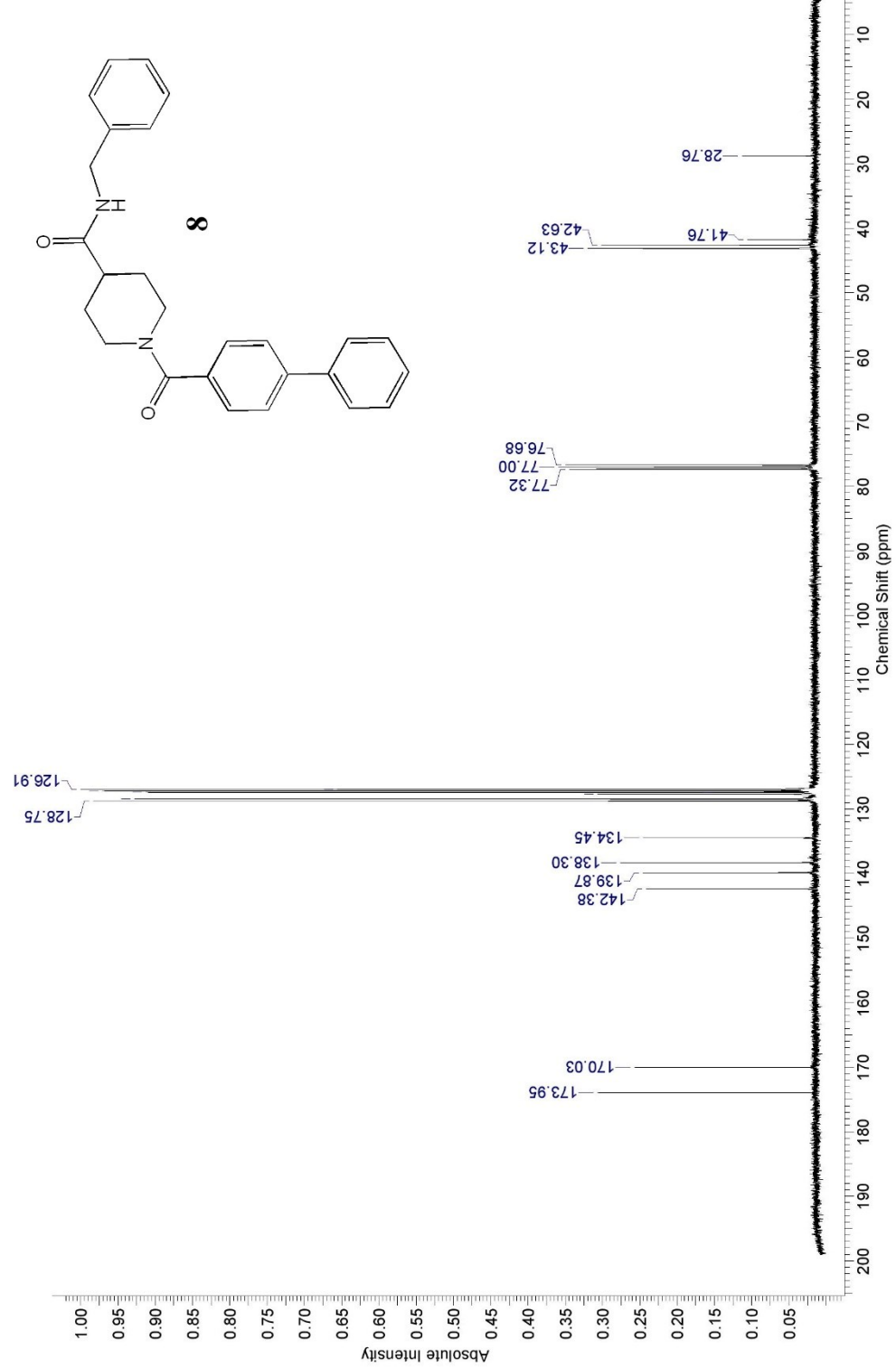
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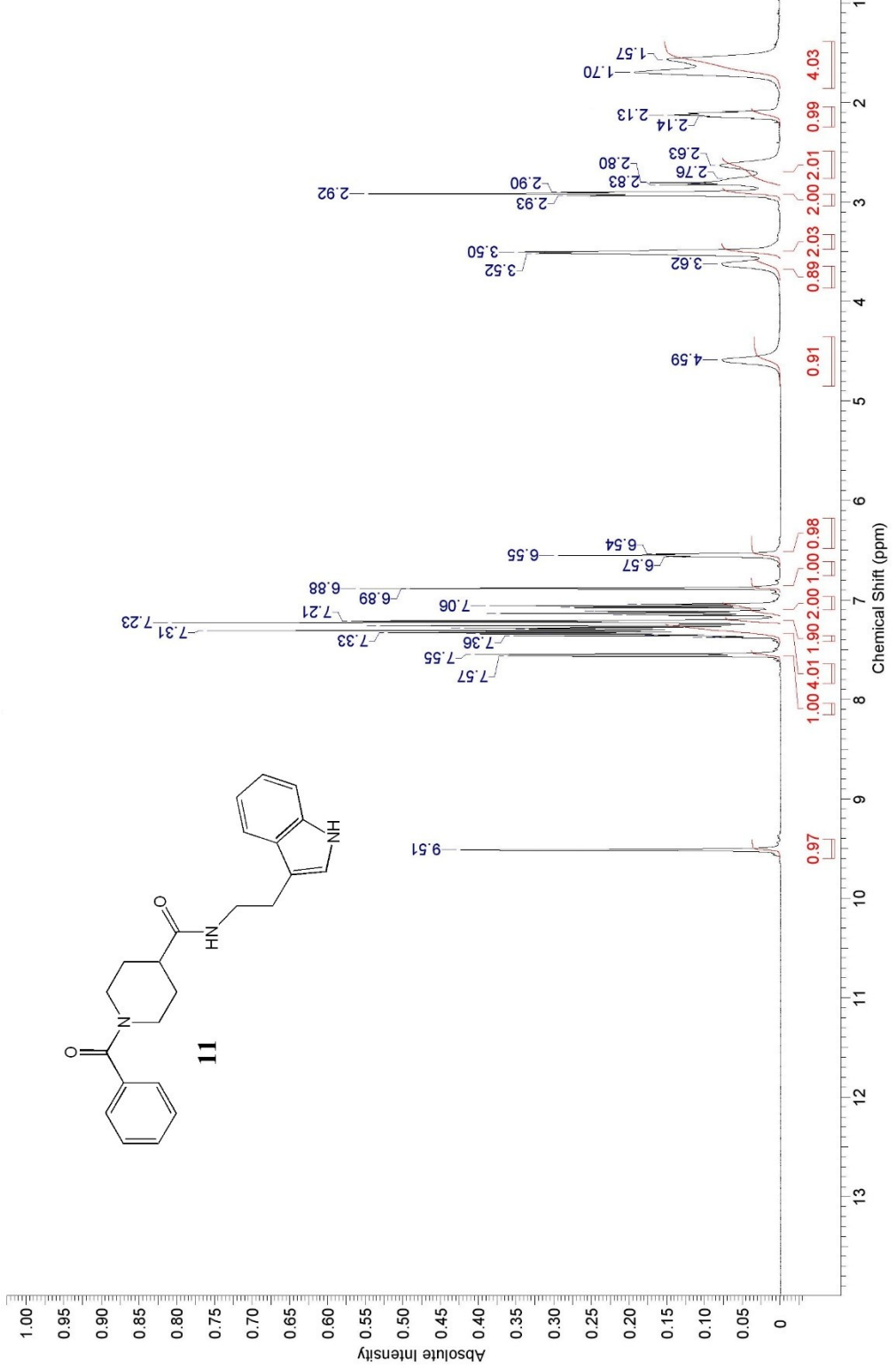
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3.50
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2.83
2.80
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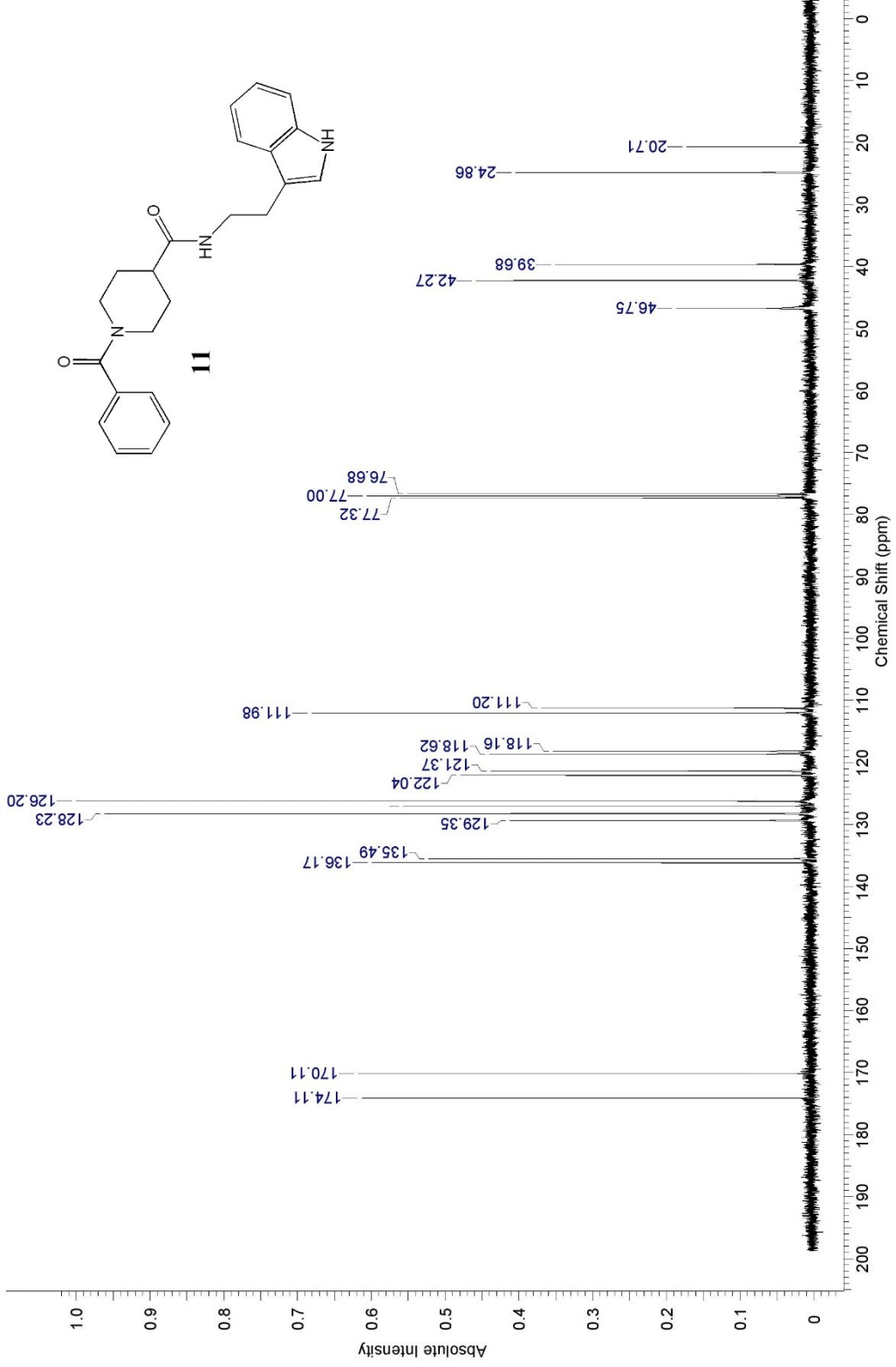
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111.98
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118.62
121.37
122.04
126.20
128.23
129.35
135.49
136.17
170.11
174.11