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New nanomolar negative modulators of AMPA receptors

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1. General

NMR spectra were recorded on the Agilent 400-MR spectrometer (400.0 MHz for ^{1}H; 100.6 MHz for ^{13}C) at room temperature; chemical shifts (δ) were measured with reference to the solvents, $CDCl_3$ (δ = 7.26 ppm for ^{1}H, δ = 77.16 ppm for ^{13}C), $(CD_3)_2SO$ (δ = 2.50 ppm for ^{1}H, δ = 39.52 ppm for ^{13}C), CD_3OD (δ = 3.31 ppm for ^{1}H, δ = 49.00 ppm for^{13}C). Chemical shifts (δ) are given in ppm; *J* values are given in Hz. When necessary, assignments of signals in NMR spectra were made using 2D techniques. Accurate mass measurements (HRMS) were performed on a Bruker micrOTOF II instrument using electrospray ionization (ESI). The measurements were done in a positive ion mode (interface capillary voltage 4500 V) or in a negative ion mode (3200 V). Analytical thin layer chromatography was carried out with Silufol silica gel plates (supported on aluminum); the detection was done by UV lamp (254 and 365 nm) and chemical staining (5% aqueous solution of $KMnO_4$). Column chromatography was performed on silica gel (230–400 mesh, Merck). All other starting materials were commercially available. All reagents except commercial products of satisfactory quality were purified by literature procedures prior to use.

2. Synthetic procedures

*General procedures for the synthesis of compounds **1-3***

To a solution of appropriate acyl chloride (2 mmol) in anhydrous acetonitrile (20 ml), anhydrous potassium carbonate (0.35 g, 2.5 mmol) was added and the mixture was stirred for 15 min at room temperature. Then 1,4-bis(aminomethyl)benzene (1 mmol) was added in several portions and the resulting mixture was stirred vigorously at room temperature for 1.5 h (TLC control). After completion of the reaction, the mixture was filtered, washed with cold acetonitrile (2×20 ml) and concentrated *in vacuo*. The crude mixture was purified by column chromatography on silica gel (CH_2Cl_2/CH_3OH = 50/1).

N,N'-*[1,4-Phenylenebis(methylene)]dicyclopropanecarboxamide*, ***(1)***

Yield 0.18 g (63%); colorless solid, mp 254-256°C; R_f= 0.31 (CH_2Cl_2/CH_3OH, 20/1); δ_H (400 MHz, CD_3OD): 0.73-0.78 (m, 4H, $2CH_2$), 0.84-0.89 (m, 4H, $2CH_2$), 1.60 (tt, 2H, CHCO, *J* 4.6, 8.0 Hz), 4.35 (s, 4H, $2CH_2$), 7.25 (s, 4H, 4CH); δ_C (101 MHz, CD_3OD): 7.3 ($4CH_2$), 14.8 (2CH), 44.0 ($2CH_2NH$), 128.7 (4CH), 139.2 (2C), 176.4 (2C=O). HRMS $[M+H]^+$: calcd. for $C_{16}H_{21}N_2O_2$ 273.1599, found 273.1598.

N,N'-*[1,4-Phenylenebis(methylene)]dicyclobutanecarboxamide*, ***(2)***

Yield 0.16 g (52%); colorless solid, mp 230-231 °C; R_f= 0.23 (CH_2Cl_2/CH_3OH, 20/1); δ_H(400 MHz, CD_3OD): 1.79-1.90 (m, 2H, $2CH_2$), 1.91-2.05 (m, 2H, $2CH_2$), 2.07-2.17 (m, 4H, $4CH_2$), 2.19-2.32 (m, 4H, $4CH_2$), 3.11 (quin d, *J* 8.6, 1.0 Hz, 2H, 2CH), 4.31 (s, 4H, $2CH_2$), 7.21 (s, 4H, 4CH); δ_C(101 MHz, CD_3OD+$CDCl_3$): 19.0 ($2CH_2$), 26.1 ($4CH_2$), 40.7 (2CH), 43.6 ($2CH_2NH$), 128.6 (4CH), 139.0 (2C), 177.5 (2C=O). HRMS $[M+H]^+$: calcd. for $C_{18}H_{24}N_2O_2$ 301.1908, found 301.1911.

N,N'-*[1,4-Phenylenebis(methylene)]dicyclopentanecarboxamide*, ***(3)***

Yield 0.22 g (67%); colorless solid, mp 258-260 °C; R_f= 0.44 (CH_2Cl_2/CH_3OH, 20/1); δ_H (400 MHz, CD_3OD): 1.53-1.64 (m, 4H, $2CH_2$), 1.65-1.79 (m, 8H, $4CH_2$), 1.80-1.91 (m, 4H, $2CH_2$), 2.61-2.70 (m, 2H, 2CHCO), 4.33 (s, 4H, $2CH_2$), 7.23 (s, 4H, 4CH); δ_C (101 MHz, CD_3OD+$CDCl_3$): 26.8 ($4CH_2$), 31.3 ($4CH_2$), 43.6 (2CH), 46.2 ($2CH_2NH$), 128.4 (4CH), 138.7 (2C), 178.8 (2C=O). HRMS $[M+Na]^+$: calcd. for $C_{20}H_{28}N_2O_2Na$ 351.2044, found 351.2043.

3. Patch clamp studies

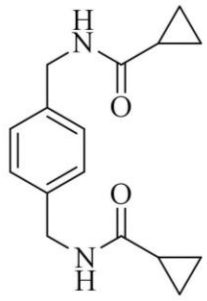
In vitro electrophysiological experiments were carried out using a patch clamp technique with local fixation of potential as described.[1] Freshly isolated single Purkinje neurons from the cerebellum of 12-16 day old Wistar rats were used as a test system. Transmembrane currents were induced by the activation of AMPA receptors with a solution of their partial agonist kainic acid using fast superfusion of solutions, where 30 μL of the agonist buffer were added to the neuron washing buffer at a constant rate, and the agonist concentration was varied in the range of 10^{-6}–10^{-4} M. The transmembrane currents for individual neurons were recorded using 2.5–5.5 MΩ borosilicate microelectrodes in a whole-cell configuration with an EPC-9 device from HEKA, Germany. The data were processed by a Pulsfit program from HEKA, Germany. Cyclothiazide (CTZ) as a well-known positive allosteric modulator of AMPA receptors was used as a reference ligand. The experimental results for compounds **1** – **3** are presented in Table 1 in the main text.

References

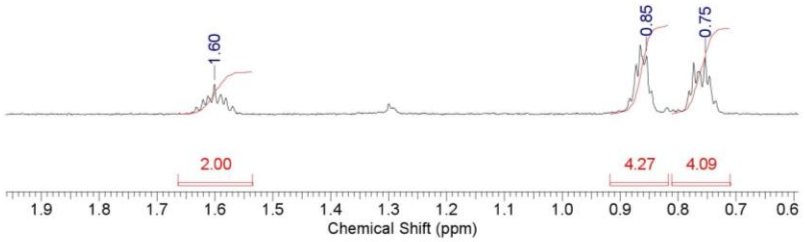
1. M. I. Lavrov, D. S. Karlov, T. A. Voronina, V. V. Grigoriev, A. A. Ustyugov, S. O. Bachurin and V. A. Palyulin, *Mol. Neurobiol.*, 2020, **57**, 191.

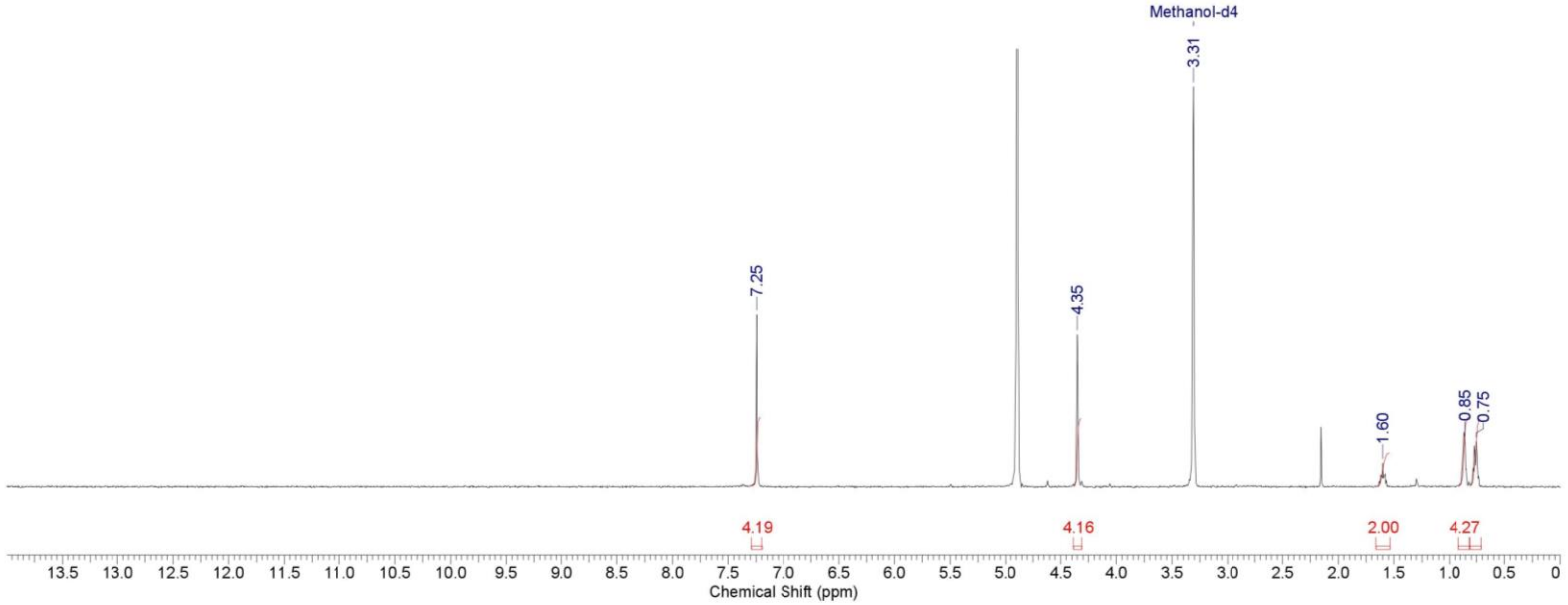
4. NMR spectra

***N,N′*-[1,4-Phenylenebis(methylene)]dicyclopropanecarboxamide (1)**

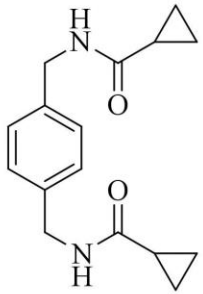


^{1}H NMR

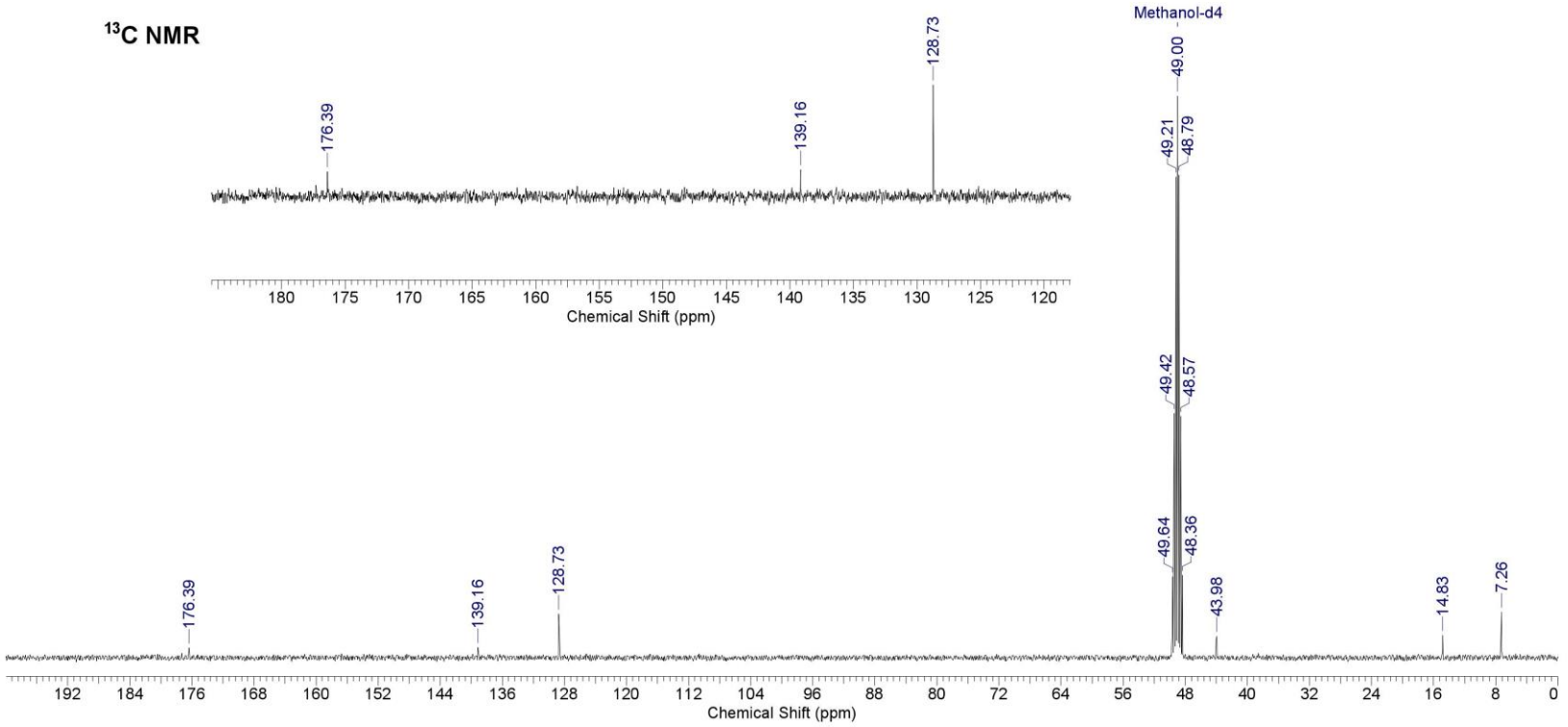


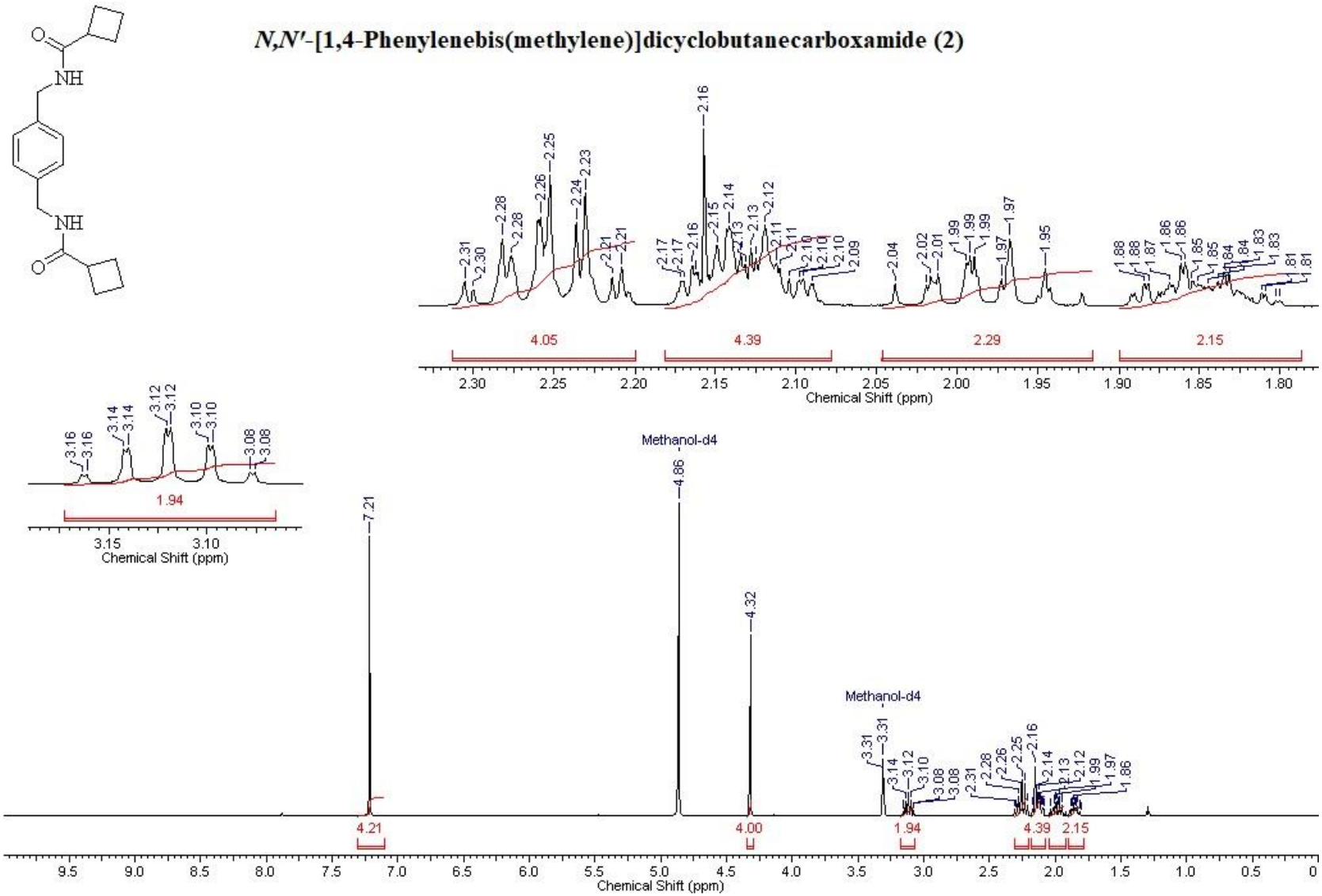


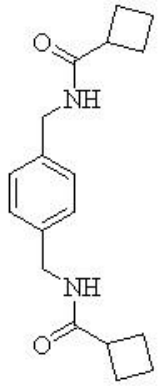
_N,N′_-[1,4-Phenylenebis(methylene)]dicyclopropanecarboxamide (1)



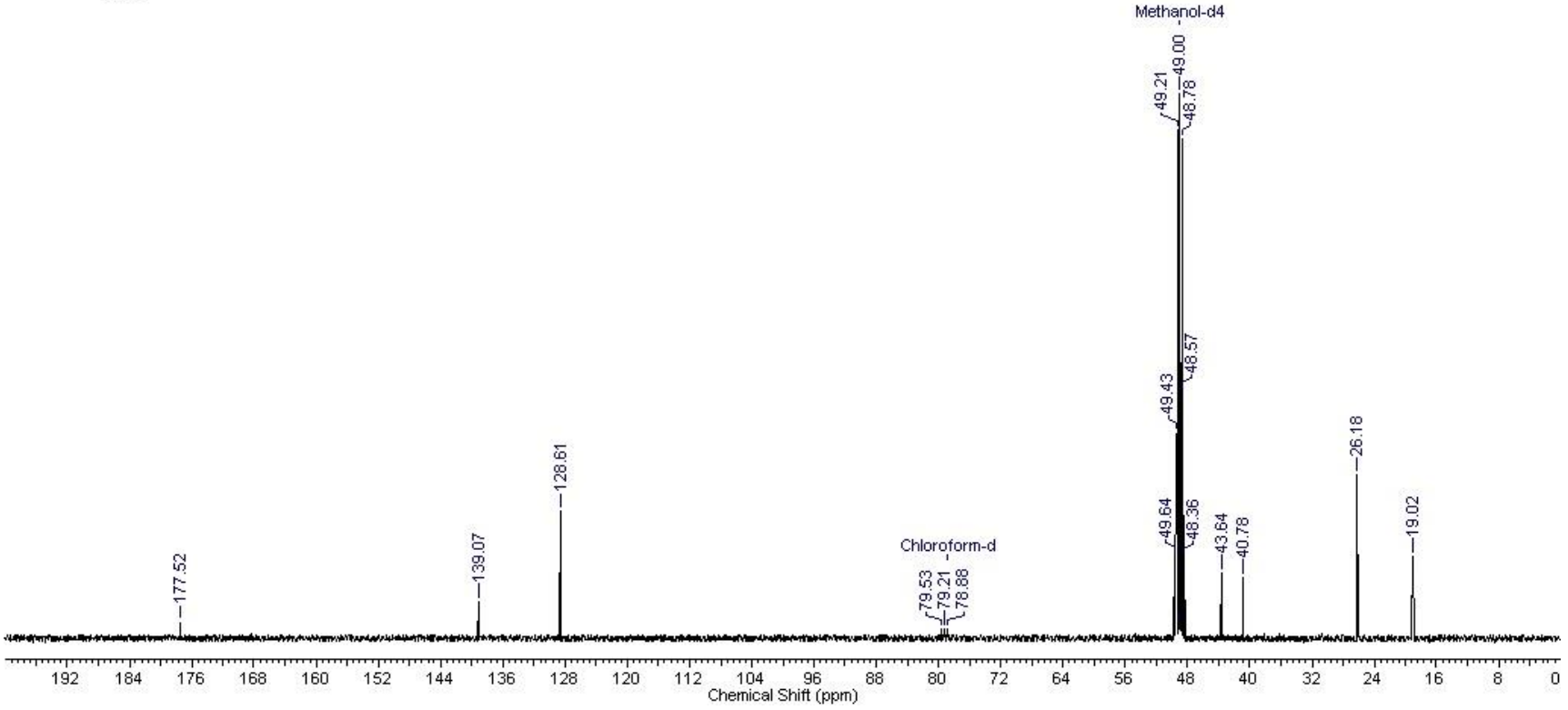
^{13}C NMR

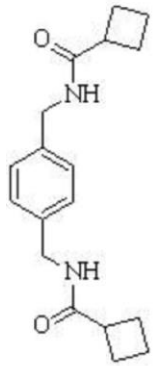



N,N′-[1,4-Phenylenebis(methylene)]dicyclobutanecarboxamide (2)
NH
NH
Methanol-d4
Methanol-d4
Chemical Shift (ppm)
Chemical Shift (ppm)
Chemical Shift (ppm)



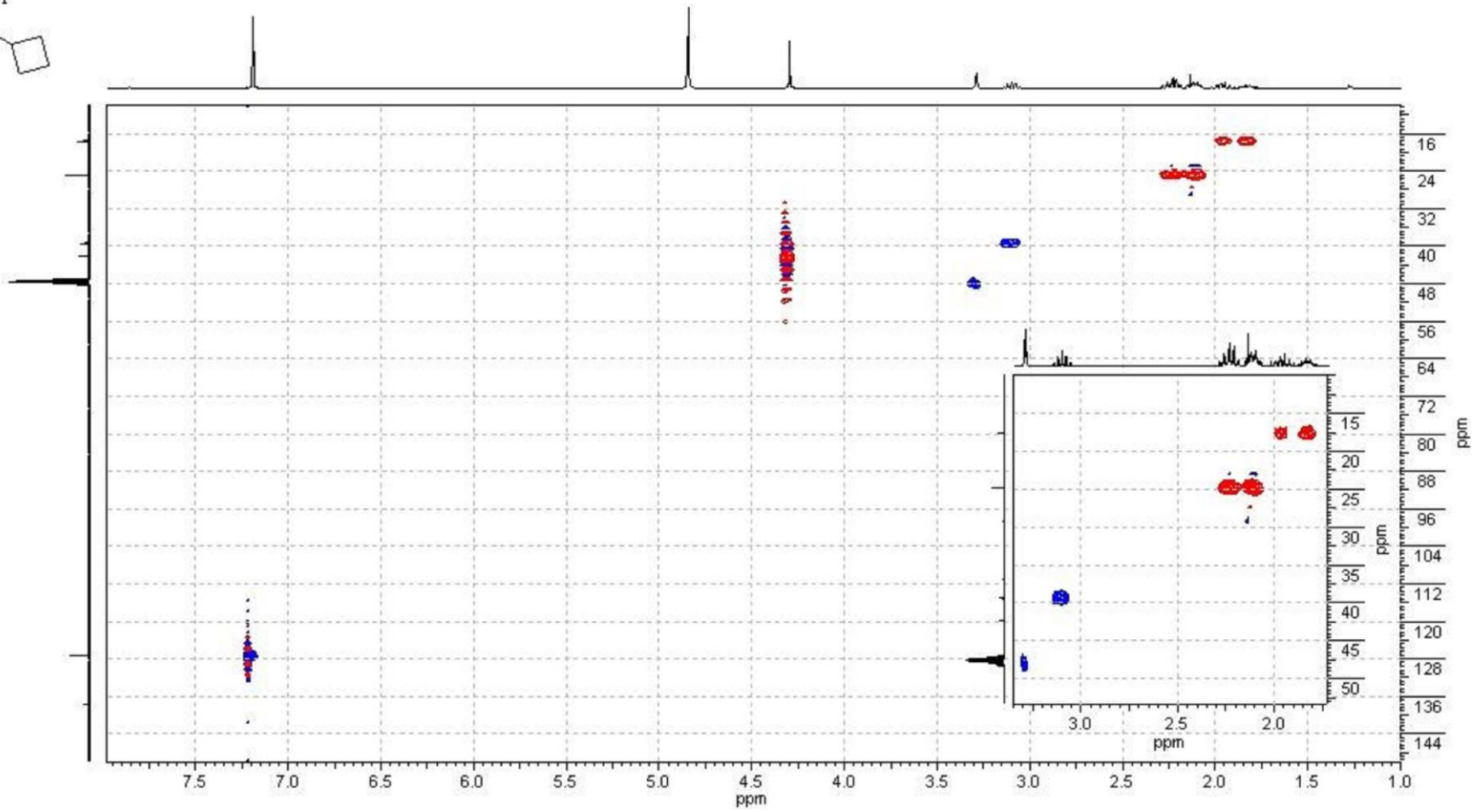
_N,N′_-[1,4-Phenylenebis(methylene)]dicyclobutanecarboxamide (2)



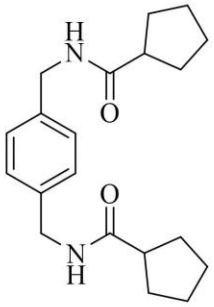


***N,N′*-[1,4-Phenylenebis(methylene)]dicyclobutanecarboxamide (2)**

HSQC

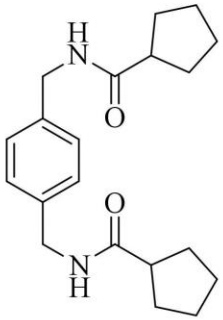


_N,N′_-[1,4-Phenylenebis(methylene)]dicyclopentanecarboxamide (3)



^{1}H NMR

_N,N′_-[1,4-Phenylenebis(methylene)]dicyclopentanecarboxamide (3)



^{13}C NMR