

Supplementary Information

Focused microwave irradiation-assisted synthesis of *N*-cyclohexyl-1,2,4-oxadiazole derivatives with antitumor activity

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In honor of Professor Rajendra Mohan Srivastava on the occasion of his 80th birthday

Experimental

Microwave reactions were conducted in a focused microwave (FMW) power delivery system, using a CEM Discover Synthesis (Model 908005, 0-300 W, 2455 MHz, CEM Corporation). For the synthesis the power was 150 W and the temperature achieved during the procedure was 120 °C. The reactions were performed in sealed glass vessels (capacity 10 mL). Melting points were determined in a capillary tube and performed with a PFM II BioSan instrument. HRMS analyses were performed on a LC-MS/ESI(-)TOF spectrometer (Model Xevo G2-XS QTof, Waters). NMR spectra were obtained with Varian Unity Plus-300 or 400 spectrometers using CDCl₃ as solvents, and calibrated for the solvent signal. The purification was performed by column chromatography on Merck silica gel 60 (70-230 mesh). The arylamidoximes **1a-i** were prepared according to conventional protocol or ultrasound irradiation.^[1]

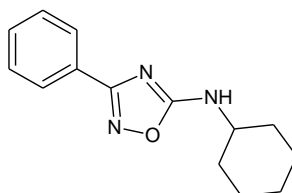
Synthesis of 3-aryl-5-amine-cyclohexyl-1,2,4-oxadiazole (2a-i)

To a solution of arylamidoximes **1a-i** (1 mmol) in DMF (0.5 mL) dicyclohexylcarbodiimide DCC (1.5 or 2.0 mmol) was added. The mixture was irradiated in a closed vessel at 120 °C and 150 W for a specific time. The reaction was monitored each 5 min using TLC (hexane:ethyl acetate 7:3). After it cooled, the residue was evaporated under reduced pressure. The crude material was purified by column chromatography on silica gel (eluent, hexane:EtOAc gradient from 8:2 to 7:3) to give the products **2a-i**.

N-Cyclohexyl-3-(phenyl)-1,2,4-oxadiazol-5-amine (**2a**)

CAS RN 109879-46-1

100 mg (0.734 mmol) of arylamidoxime **1a** and 303 mg (1.47 mmol; 2.0 equiv) of DCC **4** were mixed; after 10 min of reaction under MW irradiation and purification, the product **2a** was obtained.

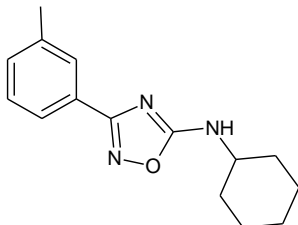


Yield 68% (118 mg); colorless solid; mp 124-126 °C [Lit. 126.7-127.7 °C]^[4]; R_f 0.7 (hexane:EtOAc 6:4). The NMR spectroscopy was recorded and compared to previously reported data.^[2,3] The NH proton was observed in δ 5.15 ppm as a broad duplet ($J = 7.4$ Hz).

N-Cyclohexyl-3-(3-methylphenyl)-1,2,4-oxadiazol-5-amine (**2b**)

CAS RN 1259065-33-2

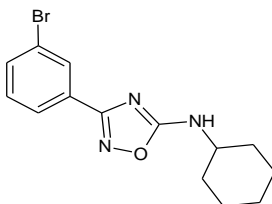
150 mg (1.0 mmol) of arylamidoxime **1b** and 309.5 mg (1.5 mmol) of DCC were mixed; the mixture was left for 10 min under MW irradiation. Purification gave the compound **2b**.



Yield 61% (157.3 mg); colorless solid; mp 108-110 °C; R_f 0.8 (hexane:EtOAc 7:3). ^1H NMR (300 MHz, CDCl_3): δ 1.19-1.49 (m, 4H, $\text{H}_{\text{cyclohexyl}}$), 1.64-1.69 (m, 2H, $\text{H}_{\text{cyclohexyl}}$), 1.75-1.81 (m, 2H, $\text{H}_{\text{cyclohexyl}}$), 2.09-2.13 (m, 2H, $\text{H}_{\text{cyclohexyl}}$), 2.41 (s, 3H, CH_3), 3.66-3.75 (m, 1H, $\text{H}_{\text{cyclohexyl}}$), 5.12 (d, $J = 7.8$ Hz, 1H, NH), 7.29-7.36 (m, 2H, H_{Ar}), 7.80 (t, $J = 7.5$ Hz, 2H, H_{Ar}). ^{13}C NMR (75.5 MHz, CDCl_3): δ 21.3, 24.6, 25.3, 33.2, 52.8, 124.3, 127.5, 127.7, 128.5, 131.5, 138.3, 168.5, 170.5. ESI-HRMS calcd for $[(\text{C}_{15}\text{H}_{19}\text{N}_3\text{O})+\text{H}]^+$: 258.16009; found: 258.1690.

N-Cyclohexyl-3-(3-bromophenyl)-1,2,4-oxadiazol-5-amine (2c)

100 mg (0.465 mmol) of arylamidoxime **1c** and 143.9 mg (0.698 mmol, 1.5 equiv) of DCC **4** were mixed; after 10 min of reaction under MW irradiation and purification, the compound **2c** was obtained.



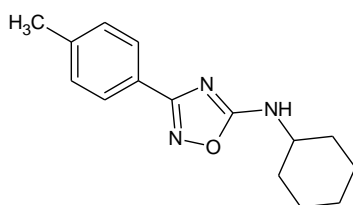
Yield 81% (121.4 mg); colorless solid; mp 110-112 °C; R_f 0.8 (hexane:EtOAc 7:3). ^1H NMR (300 MHz, CDCl_3): δ 1.21-1.49 (m, 4H, $\text{H}_{\text{cyclohexyl}}$), 1.63-1.81 (m, 4H, $\text{H}_{\text{cyclohexyl}}$), 2.09-2.13 (m, 2H, $\text{H}_{\text{cyclohexyl}}$), 3.61-3.74 (m, 1H, $\text{H}_{\text{cyclohexyl}}$), 5.22 (d, $J = 7.8$, 1H, NH), 7.32 (t, $J = 7.8$ Hz, 1H, H_{Ar}), 7.58-7.61 (m, 1H, H_{Ar}), 7.92-7.95 (m, 1H, H_{Ar}), 8.82 (t, J

= 1.8 Hz, 1H, H_{Ar}). ¹³C NMR (75.5 MHz, CDCl₃): δ 24.6, 25.3, 33.2, 53.0, 122.6, 125.7, 129.7, 130.2, 133.6, 167.3, 170.6. ESI-HRMS calcd for [(C₁₄H₁₆N₃OBr)+H]⁺: 322.0549; found: 322.0653.

***N*-Cyclohexyl-3-(4-methylphenyl)-1,2,4-oxadiazol-5-amine (2d)**

CAS RN 1041005-76-8

100 mg (0.666 mmol) of arylamidoxime **1d** and 274.8 mg (1.332 mmol; 2 equiv) of DCC **4** were mixed. The reaction was finished after 10 min and the residual crude material was purified to give the compound **2d**.

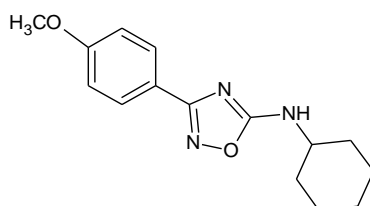


Yield 62% (106.3 mg); colorless solid; mp 142-144 °C {Lit. 146-147 °C}^[3]; R_f 0.8 (hexane:EtOAc 7:3). The NMR spectroscopy was recorded and compared to previously reported data.^[3,5]

***N*-Cyclohexyl-3-(4-methoxyphenyl)-1,2,4-oxadiazol-5-amine (2e)**

CAS RN 1023375-50-9

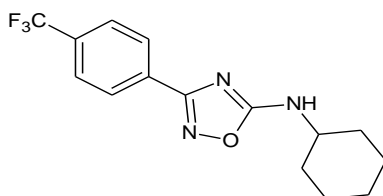
100 mg (0.602 mmol) of arylamidoxime **1e** and 186 mg (1.2 mmol; 2 equiv) of DCC **4** were mixed in DMF. After 15 min of reaction under MW irradiation and purification, the compound **2e** was obtained.



Yield 66% (108.5 mg); orange solid; mp 107-109 °C {Lit. 112.5-114 °C}^[3]; R_f 0.7 (hexane:EtOAc 7:3). The NMR spectroscopy was recorded and compared to previously reported data.^[4]

N-Cyclohexyl-3-(4-trifluoromethylphenyl)-1,2,4-oxadiazol-5-amine (2f)

100 mg (0.490 mmol) of arylamidoxime **1f** and 151.6 mg (0.735 mmol; 1.5 equiv) of DCC **4** were mixed; after 10 min of reaction under MW irradiation and purification, the compound **2f** was obtained.

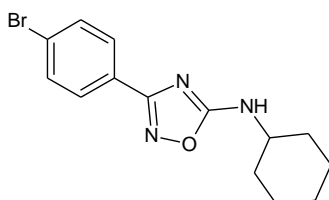


Yield 73% (111 mg); colorless solid; mp 165-167 °C; R_f 0.8 (hexane:EtOAc 7:3). ¹H NMR (300 MHz, CDCl₃): δ 1.19-1.49 (m, 4H, H_{cyclohexyl}), 1.64-1.81 (m, 4H, H_{cyclohexyl}), 2.09-2.13 (m, 2H, H_{cyclohexyl}), 3.67-3.75 (m, 1H, H_{cyclohexyl}), 5.24 (d, *J* = 8.2 Hz, 1H, NH), 7.71 (d, *J* = 8.2 Hz, 2H, H_{Ar}), 8.82 (d, *J* = 7.8 Hz, 2H, H_{Ar}). ¹³C NMR (75.5 MHz, CDCl₃): δ 24.6, 25.3, 33.2, 53.0, 122.5, 125.2, 125.5, 127.5, 131.2, 132.6, 167.4, 170.7. ESI-HRMS calcd for [(C₁₅H₁₆N₃OF₃)+H]⁺: 312.1318; found: 312.1419.

N-Cyclohexyl-3-(4-bromophenyl)-1,2,4-oxadiazol-5-amine (2g)

CAS RN 1161923-84-7

100 mg (0.465 mmol) of arylamidoxime **1g** and 144 mg (0.697 mmol; 1.5 equiv) of DCC **4** were mixed. The reaction was finished after 10 min and the residual crude material was purified to give the compound **2g**.

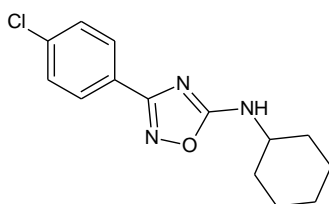


Yield 81% (121 mg); colorless solid; mp 190-191 °C {Lit. 186-187 °C}^[5]; R_f 0.8 (hexane:EtOAc 7:3). The NMR spectroscopy was recorded and compared to previously reported data.^[5]

***N*-Cyclohexyl-3-(4-chlorophenyl)-1,2,4-oxadiazol-5-amine (2h)**

CAS RN 1161923-83-6

100 mg (0.586 mmol) of arylamidoxime **1h** and 181.4 mg (0.879 mmol; 1.5 equiv) of DCC **4** were mixed. The reaction was finished after 10 min and the residual crude material was purified to give the compound **2h**.

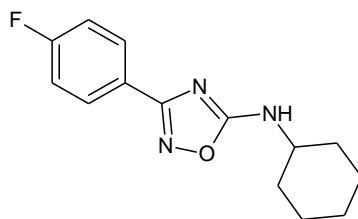


Yield 71% (115.6 mg); yellowish solid; mp 188-189 °C {Lit. 176-178 °C}^[5]; R_f 0.8 (hexane:EtOAc 7:3). The NMR spectroscopy was recorded and compared to previously reported data.^[5]

***N*-Cyclohexyl-3-(4-fluorophenyl)-1,2,4-oxadiazol-5-amine (2i)**

CAS RN 1220511-99-8

154 mg (1.0 mmol) of arylamidoxime **1i** and 309 mg (1.5 mmol) of DCC were mixed; after 10 min of reaction under MW irradiation and purification, the compound **2i** was obtained.

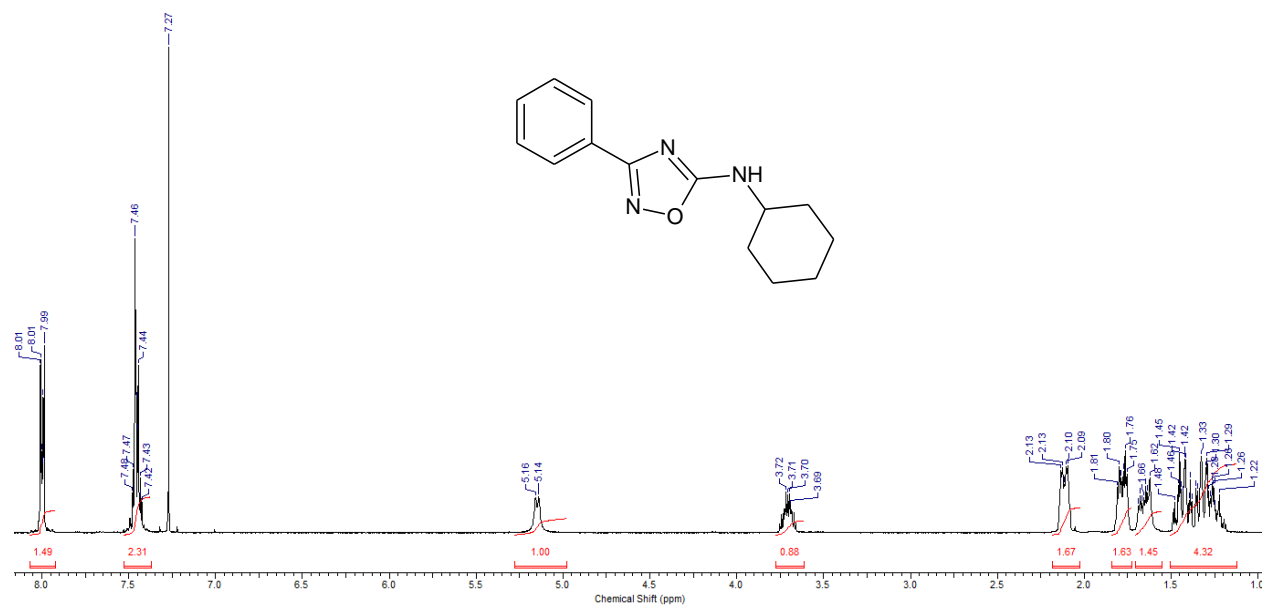


Yield 67% (175 mg); brownish solid; mp 122-124 °C; R_f 0.8 (hexane:EtOAc 7:3). ^1H NMR (300 MHz, CDCl_3): δ 1.20-1.49 (m, 5H, $\text{H}_{\text{cyclohexyl}}$), 1.64-1.69 (m, 2H, $\text{H}_{\text{cyclohexyl}}$), 1.76-1.81 (m, 2H, $\text{H}_{\text{cyclohexyl}}$), 2.09-2.14 (m, 2H, $\text{H}_{\text{cyclohexyl}}$), 3.66-3.76 (m, 1H, $\text{H}_{\text{cyclohexyl}}$), 5.15 (d, $J = 7.9$ Hz, 1H, NH), 7.10-7.16 (m, 2H, H_{Ar}), 7.97-8.02 (m, 2H, H_{Ar}). ^{13}C NMR (75.5 MHz, CDCl_3): δ 24.6, 25.3, 33.2, 52.9, 115.7 (d, *ortho* $J_{\text{C-F}} = 22$ Hz), 123.9, 129.3 (d, *meta* $J_{\text{C-F}} = 8.1$ Hz), 165.9 and 162.6 (d, *ipso* $J_{\text{C-F}} = 250.9$ Hz), 167.5, 170.5. ESI-HRMS calcd for $[(\text{C}_{14}\text{H}_{16}\text{N}_3\text{OF})+\text{H}]^+$: 262.1350; found: 262.1348.

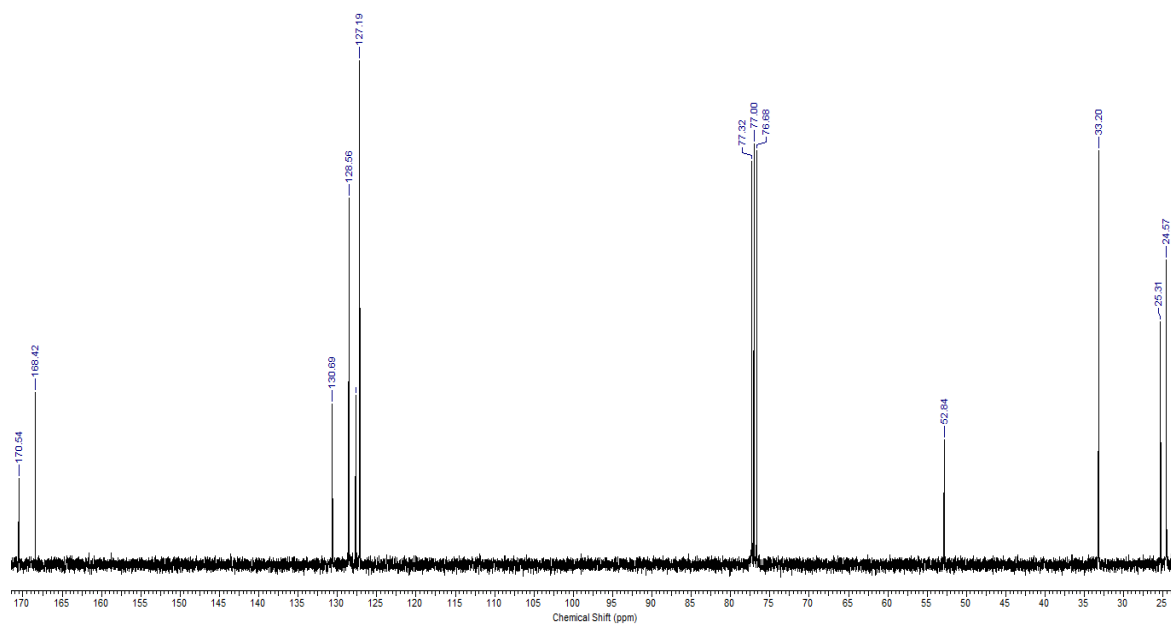
References

- [1] Barros, C. J. P.; de Freitas, J. J. R.; de Oliveira, R. N.; de Freitas Filho, J. R.. Synthesis of amidoximes using an efficient and rapid ultrasound method. *J. Chil. Chem. Soc.* **2011**, 56, 721-722.
- [2] Kawashima, E.; Tabei, K. Reaction of benzamide oxime with DCC. *J. Heterocyclic Chem.* **1986**, 23, 1657-1660.
- [3] Ispikoudi, M.; Amvrazis, M.; Kontogiorgis, C.; Koumbis, A. E.; Litinas, K. E.; Hadjipavlou-Litina, D.; Fylaktakidou, K. C. Convenient synthesis and biological profile of 5-amino-substituted 1,2,4-oxadiazole derivatives. *Eur. J. Med. Chem.* **2010**, 45, 5635-5645.
- [4] Mercalli, V.; Massarotti, A.; Varese, M.; Giustiniano, M.; Meneghetti, F.; Novellino, E.; Tron, G. C. Multicomponent Reaction of Z-Chlorooximes, Isocyanides, and Hydroxylamines as Hypernucleophilic Traps. A One-Pot Route to Aminodioximes and Their Transformation into 5-Amino-1,2,4-oxadiazoles by Mitsunobu–Beckmann Rearrangement *J. Org. Chem.* **2015**, 80, 9652-9661.
- [5] Dürüst, Y.; Yildirim, M. Aycan, A. An efficient one-pot synthesis of 5-(substituted amino)-1,2,4-thia- and -oxa-diazoles. *J. Chem. Res.* **2008**, 235-239.

***N*-Cyclohexyl-3-(phenyl)-1,2,4-oxadiazol-5-amine (2a)**

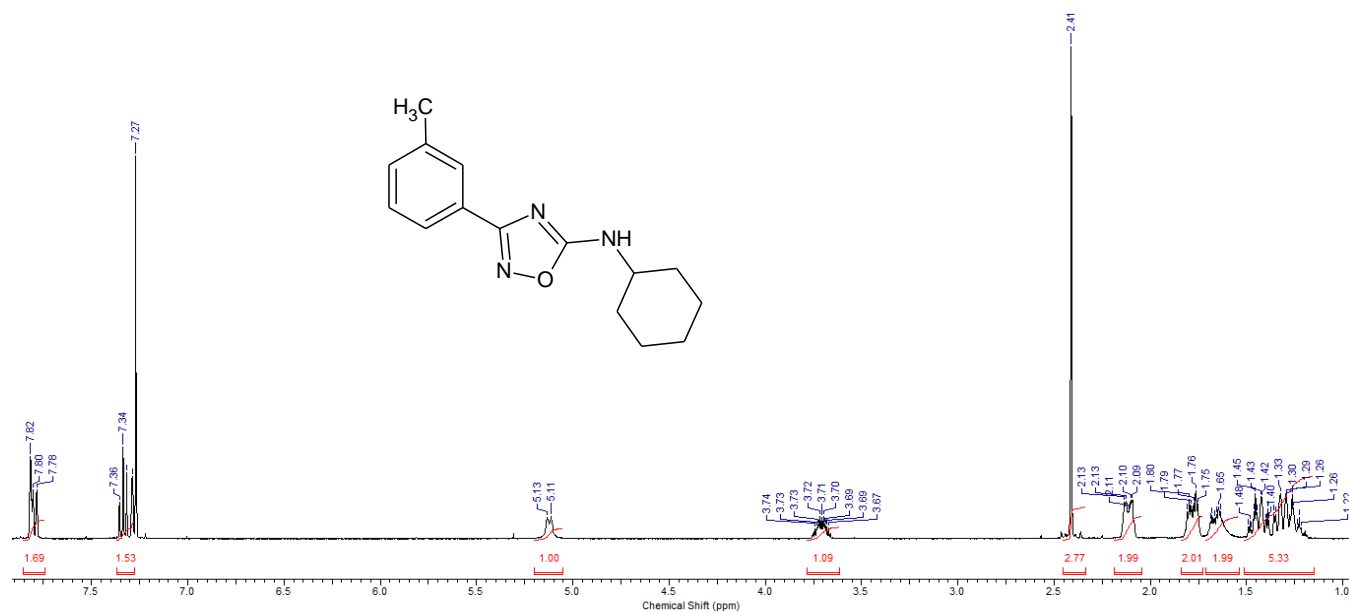


¹H NMR (CDCl₃, 300 MHz) of the compound (2a)

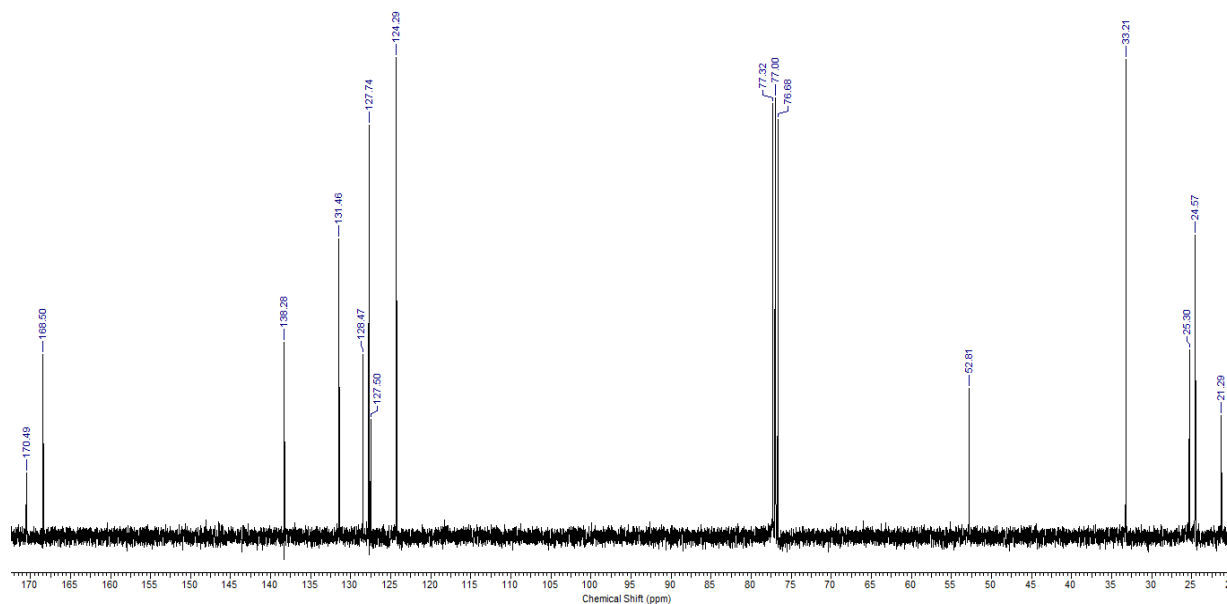


¹³C NMR (CDCl₃, 75.5 MHz) of the compound (2a)

***N*-Cyclohexyl-3-(3-methylphenyl)-1,2,4-oxadiazol-5-amine (2b)**

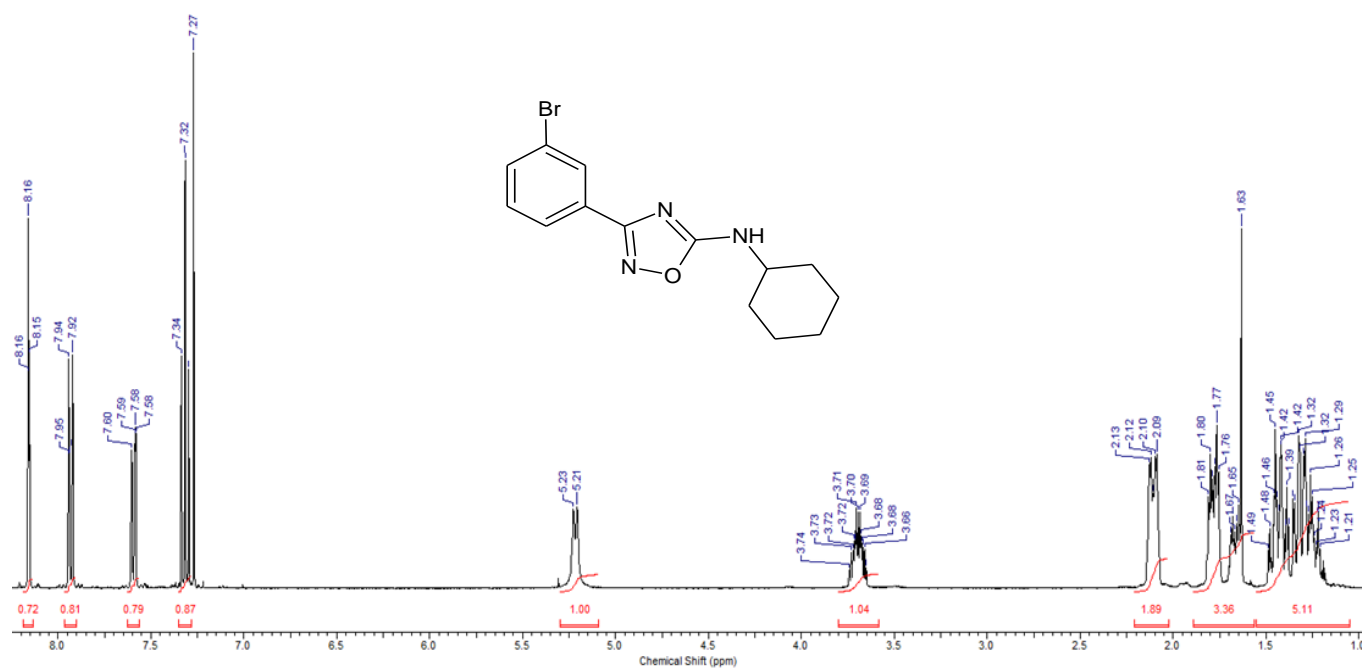


¹H NMR (CDCl₃, 300 MHz) of the compound (2b)

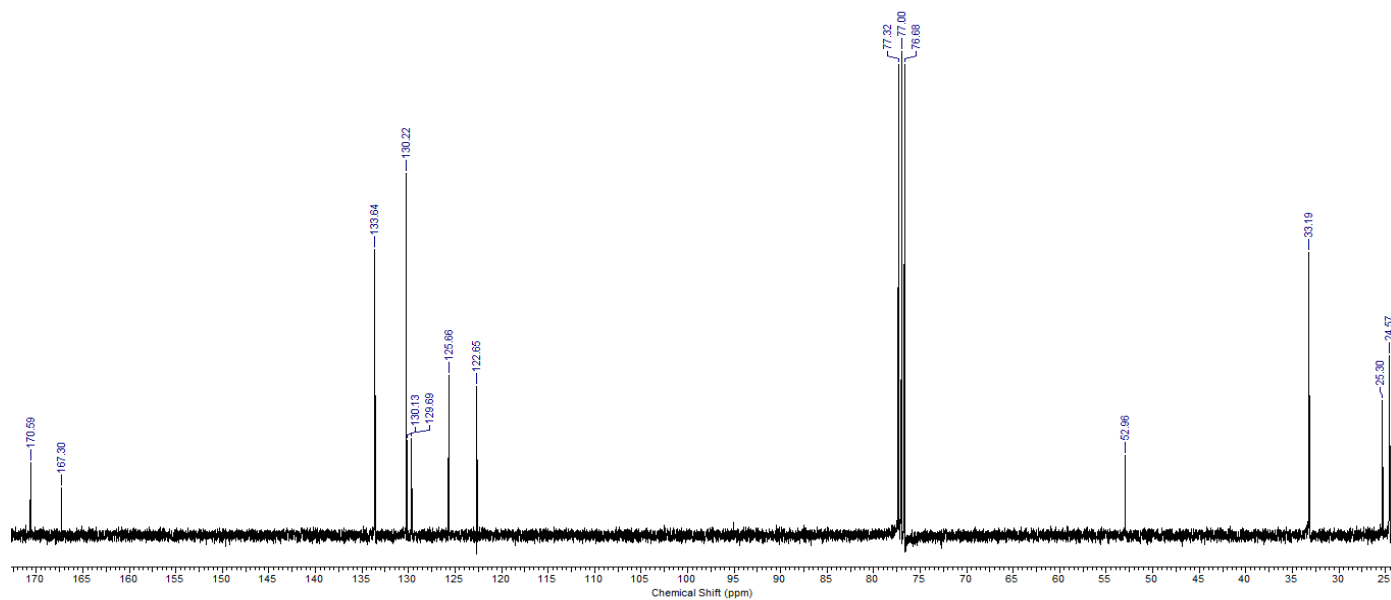


¹³C NMR (CDCl₃, 75.5 MHz) of the compound (2b)

***N*-Cyclohexyl-3-(3-bromophenyl)-1,2,4-oxadiazol-5-amine (2c)**

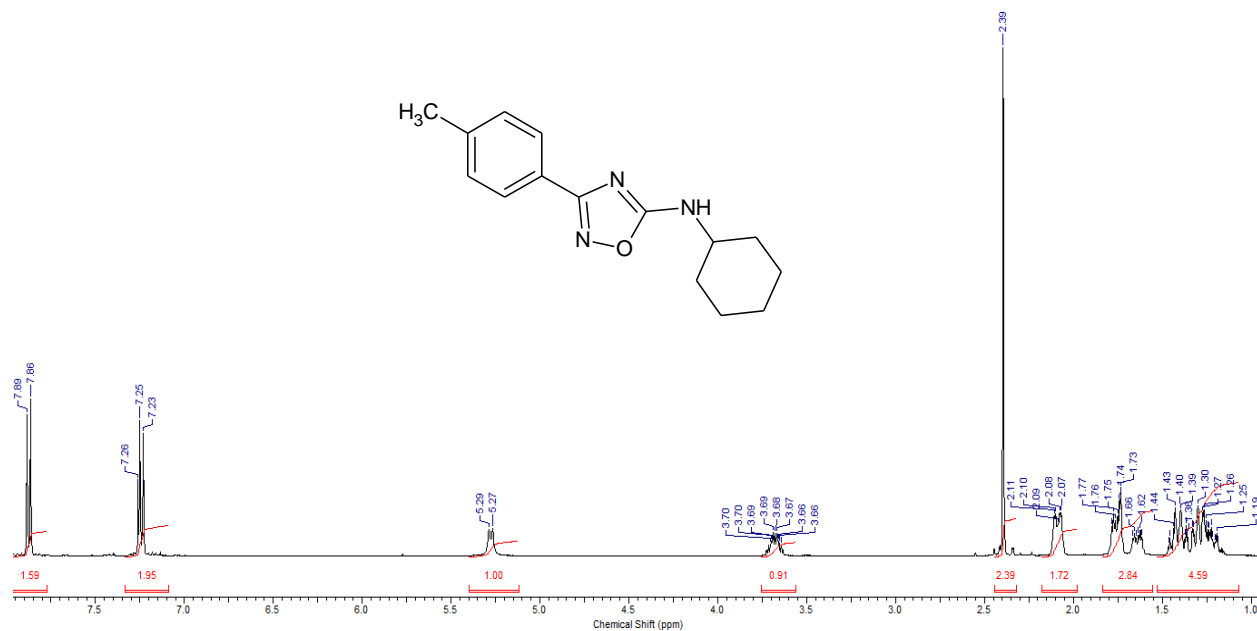


¹H NMR (CDCl₃, 300 MHz) of the compound (2c)

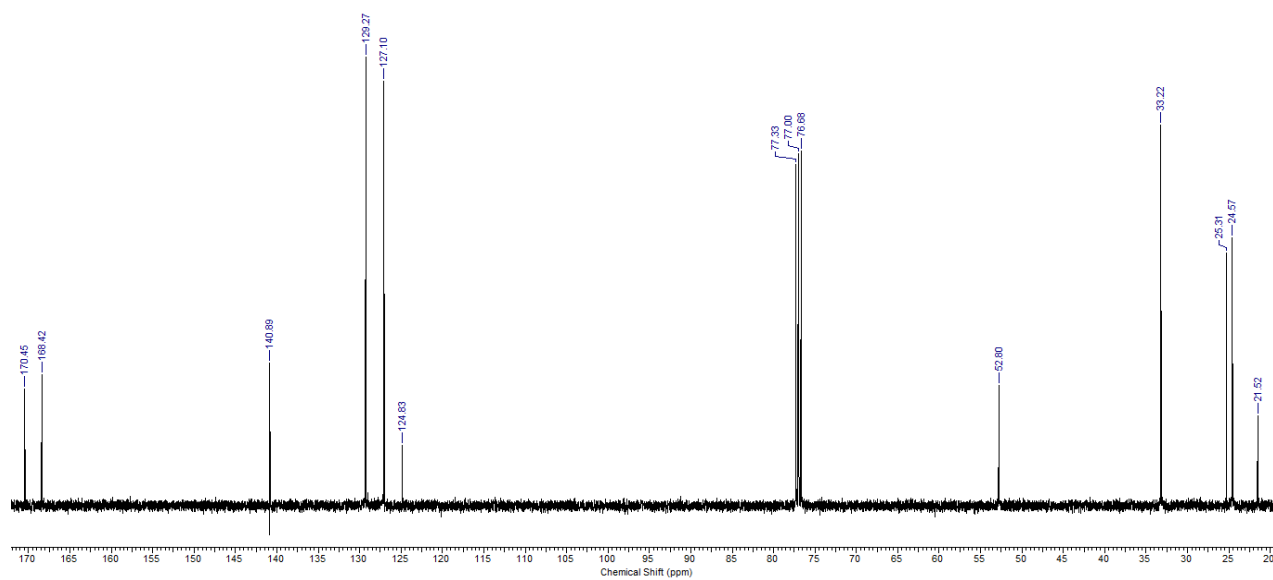


¹³C NMR (CDCl₃, 75.5 MHz) of the compound (2c)

***N*-Cyclohexyl-3-(4-methylphenyl)-1,2,4-oxadiazol-5-amine (2d)**

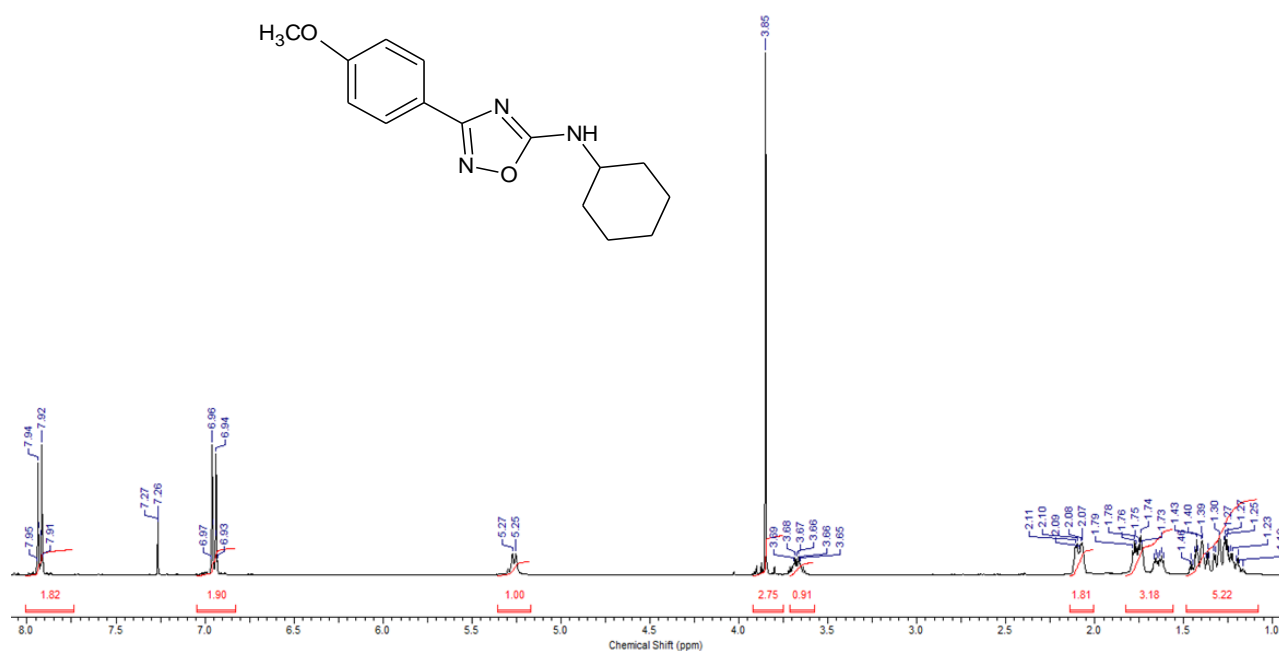


¹H NMR (CDCl₃, 300 MHz) of the compound (2d)

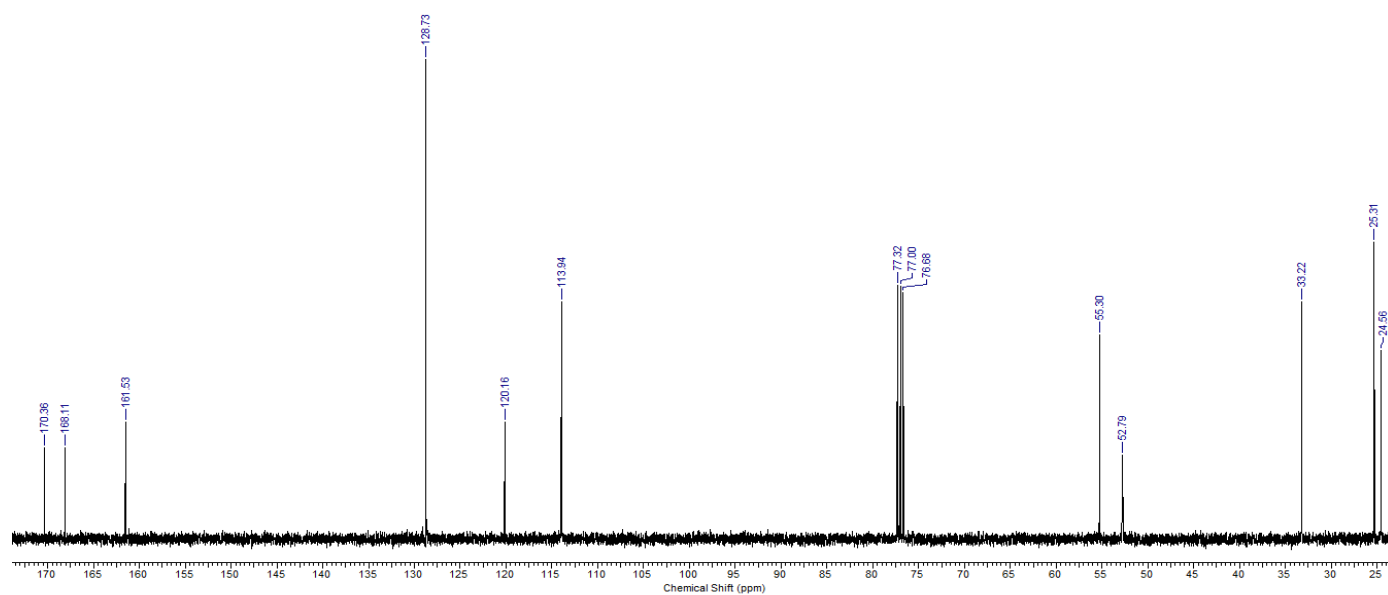


¹³C NMR (CDCl₃, 75.5 MHz) of the compound (2d)

***N*-Cyclohexyl-3-(4-methoxyphenyl)-1,2,4-oxadiazol-5-amine (2e)**

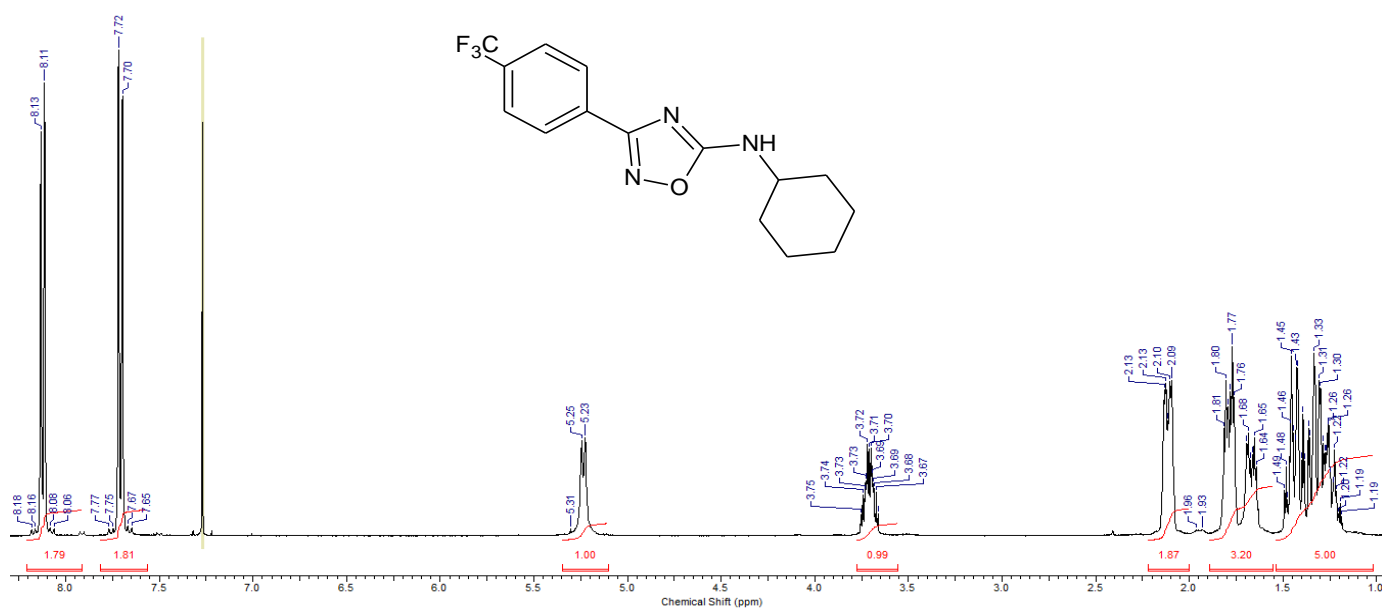


¹H NMR (CDCl₃, 300 MHz) of the compound (2e)

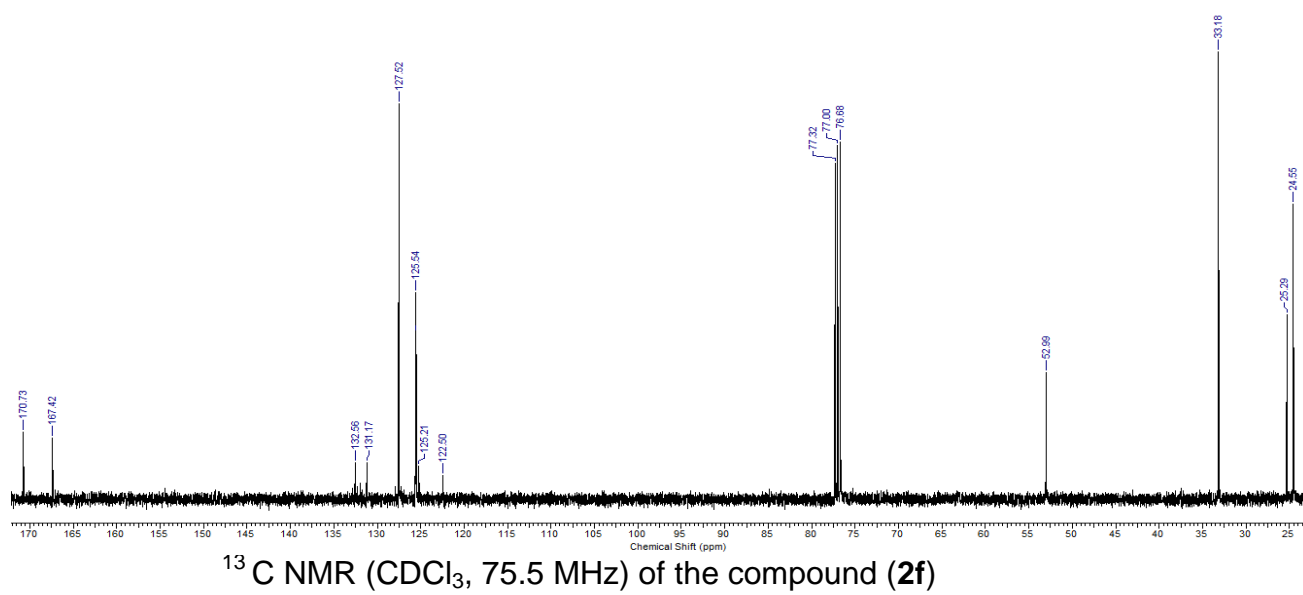


¹³C NMR (CDCl₃, 75.5 MHz) of the compound (2e)

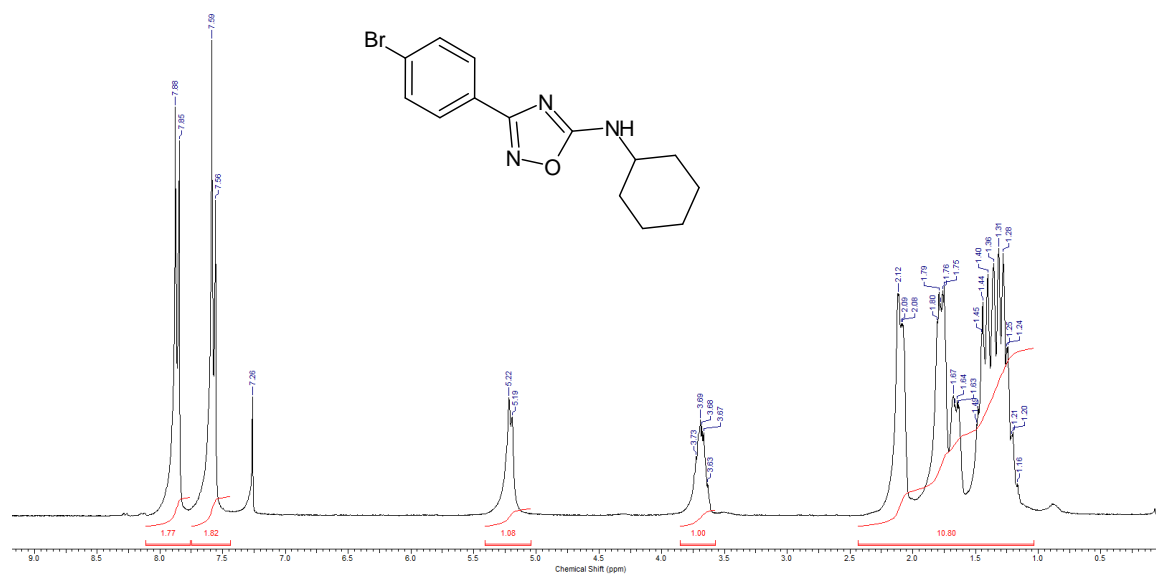
***N*-Cyclohexyl-3-(4-trifluoromethylphenyl)-1,2,4-oxadiazol-5-amine (2f)**



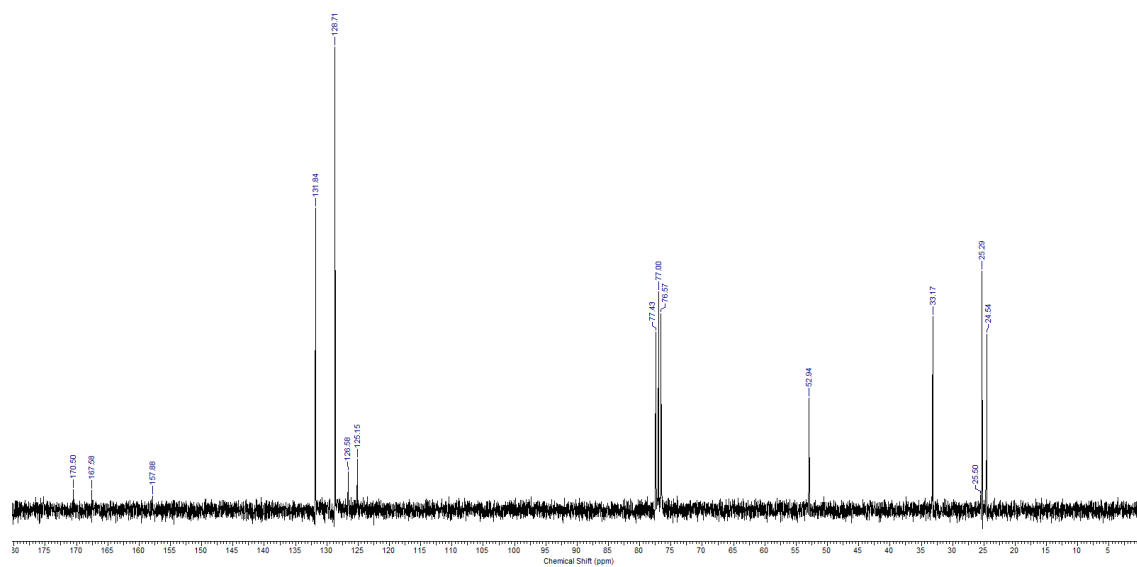
¹H NMR (CDCl₃, 300 MHz) of the compound (2f)



***N*-Cyclohexyl-3-(4-bromophenyl)-1,2,4-oxadiazol-5-amine (2g)**

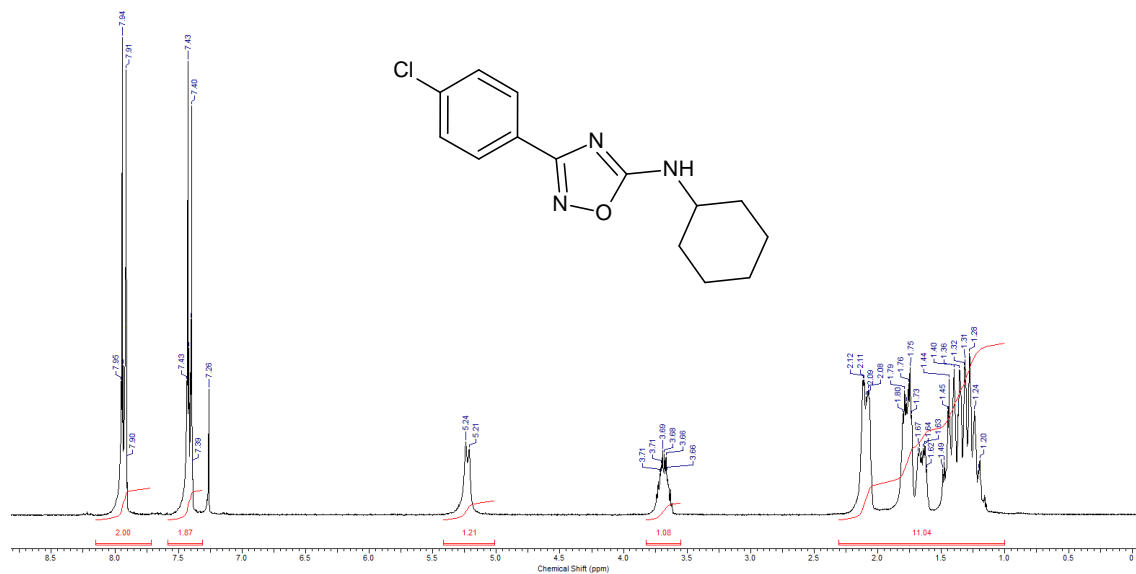


¹H NMR (CDCl₃, 300 MHz) of the compound (2g)

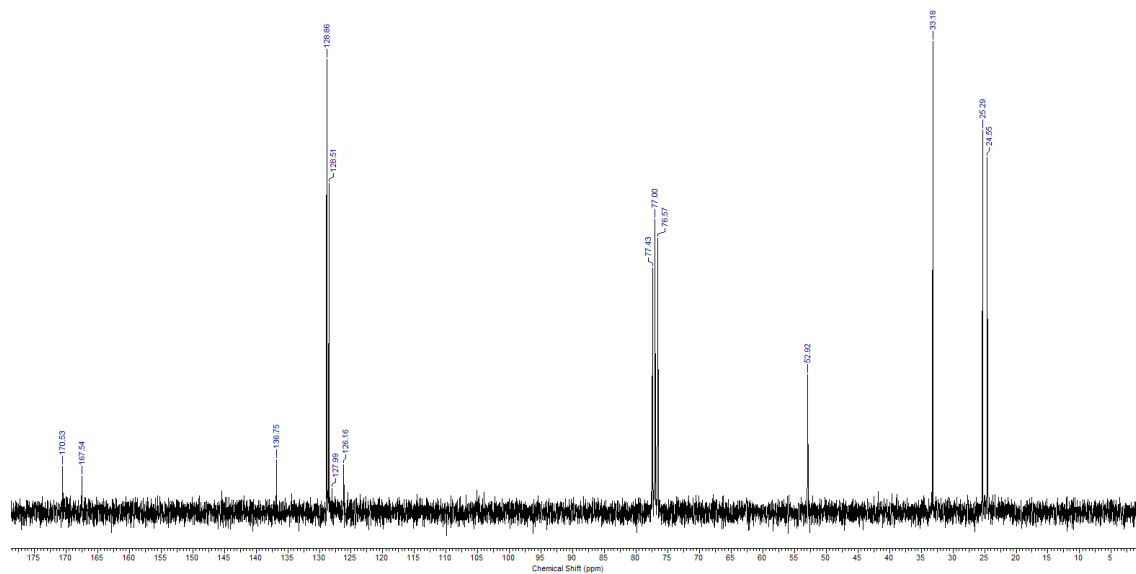


¹³C NMR (CDCl₃, 75.5 MHz) of the compound (2g)

***N*-Cyclohexyl-3-(4-chlorophenyl)-1,2,4-oxadiazol-5-amine (2h)**

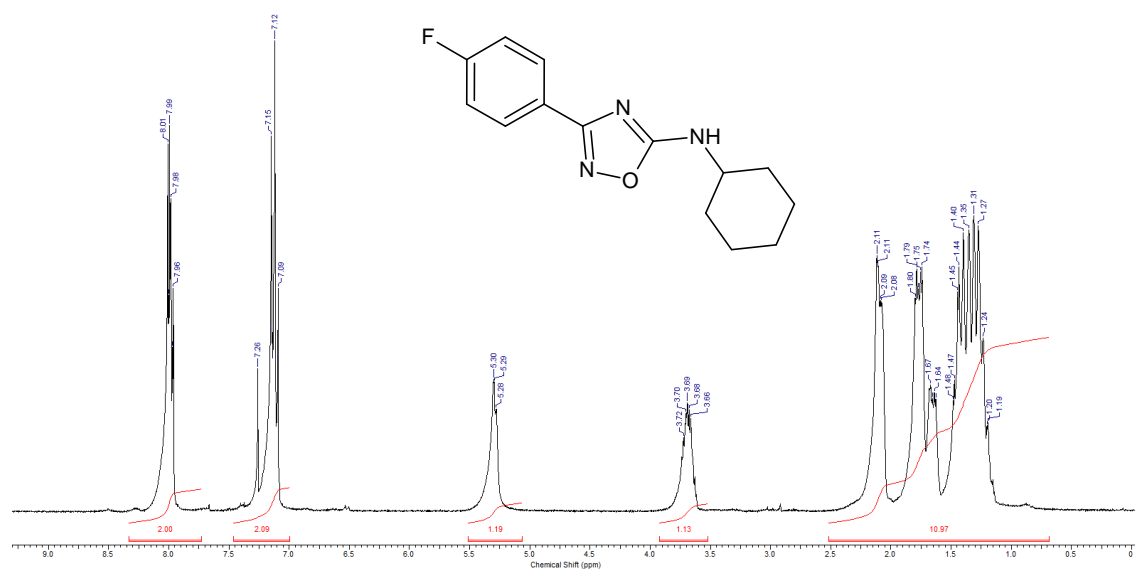


¹H NMR (CDCl₃, 300 MHz) of the compound (2h)

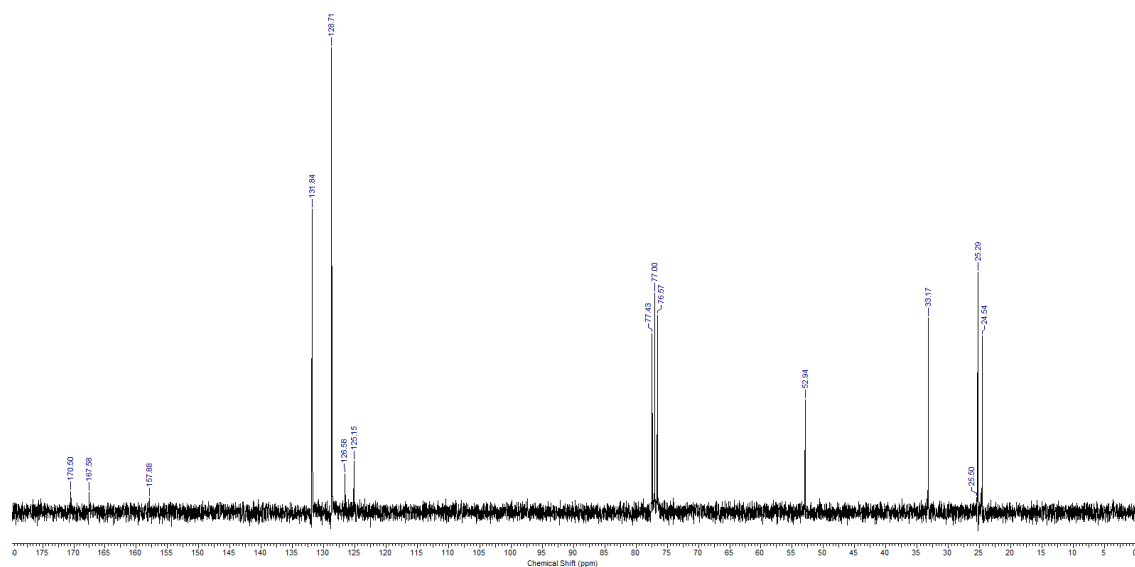


¹³C NMR (CDCl₃, 75.5 MHz) of the compound (2h)

***N*-Cyclohexyl-3-(4-fluorophenyl)-1,2,4-oxadiazol-5-amine (2i)**



¹H NMR (CDCl₃, 300 MHz) of the compound (2i)



¹³C NMR (CDCl₃, 75.5 MHz) of the compound (2i)