

Electronic supplementary information

**FERROCENE-CONTAINING MESOIONIC OXADIAZOLES:
SYNTHESIS AND PHYTOACTIVITY**

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General information

The NMR spectra were recorded on a Bruker AvanceTM 400 spectrometer with an operating frequency of 400.13 MHz for ¹H and 100.62 MHz for ¹³C. The chemical shifts are reported in ppm relative to tetramethylsilane using the solvent resonance as an internal standard (deuteriochloroform: δ 7.28 ppm for ¹H and 77.0 ppm for ¹³C, dimethyl sulfoxide: δ 2.50 ppm for ¹H and 39.5 ppm for ¹³C). The data are reported as follows: chemical shifts (δ), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, br = broad, m = multiplet), coupling constants (*J*, Hz). The elemental analyses (except for Fe) were performed using a Carlo-Erba CE-1106 elemental analyzer; the contents of Fe were determined by X-ray fluorescence (XRF) using a VRA-30 XRF spectrometer. The melting points were determined with an Electrothermal 1002 MEL-TEMP[®] capillary melting point apparatus and were uncorrected. All reactions with organometallic compounds were carried out in a dry argon atmosphere in absolute solvents (THF was distilled over sodium benzophenone ketyl). The reaction course was monitored by thin layer chromatography on Sorbfil (Russia) and Silufol UV-254 (Czech Republic) plates, and spots were visualized by UV light at 254 nm and in camera with iodine. Column chromatography on silica gel 30–70 mesh was used for the routine purification of reaction products. All commercially available ABCR and Aldrich reagents were used without preliminary purification.

Experimental procedures and characterization

The starting sydnone and sydnone imines were prepared according to the published methods [S1, S2].

3-(Dimethylamino)-*N*- α -haloacetylsydnone imine. Yield 78%, white crystals, m.p. 83–84 °C. ¹H NMR (400 MHz, CDCl₃): δ 8.03 (s, 1H, H(4)), 4.20 (s, 2H, -CH₂-), 3.26 (s, 6H, -N(CH₃)₂). ¹³C NMR (101 MHz, CDCl₃): δ 174.9, 173.1, 100.7, 46.7, 45.3. Anal. Calcd for C₆H₉ClN₄O₂: C, 35.22; H, 4.43; Cl, 17.33; N, 27.38. Found: C, 35.03; H, 4.68; Cl, 17.39; N, 27.51%.

Synthesis of *N*-substituted *N*-(sydnon-4-yl-methyl)ferrocenecarboxamides (2a–c) (general procedure). *N,N*-Diisopropylethylamine (DIPEA) (3.0 mmol) was added dropwise to a solution of 4-*N*-monosubstituted aminomethylsydnone (1.0 mmol) and ferrocenoyl chloride (1.1 mmol) in 10 mL of anhydrous dichloromethane at 20 °C. The mixture was warmed to room temperature and was left for 1 h. The reaction mixture was washed with water (2×10 mL) and passed through a layer of Al₂O₃ (2×3 cm) using an ethyl acetate–chloroform mixture (1:1) as an eluent. The solvent was evaporated under reduced pressure. The residue was recrystallized from toluene–petroleum ether.

***N*-Methyl-*N*-(3-phenyl-sydnon-4-yl-methyl)ferrocenecarboxamide (2a).** Yield 98%, dark brown crystals, m.p. 197–198 °C. ¹H NMR (400 MHz, CDCl₃): δ 7.65 (s, 5H, -C₆H₅), 4.55 (s, 4H, Fc), 4.32 (s, 2H, -CH₂-), 4.19 (s, 5H, Fc), 3.36 (s, 3H, -CH₃). ¹³C NMR (101 MHz, CDCl₃): δ 171.1, 168.5, 133.7, 132.4, 130.1, 124.8, 105.0, 77.3, 70.7, 69.9, 69.8, 41.2, 38.6. Anal. Calcd for C₂₁H₁₉FeN₃O₃: C, 60.45; H, 4.59; Fe, 13.38; N, 10.07. Found: C, 60.29; H, 4.72; Fe, 13.42; N, 9.82%.

***N*-(2-Methoxyethyl)-*N*-(3-phenyl-sydnon-4-yl-methyl)ferrocenecarboxamide (2b).** Yield 74%, dark brown crystals, m.p. 134–135 °C. ¹H NMR (400 MHz, CDCl₃): δ 7.86–7.52 (m, 5H, -C₆H₅), 4.58 (s, 2H, -CH₂-), 4.53 (s, 2H), 4.30 (s, 2H) and 4.23 (s, 5H, Fc), 4.17–3.87 (br. m, 2H, -NCH₂-), 3.58 (t, *J* = 5.2 Hz, 2H, -OCH₂-), 3.30 (s, 3H, -CH₃). ¹³C NMR (101 MHz, CDCl₃): δ 171.3, 168.2, 133.9, 132.2, 130.0, 125.0, 105.6, 77.3, 71.5, 70.6, 70.0, 69.7, 59.1, 49.4, 40.1. Anal. Calcd for C₂₃H₂₃FeN₃O₄: C, 59.89; H, 5.03; Fe, 12.11; N, 9.11. Found: C, 59.95; H, 4.89; Fe, 12.17; N, 9.34%.

***N*-Benzyl-*N*-(3-phenyl-sydnon-4-yl-methyl)ferrocenecarboxamide (2c).** Yield 83%, dark brown crystals, m.p. 167–168 °C. ¹H NMR (400 MHz, CDCl₃): δ 7.81–7.60 (m, 5H, -C₆H₅), 7.44–7.34

(m, 2H), 7.34–7.27 (m, 1H) and 7.26–7.20 (m, 2H, $-\text{CH}_2-\text{C}_6\text{H}_5$), 5.33 (s, 2H, $-\text{CH}_2-\text{C}_6\text{H}_5$), 4.54 (s, 2H, Fe), 4.39 (s, 2H, $-\text{CH}_2-$), 4.30 (s, 2H) and 4.26 (s, 5H, Fe). ^{13}C NMR (101 MHz, CDCl_3): δ 172.0, 168.0, 137.2, 133.8, 132.2, 130.0, 129.0, 127.6, 126.7, 125.1, 105.7, 77.1, 70.6, 70.2, 70.0, 54.2, 40.1. Anal. Calcd for $\text{C}_{27}\text{H}_{23}\text{FeN}_3\text{O}_3$: C, 65.73; H, 4.70; Fe, 11.32; N, 8.52. Found: C, 65.84; H, 4.58; Fe, 11.41; N, 8.71%.

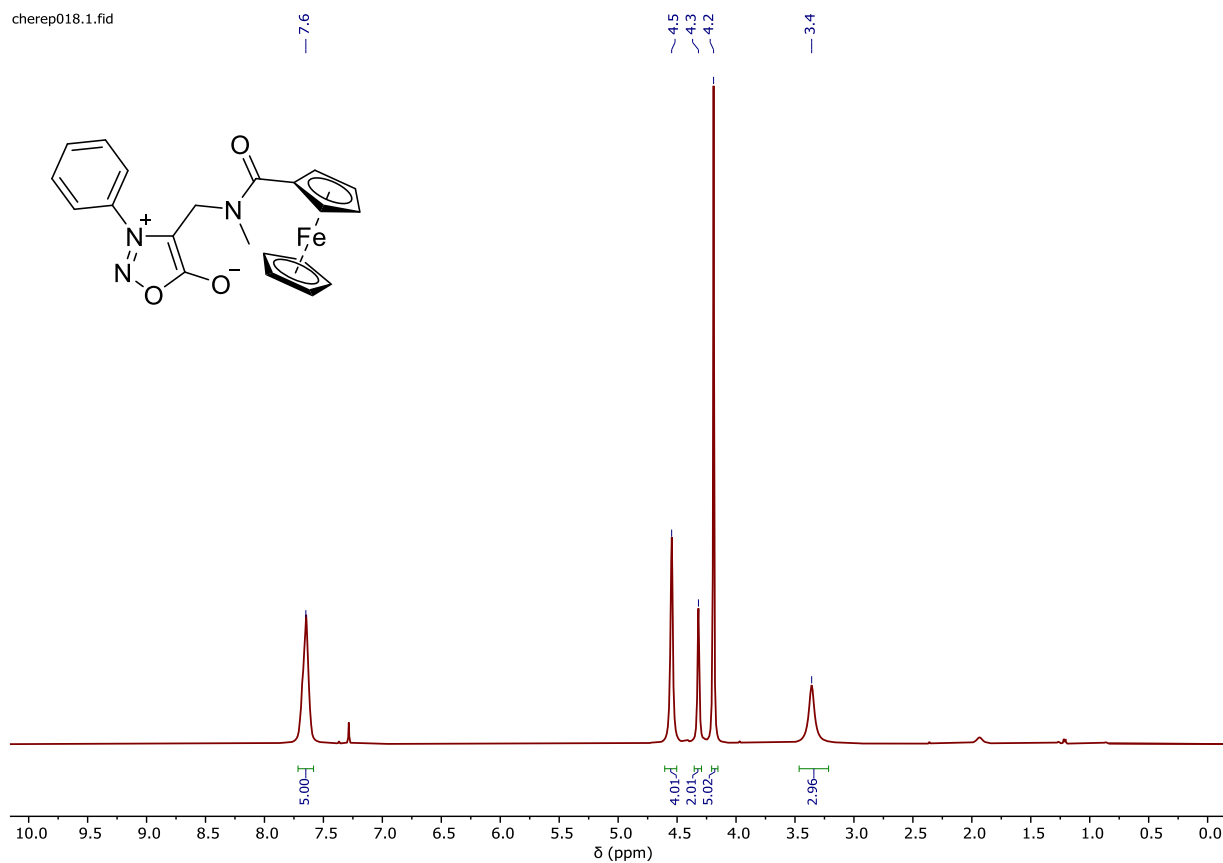
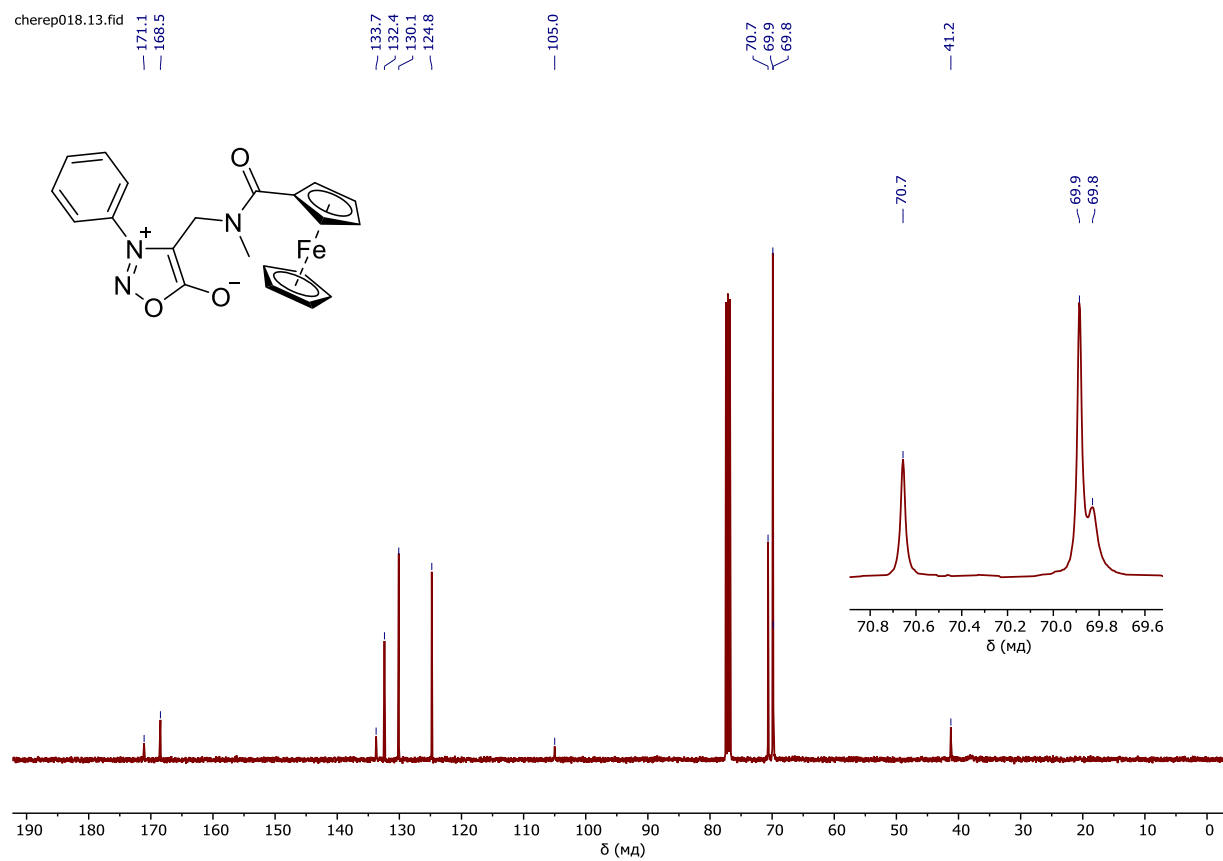
Synthesis of Preparation of N_6 -(2-methyl(ferrocenylmethyl)amino)acetylsydnone imines (4a–c) (general procedure). *N,N*-Diisopropylethylamine (DIPEA) (3.0 mmol) was added dropwise to a solution of N_6 - α -haloacetylsydnone imine (1.0 mmol) and *N*-methyl-*N*-(ferrocenylmethyl)amine (1.1 mmol) in 10 mL of methanol. The mixture was warmed to 60 °C and left for 20 min. The reaction mixture was washed with water (2×10 mL) and passed through a layer of Al_2O_3 (2×3 cm) using an ethyl acetate–chloroform mixture (1:1) as an eluent. The solvent was evaporated under reduced pressure. The residue was recrystallized from toluene–petroleum ether.

3-Ethyl- N_6 -[2-methyl(ferrocenylmethyl)amino]acetylsydnone imine (4a). Yield 58%, yellow crystals, m.p. 118–119 °C. ^1H NMR (400 MHz, CDCl_3): δ 8.11 (s, 1H, H(4)), 4.52 (q, $J = 7.5$ Hz, 2H, $-\text{CH}_2\text{CH}_3$), 4.18 (br. s, 2H) and 4.09 (br. s, 7H, Fe), 3.56 (s, 2H, $-\text{CH}_2-$), 3.31 (s, 2H, $-\text{CH}_2\text{Fc}$), 2.29 (s, 3H, $-\text{CH}_3$), 1.68 (t, $J = 7.3$ Hz, 3H, $-\text{CH}_2\text{CH}_3$). ^{13}C NMR (101 MHz, CDCl_3): δ 180.0, 173.9, 105.4, 82.6, 70.4, 68.4, 68.0, 62.4, 56.7, 48.9, 42.1, 14.2. Anal. Calcd for $\text{C}_{18}\text{H}_{22}\text{FeN}_4\text{O}_2$: C, 56.56; H, 5.80; Fe, 14.61; N, 14.66. Found: C, 56.34; H, 5.91; Fe, 14.87; N, 14.31%.

3-Isopropyl- N_6 -[2-methyl(ferrocenylmethyl)amino]acetylsydnone imine (4b). Yield 60%, yellow crystals, m.p. 109–110 °C. ^1H NMR (400 MHz, CDCl_3): δ 8.02 (s, 1H, H(4)), 4.87 (hept, $J = 6.6$ Hz, 1H, $-\text{CH}(\text{CH}_3)_2$), 4.19 (br. s, 2H) and 4.09 (br. s, 7H, Fe), 3.58 (s, 2H, $-\text{CH}_2-$), 3.31 (s, 2H, $-\text{CH}_2\text{Fc}$), 2.30 (s, 3H, $-\text{CH}_3$), 1.70 (d, $J = 6.8$ Hz, 6H, $-\text{CH}(\text{CH}_3)_2$). ^{13}C NMR (101 MHz, CDCl_3): δ 180.2, 173.8, 103.5, 82.7, 70.4, 68.4, 67.9, 62.2, 58.2, 56.5, 42.0, 21.9. Anal. Calcd for $\text{C}_{19}\text{H}_{24}\text{FeN}_4\text{O}_2$: C, 57.59; H, 6.10; Fe, 14.09; N, 14.14. Found: C, 57.71; H, 6.31; Fe, 13.93; N, 14.24%.

3-Dimethylamino- N_6 -[2-methyl(ferrocenylmethyl)amino]acetylsydnone imine (4c). Yield 62%, yellow crystals, m.p. 92–93 °C. ^1H NMR (400 MHz, CDCl_3): δ 8.01 (s, 1H, H(4)), 4.21 (s, 2H) and 4.11 (s, 7H, Fe), 3.62 (s, 2H, CH_2-), 3.34 (s, 2H, $-\text{CH}_2\text{Fc}$), 3.25 (s, 6H, $-\text{N}(\text{CH}_3)_2$), 2.34 (s, 3H, $-\text{CH}_3$). ^{13}C NMR (101 MHz, CDCl_3): δ 180.0, 172.9, 100.4, 82.5, 70.4, 68.4, 68.0, 62.1, 56.5, 45.5, 41.9. Anal. Calcd for $\text{C}_{18}\text{H}_{23}\text{FeN}_5\text{O}_2$: C, 54.42; H, 5.84; Fe, 14.06; N, 17.63. Found: C, 54.51; H, 5.96; Fe, 14.14; N, 17.31%.

NMR spectra of the products obtained

Figure S1. ^1H NMR spectrum of **2a**, CDCl_3 , 400 MHz.Figure S2. ^{13}C NMR spectrum of **2a**, CDCl_3 , 101 MHz.

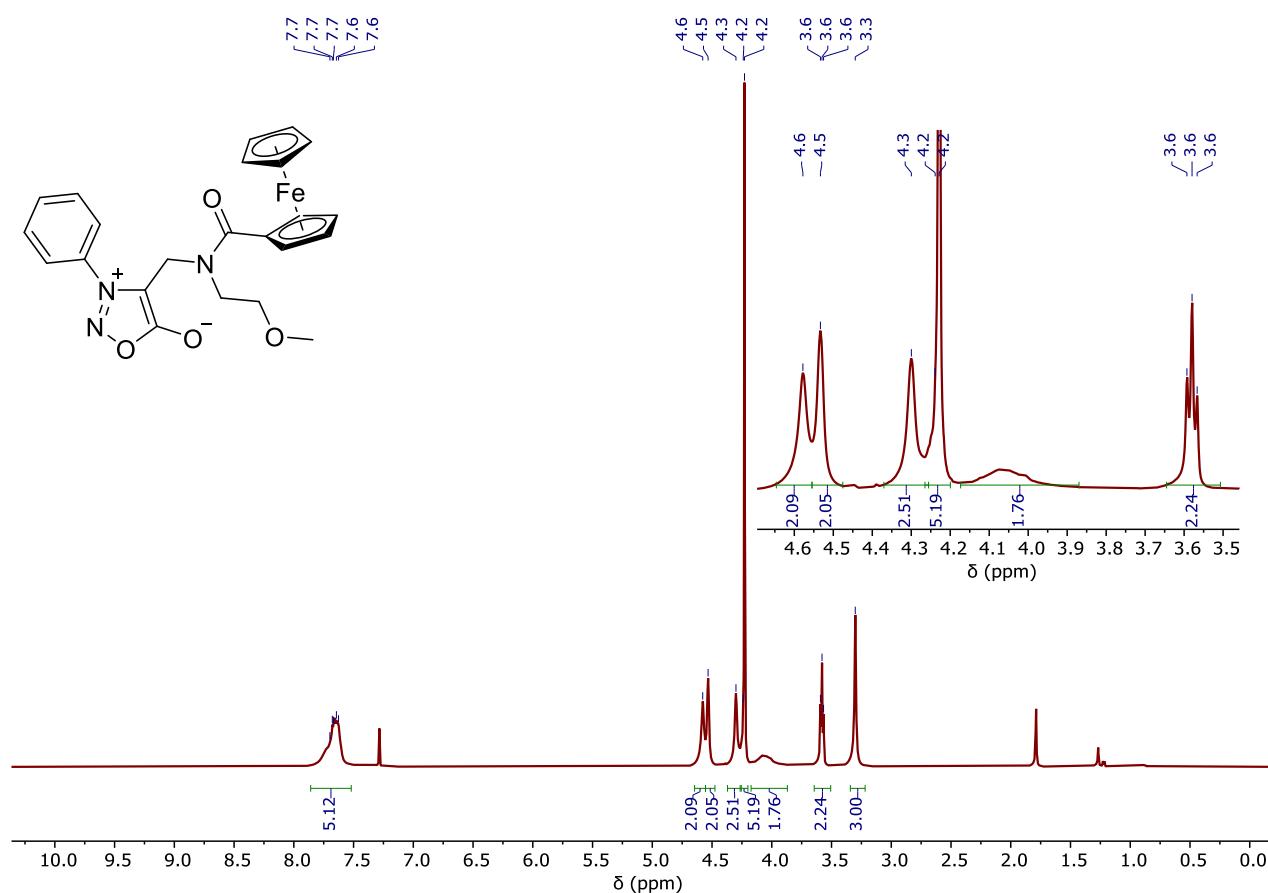


Figure S3. ¹H NMR spectrum of **2b**, CDCl₃, 400 MHz.

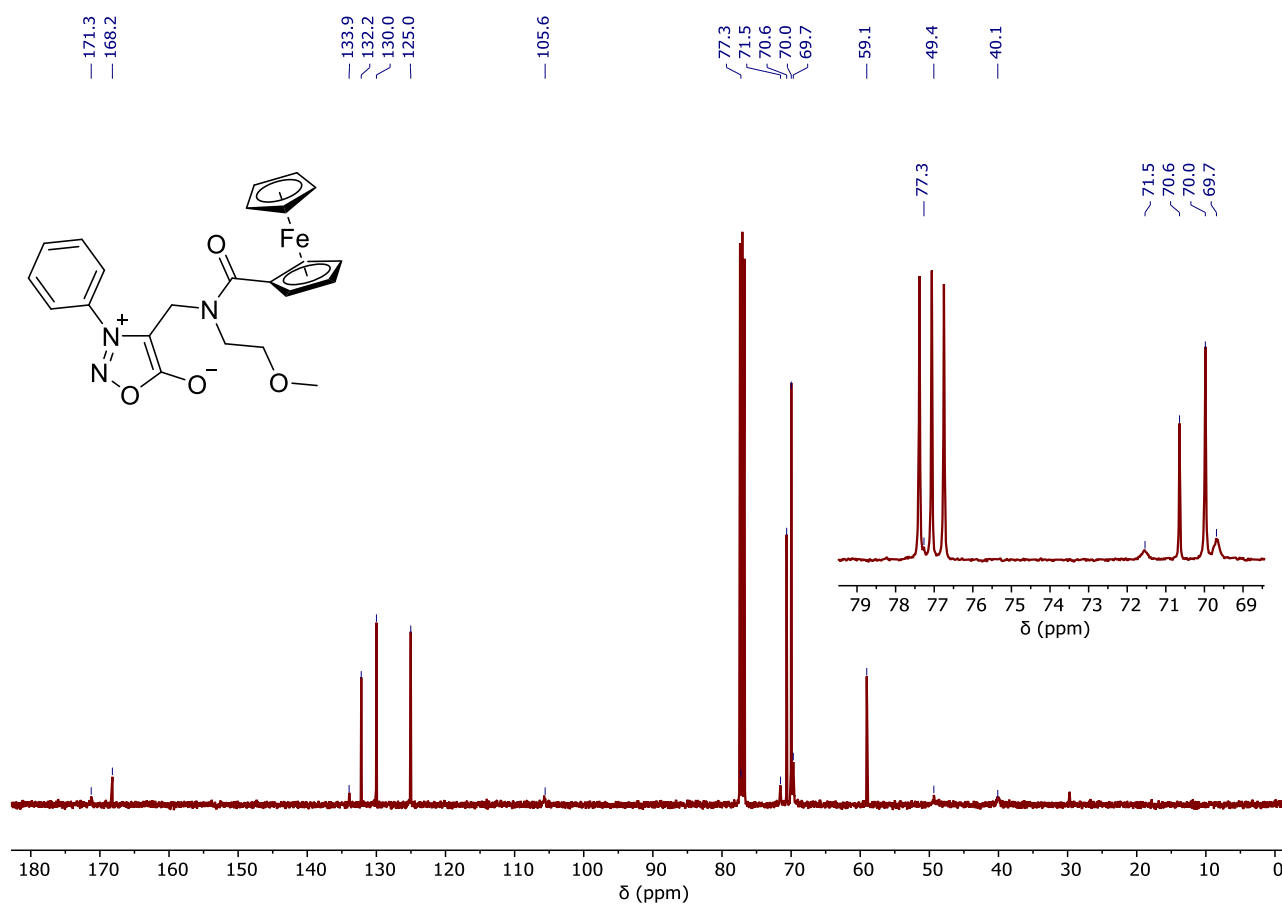


Figure S4. ¹³C NMR spectrum of **2b**, CDCl₃, 101 MHz.

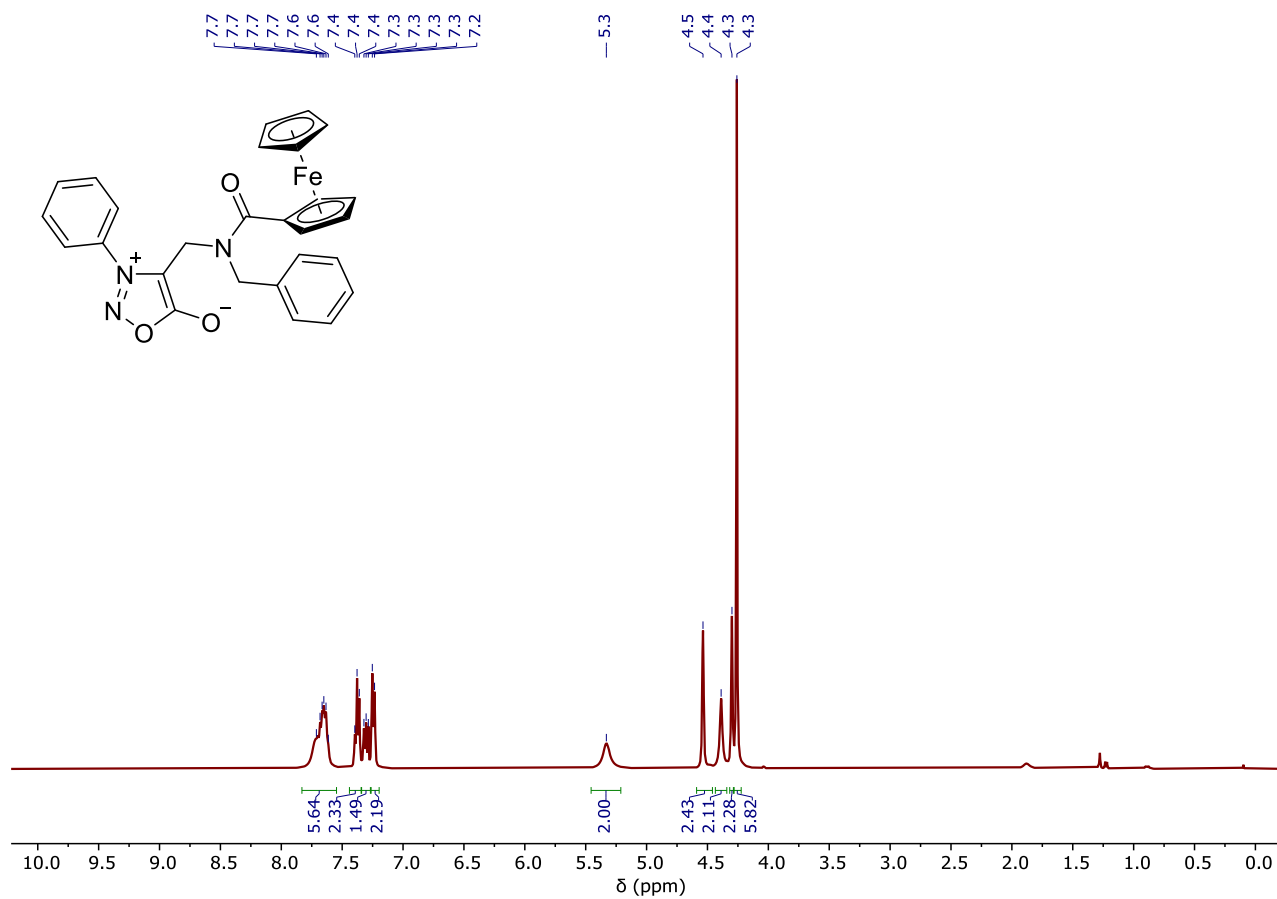


Figure S5. ¹H NMR spectrum of **2c**, CDCl₃, 400 MHz.

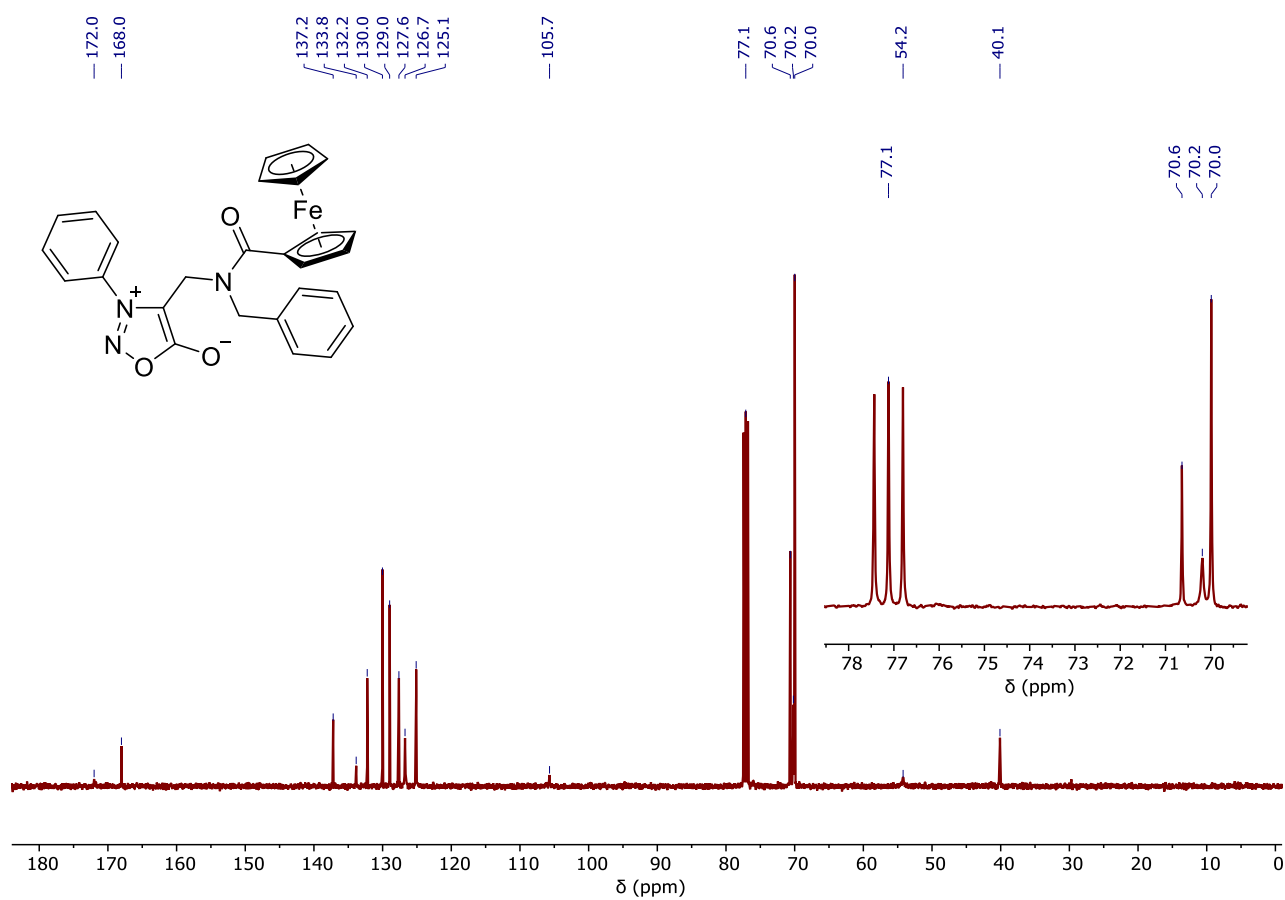
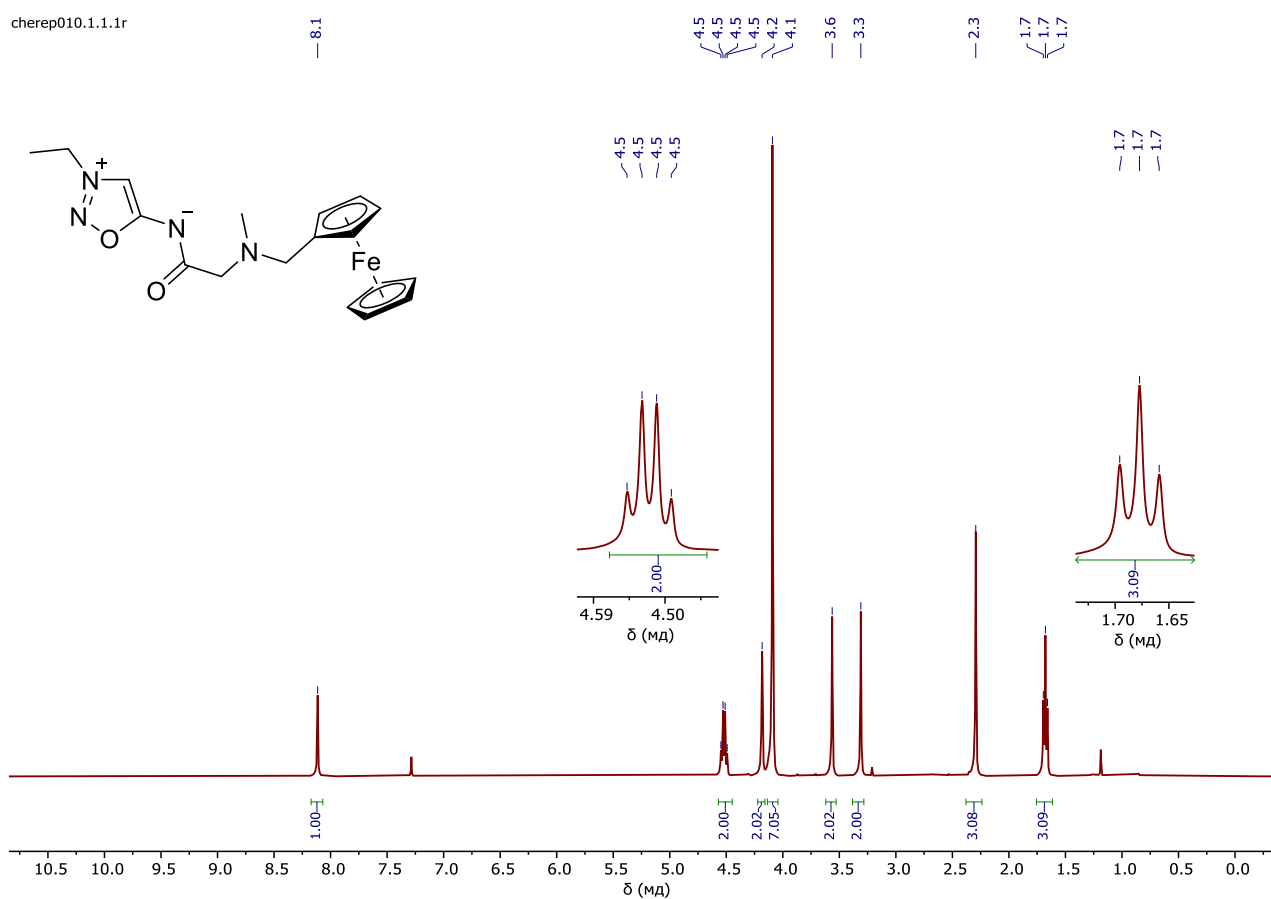
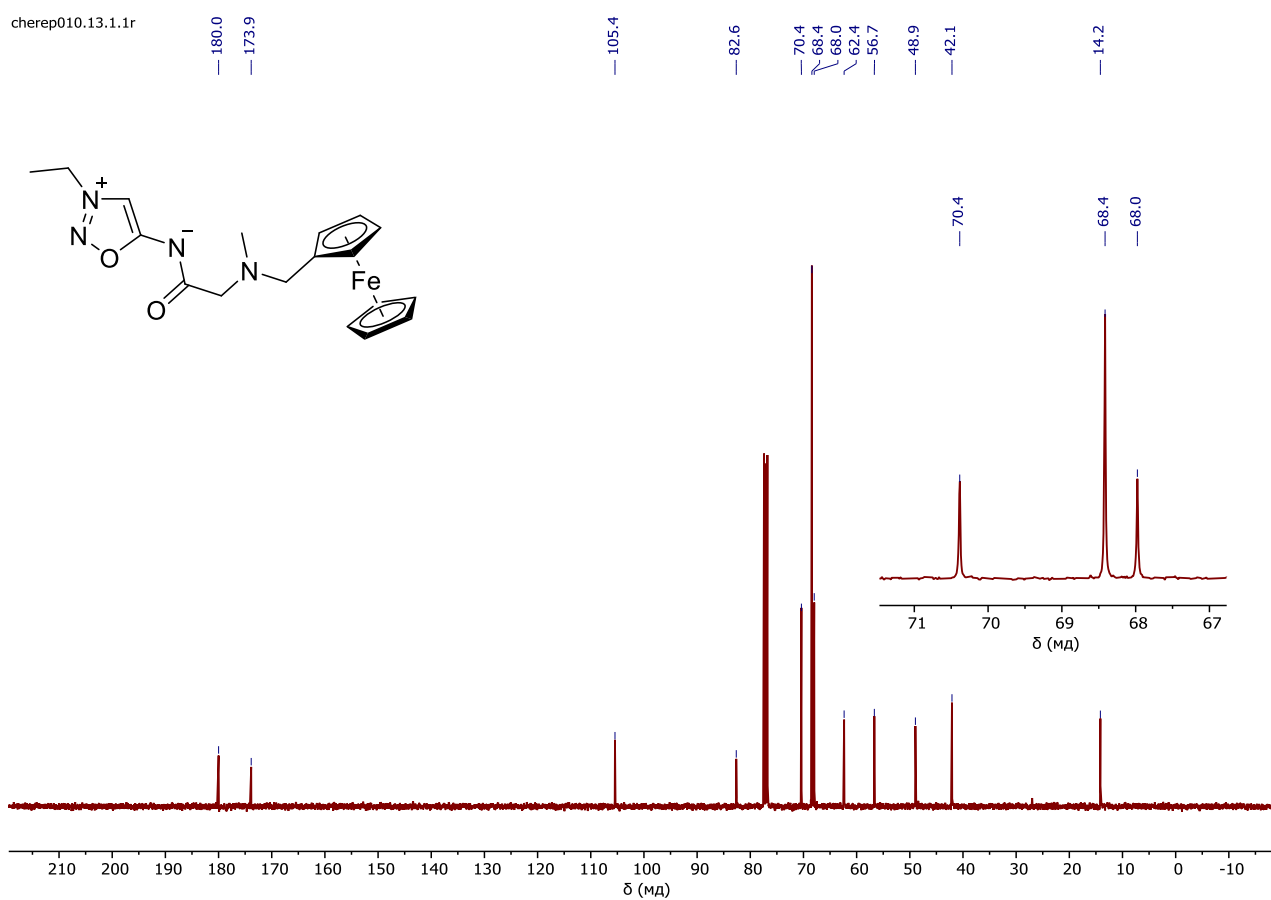


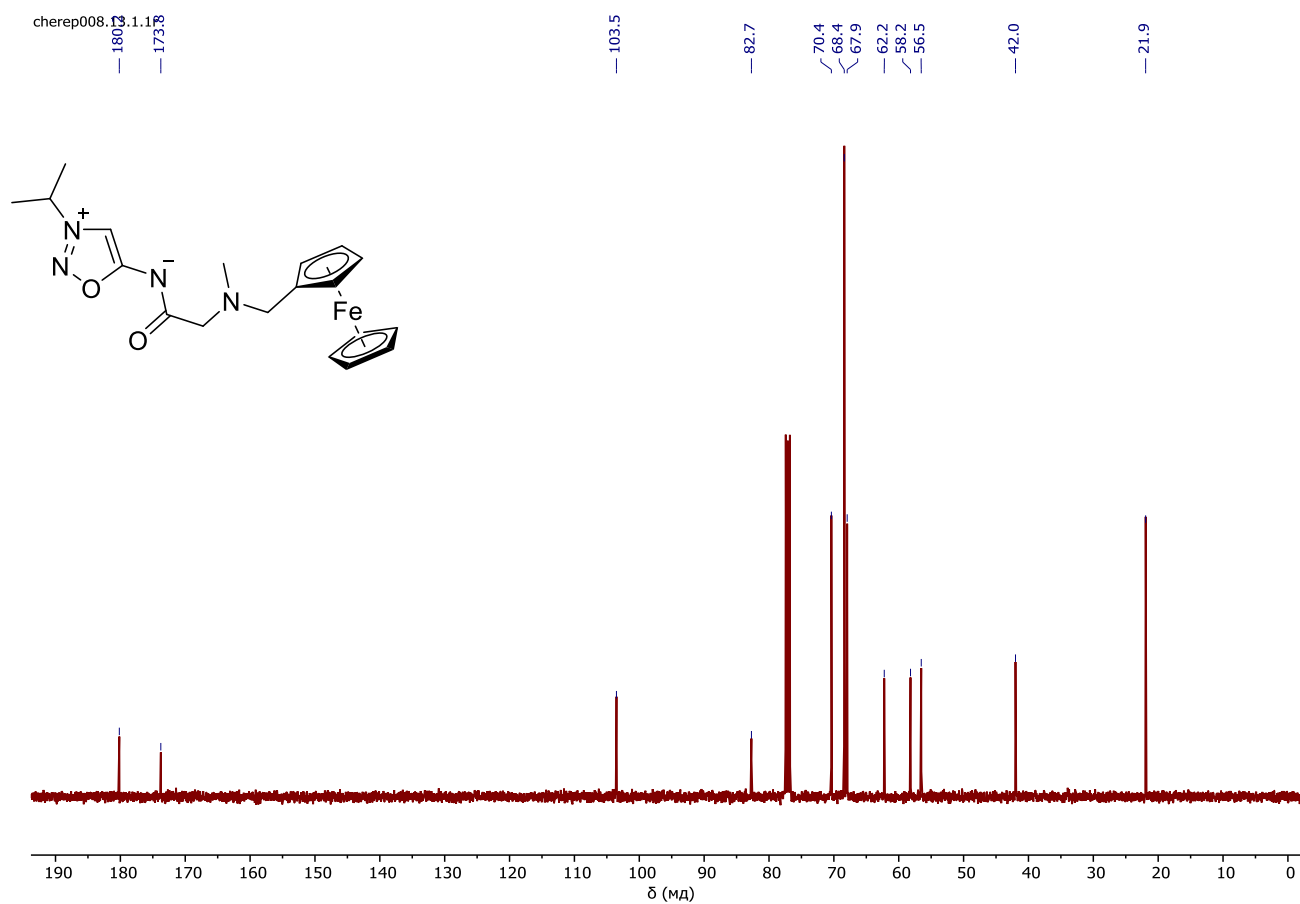
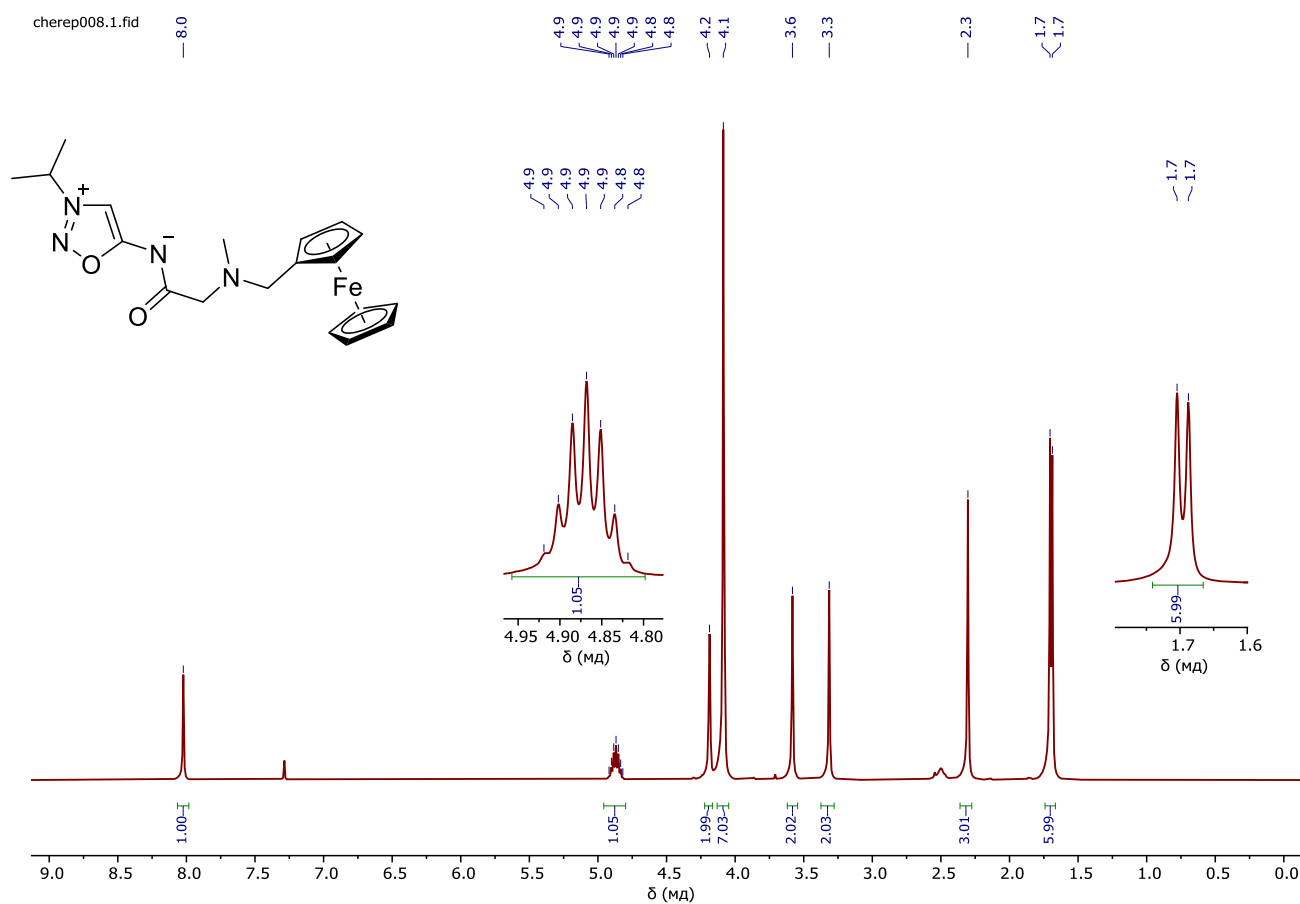
Figure S6. ¹³C NMR spectrum of **2c**, CDCl₃, 101 MHz.

cherep010.1.1.1r

Figure S7. ^1H NMR spectrum of **4a**, CDCl_3 , 400 MHz.

cherep010.13.1.1r

Figure S8. ^{13}C NMR spectrum of **4a**, CDCl_3 , 101 MHz.



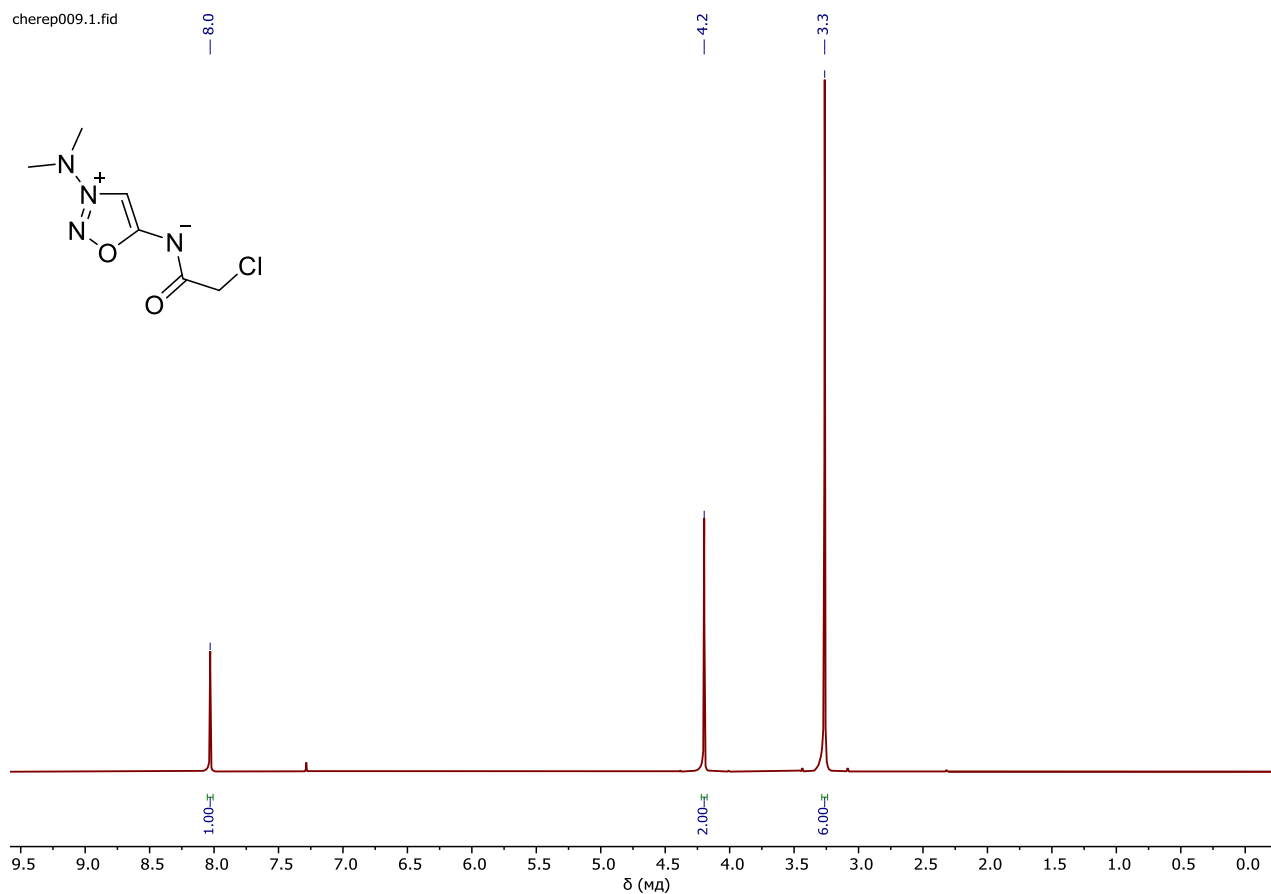


Figure S11. ^1H NMR spectrum of 3-(dimethylamino)-*N*₆-α-haloacetylsydnone imine, CDCl_3 , 400 MHz.

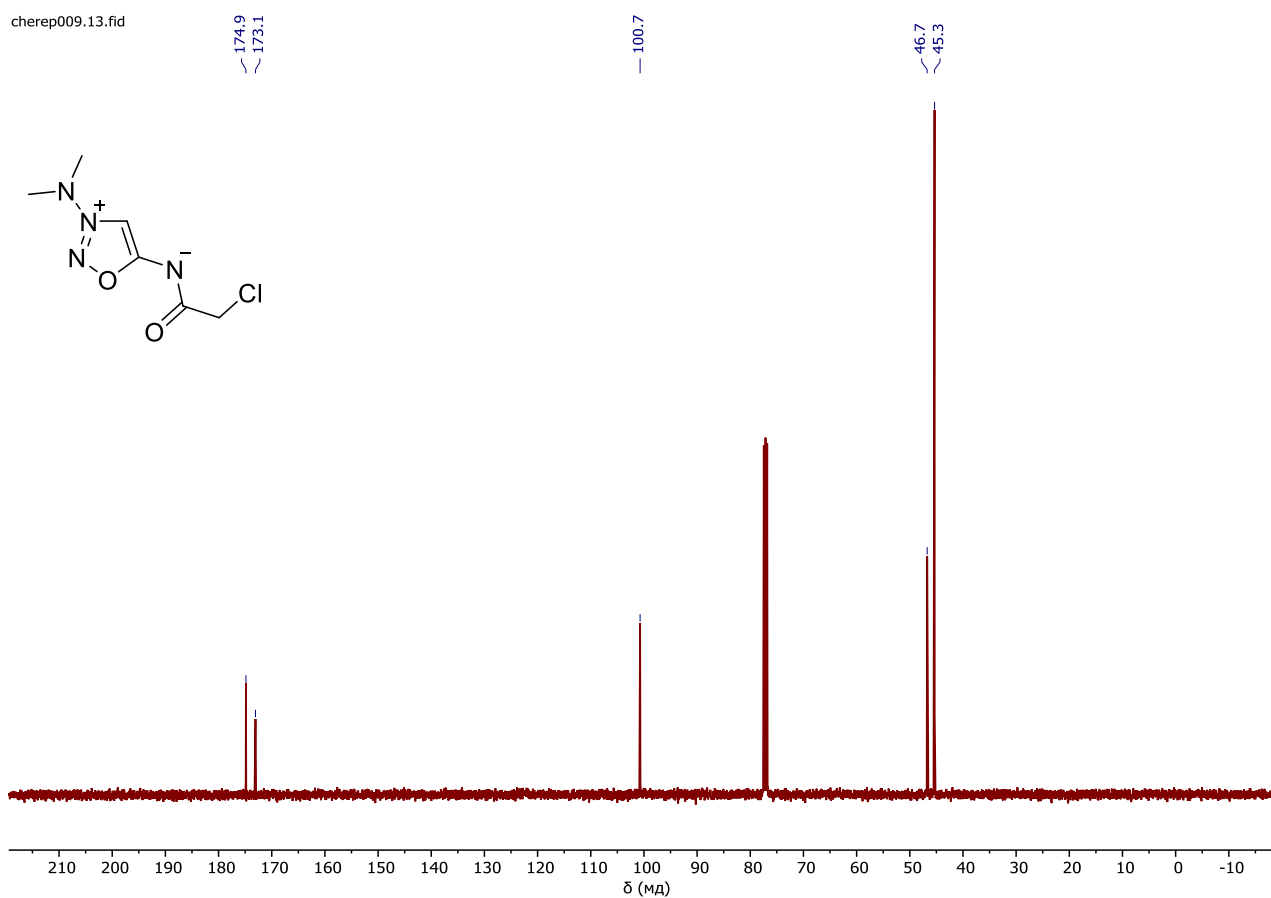
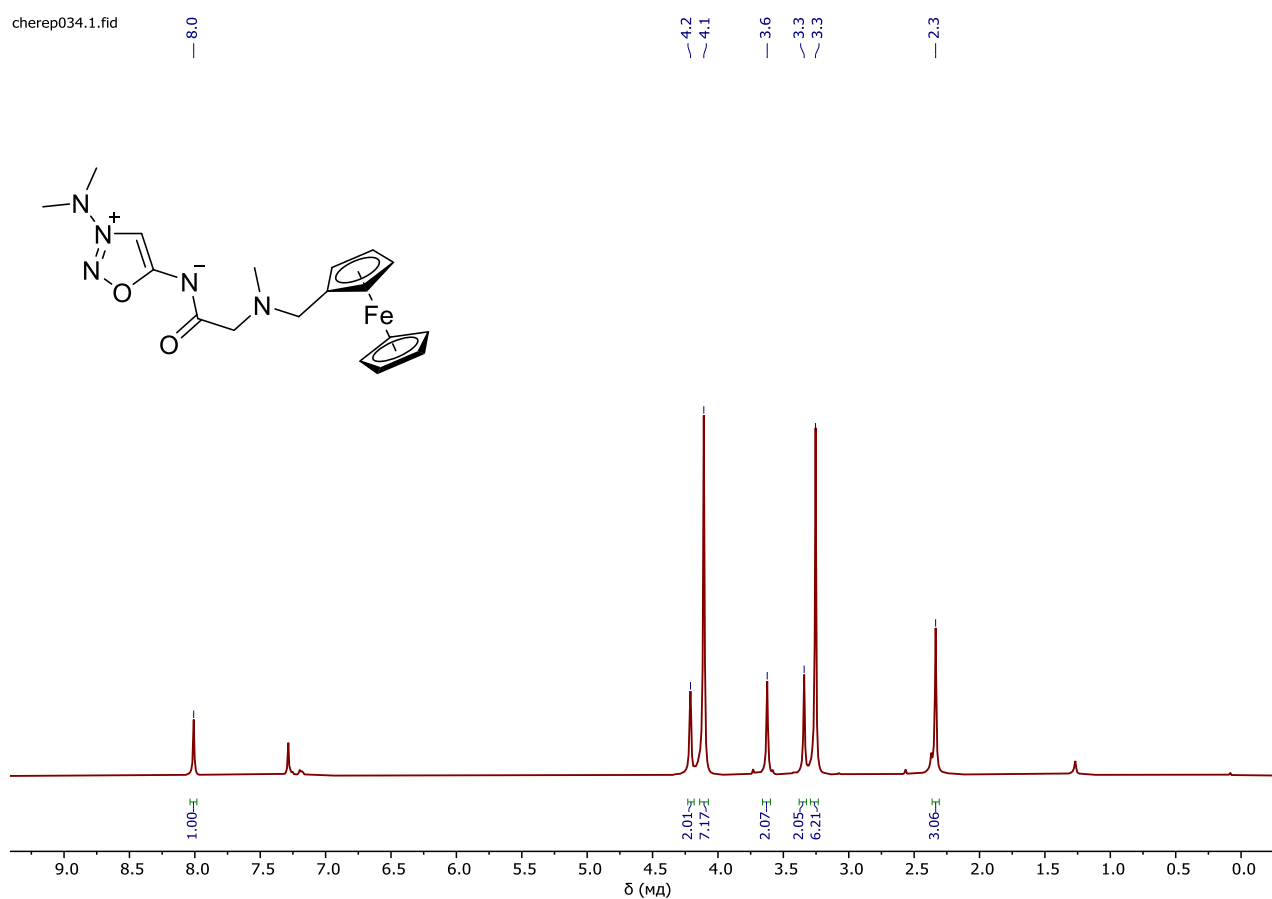
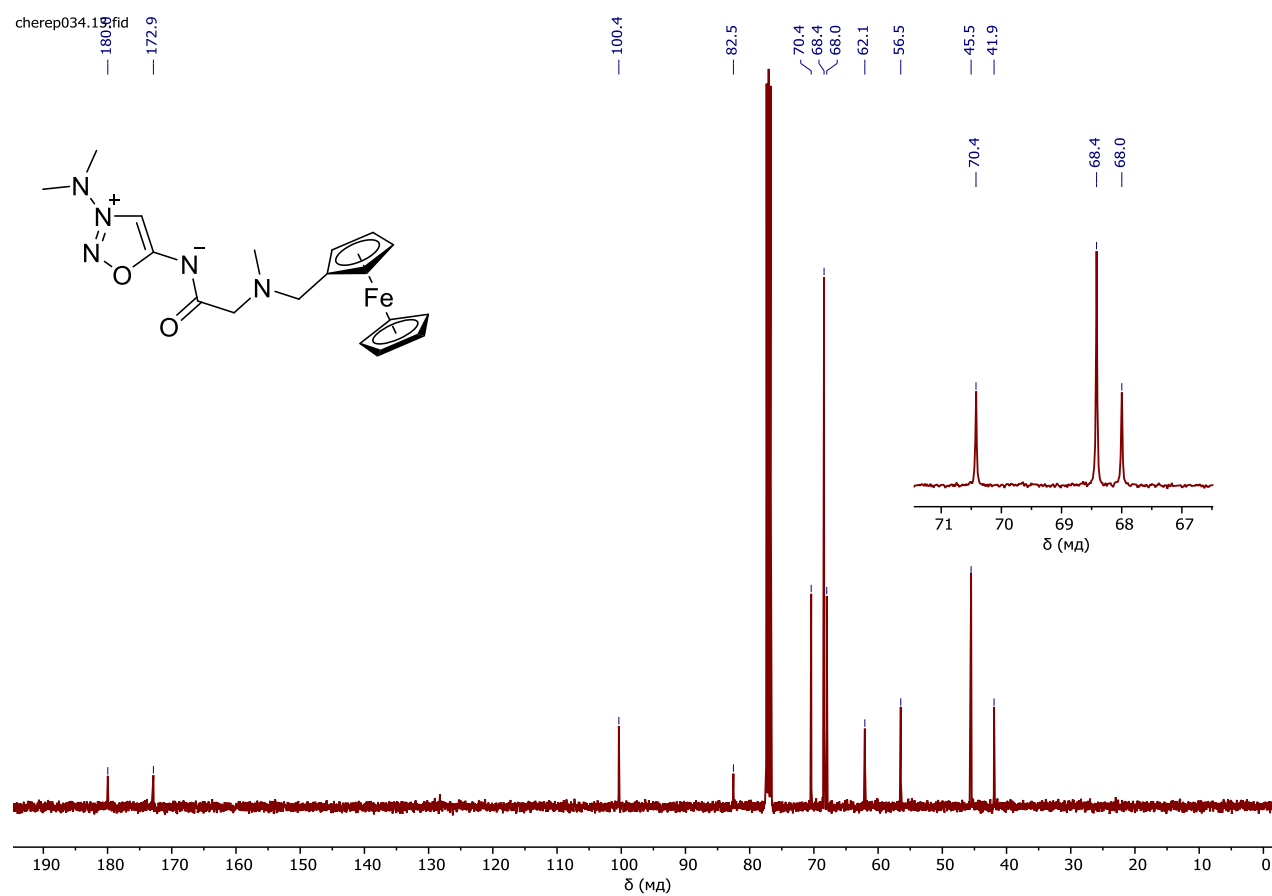


Figure S12. ^{13}C NMR spectrum of 3-(dimethylamino)-*N*₆-α-haloacetylsydnone imine, CDCl_3 , 101 MHz.

cherep034.1.fid

Figure S13. ¹H NMR spectrum of **4c**, CDCl₃, 400 MHz.

cherep034.19.fid

Figure S14. ¹³C NMR spectrum of **4c**, CDCl₃, 101 MHz.

Biological experiments

Corn seeds of the Krasnodar 291 AMV cultivar were germinated in Petri dishes (diameter 9 cm) with round paper filters of the appropriate size. 16 pieces of seeds (average weight 5.5 g) were placed in Petri dishes to which the following was added: 5 mL of distilled water, or 5 mL of an aqueous solution of the herbicide Zinger WP (60% metsulfuron-methyl, Metsulfuron-Methyl, Zinger WP, CAS 74223-64-6) with a concentration of $1.7 \cdot 10^{-7}$ M [at a dose of $2.6 \cdot 10^{-4}$ mol·t⁻¹], or 5 mL of an aqueous solution of tested compounds **2a–c**, **4a–c** with a concentration of $2.75 \cdot 10^{-6}$ M [at a dose of $2.5 \cdot 10^{-3}$ mol·t⁻¹], or 5 mL of an aqueous solution containing compounds **2a–c**, **4a–c** with a concentration of $2.75 \cdot 10^{-6}$ M and the herbicide Zinger WP with a concentration of $1.7 \cdot 10^{-7}$ M. Each experiment was repeated seven times. The Petri dishes with corn seeds were thermostated at 25 °C and 100% humidity for 96 h. After 96 h, the sum mass of roots and sprouts separately of 10 corn seeds with the best growth in each Petri dishes was measured. The resulting data are given in Tables S1, S2. The sample standard deviation was calculated using the following formula

$$S^2 = \sqrt{\frac{\sum (x - \bar{x})^2}{n - 1}}$$

where n is the number of observations obtained in the sample, x_1, x_2, \dots, x_n are the obtained observations and \bar{x} is the sample mean. The standard error of mean was calculated as follows

$$S_x = \sqrt{\frac{S^2}{n}}$$

The 95% confidence interval for the mean, was calculated from a sample with $n = 7$

$$\bar{x} \pm t_{0.05} \cdot S_x$$

The relative measurement error of the sample mean was calculated as:

$$S_{x\%} = \frac{t_{0.05} \cdot S_x}{\bar{x}}$$

Table S1. Measurement data for compounds **2a–c**

	Water (Exp.1)		2a		2b		Water (Exp.2)		2c		Zinger (Exp.1)		2a + Zinger		2b + Zinger		Zinger (Exp.2)		2c + Zinger	
n	$\sum m_{\text{roots}}$	$\sum m_{\text{sprouts}}$	$\sum m_{\text{roots}}$	$\sum m_{\text{sprouts}}$	$\sum m_{\text{roots}}$	$\sum m_{\text{sprouts}}$	$\sum m_{\text{roots}}$	$\sum m_{\text{sprouts}}$	$\sum m_{\text{roots}}$	$\sum m_{\text{sprouts}}$	$\sum m_{\text{roots}}$	$\sum m_{\text{sprouts}}$	$\sum m_{\text{roots}}$	$\sum m_{\text{sprouts}}$	$\sum m_{\text{roots}}$	$\sum m_{\text{sprouts}}$	$\sum m_{\text{roots}}$	$\sum m_{\text{sprouts}}$	$\sum m_{\text{roots}}$	$\sum m_{\text{sprouts}}$
1	1246	656	1150	485	1198	621	1032	412	994	273	812	355	671	349	890	546	663	401	788	331
2	1127	620	948	414	1148	647	1037	462	1026	494	705	490	824	455	783	382	680	436	711	296
3	1298	643	995	523	1201	518	1065	598	1157	650	711	470	790	390	708	426	703	400	765	297
4	1163	644	1101	541	1172	505	973	550	1140	628	913	403	793	384	782	333	788	311	694	486
5	1136	611	1155	672	1135	534	949	532	931	300	798	523	861	420	799	362	764	337	725	222
6	1000	566	1220	618	1182	886	1068	607	1148	500	782	351	712	398	678	395	812	386	745	302
7	1119	719	1005	496	1253	501	1160	498	1142	356	723	606	845	452	701	462	672	367	662	495
\bar{x}	1156	637	1082	536	1184	602	1041	523	1077	457	778	457	785	407	763	415	726	377	727	347
$t_{0.05} S_x$	89	43	93	80	36	128	64	66	85	141	65	87	65	35	68	66	40	39	40	96
$S_{x\%}$	8	7	9	15	3	21	6	13	8	31	9	19	8	9	9	16	8	10	5	28

Table S2. Measurement data for compounds **4a–c**

	Water	4a	4b	4c	Zinger WP	4a + Zinger	4b + Zinger	4c + Zinger
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n	$\sum m_{\text{roots}}$	$\sum m_{\text{sprouts}}$	$\sum m_{\text{roots}}$	$\sum m_{\text{sprouts}}$	$\sum m_{\text{roots}}$	$\sum m_{\text{sprouts}}$	$\sum m_{\text{roots}}$	$\sum m_{\text{sprouts}}$	$\sum m_{\text{roots}}$	$\sum m_{\text{sprouts}}$	$\sum m_{\text{roots}}$	$\sum m_{\text{sprouts}}$	$\sum m_{\text{roots}}$	$\sum m_{\text{sprouts}}$	$\sum m_{\text{roots}}$	$\sum m_{\text{sprouts}}$
1	1246	656	1150	485	1198	621	1223	652	812	355	671	349	890	546	738	338
2	1127	620	948	414	1148	647	1367	525	705	490	824	455	783	382	716	449
3	1298	643	995	523	1201	518	1138	735	711	470	790	390	708	426	940	370
4	1163	644	1101	541	1172	505	1201	739	913	403	793	384	782	333	665	568
5	1136	611	1155	672	1135	534	1346	597	798	523	861	420	799	362	881	550
6	1000	566	1220	618	1182	886	1098	769	782	351	712	398	678	395	757	453
7	1119	719	1005	496	1253	501	1114	598	723	606	845	452	701	462	920	572
\bar{x}	1156	637	1082	536	1184	602	1212	659	778	457	785	407	763	415	802	471
$t_{0.05}S_x$	89	43	93	80	36	128	100	84	65	87	65	35	68	66	101	88
$S_{x\%}$	8	7	9	15	3	21	8	13	9	19	8	9	9	16	13	19

Table S3 shows the experimental data on the effects of the compounds **2a–c** and **4a–c**, the herbicide control, and their combination on the seed germination.

Table S3. Growth-regulating activity of compounds **2a–c**, **4a–c** at a dose of $2.5 \cdot 10^{-3}$ mol·t⁻¹ of seeds, the herbicide Zinger WP at a dose of $2.6 \cdot 10^{-4}$ mol·t⁻¹ of seeds, and their combined effect on germination of the corn seeds of the Krasnodar 291 AMV cultivar

Comp.	Weight of sprouts and roots in relation to the water control, % ^a											
	Compound				Zinger WP				Compound + Zinger WP			
	Roots		Sprouts		Roots		Sprouts		Roots		Sprouts	
		LSD ^b		LSD ^b		LSD ^b		LSD ^b		LSD ^b		LSD ^b
2a	107	10	103	12	67	9	72	14	64	7	71	14
2b	106	12	109	14	67	9	72	14	68	8	78	15
2c	103	9	87	26	70	7	72	13	70	6	66	18
4a	94	10	84	13	67	9	72	14	68	7	64	13
4b	102	7	94	19	67	9	72	14	66	7	65	15
4c	105	10	103	13	67	9	72	14	69	9	74	17

^a weight of sprouts and roots in the water control were taken as 100%;

^b LSD is the least significant difference 95% confidence.

The antidote activity of the tested compounds was assessed by comparing the values of E_{exp} and E_{calc} . E_{exp} is the experimental growth-regulating effect of a combination of the tested compound and metsulfuron-methyl. E_{calc} is the expected value calculated according to formula (1) [S3].

$$E_{\text{calc}} = (x \times y) : 100 \quad (1),$$

where E_{calc} is the expected growth regulation value on the combined action of the tested compound and the herbicide, expressed as a percentage of the reference value; x is the experimental growth regulation value in the presence of the tested compound expressed as a percentage of the reference value; y is the experimental growth regulation value in the presence of the herbicide, expressed as a percentage of the reference value.

Equation (1) suggests that the two active substances affect the plant growth independently. A significant difference between the experimental and calculated values indicates the effect of one compound on the activity of the other.

Table S4 summarizes the experimental (E_{exp}) and calculated (E_{calc}) values of the growth regulating effect for the combinations of compounds **2**, **4** and the herbicide Zinger WP, as well as the difference [$E_{\text{exp}} - E_{\text{calc}}$]. The large values of the difference indicate antagonism or synergism between the compound and the herbicide, with the positive difference meaning that the compound under study is an antagonist of the herbicide.

Table S4. Antidote effects of compounds **2a–c**, **4a–c** in the presence of Zinger WP on the growth of corn seeds (Krasnodar 291 AMV cultivar).

Compound	Weight of roots, % ^a					Weight of sprouts, % ^a				
	Zinger SP	Compound	E_{calc}	E_{exp}	$E_{\text{exp}} - E_{\text{calc}}$, %	Zinger SP	Compound	E_{calc}	E_{exp}	$E_{\text{exp}} - E_{\text{calc}}$, %
2a	67	107	72	64	-8	72	103	74	71	-3
2b	67	106	71	68	-3	72	109	78	78	0
2c	70	103	72	70	-2	72	87	63	66	3
4a	67	94	63	68	5	72	84	60	64	4
4b	67	102	69	66	-3	72	94	68	65	-3
4c	67	105	71	69	-1	72	103	74	74	0

^a weight of sprouts and roots in the water control were taken as 100%.

References

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