Electronic Supplementary Material

Near-infrared benzodiazoles as small molecule environmentally-sensitive fluorophores

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Characterisation of library of C-bridged benzodiazoles

HPLC-MS analysis was performed on a Waters Alliance 2695 separation module connected to a Waters PDA2996 photodiode array detector and a ZQ Micromass mass spectrometer (ESI-MS) with a Phenomenex® column (C_{18}, 5 µm, 4.6 × 150 mm). A linear gradient of 0% to 100% (8 min, flow 1 mL min^{-1}) using H_2O (0.1% HCOOH) and ACN (0.1% HCOOH) as eluents was used, with the coupled mass spectrometer in the positive mode.

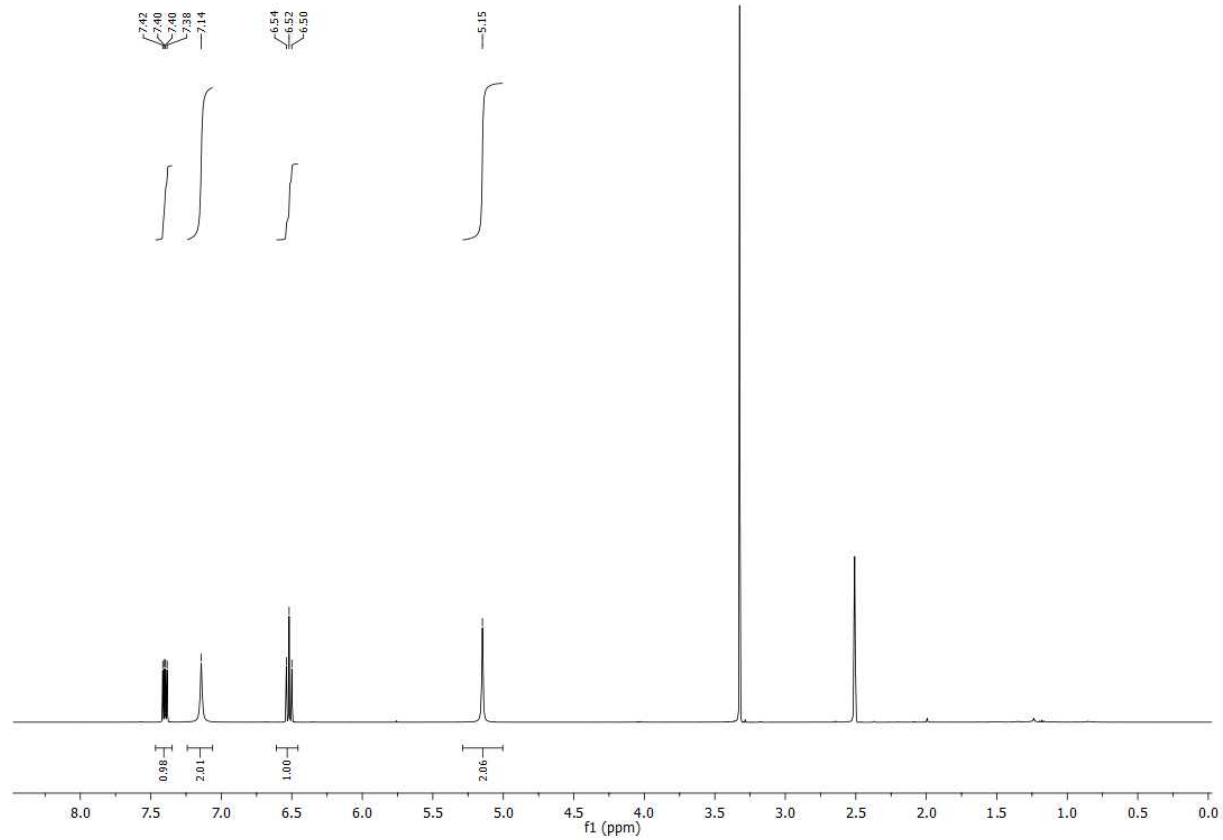
Table S1. Chemical characterisation of the library of compounds **3a**-**3k**.

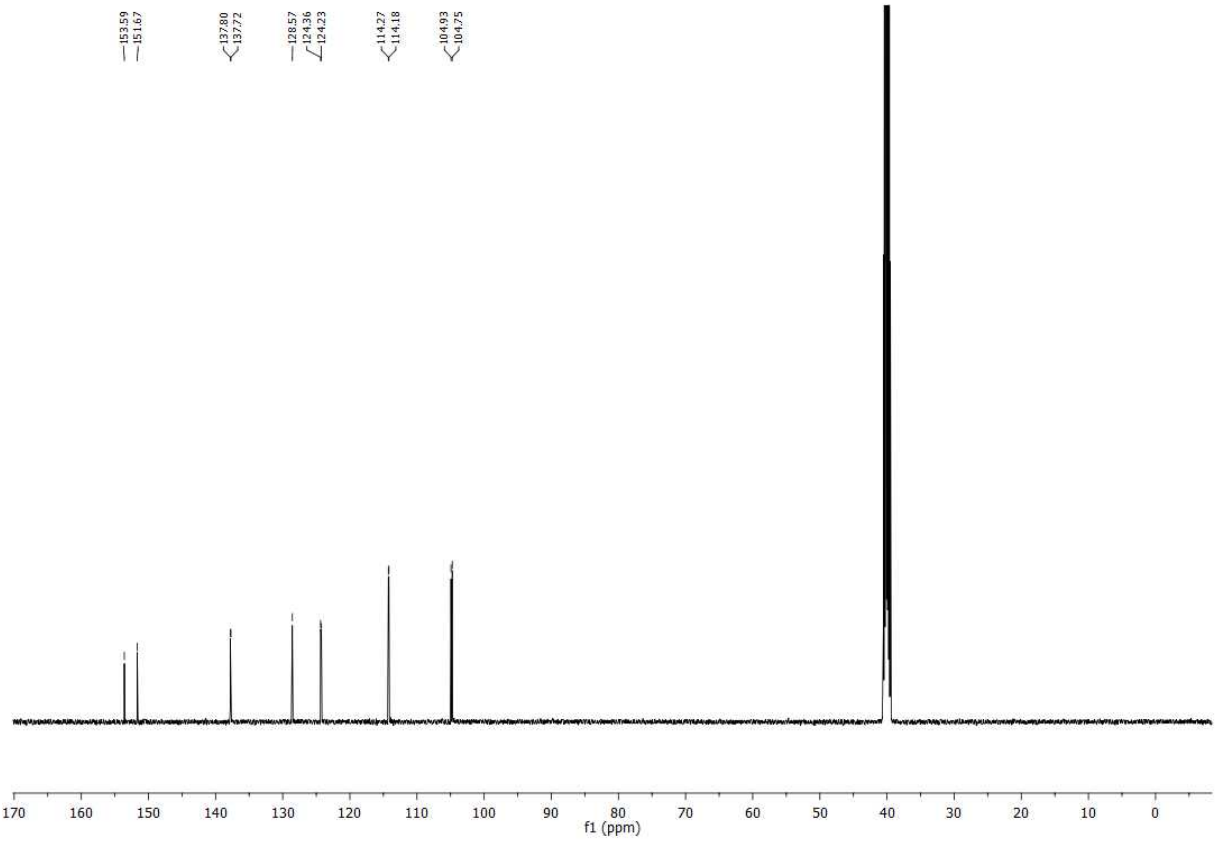
Compound	t_R (min)	HPLC purity	λ_{abs} (nm)	λ_{em} (nm)	HRMS calc. $[M+H]^+$	HRMS exp. $[M+H]^+$
3a	4.50	>99%	562	670	263.1503	263.1506
3b	6.73	97%	568	688	325.1586	325.1590
3c	7.70	97%	580	692	371.1437	371.1439
3d	4.98	>99%	574	680	249.1346	249.1346
3e	4.43	>99%	562	675	321.1557	321.1552
3f	5.28	>99%	568	672	275.1503	275.1505
3g	5.07	>99%	591	676	261.1346	261.1350
3h	6.20	96%	595	675	317.1972	317.1977
3i	4.10	93%	598	675	277.1295	277.1301
3j	4.75	93%	528	687	362.1823	362.1823
3k	6.32	>99%	480	658	402.1925	402.1926

Table S2. Summary of the standard Z-score values calculated for each pair analyte/probe. Z-score values that are greater than 2 are highlighted in bold.

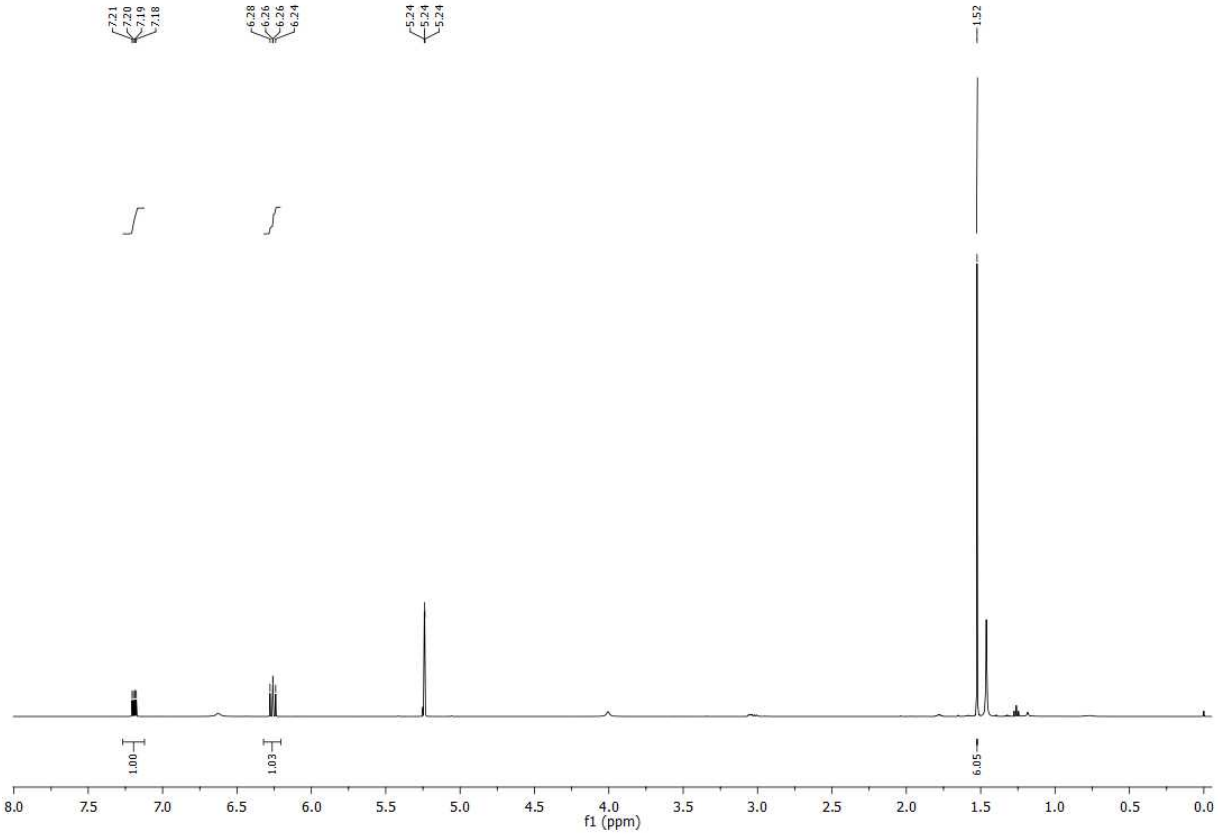
Analyte	Concentration analyte	2a	3a	3d	3e	3f	3g	3h	3i	3j	3k
Estirol	100 μM	-0.17	-0.84	-0.73	-0.16	0.00	-0.60	-1.23	-0.92	-1.00	-1.26
Choric acid	100 μM	-0.27	-0.99	-0.03	1.62	1.39	-0.69	-1.25	-0.98	-1.01	-1.31
Dexamethasone	100 μM	-0.22	-0.93	**4.78**	**3.53**	0.10	-0.65	-1.21	-0.97	-1.00	-1.34
Estrone	100 μM	-0.20	-0.89	-0.72	0.13	1.80	-0.60	-1.22	-0.98	-0.99	-1.21
Beta-estradiol	100 μM	-0.17	-0.89	0.51	-0.28	0.32	-0.29	-0.26	-0.78	-0.51	-0.24
4-Androsterene-3,17-dione CIIIN	100 μM	-0.16	-0.96	-0.33	**5.23**	**6.96**	-0.43	-1.21	-0.30	0.11	-1.24
Arabinose	1 mM	0.92	1.22	0.53	0.50	0.24	-0.11	-0.29	-0.43	-0.68	0.47
Glucose	1 mM	0.99	0.00	0.61	-0.37	-0.01	0.28	-0.13	-0.53	-0.71	-0.10
Fructose	1 mM	0.24	-0.18	0.27	-0.57	-0.11	-0.21	-0.71	-0.81	-1.05	-0.10
Galactose	1 mM	1.41	1.61	0.27	0.92	0.16	0.37	-0.03	-0.53	-0.67	0.08
Sucrose	1 mM	0.40	1.03	0.81	-0.29	-0.06	-0.30	-0.24	-0.62	-0.70	0.20
Maltose	1 mM	1.31	1.46	0.50	0.59	-0.09	0.59	-0.32	-0.63	-0.68	0.53
Mannose	1 mM	1.13	1.38	-0.09	0.96	0.29	-0.38	**2.57**	-0.54	-0.49	0.35
Glycogen	1 mM	0.14	-1.12	-0.34	-0.38	-0.32	-0.44	-0.75	-1.26	-1.26	-0.63
Cysteine	1 mM	-0.51	-0.18	-0.11	0.12	-0.17	0.05	0.17	0.87	0.96	0.62
Homocysteine	1 mM	-0.35	-0.81	-0.07	-0.55	-0.44	-0.59	-0.29	0.32	0.94	0.17
GSH	1 mM	-0.59	-0.78	-0.16	-0.45	-0.47	-0.10	-0.18	0.89	0.69	-0.45
GSSG	1 mM	-0.19	0.26	-0.24	0.24	-0.23	-0.51	0.10	0.57	1.18	0.74
H_2O_2	1 mM	-0.27	0.07	-0.35	-0.17	-0.24	-0.44	0.04	0.83	1.19	0.75
OCl^-	1 mM	-0.25	0.54	-0.50	-0.22	-0.24	0.04	0.27	0.67	1.06	0.75
$^{\cdot}OH$	1 mM	-0.16	0.48	-0.18	-0.12	-0.39	-0.48	0.07	0.52	1.27	0.33
$^1O_2$	1 mM	-0.56	-1.30	-0.62	-0.56	-0.51	-0.46	-0.69	-0.49	0.17	-0.91
NO	1 mM	-0.46	0.64	0.61	-0.35	-0.21	0.11	0.38	0.65	1.39	0.81
NaSH	1 mM	-0.42	-0.25	-0.28	-0.64	-0.31	0.14	-0.15	1.13	1.26	0.24
$^{\cdot}O_2$	500 μM	-0.27	-1.29	-0.89	-0.61	-0.37	-0.54	-0.79	-0.67	-1.23	-1.38
BSA	200 μg/mL	**6.36**	**3.45**	**3.70**	-0.14	0.27	**6.92**	**5.18**	**4.02**	-0.52	-1.97
HSA	200 μg/mL	1.06	-1.15	-1.16	-0.91	0.18	-0.56	-0.84	**3.43**	-1.57	**3.64**
Lysozyme	200 μg/mL	1.15	0.95	0.45	-0.05	0.05	0.11	-0.25	-0.58	-0.72	0.20
Peroxidase	200 μg/mL	-0.42	-1.73	-1.29	-0.92	-0.58	-0.67	-1.53	-1.33	-1.58	-2.04
Asp	1 mM	-0.43	-0.78	-0.67	-0.36	-0.26	-0.33	-0.10	-0.20	-0.54	-0.18
Ile	1 mM	-0.21	-0.60	-0.31	-0.45	-0.36	0.05	0.32	0.00	-0.39	0.21
Tyr	1 mM	-0.19	0.32	**2.05**	0.22	-0.05	-0.52	1.12	1.23	0.08	0.58
Lys	1 mM	-0.26	0.39	0.10	-0.53	-0.22	-0.32	-0.43	-0.42	-0.29	-0.21
Trp	1 mM	-0.58	-0.66	-0.45	-0.50	-0.29	0.54	-0.10	-0.29	-0.47	-0.14
Ala	1 mM	-0.43	-0.18	0.24	-0.67	-0.23	-0.09	0.09	-0.43	-0.50	-0.12
Arg	1 mM	-0.55	-0.34	-0.07	-0.50	-0.32	-0.38	-0.13	-0.35	-0.60	-0.39
Asn	1 mM	-0.47	-0.31	-0.31	-0.32	-0.18	-0.30	0.09	-0.59	-0.55	-0.53
Phe	1 mM	-0.26	-0.08	-0.63	-0.44	-0.20	0.32	-0.29	0.02	-0.07	0.54
Met	1 mM	-0.62	0.06	-0.08	-0.71	-0.31	-0.28	-0.05	-0.30	-0.31	-0.21
Pro	1 mM	-0.47	-0.53	-0.39	-0.56	-0.18	-0.24	-0.40	-0.06	-0.55	-0.42
Val	1 mM	-0.43	0.01	-0.61	-0.17	-0.17	0.25	-0.20	-0.25	-0.07	0.10
Gln	1 mM	-0.55	-0.67	-0.70	-0.69	-0.25	-0.01	-0.44	-0.14	-0.36	-0.46
His	1 mM	-0.53	-0.57	-0.36	-0.37	-0.16	-0.11	0.00	-0.24	-0.21	-0.37
Leu	1 mM	-0.59	-0.11	-0.53	-0.26	-0.34	-0.44	-0.29	-0.33	1.26	-0.13
Glu	1 mM	-0.50	-0.29	-0.59	-0.54	-0.27	0.39	0.17	-0.24	-0.30	-0.21
Ser	1 mM	-0.55	-0.39	-0.49	-0.33	-0.30	0.23	0.05	-0.08	-0.58	-0.09
Gly	1 mM	0.13	0.42	-0.34	-0.34	-0.33	0.08	0.18	-0.04	-0.26	-0.51
Thr	1 mM	-0.46	-0.49	-0.69	-0.72	-0.34	-0.09	-0.22	-0.32	-0.42	-0.20
Fe3+	1 mM	0.02	0.68	0.17	0.70	-0.21	0.13	0.58	1.06	1.07	0.99
Fe2+	1 mM	0.91	1.69	0.86	0.87	0.07	0.77	1.29	**2.50**	**2.14**	1.75
Cu2+	1 mM	-0.08	1.10	0.21	0.36	-0.19	0.30	0.87	0.37	1.84	**2.18**
Cu+	1 mM	-0.21	0.88	-0.04	-0.10	-0.18	1.13	0.75	-0.77	1.60	1.08
Ca2+	1 mM	-0.20	1.40	0.20	0.34	-0.05	0.12	0.53	0.17	1.16	0.91
Mg2+	1 mM	-0.10	0.64	0.40	0.59	0.00	0.02	1.22	0.49	1.00	0.80
Zn+	1 mM	-0.07	1.15	0.06	0.42	-0.15	0.40	0.47	-0.02	1.88	1.08
Hg2+	1 mM	-0.09	0.98	0.14	0.18	-0.26	-0.41	0.11	0.22	1.31	0.47
Sn2+	1 mM	-0.23	0.69	0.48	0.63	-0.12	0.25	0.73	0.37	1.70	0.84
Pd2+	1 mM	-0.84	-1.99	-1.50	-1.14	-0.71	-0.73	-1.64	-1.36	-1.51	-2.30
DNA total	310 pg/μL	-0.61	-1.24	-1.12	-0.72	-0.41	-0.29	0.53	-0.56	-0.90	-0.75

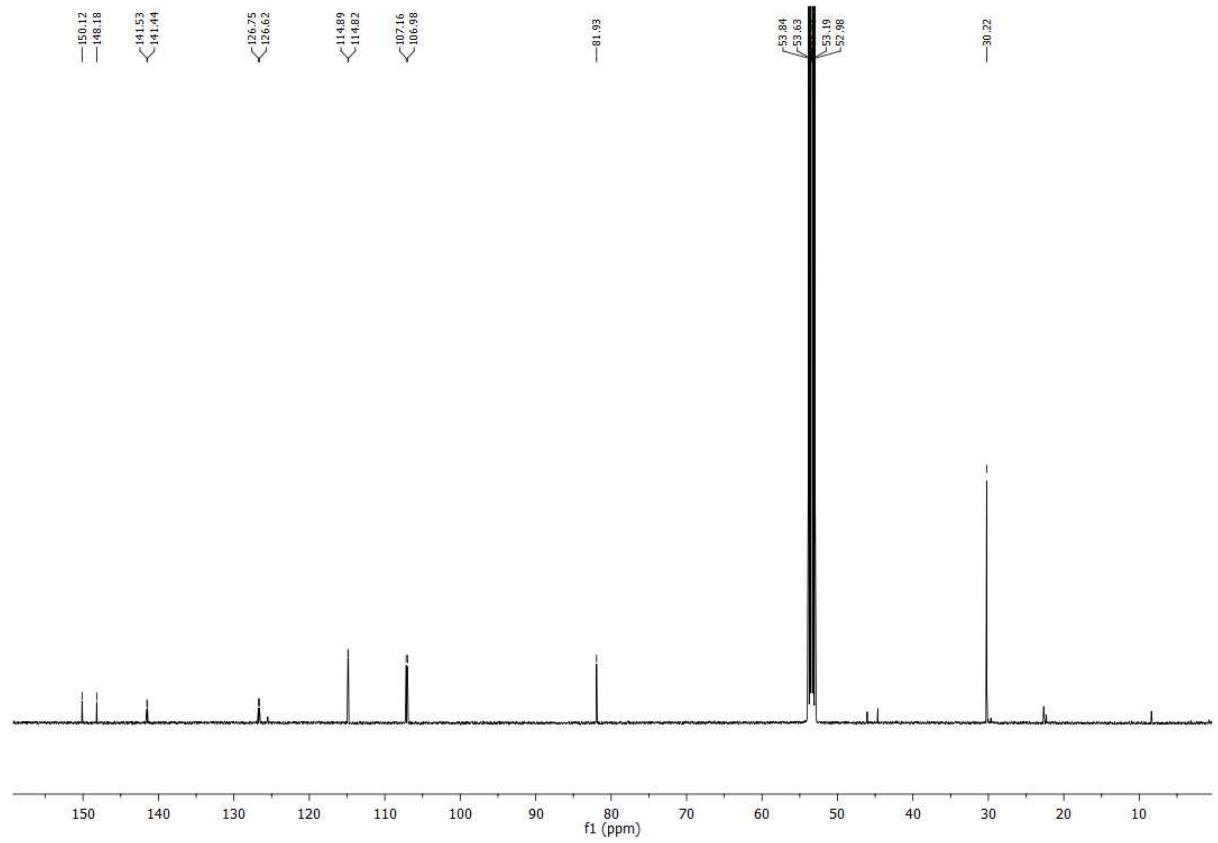
NMR spectra for compound 1



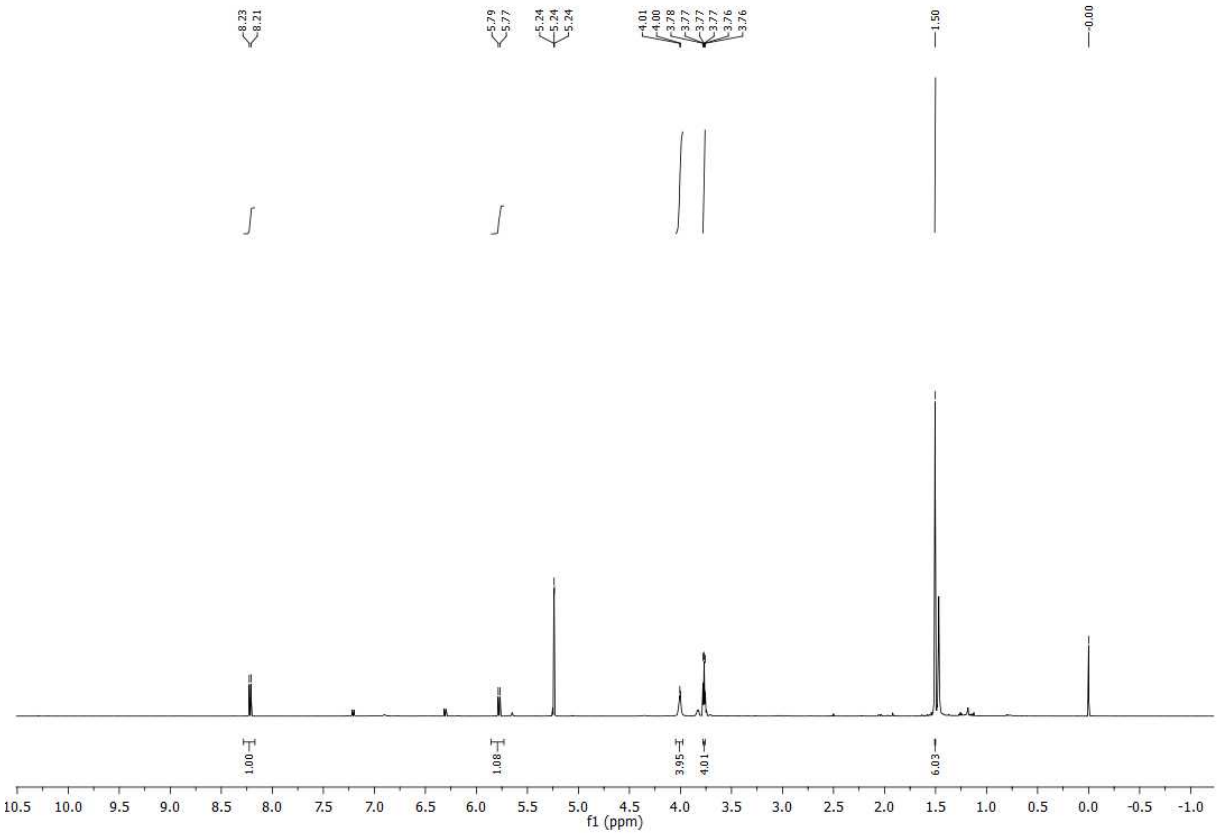


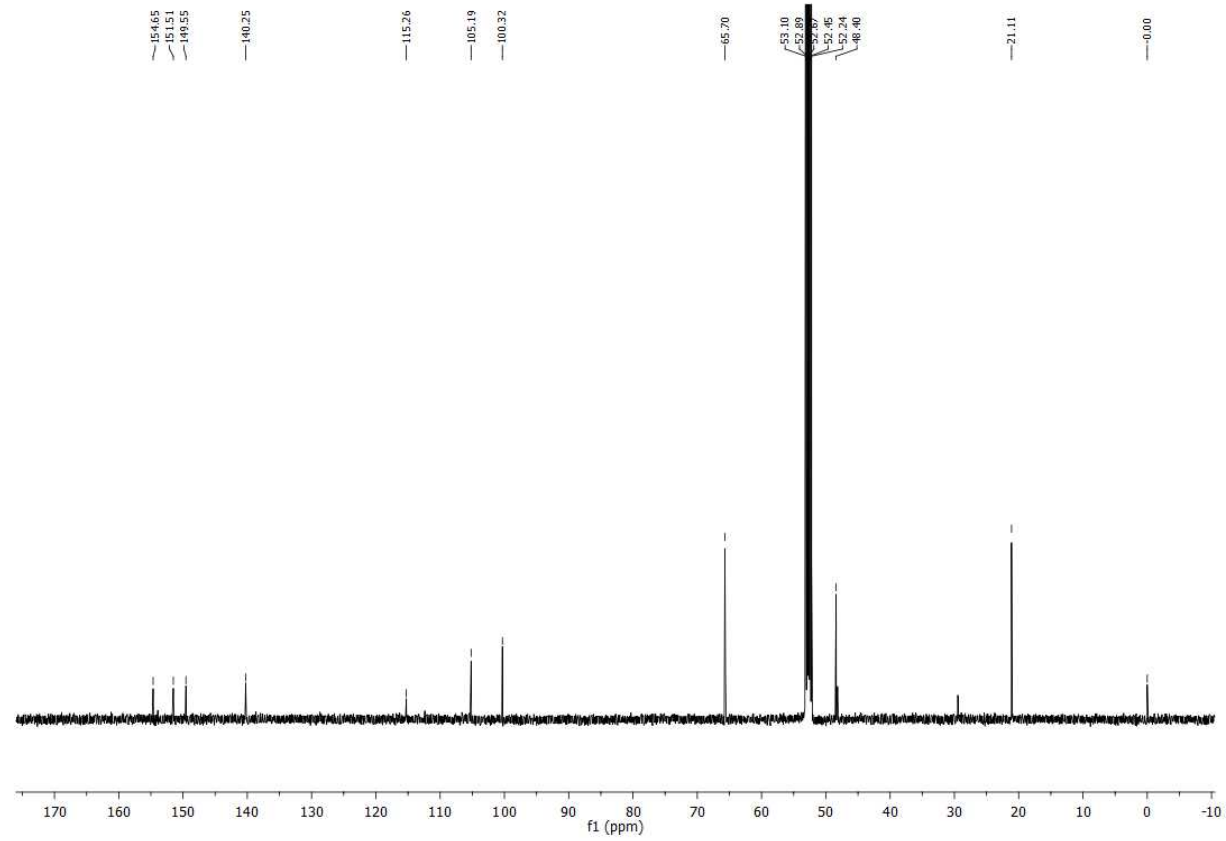
NMR spectra for compound 2a





NMR spectra for compound 3i





Additional figures

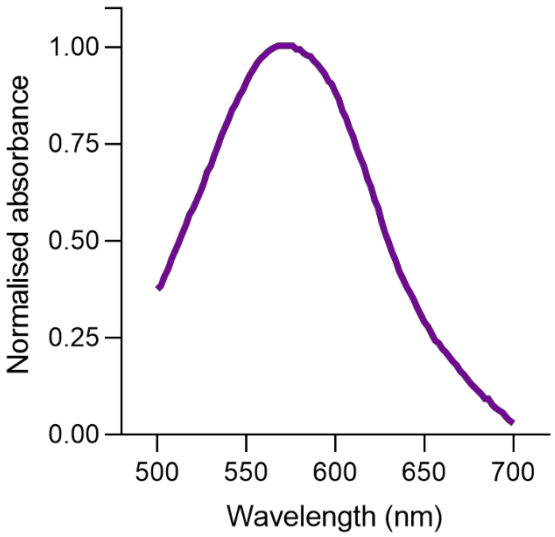


Figure S1. Absorbance spectrum of the hit compound **3i** (200 μM) in water.

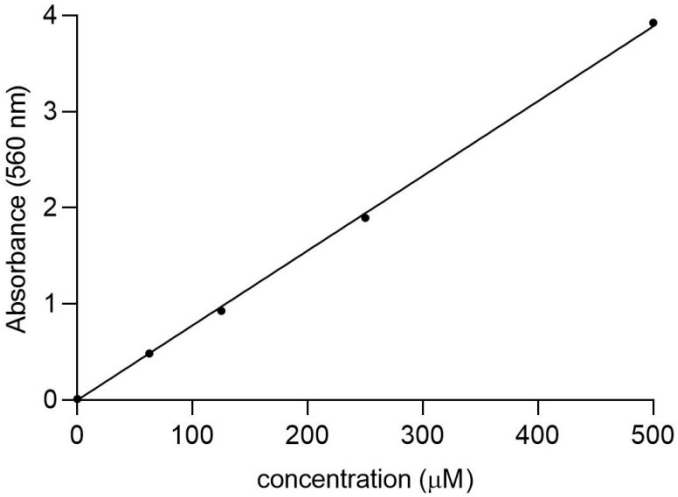


Figure S2. Determination of the extinction coefficient of compound **3i** in DMSO. Data presented as mean values from 3 independent experiments. ε: 7,800 $M^{-1}cm^{-1}$.

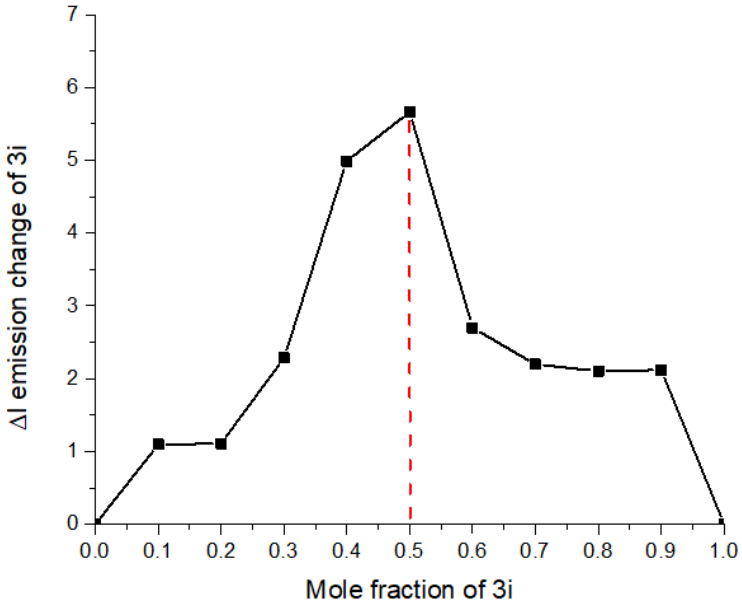


Figure S3. Job plot analysis of the interaction between compound **3i** and Fe^{2+} ions.