Supporting Information For:

A Deferasirox Derivative that Acts as a Multifaceted Platform for the Detection and Quantification of Fe^{3+}

Axel Steinbrueck, Adam C. Sedgwick, Suh Mi Hwang, Sajal Sen, Michael Y. Zhao, Dan Ying Huang, Daniel M. Knoll, Yu-Ying Wang, and Jonathan L. Sessler*

Department of Chemistry, The University of Texas at Austin, 105 E 24th street A5300, Austin, TX 78712-1224 (USA)

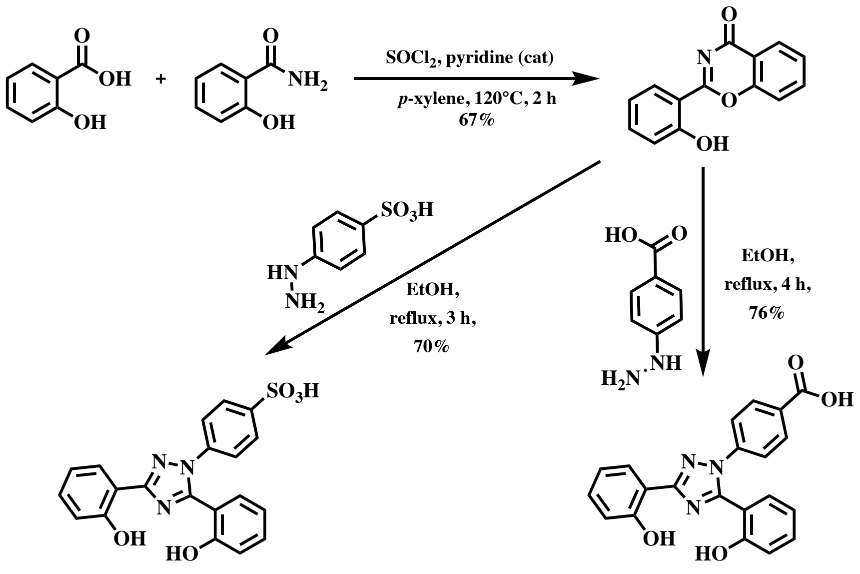
Emails:

sessler@cm.utexas.edu

Common abbreviations used:

a.u. = arbitrary units (intensity scale in fluorescence spectra)
Ac = acetyl
A549 = human lung cancer cell line
DCM = dichloromethane
Et = ethyl
FBS = fetal bovine serum (additive in cell growth media for MTT assays)
HSA = human serum album
Me = methyl
P/S = penicillin/streptomycin
r.t. = room temperature
TEA = triethylamine

1. Synthesis



ExSO$_3$H (2)

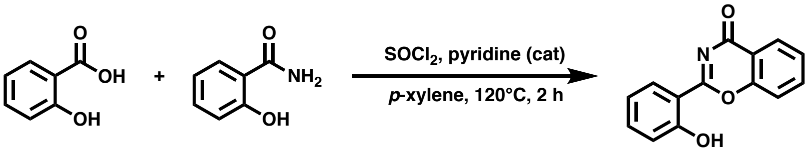
Deferasirox (1)

Scheme S1 – Synthetic scheme for deferasirox (**1**) and its sulfonated derivative **2**.

1.1. Oxazinone Intermediate

2-(2-Hydroxyphenyl)-4H-benzo[e][1,3]oxazin-4-one

This synthesis was adapted from the literature.[1]



In a 250 ml round bottom flask equipped with a reflux condenser, salicylic acid (11.1 g, 80.2 mmol, 1.1 eq), salicylamide (10.0 g, 72.9 mmol, 1.0 eq) and pyridine (0.5 ml, catalyst) were dissolved in *o*-xylene (50 ml). The resulting solution was heated to 80°C. $SOCl_2$ (5.0 ml, 69.3 mmol 0.9 eq) was added dropwise over the course of 5 min. The reaction mixture was heated further to 120°C, stirred for 1 h, and then another aliquot of $SOCl_2$ (5.0 ml, 69.3 mmol 0.9 eq) was added dropwise over the course of 5 min. The reaction mixture was stirred for another 1 h at 120°C. After cooling, the volatiles were removed under reduced pressure and to the residue was added ethanol (50 mL) and acetic acid (1 mL). The resulting suspension was cooled to 4°C in the fridge for 10 min and the precipitate was filtered off, washed with cold ethanol (three times, 50 mL each) and dried under vacuum to yield the product as yellow-green powder (17.9 g, 93%).

^{1}H-NMR (400 MHz, $CDCl_3$):

δ 12.73 (s., 1 H, O-*H*), 8.22 (dd, *J* = 1.7, 8.2 Hz, 1 H, Ar*H*), 8.12 (ddd , *J* = 0.4, 1.7, 8.1 Hz, 1 H, Ar*H*), 7.81 (ddd, *J* = 1.7, 7.4, 8.4 Hz, 1 H, Ar*H*), 7.51-7.57 (m, 3 H, Ar*H*), 7.10 (ddd, *J* = 0.4, 1.2, 8.5 Hz, 1 H, Ar*H*), 7.01 (ddd, *J* = 1.2, 7.1, 8.2 Hz, 1 H, Ar*H*)

^{13}C-NMR (100.6 MHz, $CDCl_3$):

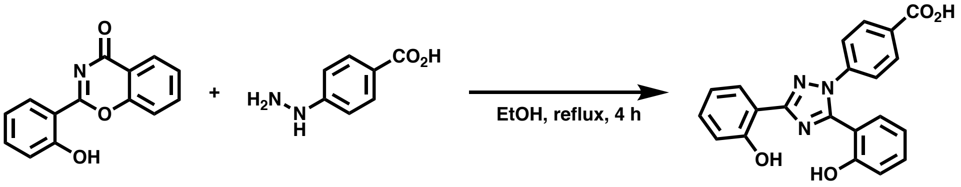
δ 165.3, 164.0, 163.2, 154.2, 136.9, 135.8, 128.7, 128.1, 127.3, 119.5, 118.9, 118.4, 117.0, 111.3

HR-MS (ESI): $C_{14}H_9NO_3$
Calculated ($[M+H]^+$): 240.0661
Found: 240.0658

*1.2. Deferasirox **1***

4-(3,5-Bis(2-hydroxyphenyl)-1*H*-1,2,4-triazol-1-yl)benzoic acid

This synthesis was adapted from the literature.[1]



In a 250 mL round bottom flask, 4-hydrazinobenzoic acid (10.5 g, 43.9 mmol) was heated at reflux in ethanol (100 mL) for 10 min. Then, 2-(2-hydroxyphenyl)-4H-benzo[e][1,3]oxazin-4-one (7.40 g, 48.7 mmol, 1.1 eq.) was added all at once through a solid addition funnel and the reaction was stirred at reflux for 4 h. After cooling to room temperature, the reaction mixture was filtered off and the filter cake was washed once with cold ethanol (50 mL) and then twice with cold methanol (50 mL each). After drying under vacuum, the product was obtained as a pale brown powder (12.5 g. 33.5 mmol, 76%).

For biological studies, a portion of the product (5.0 g, 13 mmol) was recrystallized from ethanol (400 mL) to produce a white fluffy powder (2.9 g, 10 mmol, 58%).

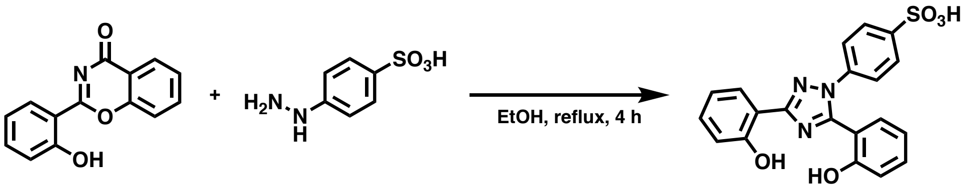
^{1}H-NMR (500 MHz, DMSO-*d*$_6$):
δ 13.09 (s, 1 H, COO-*H*), 10.81 (s, 1 H, O-*H*), 10.06 (s, 1 H, O-*H*), 8.06 (dd, *J* = 1.7, 7.8 Hz, 1 H, Ar*H*), 7.98-8.01 (m, 2 H, Ar*H*), 7.53 - 7.58 (m, 3 H, Ar*H*), 7.35 - 7.42 (m, 2 H, Ar*H*), 6.96 - 7.05 (m, 3 H, Ar*H*), 6.87 (dd, *J* = 1.0, 8.3 Hz, 1 H, Ar*H*)

^{13}C-NMR (125.75 MHz, DMSO-*d*$_6$):
δ 166.9, 160.4, 156.8, 155.6, 152.5, 141.7, 133.0, 131.9, 131.5, 131.0, 130.8, 127.2, 123.8, 120.2, 119.9, 117.5, 116.6, 114.9, 114.1

HR-MS (ESI): $C_{21}H_{15}N_3O_4$
Calculated ([M+H]$^+$): 374.1141
Found: 374.1143

1.3. *ExSO$_3$H* ***2***

4-(3,5-Bis(2-hydroxyphenyl)-1*H*-1,2,4-triazol-1-yl)benzenesulfonic acid



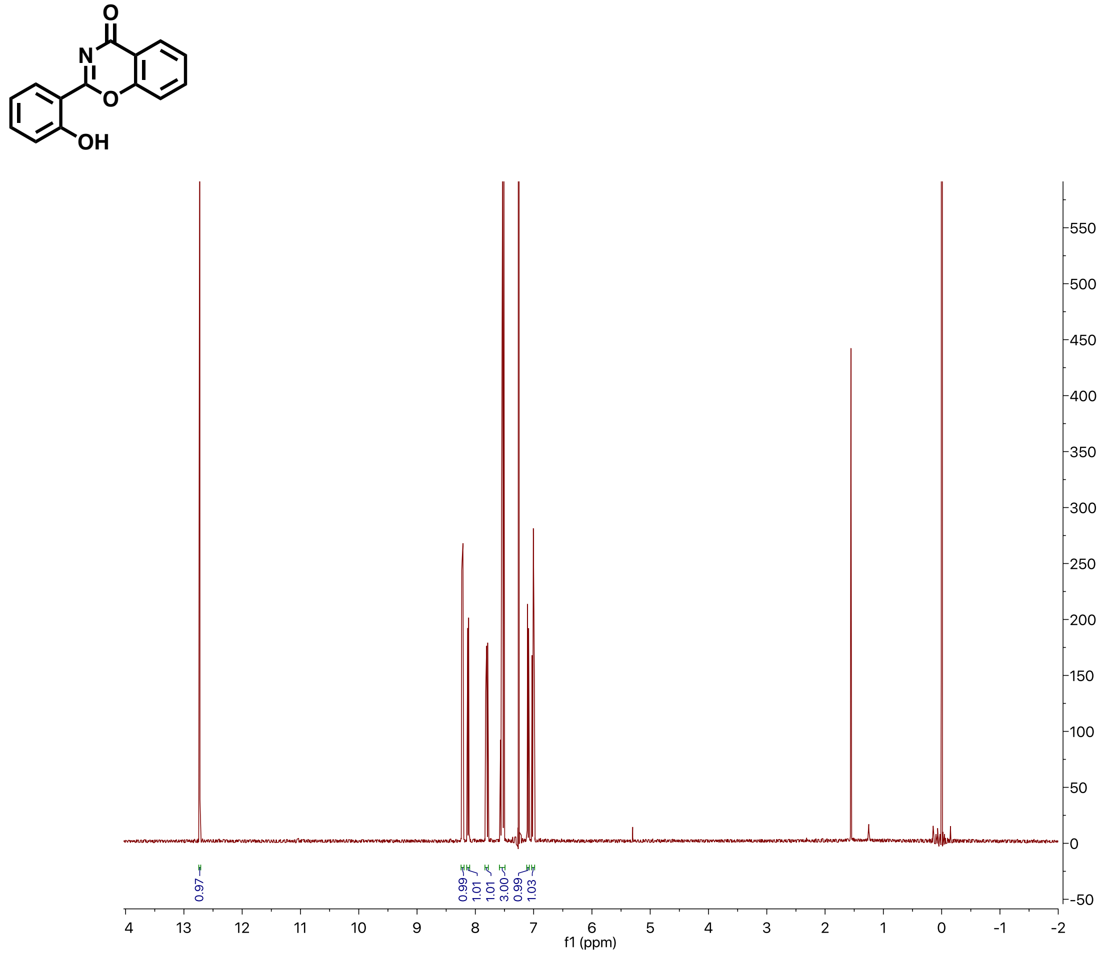
In a 250 mL round bottom flask, 4-hydrazinbenzosulfonic acid (8.7 g, 46.0 mmol, 1.1 eq.) was heated at reflux in ethanol (100 mL) for 2 min. Then, 2-(2-hydroxyphenyl)-4H-benzo[e][1,3]oxazin-4-one (10.0 g, 41.8 mmol, 1.0 eq.) was added at once through a solid addition funnel and the reaction was stirred at reflux for 3 h. The reaction was cooled to room temperature and the volatiles were removed from the reaction mixture on a rotary evaporator (60 mbar, 50°C water bath). To the brown residue was added methanol (100 mL), and the resulting solid mass collected on a glass filter frit. The filter cake, containing the product, was washed with cold methanol (4°C, 4 times with 50 mL MeOH each) and acetone (4°C, 1 time with 50 mL) followed by drying under vacuum to yield the product (13.4 g, 70%) as white, bulky powder.

^{1}H-NMR (500 MHz, DMSO-*d*$_6$):
8.04 (dd, *J* = 1.8, 7.8 Hz, 1 H, Ar*H*), 7.65 (d, *J* = 8.6 Hz, 2 H, Ar*H*), 7.51 (dd, , *J* = 1.7, 7.6 Hz, 1 H, Ar*H*), 7.35-7.43 (m, 4 H, Ar*H*), 6.94 - 7.05 (m, 3 H, Ar*H*), 6.87 (dd, *J* = 1.0, 8.3 Hz, 1 H, Ar*H*)

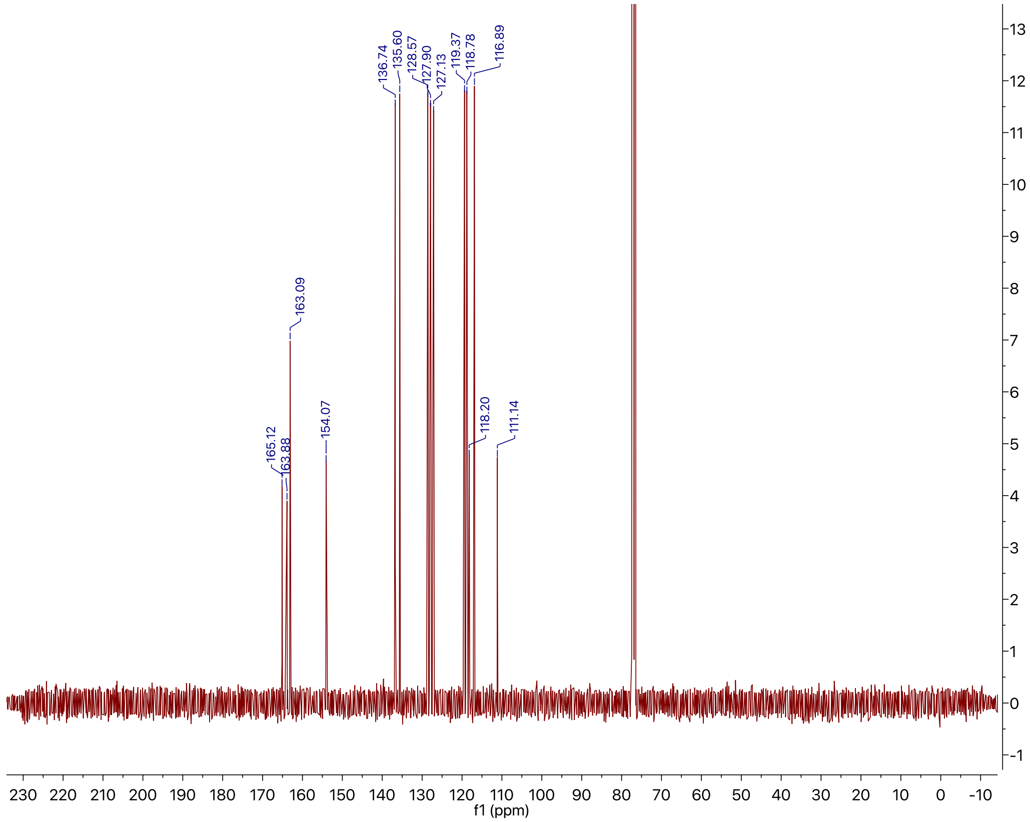
^{13}C-NMR (125.75 MHz, DMSO-*d*$_6$):
δ 159.2, 156.3, 155.3, 151.7, 148.3, 137.6, 132.5, 131.5, 131.1, 126.9, 126.5, 123.1, 119.7, 119.4, 117.0, 116.2, 114.2, 113.6

HR-MS (ESI): $C_{20}H_{15}N_3O_5S$
Calculated ([M+H]$^+$): 410.0811
Found: 410.0806

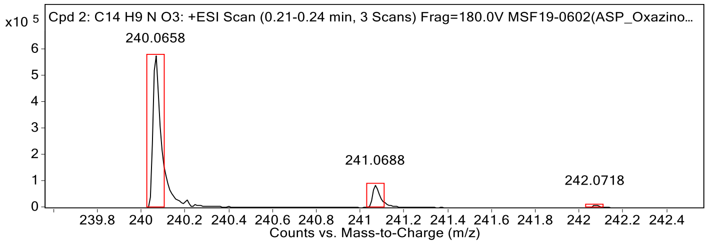
2. ^{1}H NMR, ^{13}C NMR and HRMS spectra; liquid chromatograms



^{1}H NMR spectrum of 2-(2-hydroxyphenyl)-4H-benzo[e][1,3]oxazin-4-one (400 MHz, $CDCl_3$)



^{13}C NMR spectrum of 2-(2-hydroxyphenyl)-4H-benzo[e][1,3]oxazin-4-one (100.6 MHz, $CDCl_3$)



MS Spectrum Peak List

Obs. m/z	Calc. m/z	Charge	Abundance	Formula	Ion Species	Tgt Mass Error (ppm)
240.0658	240.0655	1	581677	C14H9NO3	(M+H)+	-1.24
241.0688	241.0688	1	88067	C14H9NO3	(M+H)+	-0.37
242.0718	242.0712	1	11116	C14H9NO3	(M+H)+	-2.31
243.0168	243.0738	1	502	C14H9NO3	(M+H)+	234.28

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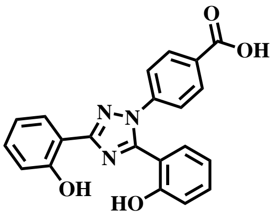
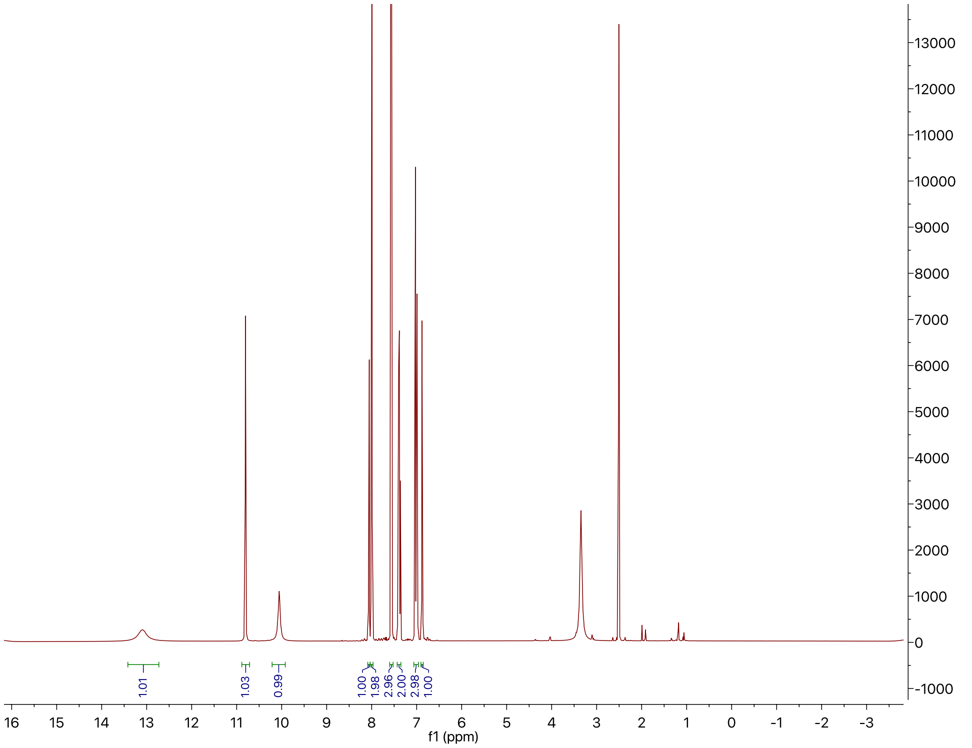
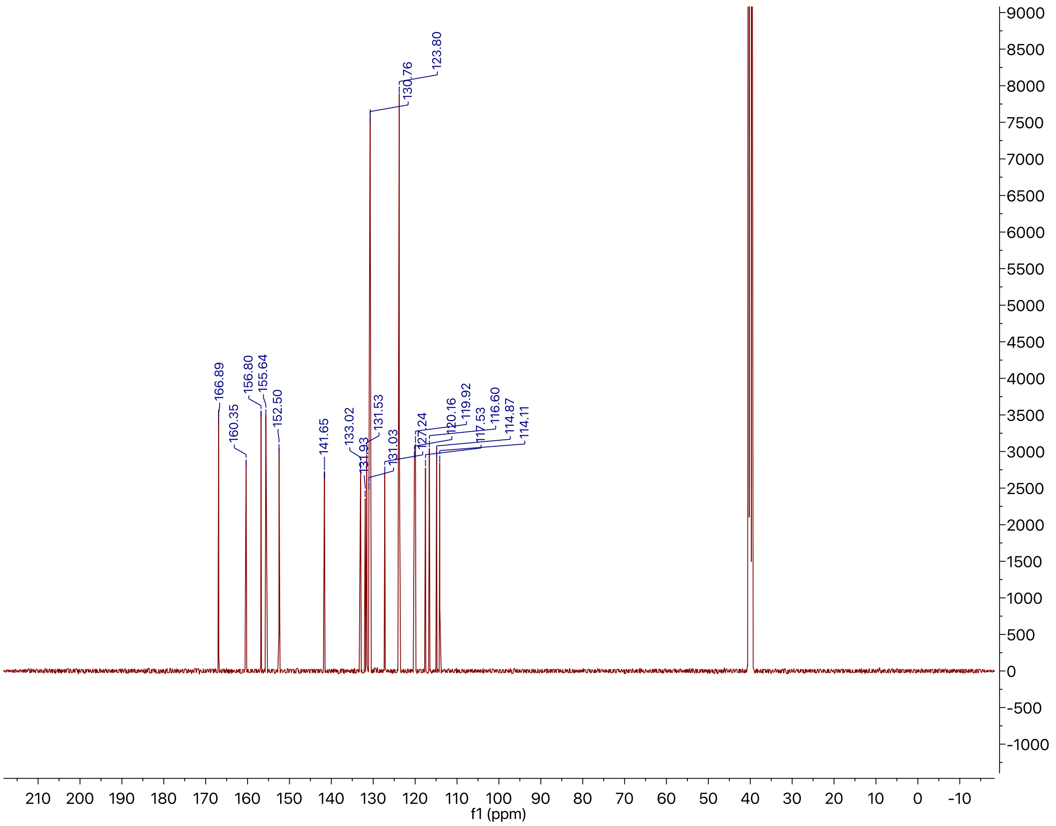
HRMS (ESI) analysis of 2-(2-hydroxyphenyl)-4H-benzo[e][1,3]oxazin-4-one



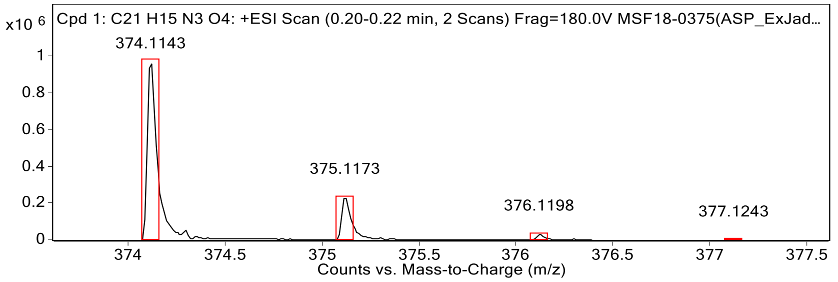
Deferasirox (1)



^{1}H NMR spectrum of **1** (400 MHz, DMSO-d_6)



^{13}C NMR spectrum of **1** (100.6 MHz, DMSO-d_6)



MS Spectrum Peak List

Obs. m/z	Calc. m/z	Charge	Abundance	Formula	Ion Species	Tgt Mass Error (ppm)
374.1143	374.1135	1	983483	C21H15N3O4	(M+H)+	-2.06
375.1173	375.1166	1	236734	C21H15N3O4	(M+H)+	-1.68
376.1198	376.1193	1	36037	C21H15N3O4	(M+H)+	-1.28
377.1243	377.1218	1	3565	C21H15N3O4	(M+H)+	-6.62
378.0973	378.1244	1	720	C21H15N3O4	(M+H)+	71.53

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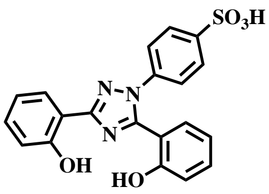
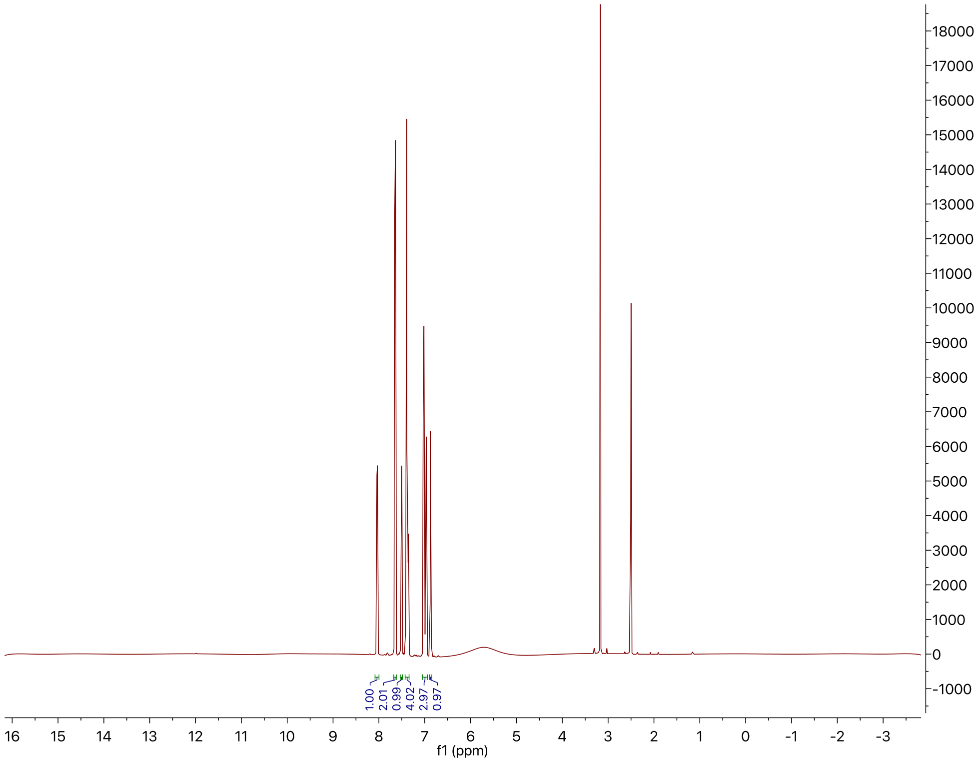
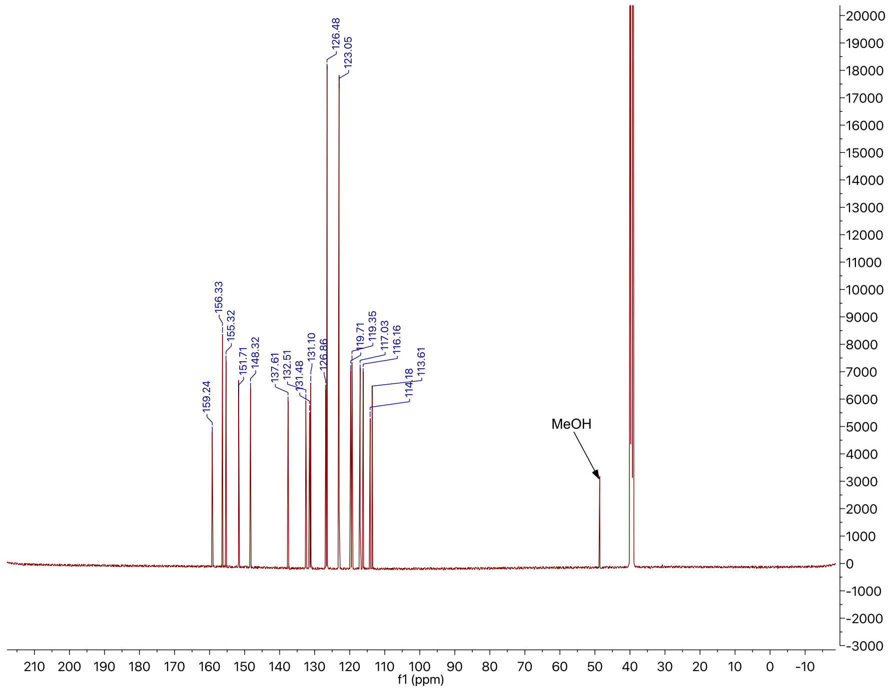
HRMS (ESI) analysis of **1**



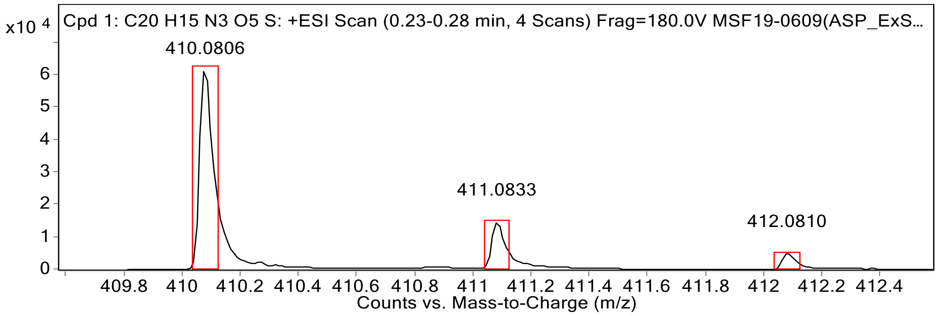
ExSO$_3$H (2)



^{1}H NMR spectrum of **2** (400 MHz, DMSO-d_6)



^{13}C NMR spectrum of **2** (100.6 MHz, DMSO-d_6)



MS Spectrum Peak List

Obs. m/z	Calc. m/z	Charge	Abundance	Formula	Ion Species	Tgt Mass Error (ppm)
410.0806	410.0805	1	62694	C20H15N3O5S	(M+H)+	-0.31
411.0833	411.0835	1	14926	C20H15N3O5S	(M+H)+	0.56
412.0810	412.0807	1	5211	C20H15N3O5S	(M+H)+	-0.64
413.0838	413.0821	1	965	C20H15N3O5S	(M+H)+	-3.99
922.0098			332012			

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HRMS (ESI) analysis of **2**

3. UV-Vis and Fluorescence Spectroscopic Experiments

Stock solutions of 5 mM **1**, **2** and $FeCl_3$*6 H_2O, respectively, were prepared in dimethyl sulfoxide (DMSO) and used for all spectroscopic experiments unless otherwise indicated.

5 mM stock solutions in deionized water were prepared for the following metal salts and used to screen the putative interactions with various divalent and trivalent metal cations, namely $FeSO_4$*7 H_2O, $CuSO_4$*5 H_2O, $ZnSO_4$*7 H_2O, $MnSO_4$*H_2O, $MgSO_4$*7 H_2O, $CaCl_2$, $AlCl_3$, $Cr(NO_3)_3$*9 H_2O.

pH 2-5 buffers were made from 0.1 M aqueous AcOH/AcONa that was adjusted to the desired pH using appropriate amounts of 0.01 M NaOH and 0.01 M HCl, respectively.

For each fluorescence experiment, a 1 mM stock solution of HSA was freshly prepared in deionized water.

The effect of DMSO on the fluorescence emission was investigated by adding DMSO into deionized water containing 20 µM HSA and 10 µM **2**. The results are shown in Figure S1. Throughout all experiments conducted over the course of this investigation, the amount of DMSO in water never exceeded 2.5V%. Based on these results, the fluorescence quenching effect that may arise through the addition of DMSO from the stock solutions was considered small as compared to the quenching effect of added Fe^{3+}.

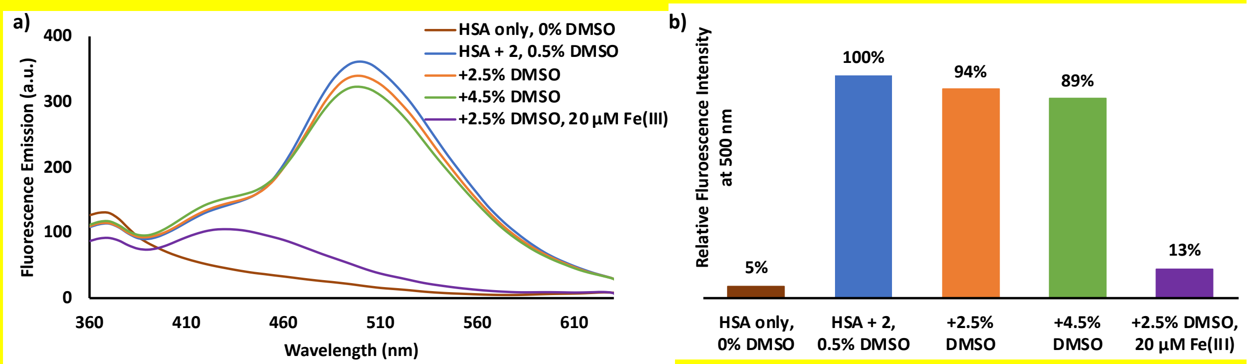

Figure S1. a) Emission profiles of 20 µM HSA and 10 µM **2** in deionized water before and after addition of DMSO. The emission profiles of HSA without **2**, as well as in the presence of 10 µM **2** and 20 µM Fe^{3+}, are included as reference. All experiments were conducted with excitation at 330 nm and with excitation and emission slit widths of 5 nm, respectively. b) Bar chart showing the relative fluorescence intensities of selected samples at 500 nm.

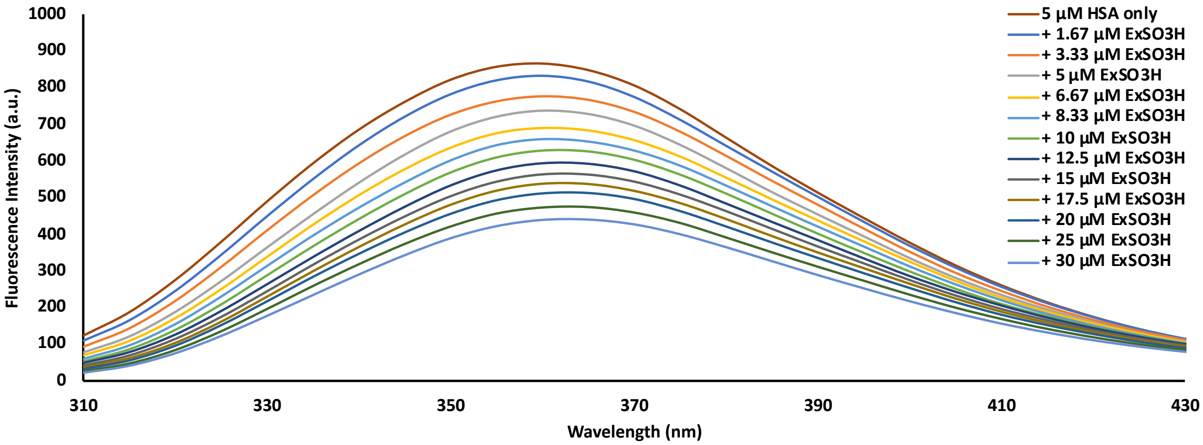

Figure S2. Emission profiles of 5 µM HSA in deionized water titrated with **2**. Experiments were conducted with excitation at 290 nm. The excitation and emission slit widths were 5 nm, respectively. From the fluorescence intensity at 360 nm a Stern-Volmer plot and a corresponding double logarithm plot were constructed. The results are shown in Figure 7 in the main text.

4. Reference

1 S. Steinhauser, U. Heinz, M. Bartholomä, T. Weyhermüller, H. Nick and K. Hegetschweiler, *Eur. J. Inorg. Chem.*, 2004, **2004**, 4177–4192.