Electronic Supplementary Material (ESI) for Analyst.

Supplementary Information for

A library-screening approach to developing a fluorescent sensing array for the detection of metal ions

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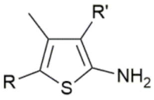
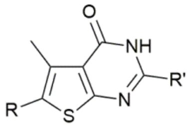
General methods and materials

All solvents used were laboratory grade and were dried over appropriate drying agents when required. MilliQ water was used to prepare all aqueous solutions. Merck 230-400 mesh Kieselgel 60 0.25 mm F254 precoated sheets were used for analytical thin layer chromatography. Chemicals were obtained from Sigma-Aldrich, Alfa Aesar and Combi-Blocks and used as received. Greiner black polypropylene 96-well plates were used for plate reader measurements. Fluorescence measurements were collected using a bench top Perkin Elmer Enspire Multimode Plate Reader.

Synthetic Procedures



(a)



(b)

General procedure for class (a):
A mixture of a ketone (0.1 mol), active methylene (0.1 mol) and elemental sulfur (0.1 mol) in ethanol (10 ml) was treated with morpholine (2 mL). The reaction mixture was refluxed for 2 hr and subsequently cooled to room temperature. Crystals were obtained which were filtered, washed with ethanol, and dried.

General procedure for class (b):
A mixture of a 2-amino-thiophene-3-carboxamide (0.05 mol) and the appropriate aldehyde (0.05 mol) were dissolved in methanol, 5 drops of concentrated HCl were added and the solution stirred for 4 hr. The precipitate obtained was filtered, washed with ethanol, and dried.

TAP-6
^{1}H NMR (300 MHz, DMSO): δ 7.99 (s, 1H), 2.91-2.82 (m, 2H), 2.78-2.67 (m, 2H), 1.85-1.69 (m, 4H). ^{13}C NMR (75 MHz, DMSO): δ 162.4, 157.7, 144.8, 132.1, 130.8, 122.7, 25.3, 24.4, 22.5, 21.8; MS (ESI) $[M-H]^-$ 205.1.

TAP-14
^{1}H NMR (300 MHz, DMSO): δ 8.20 (s, 1H), 4.30 (q, *J* = 7.1, 2H), 2.81 (s, 3H), 1.30 (t, *J* = 7.1, 3H). ^{13}C NMR (75 MHz, DMSO): δ 165.8, 161.9, 158.6, 148.6, 143.4, 123.8, 121.3, 14.9, 14.1; MS (ESI) $[M+H]^+$ 239.2.

TAP-27
^{1}H NMR (300 MHz, DMSO): δ 7.94 (d, *J* = 16.1, 1H), 7.70-7.62 (m, 2H) 7.56-7.36 (m, 7H), 7.02 (d, *J* = 16.1, 1H), 4.40 (s, br, 1H), 2.57 (s, 3H); ^{13}C NMR (75 MHz, DMSO): δ 161.0, 154.2, 152.0, 134.9, 132.7, 132.4, 129.2, 129.1, 129.0, 127.9, 127.7, 125.8, 14.9; MS (ESI) $[M-H]^-$ 343.2.

TAP-36
^{1}H NMR (300 MHz, DMSO): δ 8.71 (ddd, J = 4.8, 1.5, 0.9, 1H), 8.42 (s, 1H), 8.07 (d, *J* = 7.6, 1H), 7.96 (td, *J* = 7.6, 1.5, 1H), 7.52 (ddd, *J* = 7.6, 4.8, 1.5, 1H), 7.48 (br s, 2H), 2.76-2.66 (m, 4H) , 1.83-1.66 (m, 4H); ^{13}C NMR (75 MHz, DMSO): δ 164.9, 156.2, 153.2, 150.2, 148.4, 137.2, 135.7, 133.0, 132.3, 125.7, 122.3, 25.4, 25.0, 22.6, 22.0; MS (ESI) $[M+Na]^+$ 308.1.

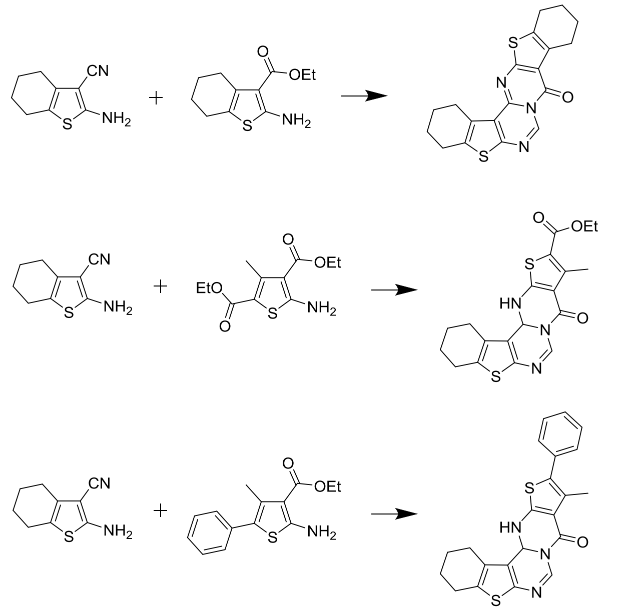
Array Measurements

The initial screening was performed using a final probe concentration of 100 µM and final metal concentration of 1 mM. The more detailed investigation of the top twelve performing probes was performed using a probe concentration of 10 µM and metal concentration of 100 µM. The final lake-water experiment was performed using a probe concentration of 100 µM and metal concentration of 1 mM. Metal salts used were $AlCl_3$, $Cd(NO_3)_2$, $[(CH_3CN)_4Cu]PF_6$, $Cu(NO_3)_2$, $K_2Cr_2O_7$, $(NH_4)_2Fe(SO_4)_2$, $Hg(NO_3)_2$, $Ni(NO)_2$, $Zn(NO)_2$ and 10 mM stock solutions were prepared in water (acetonitrile for the copper(I) salt). Stock solutions of the probes were made in DMSO, at a concentration of 2, 5 or 10 mM depending upon their solubility. Lake water was obtained from Lake Northam in Victoria Park, Sydney.

During the initial screening of the fifty-five probes against all metals, matrix scans or contour plots were obtained. Multiple spectra were taken for each condition, exciting in steps of every 10 nm from 230 – 460 nm and collecting emission every 10 nm up to 700 nm. The starting emission wavelength was 20 nm higher than the excitation wavelength used – the minimum separation allowed by the instrument.

For the second screening of the selected top twelve probes, and final lake-water array of the four selected probes, individual excitation/emission points were taken. These were chosen based on the point where maximum change was observed for each probe during round one.

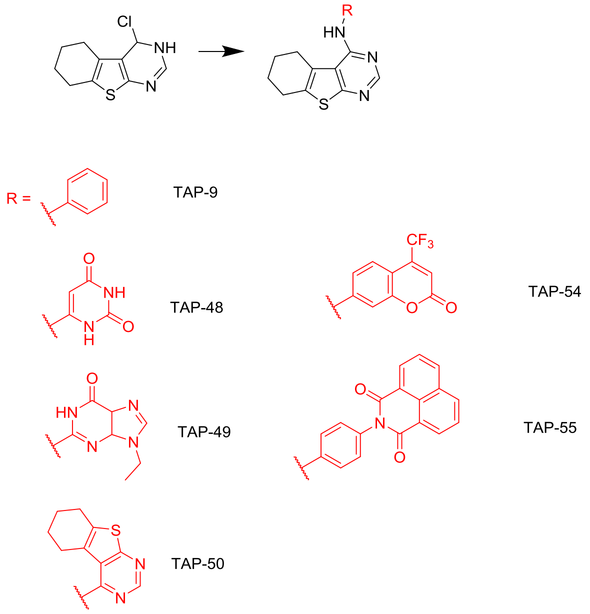
Principal Component Analysis and Linear Discriminant Analysis were performed using SPSS.

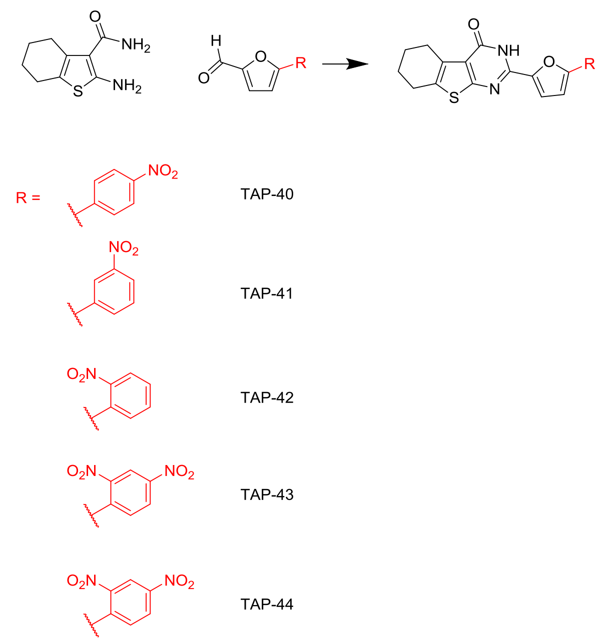


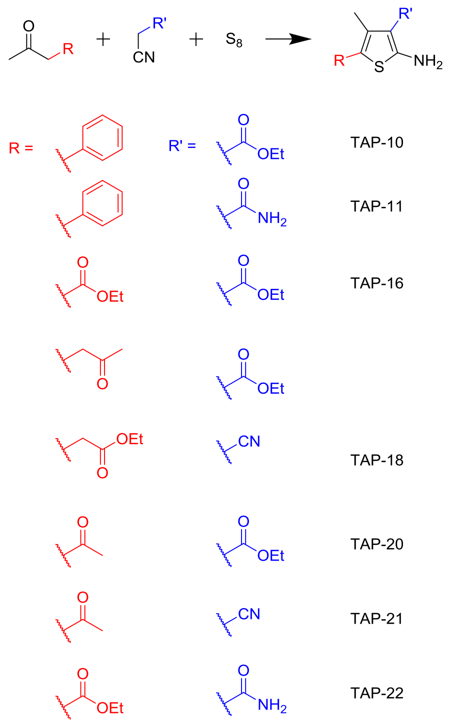
TAP-51

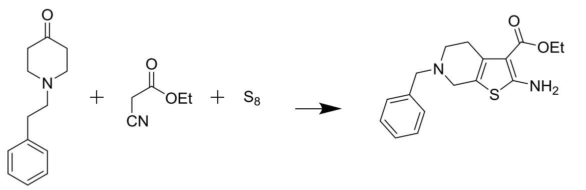
TAP-52

TAP-53

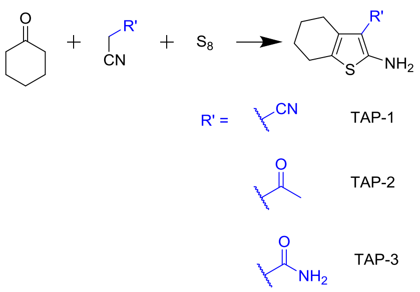


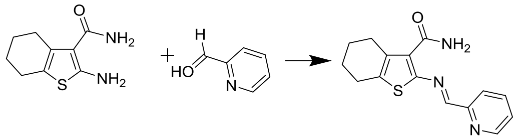




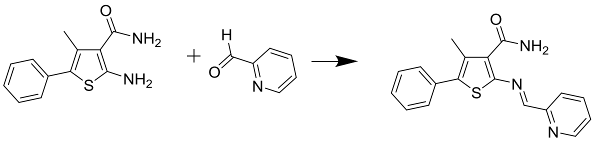


TAP-38

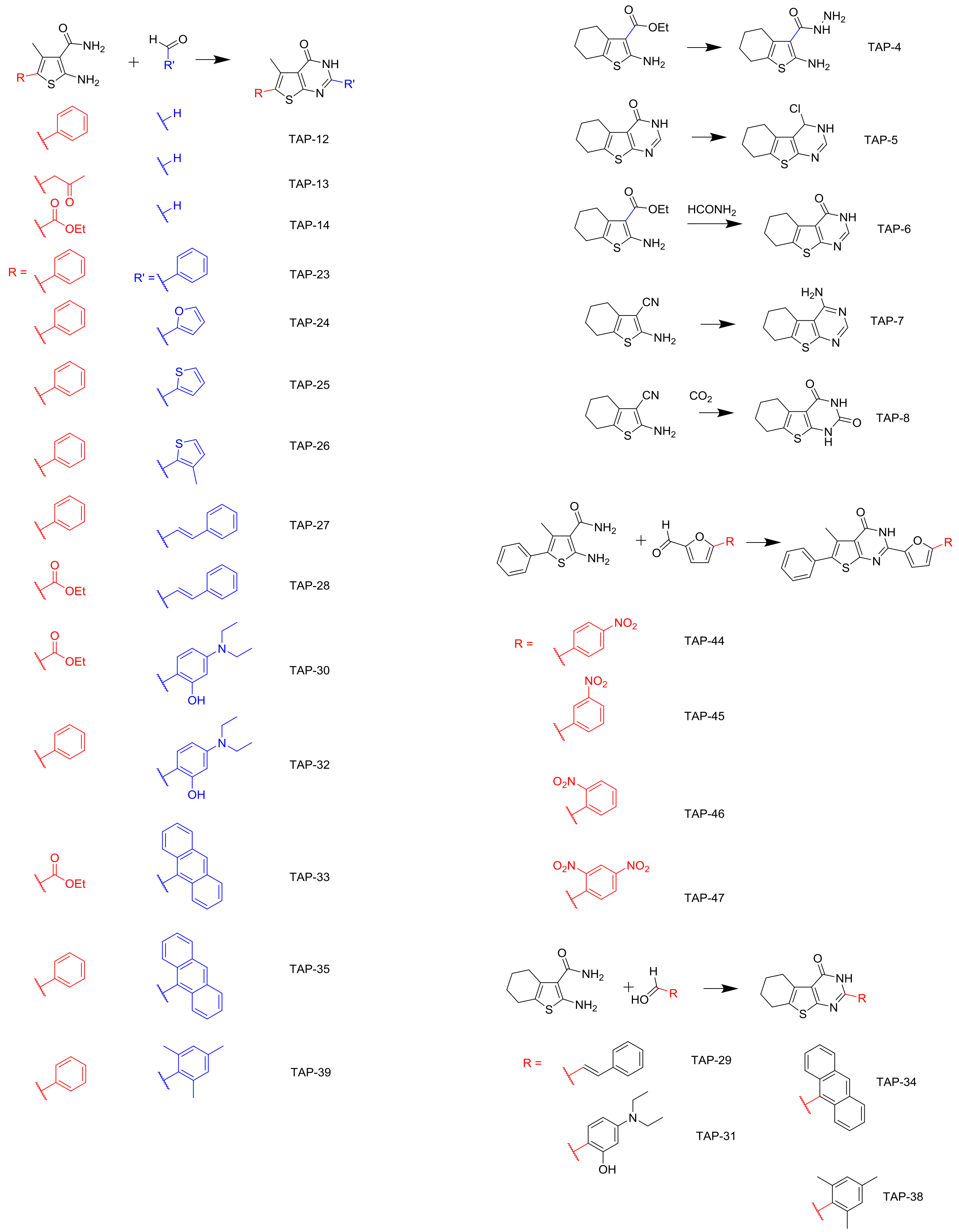




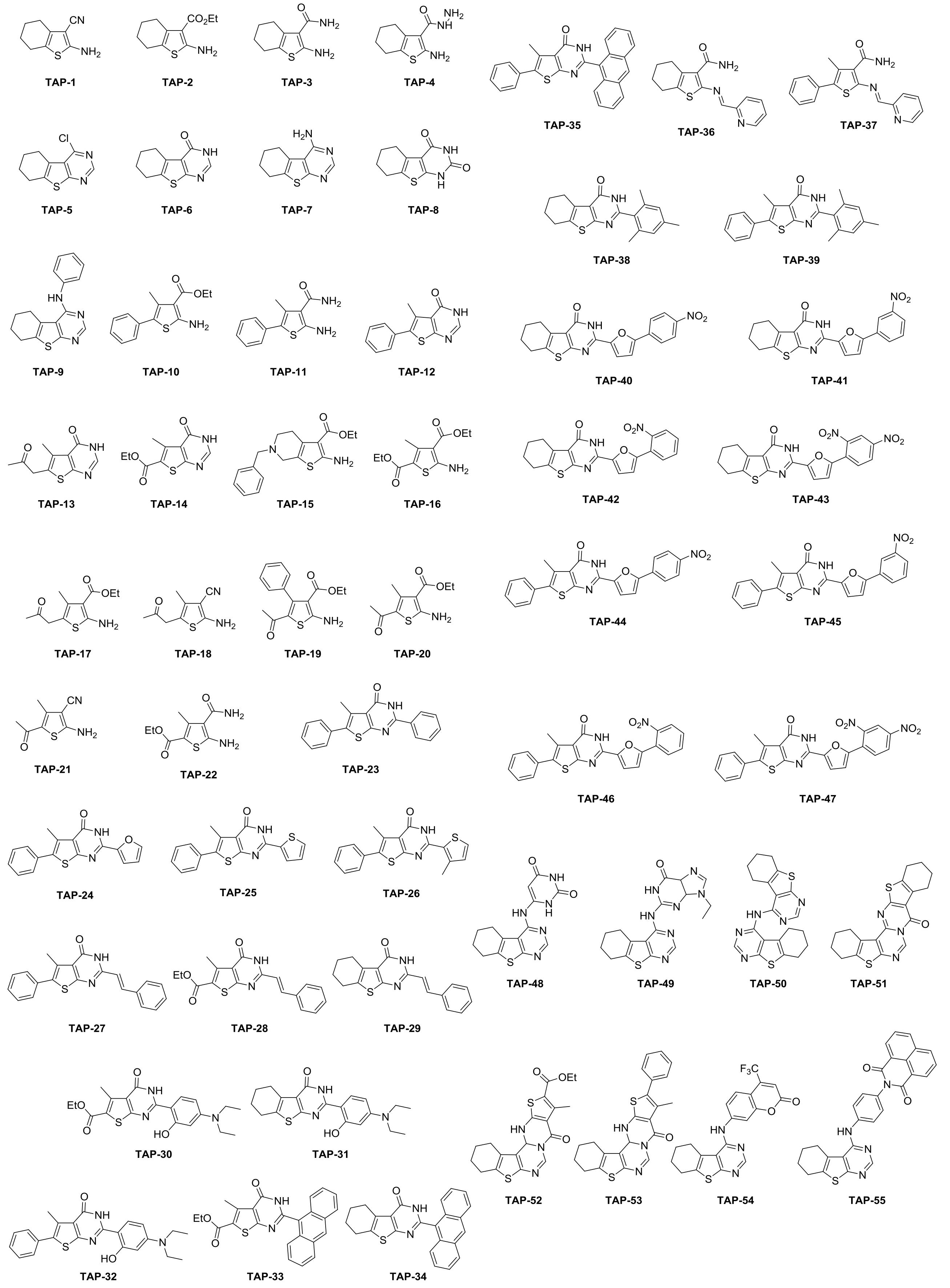
TAP-36



TAP-37



Scheme S1: Overall synthetic scheme for **TAP-1** to **55**



Scheme S2: The structures of **TAP-1** to **55**

Table S3: The optimum excitation and emission wavelength pair for each probe in the top twelve. These were used as the scanning points for the final twelve array.

Probe	λ_{ex} / λ_{em}
TAP-6	360 / 410
TAP-14	310 / 400
TAP-17	460 / 550
TAP-19	360 / 410
TAP-27	300 / 510
TAP-28	330 / 410
TAP-29	300 / 510
TAP-31	430 / 550
TAP-32	360 / 410
TAP-36	360 / 480
TAP-37	360 / 480
TAP-55	340 / 410

Table S4: Ranked responses of the probes to the metals tested. (A) an apparent change in excitation or emission wavelength, (B) a large change in emission intensity, (C) a small change in emission intensity and (D) no change. Each probe was awarded points based on the following scoring system, and then ranked in descending order. Each

(A) resulted in 3 points, (B) = 2, (C) = 1 and (D) = 0. Bonus points, to reward a varied response were awarded to probes which included at least one (A) + one (B) = 3 points, (A) + (C) = 2 points and (B) + (C) = 1 point. Final bonus points were awarded to probes which showed the greatest breadth of response to the range of metals. 3 points were awarded if a probe responded to more than seven metals, 2 points if more than five, and 1 point if more than 3.

	Al(III)	Cd(II)	Cu(I)	Cu(II)	Cr(VI)	Fe(II)	Hg(II)	Ni(II)	Zn(II)
TAP-32	A	B	C	C	A	D	A	D	B
TAP-28	B	C	C	B	D	A	C	B	C
TAP-36	C	A	C	B	C	C	C	C	A
TAP-27	C	B	B	D	B	C	C	B	B
TAP-6	D	A	B	D	B	D	A	B	B
TAP-31	C	D	C	C	C	B	C	D	C
TAP-14	C	D	B	C	B	C	B	C	D
TAP-29	C	B	B	D	D	C	D	C	B
TAP-19	B	A		A	D	D	D	D	D
TAP-11	C	D	D	D	C	C	B	D	B
TAP-37	D	C	C	C	D	D	B	B	C
TAP-55	D	D	C	B	D	C	A	D	D
TAP-17	D	D	B	D	D	A	B	D	D
TAP-2	C	D	D	B	B	D	D	D	D
TAP-4	D	D	B	D	D	C	B	D	D
TAP-39	D	C	B	B	B	D	D	D	D
TAP-23	D	C	C	C	D	C	D	D	C
TAP-7	B	D	C	D	D	C	C	D	D
TAP-22	B	D	D	C	D	C	C	D	D
TAP-33	D	D	B	D	D	D	D	C	D
TAP-15	B	B	D	D	D	D	B	D	D
TAP-44	A	D	D	A	D	D	D	A	D
TAP-5	B	D	D	D	D	B	C	D	D
TAP-40	C	D	B	D	D	D	B	D	D
TAP-10	D	D	D	D	D	A	D	D	D
TAP-18	D	B	D	D	D	A	D	D	D
TAP-48	A	D	B	D	D	D	D	D	D
TAP-12	D	D	B	C	D	D	D	D	D
TAP-26	D	D	A	D	D	D	A	C	D
TAP-1	B	D	D	C	D	D	D	D	D
TAP-30	B	D	D	D	D	D	D	D	C
TAP-51	C	D	D	D	D	D	B	D	D
TAP-13	C	D	D	C	D	D	C	C	D
TAP-25	D	D	D	D	D	C	D	D	C
TAP-38	D	C	D	C	C	D	D	D	C
TAP-49	D	D	D	D	D	C	D	D	A
TAP-3	C	D	D	C	D	D	D	D	D
TAP-16	D	D	D	D	D	D	C	D	C
TAP-34	D	C	D	D	D	D	D	D	C
TAP-24	D	D	D	D	B	D	D	D	D
TAP-21	D	D	D	D	D	C	D	D	D
TAP-50	D	D	D	D	D	C	D	D	D
TAP-8	D	D	D	D	D	D	D	D	D
TAP-9	D	D	D	D	D	D	D	D	D
TAP-20	D	D	D	D	D	D	D	D	D
TAP-35	D	D	D	D	D	D	D	D	D
TAP-41	D	D	D	D	D	D	D	D	D
TAP-42	D	D	D	D	D	D	D	D	D
TAP-43	D	D	D	D	D	D	D	D	D
TAP-45	D	D	D	D	D	D	D	D	D
TAP-46	D	D	D	D	D	D	D	D	D
TAP-47	D	D	D	D	D	D	D	D	D
TAP-52	D	D	D	D	D	D	D	D	D
TAP-53	D	D	D	D	D	D	D	D	D
TAP-54	D	D	D	D	D	D	D	D	D

Table S5: Raw fluorescence data for the array performed on the top twelve ranking probes. Each probe was scanned at the wavelengths shown in the previous SI figure. The value represented is the mean of three sets of 50 flashes. The five replicates are shown.

	TAP-6	TAP-14	TAP-17	TAP-19	TAP-27	TAP-28	TAP-29	TAP-31	TAP-32	TAP-36	TAP-37	TAP-55
-	1,411	10,038	630	359	10,907	1,939	6,497	220	122	205	126	489
-	1,414	10,196	597	336	11,060	1,928	6,331	233	109	190	132	457
-	1,409	10,101	702	365	10,860	1,927	6,292	258	112	223	151	471
-	1,267	10,277	711	356	10,943	1,981	6,238	255	140	184	137	476
-	1,415	10,306	752	-	11,232	2,000	6,790	226	144	218	161	488
Al	1,211	6,296	313	281	12,328	1,162	5,530	244	77	143	92	396
Al	1,247	7,957	352	293	12,350	1,183	5,930	264	110	138	101	415
Al	1,176	8,166	316	263	12,279	1,207	6,149	241	70	145	96	365
Al	1,167	8,330	329	308	12,294	2,091	5,964	272	113	162	124	436
Al	1,138	8,460	329	253	12,940	1,210	6,676	283	66	178	109	399
Cd	1,394	10,914	396	346	10,802	1,924	6,218	180	102	1,875	358	445
Cd	1,412	10,658	413	348	10,797	1,894	6,137	176	117	1,830	321	467
Cd	1,390	10,664	419	343	10,734	1,914	6,131	184	85	1,889	323	484
Cd	1,391	11,005	404	358	10,537	1,964	6,361	187	112	1,886	348	461
Cd	1,383	11,202	422	351	10,655	2,005	6,936	208	119	2,158	357	473
Cu(I)	729	6,867	303	231	9,354	1,449	5,345	209	99	153	78	200
Cu(I)	718	6,936	352	224	9,510	1,341	5,564	224	89	146	77	207
Cu(I)	668	6,709	335	217	9,602	1,402	5,372	233	107	130	84	210
Cu(I)	673	7,154	372	246	9,390	1,442	5,755	227	86	135	61	219
Cu(I)	689	7,221	367	209	9,335	1,461	5,799	224	92	169	101	227
Cu(II)	1,340	9,431	499	305	9,929	1,745	5,738	61	124	86	56	413
Cu(II)	1,346	9,488	483	320	10,008	1,753	5,844	45	150	89	11	448
Cu(II)	1,344	9,454	508	330	10,520	1,746	6,332	83	160	80	-	436
Cu(II)	1,297	9,574	479	306	10,687	1,731	6,189	63	131	96	23	427
Cu(II)	1,318	9,192	517	322	10,255	1,363	6,586	58	170	78	35	450
Cr	1,236	8,601	567	299	9,343	1,519	6,044	44	133	92	3	404
Cr	1,282	8,743	619	313	9,474	1,543	5,386	50	137	77	10	458
Cr	1,259	8,635	690	323	9,704	1,580	5,492	34	128	76	52	463
Cr	1,303	9,074	843	331	9,763	1,483	5,327	35	123	107	21	447
Cr	1,282	8,961	778	313	9,652	1,516	6,238	49	117	85	33	454
Fe	1,022	6,552	422	267	7,620	2,027	4,279	77	130	143	55	335
Fe	1,013	6,387	426	250	7,666	2,042	4,126	42	85	155	80	337
Fe	977	6,638	410	243	7,631	2,061	4,161	69	130	149	31	355
Fe	961	6,801	455	250	7,716	2,389	4,122	88	103	134	63	345
Fe	1,033	7,017	443	253	7,891	2,375	4,779	88	129	130	48	351
Hg	609	1,686	488	343	7,889	123	4,916	94	122	109	39	312
Hg	600	1,771	466	328	8,073	97	4,954	132	156	90	31	298
Hg	587	1,805	502	328	7,969	92	4,985	145	144	90	27	338
Hg	582	2,129	625	332	8,104	176	5,043	138	155	104	44	316
Hg	591	2,222	501	364	7,899	145	5,652	127	151	103	5	337
Ni	1,388	10,053	335	336	10,478	1,868	5,887	125	105	96	63	455
Ni	1,409	9,975	345	316	10,524	1,944	6,135	164	79	115	17	437
Ni	1,380	9,877	359	341	10,631	1,911	6,144	128	95	100	32	473
Ni	1,397	10,332	322	338	10,646	1,894	6,224	141	109	113	60	467
Ni	1,376	10,068	359	327	10,652	1,949	6,714	140	105	79	25	530
Zn	1,436	10,460	479	336	10,803	1,914	5,943	260	135	3,082	1,016	453
Zn	1,422	10,496	527	361	10,369	1,923	6,441	225	152	3,197	1,002	462
Zn	1,403	10,330	482	360	11,007	1,345	6,104	234	158	3,205	1,024	432
Zn	1,391	10,542	514	347	10,853	1,752	6,530	245	152	3,373	1,077	451
Zn	1,396	10,584	522	328	10,923	2,001	6,648	245	141	3,786	1,037	468

Table S6: Raw fluorescence data for the array performed using the final four probes in lake water. Each probe was scanned at each of the optimum wavelengths for each of the probes as shown in the header. The values represented are the mean of three sets of 50 flashes. The four replicates are shown.

	TAP-6 (λex / λem)				TAP-14 (λex / λem)				TAP-27 (λex / λem)				TAP-36 (λex / λem)			
	300 / 510	310 / 400	360 / 410	360 / 480	300 / 510	310 / 400	360 / 410	360 / 480	300 / 510	310 / 400	360 / 410	360 / 480	300 / 510	310 / 400	360 / 410	360 / 480
Blank	1,453	3,030	3,045	1,416	927	77,219	3,880	670	11,931	765	381	4,933	739	846	540	697
Blank	1,514	3,097	3,175	1,503	1,053	84,643	4,217	769	12,422	808	414	5,177	832	896	623	868
Blank	1,483	3,029	3,027	1,435	1,175	92,070	4,008	734	12,552	814	433	5,194	859	1,006	652	908
Blank	1,525	3,175	3,187	1,493	1,126	92,498	3,932	750	12,347	839	440	5,110	840	984	684	926
Al(III)	1,339	2,828	2,914	1,434	428	3,087	644	560	11,263	688	432	5,497	787	1,097	933	1,079
Al(III)	1,516	3,248	3,485	1,721	444	3,323	720	592	11,836	746	481	5,389	786	1,036	862	1,032
Al(III)	1,665	3,677	3,965	1,920	436	3,040	666	591	12,307	719	430	5,705	839	1,075	944	1,095
Al(III)	1,490	3,280	3,472	1,666	445	3,194	1,067	841	12,195	743	477	5,915	805	1,074	951	1,093
Cd(II)	1,389	2,871	2,933	1,370	1,023	100,089	2,920	611	11,123	720	390	4,798	3,539	922	1,787	12,730
Cd(II)	1,378	2,997	3,054	1,465	1,106	110,537	3,090	636	11,252	751	417	4,935	3,545	929	1,767	12,921
Cd(II)	1,277	2,793	2,808	1,361	1,113	110,096	2,685	629	10,691	769	391	4,836	4,176	1,158	2,362	16,565
Cd(II)	1,339	2,797	2,857	1,382	1,046	108,512	2,639	639	11,256	769	413	5,183	4,174	1,164	2,418	16,693
Cu(I)	829	1,958	3,150	1,348	187	2,993	243	153	7,645	301	195	3,900	157	471	234	148
Cu(I)	893	2,264	3,551	1,517	162	2,926	286	146	5,953	297	224	3,128	162	480	253	139
Cu(I)	912	2,342	4,615	2,185	189	3,024	257	143	5,690	283	272	4,668	180	577	304	186
Cu(I)	774	1,978	3,843	1,767	179	3,260	252	153	5,225	346	291	4,415	175	573	317	180
Cu(II)	472	1,474	2,306	1,028	101	4,764	348	155	6,453	182	211	4,758	59	352	215	119
Cu(II)	557	1,655	2,728	1,132	111	4,970	372	149	6,082	202	186	4,268	108	372	264	162
Cu(II)	552	1,590	2,468	1,022	134	4,939	346	180	5,330	251	267	4,141	153	568	272	167
Cu(II)	526	1,564	2,436	1,029	123	5,277	369	186	5,155	264	244	3,634	130	561	283	170
Cr(VI)	398	522	244	171	195	1,938	72	60	3,559	170	47	578	273	149	89	125
Cr(VI)	480	626	323	190	212	2,119	65	64	3,926	133	51	676	285	183	65	121
Cr(VI)	429	533	261	161	221	1,775	89	104	3,685	154	73	520	276	189	88	134
Cr(VI)	494	640	298	178	195	2,015	84	100	3,923	146	92	583	297	208	107	148
Fe(II)	902	1,921	2,124	1,008	517	1,602	361	290	8,350	426	249	3,333	173	541	257	148
Fe(II)	953	2,014	2,307	1,056	575	1,670	371	307	8,443	399	241	3,408	196	499	213	160
Fe(II)	872	1,857	2,071	985	522	1,521	356	295	7,132	404	274	3,014	177	451	239	148
Fe(II)	961	2,075	2,270	1,041	559	1,562	381	311	7,212	363	256	3,069	176	432	200	160
Hg(II)	457	759	786	449	167	3,300	235	189	3,893	235	152	1,424	192	248	144	184
Hg(II)	460	806	792	465	158	3,331	253	190	3,822	248	167	1,439	213	291	143	191
Hg(II)	459	760	811	533	199	3,265	289	227	2,991	307	215	1,337	211	312	224	217
Hg(II)	436	727	768	503	199	3,539	285	222	3,054	308	212	1,296	252	375	194	251
Ni(II)	1,046	2,497	2,565	1,146	650	64,752	3,011	379	10,845	434	262	4,422	407	435	521	900
Ni(II)	1,089	2,534	2,711	1,191	701	70,767	3,124	411	10,829	463	241	4,587	334	445	208	223
Ni(II)	1,180	2,730	2,949	1,328	706	74,828	2,987	408	10,690	480	280	4,529	349	493	238	254
Ni(II)	1,102	2,579	2,733	1,237	780	79,602	3,108	423	11,157	472	282	4,758	348	476	259	274
Zn(II)	1,296	2,687	2,723	1,287	855	49,158	2,027	600	10,714	651	403	4,732	4,572	1,184	2,847	18,814
Zn(II)	1,358	2,854	2,924	1,401	982	51,707	1,992	593	11,225	693	467	4,931	4,403	1,187	2,773	18,237
Zn(II)	1,223	2,659	2,751	1,309	1,033	56,137	1,886	592	10,531	677	398	4,735	4,999	1,229	3,197	21,723
Zn(II)	1,308	2,850	2,914	1,330	1,070	57,019	1,902	597	10,861	678	410	4,948	4,884	1,211	3,122	21,401