Supplementary Information

Discovery of Novel Zebrafish Neural Tracers by Organism-Based Screening of Rosamine Library

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General remarks

Medium-to-high throughput screening.

Prior to the staining, the Zebrafish larvae of 3-5 dpf were put into the clear U-bottom 96-well plates with 4-6 larvae per well. Embryo media containing RS compounds at 2μM final concentration were added to each well. After one hour staining, media were replaced with fresh media containing Ethyl 3-aminobenzoate (MS-222, 0.015% final concentration) so that the larvae were immobilized for live image. For the primary screening, images were taken with Nikon SMZ1500 stereoscopic zoom microscope equipped with a Nikon C-HGFI fluorescence lamp, images of larvae in compound were taken with both FITC and TRITC filter sets (FITC: Ex. 470, Em. 515; TRITC: Ex. 546/10, Em. 590 long pass).

High resolution imaging and optical sectioning.

For representative compounds, imaging of labeled larvae was conducted using a Nikon upright microscope, the multi-purpose zoom microscope Multizoom AZ100, equipped with a Nikon C-HGFI fluorescence lamp. Larvae after compound application were anaesthetized with MS-222 and embedded in 1.5% low gelling agarose (Type VII) for live imaging. Images were taken with slightly different FITC and TRITC filter sets (FITC: Ex. 460-500, Em. 510-560; TRITC: Ex. 540/25, Em. 605/55). Optical sections were carried out using a Nikon inverted microscope Eclipse Ti equipped with the Nikon A1R/ A1 scanner. Larvae after compound application were anaesthetized and embedded with agarose at the bottom of the thin-glass bottomed petri dish for imaging. Laser at 488nm or 560nm was used for excitation. Images were acquired with FITC and TRITC emission

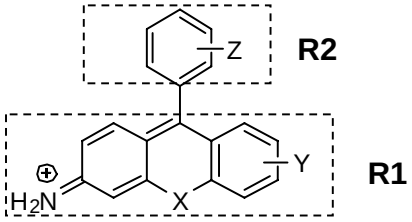
filter sets with 525/50 and 595/50 emission wavelengths, respectively. Optical sections were acquired at 2 to 5μm intervals; reconstruction was performed with the Nikon image acquisition and analysis software, NIS-Elements.

Effect of lead compound on development and acute ototoxicity induced by aminoglycoside.

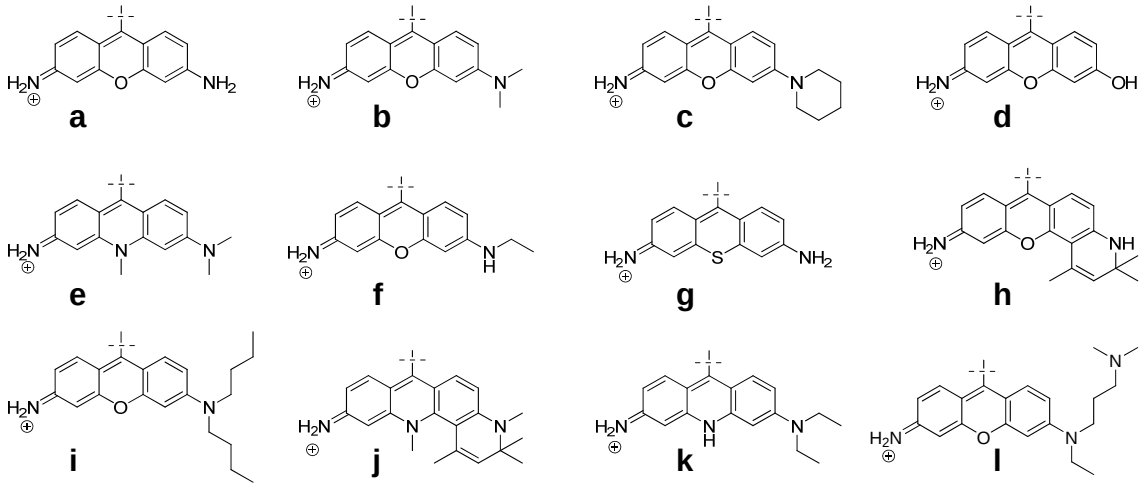
To evaluate the application of our novel neural tracers in aquatic surveillance, larvae were raised in embryo media containing 0.1 mM lead chloride from birth. At 4 dpf, routine compound staining and imaging was carried out. To evaluate the application of our tracers in acute ototoxocity induced by aminoglicoside, 200μM gentamicin was added together with the staining compound for an hour in the medium.

Structure-activity analysis of RS compounds

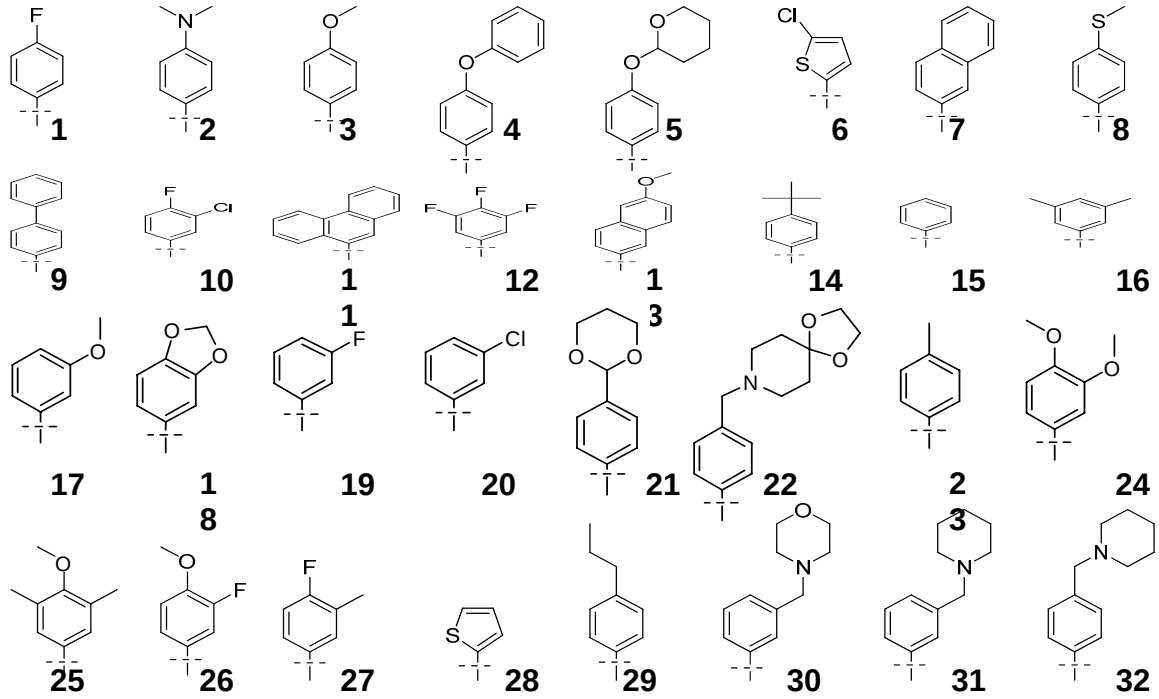
As illustrated, the diversity of the RS library is conveyed by changing X, Y and Z.



The building blocks (R1) is from one of the structures below:



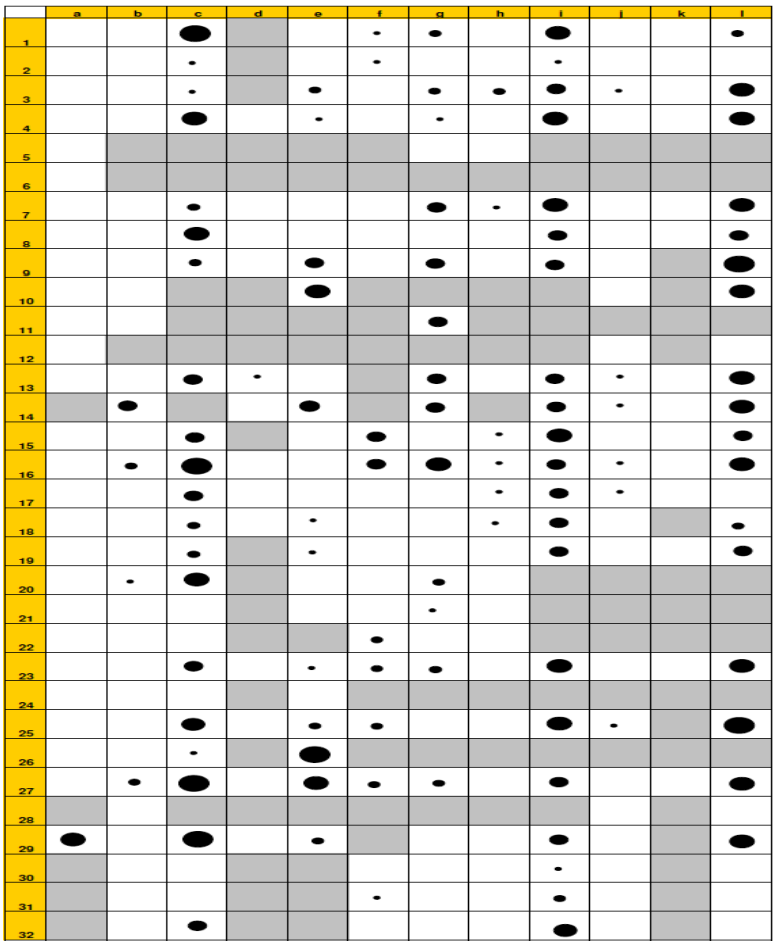
R2 is listed below:

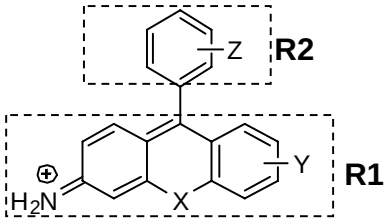


Semi-quantification of labeling intensity and its relation to the chemical structure.

Since the RS library is constructed in a combinatorial fashion (S1), we sought to find out the influence of R1 and R2 on the labeling intensity and thus the labeling intensity was graded from 1-5 by visual inspection and plotted against R1 and R2. There exists a clear "column wise" distribution of the labeling intensity, whereas row wise it appears random (Table S1). The most intense labeling was observed when R1 is c, i or l, whereas little or no labeling intensity was observed when R1 is j or k, indicating that 1) R1 has a much stronger influence than R2 to the staining intensity; 2) long alkyl chain that is hydrophobic or piperidine helps for the labeling, and 3) the addition of positive charge may have little effect (i versus l).

Table S1. Influence of R1 and R2 on the overall labeling intensity. Size of the circle represents the graded labeling intensity (1-5). Rosamine scaffold (S1) is shown at the right side of the table that contains R1 and R2 building blocks.



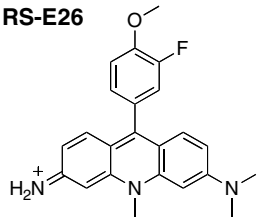


(S1) Ahn, Y. H.; Lee, J. S.; Chang, Y. T. *J. Am. Chem. Soc.* **2007**, *129*, 4510-451

Spectra of ZeN-Green (RS-E26) and ZeN-Red (RS-C20).

NMR Characterization

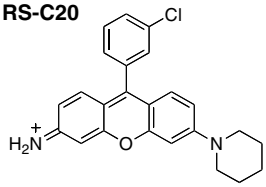
RS-E26



Chemical Formula: $C_{23}H_{23}FN_3O^+$
Molecular Weight: 376.45

^{1}H NMR (300 MHz, MeOH-d4) δ = 3.25 (s, 6H), 3.96 (s, 3H), 4.13 (s, 3H), 6.75 (m, 1H), 6.87 (d, 1H, *J* = 9.06 Hz), 6.96 (s, 1H), 7.12 (m, 3H), 7.32 (m, 2H), 7.43 (d, 1H, *J* = 9.65 Hz). ^{13}C NMR (300 MHz, MeOH-d4) δ = 12.79, 20.22, 29.75, 32.52, 68.54, 68.86, 90.13, 92.89, 93.15, 94.35, 96.55, 97.96, 113.91, 114.42, 117.01, 117.63, 117.89, 126.45, 126.51, 131.58, 132.14, 133.84, 141.76. ESI-MS: *m/z* found = 376.0 HRMS(ESI) Calculated for $C_{23}H_{23}FN_3O^+$ = 376.45 found: 376.1824

RS-C20

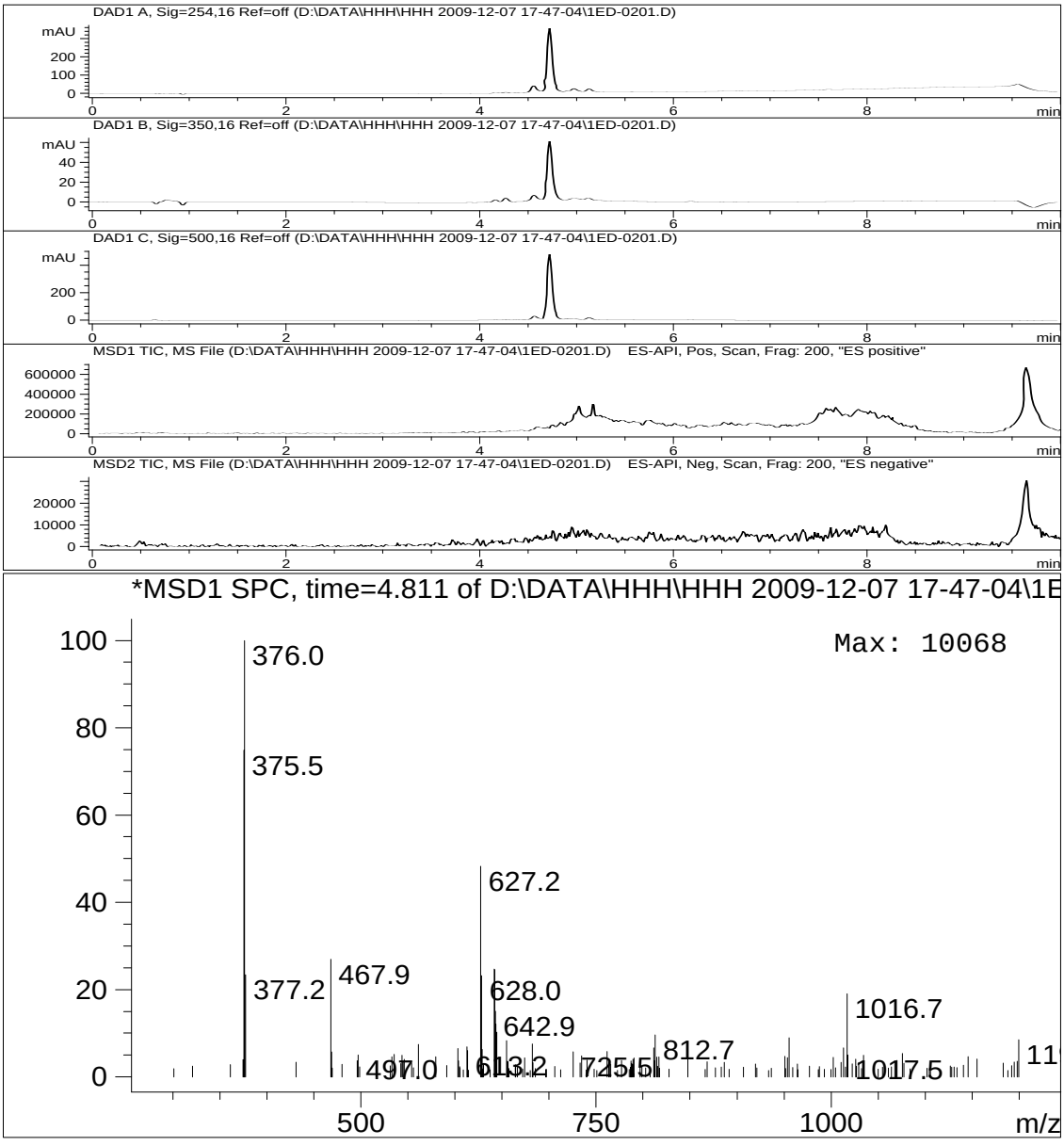


Chemical Formula: $C_{24}H_{22}ClN_2O^+$
Molecular Weight: 389.90

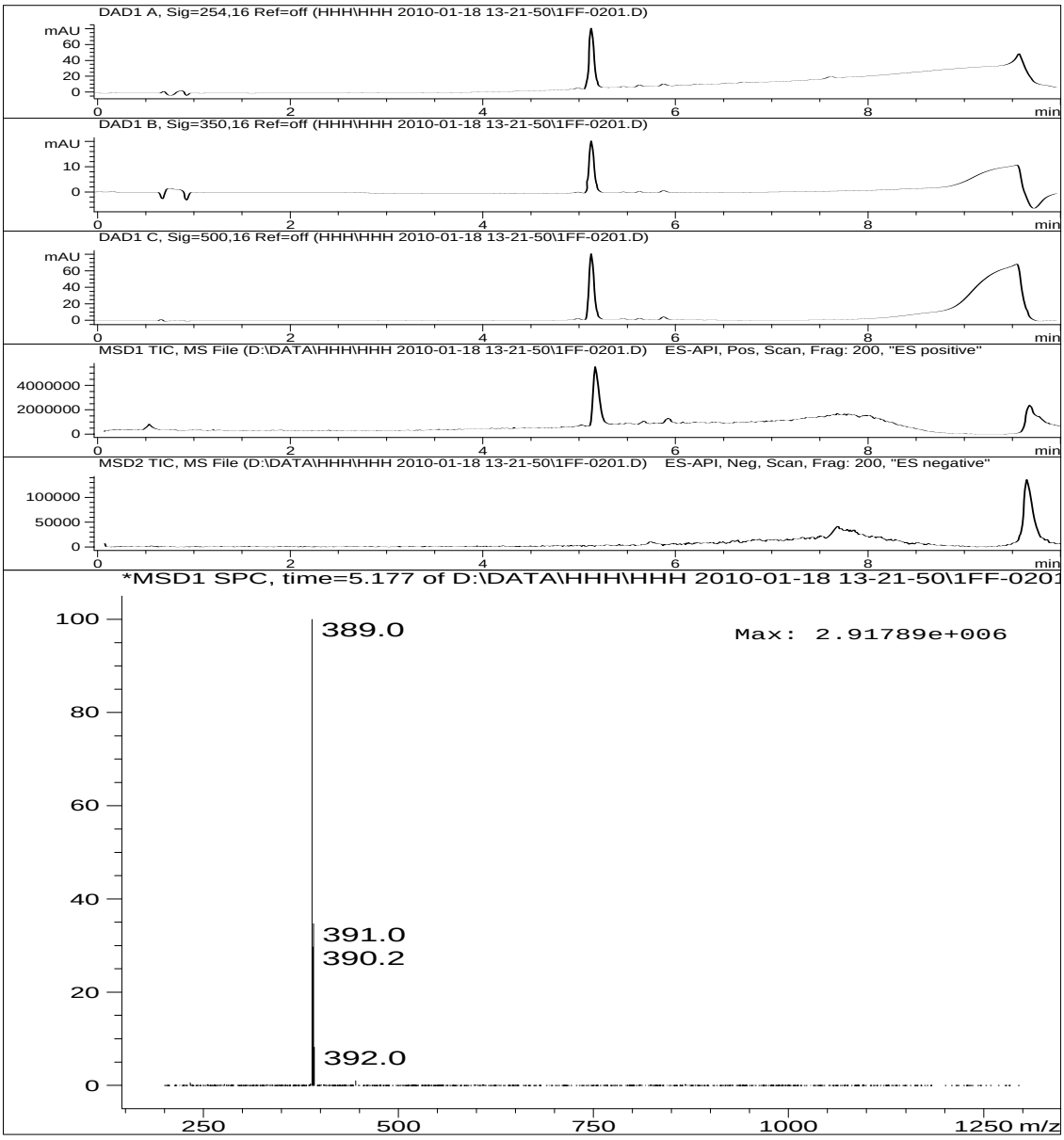
^{1}H NMR (300 MHz, MeOH-d4) δ = 1.69 (m, 6H), 3.71 (m, 4H), 6.75 (d, 1H, *J* = 2.05 HZ), 6.80 (dd, 1H, *J* = 2.05, 9.06 Hz), 7.06 (d, 1H, *J* = 2.05 Hz), 7.20 (m, 3H), 7.33 (d, 1H, *J* = 7.01 Hz), 7.46 (s, 1H), 7.61 (m, 2H). ^{13}C NMR (300 MHz, MeOH-d4) δ = 20.34, 21.38, 24.27, 26.11, 57.69, 81.25, 96.78, 97.18, 97.66, 115.11, 117.11, 126.46, 128.10, 129.37, 130.37, 130.68, 131.80, 132.17, 133.82, 142.67, 148.84, 157.86. ESI-MS: *m/z* found = 389.0 HRMS(ESI) Calculated for $C_{24}H_{22}ClN_2O^+$ = 389.90 found: 389.1416

LC-MS Characterization

RS-E26



RS-C20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DAD1 B, Sig=350,16 Ref=off (HHH\HHH 2010-01-18 13-21-50\1FF-0201.D)
mAU
DAD1 C, Sig=500,16 Ref=off (HHH\HHH 2010-01-18 13-21-50\1FF-0201.D)
mAU
MSD1 TIC, MS File (D:\DATA\HHH\HHH 2010-01-18 13-21-50\1FF-0201.D) ES-API, Pos, Scan, Frag: 200, "ES positive"
MSD2 TIC, MS File (D:\DATA\HHH\HHH 2010-01-18 13-21-50\1FF-0201.D) ES-API, Neg, Scan, Frag: 200, "ES negative"
min
*MSD1 SPC, time=5.177 of D:\DATA\HHH\HHH 2010-01-18 13-21-50\1FF-0201
389.0
Max: 2.91789e+006
391.0
390.2
392.0
m/z

HR-MS Data

RS-E26

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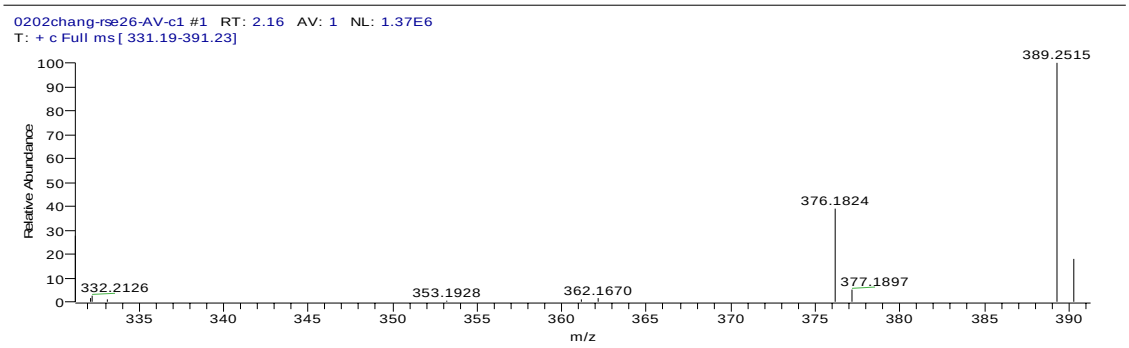
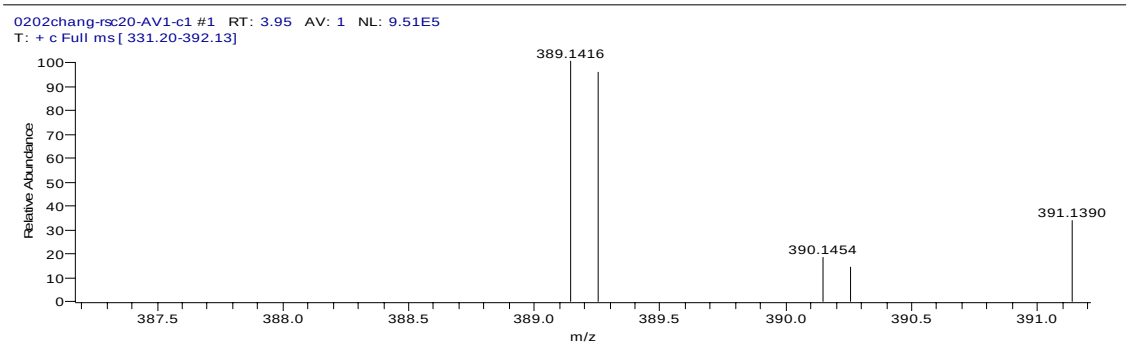


0202chang-rse26-AV-c1#1 RT: 2.16
T: + c Full ms [331.19-391.23]
m/z= 375.68-376.40

m/z	Intensity	Relative	Theo. Mass	Delta (ppm)	RDB equiv.	Composition
376.1824	530089.0	100.00	376.1820	1.04	13.5	$C_{23}H_{23}O_1N_3F_1$
			376.1808	4.08	17.5	$C_{26}H_{22}N_3$
			376.1843	-5.14	6.0	$C_{17}H_{27}O_5N_3{}^{23}Na_1$

RS-C20

A:\03feb10_esihr\0202chang-rsc20-AV1-c1 02/02/2010 03:21:53 PM RS-C20

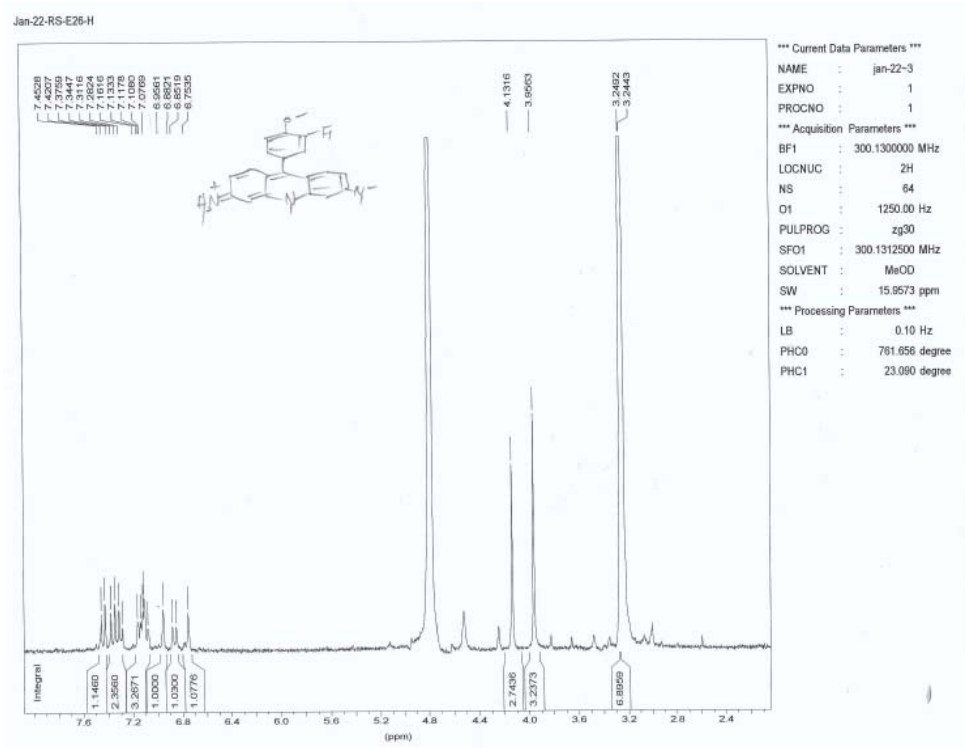


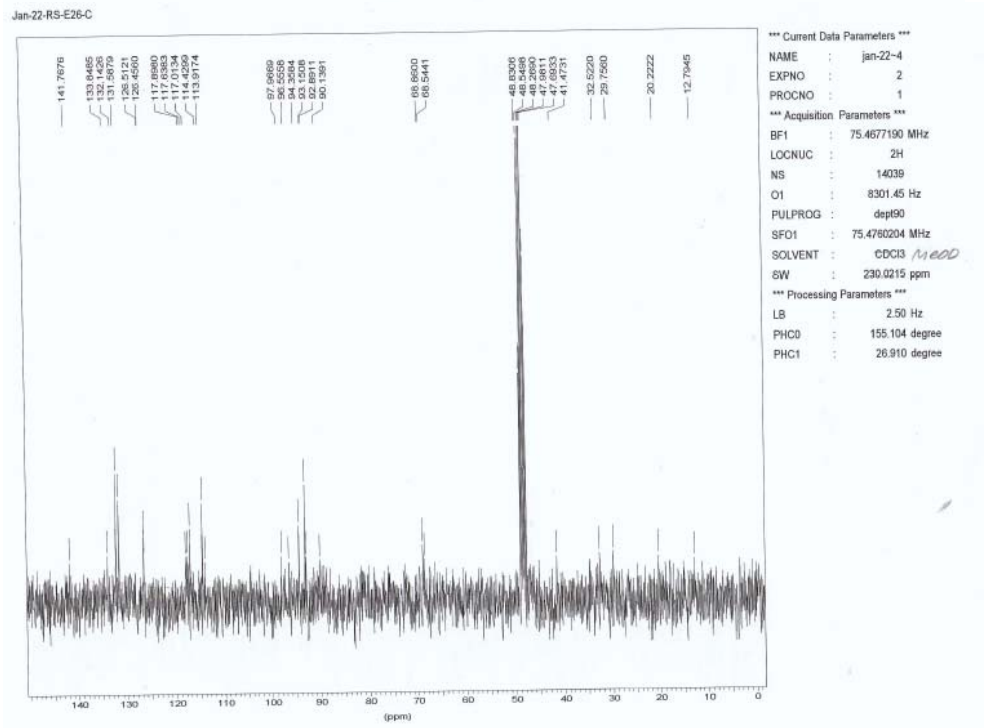
0202chang-rsc20-AV1-c1#1 RT: 3.95
T: + c Full ms [331.20-392.13]
m/z= 389.09-389.15

m/z	Intensity	Relative	Theo. Mass	Delta (ppm)	RDB equiv.	Composition
389.1416	950674.0	100.00	389.1415	0.29	14.5	$C_{24}H_{22}O_1N_2{}^{35}Cl_1$
			389.1432	-3.92	8.0	$C_{16}H_{22}O_6N_4{}^{23}Na_1$

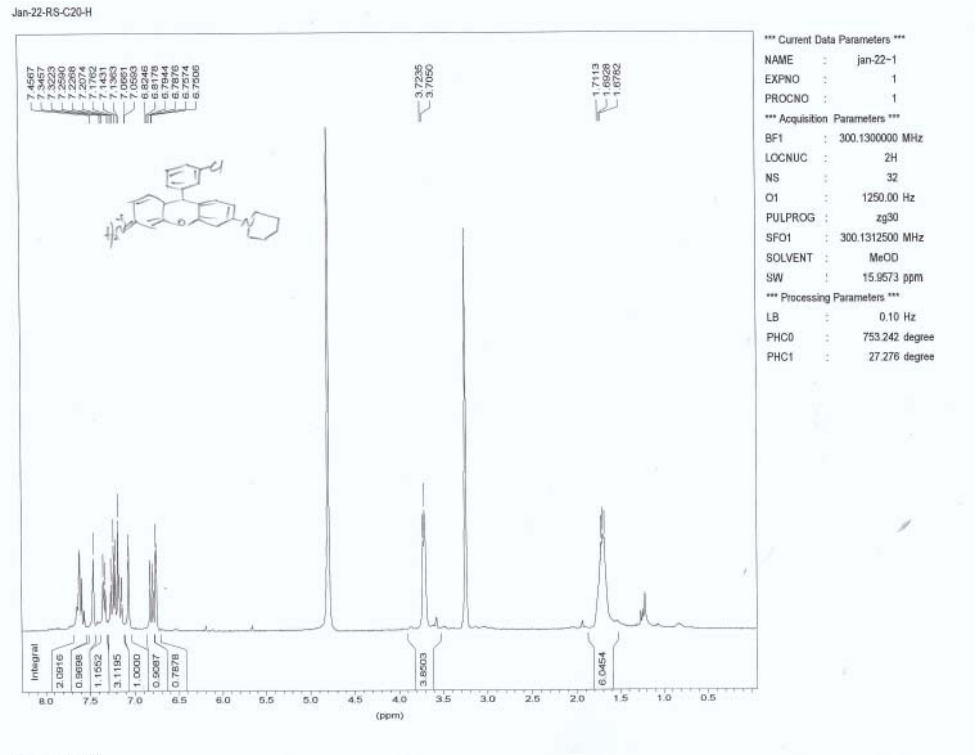
1H NMR and 13 C NMR Spectra

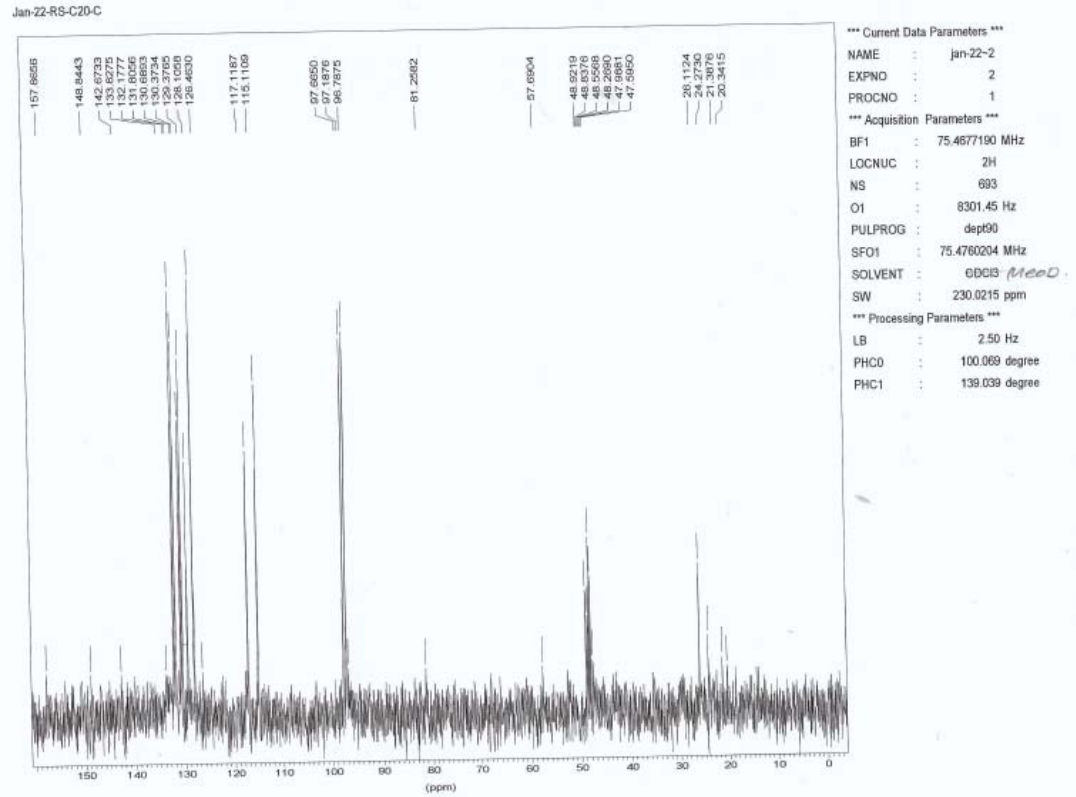
RS-E26





RS-C20





Co-labeling of ZeN-Green and ZeN-Red.

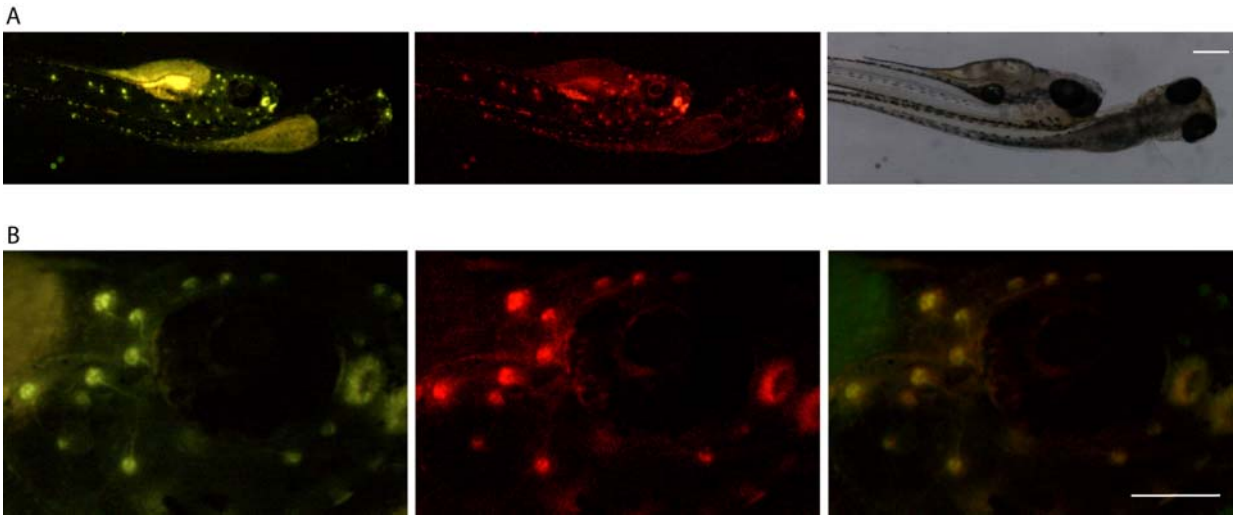


Figure S1. Co-labeling of ZeN-Green and ZeN-Red in the same neurons. (A) Lower resolution images showing the labeling of ZeN-Green and ZeN-Red is overlapping. (B) High magnification of the labeling in the ear and nose, neurons are labeled by both compounds. Scale bar: 100 μm.