Molecular Pharmacology

Supplemental Data

for

Lithium 'Hot-spots': Real-time Analysis of non-selective cation channel activity in migrating cancer cells

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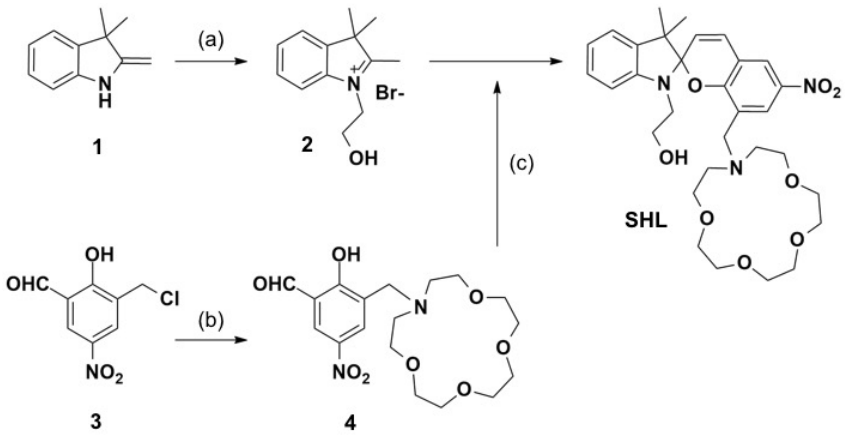
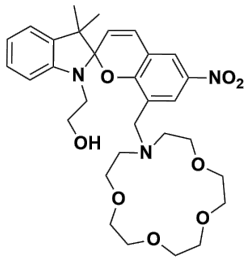
Supplemental Methods

General Methods and Materials for SHL Synthesis

All ^{13}C NMR spectra (126 MHz) and ^{1}H NMR spectra (500 MHz) were recorded on an Agilent Technologies 500 MHz NMR spectrometer with DD2 console in DMSO-d_6. Chemical shifts (δ) are reported in ppm. Chemical shifts of DMSO-d_6 (δ_C = 39.52 ppm) or TMS (δ_H = 0.0 ppm) were used as internal standards in all ^{13}C NMR and ^{1}H NMR experiments, respectively. High resolution mass spectrometry was performed on the Agilent 6230 TOF LC-MS. HPLC grade acetonitrile was used in all related experiments. Compound **2** [1] and compound **4** [2] were prepared as previously described. All metal ions used in this work were in the form of perchlorate salt. All other reagents were purchased from Sigma-Aldrich and used without further purification.

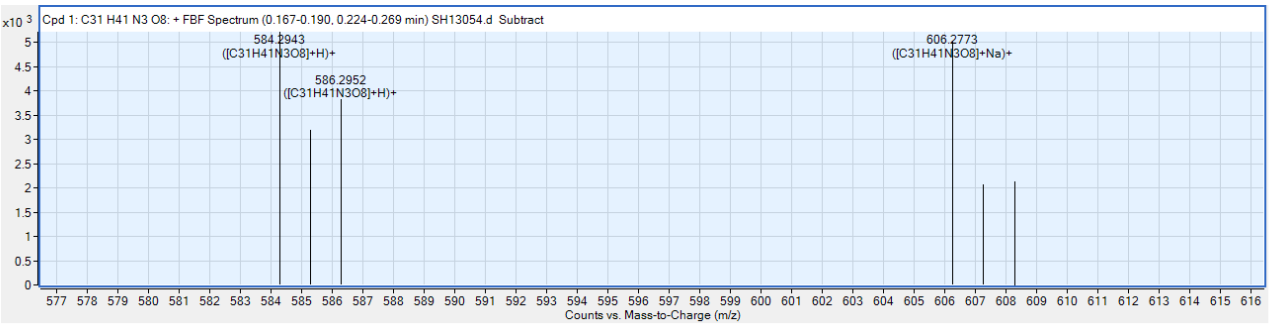


Supplemental Scheme 1. Synthesis of **SHL.** (a) 2-Bromoethanol, acetonitrile, reflux, 18 h; (b) 1-aza-15-crown-5, Et_3N, THF, 0 °C; (c) ethanol, reflux, 18 h.

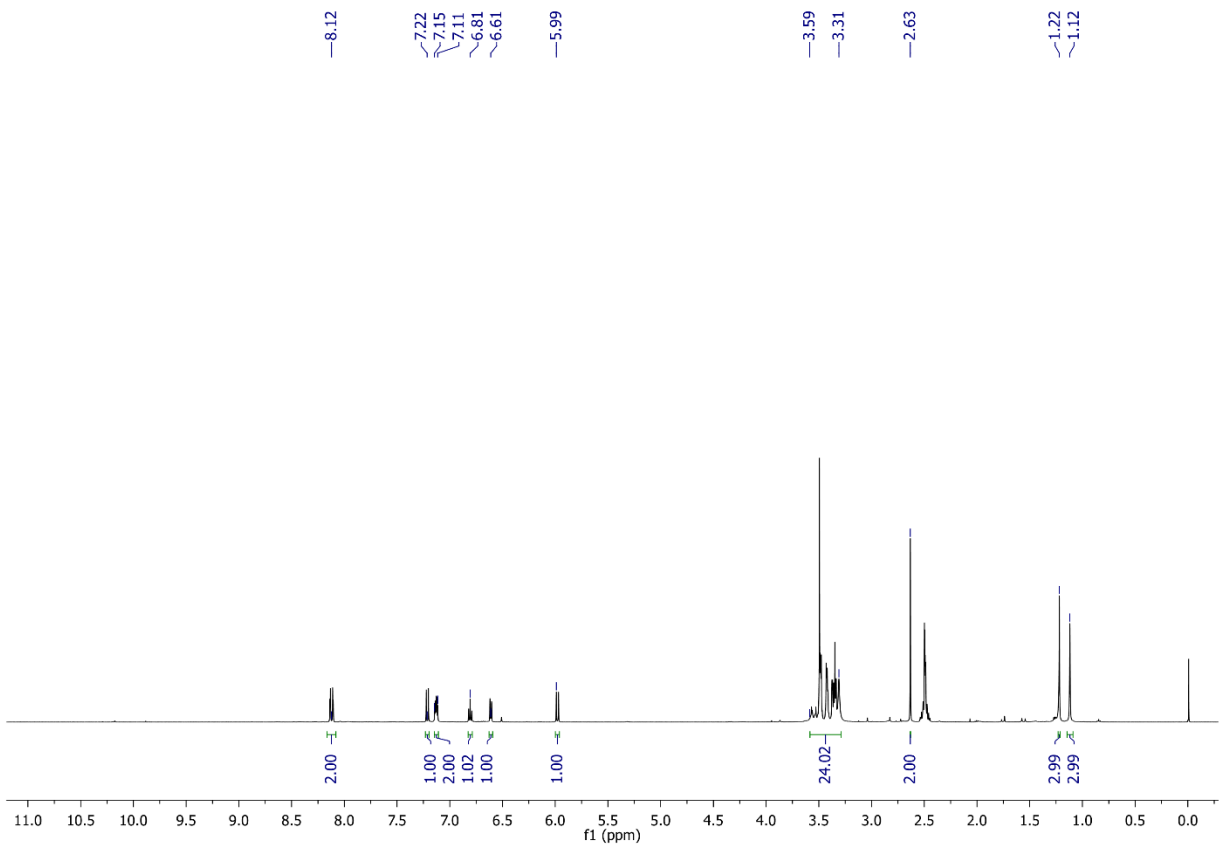


2-(8-((1,4,7,10-tetraoxa-13-azacyclopentadecan-13-yl)methyl)-3',3'-dimethyl-6-nitrospiro[chromene-2,2'-indolin]-1'-yl)ethanol (**SHL**).

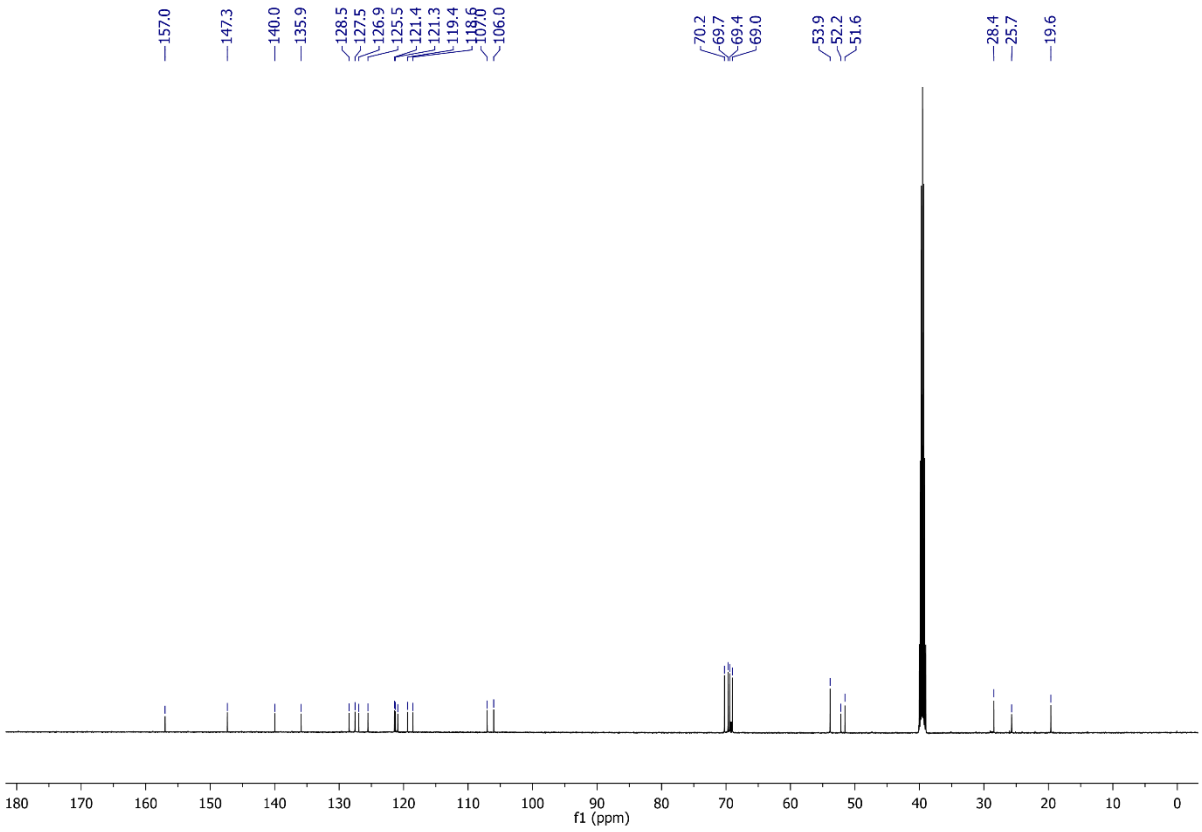
Compound **4** [2] (566 mg, 1.4 mmol) and indoline **2** [1] (230 mg, 1.1 mmol) were combined and dissolved in ethanol (10 mL) and the mixture was heated to reflux for 18 h. Excess solvent was removed *in vacuo* to give a purple crude solid (0.68 g) which was purified by C18 reverse phase silica chromatography eluting with 0 - 30% water / acetonitrile to give **SHL** as a purple solid (120 mg, 18 % yield). **^{1}H NMR (500 MHz, DMSO-d$_6$)** δ 8.12 (dd, 2H, Ar*H*, *J* = 12.5, 2.5 Hz), 7.22 (d, 1H, Ar*H*, *J* = 10.5 Hz), 7.15 – 7.12 (m, 2H, Ar*H*), 6.81 (t, 1H, Ar*H*, *J* = 7.5 Hz), 6.61 (d, 1H, Ar*H*, *J* = 7.5 Hz), 5.99 (d, 1H, Ar*H*, *J* = 10.5 Hz), 3.59 – 3.31 (m, 24H, CH_2), 2.63 (m, 2H, CH_2), 1.22 (s, 3H, CH_3), 1.12 (s, 3H, CH_3) ppm; **^{13}C NMR (126 MHz, DMSO-d$_6$)** δ 157.0, 147.3, 140.0, 135.9, 128.5, 127.5, 126.9, 125.5, 121.4, 121.3, 119.4, 118.6, 107.0, 106.0, 70.2, 69.7, 69.4, 69.0, 53.9, 52.2, 51.6, 28.4, 25.7, 19.6 ppm; **HRMS-ESI (*m/z*)** calculated for $C_{31}H_{41}N_3O_8$ [M + H]$^+$ 584.2972, found 584.2943 and [M + Na]$^+$ 606.2791, found 606.2773.



HRMS-ESI spectrum of **SHL**



^{1}H NMR spectrum of **SHL** recorded in DMSO-d_6 at 500 MHz



^{13}C NMR spectrum of **SHL** recorded in DMSO-d_6 at 126 MHz

Supplemental Spectroscopic Experiments

1. General Information

Stock solutions of **SHL** (5 mM) were prepared in HPLC-grade acetonitrile. Stock solutions of metal ion salts (10 mM) were prepared in water and diluted in acetonitrile (1 mM). Salt solutions were prepared from dried perchlorate or chloride salts and these were used interchangeably.

For the selectivity studies, solutions were prepared (in triplicate) on the same microplate tray from 2 µL **SHL** and 2 µL of ion stock solutions, such that each replicate contained a 1:2 molar ratio of **SHL**: ion. 196 µL of HPLC grade acetonitrile was then added to dilute each replicate, such that the final concentrations of **SHL** and ions in each solution were 50 µM and 100 µM, respectively. The microplate tray was then incubated in the dark for 10 mins before reading. Absorbance and fluorescence spectra were recorded between 300-700 nm and 555-800 nm, respectively, at 25 °C using a BioTek Synergy H4 Hybrid Multi-Mode Microplate Reader scanning with a resolution of 5 nm. Fluorescence excitation was at 532 nm, with a bandgap of 9 nm and 100 gain setting. The assay tray was then removed and repetitive photoswitching was performed by exposure to 352 nm UV light from a filtered 8 W Hg lamp (UVP), or halogen white lamp for 10 min each with absorbance and fluorescence spectra obtained after each irradiation. The error bars presented represent a standard deviation about the mean value.

For the Job's Plot Analysis of Binding Stoichiometry, stock solutions of **SHL** (100 µM) and Li^+ salt (100 µM) were prepared separately in HPLC-grade acetonitrile. Solutions were prepared (in triplicate) in the same clear-bottom microplate tray from varying volumes of the **SHL** and Li^+ stock solutions until the total volume of each replicate was 200 µL, where each solution contained a constant total combined concentration of

[**SHL**]+[Li^+] = 100 μM. The microplate tray was then incubated in the dark for 10 mins before reading. Fluorescence emission spectra were recorded as described above. A Job's plot was derived by plotting the mean fluorescence at the maximum emission wavelength for each concentration ratio of ([**SHL**], [Li^+]).

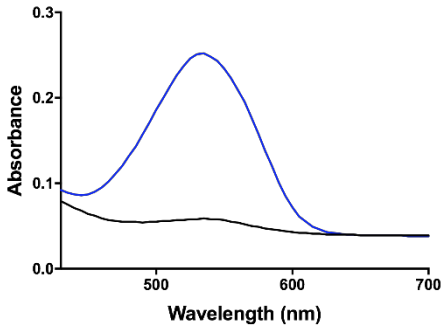


Figure S1. Absorbance spectra of **SHL** (50 μM) in the absence (black) and prescence of Li^+ (blue, 100 μM).

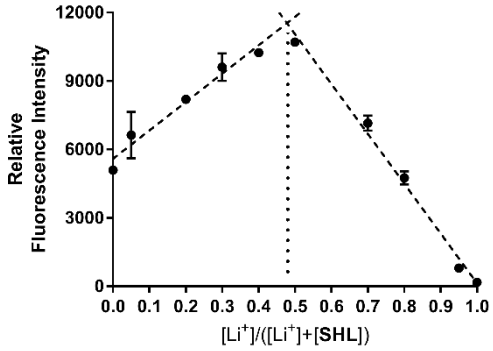


Figure S2. Job's plot analysis of binding stoichiometry of **SHL** with Li^+. Maximum fluorescence at X (= $[Li^+]/([Li^+] + [\mathbf{SHL}])$) = 0.55 depicted by vertical dotted line on the graph.

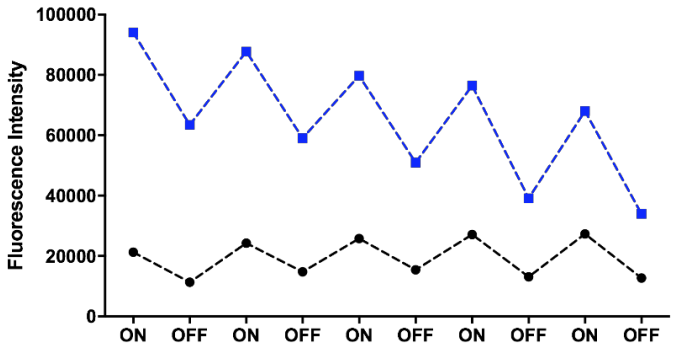


Figure S3. Photoswitching of **SHL** (50 μM) in the absence (black) and in the presence of Li^+ (100 μM, blue). Each "off-cycle" represents the fluorescence emission (λ_{em} =

approx. 635 nm) of the ring closed spiropyran without Li^+ chelated. Each “on-cycle” represents the resultant fluorescence emission of ring-opened (**SHL**)MC-Li^+.

2. Determination of Dissociation Constant (K_d) of **SHL**

Stock solutions of Li^+ salt (0.02-2 mM) and **SHL** (5 mM) were prepared separately in HPLC-grade acetonitrile. Replicate solutions were prepared (in triplicate) in the same clear-bottom microplate tray and diluted in acetonitrile such that the final concentrations of **SHL** and Li^+ in each solution were 50 μM and 1-100 μM, respectively. The microplate tray was then incubated in the dark for 10 mins before reading. Fluorescence emission spectra were recorded as described above. A concentration curve was prepared from the fluorescence emission maximum for each ion concentration. The apparent dissociation constant (K_d) of **SHL** for Li^+ was then calculated by fitting an appropriate non-linear regression in GraphPad Prism version 7.02. The ‘Hill Plot with Specific Binding’ model was selected, as it represents a saturation binding experiment, where concentration of ‘radioligand’ (i.e. Li^+) is varied and binding to the ‘recepter’ (i.e. merocyanine isomer of **SHL**) is measured, in this case as a fluorescence emission. The model uses the following equation,

$$Y = Bmax*X^h/(K_d^h + X^h)$$

Where Y is the relative fluorescence intensity at any given concentration of metal ion, Bmax is the maximum specific binding in the same units as Y (i.e. in this case the fluorescence at sensor saturation), X is the concentration of Li^+ and K_d is the Li^+ concentration needed to achieve the half-maximum binding at equilibrium, expressed in the same units as X. The parameter h is the Hill slope, and h = 1.0 when a monomer binds with no cooperativity to one site.

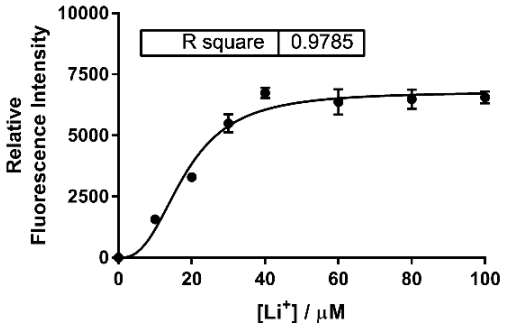


Figure S4. Metal ion titration curve of **SHL** (50 μM), in the presence of increasing concentrations of Li^+ (1-100 μM). Each data point is measured from the sensor emission maxima. **SHL**-Li^+ (K_d = 18 ± 2 μM).

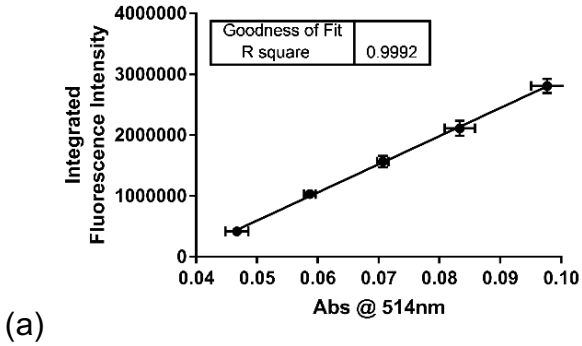
3. Determination of Quantum Yield (Φ) of **SHL**

A stock solution containing **SHL** (50 μM) and Li^+ (100 μM) was prepared in HPLC-grade acetonitrile. Solutions were prepared (in triplicate) on the same clear-bottom microplate tray from varying volumes of the combined **SHL**/Li^+ stock solution and HPLC-grade acetonitrile, until the volume of each replicate was 200 μL. The microplate tray was then incubated in the dark for 10 mins before reading. Absorbance (400-600 nm, 2 nm scanning resolution) and fluorescence emission spectra (535-800 nm, 5 nm scanning resolution, excitation 514 nm, 80 gain) were recorded at 25 °C using a BioTek Synergy H4 Plate Reader. These absorbance and fluorescence measurements was repeated for Rhodamine B (0, 1, 2, 3, 4, 5 μM), and a graph of integrated fluorescence *vs* absorbance at 514 nm (up to an approximate absorbance value of 0.1) was obtained. Quantum yield was calculated using the following equation:

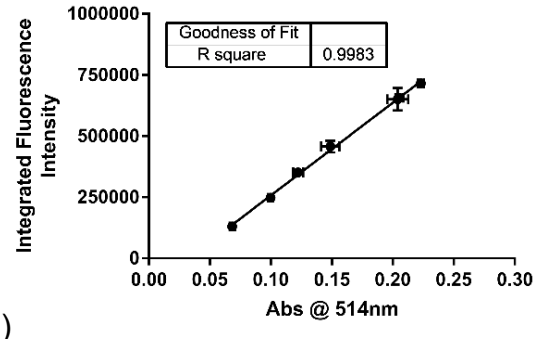
$$\Phi_x = \Phi_{ST} (Grad_x/Grad_{ST})(\eta^2_x/\eta^2_{ST})$$

Where subscripts ST and X denote standard (Rhodamine B) and the unknown (**SHL**), respectively. Φ is fluorescence quantum yield, Grad is the gradient from the plot of integrated fluorescence intensity vs absorbance and η is the refractive index of the

solvent ($\eta_{(water)}$ = 1.330, $\eta_{(acetonitrile)}$ = 1.344). The fluorescence quantum yield of Rhodamine B in water at λ_{ex} = 514 nm is 0.31, as reported in the literature.[3]



(a)



(b)

Figure S5. Integrated florescence spectra after excitation at 514 nm versus the absorbance at 514 nm for (a) Rhodamine B and (b) **SHL** -Li^+ (Φ = 0.03).

Supplemental Video

Time lapse recording of Li^+ 'hot spots' for 300 s duration with 3 s interval. Li^+ signal was increasing during the early stage of the video then dynamically turned on and off during the late stage of the video.