Title

Correction to Organohalogens Naturally Biosynthesized in Marine Environments and Produced As Disinfection Byproducts Alter Sarco/Endoplasmic Reticulum Ca^{2+} Dynamics.

Journal

Environmental science & technology, 52(13)

ISSN

0013-936X

Authors

Zheng, Jing
McKinnie, Shaun MK
El Gamal, Abrahim
et al.

Publication Date

2018-07-01

DOI

10.1021/acs.est.8b03174

Peer reviewed

Supporting Information

Organohalogens naturally biosynthesized in the marine environment and produced as disinfection by-products alter sarco/endoplasmic reticulum Ca^{2+} dynamics

Jing Zheng [1, 2], Shaun M. K. McKinnie [3], Abrahim El Gamal [3], Wei Feng [1], Yao Dong [1], Vinayak Agarwal [3#], Bill Fenical[3], Abdhesh Kumar[3], Zhengyu Cao[1, 2], Bradley S. Moore [3,4] and Isaac N. Pessah [1]

[1]Department of Molecular Biosciences, School of Veterinary Medicine, University of California, Davis, USA;

[2]Department of TCM Pharmacology, School of Traditional Chinese Medicines, China Pharmaceutical University, Nanjing, China

[3]Center for Oceans and Human Health, Scripps Institution of Oceanography, Unviersity of California, San Diego, USA

[4]Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California, San Diego, USA

[3#]current location: Parker H. Petit Institute for Bioengineering & Bioscience, Georgia Institute of Technology, Atlanta, USA

MATERIALS AND METHODS

RyR1-Enriched Microsomal Preparations

Junctional sarcoplasmic reticulum (JSR) was isolated from fast-twitch skeletal muscles of 1 year old male (2-2.5 kg) New Zealand White rabbits, as previously described[1]. Briefly, 250 g muscle was ground and homogenized by a blender in buffer containing: 300 mM sucrose, 5 mM imidazole, 100 μM phenylmethylsulfonyl fluoride (PMSF) and 5 μg/mL leupeptin, pH 7.4. The fraction enriched with JSR was then isolated through a discontinuous sucrose gradient. The JSR preparation was re-suspended in storage buffer containing 300 mM sucrose and 10 mM HEPES, pH 7.4 and flash frozen in liquid nitrogen followed by transferring to -80℃ until use. Protein concentrations were determined using BCA assay protocol (Fisher).

Equilibrium [^{3}H] Ryanodine Binding Assay

[^{3}H] ryanodine (56.6 Ci/mmole) binding was performed as previously described [2]. Briefly, 50 μg proteins and 1 nM [^{3}H] ryanodine were utilized in the presence of 1% vehicle or 0.05-20 μM selected HOCs, and incubated with 500 μL of buffer containing 250 mM KCl, 15 mM NaCl, 20 mM HEPES and 2 μM free Ca^{2+}, pH 7.4 for 3 hr at 37˚C. Non-specific binding was determined with the addition of 1000-fold excessive unlabeled ryanodine.

Measurement of Ca^{2+} Flux and Ca^{2+} -Induced Ca^{2+} Release (CICR)

Agilent 8453 UV-visible Spectroscopy System (Agilent Technologies, CA) equipped with a temperature controlled multi-cuvette stage was used to record the calcium flux into and out of skeletal muscle SR membrane vesicles by measuring the differential absorbance of metallochromic Ca^{2+} indicator Arsenazo III at 650-700 nm at 35℃. Measurement closely paralleled those previously reported with Antipyrylazo III[3]. Ca^{2+} loading and releasing were monitored in real-time at 1 Hz. The cuvettes containing 1.5 mL reaction mixture were gently and constantly stirred by magnetic bars. The assay solution contained 100 μg/mL of rabbit skeletal muscle JSR, coupling enzyme (2 mM MgATP, 10 mM phosphocreatine; 20 μg/mL creatine phosphokinase) and Arsenazo III buffer (KCl: 100 mM; Arsenazo III: 250 μM; MOPS: 20 mM; $Na_4P_2O_7$: 6 mM, pH7.4 KOH). Ca^{2+} loading of the SR vesicles was accomplished by 3-4 sequential additions of 45 nmole $CaCl_2$. 1-2 min after the final bolus of Ca^{2+} was accumulated into the vesicles, vehicle or test compounds was added, for the CICR assay, 90nmole Ca^{2+} was added after the final bolus of Ca^{2+} with vehicle/compound was accumulated into the vesicles to determine the sensitization of test compounds to CICR.

Measurement of Sarco/Endoplasmic Reticulum Ca^{2+} ATPase (SERCA1a) Activity

Activity of SERCA1a from skeletal JSR was measured using a coupled enzyme assay that monitors the rate of oxidation of NADH at 340 nm as described previously [4]. In brief, each

cuvette containing 1.5 mL of assay buffer consisted of 25 μg/mL JSR protein, 7 mM HEPES, 143 mM KCl, 7 mM $MgCl_2$, 0.085 mM EGTA, 0.048 mM free calcium, 0.43 mM sucrose, pH 7.0 (KOH), 1 mM phosphoenolpyruvate and 15 μL of coupling enzyme mixture (600-1,000 units/mL pyruvate kinase, 900-1400 units/mL lactic dehydrogenase). After the baselines were established in about 1-2 min, 0.3 mM NADH was added to each cuvette followed by addition of 0.4 mM Na_2ATP to initiate the oxidation reaction. The vehicle (0.2% DMSO), TG or test compounds was introduced before the establishment of baseline, among which 20 μM of TG (thapsigargin) was used as a negative control to determine SERCA-independent ATPase activity and verification of the assay system.

Data Analysis and Statistics

For [^{3}H]Ry binding assay, specific binding was achieved by subtracting the non-specific binding from the total binding as measured disintegrations per minute (DPM), concentration responses were tested in at least two different JSR preparations ($n \geq 2$) in triplicate or quintuplicates per experiment. Curves were fitted using Graph Pad 7.03 three-parameter nonlinear regression. For Ca^{2+} transport and SERCA activity assays, 4-6 repetitions were conducted from 2 independent JSR preparations. One-way ANOVA followed by *post hoc* Dunnett's test was used to analyze the data at 95% confidence intervals.

For figures whose potency values were determined by nonlinear regression with three-parameter equation using Prism Graph Pad 7.03. The equation used is followed as:

$$y = \frac{A_1 - A_2}{1 + 10^{\log EC_{50} - X}} + A_2$$

Where,

The y represents the Response, which increase as X increases; X represents the log of dose or concentration; A1=Maximum (Bound [3H]ryanodine/protein); A2= Minimum (Bound [3H]ryanodine/protein); logEC50: same log unit as X

For curves fitted with bell-shaped using Prism Graph Pad 7.03. The equation used is followed as:

$$\mathbf{Span1 = Plateau1 - Dip}$$

$$\mathbf{Span2 = Plateau2 - Dip}$$

$$\mathbf{Section1} = \frac{\mathbf{Span1}}{\mathbf{(1 + 10^{(LogEC50\_1 - X) * nH1})}}$$

$$\mathbf{Section2} = \frac{\mathbf{Span2}}{\mathbf{(1 + 10^{(X - LogEC50\_2) * nH2})}}$$

$$\mathbf{Y = Dip + Section1 + Section2}$$

Where,

X: Log of the dose or concentration;
Y: Response in user-specific units;
Plateau1 and plateau2: Initial and final plateau levels in same units as Y;
Dip: Plateau level between phase, in the same units as Y;
LogEC50_1 and LogEC50_2: Same units as X;
nH1 and nH2: Unitless slope factors.

Synthetic Procedures

Molecules **1**, **2**, and **7** were isolated from *Dysidea* sp. sponges as previously described [5]. Molecules **5**, **6**, and **8** were obtained commercially (Sigma-Aldrich). Hexahalogenated **3** and **4** were synthesized from reacting **1** with 2.5 equivalents of *N*-bromosuccinimide and *N*-iodosuccinimide, respectively, as has been previously described [5, 6]. **3**: ^{1}H-NMR (600 MHZ, CD_3OD): δ 7.81 (d, *J* = 2.3 Hz, 1H, 3'-H), 7.35 (dd, *J* = 8.8, 2.2 Hz, 1H, 5'-H), 6.42 (d, *J* = 8.8 Hz, 1H, 6'-H); HRMS (ESI) Calculated for $C_{12}H_3{}^{79}Br_6O_2$ 652.5239, found 652.5217 $(M-H)^-$. **4**: ^{1}H-NMR (600 MHZ, CD_3OD): δ 7.81 (d, *J* = 2.3, 1H, 3'-H), 7.34 (dd, *J* = 8.8, 2.1 Hz, 1H, 5'-H), 6.37 (d, *J* = 8.8 Hz, 1H, 6'-H); Calculated for $C_{12}H_3{}^{79}Br_4I_2O_2$ 748.4961, found 748.4997 $(M-H)^-$.

3,3',4,4',5,5'-hexabromo-1,1'-dimethyl-2,2'-bipyrrole (**9**):

Phenyliodine bis(trifluoroacetate) (PIFA) (211 mg, 0.49 mmol) and bromotrimethylsilane (TMSBr) (150 mg, 0.98 mmol) were quickly added to a stirred solution of pyrrole (100 mg, 1.49 mmol) in dichloromethane (15 mL) at -78 °C. The reaction mixture was then stirred for 1 h, while the reaction temperature was maintained below -40 °C. After the reaction completion, it was heated to room temperature and poured into saturated aqueous sodium bicarbonate solution (20 mL) and then stirred for 10 min. The product was extracted with dichloromethane. The combined organic layers were dried with anhydrous sodium sulfate, concentrated under vacuum and purified by column chromatography (SiO_2 (neutral)/*n*-hexane-ethyl acetate) to give the pure 2,2'-bipyrrole (52 mg, 74%) as an amorphous solid. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 8.25 (bs, 2H), 6.79-6.78 (m, 2H), 6.27-6.25 (m, 2H), 6.23-6.22 (m, 2H), MS (ESI), m/z: 132 (M+). This material was identical by direct comparison with the previously reported material [7] by NMR and mass spectroscopy.

To a solution of above synthesized 2,2'-bipyrrole (50 mg, 0.38 mmol) dissolved in anhydrous DMF (10 mL), 60% mineral oil dispersion of sodium hydride (NaH) (45 mg, 1.89 mmol) was added at 0 ^{o}C and the reaction mixture was stirred for 30 min at room temperature, then iodomethane (118 mg, 0.83 mmol) was added at 0 ^{o}C. The reaction mixture was stirred for 1 h, and then poured onto crushed ice and extracted with dichloromethane (20 mL). The dichloromethane layers were dried (Na_2SO_4) and evaporated under reduced pressure. The residue was purified by chromatography using hexane/ethyl acetate (98:2) as an eluent, giving 1,1'-dimethyl-2,2'-bipyrrole (56 mg, 92%) as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$): δ 6.74-6.73 (m, 2H), 6.21 -6.20 (m, 2H), 6.18–6.17 (m, 2H), 3.53 (s, 2 × CH_3); ^{13}C NMR ($CDCl_3$): δ 125.3, 122.8, 110.7, 107.6, 34.6; MS (ESI), m/z: 160 (M+). This material was identical by direct comparison with the previously synthesized compound [8] by NMR and mass spectroscopy.

A solution of *N*-bromosuccinimide (389 mg, 2.19 mmol) in anhydrous MeCN (10 mL) was added to a stirred solution of above synthesized 1,1'-dimethyl-2,2'-bipyrrole (50 mg, 0.315 mmol) in anhydrous MeCN (15 mL) at –40 °C. The resulting green solution was warmed slowly to room temperature and stirred for 15 h. Removal of the solvent in vacuum and purification by chromatography (Ethyl acetate:hexane, 1:99) on silica gel provided compound **3** (178 mg, 90%) as a pale yellow solid. UV (MeOH): λ = 256 nm. IR (KBr): 2990, 2935, 1485, 1437, 1320, 1087, 970 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 3.47 (s, 6H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 122.4, 107.0, 103.5, 101.4, 35.6; MS (ESI): *m*/*z* (%) = 640 (7), 638 (44), 636 (81), 634 (100), 632 (84), 630 (47), 628 (7) [M+], 516 (10), 514 (23), 512 (24), 510 (11), 476 (29), 474 (46), 472 (31), 395 (15), 393 (15). This material was identical by direct comparison with the previously synthesized compound [8] by NMR and mass spectroscopy.

3,3',4,4',5,5'-hexachloro-1,1'-dimethyl-2,2'-bipyrrole (**10**):

A solution of *N*-chlorosuccinimide (260 mg, 1.94 mmol) in anhydrous MeCN (10 mL) was added to a stirred solution of above synthesized 1,1'-dimethyl-2,2'-bipyrrole (50 mg, 0.312 mmol) in anhydrous MeCN (15 mL) at –40 °C. The resulting dark black solution was warmed slowly to room temperature and stirred for 2 h. Removal of the solvent in vacuum and purification by chromatography (Ethyl acetate:hexane, 1:99) on silica gel provided compound **4** (95 mg, 83%) as colorless crystals. UV (MeOH): λ = 230, 260 nm. IR (KBr): 2921, 2845, 1622, 1471, 1320, 1093, 977 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 3.43 (s, 6H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 116.8, 116.6, 113.8, 108.6, 33.0; MS (ESI): *m*/*z* (%) = 370 (30), 368 (78), 366 (100), 364 (47) [M+], 331 (11), 296 (16), 294 (17), 292 (16), 290 (26), 288 (16). This material was identical by direct comparison with the previously synthesized compound [8] by NMR and mass spectroscopy.

2,3,3',4,4',5,5'-heptabromo-1'-methyl-1'*H*-1,2'-bipyrrole (**11**):

N-chlorosuccinimide (330 mg, 2.45 mmol) and sodium bicarbonate (414 mg, 4.92 mmol) were added in the solution of 1-methylpyrrole (200 mg, 2.45 mmol) in chloroform (20 mL) and the reaction mixture was stirred under dark conditions at room temperature for 8 h. After the reaction completion, the reaction mass was washed with water. The chloroform was removed under reduced pressure and the product was purified over silica gel using dichloromethane/methanol as eluent, yielding 1-(1-methyl-1*H*-pyrrol-2-yl)pyrrolidine-2,5-dione (280 mg, 64%) as a white solid. ^{1}H NMR (500 MHz, CDCl3): δ 6.68-6.67 (m, 1H), 6.21-6.20 (m, 1H), 6.11-6.10 (m, 1H), 3.42 (s, CH_3), 2.93 (s, 2 x CH_2). MS (ESI), m/z: 179 $(M+H)^+$. This material was identical by direct comparison with the previously synthesized compound [9] by NMR and mass spectroscopy.

N-Bromosuccinimide (900 mg, 5.06 mmol) was added to a stirred solution of the

above synthesized compound 1-(1-methyl-1H-pyrrol-2-yl)pyrrolidine-2,5-dione (250 mg, 1.40 mmol) in anhydrous THF (15 mL) at –78 °C. The solution was warmed slowly to room temperature and stirred for 3 h. The solvent was evaporated at reduced pressure, and the residue was purified on silica gel, eluting with hexane/DCM to give 500 mg (86 %) of compound 1-(3,4,5-tribromo-1-methyl-1*H*-pyrrol-2-yl)pyrrolidine-2,5-dione. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 3.44 (s, CH_3), 3.01-2.98 (m, 2 x CH_2). MS (ESI), m/z (%): 414 (100), 412 (97), 412 (34) (M^+).

The above synthesized compound 1-(3,4,5-tribromo-1-methyl-1H-pyrrol-2-yl)pyrrolidine-2,5-dione (100 mg, 0.24 mmol), titanium (iv) isopropoxide (6.8 mg, 10 mol%) and triethoxysilane (198 mg, 1.20 mmol) were dissolved in dry benzene under an inert atmosphere and the reaction mixture was heated at 65 ^{o}C for 4 h. The reaction mass was purified directly over silica gel using pentane as mobile phase and gave compound 3',4',5'-tribromo-1'-methyl-1'*H*-1,2'-bipyrrole (55 mg, 60 %) as an oil. ^{1}H NMR (500 MHz, CDCl3): δ 6.71 (t, *J* = 5.0 Hz, 2H), 6.37 (t, *J* = 5.0 Hz, 1H), 3.34 (s, CH_3).

A solution of bromine (157 mg, 0.98 mmol) in chloroform (1 mL) was added to a solution of above synthesized compound 3',4',5'-tribromo-1'-methyl-1'*H*-1,2'-bipyrrole (50 mg, 0.13 mmol) in chloroform (10 mL) at room temperature and stirred for 4 h. The reaction mass was washed with water and extracted with chloroform. After the solution was dried over Na_2SO4 and concentrated, the residue was chromatographed on silica gel, eluting with hexane to give 60 mg (66 %) of **11** as a white solid. UV (MeOH): λ = 232. IR (KBr): 2941, 2790, 1567, 1506, 1567, 1444, 1327, 1286, 1093, 970 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.37 (s, CH_3). ^{13}C NMR (125 MHz, $CDCl_3$): δ 123.9, 106.6 (2 x C), 105.4, 104.5 (2 x C), 100.9, 100.5, 34.4; MS (EI): *m/z* (%) 699 ([M^+]), 618 (100), 538, 459, 378, 298, 248, 222, 208. This material was identical by direct comparison with the previously synthesized compound [10] by NMR and mass spectroscopy.

2,3,3',4,4',5,5'-heptachloro-1'-methyl-1'*H*-1,2'-bipyrrole (**12**):

1-(1-methyl-1*H*-pyrrol-2-yl)pyrrolidine-2,5-dione (200 mg, 1.12 mmol) (as synthesized in synthesis of compound **11**), titanium (iv) isopropoxide (32 mg, 10 mol%) and triethoxysilane (922 mg, 5.62 mmol) were dissolved in dry benzene under an inert atmosphere and the reaction mixture was heated at 65 ^{o}C for 12 h. The reaction mass was purified directly over silica gel using pentane as mobile phase and gave 1'-methyl-1'*H*-1,2'-bipyrrole (70 mg, 43 %) as an oil. ^{1}H NMR (500 MHz, $CDCl_3$): δ 6.67 (t, *J* = 5.0 Hz, 2H), 6.58 (t, *J* = 5.0 Hz, 1H), 6.30 (t, *J* = 5.0 Hz, 2H), 6.14-6.11 (m, 2H), 3.38 (s, CH_3). MS, m/z: 146 (M+).

A solution of 1.0 M sulfuryl chloride (2.74 mL, 2.74 mmol) was added slowly to the above synthesized compound 1'-methyl-1'*H*-1,2'-bipyrrole (40 mg, 69 mmol) in 10 mL of dichloromethane and was stirred for 3 h, poured into water, and extracted with

dichloromethane. The solvent was removed at reduced pressure and the residue was chromatographed on silica gel, eluting with hexane to give compound **12** (23 mg, 17 %) as a white solid. UV (MeOH): λ = 220. IR (KBr): 2928, 2845, 1588, 1540, 1348, 1293, 1018 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ 3.34 (s, CH_3). MS, m/z: 386 ([M^+]), 371, 351 (100), 336, 316, 281, 266, 244, 208. This material was identical by direct comparison with the previously synthesized compound [11] by NMR and mass spectroscopy.

2,3-dichloroindole (**13**):

Compounds **13** & **14** were synthesized following a literature protocol [12]. To an ether solution of 0.29 g (2.5 mM) of indole at 0 °C, 0.4 mL (5 mM) sulfuryl chloride was added dropwise. The mixture was stirred at 0 ºC for 5 h. It was then washed successively with cold $NaHCO_3$ and NaCl solutions, dried over Na_2SO_4, and the ether was removed at reduced pressure. The residue was chromatographed on silica gel, eluting with hexane to give **13** (380 mg, 78%) as a colorless compound. Recrystallization from hexane gave needles. IR (KBr): 3445, 3032, 1408, 1350, 1330 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ 12.4 (s, 1H), 7.43-7.34 (m, 2H), 7.22-7.12 (m, 2H).

2,3-dibromoindole (**14**):

To a pyridine solution of 500 mg (17 mM) of indole cooled in an ice bath was added dropwise and with stirring a pyridine solution of 1.73 g (18 mM) of pyridinium bromide perbromide. After complete addition, ice water was added, and the mixture was extracted with ether. The ether extracts were washed successively with ice cold 6 N HC1, 5% $NaHCO_3$, and NaCl solutions. The ether layer was dried (Na_2SO_4) and evaporated to give 3-bromoindole (650 mg, 80%). Recrystallization from hexane gave pale pink crystals. IR (KBr): 3455, 3030, 1408, 1325, 1290 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 11.47 (s, 1H) , 7.53 (s, 1H), 7.52-7.38 (m, 2H), 7.17-7.08 (m, 2H).

To 392 mg (2 mM) of above synthesized 3-bromoindole dissolved in 10 ml of CH_2C1_2 at 0 °C was added dropwise 320 mg (2 mM) of bromine in CH_2C1_2. After complete addition, the mixture was washed with cold aqueous $NaHCO_3$ and saturated NaCl solutions and dried over Na_2SO_4. Removal of the solvent gave a quantitative yield. Recrystallization from hexane gave compound **14 (**430 mg 72%). IR (KBr): 3452, 3036, 1405, 1340, 1315, cm-l; ^1H NMR (500 MHz, $CDCl_3$): δ 12.4 (s, 1H), 7.41-7.32 (m, 2H), 7.20-7.13 (m, 2H).

2-bromo-4-(2,4-dibromophenoxy)phenol (**15**):

This compound was synthesized following a literature protocol [13]. 4-Fluorobenzaldehyde (500 mg, 4.03 mmol) and 2,4-dibromophenol (1012 mg, 4.03 mmol) were dissolved in 20 mL of dimethylacetamide (DMAC), then anhydrous sodium carbonate (427 mg, 4.03 mmol) was added to the system and heated at reflux for 8 h. Water (200 mL) was added to the cooled

reaction mixture, and the reaction mixture was extracted with ethyl acetate (3 x 200 mL). The organic layer was separated and washed with aqueous NaOH (1M, 2 x 60 mL) and water (2 x 60 mL). The organic layer was dried with anhydrous Na_2SO_4 and the solvent was removed under vacuum, and the residue was purified by silica gel chromatography to give intermediate 4-(2,4-dibromophenoxy)benzaldehyde (1119 mg, 78%) as an oil. ^{1}H NMR (500 MHz, $CDCl_3$): δ 9.94 (s, 1H), 7.88-7.83 (m, 3H), 7.50-7.48 (m, 1H), 7.27-7.0 (m, 3H).

The above synthesized compound 4-(2,4-dibromophenoxy)benzaldehyde (200 mg, 0.56 mmol) was dissolved in 20 mL of CH_2Cl_2 and KH_2PO_4 (1528 mg, 11.24 mmol) was added. The reaction mixture was cooled to 0 °C, a solution of trifluoroperacetic acid (TFPA) was added dropwise over 0.5 h, and the mixture was stirred at 0 °C for 1 h. The TFPA solution was prepared by dropwise addition of trifluoroacetic anhydride (885 mg, 4.2 mmol) to aqueous hydrogen peroxide (30%, 28.65 mg, 0.84 mmol) in CH_2Cl_2 (4 mL) at 0 °C, and the mixture was stirred at 0 °C for 1 h. The reaction mixture was diluted with brine (25 mL) and aqueous $NaHSO_3$ (20%, 15 mL) at 0 °C. The phases were separated and the aqueous layer was extracted once with an equal volume of CH_2Cl_2. The combined organic layers were washed once with an equal volume of saturated $NaHCO_3$ and water. The crude formate ester was dissolved in MeOH (20 mL) containing one drop of concentrated hydrochloric acid. The solution was stirred for 15 h and then concentrated in vacuo. The residue was purified by silica gel chromatography to give compound 4-(2,4-dibromophenoxy)phenol as a white amorphous solid (183 mg, 95%).^{1}H NMR (500 MHz, $CDCl_3$): δ 7.76 (d, J = 5.0 Hz, 1H), 7.32 (d, J = 5.0 Hz, 1H), 6.92-6.90 (m, 2H), 6.85-6.83 (m, 2H), 6.71 (d, J = 5.0 Hz, 1H).

To a solution of above synthesized compound 4-(2,4-dibromophenoxy)phenol (50 mg, 0.145 mmol) dissolved in acetic acid (4 mL), a solution of bromine (23 mg, 0.145 mmol) in acetic acid (0.5 mL), was slowly added at room temperature and the reaction mixture was stirred for 1 h. The progress of the reaction was monitored by TLC and on completion of the reaction, 10% aqueous sodium metabisulfite solution (5 mL) was poured into the reaction mixture. The product was extracted with ethyl acetate (2 × 5 mL), and the organic layer was collected, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The resulting residue was purified by chromatography (ethyl acetate:hexane, 1:99) on silica gel provided compound **15** (55 mg, 90%) as an oil. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.78 (d, J = 5.0 Hz, 1H), 7.37 (dd, J = 10.0, 5.0, 1H), 7.14 (d, J = 5.0 Hz, 1H), 7.02 (d, J = 10.0 Hz, 1H), 6.91 (dd, J = 10.0, 5.0, 1H), 6.76 (d, J = 5.0 Hz, 1H), 5.37 (s, 1H). MS, m/z: 421 (M^-).

2,4-dibromo-1-(3-bromo-4-methoxyphenoxy)benzene (**17**):

To a solution of compound **15** (50 mg, 0.118 mmol) dissolved in anhydrous DMF (5 mL), 60% mineral oil dispersion of sodium hydride (NaH) (5 mg, 0.118 mmol) was added at 0 ^{o}C and the reaction mixture was stirred for 30 min at room temperature. Next iodomethane (17 mg, 0.118 mmol) was added at 0 ^{o}C. The reaction mixture was stirred for 1 h, and then

poured onto crushed ice and extracted with ethyl acetate (20 mL). The ethyl acetate layers were dried (Na_2SO_4) and the solvent removed under reduced pressure. The residue was purified by silica chromatography using *n*-hexane as an eluent, to give compound **17** (48 mg, 93%) as white amorphous solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.77 (d, *J* = 5.0 Hz, 1H), 7.37 (dd, *J* = 10.0, 5.0, 1H), 7.23 (d, *J* = 5.0 Hz, 1H), 6.94 (dd, *J* = 10.0, 5.0, 1H), 6.89 (d, *J* = 5.0 Hz, 1H), 6.75 (d, J = 10.0 Hz, 1H), 3.90 (s, 3H).

pentabromopseudilin (**16**):

To a solution of 2-bromoanisole (500 mg, 2.67 mmol) and *N*-Boc-2-pyrroleboronic acid (564 mg, 2.67 mmol) in 20 mL of mixture of toluene and methanol (5:1), tetrakis(triphenylphosphine)palladium (62 mg, 0.053 mmol) and an aqueous 2 M solution of sodium carbonate (570 mg 5.38 mmol) were added, and the mixture was stirred. The mixture was then warmed at 80 ^{o}C for 6 h. The mixture was diluted with water (50 mL) and extracted with ethyl acetate (2 × 50 mL). The combined organic fractions were washed with brine (50 mL), dried over sodium sulfate, and concentrated under vacuum. The crude product was purified by column chromatography over silica gel by using hexane/ ethyl acetate (9:1) as an eluent to give compound *tert*-butyl 2-(2-methoxyphenyl)-1*H*-pyrrole-1-carboxylate (482 mg, 66%) as an oil. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.38-7.36 (m, 1H), 7.34-7.31 (m, 1H), 7.29-7.27 (m, 1H), 7.0-6.96 (m, 1H), 6.89-6.87 (m, 1H), 6.27-6.26 (m, 1H), 6.17-6.16 (m, 1H), 3.77 (s, 3H), 1.34 (s, 9H).

To a solution of the above synthesized compound *tert*-butyl 2-(2-methoxyphenyl)-1*H*-pyrrole-1-carboxylate (200 mg, 0.73 mmol) in THF (10 mL), a 4.6 N solution of CH_3ONa (120 mg, 2.20 mmol) in methanol was slowly added, and the reaction mixture was heated at 40 ^{o}C for 2h. After the reaction completion, the reaction mass was suspended in water (25 mL) and extracted with ethyl acetate (2 × 30 mL). The combined organic layers were dried on Na_2SO_4, filtered, and concentrated to dryness under reduced pressure. The crude product purified by column chromatography over silica gel by using hexane/ethyl acetate as an eluent to give compound 2-(2-methoxyphenyl)-1*H*-pyrrole (83 mg, 66%) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 9.86 (bs, 1H), 7.70-7.68 (m, 1H), 7.01-7.0 (m, 1H), 6.98-6.97 (m, 1H), 6.88 (s, 1H), 6.64 (s, 1H), 6.31 (s, 1H), 3.98 (s, 3H).

To a stirred solution of above synthesized compound 2-(2-methoxyphenyl)-1*H*-pyrrole (120 mg, 0.693 mmol) in *N*-methylpyrrolidone (NMP, 10 mL), sodium sulfide (323 mg, 4.14 mmol) was added at room temperature and the mixture was heated at 160 °C for 2.5 h. Upon cooling, the mixture was poured into a 1 M HCl solution and thoroughly extracted with ethyl acetate. The combined organic phases were washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure. The crude product was purified by column chromatography over silica gel by using hexane/ethyl acetate to give compound 2-(2-hydroxyphenyl)-1*H*-pyrrole as a dark blue-grey solid. ^{1}H NMR (500

MHz, $CDCl_3$): δ 9.45 (bs, 1H), 7.56-7.54 (m, 1H), 7.12-7.09 (m, 1H), 6.99-6.98 (m, 1H), 6.88-6.87 (m, 1H), 6.59 (s, 1H), 6.33 (s, 1H). The spectroscopic data obtained for this compound were consistent with those reported in the literature [8].

To a stirred solution of above synthesized compound 2-(2-hydroxyphenyl)-1*H*-pyrrole (87 mg, 546 μmol) in absolute ethanol (5 mL), pyridinium tribromide (1.05 g, 3.29 mmol) was added at room temperature and the mixture was stirred at room temperature for 3 d. The solvent was evaporated. Purification of the residue by chromatography on a silica gel column afforded **16** (63.7 mg, 46%) as a purple solid.^{1}H NMR (500 MHz, $CDCl_3$): δ 9.51 (s, 1H), 8.11 (s, 1H), 7.58 (s, 1H), 6.06 (s, 1H). The spectroscopic data obtained for this compound were consistent with those reported in the literature [8].

2,6-dibromo-4-(2,4,6-tribromophenoxy)phenol (**18**):

This compound was synthesized following a literature protocol [14 15] .A 50 mL flask is charged with a magnetic stirrer bar, 2,4,6-tribromophenol (760 mg, 1.82 mmol), 4-(tert-butyldimethylsilyloxy)phenylboronic acid (458 mg, 1.82 mmol), anhydrous $Cu(OAc)_2$ (330 mg, 1.82 mmol), triethylamine (918 mg, 9.1 mmol), powdered 4Å molecular sieves and dichloromethane (20 mL) at room temperature and the mixture was stirred for 6 h. The progress of the reaction was monitored by TLC and on completion of the reaction, the reaction mixture was filtered and concentrated under reduced pressure to give the crude product. The crude product was purified by column chromatography (hexane) on silica gel to afford the compound *tert*-butyldimethyl(4-(2,4,6-tribromophenoxy)phenoxy)silane (320 mg, 33%) as an oil. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.74 (s, 2H), 6.76-6.74 (m, 2H), 6.69-6.66 (m, 2H), 0.97 (s, 9H), 0.18 (s, 6H).

To a magnetically stirred solution of above synthesized compound *tert*-butyldimethyl(4-(2,4,6-tribromophenoxy)phenoxy)silane (200 mg, 0.37 mmol) in CH_3CN (3.4 mL) and H_2O (0.18 mL) was added 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (56 mg, 0.37 mmol). After the starting material disappeared (TLC), saturated aq NH_4Cl solution (5 mL) was poured into the reaction mixture. The mixture was extracted with DCM (2 × 5 mL), and the organic layer was collected, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The resulting residue was purified by chromatography (ethyl acetate:hexane, 2:98) on silica gel provided compound 4-(2,4,6-tribromophenoxy)phenol (150 mg, 95%) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.75 (s, 2H), 6.78-6.75 (m, 2H), 6.71-6.69 (m, 2H).

To a solution of above synthesized compound 4-(2,4,6-tribromophenoxy)phenol (40 mg, 0.095 mmol) dissolved in acetic acid (4 mL), a solution of bromine (30 mg, 0.19 mmol) in acetic acid (1 mL), was slowly added at room temperature and the reaction mixture was stirred for 1 h. The progress of the reaction was monitored by TLC and on completion of the reaction, 10% aqueous sodium metabisulfite solution (5 mL) was poured into the reaction

mixture. The product was extracted with ethyl acetate (2 × 5 mL), and the organic layer was collected, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The resulting residue was purified by chromatography (ethyl acetate:hexane, 1:99) on silica gel provided compound **18** (50 mg, 91%) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.77 (s, 2H), 6.94 (s, 2H), 5.64 (s, 1H).

1,3,5-tribromo-2-(3,5-dibromo-4-methoxyphenoxy)benzene (**19**):
To a solution of compound **18** (40 mg, 0.069 mmol) dissolved in acetone (4 mL), anhydrous K_2CO_3 (19 mg, 0.14 mmol) and iodomethane (10 mg, 0.069 mmol) were added at room temperature and the reaction mixture was stirred for 1 h. The progress of the reaction was monitored by TLC and on completion of the reaction, the reaction mixture was filtered and concentrated under reduced pressure to give the crude product. The crude product was purified by column chromatography (hexane) on silica gel to afford compound **19** (39 mg, 95%) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.78 (s, 2H), 6.96 (s, 2H), 3.87 (s, 3H). MS (ESI): *m*/*z* (%) = 593 (100), 595 (97), 591 (51).

1,5-dibromo-2-(2,4-dibromophenoxy)-3-methoxybenzene (**20**):
To a solution of compound **1** (isolated from *Dysidea* sp. sponges as described above) (5 mg, 0.01 mmol) dissolved in acetone (1 mL), anhydrous K_2CO_3 (6 mg, 0.02 mmol) and iodomethane (2 mg, 0.015 mmol) were added at 30 ^{o}C and the reaction mixture was stirred for 1 h. The progress of the reaction was monitored by TLC and on completion of the reaction, the reaction mixture was filtered and concentrated under reduced pressure to give the crude product. The crude product was purified by column chromatography (hexane) on silica gel to afford compound **20** (5 mg, 85%) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.75 (d, *J* = 2.5 Hz, 1H), 7.41 (d, *J* = 2.5 Hz, 1H), 7.24 (dd, *J* = 10.0, 5.0 Hz, 1H), 6.32 (d, *J* = 10.0 Hz, 1H), 3.77 (s, 3H).

1,5-dibromo-3-(2,4-dibromophenoxy)-2-methoxybenzene (**21**):
To a solution of compound **2** (isolated from *Dysidea* sp. sponges as described above) (10 mg, 0.02 mmol) dissolved in acetone (1 mL), anhydrous K_2CO_3 (6 mg, 0.04 mmol) and iodomethane (3 mg, 0.02 mmol) were added at 30 ^{o}C and the reaction mixture was stirred for 1 h. The progress of the reaction was monitored by TLC and on completion of the reaction, the reaction mixture was filtered and concentrated under reduced pressure to give the crude product. The crude product was purified by column chromatography (hexane) on silica gel to afford compound **21** (10 mg, 86%) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.80 (d, *J* = 2.5 Hz, 1H), 7.51 (d, *J* = 2.5 Hz, 1H), 7.40 (dd, *J* = 9.0, 2.5 Hz, 1H), 6.93 (d, *J* = 2.5 Hz, 1H), 6.76 (d, *J* = 9 Hz, 1H), 3.91 (s, 3H).

1,2,3,4-tetrabromo-5-(2,4-dibromophenoxy)-6-methoxybenzene (**22**):

To a solution of compound **3** (10 mg, 0.015 mmol) dissolved in acetone (1 mL), anhydrous K_2CO_3 (4 mg, 0.03 mmol) and iodomethane (3 mg, 0.015 mmol) were added at 30 ^oC and the reaction mixture was stirred for 1 h. The progress of the reaction was monitored by TLC and on completion of the reaction, the reaction mixture was filtered and concentrated under reduced pressure to give the crude product. The crude product was purified by column chromatography (hexane) on silica gel to afford compound **22** (10 mg, 98%) as a white solid. ^1H NMR (500 MHz, $CDCl_3$): δ 7.80 (d, J = 2.0 Hz, 1H), 7.29 (dd, *J* = 9.0, 2.5 Hz, 1H), 6.34 (d, *J* = 9 Hz, 1H), 3.88 (s, 3H).

1,3-dibromo-4-(2,4-dibromophenoxy)-2,6-diiodo-5-methoxybenzene (**23**):

To a solution of compound **4** (10 mg, 0.013 mmol) dissolved in acetone (1 mL), anhydrous K_2CO_3 (3 mg, 0.026 mmol) and iodomethane (3 mg, 0.013 mmol) were added at 30 ^oC and the reaction mixture was stirred for 1 h. The progress of the reaction was monitored by TLC and on completion of the reaction, the reaction mixture was filtered and concentrated under reduced pressure to give the crude product. The crude product was purified by column chromatography (hexane) on silica gel to afford compound **23** (9 mg, 92%) as a white solid. ^1H NMR (500 MHz, $CDCl_3$): δ 7.77 (d, *J* = 2.3 Hz, 1H), 7.26 (dd, *J* = 8.8, 2.1 Hz, 1H,), 6.29 (d, *J* = 8.8 Hz, 1H), 3.84 (s, 3H).

4,5-dibromopyrrole-2-carboxylic acid (**29**)

This compound was synthesized following a modified literature protocol [16]. Briefly, a suspension of pyrrole-2-carboxylic acid (1.00 g, 9.00 mol) in anhydrous chloroform (10 mL) and glacial acetic acid (2 mL) had bromine (0.97 mL, 18.9 mmol) added over 5 minutes. The mixture was heated to 50 °C for 5 h, then cooled to room temperature and diluted with water (30 mL) and chloroform (30 mL). The phases were separated and the organic phase was washed with water (2 x 30 mL). Brominated carboxylic acid products were extracted with an aqueous solution of 10% K_2CO_3 (30 mL), washed with chloroform (2 x 30 mL), and then acidified to pH 3 with 4N HCl. The precipitate was collected by vacuum filtration and rinsed with aqueous 0.1N HCl. Further purification by semi-preparative RP-HPLC (Phenomenex Luna 5u C18(2) 100 A, 250 x 10.00 mm, was done to separate di- and tribromopyrrole-2-carboxylic acids using a gradient of 55 – 100% B over 15 minutes (A = 0.1% aqueous trifluoroacetic acid, B = 0.1% trifluoroacetic acid in acetonitrile). Pooled fractions were concentrated *in vacuo,* generating **29** as an off white solid (0.157 g, 0.58 mmol, 7%). R_f 0.13 (hexanes/EtOAc 4:1); ^1H-NMR (600 MHz, d_6-DMSO): δ 13.30 (br s, 2H, COO*H,* N*H*); ^{13}C-NMR (150 MHz, d_6-DMSO): δ 160.3, 125.0, 116.9, 106.8, 98.8; HRMS (ESI) Calculated for $C_5H_2{}^{79}Br_2NO_2$ 265.8458, found 265.8460 $(M-H)^-$.

3,4,5-tribromopyrrole-2-carboxylic acid (**30**)

Tribrominated **30** was synthesized and purified in the same manner as **29**, with the only difference involving the addition of 2.5 molar equivalents of bromine (1.15 mL, 22.5 mmol) over 5 minutes. Compound **30** was isolated as an off white solid (0.328 g, 0.94 mmol, 11%). R_f 0.15 (hexanes/EtOAc 4:1); ^{1}H-NMR (600 MHz, d_6-DMSO): δ 12.99 (s, 1H, COO*H*), 12.81 (s, 1H, N*H*), 6.84 (s, 1H, C3-*H*); ^{13}C-NMR (150 MHz, d_6-DMSO): δ 159.5, 122.6, 106.8, 104.3, 103.4; HRMS (ESI) Calculated for $C_5H^{79}Br_3NO_2$ 343.7563, found 343.7566 $(M-H)^-$.

tetrabromopyrrole (**31**)

This compound was synthesized following a modified literature protocol [17]. A solution of recrystallized *N*-bromosuccinimide (4.00 g, 22.47 mmol) in dry THF (60 mL) was added over 45 minutes to a solution of freshly distilled pyrrole (0.39 mL, 5.62 mmol) in dry THF (20 mL) at -78 °C under an Ar atmosphere. The reaction mixture was warmed to -10 °C, stirred for 2 h, and concentrated *in vacuo*. The reaction mixture was resuspended in water (25 mL) and ethyl acetate (25 mL), the phases were separated, and the organic layer was further washed with water (25 mL), brine (25 mL), and dried over Na_2SO_4. Following filtration and concentration *in vacuo*, the crude reaction mixture was purified by silica flash chromatography (20:1 hexanes:EtOAc). Compound **31** was isolated as a light grey solid (1.41 g, 3.68 mmol, 65%). R_f 0.21 (hexanes/EtOAc 9:1); ^{1}H-NMR (600 MHz, d_6-DMSO): δ 13.17 (s, 1H, N*H*); ^{13}C-NMR (150 MHz, d_6-DMSO): δ 100.4, 100.0; HRMS (ESI) Calculated for $C_4{}^{79}Br_4N$ 377.6770, found 377.6769 $(M-H)^-$.

ethyl 4,5-dibromopyrrole-2-carboxylate (**32**)

The synthesis of **32** was adapted from a literature protocol [18]. A solution of bromine (71 μL, 1.38 mmol) in glacial acetic acid (5 mL) was added over 10 minutes to a solution of ethyl 4-bromopyrrole-2-carboxylate (0.301 g, 1.38 mmol) in glacial acetic acid (10 mL). This was stirred at room temperature for 6.5 h, and then diluted with saturated aqueous $NaHCO_3$ until a neutral pH (~35 mL). Organic compounds were extracted using EtOAc (3 x 25 mL), pooled organic layers were washed with brine (25 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude reaction mixture was purified by silica flash chromatography (4:1 hexanes:EtOAc), yielding compound **32** as a light white powder (0.363 g, 1.22 mmol, 89%). R_f 0.70 (hexanes/EtOAc 2:1); ^{1}H-NMR (600 MHz, d_6-DMSO): δ 13.14 (s, 1H, N*H*), 6.89 (s, 1H, C3-*H*), 4.23 (q, J = 7.1 Hz, 2H, OCH_2), 1.26 (t, J = 7.1 Hz, 3H, -CH_3); ^{13}C-NMR (150 MHz, d_6-DMSO): δ 158.9, 124.0, 117.1, 107.6, 99.0, 60.3, 14.3; HRMS (ESI) Calculated for $C_7H_6{}^{79}Br_2NO_2$ 293.8771, found 293.8773 $(M-H)^-$.

ethyl 3,4,5-tribromopyrrole-2-carboxylate (**33**)

Tribrominated **33** was synthesized and purified in the same manner as **32**, with the only difference involving the addition of 2.0 molar equivalents of bromine (141 μL, 2.76 mmol) over 10 minutes. Compound **33** was isolated as a white solid (0.486 g, 1.29 mmol, 94%). R_f 0.65 (hexanes/EtOAc 2:1); ^{1}H-NMR (600 MHz, d_6-DMSO): δ 13.51 (s, 1H, N*H*), 4.27 (q, J = 7.1 Hz, 2H, OCH_2), 1.29 (t, J = 7.1 Hz, 3H, -CH_3); ^{13}C-NMR (150 MHz, d_6-DMSO): δ 158.0, 121.7, 107.5, 104.8, 103.7, 60.6, 14.2; HRMS (ESI) Calculated for $C_7H_5{}^{79}Br_3NO_2$ 371.7876, found 371.7878 $(M-H)^-$.

2,3,5-tribromopyrrole (**34**)

Tribrominated **34** was prepared in the same manner as **31**, with the only difference involving the addition of 3.0 molar equivalents of *N*-bromosuccinimide (3.00 g, 16.84 mmol). The workup was adapted from two literature references [17, 19] to maximize the stability of **34**. Following the 2 h reaction time at -10 °C, the reaction was diluted with water (30 mL) and EtOAc (30 mL) and maintained under an Ar atmosphere as best as possible during work up. The phases were separated and the organic layer was washed with water (30 mL), brine (30 mL), dried over Na_2SO_4 and filtered. To maximize the stability of **34**, 0.5 molar equivalents of tri-*n*-butylamine (0.67 mL, 2.81 mmol,) was added to the organic phase and was concentrated to remove ~90% of the solvent *in vacuo*. Dry DMSO (3 mL) was added to the flask and further concentrated to remove additional EtOAc. While concentrating, Ar gas was used to flush the system afterwards and care was taken to not completely dry the product, both of which contribute to rapid decomposition. Characterization of the dark red DMSO solution of **34** indicated that the correct molecule was prepared in >90% purity and in 71% yield following NMR standardization to tri-*n*-butylamine. No further purification was attempted due to product decomposition. ^{1}H-NMR (600 MHz, d_6-DMSO): δ 6.29 (s, 1H, C3-*H*); ^{13}C-NMR (150 MHz, d_6-DMSO): δ 113.3, 100.7, 99.3, 98.1; HRMS (ESI) Calculated for $C_4H^{79}Br_3N$ 299.7665, found 299.7667 $(M-H)^-$.

SUPPLEMENTAL RESULTS

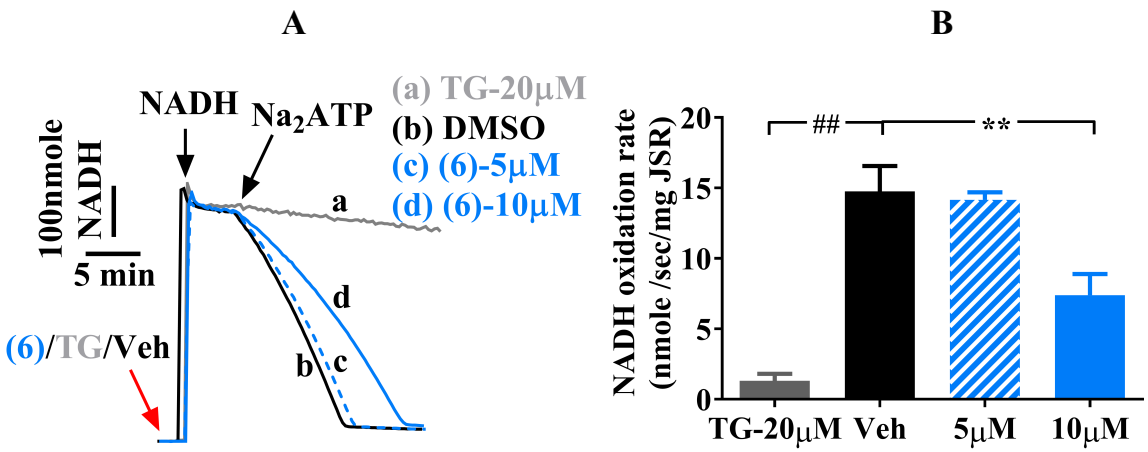


Figure SI 1. Compound 6 exhibited an inhibitory effect on Ca^{2+} ATPase activity.
(A) Representative traces of absorbance detected at 340-400 nm. Veh (0.1% DMSO), TG (thapsigargin, 20 μM) or compound **6** was added into one of the cuvettes before addition of NADH, Na_2ATP was eventually added to initiate the oxidation reactions. The oxidation rate of NADH in the presence of TG, Veh or test compounds were summarized in bar graph (B). Two different JSR preparations were used to achieve the data for each group in triplicates or quintuplicates, n=6-10. Data were shown as Mean ± SD and one-way ANOVA followed by Dunnett's multiple comparisons test was performed using Graph Pad 7.03. $^{**}p < 0.01$ *v.s.* Veh, $^{\#\#}p < 0.01$ *v.s.* TG.

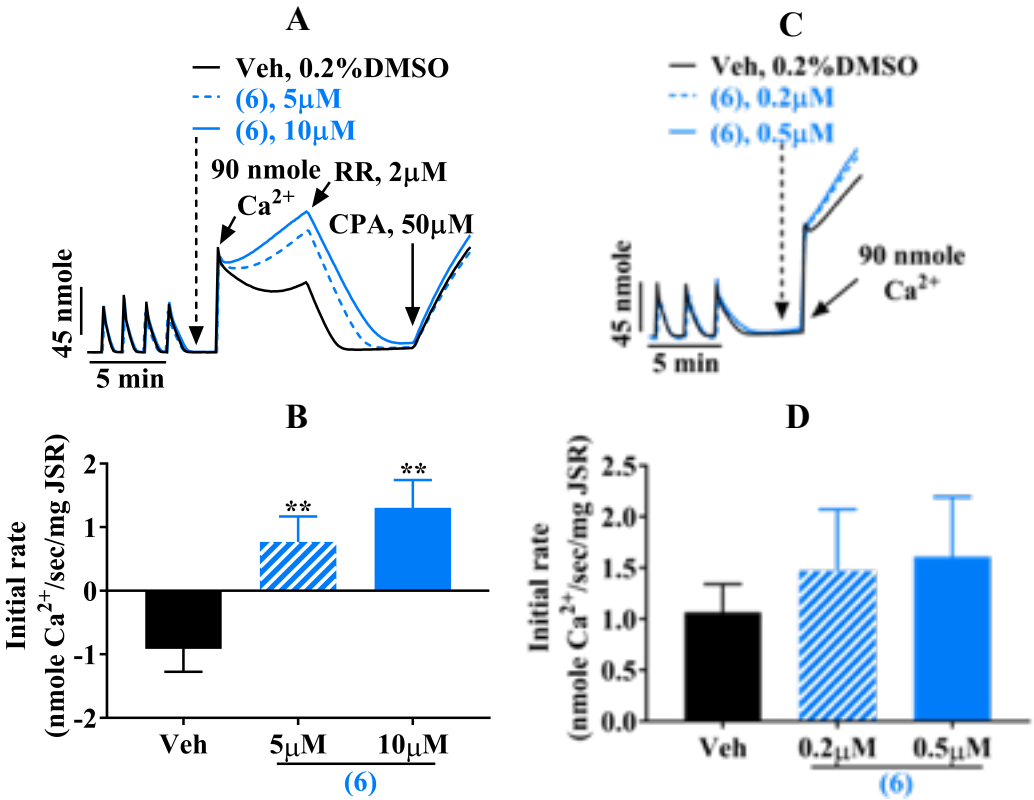


Figure SI 2. Compound 6 sensitized the Ca^{2+} induced calcium release (CICR) at micromolar level rather than nanomolar level. Actively Ca^{2+} uptake and release traces were shown in (A) and (C). The Ca^{2+} release rate of initial 60s upon the addition of 90nmole Ca^{2+} in the presence of Veh, micromolar or nanomolar compound **6** were summarized in (B) and (D), respectively. Two different JSR preparations were used to perform the experiments in duplicates or triplicates for each group, n=4-6. Data were shown as Mean ± SD and one-way ANOVA followed by Dunnett's multiple comparisons test was used to determine the significance using Graph Pad 7.03. ** p <0.01 compared with Veh. Veh indicates 0.2% DMSO.

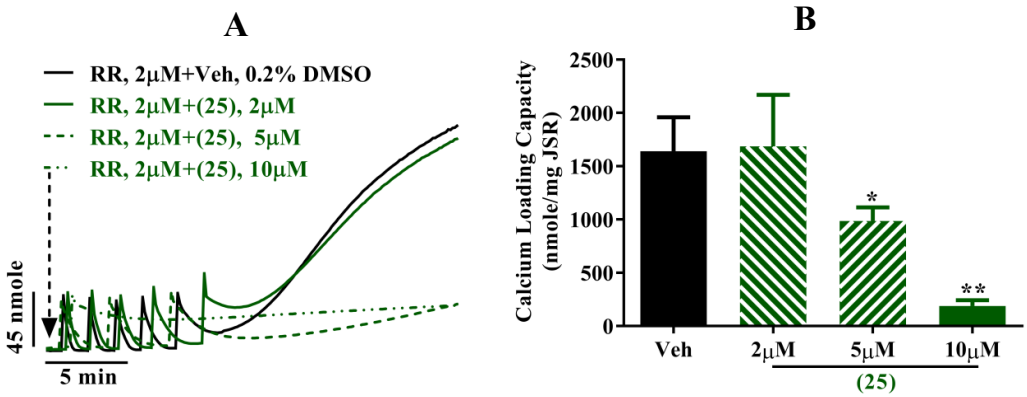
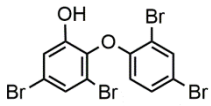
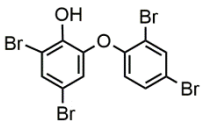
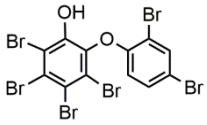
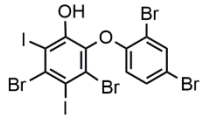
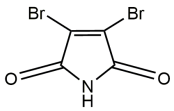
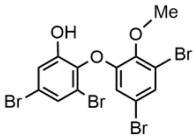
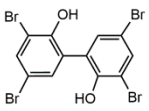
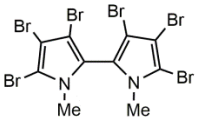
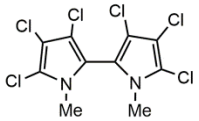
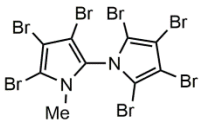
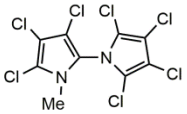
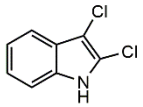
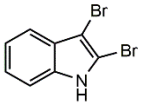
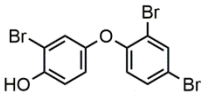
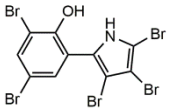
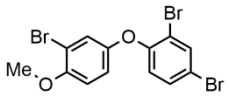
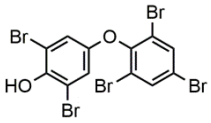
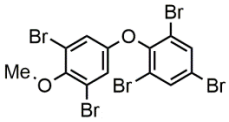
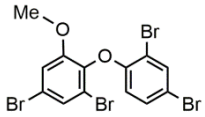
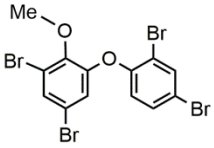
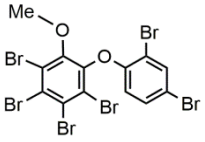
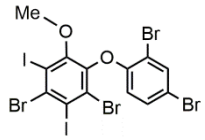


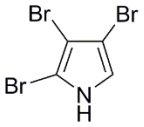
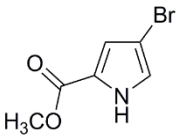
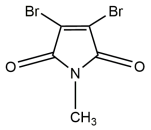
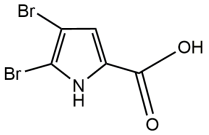
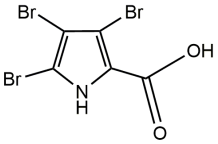
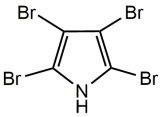
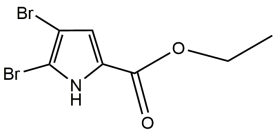
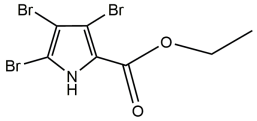
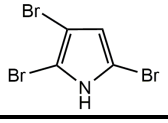
Figure SI 3. Pretreatment of compound 25 diminished the loading capacity of calcium into JSR vesicles dose dependently. (A) The representative traces of loading capacity measured using Ca^{2+} dye Arsenazo III. After the establishment of baseline, RR (Ruthenium Red, 2μM), a RyR1 blocker, was added to each cuvette followed by the addition of corresponding concentrations of compound **25**. A sequential of 45 nmole of $CaCl_2$ was added to each cuvette after 1min of incubation. For each cuvette, the next bolus of $CaCl_2$ could be added after the former bolus of $CaCl_2$ was completely accumulated into vesicles. (B) The amount of Ca^{2+} actively loaded into vesicles were calculated and summarized in bar graph. 3-5 data were achieved from 2 different JSR preparations for each group (n=3-5). Data were shown as Mean ± SD and one-way ANOVA followed by Dunnett's multiple comparisons test using Graph Pad 7.03. * $p<0.05$, ** $p<0.01$ *v.s.* Veh. Veh represents 0.2% DMSO.

Table SI 1. Structural Overview of Screened Halogens

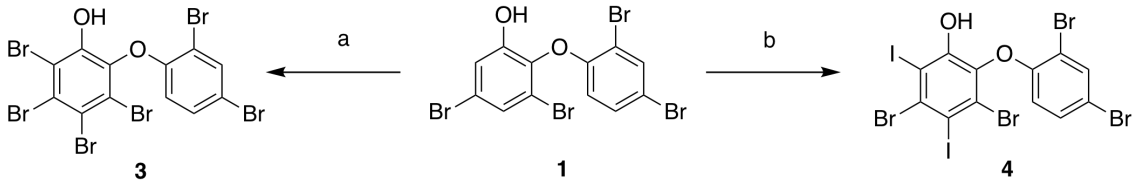
Number	Chemical Name/CAS number	Structure
1	3,5-dibromo-2-(2,4-dibromophenoxy)phenol 79755-43-4	
2	2,4-dibromo-6-(2,4-dibromophenoxy)phenol 80246-25-9	
3	2,3,4,5-tetrabromo-6-(2,4-dibromophenoxy)phenol 111863-67-3	
4	3,5-dibromo-2-(2,4-dibromophenoxy)-4,6-diiodophenol	
5	2,4,6-tribromophenol 118-79-6	
6	3,4-dibromo-1*H*-pyrrole-2,5-dione (2,3-dibromomaleimide) 1122-10-7	
7	3,5-dibromo-2-(3,5-dibromo-2-methoxyphenoxy)phenol 80246-35-1	
8	3,3',5,5'-tetrabromo-[1,1'-biphenyl]-2,2'-diol (bromophene) 21987-62-2	

9	3,3',4,4',5,5'-hexabromo-1,1'-dimethyl-1*H*,1'*H*-2,2'-bipyrrole 253798-63-9	
10	3,3',4,4',5,5'-hexachloro-1,1'-dimethyl-1*H*,1'*H*-2,2'-bipyrrole 253798-66-2	
11	2,3,3',4,4',5,5'-heptabromo-1'-methyl-1'*H*-1,2'-bipyrrole 873956-95-7	
12	2,3,3',4,4',5,5'-heptachloro-1'-methyl-1'*H*-1,2'-bipyrrole 428442-17-5	
13	2,3-dichloroindole 101495-59-4	
14	2,3-dibromoindole 108438-54-6	
15	2-bromo-4-(2,4-dibromophenoxy)phenol 862807-89-4	
16	2,4-dibromo-6-(3,4,5-tribromo-1*H*-pyrrol-2-yl)phenol (pentabromopseudilin) 10245-81-5	

17	2,4-dibromo-1-(3-bromo-4-methoxyphenoxy)benzene	
18	2,6-dibromo-4-(2,4,6-tribromophenoxy)phenol 91370-78-4	
19	1,3,5-tribromo-2-(3,5-dibromo-4-methoxyphenoxy)benzene 678988-40-4	
20	1,5-dibromo-2-(2,4-dibromophenoxy)-3-methoxybenzene 102739-99-1	
21	1,5-dibromo-3-(2,4-dibromophenoxy)-2-methoxybenzene 96920-28-4	
22	1,2,3,4-tetrabromo-5-(2,4-dibromophenoxy)-6-methoxybenzene 169901-73-9	
23	1,3-dibromo-4-(2,4-dibromophenoxy)-2,6-diiodo-5-methoxybenzene	
24	pyrrole 109-97-7	
25	3,3',4,4',5,5'-hexabromo-1*H*,1'*H*-2,2'-bipyrrole 54705-15-6	

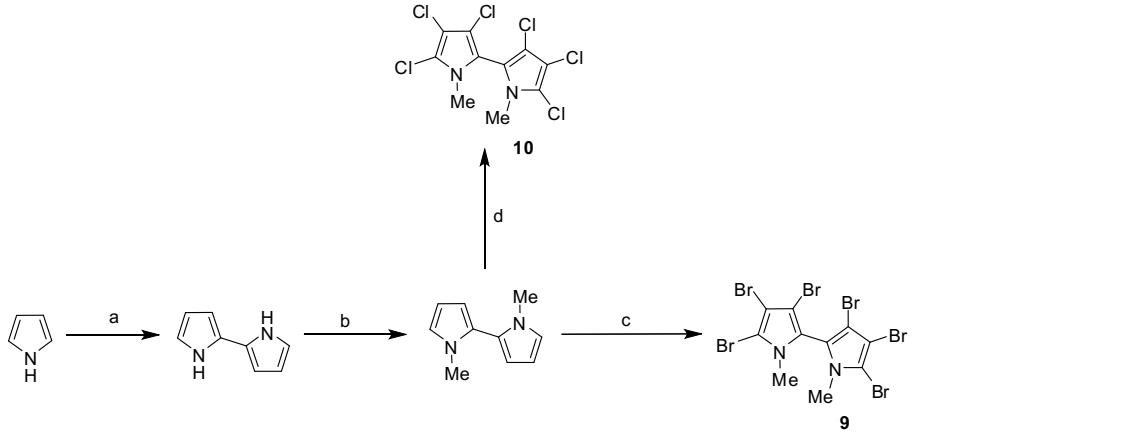
26	2,3,4-tribromopyrrole 69624-12-0	
27	methyl 4-bromopyrrole-2-carboxylate 934-05-4	
28	3,4-dibromo-1-methyl-1*H*-pyrrole-2,5-dione (2,3-dibromo-*N*-methyl-maleimide) 3005-27-4	
29	4,5-dibromopyrrole-2-carboxylic acid 34649-21-3	
30	3,4,5-tribromopyrrole-2-carboxylic acid 74039-30-8	
31	2,3,4,5-tetrabromopyrrole 54705-14-5	
32	ethyl 4,5-dibromopyrrole-2-carboxylate 516465-86-4	
33	ethyl 3,4,5-tribromopyrrole-2-carboxylate 740813-36-9	
34	2,3,5-tribromopyrrole 77124-07-3	

Syntheses of compounds **3** and **4**:



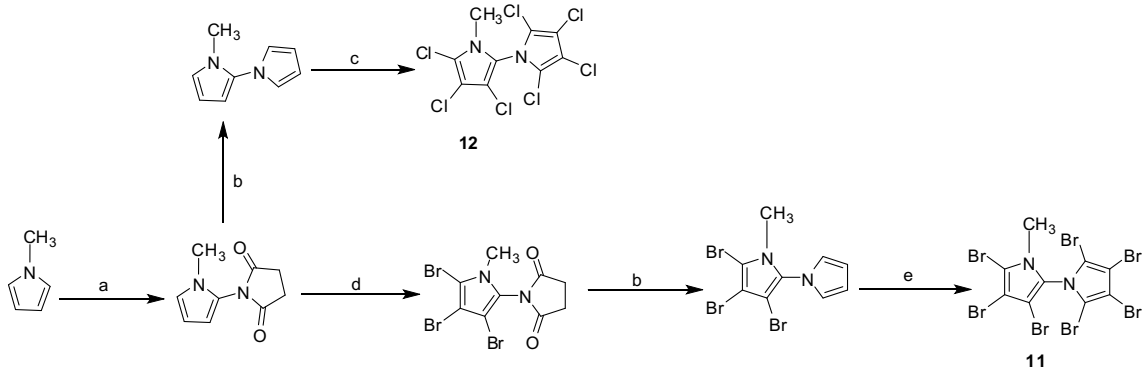
Scheme 1. Reagents and conditions: (a) NBS (2.5 equiv.) rt, (b) NIS (2.5 equiv.)

Syntheses of compounds **9** and **10**:



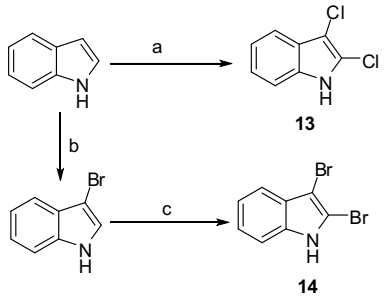
Scheme 2. Reagents and conditions: (a) pyrrole (3 equiv.), PIFA (1 equiv.), TMSBr (2 equiv.), DCM, -78 ^{o}C to -40 ^{o}C, 1h (b) CH_3I (2.2 equiv.), NaH (3.0 equiv.), DMF, 0 ^{o}C, 1h (c) NBS (7.0 equiv), CH_3CN, –40 °C to 25 °C, 15 h. (d) NCS (6.2 equiv.), CH_3CN, –40 °C to 25 °C, 90 min.

Syntheses of compounds **11** and **12**:



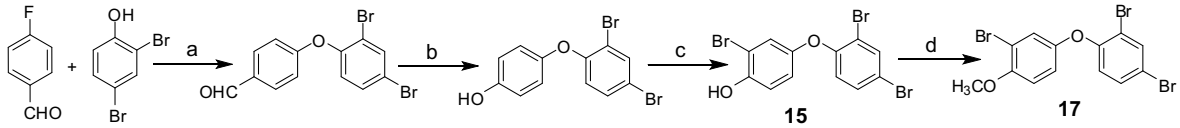
Scheme 3. Reagents and conditions: (a) NCS, $NaHCO_3$, DCM, dark, rt, 4 h (b) $HSi(OEt)_3$, $Ti(OiPr)_4$, benzene, 65 ^{o}C (c) SO_2Cl_2, DCM, rt, 3 h (d) NBS (3.6 equiv.), THF, –78 °C to 25 °C, 3 h (e) bromine, chloroform, rt, 4 h.

Syntheses of compounds **13** and **14**:



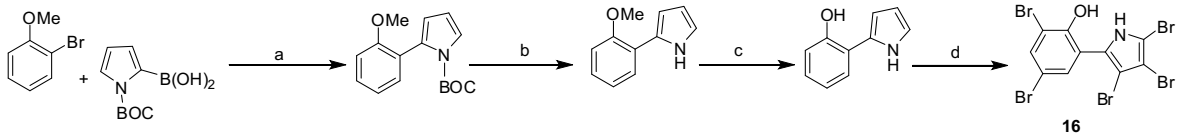
Scheme 4. Reagents and conditions: (a) SO_2Cl_2, ether, 0 ºC, 5h (b) pyridinium bromide perbromide, pyridine (c) bromine, CH_2C1_2, 0 ºC.

Syntheses of compounds **15** and **17**:



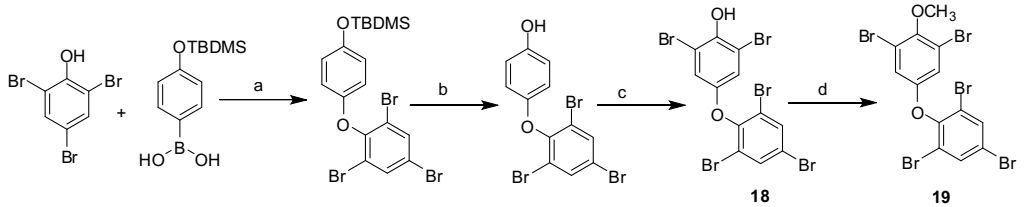
Scheme 5. Reagents and conditions: (a) DMAC, Na_2CO_3 (b) KH_2PO_4, TFPA, CH_2Cl_2, 0 °C, 1h (c) bromine, acetic acid, rt, 1h (d) NaH, DMF, CH_3I, 0 ^oC to rt.

Synthesis of compound **16**:



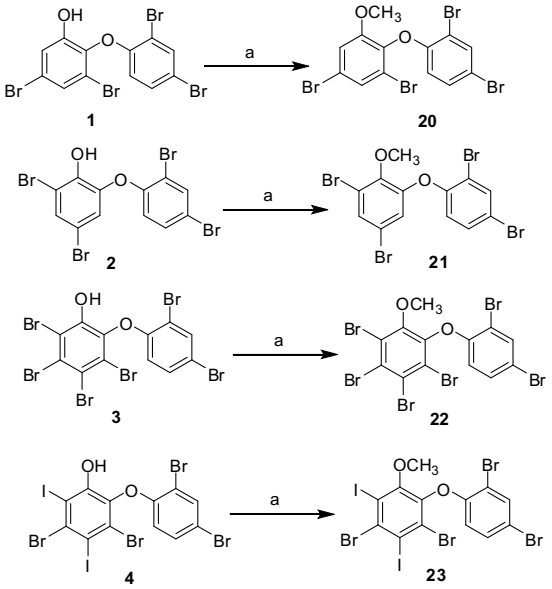
Scheme 6. Reagents and conditions: (a) $Pd(PPh_3)_4$, Na_2CO_3, CH_3Ph/MeOH, 80 ^oC, 15-17 h. (b) CH_3ONa, THF, 40 ^oC (c) Na_2S, NMP, 160 ^oC, 2.5 h (d) Py·HBr·Br_2, EtOH, rt, 3 days

Syntheses of compounds **18** and **19**:



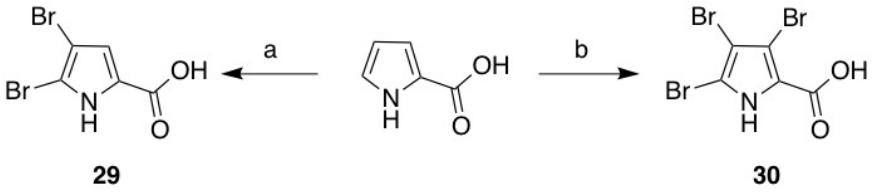
Scheme 7. Reagents and conditions: (a) $Cu(OAc)_2$ (1.0 equiv.), triethylamine (5.0 equiv.), 4 Å molecular sieves, DCM. (b) DBU (1.0 equiv.), CH_3CN–H_2O (95:5) r.t., 1 h. (c) Br_2 (2.0 equiv.), AcOH, rt, 1 h. (d) CH_3I (1.0 equiv.), K_2CO_3 (1.2 equiv.), acetone, rt.

Syntheses of compounds **20, 21, 22** and **23**:



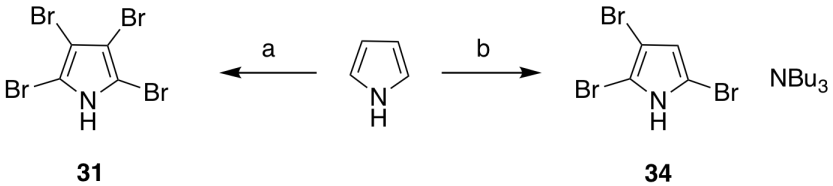
Scheme 8. Reagents and conditions: (a) CH_3I (1.0 equiv.), K_2CO_3 (1.2 equiv.), acetone, rt to 30 ^oC.

Syntheses of compounds **29** and **30**:



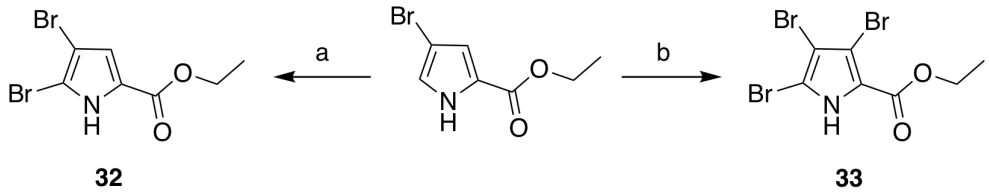
Scheme 9. Reagents and conditions: (a) bromine (2 equiv.), chloroform, AcOH, 50 °C, 5 h. (b) bromine (3 equiv.), chloroform, AcOH, 50 °C, 5 h.

Syntheses of compounds **31** and **34**:



Scheme 10. Reagents and conditions: (a) NBS (4 equiv.), THF, -78 °C to -10 °C, 2h (b) NBS (3 equiv.), THF, -78 °C to -10 °C, 2h, then tri-*n*-butylamine (0.5 equiv).

Syntheses of compounds **32** and **33**:



32 **33**

Scheme 11. Reagents and conditions: (a) bromine (2 equiv.), AcOH, rt, 6.5 h. (b) bromine (3 equiv.) AcOH, rt, 6.5 h

REFERENCE

1. Pessah, I. N.; Francini, A. O.; Scales, D. J.; Waterhouse, A. L.; Casida, J. E., Calcium-ryanodine receptor complex. Solubilization and partial characterization from skeletal muscle junctional sarcoplasmic reticulum vesicles. *J Biol Chem* **1986,** *261*, (19), 8643-8.
2. Pessah, I. N.; Hansen, L. G.; Albertson, T. E.; Garner, C. E.; Ta, T. A.; Do, Z.; Kim, K. H.; Wong, P. W., Structure-activity relationship for noncoplanar polychlorinated biphenyl congeners toward the ryanodine receptor-Ca2+ channel complex type 1 (RyR1). *Chem Res Toxicol* **2006,** *19*, (1), 92-101.
3. Wong, P. W.; Pessah, I. N., Ortho-substituted polychlorinated biphenyls alter calcium regulation by a ryanodine receptor-mediated mechanism: structural specificity toward skeletal- and cardiac-type microsomal calcium release channels. *Mol Pharmacol* **1996,** *49*, (4), 740-51.
4. Haak, L. L.; Song, L. S.; Molinski, T. F.; Pessah, I. N.; Cheng, H.; Russell, J. T., Sparks and puffs in oligodendrocyte progenitors: cross talk between ryanodine receptors and inositol trisphosphate receptors. *J Neurosci* **2001,** *21*, (11), 3860-70.
5. Agarwal, V.; Li, J.; Rahman, I.; Borgen, M.; Aluwihare, L. I.; Biggs, J. S.; Paul, V. J.; Moore, B. S., Complexity of naturally produced polybrominated diphenyl ethers revealed via mass spectrometry. *Environ Sci Technol* **2015,** *49*, (3), 1339-46.
6. Göran Marsh, R. S., Åke Bergman, Synthesis of Hydroxylated and Methoxylated Polybrominated Diphenyl Ethers − Natural Products and Potential Polybrominated Diphenyl Ether Metabolites. *European Journal of Organic Chemistry* **2003,** *2003*, (14), 2556-2576.
7. Dohi, T.; Morimoto, K.; Maruyama, A.; Kita, Y., Direct synthesis of bipyrroles using phenyliodine bis(trifluoroacetate) with bromotrimethylsilane. *Org Lett* **2006,** *8*, (10), 2007-10.
8. Martin, R. J., A.; Knölker, H., Transition Metals in Organic Synthesis, Part 97: [1] Silver-Catalyzed Synthesis of Hexahalogenated 2,2´-Bipyrroles. *Synlett* **2011,** *2011*, (19), 2795-2798.
9. Rosa, M. D. N., G.C.; Gago, F.F, Effect of N-chloro structure and 1-substituent on .sigma.-substitution (addition-elimination) in pyrroles. *Journal of Organic Chemistry* **1989,** *54*, (22), 5347–5350.
10. Fu, L. G., G.W, A SHORT SYNTHESIS OF THE NATURALLY OCCURRING 2,3,3´,4,4´,5,5´-HEPTACHLORO- (“Q1”) AND HEPTABROMO-1´-METHYL-1,2´-BIPYRROLES. *Organic Preparations and Procedures International* **2008,** *40*, (6), 561-566.
11. Jun Wu, W. V., Gordon W. Gribble, John S. Schneekloth, Jr., David H. Blank and Helmar Görls, Structure and Synthesis of the Natural Heptachloro-1´-methyl-1,2´-bipyrrole (Q1).

Angewandte International Edition Chemie **2002**, 1740-1743.
12. Brennan, M. R. E., K.L.; Szmalc, F. S.; Tansey, M.J..; Thornton, J.M., The Preparation and Spectral Characterization of 2-Haloindoles, 3-Haloindoles, and 2,3-Dihaloindoles. *Heterocycles* **1986,** *24*, (10), 2879 - 2885.
13. Marsh, G. S., R.; Bergman, A., Synthesis of Hydroxylated and Methoxylated Polybrominated Diphenyl Ethers − Natural Products and Potential Polybrominated Diphenyl Ether Metabolites. *European Journal of Orgnic Chemistry* **2003,** *2003*, (14), 2566-2576.
14. Evans, D. A.; Katz, J. L.; West, T. R., Synthesis of diaryl ethers through the copper-promoted arylation of phenols with arylboronic acids. An expedient synthesis of thyroxine. *Tetrahedron Letters* **1998,** *39*, (19), 2937-2940.
15. Yeom, C. K., H.W.; Lee, S.Y; Kim, B.M, DBU-Mediated Mild and Chemoselective Deprotection of Aryl Silyl Ethers and Tandem Biaryl Ether Formation. *Synlett* **2007,** *2007*, (1), 146-150
16. El Gamal, A.; Agarwal, V.; Diethelm, S.; Rahman, I.; Schorn, M. A.; Sneed, J. M.; Louie, G. V.; Whalen, K. E.; Mincer, T. J.; Noel, J. P.; Paul, V. J.; Moore, B. S., Biosynthesis of coral settlement cue tetrabromopyrrole in marine bacteria by a uniquely adapted brominase-thioesterase enzyme pair. *Proc Natl Acad Sci U S A* **2016,** *113*, (14), 3797-802.
17. H. M. Gilow, D. E. B., Bromination and chlorination of pyrrole and some reactive 1-substituted pyrroles. *Journal of Organic Chemistry* **1981,** *46*, (11), 2221-2225.
18. Li, Q.; Hurley, P.; Ding, H.; Roberts, A. G.; Akella, R.; Harran, P. G., Exploring symmetry-based logic for a synthesis of palau'amine. *J Org Chem* **2009,** *74*, (16), 5909-19.
19. John, E. A.; Pollet, P.; Gelbaum, L.; Kubanek, J., Regioselective syntheses of 2,3,4-tribromopyrrole and 2,3,5-tribromopyrrole. *J Nat Prod* **2004,** *67*, (11), 1929-31.