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# Novel steroidal saponins, Sch 725737 and Sch 725739, from a marine starfish, *Novodinia antillensis*

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**Abstract**—Bioassay-guided fractionation of an active fraction from an extract of a marine starfish, *Novodinia antillensis*, led to the isolation and identification of two new saponins, Sch 725737 (**1**) and Sch 725739 (**2**). Compound **1** was identified as the NaV1.8 inhibitor with $IC_{50}$ of ~9 μM. The purification and the structure elucidation of these two saponins are described.


NaV1.8 is a member of the voltage-gated sodium channel family. This sodium channel has received much attention over recent years due to its unusual resistance to the potent sodium channel blocker, tetrodotoxin (TTX), its atypical biophysical properties, and because it is expressed, almost exclusively, in a subpopulation of sensory neurons of the peripheral nervous system. These characteristics, together with other evidence, have led to the suggestion that NaV1.8 may be implicated in the perception and processing of pain sensations. Agents that prevent NaV1.8 activity may, therefore, show promise as novel analgesics.[1]

Marine organisms are an excellent source of structurally diverse molecules which are potentially valuable for drug discovery.[2,3] Many marine natural products have been designated as lead compounds in various therapeutical areas.[2] Among them, several compounds are currently under clinical studies, such as dolastatin 10, ecteinascidin 743 (ET-743), aplidine, discodermolide, kahalalide F, and squalamine, mainly in the anti-cancer area.[3] Among marine natural products, steroidal glycosides are the major chemical constituents of starfishes.[4,5] Marine asterosaponins or sterol sulfates have been described to exhibit various biological activities, including hemolytic,[4] cytotoxic,[5] anti-HIV,[6] and antimicrobial activities.[7]

In our marine natural product research program, we have generated a marine fraction library (MFL), derived from various marine collections, for high throughput screening (HTS) assays in various biological targets.[8] The active fractions in preliminary screening tests were followed up using bioassay-guided fractionation to identify the active compounds.[9,10] Several fractions were identified as active 'hits' in a NaV1.8 sodium channel assay.

Described herein are the results of the isolation and structure elucidation of active principles derived from the marine starfish *Novodinia antillensis*. In this investigation, a fraction was identified to be active in the NaV1.8 HTS assay. Followed by bioassay-guided purification of this active fraction using reverse phase HPLC, two new saponins, Sch 725737 (**1**) and Sch 725739 (**2**), were isolated and identified.

The organism was identified as *N. antillensis* [Phylum Echinodermata, Class Asteroidea; Order Brisingida; Family Brisingidae].[11] The specimen (HBOI Cat

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Number 4-IV-89-4-003) was collected using the Johnson-Sea-Link II submersible at a depth of 587 meters, west of Isle de Ronde, Grenada, Grenadines (Latitude 12° 19.38′ N, Longitude 61° 37.19′ W).

In the preliminary HTS assay, a sample in the MFL from the marine starfish (*N. antillensis*) showed 88% inhibitory activity in the NaV1.8 assay at 20 μg/mL. Therefore, the active fraction was subjected to bioassay-guided fractionation. The general procedure for generation of the MFL was described previously[8] and was summarized as follows: the crude extract of marine sample was obtained by grinding and extracting with ethanol, and was further fractionated on a CG161 column to generate six fractions eluting with aqueous acetonitrile ($CH_3CN$) stepwise-gradient solution (10%, 25%, 50%, 75%, 100%, methanol–EtOAc wash). All fractions were dried and a portion of the material was submitted for the HTS assay. In this particular study, fraction 3 (157 mg from the 50% $CH_3CN$ elution) was active in the NaV1.8 assay. Fraction 3 (157 mg) was further purified on an HPLC semi-preparative ODS-A column (YMC, 120 Å, S-7, 2 × 25 cm). The column was eluted with a gradient of aqueous $CH_3CN$ (3–50% $CH_3CN$ over 70 min), to yield 100 fractions (13 mL/fraction). Two pure compounds **1** (9.5 mg) and **2** (5.3 mg) were obtained from fractions 53 and 55, respectively.

The structure of compound **1** was determined based on extensive NMR and HRMS analyses.[12] From the high-resolution negative ESI-MS, the molecular formula of **1** was established as $C_{61}H_{101}O_{30}S^-$ (obsd 1345.6069; calcd 1345.6103 for $[M]^-$), suggesting a possible sulfate functionality. The fragment ions *m/z* 1202 ($M-C_6H_9O_4$) and *m/z* 1056 ($M-2\times C_6H_9O_4$) observed in ESI-MS indicated saponin type structure and at least two deoxy sugars present in the molecule. The $^1H$ NMR spectrum showed highly overlapped signals in the upfield region as well as in the oxygenated methine region. Of 61 carbon signals in the $^{13}C$ NMR spectrum, 27 were assigned to an aglycone moiety, and the remainder to six sugars (Table 1). Six characteristic anomeric proton/carbon pairs were observed in the $^1H$ and $^{13}C$ NMR spectra, respectively. Through analyses of HSQC and HSQC–TOCSY data, the $^1H$ and $^{13}C$ data of the six sugars were correlated and assembled. The correlation data are summarized and represented in Figure 1.

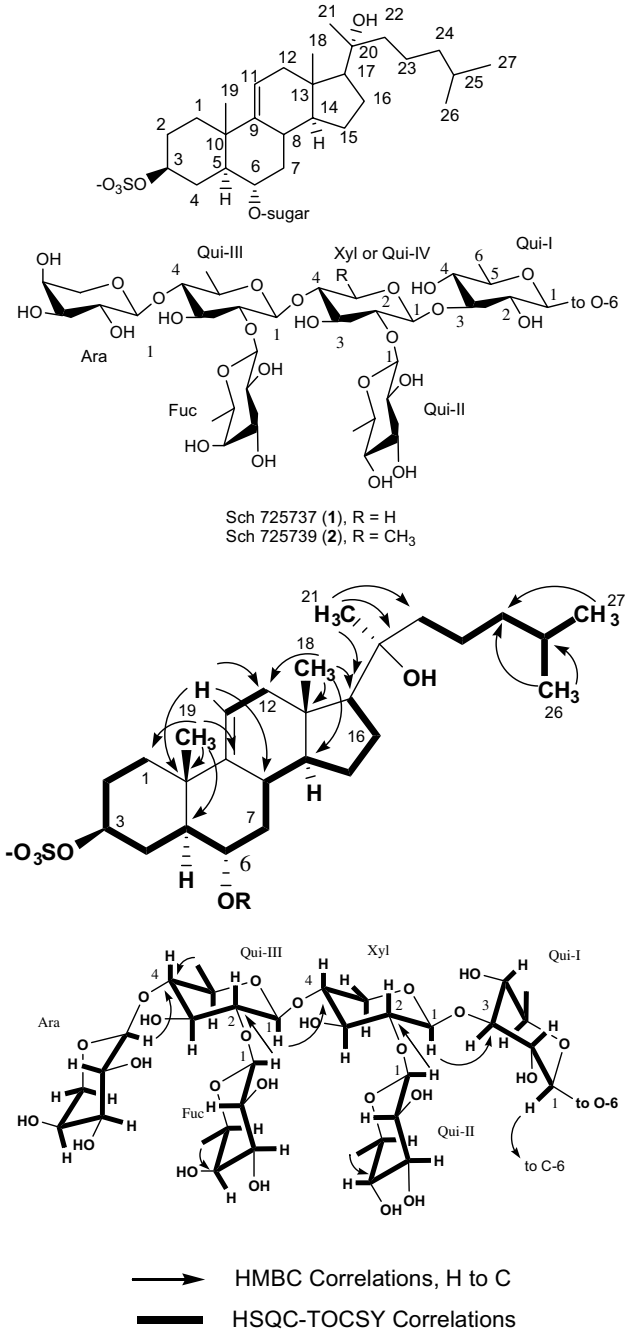


**Figure 1.** HMBC and HSQC–TOCSY correlations of **1**.

The stereochemistry of the sugars was further established based on the coupling constants, coupling patterns, and NOE correlations of the oxygenated methine protons, observed in the 1D $^1H$ NMR or in the 2D $^1H$–$^1H$ COSY, HSQC, HSQC–TOCSY, or HSQC–ROESY spectra. For instance, three quinovose and one xylose sugars were identified on the basis of the large coupling constants for all of the methine protons (H-1 to H-5) observed in the HSQC spectrum. Arabinose moiety was identified based on the $^{13}C$ NMR data and the coupling patterns of H-1 ($\delta$ 4.19, d, $J$ = 7.3 Hz), $H_2$-5 ($\delta$ 3.44, br d, $J$ = 12.4 Hz; $\delta$ 3.72, br dd, $J$ = 12.4 and 2.6 Hz), and H-4 ($\delta$ 3.61, small couplings observed in HSQC). The stereochemistry of arabinose was further confirmed by NOE correlations among the axial protons, H-1, H-3 ($\delta$ 3.32), and H-5 and between H-3 and H-4 observed in the HSQC–ROESY spectrum. Finally, the last six-carbon deoxy sugar was assigned to fucose based on the coupling patterns of H-1 ($\delta$ 4.27, d, $J$ = 7.1 Hz), H-2 ($\delta$ 3.26, two large coupling constants), and H-4 ($\delta$ 3.36, small coupling constants) and NOE correlations among the axial protons H-1, H-3 ($\delta$ 3.28), and H-5 ($\delta$ 3.48) and between H-4 and $H_3$-6 ($\delta$ 1.10). The linkage between these sugars was determined by analysis of HMBC long-range correlations through the anomeric protons, detailed as follows: H-1 of Xyl to C-3 of Qui-I; H-1 of Qui-II to C-2

of Xyl; H-1 of Qui-III to C-4 of Xyl; H-1 of Fuc to C-2 of Qui-III; H-1 of Ara to C-4 of Qui-III. This sequence of the polysaccharide was further confirmed by the analysis of NOE data acquired from HSQC–ROESY spectrum, summarized in Figure 2. Thus the polysaccharide moiety was established.

The remaining 27 carbon resonances resembled a steroidal skeleton. The carbon skeleton was connected based on HMBC and HSQC–TOCSY data. The connection of the consecutive protonated carbons in the sterol skeleton was completed through two-bond H–C correlations by analysis of the HSQC–TOCSY data. The connectivity of the quaternary carbons C-9 ($\delta$ 145.4), C-10 ($\delta$ 38.0), C-13 ($\delta$ 40.9), and C20 ($\delta$ 73.4) to their neighboring carbons was determined by HMBC correlations with H-11 ($\delta$ 5.21), $H_3$-19 ($\delta$ 0.85), $H_3$-18 ($\delta$ 0.66), and $H_3$-21 ($\delta$ 1.09), respectively, as shown in Figure 1. The protonated carbons including two oxygenated carbons (C-3 and C-6) and one double bond ($\Delta$ 9,11) were connected unambiguously through the analysis of the HSQC–TOCSY data as depicted in Figure 1 with bolded lines. The full skeleton of the aglycone was thus established. The sulfate ester moiety was assigned to C-3 based on its carbon chemical shift ($\delta$ 75.9 in DMSO-$d_6$–$D_2O$ and $\delta$ 80.0 in $CD_3OD$), by comparison to that of known sterol sulfate derivatives.[13–19] The linkage between the glycone and aglycone was unambiguously identified based on the correlation between C-6 ($\delta$ 78.5) and H-1 ($\delta$ 4.28) of Qui-I.

The stereochemistry of H-3 and H-6 was determined as $\alpha$ and $\beta$ orientation, respectively, by the analysis of their coupling patterns (H-3, $\delta$ 3.84, tt, $J$ = 5, 5, 10.5, 10.5 Hz; H-6, $\delta$ 3.42, m, small coupling constants). The $^1$H and $^{13}$C NMR data confirmed the *trans* configuration of the ring junctions and stereochemistry of C-20 (*S*), which were consistent with those of known sterols.[13–20]

The aglycone moiety of **1** is not common but has been reported previously derived from starfish saponins. The NMR data of the aglycone were identical to those of the known saponins, (20*S*)-20-hydroxy-6α-(4-*O*-sulfo-β-D-quinovopyranosyloxy)-3β-sulfooxy-5α-cholest-9(11)-ene [13] and ruberoside C.[14]

Compound **2** showed a molecular ion *m*/*z* 1359 $[M]^-$ in the negative ESI-MS, suggesting a molecular formula $C_{62}H_{103}O_{30}S^-$. The NMR data of **2** were almost identical to those of **1**, except the $^1$H- and $^{13}$C-chemical shifts around the xylose moiety of **1**. Therefore, the sterol moiety of **2** was established as identical to that of **1**, and the remainder of the structure elucidation of **2** was focused on the second sugar in the oligosaccharide moiety. In the $^1$H and $^{13}$C NMR spectra, additional $CH_3$ and oxygenated CH signals ($\delta$ 18.0 q; $\delta$ 70.8 ppm, respectively) were observed and the original $CH_2$ signal of xylose in **1** was not detected. These data indicated that the six-carbon sugar instead of the five-carbon sugar was present in **2**. From analysis of the HMBC data of **2**, this additional $CH_3$ signal had significant correlations to the additional CH ($\delta$ 70.8) and to another CH ($\delta$ 84.5, C-4 of Qui-IV) resonance. Finally, the large coupling constants of all of the sugar methine protons (H-1 to H-4) confirmed the replacement of the xylose moiety of **1** with a quinovose in **2**. Thus, the structure elucidation of **2** was completed. The glycone of the starfish saponin, pectinifera G,[21] reported previously possesses similar sugar component and sequence to that of **2**. The only distinction between polysaccharides of the two compounds is that **2** has 6-deoxyglucose (Qui-III) instead of glucose in pectinifera G.

Compound **1** exhibited robust inhibitory activity in the NaV1.8 assay. The NaV1.8 assay was carried out with an ND7/23 clonal line engineered to permanently express rat NaV1.8 sodium channels[22] and activity was assayed using a membrane-potential-sensitive dye and a fluorometric imaging plate reader (FLIPR384; Molecular Devices Corp.). For the FLIPR experiments, cells were dislodged from propagation flasks and re-plated (~5000 cells per well) into 384-well plates (BD Biocoat) 48 h prior to use. On the day of the assay, growth medium was removed from all wells and replaced with a simple sodium-free assay buffer (20 μl) having the following composition ([mM]): *N*-methyl-D-glucamine, 150; KCl, 3.25; $CaCl_2$, 2; $MgCl_2$, 2; $CdCl_2$, 0.3; Hepes, 10; D-glucose 11. The assay buffer solution was supplemented with 300 nM TTX (to prevent sodium channel activity other than that due to NaV1.8), 3 μM cypermethrin (to 'lock' NaV1.8 channels in their open configuration;

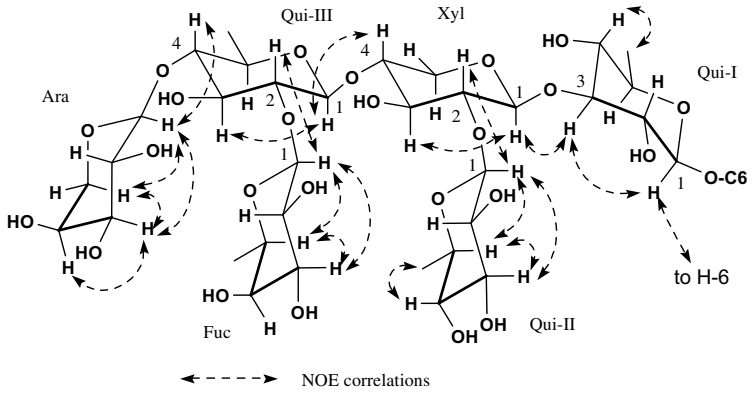


**Figure 2.** Selected NOE correlations of **1**, acquired from HSQC–ROESY.

**Table 1.** NMR Spectral data of Sch 725737 (**1**) and Sch 725739 (**2**), recorded in DMSO-$d_6$–$D_2O$ (100:15); $\delta$ in ppm; $J$ in Hz

| | **1** | | | | | **2** | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| No. | $^{13}C$ | $^{1}H$ ($J$) | No. | $^{13}C$ | $^{1}H$ ($J$) | No. | $^{13}C$ | $^{1}H$ ($J$) | No. | $^{13}C$ | $^{1}H$ ($J$) |
| 1 | 35.5 t | 1.23 m; 1.58 m | 15 | 24.8 t | 1.06 m; 1.61 m | 1 | 35.7 t | 1.23 m; 1.58 m | 15 | 25.0 t | 1.06 m; 1.61 m |
| 2 | 28.6 t | 1.36 m; 2.07 m | 16 | 22.4 t | 1.50 m; 1.76 m | 2 | 28.8 t | 1.36 m; 2.04 m | 16 | 22.6 t | 1.50 m; 1.74 m |
| 3 | 75.9 d | 3.84 tt (5, 5, 10.5, 10.5) | 17 | 57.8 d | 1.39 m | 3 | 76.4 d | 3.84 tt (5, 5, 10.5, 10.5) | 17 | 58.0 d | 1.39 m |
| 4 | 29.8 t | 1.04 m; 2.32 m | 18 | 13.4 q | 0.66 s | 4 | 29.9 t | 1.05 m; 2.31 m | 18 | 13.6 q | 0.65 s |
| 5 | 48.7 d | 1.04 m | 19 | 19.4 q | 0.85 s | 5 | 48.9 d | 1.04 m | 19 | 19.5 q | 0.84 s |
| 6 | 78.5 d | 3.42 m | 20 | 73.4 s | | 6 | 78.8 d | 3.43 m | 20 | 73.6 s | |
| 7 | 40.8 t | 0.77 m; 2.22 m | 21 | 25.9 q | 1.09 s | 7 | 41.0 t | 0.79 m; 2.21 m | 21 | 26.1 q | 1.09 s |
| 8 | 34.9 d | 1.94 m | 22 | 44.1 t | 1.17 m; 1.28 m | 8 | 35.2 d | 1.94 m | 22 | 44.4 t | 1.17 m; 1.28 m |
| 9 | 145.4 s | | 23 | 21.8 t | 1.15 m; 1.20 m | 9 | 145.6 s | | 23 | 22.0 t | 1.14 m; 1.19 m |
| 10 | 38.0 s | | 24 | 39.6 t | 1.05 m | 10 | 38.2 s | | 24 | 39.8 t | 1.04 m |
| 11 | 116.3 d | 5.21 br d (5.5) | 25 | 27.6 d | 1.46 m | 11 | 116.6 d | 5.21 br d (5) | 25 | 27.8 d | 1.45 m |
| 12 | 42.0 t | 1.93 br d (15.2); 2.10 br dd (4.8, 15.2) | 26 | 22.6 q | 0.80 d (6.6) | 12 | 42.2 t | 1.93 br d (15.5); 2.10 br dd (5, 15.5) | 26 | 23.0 q | 0.80 d (6.5) |
| 13 | 40.9 s | | 27 | 23.1 q | 0.81 d (6.6) | 13 | 40.9 s | | 27 | 23.1 q | 0.80 d (6.5) |
| 14 | 53.6 d | 1.12 m | | | | 14 | 53.8 d | 1.12 m | | | |
| Qui-I | | | Qui-III | | | Qui-I | | | Qui-III | | |
| 1 | 103.0 d | 4.28 d (7.8) | 1 | 99.7 d | 4.49 d (7.4) | 1 | 103.1 d | 4.29 d (7.7) | 1 | 100.8 d | 4.49 d (6.7) |
| 2 | 73.3 d | 3.12 t[a] | 2 | 82.9 d | 3.24 t[a] | 2 | 73.2 d | 3.12 t[a] | 2 | 82.3 d | 3.33 t[a] |
| 3 | 88.2 d | 3.25 t[a] | 3 | 74.2 d | 3.47 t[a] | 3 | 89.2 d | 3.24 t[a] | 3 | 74.6 d | 3.50 t[a] |
| 4 | 73.2 d | 2.88 t (9.1, 9.1) | 4 | 83.4 d | 3.09 t[a] | 4 | 73.6 d | 2.89 t (9, 9) | 4 | 83.6 d | 3.12 t[a] |
| 5 | 71.0 d | 3.23 m | 5 | 70.5 d | 3.41 m | 5 | 71.2 d | 3.25 m | 5 | 70.7 d | 3.48 m |
| 6 | 18.2 q | 1.12 d (6.1) | 6 | 17.7 q | 1.22 d (6.1) | 6 | 18.3 q | 1.12 d (6) | 6 | 17.6 q | 1.22 d (6) |
| Xyl | | | Ara | | | Qui-IV | | | Ara | | |
| 1 | 102.8 d | 4.48 d (7.4) | 1 | 103.8 d | 4.19 d (7.3) | 1 | 102.2 d | 4.50 d (6.8) | 1 | 104.1 d | 4.19 d (7.6) |
| 2 | 83.1 d | 3.31 t[a] | 2 | 70.7 d | 3.33 m | 2 | 83.3 d | 3.34 t[a] | 2 | 71.0 d | 3.32 m |
| 3 | 74.0 d | 3.51 t (9, 9) | 3 | 72.6 d | 3.32 m | 3 | 73.8 d | 3.57 t (9, 9) | 3 | 72.8 d | 3.33 m |
| 4 | 76.7 d | 3.58 m | 4 | 67.8 d | 3.61 m[b] | 4 | 84.5 d | 3.14 t[a] | 4 | 68.1 d | 3.62 m[b] |
| 5 | 63.2 t | 3.26 t[a]; 3.93 br dd (4.6, 11.5) | 5 | 66.2 t | 3.44 br d (12.4); 3.72 br dd (12.4, 2.6) | 5 | 70.8 d | 3.45 m | 5 | 66.5 t | 3.45 br d (12.5); 3.71 br d (12.5) |
| | | | | | | 6 | 18.0 q | 1.27 d (6) | | | |
| Qui-II | | | Fuc | | | Qui-II | | | Fuc | | |
| 1 | 104.9 d | 4.41 d (7.7) | 1 | 105.3 d | 4.27 d (7.1) | 1 | 105.1 d | 4.40 d (7.5) | 1 | 105.3 d | 4.34 d (7.5) |
| 2 | 75.0 d | 3.03 dd (7.8, 9.2) | 2 | 72.2 d | 3.26 t[a] | 2 | 75.3 d | 3.02 t (8.5, 8.5) | 2 | 72.3 d | 3.26 t[a] |
| 3 | 75.6 d | 3.10 t[a] | 3 | 73.1 d | 3.28 m | 3 | 75.7 d | 3.11 t[a] | 3 | 73.4 d | 3.28 m |
| 4 | 74.8 d | 2.84 t (9.1, 9.1) | 4 | 71.1 d | 3.36 m[b] | 4 | 75.0 d | 2.85 t (9.2, 9.2) | 4 | 71.3 d | 3.37 m[b] |
| 5 | 72.8 d | 3.20 m | 5 | 70.7 d | 3.48 m | 5 | 73.0 d | 3.19 m | 5 | 71.0 d | 3.47 m |
| 6 | 17.6 q | 1.16 d (6.1) | 6 | 16.9 q | 1.10 d (6.4) | 6 | 17.8 q | 1.15 d (6) | 6 | 16.9 q | 1.07 d (6.5) |

[a] Triplet observed in HSQC, showing two large coupling constants.
[b] Multiplet, showing only small coupling constants in HSQC.

Calbiochem), a membrane-potential-sensitive dye (Molecular Devices, product R8034), and the test compound of interest (or an equivalent volume of sodium-free buffer) The final well-volume was 20 μl in all experiments. Dye loading was allowed to proceed for 1 h at room temperature, and plates were then transferred to a FLIPR384 for processing. A membrane potential response to sodium flux through cypermethrin-modified NaV1.8 channels was initiated by the addition of 6.67 μl of 600 mM NaCl (a 4 × concentration) to each well (final $Na^+$ concentration = 150 mM). Fluorescence (RFU, max–min) was monitored for 150 s. All observations were performed using duplicate wells with the effect of test compounds being expressed as a % control signal.

The $IC_{50}$ value of **1** for NaV1.8 channel block was determined to be ~9 μM, shown in Figure 3. Compound **2** was not tested due to the limited quantity of material. To our best knowledge this is the first report of saponin type natural product possessing inhibitory activity of NaV1.8 channel.

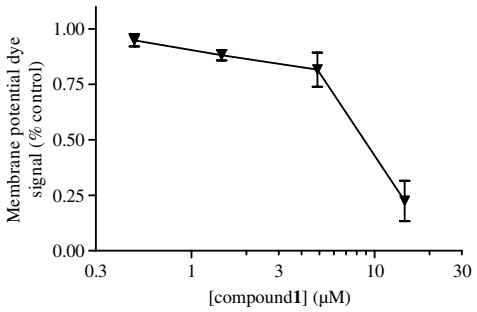


**Figure 3.** Dose–response curve for compound **1** in the NaV1.8 assay.

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