Inhibition of N-Type Calcium Channels by Fluorophenoxyanilide Derivatives

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Abstract: A set of fluorophenoxyanilides, designed to be simplified analogues of previously reported ω-conotoxin GVIA mimetics, were prepared and tested for N-type calcium channel inhibition in a SH-SY5Y neuroblastoma FLIPR assay. N-type or Ca\textsubscript{v}2.2 channel is a validated target for the treatment of refractory chronic pain. Despite being significantly less complex than the originally designed mimetics, up to a seven-fold improvement in activity was observed.

Keywords: N-type calcium channel; Ca\textsubscript{v}2.2; channel blocker; pain; FLIPR
1. Introduction

Neuropathic pain, which results from nerve damage caused by surgery, trauma, infection or disease, often does not respond to existing therapies [1,2]. Various estimates put the proportion of the world’s population afflicted by this condition to be at least 3%, with up to 5% of postoperative patients being affected. Safe and effective therapies for neuropathic pain are therefore a major unmet medical need. N-Type calcium channels (Ca\textsubscript{2.2} channels) are strongly implicated in chronic and neuropathic pain and their inhibitors have been widely pursued [1–4]. This approach has been best validated by Ziconotide (Prialt\textsuperscript{®}), a synthetic version of the peptide ω-conotoxin MVIIA found in the venom of a fish-hunting marine cone snail *Conus magnus*. This peptide selectively targets Ca\textsubscript{2.2} channels and is one of the very few effective drugs used to treat intractable chronic pain [5]. However, its intrathecal mode of delivery and narrow therapeutic window make it less than ideal as a treatment option.

We as well as others have been developing small-molecule inhibitors of Ca\textsubscript{2.2} channels as possible alternatives to Ziconotide [6–29]. Recently clinical development of Z160 (1, Figure 1), a reformulated form of NP118809 [8], was discontinued after Z160 (1) failed to meet the primary endpoint in Phase II clinical studies [30]. As a result there is only one compound that targets Ca\textsubscript{2.2} channels currently in clinical trials for the treatment of chronic pain, CNV2197944, the structure of which is yet to be disclosed [24].

![Figure 1. Chemical structure of Z160/NP118809 (1).](image)

As part of an ongoing program to develop new small-molecule inhibitors of Ca\textsubscript{2.2} channels, the pharmacophore of ω-conotoxin GVIA, a 27 residue peptide present in the venom of the cone snail *Conus geographus*, has been investigated. This peptide binds essentially irreversibly to the Ca\textsubscript{2.2} channel, making it unattractive as a therapeutic, however its well-defined structure has facilitated the development of peptidomimetics. A number of such mimetics have been disclosed [25–29], developed using the α,β-bond vector strategy described by Bartlett and Lauri [31], combined with interactive de novo design [32]. In these mimetics the biologically relevant tyrosine, lysine and arginine side chain mimics are projected from a central scaffold, as illustrated by the anthranilamide derivative (2) (Figure 2) [27,32]. Guided by the results of a radioligand-displacement assay, it has been concluded that with these anthranilamide-based mimetics (Compounds 2–4, Figure 2) the optimum length of the alkyl side chains is \( n = 7 \) for the lysine mimic and \( n = 3 \) for the arginine mimic. It was also found that the replacement of the phenol functionality with fluorine is well tolerated [25]. The most potent fluorinated analogue in this series was found to be the diguanidino compound (3) while the corresponding diamino compound (4) had comparatively weak activity. This is consistent with previous results where truncation of the arginine side chain mimic to an amine in ω-conotoxin GVIA mimetics is typically detrimental to activity at the N-type channel [25–29].
Figure 2. Structures of previously synthesised anthranilamide-based ω-conotoxin GVIA mimetics (2–4) [25,27].

Figure 3. Analogues 5a–c and 6a–c targeted in this study.
In order to transition conotoxin mimics towards more drug-like compounds, a number of their physiochemical properties need to be adjusted. Marketed central nervous system (CNS) active drugs, for example, tend to have much lower molecular weights, percentage polar surface areas, total number of nitrogen and oxygen atoms, and hydrogen bond acceptors and donors than are found in mimetics like 2. We have therefore embarked on a program of molecular modifications aimed at improving the physiochemical properties of this class of conotoxin mimics while retaining activity at the N-type calcium channel. A major priority has been to reduce overall molecular weight. Encouraged by favourable results obtained with the simplification of a benzothiazole class of mimetics, which involved the deletion of one of the amino acid side chain mimics [28], a similar strategy has been pursued with the anthranilamides. Thus, in the study described here, the effect on activity of the deletion of the lysine side chain mimic in compounds 2–4 has been investigated, together with the SAR related to the substitution pattern of the central aromatic ring (ortho, meta or para). The amino analogues and their corresponding monoguanidino analogues that were synthesised and tested in this study are shown in Figure 3, compounds 5a–c and 6a–c.

2. Results and Discussion

2.1. Chemistry

The previously described synthetic route to the anthranilamide-based mimics (3 and 4) [25] was modified to allow incorporation of the chloropropoxybenzamide moiety and its subsequent derivatisation. The ortho and para phenoxy anilines (10a [33,34] and 10c [25]) were readily available and the required meta phenoxy aniline (10b) was synthesized in two steps from 4-fluorophenyl boronic acid (7) and meta-nitrophenol (8) via the intermediate phenoxy nitrobenzene (9) (Scheme 1).

![Scheme 1. Synthesis of meta-(4-fluorophenoxy)aniline (10b). Reagents and conditions: (a) Cu(OAc)$_2$, Et$_3$N, air, 4 Å molecular sieves, dichloromethane (DCM), room temperature (RT), 24 h 83%; (b) Pd/C, NH$_2$NH$_2$·H$_2$O, EtOH, RT, 4 h, 95%.](image)

With the required 4-fluorophenoxyanilines (10a–c) in hand, the desired ortho, meta and para amino phenoxy anilides (5a–c) and monoguanidino phenoxy anilides (6a–c) were synthesised, as outlined in Scheme 2. Reaction of the phenoxy aniline (10a–c) with 4-(3-chloropropoxy)benzoic acid [35,36], using either carbodiimide activation [37] or formation of the acid chloride, gave the desired chloro compounds (11a–c). Subsequent conversion to the azide (12a–c) with sodium azide, followed by a transfer-hydrogenation reaction provided the corresponding amines (5a–c). Treatment of amines (5a–c) with 1H-pyrazole-carboxamidine [38] furnished the phenoxy anilides (6a–c) as hydrochloride salts.
Scheme 2. Synthesis of phenoxy anilides (5a–c and 6a–c). Reagents and conditions: (a) 4-(3-chloropropoxy)benzoic acid, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC·HCl), 4-dimethylaminopyridine (DMAP), Et3N, DCM/tetrahydrofuran (THF), (11a) 47%, (11b) 81% or 4-(3-chloropropoxy)benzoyl chloride, THF, (11c) 70%; (b) NaN₃, dimethyl sulfoxide (DMSO), 70 °C, (12a) 95%, (12b) 99%, (12c) 92%; (c) Pd/C, NH₂NH₂·H₂O, MeOH, (5a–c) quant.; (d) 1H-pyrazole-1-carboximidide hydrochloride, N,N-diisopropylethylamine (DIPEA), dimethylformamide (DMF), (6a–c) quant.

2.2. Biology

It has been shown that SH-SY5Y neuroblastoma cells endogenously express human Caᵥ₂.2 channels allowing the rapid screening of potential channel blockers by means of a FLIPR assay [39]. The synthesised compounds, the amino (5a–c) and the guanidinium (6a–c) analogues, as well as the anthranilamide-based mimetic (3), were evaluated for their ability to inhibit Caᵥ₂.2 calcium responses in SH-SY5Y cells in the presence of the L-type calcium channel blocker nifedipine. It was found that Ca²⁺ ion channel responses elicited by KCl-mediated depolarization were inhibited in a dose-dependent manner (Table 1). Compound 3 only partially inhibited responses at a concentration of 1 mM, resulting in an estimated IC₅₀ value of 1452 µM. In contrast, 5a and 5b fully blocked KCl-induced Ca²⁺ responses with IC₅₀ values of 46 µM and 35 µM, respectively. In the guanidinium series, 6a and 6b retained weaker activity with IC₅₀ values of 124 µM and 185 µM respectively.[40] Both para substituted compounds, 5c and 6c, were only weakly active and partially inhibited responses with IC₅₀ values of 764 µM and 723 µM, respectively.

Compared to compound 3, compounds 5a–c and 6a–c are significantly less complex and have molecular weights reduced by 33%–45%. It is encouraging, therefore, to find that all but 5c and 6c are considerably more active than 3. A relationship between the substitution pattern around the central aromatic ring and biological activity can also be clearly seen, with the ortho and meta analogues...
showing considerably stronger activity than the *para* analogues. It is also interesting to note that the amino compounds 5a–b are three to five fold more active than the guanidino compounds 6a–b.

Table 1. Functional inhibition of calcium channels by compounds 3, 5a–c, 6a–c.

| Compound          | logIC₅₀ | SEM | IC₅₀ (µM) | 95% CI (µM) |
|-------------------|---------|-----|-----------|-------------|
| 3                 | ~−2.84  | 0.29| 1452 ᵃ     | 380–5550    |
| 5a                | −4.34   | 0.01| 46 ᵃ      | 44–48       |
| 6a                | −3.90   | 0.03| 124 ᵃ     | 107–150     |
| 5b                | −4.45   | 0.01| 35 ᵃ      | 33–37       |
| 6b                | −3.73   | 0.02| 185 ᵃ     | 169–203     |
| 5c                | ~−3.12  | 0.06| 764 ᵄ     | 575–1020    |
| 6c                | −3.14   | 0.10| 723 ᵄ     | 447–1170    |

ᵃ This compound gave an IC₅₀ of 6 µM in a radioligand displacement assay with ¹²⁵I-GVIA [25] but it has since been established that it is less potent in an assay of functional activity at Ca₂⁺.² [29]; ᵃ Max inhibition, 15% at 300 µM; ᵄ Max inhibition, 65% at 300 µM; ᵄ Max inhibition, 30% at 300 µM; ᵄ Max inhibition, 19% at 300 µM.

3. Experimental Section

3.1. Chemistry

3.1.1. General Experimental Procedures

Starting materials and reagents were purchased from Sigma-Aldrich (Sydney, Australia) and used without purification. Solvents were dried, when necessary, using standard methods. Normal phase flash chromatography was performed on Merck silica gel No. 9385. Spectra were recorded on a Bruker Av400 or Av600 spectrometer (Fallanden, Switzerland). NMR spectra were referenced to residual solvent peak [chloroform (δH 7.26, δC 77.2), methanol (δH 4.87, 3.30, δC 49.0)]. The units for all coupling constants (J) are in hertz (Hz). ‡ Denotes signals only observed in 2D NMR. Mass spectrometry (APCI) was performed on a Thermo Scientific Q-Exactive FTMS. High-resolution mass spectra were recorded on a Waters Q-TOF II (Manchester, UK) or Thermo Scientific Q-Exactive FTMS mass spectrometer (Bremen, Germany). Melting points were recorded on a Stuart Scientific Melting Point Apparatus SMP3. Infrared spectra were recorded on a Perkin-Elmer RXI FTIR Spectrometer as thin films. Preparative HPLC was performed on a Waters Prep LC 4000 System using an Alltima C18 column (22 × 250 mm, 5 micron), detection at 237 nm. Mobile phase 12 mL/min 30% CAN/H₂O/0.2% TFA isocratic for 135 min then 115 min gradient to 100% ACN containing 0.2% TFA.

3.1.2. Synthesis

4-(3-Chloropropoxy)-N-(2-(4-fluorophenoxy)phenyl)benzamide (11a)

Alkyl chloride 11a was synthesised using a modified procedure outlined by Altin *et al.* [37]. A solution of 4-(3-chloropropoxy)benzoic acid [36] (1.27 g, 5.91 mmol) in dry THF (50 mL) was stirred under N₂ at room temperature. Triethylamine (0.80 mL, 600 mg, 6.22 mmol) and DMAP (340 mg, 2.79 mmol) were added to the reaction mixture, followed by EDC·HCl (867 mg, 4.54 mmol). After
15 min a solution of the 2-(4-fluorophenoxy)aniline 10a [33,34] (800 mg, 3.94 mmol) in dry DCM (20 mL) was added and the reaction mixture was stirred under N\textsubscript{2} atmosphere at room temperature. After 48 h the THF was removed in vacuo before the residue was taken up in DCM (50 mL). The organic layer was washed with saturated NaHCO\textsubscript{3} (3 × 50 mL), dried with Na\textsubscript{2}SO\textsubscript{4} and concentrated to afford a brown oil. Purification by column chromatography (hexanes: EtOAc; 7:2) yielded the alky chloride 11a as a colourless solid (711 mg, 47%). Mp: 101.5–103.5 °C. IR (ATR): 3333 s, 3113 w, 2942 w, 2873 s, 265 s, 1497 s, 1446 s, 1310 m, 1250 s, 1196 s, 1173 s, 1036 m, 829 m, 764 m cm\textsuperscript{-1}.\textsuperscript{1}\textsuperscript{H} NMR (400 MHz, CDCl\textsubscript{3}): \(\delta\) 8.59 (dd, \(J = 8.2, 1.5\) Hz, 1H), 8.41 (br s, 1H), 7.81–7.77 (m, 2H), 7.19–7.14 (m, 1H), 7.09–7.01 (m, 5H), 6.97–6.94 (m, 2H), 6.83 (dd, \(J = 8.2, 1.5\) Hz), 4.17 (t, \(J = 6.0\) Hz, 2H), 3.75 (t, \(J = 6.0\) Hz, 2H). \textsuperscript{13}C NMR (100 MHz, CDCl\textsubscript{3}): 165.0, 161.9, 159.4 (d, \(J = 241.3\) Hz), 152.3, 146.4, 130.0, 129.2, 127.5, 124.3, 124.1, 121.1, 120.5 (d, \(J = 8.3\) Hz), 117.3, 116.8 (d, \(J = 23.5\) Hz), 114.7, 64.7, 41.4, 32.2. \textsuperscript{19}F NMR (376 MHz, CDCl\textsubscript{3}): \(\delta\) −119.4. LRMS (APCI): \(m/z\) 407.2 [M + H]\textsuperscript{+} (100%), 288.1 [M − C\textsubscript{6}H\textsubscript{4}F\textsubscript{2}]\textsuperscript{+} (48%). HRMS (APCI): \(m/z\) calcd. For C\textsubscript{22}H\textsubscript{19}F\textsubscript{5}ClFNO\textsubscript{3} [M + H]\textsuperscript{+}: 399.1032, found: 399.1032.

4-(3-Azidopropoxy)-N-(2-(4-fluorophenoxy)phenyl)benzamide (12a)

The azide 12a was synthesised according to a procedure outlined by Alvarez et al. [41] Sodium azide (95 mg, 1.5 mmol) was added to a solution of alkyl chloride 11a (0.45 g, 1.1 mmol) in dry DCM (3 mL) and stirred under N\textsubscript{2} atmosphere at 70 °C. After 48 h, the reaction mixture was allowed to cool in vacuo before the residue was taken up in DCM (50 mL), dried with Na\textsubscript{2}SO\textsubscript{4} and concentrated to provide azide 12a as a pale brown oil (377 mg, 95%). \textsuperscript{1}\textsuperscript{H} NMR spectroscopy deemed this solid to be pure enough to use in the next step. Mp: 69.2–71.2 °C. IR (ATR): 3333 s, 3113 w, 2942 w, 2873 s, 265 s, 1497 s, 1446 s, 1310 m, 1250 s, 1196 s, 1173 s, 1036 m, 829 m, 764 m cm\textsuperscript{-1}.\textsuperscript{1}\textsuperscript{H} NMR (400 MHz, CDCl\textsubscript{3}): \(\delta\) 8.59 (dd, \(J = 8.2, 1.6\) Hz, 1H), 8.41 (br s, 1H), 7.81–7.77 (m, 2H), 7.18–7.14 (m, 1H), 7.09–7.00 (m, 5H), 6.96–6.93 (m, 2H), 6.83 (dd, \(J = 8.2, 1.4\) Hz, 1H), 4.10 (t, \(J = 6.0\) Hz, 2H), 3.52 (t, \(J = 6.0\) Hz, 2H), 2.07 (quin, \(J = 6.0\) Hz, 2H). \textsuperscript{13}C NMR (100 MHz, CDCl\textsubscript{3}): \(\delta\) 166.9, 161.7, 159.3 (d, \(J = 241.4\) Hz), 152.3, 146.4, 129.9, 129.1, 127.5, 124.3, 124.1, 121.0, 120.4 (d, \(J = 8.3\) Hz), 117.2, 116.7 (d, \(J = 23.3\) Hz), 114.6, 64.9, 48.2, 28.8. \textsuperscript{19}F NMR (376 MHz, CDCl\textsubscript{3}): \(\delta\) −119.4. LRMS (APCI): \(m/z\) 407.2 [M + H]\textsuperscript{+} (100%), 288.1 [M − C\textsubscript{6}H\textsubscript{4}F\textsubscript{2}]\textsuperscript{+} (48%). HRMS (APCI): \(m/z\) calcd. For C\textsubscript{22}H\textsubscript{19}F\textsubscript{5}ClFNO\textsubscript{3} [M + H]\textsuperscript{+}: 399.1032, found: 406.1438.

4-(3-Aminopropoxy)-N-(2-(4-fluorophenoxy)phenyl)benzamide (5a)

A solution of azide 12a (100 mg, 0.25 mmol), 10% Pd/C (12 mg) and hydrazine monohydrate (30 µL, 30 mg, 0.62 mmol) in MeOH (3 mL) was vigorously stirred under N\textsubscript{2} at room temperature. After 20 min, the reaction mixture was filtered through Celite™ and concentrated to provide the amino compound 5a as yellow oil in a quantitative crude yield. A small amount was purified by reversed-phase HPLC to give a sample for biological testing. IR (ATR): 3432 br, 3071 w, 2942 w, 2873 w, 1653 s, 1493 s, 1378 m, 1248 s, 1203 s, 1173 s, 838 m, 758 m cm\textsuperscript{-1}.\textsuperscript{1}\textsuperscript{H} NMR
(600 MHz, CD$_2$OD): $\delta$ 7.90–7.89 (m, 1H), 7.76–7.74 (m, 2H), 7.18–7.17 (m, 2H), 7.05–6.99 (m, 7H), 4.08 (t, $J = 6.2$ Hz, 2H), 2.81 (t, $J = 7.0$ Hz, 2H), 1.95–1.91 (m, 2H). $^{13}$C NMR (150 MHz, CD$_2$OD): $\delta$ 168.2, 163.5, 160.7 (d, $J = 238.9$ Hz), 154.3, 150.8, 130.5, 130.4, 127.6, 127.4, 126.7, 125.0, 120.8 (d, $J = 7.8$ Hz), 120.0, 117.2 (d, $J = 23.6$ Hz), 115.3, 67.3, 39.6, 33.3. $^{19}$F NMR (376 MHz, CD$_2$OD): $\delta$ −120.5. LRMS (APCI): $m/z$ 381.2 [M + H]$^+$ (50%), 380.2 [M]$^+$ (100%). HRMS (APCI): $m/z$ calc. For C$_{22}$H$_{21}$FN$_2$O$_3$ [M]$^{3+}$: 380.1531, found: 380.1532.

N-(2-(4-Fluorophenoxy)phenyl)-4-(3-guanidinopropoxy)benzamide hydrochloride (6a)

The guanidinylated compound 6a was synthesised according to a modified procedure by Bernatowicz et al. [38]. Amine 5a (102 mg, 0.24 mmol), DIPEA (42 µL, 31 mg, 0.24 mmol), 1H-pyrazole-1-carboximidic hydrochloride (35 mg, 0.24 mmol) and DMF (2 mL) were combined and stirred vigorously under N$_2$ atmosphere at room temperature. After 18 h TLC analysis revealed that starting material remained, additional 1H-pyrazole-1-carboximidic hydrochloride (9 mg, 0.06 mmol) and DIPEA (10 µL, 8 mg, 0.06 mmol) were added. After a further 24 h the solvent was removed in vacuo to yield the guanidinylated compound 6a as crystalline product. This solid was dissolved in MeOH (1 mL) and the product precipitated by addition of Et$_2$O (10 mL). After removal of the residual solvent the product was obtained as a crystalline solid (47 mg, 39%) IR (ATR): 3307 br s, 3251 br s, 1593, 1513, 1497, 1434, 1245 s, 1196 s, 1174 s, 840 m, 759 m cm$^{-1}$. $^1$H NMR (400 MHz, CD$_2$OD): $\delta$ 7.88–7.85 (m, 1H), 7.78–7.76 (m, 2H), 7.21–7.18 (m, 2H), 7.07–6.98 (m, 6H), 6.97–6.94 (m, 1H), 4.13 (t, $J = 5.9$ Hz, 2H), 3.41 (t, $J = 6.8$ Hz, 2H), 2.11–2.05 (m, 2H). $^{13}$C NMR (100 MHz, CD$_2$OD): $\delta$ 168.2, 163.2, 160.2 (d, $J = 239$ Hz), 158.7, 154.4, 151.0, 130.5, 130.4, 127.9, 127.6, 126.9, 125.0, 120.8 (d, $J = 8.3$ Hz), 120.1, 117.2 (d, $J = 23.4$ Hz), 115.4, 66.3, 39.5, 29.5. $^{19}$F NMR (376 MHz, CD$_2$OD): $\delta$ −120.6. LRMS (APCI): $m/z$ 422.2 [M]$^{3+}$ (62%), 380.15 [M − CH$_2$N$_2$]$^+$ (100%). HRMS (ESI): $m/z$ calc. For C$_{23}$H$_{23}$FN$_2$O$_3$ [M]$^{3+}$: 422.1749, found: 422.1750.

1-(4-Fluorophenoxy)-3-nitrobenzene (9)

The fluoro diaryl ether 9 was synthesised according to a general procedure outlined by Evans et al. [42] 4-Fluorophenyl boronic acid 7 (300 mg, 2.16 mmol), m-nitrophenol 8 (200 mg, 1.44 mmol), Cu(OAc)$_2$ (260 mg, 1.44 mmol) and freshly activated powdered 4 Å molecular sieves were added to dry DCM (15 mL). Et$_3$N (1.0 mL, 730 mg, 7.19 mmol) was then added to the reaction mixture. The reaction left open to air through a drying tube and stirred at room temperature, and reaction progress was monitored by TLC (hexanes: EtOAc; 30:1). After 24 h, the resulting slurry was filtered through Celite™ and concentrated to give a brown oil. The crude product was purified by flash column chromatography (hexanes: EtOAc; 30:1) to give the fluoro diaryl ether 9 as a yellow oil (280 mg, 83%). IR (ATR): 1526 s, 1497 s, 1473 s, 1352 s, 1212 s, 1187 s, 811 s, 734 s cm$^{-1}$; $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 7.93 (ddd, $J = 8.2$, 2.3, 0.9 Hz, 1H), 7.75 (t, $J = 2.3$ Hz, 1H), 7.48 (t, $J = 8.2$ Hz, 1H) 7.29 (ddd, $J = 8.2$, 2.3, 0.9 Hz, 1H), 7.13–7.08 (m, 2H), 7.07–7.02 (m, 2H). $^{13}$C NMR (100 MHz, CDCl$_3$) 159.8 (d, $J = 242.4$ Hz), 159.0, 151.3, 149.5, 130.5, 123.8, 121.7 (d, $J = 8.4$ Hz), 117.8, 117.1 (d, $J = 23.2$ Hz), 112.4. $^{19}$F NMR (376 MHz, CDCl$_3$): $\delta$ −118.1. LRMS (APCI): $m/z$ 234.1 [M + H]$^+$ (100%), 204.1 [M − O$_2$ + 2H]$^+$ (32%), 188.1 [M − NO$_2$]$^+$ (24%). HRMS (APCI): $m/z$ calc. for C$_{12}$H$_{10}$FNO$_3$ [M + H]$^+$: 234.0561, found: 234.0562.
3-(4-Fluorophenoxy)aniline (10b)

The m-aniline 10b was prepared following a procedure similar to that of Bavin et al. [43] Hydrazine monohydrate (790 mg, 0.77 mL, 16 mmol) was added to a deoxygenated mixture of the nitroarene 9 (0.50 g, 2.1 mmol), 10% Pd/C (50 mg) and EtOH (25 mL). This reaction mixture was then refluxed under N₂ until TLC analysis (hexanes:EtOAc; 4:1) showed no starting material (typically 4 h). The reaction mixture was allowed to cool, filtered through Celite™, concentrated in vacuo and the colourless residue purified by column chromatography (hexanes: EtOAc; 4:1) to yield the m-aniline 10b as a colourless oil (410 mg, 95%). All spectral data was in accordance with that in the literature [44].

IR (ATR): 3462 s, 3317 s, 1619 m, 1584 m, 1485 s, 1282 m, 1193 s, 1141 s, 832 m, 772 m cm⁻¹.

1H NMR (400 MHz, CDCl₃): δ 7.08 (t, J = 8.0, 1H) 7.04–6.96 (m, 4H) 6.42 (ddd, J = 8.0, 2.2, 0.8 Hz, 1H), 6.35 (ddd, J = 8.0, 2.2, 1.8, 1H), 6.30 (t, J = 2.2 Hz, 1H), 3.70 (bs, 2H).

13C NMR (100 MHz, CDCl₃): δ 159.0, 158.7 (d, J = 8.3 Hz), 116.3 (d, J = 23.1 Hz), 110.1, 108.3, 105.0.

19F NMR (376 MHz, CDCl₃): δ −120.5. LRMS (APCI): m/z 204.1 [M + H]+ (100%). HRMS (APCI): m/z calcd. for C₁₂H₁₀FNO [M + H]+: 204.0819, found: 204.0820.

4-(3-Chloropropoxy)-N-(3-(4-fluorophenoxy)phenyl)benzamide (11b)

The alkyl chloride 11b was synthesised using a modified procedure outlined by Altin et al. [37]. A solution of 4-(3-chloropropoxy)benzoic acid [33,34] (910 mg, 4.24 mmol), in dry THF (50 mL) was stirred under N₂ at room temperature. EtsN (0.87 mL, 630 mg, 6.22 mmol), and DMAP (340 mg, 2.79 mmol) were then added to the reaction mixture, followed by EDC·HCl (867 mg, 4.53 mmol). After 15 min, a solution of the 3-(4-fluorophenoxy)aniline 10b (575 mg, 2.83 mmol) in dry DCM (20 mL) was added, and the reaction mixture was stirred under a N₂ atmosphere at room temperature. After 48 hours the THF was removed in vacuo before being taken up into DCM (50 mL). The organic layer was then washed with NaHCO₃ (3 × 50 mL), dried with Na₂SO₄ and concentrated to afford a light brown oil. Purification by column chromatography (hexanes: EtOAc; 7:2) yielded the alkyl chloride 11b as a colourless solid (917 mg, 81%). Mp: 116.5 °C. IR (ATR): 3366 s, 1652 s, 1601 s, 1528 m, 1500 s, 1442 s, 1267 m, 1246 s, 1196 s, 846 m, 773 s cm⁻¹.

1H NMR (400 MHz, CDCl₃): δ 7.82–7.89 (m, 2H), 7.75 (bs, 1H), 7.35–7.27 (m, 3H), 7.06–7.00 (m, 4H), 6.98–6.94 (m, 2H), 6.74 (dt, J = 7.2, 2.0 Hz, 1H), 4.18 (t, J = 6.0 Hz, 2H), 3.76 (t, J = 6.0 Hz, 2H), 2.27 (quin, J = 6.0 Hz, 2H).

13C NMR (100 MHz, CDCl₃): δ 165.2, 161.9, 159.1 (d, J = 240.4 Hz), 158.5, 152.7, 139.7, 130.3, 129.1, 127.3, 121.0 (d, J = 8.3 Hz), 116.5 (d, J = 23.0 Hz), 114.8, 144.7, 141.4, 110.2, 64.7, 41.4, 32.2.

19F NMR (376 MHz, CDCl₃): δ −120.2. LRMS (APCI): m/z 400.1 [M + H]+ (100%). HRMS (APCI): m/z calcd. for C₂₂H₂₀ClFNO₃ [M + H]+: 400.1110, found: 400.1113.

4-(3-Azidopropoxy)-N-(3-(4-fluorophenoxy)phenyl)benzamide (12b)

The azide 12b was synthesised according to a procedure outlined by Alvarez et al. [41]. Sodium azide (219 mg, 3.38 mmol) was added to a solution of the alkyl chloride 11b (900 mg, 2.25 mmol) in DMSO (4 mL) and stirred under N₂ at 70 °C. After 48 h, the reaction was allowed to cool and DCM (40 mL) was added. (CAUTION: It is recommended that DCM be substituted with diethyl ether for larger scale reactions, to avoid the formation of hazardous side products, such as azido-chloromethane
and diazidomethane) The organic layer was washed with brine (5 × 100 mL), dried with Na₂SO₄ and concentrated to provide the azide 12b as a colourless solid (902 mg, 99%), which was deemed sufficiently pure by ¹H NMR spectroscopy for use in the next step. Mp: 104.6–106.2 °C. IR (ATR): 3366 s, 2104 s, 1640 s, 1593 s, 1499 s, 1441 s, 1248 s, 1199 s, 848 m, 770 m cm⁻¹. ¹H NMR (400 MHz, CDCl₃): δ 7.82–7.79 (m, 2H), 7.76 (bs, 1H), 7.35–7.27 (m, 3H), 7.06–7.00 (m, 4H), 6.97–6.93 (m, 2H), 6.74 (dt, J = 7.0, 2.2 Hz, 1H), 4.11 (t, J = 6.3 Hz, 2H), 3.53 (t, J = 6.3 Hz, 2H), 2.08 (quin, J = 6.3 Hz, 2H). ¹³C NMR (100 MHz, CDCl₃): δ 165.3, 161.9, 159.1 (d, J = 240.5 Hz), 158.6, 152.7, 139.7, 130.3, 129.1, 127.3, 121.0 (d, J = 8.4 Hz), 116.5 (d, J = 23.2 Hz), 114.8, 114.7, 114.2, 110.2, 65.0, 48.3, 28.9. ¹⁹F NMR (376 MHz, CDCl₃): δ –120.2. LRMS (APCI): m/z 407.2 [M + H]⁺ (100%). HRMS (APCI): m/z calcd. For C₂₂H₂₀FN₅O₃ [M + H]⁺: 407.1514, found: 407.1514.

4-(3-Aminopropoxy)-N-(3-(4-fluorophenoxo)phenyl)benzamide (5b)

A solution of the azide 12b (100 mg, 0.25 mmol), 10% Pd/C (12 mg) and hydrazine monohydrate (30 µL, 30 mg, 0.62 mmol) in MeOH (3 mL) were vigorously stirred under a N₂ atmosphere at room temperature. After 20 mins, the reaction mixture was filtered through Celite™ and concentrated to provide the amino compound 5b as a colourless oil in a quantitative crude conversion. IR (ATR): 3280 br s, 1647 s, 1597 m, 1540 m), 1484 s, 1244 s, 1195 s, 837 s, 779 s, 760 s, 684 s cm⁻¹. ¹H NMR (400 MHz, CD₂OD): δ 7.90–7.87 (m, 2H), 7.45 (t, J = 2.0 Hz, 1H), 7.39 (ddd, J = 8.0, 2.1, 1.0 Hz, 1H), 7.31 (t, J = 8.0 Hz, 1H), 7.13–7.01 (m, 6H), 6.73 (ddd, J = 8.0, 2.1, 1.0 Hz, 1H), 4.13 (t, J = 6.6 Hz, 2H), 2.84 (br t, J = 6.6 Hz, 2H), 1.96 (quin, 6.6 Hz, 2H). ¹³C NMR (100 MHz, CD₂OD): δ 168.4, 163.5, 160.3 (d, J = 238.9 Hz), 159.5, 154.3, 141.6, 130.9, 130.6, 128.0, 121.9 (d, J = 8.3 Hz), 117.3 (d, J = 23.5 Hz), 116.7, 115.3, 114.9, 112.0, 67.3, 39.6, 33.0. ¹⁹F NMR (376 MHz, CD₂OD): δ –120.5. LRMS (APCI): m/z 380.2 [M⁺] (100%), 381.2 [M + H⁺] (41%). HRMS (APCI): m/z calcd. For C₂₂H₂₁FN₅O₃ [M⁺]: 380.1532, found: 380.1531.

N-(3-(4-Fluorophenoxo)phenyl)-4-(3-guanidinopropoxy)benzamide hydrochloride (6b)

The guanidinylated compound 6b was synthesised according to a modified procedure by Bernatowicz et al. [38]. The amine 5b (47 mg, 0.12 mmol), DIPEA (35 µL, 26 mg, 0.20 mmol), 1H-pyrazole-1-carboximidic hydrochloride 27 (25 mg, 0.17 mmol) and DMF (2 mL) were added to a flask and stirred vigorously under a nitrogen atmosphere at room temperature. After 18 h, the solvent was removed in vacuo to yield the guanidinylated compound 6b as a colourless solid in quantitative conversion. IR (ATR): 3244 br s, 3150 br s, 1651 s, 1592 s, 1503 s, 1479 s, 1249 s, 1210 m, 1171 s, 826 m, 791 m, 692 m cm⁻¹. ¹H NMR (400 MHz, CD₂OD): δ 7.92–7.88 (m, 2H), 7.47 (t, J = 2.2 Hz, 1H), 7.41 (ddd, J = 8.1, 2.2, 0.9 Hz, 1H), 7.30 (t, J = 8.1 Hz, 1H), 7.11–7.01 (m, 6H), 6.72 (ddd, J = 8.1, 2.2, 0.9 Hz, 1H), 4.16 (t, J = 6.3 Hz, 2H), 3.42 (t, J = 6.3 Hz, 2H), 2.09 (quin, J = 6.3 Hz, 2H). ¹³C NMR (100 MHz, CD₂OD): 163.8, 163.2, 160.3 (d, J = 239.0 Hz), 159.9, 158.8, 154.3, 141.6, 130.9, 130.7, 128.4, 121.9 (d, J = 8.4 Hz), 117.3 (d, J = 23.3 Hz), 116.7, 115.3, 115.0, 112.0, 66.3, 39.6, 29.6. ¹⁹F NMR (376 MHz, CD₂OD): δ –120.5. LRMS (APCI): m/z 422.2 [M⁺] (32%), 381.2 [M – CHN₂]⁺ (35%), 380.2 [M – CH₂N₂]⁺ (100%). HRMS (APCI): m/z calcd. For C₂₅H₂₅FN₇O₅ [M⁺]: 422.1749, found: 422.1749.
4-(3-Chloropropoxy)-N-(4-(4-fluorophenoxy)phenyl)benzamide (11c)

4-(3-Chloropropoxy)benzoic acid (790 mg, 3.68 mmol) was taken up into SOCl₂ (2.00 mL, 3.28 g, 27.5 mmol) and the reaction mixture was stirred for 30 min. Excess SOCl₂ was removed under a stream of N₂ to provide an acid chloride intermediate. A solution of 4-(4-fluorophenoxy)aniline 10c (650 mg, 3.20 mmol) in dry THF (35 mL) was added, and the reaction mixture was stirred at room temperature for 18 h. The THF was removed in vacuo, and the resultant solid was taken up into EtOAc (60 mL) and washed with sat. NaHCO₃ (3 × 100 mL). The organic layer was dried with Na₂SO₄ and concentrated to afford the crude alkyl chloride 11c as a brown solid. The solid was recrystallised from hot EtOAc to give the desired alkyl chloride 11c as a colourless solid (900 mg, 70%). Mp: 147.3–148.1 °C. IR (ATR): 3338 s, 1642 s, 1607 m, 1496 s, 1259 s, 852 s, 764 m cm⁻¹. ¹H NMR (400 MHz, CDCl₃): δ 7.88–7.84 (m, 2H), 7.71 (br s, 1H), 7.61–7.58 (m, 2H), 7.07–6.97 (m, 8H), 4.22 (t, J = 6.0 Hz, 2H), 3.79 (t, J = 6.0 Hz, 2H), 2.30 (quin, J = 6.0 Hz, 2H). ¹³C NMR (100 MHz, CDCl₃): δ 165.3, 161.9, 158.9ⅰ (d, J = 239.7 Hz), 154.2, 153.4ⅰ, 133.7, 129.1, 127.4, 122.2, 120.2 (d, J = 8.2 Hz), 119.3, 116.4 (d, J = 23.1 Hz), 114.7, 64.7, 41.5, 32.2. ¹⁹F NMR (376 MHz, CDCl₃): δ −120.8. LRMS (APCI): m/z 400.1 [M + H]+ (75%), 399.1 [M]+ (100%). HRMS (APCI): m/z calcd. for C₂₂H₁₈ClFNO₃ [M]+: 399.1032, found: 399.1032.

4-(3-Azidopropoxy)-N-(4-(4-fluorophenoxy)phenyl)benzamide (12c)

The azide 12c was synthesised according to a procedure outlined by Alvarez et al. [41]. Sodium azide (63 mg, 0.97 mmol) was added to a solution of the alkyl chloride 11c (300 mg, 0.752 mmol) in DMSO (4 mL) and stirred under N₂ at 70 °C. After 48 h, the reaction mixture was cooled and DCM (40 mL) was added. (CAUTION: It is recommended that DCN₂ be substituted with diethyl ether for larger scale reactions, to avoid the formation of hazardous side products, such as azido-chloromethane and diazidomethane) The organic layer was washed with brine (5 × 100 mL), dried with Na₂SO₄ and concentrated to provide the azide 12c as a colourless solid (279 mg, 92%), which was deemed sufficiently pure by ¹H NMR spectroscopy for use in the next step. Mp: 120.7–122.0 °C. IR (ATR): 3325 s, 2099 s, 1648 s, 1605 m, 1494 s, 1250 s, 1208 s, 828 s, 764 m cm⁻¹. ¹H NMR (400 MHz, CDCl₃): δ 7.86–7.82 (m, 2H), 7.68 (br s, 1H), 7.60–7.56 (m, 2H), 7.05–6.95 (m, 8H), 4.13 (t, J = 6.0 Hz, 2H), 3.54 (t, J = 6.0 Hz, 2H), 2.09 (quin, J = 6.0 Hz, 2H). ¹³C NMR (100 MHz, CDCl₃): δ 165.3, 161.8, 158.8ⅰ (d, J = 261.0 Hz), 154.3, 153.4ⅰ, 133.7, 129.1, 127.4, 122.2, 120.2 (d, J = 8.3 Hz), 119.3, 116.4 (d, J = 23.2 Hz), 114.7, 65.0, 48.3, 28.9. ¹⁹F NMR (376 MHz, CDCl₃): δ −120.8. LRMS (APCI): m/z 406.1 [M + H]+ (100%). HRMS (APCI): m/z calcd. for C₂₂H₁₉ClFNO₃ [M]+: 406.1436, found: 406.1438.

4-(3-Aminopropoxy)-N-(4-(4-fluorophenoxy)phenyl)benzamide (5c)

A solution of the azide 11c (100 mg, 0.25 mmol), 10% Pd/C (12 mg) and hydrazine monohydrate (30 μL, 30 mg, 0.62 mmol) in MeOH (3 mL) were vigorously stirred under a N₂ atmosphere at room temperature. After 20 mins, the reaction mixture was filtered through Celite™ and concentrated to provide the amino compound 5c as a yellow oil in a quantitative crude conversion. IR (ATR): 3307 m, 3282 m, 1632 s, 1606 m, 1494 s, 1248 m, 1208 s, 820 s, 758 s cm⁻¹. ¹H NMR (400 MHz, CD₃OD): δ 7.92–7.89 (m, 2H), 7.66–7.62 (m, 2H), 7.10–6.95 (m, 8H), 4.14 (t, J = 6.2 Hz, 2H), 2.84 (t, J = 7.0 Hz,
N-(4-(4-Fluorophenoxy)phenyl)-4-(3-guanidinopropoxy)benzamide hydrochloride (6c)

The guanidinylated compound 6c was synthesised according to a modified procedure by Bernatowicz et al. [38]. The amine 5c (47 mg, 0.12 mmol), DIPEA (35 µL, 26 mg, 0.20 mmol), 1H-pyrazole-1-carboximidamide hydrochloride (25 mg, 0.17 mmol) and DMF (2 mL) were added to a flask and stirred vigorously under a nitrogen atmosphere at room temperature. After 18 h, the solvent was removed in vacuo to yield the guanidinylated compound 6c as a yellow oil with quantitative conversion. IR (ATR): 3272 m, 3185 m, 1631 s, 1601 s, 1495 s, 1437 s, 1393 s, 1247 s, 1208 s, 1177 s, 1024 s, 789 m. 1H NMR (600 MHz, CD3OD): δ 7.94–7.92 (m, 2H), 7.65–7.63 (m, 2H), 7.10–6.99 (m, 6H), 6.98–6.95 (m, 2H), 4.16 (t, J = 5.9 Hz, 2H), 3.43 (t, J = 6.8 Hz, 2H), 2.13–2.07 (m, 2H). 13C NMR (150 MHz, CD3OD): δ 168.3, 163.1, 159.5 (d, J = 240.9 Hz), 158.7, 155.5, 154.9, 135.4, 130.6, 128.3, 124.2, 121.2 (d, J = 8.0 Hz), 119.8, 117.2 (d, J = 24.1 Hz), 115.3, 66.3, 39.5, 29.6. 19F NMR (376 MHz, CD3OD): δ −120.9. LRMS (APCI): m/z 422.2 [M]+ (14%), 381.2 [M − CN3H]+ (30%), 380.2 [M − CH2N2]+ (100%), 323.1 [M-C3H9N3]+ (31%). HRMS (APCI): m/z calcd. for C23H25F3N4O3 [M]+: 422.1749, found: 422.1750.

3.2. Biology

Fluorescence Measurement of Calcium Responses

SH-SY5Y cells were plated at a density of 30,000–50,000 cells/well on 384-well black-walled imaging plates and loaded for 30 min at 37 °C with Calcium 4 no-wash dye (Molecular Devices, Sunnyvale, CA, USA) diluted in physiological salt solution (PSS; composition: 140 mM NaCl, 11.5 mM glucose, 5.9 mM KCl, 1.4 mM MgCl2, 1.2 mM NaH2PO4, 5 mM NaHCO3, 1.8 mM CaCl2, 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), pH 7.4). Calcium responses, elicited by addition of 90 mM KCl and 5 mM CaCl2 in the presence of 10 µM nifedipine, were measured using a FLIPRTETRA fluorescent plate reader (excitation, 470–495 nm; emission, 515–575 nm) after 5 min pre-treatment with test compounds. Fluorescent responses were plotted as response over baseline using ScreenWorks (Molecular Devices, version 3.1.1.4). Concentration-response curves of Calcium responses, normalized to control responses, were generated using GraphPad Prism (Version 4.00, San Diego, CA, USA) using a 4-parameter Hill equation with variable Hill slope and bottom >0 fitted to the data.

4. Conclusions

Despite being significantly less complex than the originally designed anthranilamide ω-conotoxin GVIA mimetics (e.g., 2), the simplified fluorophenoxanilides described here (5a–c and 6a–e) show enhanced activity in the SH-SY5Y FLIPR assay. The compounds with para-substitution around the central ring (5c and 6c) were found to have the weakest activity, suggesting that some type of...
pre-organisation through restricted rotation might enhance the activity of the ortho and meta analogues (5a, 5b, 6a and 6b). It is also unusual for the amines to be more active than the guanidines in this class of channel blocker. While primary amines typically do not make good drugs, this observation does open the possibility of developing N-type channel blockers capable of crossing the blood brain barrier. Compounds bearing very strongly basic functional groups like guanidine are unlikely to cross the blood brain barrier [45] whereas there are many CNS-active drugs that bear tertiary amines. The mode of action of the compounds reported here is currently being investigated in patch clamp electrophysiology experiments, the results of which will be reported in due course.

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Author Contributions

E.C.G. synthesised compounds 5a–c and 6a–c, J.E.G contributed to the initial synthetic strategy for compounds 5a and 6a, S.S. synthesised compound 6a, I.V. performed the FLIPR experiments and assisted with drafting of the manuscript, R.J.L. assisted with the FLIPR experiments and with drafting of manuscript, P.J.D. jointly conducted the channel blocker development, synthetic design, drafting of manuscript, K.L.T. jointly conducted the channel blocker development, synthetic design, drafting of manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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