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Synthesis and $Ca_v2.2$ Binding Data for Non-Peptide Mimetics of ω-Conotoxin GVIA based on a 5-Amino-Anthranilamide Core

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A simple and efficient method has been developed for the synthesis of two anthranilamide-based non-peptide mimetics of ω-conotoxin GVIA. These anthranilamide derivatives aim to mimic the K2, R17, and Y13 residues of the peptide. The synthetic route described enables the rapid synthesis of anthranilamide analogues with identical alkyl chain lengths. The target compounds show affinity to rat N-type voltage gated calcium channels ($Ca_v2.2$) with EC_{50} values of 42 and 75 μM.

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Introduction

The complex venom produced by marine snails of the genus *Conus* has provided medicinal chemists with the inspiration for the development of novel therapeutics.[1] Bioactive components of this venom include both low molecular weight compounds,[2] such as 5-hydroxytryptamine and serotonin, as well as peptides including conantokin,[3–5] conanlukin,[6] conopressin,[4] contryphan,[7] and the ω-conotoxins.[8] The attention of our research group has been drawn to the ω-conotoxins owing to their highly potent inhibition[9,10] of N-type voltage gated calcium channels (VGCCs, $Ca_v2.2$), involved in the pain transmission pathway.[11,12] These ω-conotoxins are a disulfide-rich class of polypeptides that are conformationally defined by the inhibitor 'cysteine knot' motif, a cysteine four loop structure formed by three intramolecular disulfide bonds.[13]

ω-Conotoxin GVIA (Fig. 1)[14] isolated from *Conus geographus* shows the most potent N-type VGCC binding of all conotoxins and shows attenuation of response to acute pain (ED_{50} 0.12 μg kg^{-1}) at 40 times greater potency than that of morphine (ED_{50} 4.36 μg kg^{-1}).[15,16] An additional advantage of ω-conotoxin GVIA over opioid-based analgesics is that there is no development of tolerance or addiction on repeated ω-conotoxin administration.[17,18]

Despite these promising discoveries, the native peptide has limited clinical potential, owing to very strong binding to the N-type VGCC receptors.[8] However, a related peptide ω-conotoxin MVIIA (Prialt, Zinconotide), isolated from *Conus magus*, was recently approved for clinical use for chronic pain where morphine-based therapeutics are not efficacious.[19] Another ω-conotoxin, CVID, isolated from *Conus catus*, reached Phase II clinical trials and was reported to have a more favourable therapeutic index than that of ω-conotoxin MVIIA.[20] Although successful analgesics, both peptides require intrathecal administration, i.e. injection near the spinal chord, to mediate beneficial effects, owing to a poor pharmacokinetic profile typical of peptide-based therapeutics.[19,21] NeuroMed's orally available small molecule N-type VGCC blocker, NMED-160, which reached Phase II clinical trials,[11,22] has recently been abandoned.[23] This highlights the need for development of more small molecule N-type VGCC blockers. An alternative approach, undertaken by our group, is the rational design of small molecule non-peptide mimetics of ω-conotoxin GVIA that act as N-type VGCC blockers.[24–26] As the binding epitopes and defined structure of ω-conotoxin GVIA have been extensively studied, this peptide is an ideal candidate for mimicry.[14,27,28] The solution structure of the native peptide was solved and published by Pallargy and Norton in 1999,[28] and alanine scanning undertaken by Lew[27] has identified Y13 and K2 as the most important amino acids for receptor binding. These amino acids, along with R17, also identified as important for receptor affinity, are part of the suggested receptor binding site on ω-conotoxin GVIA.[14,27,29]

Fig. 1. The amino acid sequence of ω-conotoxin GVIA showing cysteine connectivity, an amidated C terminus and hydroxylated prolines represented by O.[14]

Recently our group reported the synthesis and biological activity of anthranilamide (**1**) and benzothiazole (**2**) derivatives as non-peptide mimetics (see Fig. 2)[24,25] of ω-conotoxin GVIA that were designed to project the amino acids K2, Y13, and R17 with similar αβ bond vectors to those present in the native peptide. Binding affinities for four of the non-peptide mimetics are summarized in Table 1. For benzothiazole derivative binders,

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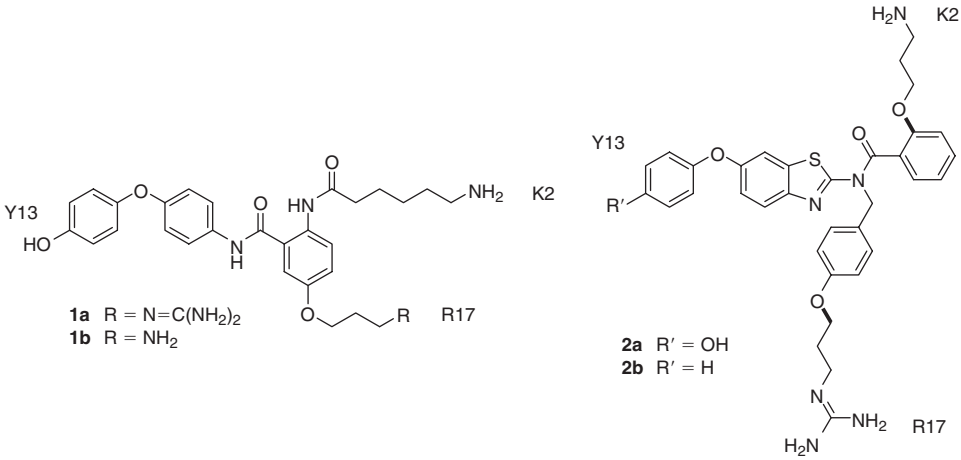


Fig. 2. ω-Conotoxin GVIA mimetics base on anthranilamide and benzothiazole cores.

Table 1. Binding potencies (μM) for ω-conotoxin GVIA and mimetics 1a, 1b, 2a, and 2b to N-type and P/Q-type voltage gated calcium channels

95% confidence intervals shown in parentheses[24,25]

Compound	EC_{50} N-type [μM]	EC_{50} P/Q-type [μM]
ω-conotoxin GVIA	3.0×10^{-5}	12 (1.4–90)
1a	3.5 (2.5–4.8)	111 (70–180)
1b	13.1 (9.5–18.1)	176 (140–220)
2a	1.9 (1.6–2.3)	42 (34–53)
2b	3.8 (2.9–4.9)	26 (20–32)

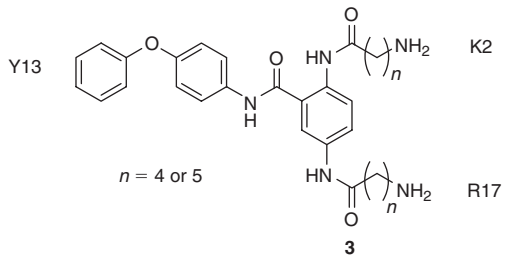


Fig. 3. The general structure of the non-peptide ω-conotoxin GVIA mimetics prepared and tested in the present study.

the omission of a hydroxyl group in the Y13 mimic resulted in little loss of binding ability. Similarly, in the anthranilamide series, an R17 mimic functionalized as an amine (**1b**) showed similar binding to that of the inhibitor with a guanidinium functionalized R17 mimic (**1a**) (Table 1).[24,25]

In the present publication, we report the synthesis and binding studies for amino-anthranilamide derivatives **3** (Fig. 3). The key modification in this class of derivatives is the incorporation of an amide linkage for the R17 mimic, replacing the ether linkage in **1**. This change enabled the rapid synthesis of inhibitors with the same K2 and R17 projection chain lengths. Other changes in design are the omission of the hydroxyl functionality of the Y13 mimic and replacement of the guanidinium group with an amine. These changes greatly simplified the synthetic path and it was anticipated that no great reduction in N-type VGCC affinity would occur, given that only a small loss in potency resulted when the hydroxyl and guanidinium functional groups were omitted in previous scaffolds.[24,25]

Mimetics with identical projection chains were synthesized in five steps in accordance with the synthetic route shown in Scheme 1. The chain lengths of $n = 4$ and 5 were chosen for projection of R17 and K2 mimics.

Results and Discussion

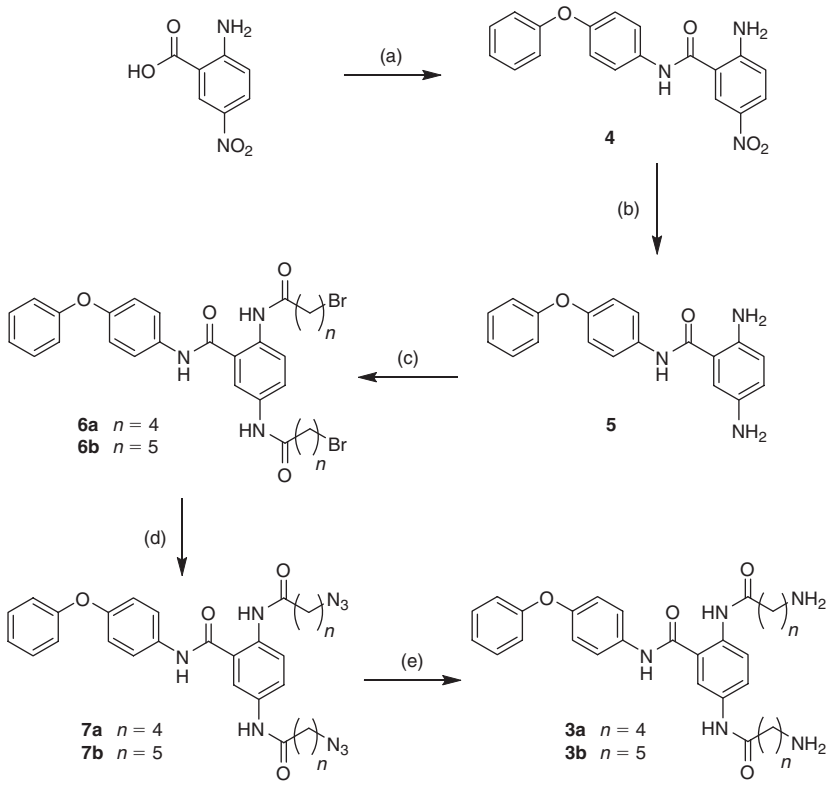
Commercially available 2-amino-5-nitrobenzoic acid was coupled with 4-phenoxyaniline using *N*,*N*′-dicyclohexylcarbodiimide (DCC) and 4-(dimethylamino)pyridine (DMAP). Owing to the low nucleophilicity of the *para*-nitroaniline moiety, its protection was not required for this step. The nitrobenzene **4** was then reduced by palladium-catalyzed hydrogenation to give the dianiline derivative **5**, which, owing to its susceptibility to oxidation was immediately treated with two equivalents of the acyl chloride, formed in situ from the corresponding acid, to give the resulting dibromide intermediates **6a** or **6b**. Treatment of the dibromides **6a** or **6b** with sodium azide and catalytic amounts of 18-crown-6 in DMF gave the consequent diazides **7a** or **7b**. Reduction of the diazides **7a** or **7b** to the target diamines **3a** or **3b** was achieved by palladium-catalyzed hydrogenation at atmospheric pressure. Reverse phase chromatography and recrystallization were used to purify both diamine targets **3a** and **3b** before biological testing.

The diamines **3a** or **3b** were tested for rat brain N-type VGCC binding affinity using a previously described radioligand displacement assay, which employs ^{125}I-GVIA as the radioligand.[30] Micromolar range N-type VGCC binding was observed for both mimetics **3a** and **3b** with EC_{50} values of 75 and 42 μM, respectively.[A] Target diamines **3a** and **3b** showed comparable but weaker binding strength with that observed for earlier mimetics (**1a**, **1b**, **2a**, and **2b**) presumably owing to the inclusion of simplified K2 and Y13 mimicking functionalities. Unfortunately the relative weakness of receptor binding shown by **3a** and **3b** made the narrowing of confidence intervals difficult.

Conclusion

Two N-type VGCC binders have been synthesized and show binding in rat brain radioligand binding assays in the micromolar

[A] 95% confidence intervals for diamines **3a** and **3b** are 11–520 and 8–220 μM, respectively.



Scheme 1. Reagents and conditions: (a) DCC, DMAP, 4-phenoxyaniline, THF, 48 h, 20%; (b) H_2, Pd/C, THF; (c) $ClOC(CH_2)_nBr$, TEA, DCM, 0°C → rt, **6a** 40%, **6b** 52% over two steps; (d) NaN_3, 18-crown-6, DMF, **7a** 98%, **7b** 98%; (e) H_2, Pd/C, MeOH, **3a** 24%, **3b** 57%.

range.[B] Thus this synthetic route provides a fast and efficient synthesis of N-type VGCC binders with varying K2 and R17 projection length with comparable binding affinity to the more synthetically demanding mimetics previously described.

Experimental

General Procedures

Melting points were recorded on a Stuart melting point apparatus and are uncorrected. Proton (^1H) NMR spectra were recorded, at 300 or 400 MHz and carbon (^{13}C) NMR spectra were acquired at 75 or 100 MHz on a Bruker AM 300 spectrometer or a Bruker Avance 400 DRX spectrometer. NMR spectra were referenced to residual solvent peaks (chloroform (δ_H 7.26, δ_C 77.0), acetone (δ_H 2.05, δ_C 29.8, 205.9), methanol (δ_H 4.87, 3.30, δ_C 49.86)). To aid the assignment of NMR spectra, correlation spectroscopy (COSY) and heteronuclear multiple quantum coherence (HMQC) spectroscopy were used. Infrared spectra were recorded on a Perkin–Elmer 1600 infrared spectrometer and refer to nujol mulls or solutions in chloroform applied as a thin film on NaCl plates. Low resolution mass spectra were recorded on a Micromass Platform spectrometer. Accurate mass determinations were carried out at high resolution on an Agilent G1969A LC-TOF system with reference and mass correction at 4000 V capillary voltage for electrospray ionization (ESI). Reverse phase silica gel used was octadecylsilane C_{18} Chromatorex DM1020T and was obtained from Fuji Silysia. Starting materials and reagents were purchased from Sigma-Aldrich and were used without purification. THF was distilled under nitrogen from sodium benzophenone ketal. Solutions were dried over anhydrous magnesium sulfate ($MgSO_4$) or sodium sulfate (Na_2SO_4).

*2-Amino-5-nitro-*N*-(4-phenoxyphenyl)benzamide* ***4***

To a vigorously stirring solution of 4-phenoxyaniline (2.45 g, 13.2 mmol), DMAP (0.33 g, 2.7 mmol) and DCC (2.82 g, 13.7 mmol) in THF (50 mL) was added 2-amino-5-nitrobenzoic acid (2.03 g, 11.2 mmol) in THF (20 mL) dropwise under nitrogen. When TLC analysis showed no remaining starting material (typically 48 h), the insoluble urea (*N*-*N*′-dicyclohexylurea) was removed by filtration and the residue rinsed with THF. The filtrate was then concentrated to one quarter of its volume and the product precipitated by addition of hexane. The solid was collected and washed with dichloromethane (DCM) to yield the aniline **4** (0.68 g, 20%) as a yellow crystalline solid, mp 210–212°C. ν_{max}/cm^{-1} 3466, 3346, 3292, 1641, 1620, 1591, 1510, 1487, 1410, 1310, 1250, 1132, 837. δ_H (400 MHz, [D_6]acetone) 9.90 (bs, 1H, NH), 8.62 (d, 1H, *J* 2.5, Ar–H), 8.07 (dd, 1H, *J* 2.6 and 9.2, Ar–H), 7.80–7.40 (m, 2H, Ar–H), 7.41 (bs, 2H, NH_2), 7.35–7.37 (m, 2H, Ar–H), 7.13–7.09 (m, 1H, Ar–H), 7.03–7.00 (m, 4H, Ar–H), 6.93 (d, 1H, *J* 9.2, Ar–H). δ_C (100 MHz, [D_6]acetone) 167.1, 158.6, 156.4, 154.3, 137.1, 135.4, 130.7 (2C), 128.4, 126.4, 123.9, 123.4 (2C), 120.0 (2C), 119.2 (2C), 117.5, 117.1. Found *m/z* 350.1144 $[M+H]^+$. $C_{19}H_{16}N_3O_4$ requires 350.1141.

*2,5-Diamino-*N*-(4-phenoxyphenyl)benzamide* ***5***

To a solution of the aniline derivative **4** (200 mg, 0.57 mmol) in THF (5 mL) was added 10% palladium on charcoal (60 mg,

[B] It has not yet been proved that the K2-Y13-R17 mimetics bind to the N-type voltage gated calcium channels in the same manner as ω-conotoxin GVIA.[24,26]

0.06 mmol). After degassing and flushing with hydrogen, the mixture was stirred under an atmosphere of hydrogen at 22°C for 4 h, then filtered through Celite to afford a green solution of the *title compound* (**9**), which was used for subsequent transformations without further purification. *m/z* (ESI, 70 eV) 320.1 $[M + H]^+$.

N,N′-*(2-(4-Phenoxyphenylcarbamoyl)-1,4-phenylene)bis(5-bromopentanamide)* ***6a***

A vigorously stirred solution of 5-bromopentanoic acid (163 mg, 0.90 mmol) in DCM (3 mL) and DMF (5 μL) was cooled to −5°C before dropwise addition of a solution of oxalyl chloride (85 μL, 0.98 mmol) in DCM (1 mL). The mixture was then allowed to warm to room temperature (RT) and after stirring for 1 h, the volatiles were evaporated under a stream of nitrogen. Following this, the residue was redissolved in DCM (10 mL), cooled to 0°C, and triethylamine (246 μL, 1.77 mmol) was added dropwise with stirring. A freshly prepared solution of 2,5-diamino-*N*-(4-phenoxyphenyl)benzamide **5** (0.30 mmol) in THF (5 mL) was then added dropwise over 20 min. The mixture was allowed to warm to RT and stirred for 15 h. The reaction was quenched with water (5 mL), the organic layer separated, and the aqueous phase washed with DCM (2 × 5 mL). The organic extracts were combined and washed with 1 M $NaOH_{(aq)}$ (3 × 10 mL), 1 M $HCl_{(aq)}$ (3 × 10 mL), and brine (2 × 15 mL). The solution was dried, filtered, and the solvent removed under vacuum to afford *title compound* **6a** (231 mg, 40%) as a solid, mp 125–132°C. ν_{max}/cm^{-1} 3284, 1715, 1652, 1592, 1538, 1506, 1488, 1410, 1246. δ_H (400 MHz, [D$_6$]DMSO) 10.37 (bs, 1H, NH), 10.01 (bs, 1H, NH), 9.90 (bs, 1H, NH), 7.88 (d, 1H, *J* 2.03, Ar–H), 7.83 (d, 1H, *J* 9.08, Ar–H), 7.70 (d, 2H, *J* 8.96, Ar–H), 7.64 (dd, 1H, *J* 1.80 and 8.56, Ar–H), 7.35–7.31 (m, 2H, Ar–H), 7.10–7.07 (m, 1H, *J* 7.4, Ar–H), 7.01–6.94 (m, 4H, Ar–H), 3.56–3.45 (m, 4H, –CH$_2$–Br), 2.35–2.16 (m, 4H, –COCH$_2$–), 1.84–1.82 (m, 4H, –C*H*$_2$–CH$_2$–Br), 1.70–1.33 (m, 4H, –COCH$_2$–C*H*$_2$–). δ_C (100 MHz, [D$_6$]acetone) 171.8, 171.6, 168.3, 157.3, 152.1, 135.0, 134.8, 131.8, 129.9 (2C), 123.4, 123.3, 121.9 (2C), 121.6, 120.1, 119.3 (2C), 119.0, 117.8 (2C), 35.3, 35.2, 34.7, 34.6, 31.7, 31.5, 23.7, 23.6. *m/z* (ESI, 70 eV) 642 [M − H. Found $[M + Na]^+$ 666.0574, required for $C_{29}H_{31}Br_2N_3NaO_4$ 666.0579.[16]

N,N′-*(2-(4-Phenoxyphenylcarbamoyl)-1,4-phenylene)bis(6-bromohexanamide)* ***6b***

Following the same method used to prepare **6a**, the title compound **6b** was prepared from 6-bromohexanoic acid (143 mg, 0.73 mmol), oxalyl chloride (68 μL, 0.78 mmol), DCM (3 mL), DMF (5 μL), triethylamine (196 μL, 1.41 mmol), 2,5-diamino-*N*-(4-phenoxyphenyl)benzamide **5** (0.28 mmol) and DCM (10 mL). The *title compound* **5a** was isolated as a solid (99 mg, 52%), mp 120–127°C. ν_{max}/cm^{-1} 3280, 1714, 1652, 1592, 1538, 1506, 1486, 1411, 1244. δ_H (400 MHz, [D$_6$]acetone) 10.40 (bs, 1H, NH), 9.82 (bs, 1H, NH), 9.20 (bs, 1H, NH), 8.40 (d, 1H, *J* 9.0, Ar–H), 8.21 (d, 1H, *J* 2.3, Ar–H), 7.81 (d, 2H, *J* 9.0, Ar–H), 7.59 (dd, 1H, *J* 2.4 and 9.0, Ar–H), 7.39–7.35 (m, 2H, *J* 7.4, Ar–H), 7.13–7.09 (m, 1H, Ar–H), 7.04–7.00 (m, 4H, Ar–H), 3.49–3.46 (m, 4H, –CH$_2$–Br), 2.40 (m, 4H, –COCH$_2$–), 1.89–1.86 (m, 4H, –C*H*$_2$–CH$_2$–Br), 1.73–1.68 (m, 4H, –COCH$_2$–C*H*$_2$–), 1.53–1.48 (m, 4H, –C*H*$_2$–CH$_2$–CH$_2$–Br). δ_C (100 MHz, [D$_6$]acetone) 171.8, 171.5, 168.0, 158.5, 154.3, 135.4, 135.3, 130.7, 130.6 (2C), 123.8, 123.5, 123.4 (2C), 123.2, 122.6, 120.0 (2C), 119.7, 119.1 (2C), 38.3, 38.0, 34.5, 34.4, 33.2, 32.9, 28.4, 28.3, 25.2, 24.2. *m/z* (ESI, 70 eV) 670.1 $[M - H]^-$. Found $[M + H]^+$ 672.1082, required for $C_{31}H_{36}Br_2N_3O_4$ 672.1073.

2,5-Bis-(5-azidopentanamido)-N-*(4-phenoxyphenyl) benzamide* ***7a***

A solution of the dihalide **6a** (270 mg, 0.42 mmol), sodium azide (135 mg, 2.08 mmol) and 18-crown-6 (1 mg, 0.004 mmol) in dry DMF (3 mL) was stirred at RT overnight. Diethyl ether (20 mL) was added and the mixture washed with sat. $NaHCO_{3(aq)}$ (3 × 10 mL). The organic layer was dried, filtered and the solvent removed under vacuum to afford *title compound* **7a** as a solid (234 mg, 98%). ν_{max}/cm^{-1} 3282, 2097, 1652, 1592, 1506, 1488, 1405, 1232. δ_H (400 MHz, [D$_6$]acetone) 10.42 (bs, 1H, NH), 9.83 (bs, 1H, NH), 9.21 (bs, 1H, NH), 8.41 (d, 1H, *J* 8.89, Ar–H), 8.21 (d, 1H, *J* 2.3, Ar–H), 7.79 (d, 2H, *J* 9.0, Ar–H), 7.57 (dd, 1H, *J* 2.4 and 9.0, Ar–H), 7.41–7.36 (m, 2H, *J* 7.4, Ar–H), 7.15–7.06 (m, 1H, Ar–H), 7.05–6.97 (m, 4H, Ar–H), 3.40–3.35 (m, 4H, CH$_2$–N$_3$), 2.46–2.41 (m, 4H, –CO–CH$_2$–), 1.80–1.72 (m, 4H, C*H*$_2$–CH$_2$–N$_3$), 1.71–1.62 (m, 4H, –CO–CH$_2$–C*H*$_2$–). δ_C (100 MHz, [D$_6$]acetone) 171.8, 171.5, 168.1, 158.6, 154.5, 135.5, 135.3, 130.7 (2C), 130.6, 124.0, 123.7, 123.4 (2C), 122.3, 122.7, 120.1 (2C), 119.7, 119.2 (2C), 51.8, 51.7, 37.6, 36.9, 29.1, 29.0, 23.4, 23.3. *m/z* (ESI, 70 eV) 568.2 $[M - H]^-$. Found $[M - H]^-$ 568.2418, required for $C_{29}H_{30}N_9O_4$ 568.2421.

2,5-Bis-(6-azidohexanamido)-N-*(4-phenoxyphenyl) benzamide* ***7b***

Title compound **7b** was prepared from a solution of the dihalide **6b** (192 mg, 0.29 mmol), sodium azide (92 mg, 1.42 mmol) and 18-crown-6 (1 mg, 0.004 mmol) in dry DMF (3 mL) following the same method used to produce diazide **7a**. The diazide **7b** was isolated as light yellow solid (176 mg, 98%). ν_{max}/cm^{-1} 3284, 2096, 1712, 1652, 1592, 1538, 1506, 1487, 1411, 1245. δ_H (400 MHz, [D$_6$]acetone) 10.41 (bs, 1H, NH), 9.83 (bs, 1H, NH), 9.20 (bs, 1H, NH), 8.40 (d, 1H, *J* 9.0, H3), 8.21 (d, 1H, *J* 2.3, H6), 7.79 (d, 2H, *J* 9.0, H3′), 7.56 (dd, 1H, *J* 2.4 and 9.0, H4), 7.39–7.35 (m, 2H, H8′), 7.14–7.09 (m, 1H, *J* 7.4, H9′), 7.03–7.00 (m, 4H, H4′ and H7′), 3.36–3.29 (m, 4H, CH$_2$–N$_3$), 2.41–2.37 (m, 4H, –CO–CH$_2$–), 1.73–1.61 (m, 4H, C*H*$_2$–CH$_2$–N$_3$), 1.46–1.29 (m, 4H, –CO–CH$_2$–C*H*$_2$–), 0.94–0.87 (m, 4H, C*H*$_2$–CH$_2$–CH$_2$–N$_3$). δ_C (100 MHz, [D$_6$]acetone) 172.0, 171.3, 168.1, 158.9, 153.9, 136.1, 135.3, 130.7 (2C), 130.6, 123.8, 123.6 (2C), 123.3, 121.8, 120.1, 119.9 (2C), 119.7, 118.9 (2C), 52.0, 51.9, 38.4, 37.3, 27.1, 27.0, 24.5, 24.4, 20.5, 20.4. *m/z* (ESI, 70 eV) 596 $[M - H]^-$. Found $[M - H]^-$ 596.2734, required for $C_{31}H_{34}N_9O_4$ 596.2739.

2,5-Bis-(5-aminopentanamido)-N-*(4-phenoxyphenyl) benzamide* ***3a***

A degassed mixture of diazide **7a** (234 mg, 0.41 mmol) and Pd/C (43 mg, 0.04 mmol) in methanol (5 mL) was stirred for 24 h under an atmosphere of hydrogen at 25°C. The reaction mix was then filtered through Celite and the solvent removed under vacuum to afford the target diamine **3a** as a light yellow oil (207 mg, 98%), which was purified by reverse phase chromatography (1:1 MeOH/H$_2$O) and recrystallized from methanol/water (51 mg, 24%). ν_{max}/cm^{-1} 3287, 2938, 1659, 1590, 1506, 1489, 1404. δ_H (400 MHz, CD$_3$OD) 8.05–8.03 (m, 2H, Ar–H), 7.64 (d, *J* 8.04, 2H, Ar–H), 7.53 (dd, *J* 1.50 and 8.53, 1H, Ar–H), 7.36–7.32 (m, 2H, Ar–H), 7.10–7.08 (m, 2H, Ar–H), 7.00–6.97 (m, 4H, Ar–H), 2.69–2.61 (m, 4H, CH$_2$–NH$_2$), 2.46–2.37 (m,

4H, –CO–CH_2–), 1.76–1.63 (m, 4H, CH_2–CH_2–NH_2), 1.58–1.49 (m, 4H, –CO–CH_2–CH_2–). δ_C (100 MHz, CD_3COD) 174.5, 174.2, 168.9, 159.0, 155.5, 136.1, 135.2, 134.5, 130.9 (2C), 128.6, 127.2, 124.5, 124.3, 123.9 (2C), 121.1, 120.3 (2C), 119.6 (2C), 42.1, 42.0, 38.1, 37.5, 33.0, 32.8, 24.1, 24.0. *m/z* (ESI, 70 eV) 518 $(M + H)^+$. Found $(M + H)^+$ 518.2761, required for $C_{29}H_{36}N_5O_4$ 518.2767.

*2,5-Bis-(6-aminohexanamido)-*N*-(4-phenoxyphenyl) benzamide **3b***

The title compound **3b** was prepared from diazide **7b** (176 mg, 0.29 mmol) and Pd/C (31 mg, 0.03 mmol) in methanol (5 mL) following the same method used to prepare diamine **3a**. Diamine **3b** was isolated as a light yellow oil (137 mg, 85%), which was purified by reverse phase chromatography (1:1 MeOH/H_2O) and recrystallized from methanol/water (90 mg, 57%). ν_{max}/cm^{-1} 3302, 2935, 1654, 1590, 1505, 1489, 1404. δ_H (400 MHz, CD_3OD) 8.06–8.02 (m, 2H, Ar–H), 7.65 (d, *J* 8.36, 2H, Ar–H), 7.53 (dd, *J* 1.69 and 8.72, 2H, Ar–H), 7.37–7.32 (m, 2H, Ar–H), 7.12–7.07 (m, 1H, Ar–H), 7.01–6.98 (m, 4H, Ar–H), 2.63–2.55 (m, 4H, CH_2–NH_2), 2.42–2.35 (m, 4H, –CO–CH_2–), 1.74–1.63 (m, 4H, CH_2–CH_2–NH_2), 1.58–1.31 (m, 8H, –CO–CH_2–CH_2–CH_2–). δ_C (100 MHz, [D_6]acetone) 172.3, 172.2, 168.1, 158.7, 154.4, 135.4, 135.3, 130.7 (2C), 124.0 (2C), 123.9, 123.3, 122.5, 120.1 (2C), 120.0 (2C), 119.4, 119.14, 119.12, 51.8 (2C), 38.1, 37.3, 33.0, 32.9, 26.97, 26.94, 25.64, 25.63. *m/z* (ESI, 70 eV) 546.3 $[M + H]^+$. Found $[M + H]^+$ 546.3078, required for $C_{31}H_{40}N_5O_4$ 546.3080.

Biological Methods

Radioligand binding assays were run in triplicate in 96-well plates at room temperature as previously described.[30] Each assay contained test compound, radiolabelled peptide (^{125}I-GVIA for N-type channels or ^{125}I-MVIIA for P/Q-type channels) and 8 μg of crude rat brain membrane added last. All dilutions were made in assay buffer (20 mM HEPES, 75 mM NaCl, 0.2 mM EDTA, 0.2 mM EGTA, 2 μM Leupeptin, 2 μL apoprotinin (to 30 mL assay buffer) and 0.1% BSA, pH 7.4). Final volume in each well was 150 μL. After shaking for 1 h, membrane was filtered (Wallac, Finland, glass fibre filters presoaked in 0.6% polyethyleneimine) and washed with 20 mM HEPES, 125 mM NaCl, pH 7.4 on a Tomtec harvester. After addition of scintillant, radioactivity bound to the filter was counted using a 1450 MicroBeta (Wallac, Finland). The data was analyzed using GraphPad Prism 2.0 (GraphPad Software, Inc., San Diego, USA).

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