Synthesis of a Series of 4-Benzyloxyaniline Analogues as Neuronal N-Type Calcium Channel Blockers with Improved Anticonvulsant and Analgesic Properties

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In this article, the rationale for the design, synthesis, and biological evaluation of a series of N-type voltage-sensitive calcium channel (VSCC) blockers is described. N-Type VSCC blockers, such as ziconotide, have shown utility in several models of stroke and pain. Modification of the previously reported lead, **1a**, led to several 4-(4-benzyloxylphenyl)piperidine structures with potent in vitro and in vivo activities. In this series, the most interesting compound, (*S*)-2-amino-1-{4-[(4-benzyloxy-phenyl)-(3-methyl-but-2-enyl)-amino]-piperidin-1-yl}-4-methyl-pentan-1-one (**11**), blocked N-type calcium channels (IC_{50} = 0.67 μM in the IMR32 assay) and was efficacious in the audiogenic DBA/2 seizure mouse model (ED_{50} = 6 mg/kg, iv) as well as the antiwrithing model (ED_{50} = 6 mg/kg, iv). Whole-cell voltage-clamp electrophysiology experiments demonstrated that compound **11** blocked N-type Ca^{2+} channels and Na^{+} channels in superior cervical ganglion neurons at similar concentrations. Compound **11**, which showed superior in vivo efficacy, stands out as an interesting lead for further development of neurotherapeutic agents in this series.

Introduction

Excessive calcium entry into depolarized neurons contributes significantly to neuronal injury. Voltage-sensitive calcium channels (VSCCs) regulate intracellular calcium concentration, which affects various important neuronal functions such as cellular excitability, neurotransmitter release, hormone secretion, intracellular metabolism, neurosecretory activity, and gene expression.[1] Neuronal VSCCs are classified into L, N, P, Q, R, and T subtypes based upon their physical and pharmacological properties. These channels differ in their protein structures, function, conductance, activation/inactivation voltage, and sensitivity to various drugs and/or toxins.[1] N-type channels are found mainly in central and peripheral neurons, being primarily located on presynaptic nerve terminals. These channels regulate the calcium flux subserving depolarization-evoked release of transmitter from synaptic endings.[1]

It has been suggested that N-type VSCCs would be ideal targets for the development of new pharmacological agents for clinical use.[2] ω-Conotoxin MVIIA,[3] a 25 amino acid residue containing peptide found in the venom of the piscivorous marine snail (*Conus magus*), is a potent and selective N-type voltage-sensitive calcium channel blocker. The synthetic version of MVIIA, ziconitide[3] has demonstrated efficacy in animal models of traumatic brain injury, focal cerebral ischemia, and pain.[2] An NDA of ziconitide will be filed for its application in pain.

Drug discovery efforts during the past decade have focused on small molecule N-type calcium channel blockers with non-peptide structures particularly for neuroprotection or analgesia.[4] Examples of known non-peptidyl Ca^{2+} channel antagonists include flunarizine,[5] fluspirilene,[6] PD-157767,[7] SB-201823,[8] SB-206284,[9] NNC-09-0026,[10] cilnipine (FRC-8653),[11] NS-649,[12] and (2*R*)-1-((*S*)-6-cyano-7-methyl-6-phenyl-octanoyl)-pyrrolidine-2-carboxylic acid benzhydryl-amide (**1c**).[13] However, most of these are nonselective and block multiple subtypes of Ca^{2+} channels as well as Na^{+} and K^{+} channels. In the process of searching for small molecule N-type calcium channel blockers, we have discovered and reported on compound **1a** as a potential chemical lead.[14,15] Compound **1a** is a potent antagonist for N-type calcium channels in the IMR32 human neuroblastoma cells (IC_{50} = 0.32 μM)[14] and showed moderate in vivo activities in the audiogenic DBA/2 seizure mouse model (protection: 100% at 30 mg/kg and 0% at 10 mg/kg, iv), as well as the antiwrithing model (protection: 75% at 30 mg and 11% at 10 mg/kg, iv). Unfortunately, due to unfavorable physicochemical properties, such as an high ClogP (6.5) and poor aqueous solubility (measured aqueous solubility < 1 μg/mL at pH 7), compound **1a** has limited therapeutic potential.

Several compounds were prepared with important structural modifications to avoid the problem encountered with antagonist **1a**.[14,15] One of the major

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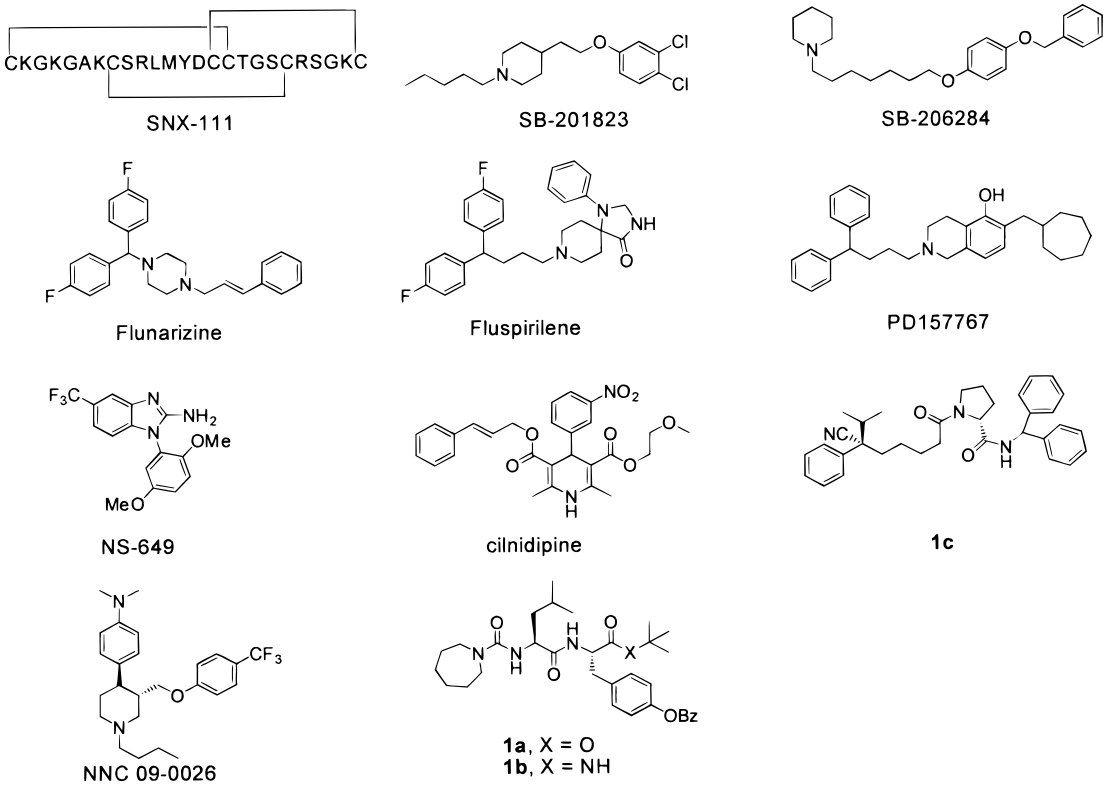
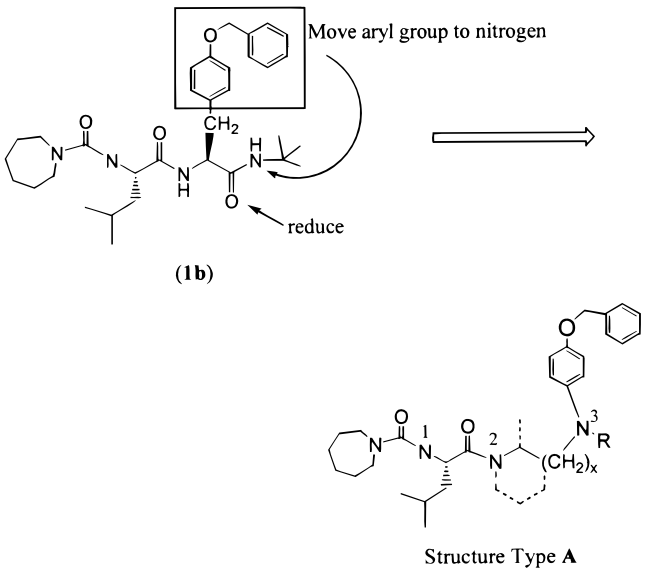
Chart 1. Selected N-Type Ca^{2+} Channel Blockers

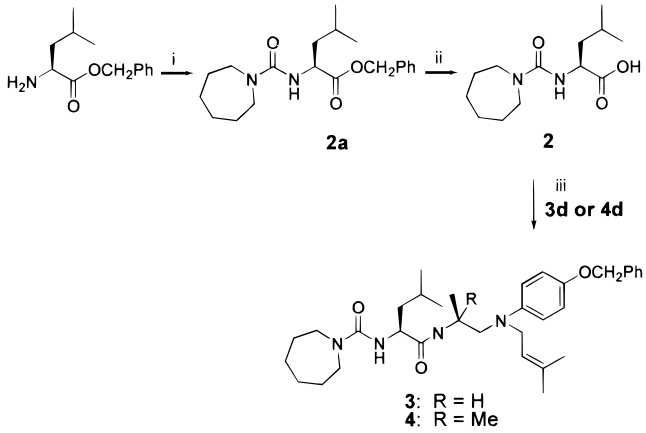


Scheme 1. Rationale for the Design of N-Type Ca^{2+} Channel Blockers



changes was to convert the *tert*-butyl ester to its *tert*-butyl amide derivative (**1b**, $IC_{50} = 0.59\ \mu M$); this change was well-tolerated for N-type Ca^{2+} channel blocking activity (Chart 1).[14,15] The previous results also suggested that some flexibility was present in terms of the electronic interaction and volume in the C-terminal region of the molecule.[14,15] Therefore, we envisioned migrating the (4-*O*-benzyl)-phenyl group of **1b** to the C-terminal end and replacing the *tert*-butyl amide with a 3-methyl-2-butenylamine to generate the structure type **A** (Scheme 1) for exploring even more active N-type Ca^{2+} channel blockers. The obvious advantages of this approach were to provide new structures with nonpeptidic motifs and an amine functionality which allowed rapid generation of analogues. In this paper, several analogues with this novel template are described. Their in vitro activities for N-type Ca^{2+} channel blockade as well as in vivo efficacy in the audiogenic DBA/2 seizure mouse model are discussed.

Scheme 2[a]

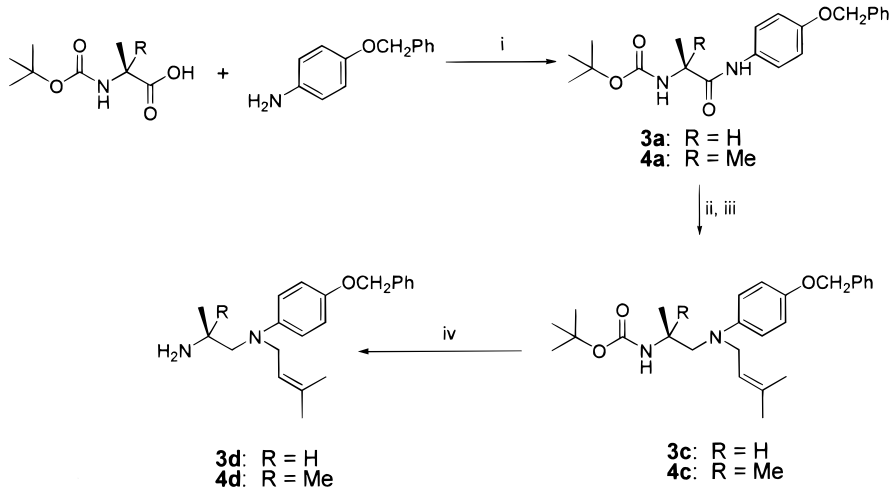


[a] Reagents and conditions: (i) triphosgene, hexamethlenimine; (ii) H_2, Pd/C; (iii) HBTU (*O*-benzotriazol-1-yl-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate), DIEA, DMF.

Methods

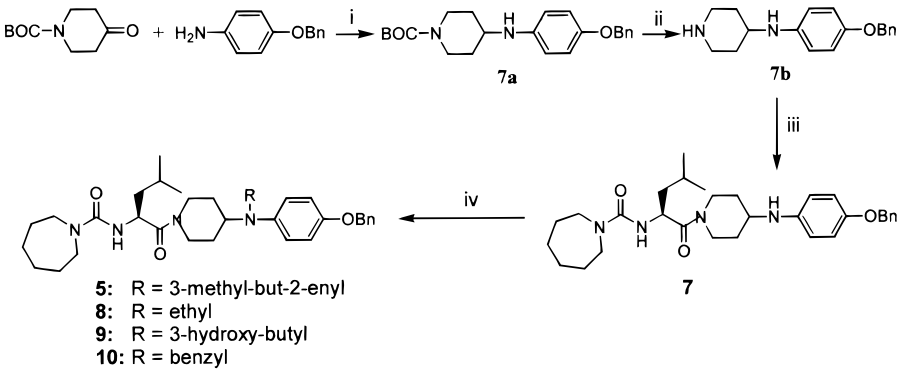
Chemistry. The preparation of (*S*)-2-[(azepane-1-carbonyl)-amino]-4-methyl-pentanoic acid (**2**), which is a common intermediate for compounds **1b**[14,15] and **3**−**10**, was carried out in two steps (Scheme 2). Condensation of triphosgene and hexamethylenimine with (*S*)-2-amino-4-methyl-pentanoic acid benzyl ester provided **2a**, which was subsequently hydrogenated to provide the acid (**2**).

Scheme 3[a]



[a] Reagents and conditions: (i) HBTU, DIEA, DMF; (ii) BH_3/THF; (iii) 4-bromo-2-methyl-2-butene, DIEA, THF; (iv) TFA, CH_2Cl_2.

Scheme 4[a]



[a] Reagents and conditions: (i) $NaBH(OAc)_3$, CH_2Cl_2; (ii) TFA, CH_2Cl_2; (iii) HBTU, DIEA, DMF, **2**; (iv) for **5**: 4-bromo-2-methyl-2-butene, THF, DIEA; for **8**−**10**: $NaBH(OAc)_3$, CH_2Cl_2 and aldehydes.

The preparation of compound **1b** was previously reported.[15] Synthesis of compound **3** was carried out by coupling the acid (**2**) with (*S*)-*N*-(4-benzyloxy-phenyl)-*N*-(3-methyl-but-2-enyl)-propane-1,2-diamine(**3d**)(Scheme 2). Compound **4** was prepared by the same method used for compound **3**, except that **4d** was the starting material. The 1,2-diamine (**3d**) was prepared by the amide formation of N^{α}-Boc-alanine and 4-benzyloxyaniline, reduction of the amide (**3a**) with diborane to generate the amine (**3b**), alkylation of the amine (**3b**) to provide compound **3c**, and deprotection of compound **3c** to yield intermediate **3d**. (Scheme 3). The preparation of intermediate **4d** was similar to that of **3d**, except that N^{α}-Boc-Aib acid was the starting material (Scheme 3).

Synthesis of compounds **5**, **7**−**10** is outlined in Scheme 4. The key compound, **7**, was prepared in three steps by the reductive amination of 4-benzyloxy-aniline with 1-*tert*-butyl-carbonyl-4-piperidone to yield **7a**, followed by deprotection with TFA to generate **7b** and the coupling of the acid (**2**) with **7b** to provide analogue **7** (Scheme 4). Further alkylating the aniline nitrogen of compound **7** resulted in **5**, **8**−**10**. Compound **6** was synthesized by reductive amination of 4-benzyloxy-aniline with 1-benzyl-3-piperidone to yield **6a**, alkylation of **6a** to generate **6b**, debenzylation of compound **6b** to provide **6c**, and coupling of **6c** with the acid (**2**) to form analogue **6** (Scheme 5).

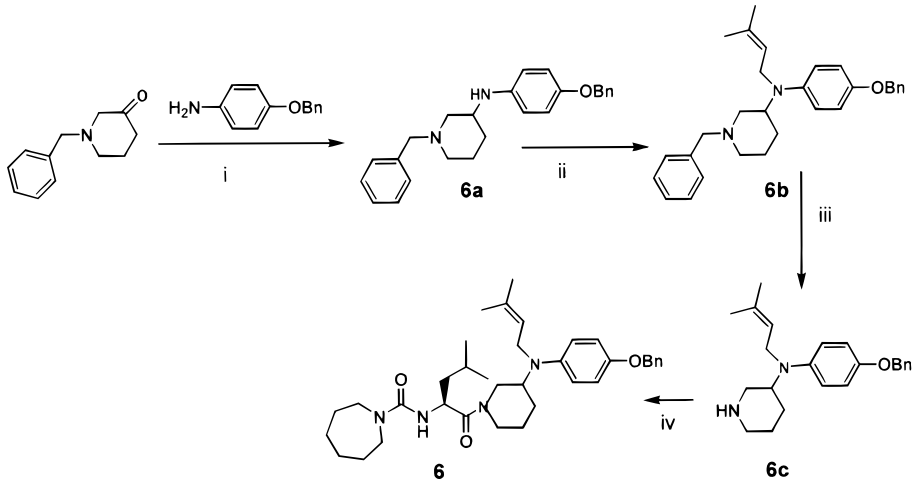
The preparation of compound **11** is illustrated in Scheme 6. Alkylation of 4-(4-benzyloxy-phenylamino)-piperidine-1-carboxylic acid *tert*-butyl ester (**7a**) with 4-bromo-2-methyl-2-butene gave **11a**. Deprotection of the Boc group of **11a** generated the amine (**11b**), which was then coupled with N^{α}-Boc-leucine to produce the amide (**11c**). Further deprotection of the Boc group of **11c** provided antagonist **11**. Finally, alkylation of compound **11** with various groups resulted in compounds **12**−**14** (Scheme 6).

Pharmacology. N-type Ca^{2+} channel blocking potencies of the compounds were determined using a fluorescence-based Ca^{2+}-flux assay in IMR32 human neuroblastoma cells. Compound **1a** was run in parallel in each assay as a standard to compare the relative potency of experimental compounds with the initial lead. The anticonvulsant effects of selected compounds were evaluated in an audiogenic seizure model using DBA/2 mice (6.5−12 g, 20−23 days of age).[16] The known N-type Ca^{2+} channel blocker, ω-conotoxin GVIA, blocked seizures in this model.[17] Data are expressed as a percentage of animals showing no tonic seizure response to the sound stimulation (Table 1).

Results and Discussion

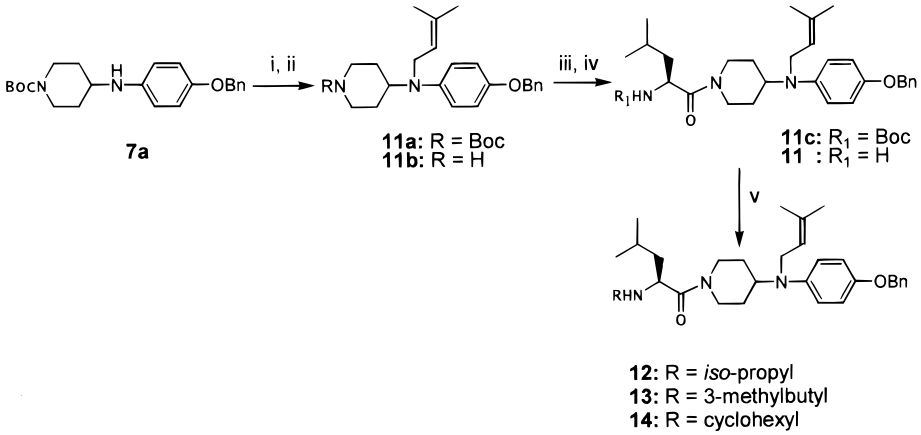
Initially, compound **3** was prepared to test the hypothesis that structure type **A** could be a potent N-type Ca^{2+} channel antagonist. Structure type **A** was derived

Scheme 5[a]



[a] Reagents and conditions: (i) $NaBH(OAc)_3$, CH_2Cl_2; (ii) 4-bromo-2-methyl-2-butene, DIEA; (iii) 1,2-dichloroethane, α-chloroethylchloroformate; (iv) HBTU, DIEA, **2b**, DMF.

Scheme 6[a]



[a] Reagents and conditions: (i) 4-bromo-2-methyl-2-butene, THF, DIEA; (ii) TFA, CH_2Cl_2, (iii) HBTU, DIEA, Boc-leucine, DMF; (iv) TFA, CH_2Cl_2; (v) $NaBH(OAc)_3$, CH_2Cl_2, aldehydes or ketones.

Table 1. Activities of N-Type Calcium Channel Blockers

compd	IMR32 IC_{50} (μM)	DBA/2 activity, iv ($n = 5$ mice/dose tested)	ClogP/solubility
1a	0.32 ± 0.04 ($n = 10$)[a]	100% @ 30 mg/kg 0% @ 10 mg/kg	6.5/0.8 μM (pH 7.4)
1b	0.59 ($n = 2$)		5.5/11 μM (pH 7.4)
3	1.0 ($n = 1$)		6.9
4	0.26 ($n = 1$)		7.2
5	0.34 ± 0.06 ($n = 4$)	0% @ 30 mg/kg	7.0
6	1.5 ($n = 1$)		7.2
7	2.5 ($n = 1$)	60% @ 30 mg/kg	5.1
8	0.83 ($n = 1$)	0% @ 30 mg/kg	6.0
9	4.6 ($n = 1$)	60% @ 30 mg/kg	5.6/4 μM (pH 7.4)
10	0.43 ($n = 1$)		7.1
11	0.67 ± 0.07 ($n = 8$)	$ED_{50} = 6$ mg/kg	5.5/87 μM (pH 7.4) and 100 mM (pH 4.0)
11b	4.7 ($n = 1$)		5.0
12	0.75 ($n = 1$)		7.0
13	0.48 ($n = 1$)	80% @ 30 mg/kg 20% @ 10 mg/kg	7.8
14	0.51 ($n = 1$)		8.1

from compound **1b** by migrating the (4-*O*-benzyl)-phenyl group of **1b** to the C-terminal and replacing the *tert*-butyl amide of **1b** with a 3-methyl-2-butenylamine. In the IMR32 assay, this non-peptidyl compound (**3**, $IC_{50} = 1.0$ μM) displayed reasonable potency: slightly less potent than the parent compound (**1b**, $IC_{50} = 0.59$ μM) or 3-fold less active than compound **1a** ($IC_{50} = 0.32$ μM). Further exploration of potent Ca^{2+} channel blockers led us to modify the 1-methyl-ethylene-1,2-diamine linker of **3** with a 1,1-dimethyl-ethylene-1,2-diamine linker resulting in analogue **4** ($IC_{50} = 0.26$ μM), which enhanced the activity by 4-fold relative to **3**. For structure–activity relationship studies, the 1-methyl-ethylene-1,2-diamine linker of **3** was also substituted with a piperidin-4-yl-amine linker obtaining compound **5** ($IC_{50} = 0.34$ μM), which showed 3-fold improvement in potency

compared to **3**. Furthermore, the piperidin-3-yl-amine analogue (**6**, $IC_{50} = 1.5\ \mu M$) was 5-fold less potent than its piperidin-4-yl-amine counterpart (**4**) and slightly less active than **3** for blocking N-type Ca^{2+} channels. From the above four analogues, it was concluded that the *N-(2),N(3)*-linker of structure type **A** was important for activity. Both 1,1-dimethyl-ethylene-1,2-diamine and piperidin-4-yl-amine moieties were advantageous for IMR32 activity in this brief set of compounds; 1-methyl-ethylene-1,2-diamine and piperidin-3-yl-amine linkers are less preferred. Compounds **4** and **5** blocked N-type Ca^{2+} channels as effectively as our screening lead, **1a**. 4-Aminopiperidine has been shown to be a successful scaffold for several known agents applied in central nervous system,[18] and this template is likely to be a good motif for design of further drug candidates. Therefore, further SAR studies in this series are all focused on the 4-piperidine analogues.

The subsequent synthesis of several *N(3)*-substituted analogues (**5**, **7**−**10**) was directed toward optimizing N-type Ca^{2+} channel activity. In the **1a**/**1b** series, the ester/amide substitution at the C-terminal of the dipeptides played a key role for blockade of N-type Ca^{2+} channels.[14] Generally speaking, lipophilic groups were advantageous for high IMR32 potency for analogues of **1a**. Here, the SAR of *N(3)*-disubstituted analogues (nitrogen of the aniline) of **5** were compared among a set of antagonists. These were hydrogen (**7**), ethyl (**8**), 3-methyl-but-2-enyl (**5**), benzyl (**10**), and 3-hydroxy-butyl (**9**). The potencies of these compounds for the blockade of N-type Ca^{2+} channels ranged from 0.34 to 4.6 μM and suggested that affinity was strongly influenced by substituent effects. A careful comparison revealed that a graded preference for 3-methyl-but-2-enyl over benzyl, ethyl, hydrogen, or 3-hydroxy-butyl existed. A lipophilic substituent with certain size favored active blockade, while the hydrophilic substituent was less preferred. This result was in agreement with our previous observations in the compound **1a**/**1b** series.

Further evaluation of compounds **5**, **7**−**9** in the audiogenic seizure model demonstrated that no anticonvulsant effect was observed for **5** and **8** at an iv dose of 30 mg/kg, while the less active compounds, **7** and **9** (IMR32 $IC_{50} = 2.5$ and 4.6 μM, respectively), exhibited moderate antiseizure activities (60% protection at 30 mg/kg, Table 1). The relatively low level of in vivo efficacy of **5** was unusual, since **5** was a rather potent N-type Ca^{2+} channel blocker in vitro (IMR32 $IC_{50} = 0.34$ μM). It was speculated that this low central nervous system activity was a consequence of the poor physicochemical properties of compound **5**. Indeed, the antagonists **7** and **9** had lower ClogP values and demonstrated improved in vivo efficacy. With the goal of enhancing the in vivo potency of this series, the azepane-1-carboxylic amide group of **5** was replaced with an NH_2, isopropylamine, 3-methylbutylamine, and cyclohexylamine. Compounds **11**−**14** displayed good activity in blocking N-type Ca^{2+} channels. Detailed analysis of these ligands (**11**−**14**) suggested a relatively flat structure−activity relationship (IC_{50} values ranged from 0.48 to 0.75 μM) and the IMR32 activities were not sensitive to the N-substitution. The free amine analogue (**11**) was chosen for further evaluation, since antagonist **11** possessed the most favorable physicochemical properties (such as solubility) in this series. Furthermore, (4-benzyloxy-phenyl)-(3-methyl-but-2-enyl)-piperidin-4-yl-amine (**11b**), a des-leucine analogue of compound **11**, was evaluated and found inactive. Therefore, it was determined that the leucine moiety was essential for blocking N-type Ca^{2+} channels in this series, even though the N-substituted analogues, compounds **11**−**14**, displayed a flat SAR.

Compounds **11** and **13** were tested in the audiogenic seizure model and displayed improved in vivo efficacy compared to **1a**. Remarkably, the free amine analogue (**11**) demonstrated a potent anticonvulsant effect with an ED_{50} of 6 mg/kg after iv administration. This is the most potent anticonvulsant agent in this report. Compound **11** was further characterized in electrophysiology assays and found to block neuronal N-type Ca^{2+} channels in superior cervical ganglion neurons with an IC_{50} of $3.5 \pm 0.62\ \mu M$ ($n = 3$) and blocked voltage-gated sodium channels with an IC_{50} of $4.2 \pm 0.9\ \mu M$ ($n = 3$). These results suggested that compound **11** was a balanced channel blocker and blocked audiogenic seizures by activity at both voltage-gated N-type calcium and sodium channels.[19a,b] Compound **11** was further evaluated in an antiwrithing model and found to be efficacious ($ED_{50} = 6$ mg/kg iv). The assessment of its cardiovascular side effects in rat indicated that **11** had no significant effect on the mean blood pressure or heart rate at 10 mg/kg iv.

Compound **11** showed improved physicochemical properties compared to **1a** (**11,** logP: measured logP = 5.7 and ClogP = 5.53, aqueous solubility: 90 μM at pH 7 and 100 mM at pH 4). Ligand **11** showed significantly improved metabolic stability in a human liver microsome assay, with a half-life ($t_{1/2}$) of 116 min, compared to the dipeptidyl compounds (**1a**/**1b** with $t_{1/2} <$ 10 min for both). This stability demonstrated that the de novo designed nonpeptidyl Ca^{2+} channel antagonists could be advantageous for in vivo application. Unfortunately, pharmacokinetic (PK) studies of compound **11** indicated that plasma concentration declined very rapidly over 10 min after a single 5 mg/kg bolus intravenous dose to rats. This appeared to be due to high clearance (>90 mL/min/kg) and large volume of distribution (>20 L/kg). This undesirable PK profile hampered the further development of compound **11** as a neuroprotection/pain candidate. Further SAR of compounds **11** and **5** will be presented elsewhere.[19c]

Conclusion

In summary, a series of 4-(4-benzyloxylphenyl)piperidine-based N-type calcium channel blockers has been described. These compounds were derived from the high-throughput screening hit (**1a**) which was a dipeptide. These new structures demonstrated potent in vitro activity in the IMR32 assay and moderate in vivo efficacy in the audiogenic seizure mouse model. From this study, a new chemical lead (**11**) was discovered with several advantages over **1a**: good aqueous solubility, potent in vivo efficacy, and increased metabolic stability. (*S*)-2-Amino-1-{4-[(4-benzyloxy-phenyl)-(3-methyl-but-2-enyl)-amino]-piperidin-1-yl}-4-methyl-pentan-1-one (**11**) was a potent N-type calcium channel antagonist ($IC_{50} = 0.67\ \mu M$ in the IMR32 assay), with anticonvulsant ($ED_{50} = 6$ mg/kg, iv) and analgesic activities ($ED_{50} = 6$

mg/kg, iv). This compound was a balanced channel blocker for both neuronal N-type calcium channels and voltage-gated sodium channels, and it stands out as an interesting lead for the future development of neuroprotective and analgesic agents.

Experimental Section

Chemistry. Melting points were determined in open capillary tubes on a Thomas-Hoover apparatus and are uncorrected. IR spectra were recorded as KBr disks on a Nicolet MX-1 F spectrophotometer. Mass spectra were recorded on a Finnigan 4500 or VG Analytical 7070E/HF mass spectrometer. Thin-layer chromatography was performed on E. Merck silica gel F_{254} (0.25 mm) glass plates. Elemental analyses and potentiometric logP measurement were performed by Robertson Laboratories. Flash chromatography was performed with E. Merck silica gel 60, 230−400 mesh ASTM. ^{1}H NMR spectra were recorded at 400 MHz on a Varian Unity 400, and the chemical shifts were reported in ppm (δ) relative to trimethylsilane (0.0 ppm). The ^{1}H NMR spectra of all compounds were consistent with their assigned structures. All Ca^{2+} antagonists were analyzed for C, H, and N elemental analyses. Compounds analyzed for exact mass were further checked for homogeneity by analytical reversed-phase HPLC on a Phenomenex C-18 column eluting with 1:1 CH_3CN/H_2O (with 0.1% TFA), detection at 254 nm.

(*S*)-2-[(Azepane-1-carbonyl)-amino]-4-methyl-pentanoic Acid Benzyl Ester (2a). A solution of triphosgene (15.7 g, 52.9 mmol) in CH_2Cl_2 (600 mL) was cooled to −10 °C. The solution was treated dropwise with a solution of leucine benzyl ester (28.1 g, 127 mmol) and pyridine (26 mL, 320 mmol) in CH_2Cl_2 (150 mL). The reaction was stirred at −10 °C for 90 min and then treated with a solution of hexamethyleneimine (22 mL, 380 mmol) in CH_2Cl_2 (75 mL). The solution was stirred for 48 h at room temperature. The reaction mixture was concentrated, and the residue was dissolved in ether and washed with 1 N HCl solution, water, and saturated aqueous $CuSO_4$ solution. The organic layer was dried over $MgSO_4$, treated with activated charcoal, and filtered. The filtrate was concentrated to $^1/_2$ volume and treated with hexane. The resulting suspension was stored overnight at −10 °C. The solid was collected by filtration, washed with hexane, and dried under vacuum to give 38.6 g (88%) of the desired product as a white solid: mp 87−88 °C; IR (KBr, cm^{-1}) 3560, 3399, 3280, 3044, 2930, 1723, 1625, 1537, 1195; ^{1}H NMR (DMSO-d_6) δ 0.83 (d, 3 H, J = 6.5 Hz), 0.89 (d, 3 H, J = 6.3 Hz), 1.38−1.72 (m, 11 H), 3.22−3.32 (m, 4 H), 4.20−4.26 (m, 1 H), 5.09 (dd, 2 H, J = 19.0, 12.5 Hz), 6.35 (d, 1 H, J = 8.0 Hz), 7.30−7.39 (m, 5 H); MS m/z 347 (M + 1 for $C_{20}H_{30}N_2O_3$).

(*S*)-2-[(Azepane-1-carbonyl)-amino]-4-methyl-pentanoic Acid (2). A solution of (*S*)-2-[(azepane-1-carbonyl)-amino]-4-methyl-pentanoic acid benzyl ester (**2a**, 8.97 g, 25.9 mmol) in THF (100 mL) was hydrogenated at 50 psi over Pd/C (0.25 g, 20% Pd) for 16 h. The reaction mixture was filtered through Celite and concentrated to dryness. The residue was heated in hexane (50 mL). The resulting suspension was cooled and the solid collected by filtration and washed with hexane. The solid was dried at room temperature under vacuum to give 6.1 g (92%) of the desired product **2** as a white solid: mp 88−89 °C; IR (KBr, cm^{-1}) 3366, 2930, 2869, 1727, 1609, 1544, 1210; ^{1}H NMR (DMSO-d_6) δ 0.79 (d, 3 H, J = 6.3 Hz), 0.84 (d, 3 H, J = 6.3 Hz), 1.39−1.44 (m, 5 H), 1.55−1.62 (m, 6 H), 3.22−3.33 (m, 4 H), 4.04−4.10 (m, 1 H), 6.10 (d, 1 H, J = 8.0 Hz), 12.20 (s, 1 H); MS m/z 258 (M + 1). Anal. ($C_{13}H_{24}N_2O_3$) C, H, N.

[1-(4-Benzyloxy-phenylcarbamoyl)-1-methyl-ethyl]-carbamic Acid *tert*-Butyl Ester (4a). 4-Benzyloxyaniline (5.05 g, 25.4 mmol) was dissolved in acetonitrile (125 mL) and treated with diisopropylethylamine (6.6 mL, 38.1 mmol), N^{α}-Boc-Aib acid (5.16 g, 25.4 mmol), and HBTU (9.63 g, 25.4 mmol). The solution was stirred for 3 h, then concentrated in vacuo. The residue was dissolved in EtOAc and filtered, then washed twice with saturated bicarbonate solution, once with brine, dried over Na_2SO_4, and concentrated. The crude material was chromatographed on silica gel eluting with 4% MeOH/CH_2Cl_2 to give 6.54 g (67%) of the desired product as a white solid: mp 183−184 °C; IR (KBr, cm^{-1}) 3314, 2979, 1688, 1531, 1511, 1232, 1168, 827; ^{1}H NMR ($CDCl_3$) δ 1.41 (s, 9 H), 1.52 (s, 6 H), 4.80 (br, 1 H), 5.01 (s, 2 H), 6.89 (d, 2 H, J = 9.0 Hz), 7.26−7.39 (m, 7 H), 8.80 (br, 1 H); MS m/z 385.1 (M + 1). Anal. ($C_{22}H_{28}N_2O_4$) C, H, N.

[2-(4-Benzyloxy-phenylamino)-1,1-dimethyl-ethyl]-carbamic Acid *tert*-Butyl Ester (4b). [1-(4-Benzyloxy-phenylcarbamoyl)-1-methyl-ethyl]-carbamic acid *tert*-butyl ester (**4a**, 6.54 g, 17.0 mmol) was dissolved in THF (50 mL) and cooled to 0 °C. A solution of diborane (51 mL, 1.0 M) in THF was added, and the reaction mixture was kept at 0 °C for 10 min and then heated to 65 °C for 18 h. The reaction was cooled to room temperature and treated with 0.1 M HCl (100 mL) and then neutralized with saturated sodium bicarbonate solution. The aqueous layer was extracted three times with EtOAc, dried over Na_2SO_4, and concentrated. The residue was chromatographed on silica gel eluting with 5:1 hexane/EtOAc to give 1.32 g (22%) of the desired product as an oil: IR (KBr, cm^{-1}) 3380, 2975, 1708, 1513, 1230, 1164, 819, 737; ^{1}H NMR ($CDCl_3$) δ 1.31 (s, 6 H), 1.39 (s, 9 H), 3.17 (s, 2 H), 3.65 (br, 1 H), 4.60 (br, 1 H), 4.95 (s, 2 H), 6.57 (d, 2 H, J = 8.8 Hz), 6.80 (d, 2 H, J = 8.8 Hz), 7.27−7.39 (m, 5 H); MS m/z 371.2 (M + 1). Anal. ($C_{22}H_{30}N_2O_3$) C, H, N.

{2-[(4-Benzyloxy-phenyl)-(3-methyl-but-2-enyl)-amino]-1,1-dimethyl-ethyl}-carbamic Acid *tert*-Butyl Ester (4c). [2-(4-Benzyloxy-phenylamino)-1,1-dimethyl-ethyl]-carbamic acid *tert*-butyl ester (**4b**, 0.6 g, 1.62 mmol) was dissolved in THF (32 mL) and treated with diisopropylethylamine (1.1 mL, 6.48 mmol) and 4-bromo-2-methyl-2-butene (0.37 mL, 3.24 mmol). The solution was heated to 40 °C overnight. The solution was cooled to room temperature, filtered, and concentrated. The residue was chromatographed on silica gel eluting with 8:1 hexane/EtOAc to give 0.56 (81%) of the desired product as an oil: IR (KBr, cm^{-1}) 2973, 1714, 1511, 1239, 1164, 733; ^{1}H NMR ($CDCl_3$) δ 1.27 (s, 6 H), 1.39 (s, 9 H), 1.62 (s, 3 H), 1.65 (s, 3 H), 3.38 (s, 2 H), 3.82 (d, 2 H, J = 5.6 Hz), 4.59 (s, 1 H), 4.95 (s, 2 H), 5.10 (br, 1 H), 6.70 (d, 2 H, J = 9.3 Hz), 6.82 (d, 2 H, J = 9.0 Hz), 7.25−7.39 (m, 5 H); MS m/z 439.1 (M + 1). Anal. ($C_{27}H_{38}N_2O_3$) C, H, N.

***N*-1-(4-Benzyloxy-phenyl)-2-methyl-*N*-1-(3-methyl-but-2-enyl)-propane-1,2-diamine (4d).** {2-[(4-Benzyloxy-phenyl)-(3-methyl-but-2-enyl)-amino]-1,1-dimethyl-ethyl}-carbamic acid *tert*-butyl ester (**4c**, 0.56 g, 1.32 mmol) was dissolved in CH_2Cl_2 (7 mL) and treated with TFA (3 mL). The solution was stirred for 2.5 h and then concentrated. The residue was twice redissolved in toluene and concentrated, then dissolved in EtOAc, washed three times with saturated sodium bicarbonate solution, once with brine, dried over Na_2SO_4, and concentrated to give 0.43 g (100%) of the desired product as an oil: IR (KBr, cm^{-1}) 2963, 1511, 1237, 813, 736; ^{1}H NMR ($CDCl_3$) δ 1.18 (s, 6 H), 1.63 (s, 3 H), 1.66 (s, 3 H), 3.16 (s, 2 H), 3.88 (s, 2 H), 4.97 (s, 2 H), 5.13 (s, 1 H), 6.78−6.86 (m, 4 H), 7.23−7.41 (m, 5 H); MS m/z 339.2 (M + 1). Anal. ($C_{22}H_{30}N_2O_1$) C, H, N.

(*S*)-Azepane-1-carboxylic Acid (1-{2-[(4-Benzyloxy-phenyl)-(3-methyl-but-2-enyl)-amino]-1,1-dimethyl-ethylcarbamoyl}-3-methyl-butyl)-amide (4). *N*-1-(4-Benzyloxy-phenyl)-2-methyl-*N*-1-(3-methyl-but-2-enyl)-propane-1,2-diamine (**4d**, 0.45 g, 1.32 mmol) was dissolved in DMF (7 mL) and treated with diisopropylethylamine (1.4 mL, 7.9 mmol), (*S*)-2-[(azepane-1-carbonyl)-amino]-4-methyl-pentanoic acid (**2**, 0.34 g, 1.32 mmol), and HBTU (0.5 g, 1.32 mmol). The reaction was stirred for 1 h, then diluted with EtOAc, washed once with saturated sodium bicarbonate solution, once with brine, dried over Na_2SO_4, and concentrated. The residue was chromatographed on silica gel eluting with 2:1 hexane/EtOAc to give 0.57 g (75%) of the desired product as a white foam: mp 104−107 °C; IR (KBr, cm^{-1}) 3283, 2928, 1650, 1619, 1542, 1511, 1239; ^{1}H NMR ($CDCl_3$) δ 0.87 (d, 3 H, J = 4.2 Hz), 0.88 (d, 3 H, J = 4.2 Hz), 1.31−1.64 (m, 22 H), 3.22−3.38 (m, 6 H), 3.77−3.80 (m, 2 H), 4.16−4.21 (m, 1 H), 4.61 (d, 2 H, J = 8.1 Hz), 4.94 (s, 2 H), 5.07−5.09 (m, 1 H), 6.31 (s, 1 H), 6.73 (d, 2 H, J

= 9.3 Hz), 6.82 (d, 2 H, *J* = 9.3 Hz), 7.25−7.39 (m, 5 H); MS *m*/*z* 577.1 (M + 1). Anal. ($C_{35}H_{52}N_4O_3$) C, H, N.

(*S*)-[1-(4-Benzyloxy-phenylcarbamoyl)-ethyl]-carbamic Acid *tert*-Butyl Ester (3a). *N*-Boc-L-alanine was subjected to the same procedure as described for compound **4a**, to give the desired product as a solid: IR (KBr, cm^{-1}) 3333, 1689, 1663, 1528, 1511, 1238, 1169, 827; ^{1}H NMR ($CDCl_3$) δ 1.28−1.41 (m, 12 H), 2.71−2.80 (m, 1 H), 4.23 (br, 1 H), 4.94 (s, 2 H), 5.32 (br d, 1 H, *J* = 6.1 Hz), 6.82 (d, 2 H, *J* = 6.8 Hz), 7.22−7.41 (m, 7 H); MS *m*/*z* 371.2 (M + 1 for $C_{21}H_{26}N_2O_4$).

(*S*)-[2-(4-Benzyloxy-phenylamino)-1-methyl-ethyl]-carbamic Acid *tert*-Butyl Ester (3b). Compound **3a** was subjected to the same procedure as described for compound **4b**, to give the desired product as an oil: IR (KBr, cm^{-1}) 3890, 2980, 1679, 1517, 1461, 1366, 1237, 1170, 1047, 812, 731; ^{1}H NMR ($CDCl_3$) δ 1.17 (d, 3 H, *J* = 6.8 Hz), 1.41 (s, 9 H), 3.00 (dd, 1 H, *J* = 12.5, 7.3 Hz), 3.07 (dd, 1 H, *J* = 12.5, 4.9 Hz), 3.88 (br, 2 H), 4.48 (br, 1 H), 4.95 (s, 2 H), 6.54 (d, 2 H, *J* = 8.8 Hz), 6.81 (d, 2 H, *J* = 9.0 Hz), 7.26−7.39 (m, 5 H); MS *m*/*z* 357.2 (M + 1 for $C_{21}H_{28}N_2O_3$).

(*S*)-{2-[(4-Benzyloxy-phenyl)-(3-methyl-but-2-enyl)-amino]-1-methyl-ethyl}-carbamic Acid *tert*-Butyl Ester (3c). Compound **3b** was subjected to the same procedure as described for compound **4c**, to give the desired product as an oil: IR (KBr, cm^{-1}) 3371, 2967, 1677, 1518, 1235, 1175, 1049, 802, 730; ^{1}H NMR ($CDCl_3$) δ 1.12 (d, 3 H, *J* = 6.3 Hz), 1.39 (s, 9 H), 1.64 (s, 3 H), 1.66 (s, 3 H), 2.99 (dd, 1 H, *J* = 13.4, 6.8 Hz), 3.26 (dd, 1 H, *J* = 14.4, 6.3 Hz), 3.71−3.84 (m, 3 H), 4.41 (br, 1 H), 4.96 (s, 2 H), 5.12 (br, 1 H), 6.70 (d, 2 H, *J* = 9.0 Hz), 6.84 (d, 2 H, *J* = 9.0 Hz), 7.23−7.40 (m, 5 H); MS *m*/*z* 425.3 (M + 1 for $C_{26}H_{36}N_2O_3$).

(*S*)-*N*^{1}-(4-Benzyloxy-phenyl)-*N*^{1}-(3-methyl-but-2-enyl)-propane-1,2-diamine (3d). Compound **3c** was subjected to the same procedure as described for compound **4d**, to give the desired product as a clear oil: IR (KBr, cm^{-1}) 2967, 2908, 2871, 1510, 1237, 813, 735, 695; ^{1}H NMR ($CDCl_3$) δ 1.09 (d, 3 H, *J* = 6.3 Hz), 1.63 (s, 3 H), 1.66 (s, 3 H), 2.61 (br, 2 H), 2.93 (dd, 1 H, *J* = 14.2, 9.0 Hz), 3.09−3.22 (m, 2 H), 3.79 (s, 2 H), 4.95 (s, 2 H), 5.13 (br, 1 H), 6.71 (d, 2 H, *J* = 9.3 Hz), 6.83 (d, 2 H, *J* = 6.8 Hz), 7.23−7.39 (m, 5 H); MS *m*/*z* 325.2 (M + 1 for $C_{21}H_{28}N_2O_1$).

[*S*-(*R,*R**)]-Azepane-1-carboxylic Acid (1-{2-[(4-Benzyloxy-phenyl)-(3-methyl-but-2-enyl)-amino]-1-methyl-ethylcarbamoyl}-3-methyl-butyl)-amide (3).** Compound **3d** was subjected to the same procedure as described for compound **4**, to give the desired product as a white solid: mp 130−132 °C; IR (KBr, cm^{-1}) 3262, 2927, 2865, 1619, 1536, 1512, 1229, 1026, 815, 734, 695; ^{1}H NMR ($CDCl_3$) δ 0.85 (dd, 6 H, *J* = 6.6, 2.0 Hz), 1.13 (d, 3 H, *J* = 6.6 Hz), 1.38−1.66 (m, 19 H), 2.96 (dd, 1 H, *J* = 14.4, 7.3 Hz), 3.29−3.34 (m, 5 H), 3.73−3.79 (m, 2 H), 4.07−4.23 (m, 2 H), 4.67 (d, 1 H, *J* = 8.3 Hz), 4.94 (s, 2 H), 5.10 (br, 1 H), 6.24 (d, 1 H, *J* = 7.1 Hz), 6.72 (d, 2 H, *J* = 9.3 Hz), 6.84 (d, 2 H, *J* = 9.0 Hz), 7.22−7.39 (m, 5 H); MS *m*/*z* 563.4 (M + 1). Anal. ($C_{34}H_{50}N_4O_3$) C, H, N.

4-(4-Benzyloxy-phenylamino)-piperidine-1-carboxylic Acid *tert*-Butyl Ester (7a). 4-Benzyloxyaniline hydrochloride salt (10 g, 42.4 mmol) was suspended in EtOAc (500 mL) and washed three times with saturated sodium bicarbonate solution, once with brine, dried over Na_2SO_4, and concentrated. The free base was dissolved in CH_2Cl_2 (250 mL), treated with 1-*tert*-butyl-carbonyl-4-piperidone (8.45 g, 42.4 mmol), stirred for 30 min and then cooled to 0 °C. $NaBH(OAc)_3$ (13.5 g, 63.6 mmol) was added, and the reaction was allowed to warm to room temperature and stir for 18 h. The reaction was diluted with CH_2Cl_2 (250 mL), washed with saturated sodium bicarbonate solution and brine, dried over Na_2SO_4, and concentrated to give 15.3 g (94%) the desired product as a white solid: mp 101−102 °C; IR (KBr, cm^{-1}) 3337, 2915, 1684, 1510, 1420, 1229, 1147, 1027, 835, 699; ^{1}H NMR ($CDCl_3$) δ 1.24−1.27 (m, 2 H), 1.43 (s, 9 H), 1.98 (br, 2 H, *J* = 12.0 Hz), 2.85 (t, 2 H, *J* = 11.5 Hz), 3.29−3.31 (m, 2 H), 4.00 (br, 2 H), 4.95 (s, 2 H), 6.54 (d, 2 H, *J* = 8.8 Hz), 6.81 (d, 2 H, *J* = 8.8 Hz), 7.27−7.39 (m, 5 H); MS *m*/*z* 383.1 (M + 1). Anal. ($C_{23}H_{30}N_2O_3$) C, H, N.

4-(4-Benzyloxy-phenylamino)-piperidine (7b). A solution of 4-(4-Benzyloxy-phenylamino)-piperidine-1-carboxylic acid *tert*-butyl ester (**7a**, 3 g, 7.84 mmol) in CH_2Cl_2 (20 mL) was treated with TFA (20 mL) and stirred for 30 min. The reaction was concentrated, diluted with EtOAc, washed with saturated sodium bicarbonate solution and brine, dried over Na_2SO_4, and concentrated to give 1.56 g (70%) of the TFA salt of the desired product as a white solid: mp 235−236 °C; IR (KBr, cm^{-1}) 3386, 2927, 1509, 1232, 1019, 698; ^{1}H NMR (DMSO-d_6) δ 1.40 (dd, 2 H, *J* = 21.5, 10.3 Hz), 1.91 (d, 2 H, *J* = 13.7 Hz), 2.82 (t, 2 H, *J* = 11.5 Hz), 3.13−3.30 (m, 4 H), 4.90 (s, 2 H), 5.17 (d, 1 H, *J* = 8.1 Hz), 6.49 (d, 2 H, *J* = 8.8 Hz), 6.73 (d, 2 H, *J* = 8.5 Hz), 7.23−7.36 (m, 5 H); MS *m*/*z* 283.1 (M + 1); HRMS *m*/*z* 283.1810 (calcd for M + 1), 283.1815 (found); HPLC purity 100%. Anal. ($C_{18}H_{22}N_2O_1$·0.5TFA·1.5H_2O) C, H, N.

(*S*)-Azepane-1-carboxylic Acid {1-[4-(4-Benzyloxy-phenylamino)-piperidine-1-carbonyl]-3-methyl-butyl}-amide (7). A solution of **7b** was subjected to the same procedure as described for compound **4**, to give the desired product as a white foam: mp 65−67 °C; IR (KBr, cm^{-1}) 3351, 2926, 2858, 1629, 1511, 1452, 1227, 697; ^{1}H NMR ($CDCl_3$) δ 0.88 (d, 3 H, *J* = 6.8 Hz), 0.96 (d, 3 H, *J* = 6.6 Hz), 1.20−1.68 (m, 13 H), 2.04−2.15 (m, 2 H), 2.76−2.89 (m, 1 H), 3.10−3.23 (m, 2 H), 3.36−3.41 (m, 5 H), 3.92 (t, 1 H, *J* = 12.2 Hz), 4.40 (dd, 1 H, *J* = 21.2, 14.4 Hz), 4.89−4.94 (m, 1 H), 4.95 (s, 2 H), 5.15−5.18, 1 H), 6.55 (d, 2 H, *J* = 8.5 Hz), 6.81 (dd, 2 H, *J* = 8.8, 2.7 Hz), 7.25−7.39 (m, 5 H); MS *m*/*z* 521.2 (M + 1). Anal. ($C_{31}H_{44}N_4O_3$) C, H, N.

(4-Benzyloxy-phenyl)-(1-benzyl-piperidin-3-yl)-amine (6a). A solution of 1-benzyl-3-piperidone was subjected to the same procedure as described for compound **7a**, to give the desired product as a solid: IR (KBr, cm^{-1}) 3262, 2930, 2807, 1506, 1452, 1295, 1217, 1045, 826, 732, 705; ^{1}H NMR ($CDCl_3$) δ 1.49 (br, 2 H), 1.66 (br, 2 H), 2.24−2.35 (m, 3 H), 2.68 (br, 1 H), 3.47 (m, 3 H), 3.74 (br, 1 H), 4.94 (s, 2 H), 6.53 (d, 2 H, *J* = 8.8 Hz), 6.79 (d, 2 H, *J* = 8.5 Hz), 7.20−7.39 (m, 10 H); MS *m*/*z* 373.1 (M + 1). Anal. ($C_{25}H_{28}N_2O_1$·0.2H_2O) C, H, N.

(4-Benzyloxy-phenyl)-(1-benzyl-piperidin-3-yl)-(3-methyl-but-2-enyl)-amine (6b). A solution of (4-benzyloxy-phenyl)-(1-benzyl-piperidin-3-yl)-amine (**6a**) was subjected to the same procedure as described for compound **10b**, to give the desired product as a gum: IR (KBr, cm^{-1}) 2934, 2797, 1509, 1237, 1027, 811, 735, 697; ^{1}H NMR ($CDCl_3$) δ 1.28−1.36 (m, 1 H), 1.57−1.91 (m, 12 H), 2.78 (d, 1 H, *J* = 10.7 Hz), 2.96 (d, 1 H, *J* = 10.3 Hz), 3.44−3.75 (m, 4 H), 4.95 (s, 2 H), 5.02 (s, 1 H), 6.66 (d, 2 H, *J* = 8.3 Hz), 6.82 (d, 2 H, *J* = 8.8 Hz), 7.20−7.40 (m, 10 H); MS *m*/*z* 441.2 (M + 1). Anal. ($C_{30}H_{36}N_2O_1$) C, H, N.

(4-Benzyloxy-phenyl)-(1-piperidin-3-yl)-(3-methyl-but-2-enyl)-amine (6c). (4-Benzyloxy-phenyl)-(1-benzyl-piperidin-3-yl)-(3-methyl-but-2-enyl)-amine (**6b**, 0.90 g, 2.04 mmol) was dissolved in 1,2-dichloroethane (20 mL), cooled to 0 °C, and treated with α-chloroethylchloroformate (0.24 mL, 2.04 mmol). The reaction was stirred at 0 °C for 15 min and then heated to 60 °C for 1 h. The reaction was cooled to room temperature, concentrated in vacuo, redissolved in MeOH (20 mL), heated to 50 °C for 1 h, and then concentrated in vacuo to give 0.86 g (>100%) of the crude material as an oil which was carried on without further purification: IR (KBr, cm^{-1}) 2928, 2803, 1511, 1452, 1220, 1039, 826, 740, 696; ^{1}H NMR ($CDCl_3$) δ 1.59 (s, 3 H), 1.65 (s, 3 H), 1.97−2.03 (m, 3 H), 2.70−2.76 (m, 2 H), 3.38−3.48 (m, 4 H), 3.73 (br, 2 H), 3.99 (br, 1 H), 4.98 (s, 2 H), 5.07 (s, 1 H), 6.84−6.89 (m, 2 H), 7.29−7.42 (m, 7 H); MS *m*/*z* 351.2 (M + 1 for $C_{23}H_{30}N_2O_1$).

(*S*,*R*/*S*)-Azepane-1-carboxylic Acid (1-{3-[(4-Benzyloxy-phenyl)-(3-methyl-but-2-enyl)-amino]-piperidine-1-carbonyl}-3-methyl-butyl)-amide (6). A solution of (4-benzyloxy-phenyl)-(1-piperidin-3-yl)-(3-methyl-but-2-enyl)-amine (**6c**) was subjected to the same procedure as described for compound **4**, to give the desired product as a gum: IR (KBr, cm^{-1}) 3337, 2928, 2863, 1734, 1632, 1509, 1453, 1406, 1375, 1284, 1240, 1212, 1025, 813, 738, 697; ^{1}H NMR (two diastereomers) ($CDCl_3$) δ 0.87−0.98 (m, 6 H), 0.98 (s, 3 H), 1.00 (s, 3 H), 1.32−

1.91 (m, 19 H), 2.58 (t, 1 H, J = 12.7 Hz), 3.03−3.13 (m, 1 H), 3.37−3.41 (m, 3 H), 3.73−3.78 (m, 3 H), 4.04 (d, 1 H, J = 13.7 Hz), 4.65−4.68 (m, 1 H), 4.92−4.95 (m, 1 H), 5.02 (s, 2 H), 5.25 (dd, 1 H, J = 13.9, 5.6 Hz), 6.32−6.35 (m, 2 H), 7.07−7.12 (m, 1 H), 7.27−7.42 (m, 4 H); MS m/z 589.3 (M + 1). Anal. ($C_{36}H_{52}N_4O_3$) C, H, N.

(*S*)-Azepane-1-carboxylic Acid (1-{4-[(4-Benzyloxy-phenyl)-(3-methyl-but-2-enyl)-amino]-piperidine-1-carbonyl}-3-methyl-butyl)-amide (5). A solution of compound **7** (0.39 g, 0.75 mmol) in THF (15 mL) was treated with diisopropylethylamine (0.4 g, 3 mmol) and 4-bromo-2-methyl-2-butene (0.149 g, 1 mmol). The reaction was heated to 40 °C for 18 h and then concentrated. The residue was chromatographed on silica gel to give the desired product as a gum: IR (KBr, cm^{-1}) 3338, 2927, 2859, 1632, 1509, 1453, 1238; ^{1}H NMR ($CDCl_3$) δ 0.87 (t, 3 H, J = 7.1 Hz), 0.96 (d, 3 H, J = 6.6 Hz), 1.32−1.67 (m, 20 H), 1.81−1.89 (m, 1 H), 2.59 (dd, 1 H, J = 22.7, 10.3 Hz), 3.01−3.08 (m, 1 H), 3.34−3.39 (m, 4 H), 3.49−3.51 (m, 1 H), 3.65 (s, 2 H), 3.97−4.00 (m, 1 H), 4.61 (t, 1 H, J = 14.4 Hz), 4.87−5.06 (m, 4 H), 5.21 (dd, 1 H, J = 22.0, 8.5 Hz), 6.70−6.76 (m, 2 H), 6.82−6.86 (m, 2 H), 7.26−7.40 (m, 5 H); MS m/z 589.2 (M + 1). Anal. ($C_{36}H_{52}N_4O_3$) C, H, N.

(*S*)-Azepane-1-carboxylic Acid (1-{4-[(4-Benzyloxy-phenyl)-ethyl-amino]-piperidine-1-carbonyl}-3-methyl-butyl)-amide (8). (*S*)-Azepane-1-carboxylic acid {1-[4-(4-benzyloxy-phenylamino)-piperidine-1-carbonyl]-3-methyl-butyl}-amide (**7**, 0.30 g, 0.28 mmol) was dissolved in CH_2Cl_2 (5 mL) and treated with acetaldehyde (32 μL, 0.58 mmol). The reaction was stirred for 30 min at room temperature, then cooled to 0 °C, treated with $NaBH(OAc)_3$, allowed to warm to room temperature as the ice melted and was stirred overnight. The reaction was then diluted with EtOAc (100 mL), washed with saturated bicarbonate solution and brine, dried over Na_2SO_4, and concentrated. The residue was chromatographed on silica gel eluting with 3:2 hexane/EtOAc to give 128 mg (61%) of the desired product as a gum: IR (KBr, cm^{-1}) 3344, 2928, 2865, 1632, 1509, 1453, 1239, 1025, 752; ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 3 H, J = 6.8 Hz), 0.96−1.0 (m, 6 H), 1.32−1.91 (m, 15 H), 2.61 (dd, 1 H, J = 23.9, 12.5 Hz), 3.00−3.10 (m, 3 H), 3.36−3.44 (m, 5 H), 3.98 (t, 1 H, J = 11.5 Hz), 4.56 (t, 1 H, J = 14.6 Hz), 4.90 (br s, 1 H), 4.97 (s, 2 H), 5.21 (dd, 1 H, J = 17.6, 8.3 Hz), 6.76−6.96 (m, 4 H), 7.28−7.41 (m, 5 H); MS m/z 549.3 (M + 1). Anal. ($C_{33}H_{48}N_4O_3$) C, H, N.

Azepane-1-carboxylic Acid (1-{4-[(4-Benzyloxy-phenyl)-(3-hydroxy-butyl)-amino]-piperidine-1-carbonyl}-3-methyl-butyl)-amide (9). A solution of 3-hydroxybutyraldehyde was subjected to the same procedure as described for compound **8**, to give the desired product as an oil: IR (KBr, cm^{-1}) 3347, 2928, 2865, 1627, 1509, 1454, 1371, 1239, 1027, 754; ^{1}H NMR ($CDCl_3$) δ 0.86 (d, 3 H, J = 6.3 Hz), 0.94 (d, 3 H, J = 9.3 Hz), 1.08 (d, 3 H, J = 5.9 Hz), 1.22−1.66 (m, 15 H), 1.82−1.87 (m, 2 H), 2.46−2.55 (m, 1 H), 2.91−3.35 (m, 8 H), 3.92−3.96 (m, 2 H), 4.40 (br, 1 H), 4.49−4.73 (m, 1 H), 4.84 (br, 1 H), 4.99 (s, 2H), 5.16 (t, 1 H, J = 7.6 Hz), 6.87−6.98 (m, 4 H), 7.29−7.41 (m, 5 H); MS m/z 593.2 (M + 1). Anal. ($C_{35}H_{52}N_4O_4$) C, H, N.

(*S*)-Azepane-1-carboxylic Acid (1-{4-[Benzyl-(4-benzyloxy-phenyl)-amino]-piperidine-1-carbonyl}-3-methyl-butyl)-amide (10). A solution of benzaldehyde was subjected to the same procedure as described for compound **8**, to give the desired product as a sticky solid: IR (KBr, cm^{-1}) 3428, 2927, 1632, 1511, 1452, 1239, 697; ^{1}H NMR ($CDCl_3$) δ 0.87 (d, 3 H, J = 6.1 Hz), 0.95 (t, 3 H, J = 5.6 Hz), 1.28−1.55 (m, 8 H), 1.65−1.67 (m, 5 H), 1.85−1.98 (m, 2 H), 2.59 (dd, 1 H, J = 18.6, 7.3 Hz), 3.01−3.14 (m, 1 H), 3.35−3.38 (m, 4 H), 3.72 (br, 1 H), 4.00 (d, 1 H, J = 12.7 Hz), 4.29 (s, 2 H), 4.61 (t, 1 H, J = 12.5 Hz), 4.86−4.89 (m, 1 H), 4.93 (s, 2 H), 5.18 (dd, 1 H, J = 15.6, 8.3 Hz), 6.70 (t, 2 H, J = 7.8 Hz), 6.80 (d, 2 H, J = 8.3 Hz), 7.17−7.38 (m, 10 H); MS m/z 611.3 (M + 1). Anal. ($C_{38}H_{50}N_4O_3$) C, H, N.

4-[(4-Benzyloxy-phenyl)-(3-methyl-but-2-enyl)-amino]-piperidine-1-carboxylic Acid *tert*-Butyl Ester (11a). 4-(4-Benzyloxy-phenylamino)-piperidine-1-carboxylic acid *tert*-butyl ester (**7a**, 5.0 g, 13.1 mmol) was dissolved in THF (65 mL) and then treated with *N*,*N*-diisopropylethylamine (9.1 mL, 52.4 mmol) and 4-bromo-2-methyl-2-butene (3.0 mL, 26.2 mmol). The reaction was heated to 40 °C for 18 h and then concentrated in vacuo. The residue was chromatographed on silica gel eluting with 5:1 hexane/EtOAc to give 5.15 g (87%) of the desired compound as an oil: IR (KBr, cm^{-1}) 2973, 2928, 2857, 1693, 1509, 1423, 1239, 1158, 1020; ^{1}H NMR ($CDCl_3$) δ 1.42 (s, 9 H), 1.43−1.56 (m, 3 H), 1.62 (s, 3 H), 1.63 (s, 3 H), 1.74 (d, 2 H, J = 11.5 Hz), 2.65 (br, 2 H), 3.41−3.45 (m, 1 H), 3.66 (d, 2 H, J = 5.4 Hz), 4.15 (br, 1 H), 4.96 (s, 2 H), 5.04 (br, 1 H), 6.71 (d, 2 H, J = 9.0 Hz), 6.84 (d, 2 H, J = 9.0 Hz), 7.27−7.40 (m, 5 H); MS m/z 451.4 (M + 1). Anal. ($C_{28}H_{38}N_2O_3$) C, H, N.

(4-Benzyloxy-phenyl)-(3-methyl-but-2-enyl)-piperidin-4-yl-amine (11b). 4-[(4-Benzyloxy-phenyl)-(3-methyl-but-2-enyl)-amino]-piperidine-1-carboxylic acid *tert*-butyl ester (**11a**, 5.0 g, 11.1 mmol) was dissolved in CH_2Cl_2 (20 mL) and treated with TFA (20 mL). The reaction was stirred for 10 min and then concentrated in vacuo. The solution was washed with saturated bicarbonate solution (2 × 400 mL) and brine (1 × 400 mL), dried over Na_2SO_4, and concentrated in vacuo to give 3.9 g (99%) of the desired product as a oil: IR (KBr, cm^{-1}) 2927, 1678, 1509, 1237, 1203, 1177, 1131, 1024, 697; ^{1}H NMR ($CDCl_3$) δ 1.60 (s, 3 H), 1.63 (s, 3 H), 1.65−1.75 (m, 2 H), 1.86 (d, 2 H, J = 11.2 Hz), 2.75 (t, 2 H, J = 12.5 Hz), 3.26 (d, 2 H, J = 12.7 Hz), 3.41−3.49 (m, 1 H), 3.67 (d, 2 H, J = 5.4 Hz), 4.62 (br, 1 H), 4.97 (s, 2 H), 5.01−5.05 (m, 1 H), 6.73 (d, 2 H, J = 9.3 Hz), 6.84 (d, 2 H, J = 9.0 Hz), 7.26−7.40 (m, 5 H); MS m/z 351.3 (M + 1). HRMS 351.2436 (calcd M + 1), 351.2444 (found); HPLC purity 100%. Anal. ($C_{23}H_{30}N_2O_1$·0.58TFA) C, H, N.

(*S*)-(1-{4-[(4-Benzyloxy-phenyl)-(3-methyl-but-2-enyl)-amino]-piperidine-1-carbonyl}-3-methyl-butyl)-carbamic Acid *tert*-Butyl Ester (11c). (4-Benzyloxy-phenyl)-(3-methyl-but-2-enyl)-piperidin-4-yl-amine (**11b**, 1.03 g, 2.94 mmol) was dissolved DMF (15 mL) and treated with DIEA (1.3 mL, 7.4 mmol), Boc-L-leucine hydrate (0.74 g, 2.94 mmol), and HBTU (1.11 g, 2.94 mmol). The reaction was stirred for 4.5 h, then diluted with EtOAc (200 mL), washed with saturated bicarbonate solution and brine, dried over Na_2SO_4, and concentrated. The crude material was chromatographed on silica gel eluting with 5% ($MeOH/CH_2Cl_2$) to give 1.17 g (71%) of the desired product as a white foam: IR (KBr, cm^{-1}) 3422, 2927, 2361, 1708, 1638, 1509, 1453, 1366, 1238, 1167, 1025, 697; ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 3 H, J = 6.3 Hz), 0.95 (dd, 3 H, J = 6.3, 2.0 Hz), 1.28−1.67 (m, 20 H), 1.81−1.91 (m, 2 H), 2.59 (dd, 1 H, J = 23.9, 12.9 Hz), 2.99−3.10 (m, 1 H), 3.49−3.53 (m, 1 H), 3.61−3.63 (m, 2 H), 3.94 (t, 1 H, J = 15.1 Hz), 4.57−4.63 (m, 2 H), 4.97 (s, 2 H), 5.02−5.05 (m, 1 H), 5.25−5.28 (m, 1 H), 6.73 (dd, 2 H, J = 11.5, 9.3 Hz), 6.84 (dd, 2 H, J = 9.0, 3.2 Hz), 7.26−7.40 (m, 5 H); MS m/z 564.5 (M + 1). Anal. ($C_{34}H_{49}N_3O_4$) C, H, N.

(*S*)-2-Amino-1-{4-[(4-benzyloxy-phenyl)-(3-methyl-but-2-enyl)-amino]-piperidin-1-yl}-4-methyl-pentan-1-one (11). (*S*)-(1-{4-[(4-Benzyloxy-phenyl)-(3-methyl-but-2-enyl)-amino]-piperidine-1-carbonyl}-3-methyl-butyl)-carbamic acid *tert*-butyl ester (**11c**, 1.07 g, 1.90 mmol) was dissolved in CH_2Cl_2 (4.5 mL) and treated with TFA (4.5 mL). The reaction was stirred for 25 min and then concentrated in vacuo. The residue was dissolved in EtOAc (175 mL), and the solution was washed twice with saturated bicarbonate solution and brine, dried over Na_2SO_4, and concentrated in vacuo to give 0.87 g (99%) of the desired product as an oil: IR (KBr, cm^{-1}) 2953, 1640, 1509, 1453, 1237, 1191, 1025; ^{1}H NMR ($CDCl_3$) δ 0.90 (d, 6 H, J = 3.9 Hz), 1.21−1.31 (m, 1 H), 1.33−1.54 (m, 5 H), 1.60 (s, 3 H), 1.62 (s, 3 H), 1.79−1.85 (m, 3 H), 2.58 (dd, 1 H, J = 20.0, 11.5 Hz), 3.01 (dd, 1 H, J = 23.7, 11.2 Hz), 3.51 (t, 1 H, J = 3.4 Hz), 3.62−3.70 (m, 3 H), 3.86 (d, 1 H, J = 13.2 Hz), 4.66 (d, 1 H, J = 12.5 Hz), 4.97 (s, 2 H), 5.03 (m, 1 H), 6.74 (t, 2 H, J = 8.8 Hz), 6.85 (d, 2 H, J = 8.8 Hz), 7.26−7.40 (m, 5 H); MS m/z 464.3 (M + 1). Anal. ($C_{29}H_{41}N_3O_2$) C, H, N.

(*S*)-1-{4-[(4-Benzyloxy-phenyl)-(3-methyl-but-2-enyl)-amino]-piperidin-1-yl}-2-isopropylamino-4-methyl-pentan-1-one (12). A solution of (*S*)-2-amino-1-{4-[(4-benzyloxy-phenyl)-(3-methyl-but-2-enyl)-amino]-piperidin-1-yl}-4-methyl-

pentan-1-one (**10**, 0.20 g, 0.43 mmol) and acetone (25 mg, 0.43 mmol) in CH_2Cl_2 (4 mL) was cooled to 0 °C, treated with $NaBH(OAc)_3$, allowed to warm to room temperature as ice melted, and stirred overnight. The reaction was diluted with EtOAc (100 mL), washed with saturated sodium bicarbonate solution and brine, dried over Na_2SO_4, and concentrated. The residue was chromatographed on silica gel eluting with 5% $MeOH/CH_2Cl_2$ to give 176 mg (81%) of the desired product as a gum: IR (KBr, cm^{-1}) 3490, 2957, 1640, 1509, 1452, 1237, 1025, 738; ^1H NMR ($CDCl_3$) δ 0.86−0.89 (m, 6 H), 0.99 (m, 5 H), 1.41−1.64 (m, 13 H), 1.83−1.86 (m, 3 H), 2.55−2.65 (m, 2 H), 2.98−3.09 (m, 1 H), 3.49−3.65 (m, 3 H), 3.92−3.95 (m, 1 H), 4.68 (d, 1 H, J = 13.4 Hz), 4.97 (s, 2 H), 5.05 (br s, 1 H), 6.74 (d, 2 H, J = 9.0 Hz), 6.85 (d, 2 H, J = 9.0 Hz), 7.26−7.40 (m, 5 H); MS m/z 506.4 (M + 1). Anal. ($C_{32}H_{47}N_3O_2$) C, H, N.

(*S*)-1-{4-[(4-Benzyloxy-phenyl)-(3-methyl-but-2-enyl)-amino]-piperidin-1-yl}-4-methyl-2-(3-methyl-butylamino)-pentan-1-one (13). A solution of isovaleraldehyde was subjected to the same procedure as described for compound **12**, to give the desired product as a gum:: IR (KBr, cm^{-1}) 2953, 1640, 1509, 1451, 1237, 1158, 1025, 738; ^1H NMR ($CDCl_3$) δ 0.82−0.89 (m, 11 H), 1.25−1.62 (m, 15 H), 1.73−1.86 (m, 3 H), 2.30−2.35 (m, 1 H), 2.44−2.50 (m, 1 H), 2.55−2.62 (m, 1 H), 3.03 (dd, 1 H, J = 29.1, 11.7 Hz), 3.50−3.55 (m, 2 H), 3.65 (s, 2 H), 3.97 (d, 1 H, J = 13.2 Hz), 4.69 (m, 1 H), 4.97 (s, 2 H), 5.05 (d, 1 H, J = 4.9 Hz), 6.74 (dd, 2 H, J = 9.0, 2.2 Hz), 6.85 (dd, 2 H, J = 9.0, 2.0 Hz), 7.26−7.40 (m, 5 H); MS m/z 534.5 (M + 1). Anal. ($C_{34}H_{51}N_3O_2$) C, H, N.

(*S*)-1-{4-[(4-Benzyloxy-phenyl)-(3-methyl-but-2-enyl)-amino]-piperidin-1-yl}-2-cyclohexylamino-4-methyl-pentan-1-one (14). A solution of cyclohexanone was subjected to the same procedure as described for compound **12**, to give the desired product as a gum: IR (KBr, cm^{-1}) 2925, 2853, 1640, 1509, 1449, 1237, 1025, 811, 738; ^1H NMR ($CDCl_3$) δ 0.86−0.89 (m, 6 H), 1.10−1.17 (m, 5 H), 1.38−1.85 (m, 20 H), 2.16 (m, 1 H), 2.54−2.66 (m, 1 H), 3.07 (dd, 1 H, J = 28.1, 13.4 Hz), 3.52 (t, 1 H, J = 11.2 Hz), 3.66 (d, 2 H, J = 5.6 Hz), 3.94 (d, 1 H, J = 12.5 Hz), 4.68 (br t, 1 H), 4.97 (d, 2 H, J = 2.7 Hz), 5.05 (s, 1 H), 6.74 (d, 2 H, J = 9.0 Hz), 6.85 (d, 2 H, J = 9.0 Hz), 7.26−7.40 (m, 5 H); MS m/z 546.5 (M + 1). Anal. ($C_{35}H_{51}N_3O_2$) C, H, N.

logP Determination. The protocol (a potentiometric titration method) for this assay has been described earlier.[20]

ClogP Determination. ClogP was calculated by Pallas software (version 1.2, released for Windows CompuDrug Chemistry Ltd., 1995).

Solubility Determination. Concentrated solution (10 mL) of individual compounds in DMSO were diluted 20-fold with PBS containing 25 mM potassium phosphate (pH 7.4) so that precipitation was observed. The suspension was stirred for 1 h at 22 °C followed by centrifugation at 20000*g* for 5 min. An aliquot of the supernatant was analyzed by HPLC; peak area and retention time were compared to those of a standard solution of the same compound in DMSO. The concentration of the supernatant (which equals solubility in 5% DMSO/95% 25 mM potassium phosphate in PBS pH 7.4) was used as a measure of aqueous solubility at neutral pH.

In Vitro Pharmacology. (1) IMR32 Assay. The IMR32 cell line was obtained from the American Type Culture Collection (Rockville, MD). Cells were grown in Eagle's Minimum Essential Medium with Earle's salts supplemented with 10% fetal bovine serum and 2 mM L-Gln and antibiotic/antimycotic mixture (Gibco). At approximately 80% confluency, differentiation was induced by the addition of 1 mM dibutyryl cAMP and 2.5 μM bromodeoxyuridine to the medium. After 7−13 days of differentiation, cells were detached using 0.5 mM EDTA and subsequently loaded with 5 μM Indo-1 acetoxymethyl ester (Molecular Probe, Eugene, OR) at 30 °C for 45 min. Dye-loaded cells at a concentration of ~10^7 cells/mL were resuspended in assay buffer (Hank's balanced salt solution with 10 mM HEPES/Tris pH 7.4 and 0.5% bovine serum albumin) and kept on ice until use. Fluorescence measurements were carried out in a Photon Technology International (PTI, South Brunswick, NJ) model RF-F3004 spectrofluorometer with dual emission monochromators using excitation at 350 nm and emission at 400 and 490 nM. The ratio of the emissions at the two wavelengths is a fraction of intracellular Ca^{2+} concentration. Different concentrations of the test compounds (dissolved and diluted in DMSO) were added to assay buffer containing approximately 3 × 10^6 loaded cells with 5 μM nitrendipine added to block L-type Ca^{2+} channels. Samples were incubated for 10 min for 30 °C, and then emission signals at 400 and 490 nm were acquired from each cuvette at 30 °C for 50 s. At 20 s after the start of reading, cells were depolarized by the addition of a high K^+ solution. Drug effects were expressed as a percentage of the amplitude of the K^+-evoked change in intracellular Ca^{2+} in drug-treated compared to control experiments. IC_{50} values in these studies were calculated from five-point concentration response curves for each compound and were estimated by fitting a four-parameter logistic function to the data using the least-squares method.

(2) Electrophysiology. The protocol for whole-cell voltage-clamp experiments on superior cervical ganglion (SCG) neurons evaluating drug actions on Ca^{2+} channels has been previously described.[21] Evaluation of Na^+ channel currents in from acutely isolated SCG neurons was done using similar techniques except solutions and voltage protocols were altered to isolate Na^+ channel currents.

After obtaining a whole-cell recording, control Ca^{2+} or Na^+ channel currents were elicited from holding potentials between −65 mV and −55 mV to a depolarized test potential (+20 mV for Ca^{2+} and 0 mV for Na^+). After stable current amplitudes were observed in control solution, the external solution was rapidly exchanged with an external solution containing test compound. Test compounds were applied until steady-state block was achieved. Data were expressed as percent inhibition of control amplitudes.

(3) Metabolic Stability in Human Liver Microsomes. The compounds were individually incubated (5 μM) with human liver microsomes (0.5 mg/mL protein) at 37 °C in duplicate or triplicate. At 0, 10, 20, and 40 min, 50 μL aliquots were removed and added to 100 μL of acetonitrile and 25 μL internal standard, PD 181210 (1 μg/mL) in acetonitrile. Standard curves were run in a similar manner with each compound at a concentration range of 1.25−5 μM. The samples were analyzed for parent concentration by LC/MS/MS to estimate in vitro metabolic half-life. These in vitro data represent the rate of oxidative and hydrolytic reaction (CYP450, FMO, esterases/amidases) but may not represent all possible metabolic routes of elimination (e.g., conjugative reactions).

In Vivo Experiments. (1) Audiogenic Seizure Model in DBA/2 Mice. For in vivo testing, compounds were dissolved in water using 1% (weight/volume) Emulphor/D5W (GAF Corp., Wayne, NJ) surfactant. Substances were administered by intravenous injection into the retro-orbital venous sinus. All testing was performed 15 min or 45 min after drug injection. All the male mice, 3−4 weeks old were obtained from Jackson Laboratories, Bar Harbor, ME. Mice were placed into an enclosed acrylic plastic chamber (21 cm height, approximately 30 cm diameter) with a high-frequency speaker (4 cm diameter) in the center of the top lid. An audio signal generator (Protek model B-8 10) was used to produce a continuous sinusoidal tone that was swept linearly in frequency between 8 and 16 kHz once each 10 ms. The average sound pressure level (SPL) during stimulation was approximately 100 dB at the floor of the chamber. Mice were placed within the chamber and allowed to acclimatize for 1 min. DBA/2 mice in the vehicle-treated group responded to the sound stimulus (applied until tonic extension occurred, or for a maximum of 60 s) with a characteristic seizure sequence consisting of wild running followed by clonic seizures, and later by tonic extension, and finally by respiratory arrest and death in 80% or more of the mice. In vehicle-treated mice, the entire sequence of seizures to respiratory arrest lasts approximately 15−20 s.

The incidence of all the seizure phases in the drug-treated and vehicle-treated mice was recorded, and the occurrence of tonic seizures was used for calculating anticonvulsant ED_{50}

values by probit analysis. Mice were used only once for testing at each time and dose point.

(2) Acetic Acid Writhing Test. The mouse acetic acid writhing test measures the acute nociceptive response elicited by injection of dilute acetic acid into the peritoneal cavity.[22] Nociceptive behavior is quantified by counting the incidence of abdominal constrictions in a fixed observation interval.

Male, CF-1 mice (26 and 30 g) were given a single, intraperitoneal injection of 0.6% acetic acid. This injection-evoked abdominal constrictions, defined as discrete episodes of torso and hind limb stretching with or without neck arching, were counted and recorded for 5 min, beginning 7 min after acetic acid injection. The mice are individually housed in Nalgene cages and allowed to move freely during the experimental period (12 min). Animals are sacrificed by CO_2 asphyxiation immediately after the 5 min observation period. Test compounds were administered by intravenous or oral routes approximately 10 min prior to administering the acetic acid. The dose−response relationship for antinociceptive effects during the acetic acid writhing test is assessed by plotting the incidence of abdominal constrictions against dose of the test compound. ED_{50} values are calculated using a four-parameter logistic function.

(3) Arterial Blood Pressure and Heart Rate Recording Procedures. Male Sprague−Dawley rats weighing between 220 and 280 g (Simonson Laboratories, Gilroy, CA) were used. Animals were acclimated to the laboratory enviroment for 5−7 days before entering the study. Approximately 2 h after recovery from isoflurane anesthesia and surgery stress, arterial blood pressure and heart rate (pulse rate) were recorded using a pressure transducer (model BP-100; CB Sciences, Inc., Dover, NH) connected to a computerized data collection system (MacLab, ADIstruments, Milford, MA). Rats were confined to their home cages during recording but were otherwise unrestrained. Animals were given intraarterial injection of 0.5−1 mL heparinized (50 IU/mL) saline, and their arterial lines were connected to the recording system. Arterial blood pressure and heart rate were recorded for at least 20 min until stable baselines were obtained prior to test article administration. Compound **11** (10 mg/kg) was intravenously infused over 10 min. Arterial blood pressure and heart rate were continuously monitored during and approximately 1 h post administration.

(4) Pharmacokinetics Study. Three Wistar rats received a 5 mg/kg bolus intravenous dose of compound **11** as a solution, and serial plasma samples were collected at various times up to 24 h postdose. Plasma samples were analyzed using direct protein precipitation with acetonitrile, and compound **11** was quantitated by Sciex LC/MS/MS system. A Betasil phenyl column (2.1 mm × 12 cm) was used with a mobile phase of acetonitrile:0.1% acetic acid (70:30, v/v).

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