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The Discovery of [1-(4-Dimethylamino-benzyl)-piperidin-4-yl]-[4-(3,3-dimethylbutyl)-phenyl]-(3-methyl-but-2-enyl)-Amine, an N-type Ca^{+2} Channel Blocker with Oral Activity for Analgesia

Lain-Yen Hu,[a,*] Todd R. Ryder,[a] Michael F. Rafferty,[a] Charles P. Taylor,[b] M. Rose Feng,[c] Be-Sheng Kuo,[c] Susan M. Lotarski,[b] George P. Miljanich,[d] Elizabeth Millerman,[d] Krista M. Siebers[a] and Balazs G. Szoke[d]

[a]*Department of Chemistry, Parke-Davis Pharmaceutical Research, Division of Warner-Lambert Company, 2800 Plymouth Road, Ann Arbor, MI 48105, USA*

[b]*Department of Neuroscience Therapeutics, Parke-Davis Pharmaceutical Research, Division of Warner-Lambert Company, 2800 Plymouth Road, Ann Arbor, MI 48105, USA*

[c]*Department of Pharmacokinetics, Dynamics and Metabolism, Parke-Davis Pharmaceutical Research, Division of Warner-Lambert Company, 2800 Plymouth Road, Ann Arbor, MI 48105, USA*

[d]*Elan Pharmaceuticals, Inc., 3760 Haven Avenue, Menlo Park, CA 94025, USA*

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Abstract—Our drug discovery efforts for N-type calcium channel blockers in the 4-piperidinylaniline series led to the discovery of an orally active analgesic agent **26**. 1-[4-Dimethylamino-benzyl)-piperidin-4-yl]-[4-(3,3-dimethyl-butyl)-phenyl]-(3-methyl-but-2-enyl)-amine (**26**) showed high affinity to functionally block N-type calcium channels ($IC_{50} = 0.7$ μM in the IMR32 assay) and exhibited high efficacy in the anti-writhing analgesia test with mice ($ED_{50} = 12$ mg/kg by po and 4 mg/kg by iv). In this report, the rationale for the design, synthesis, biological evaluation, and pharmacokinetics of this series of blockers is described.

Introduction

Voltage-sensitive calcium channels (VSCC) 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 VSCC 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 a 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.[2,3] An NDA for SNX-111 (ziconitide, an N-type VSCC administrated in vivo by an intrathecal route)[3] will be filed for its application in pain. Drug discovery efforts during the past decade have focused on small molecule N-type calcium channel for neuroprotection or analgesia.[4] Examples of known Ca^{+2} channel antagonists include flunarizine,[5] fluspirilene,[6] PD 157767,[7] SB-201823,[8] SB-206284,[9] NNC09-0026,[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 (**A**),[13] and PD 151307[14] (Chart 1).

In the process of searching for small molecule N-type calcium channel blockers, we have discovered and reported on compound **1** as a potential chemical lead[15] (Table 1). Blocker **1** was a potent antagonist for N-type calcium channels in the IMR32 human neuroblastoma cells ($IC_{50} = 0.67$ μM) and showed protection in the audiogenic DBA/2 seizure model ($ED_{50} = 6$ mg/kg, iv) as well as the antiwrithing model ($ED_{50} = 6$ mg/kg, iv).[15] However, the low oral activity and unfavorable pharmacokinetics (e.g., high volume of distribution and high clearance rate, Table 2) of **1** limited its future

*Corresponding author. Tel.: +1-734-622-5625; fax: +1-734-622-5165; e-mail: lain.yen.hu@wl.com

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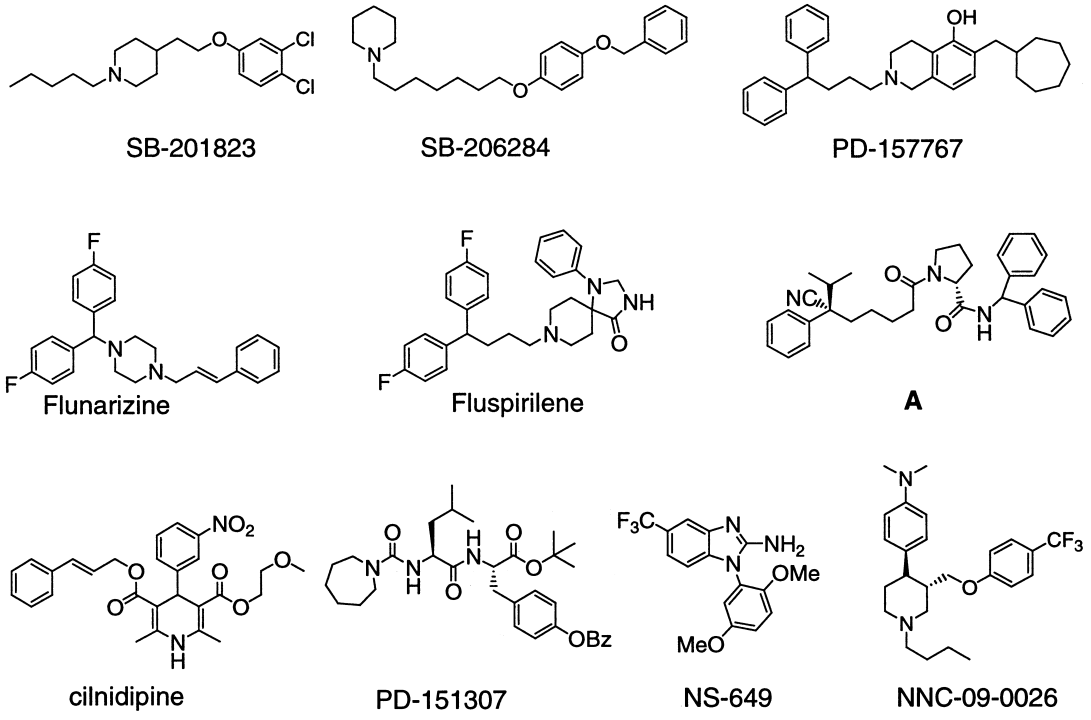


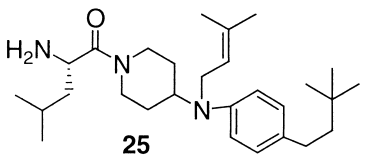
Chart 1.

development as an oral analgesic agent. Continued search for orally active N-type calcium channel blockers for analgesia led to further exploration and detailed SAR studies in this 4-piperidinylaniline series. Previously, we reported that several compounds were prepared with important structural modifications to compound **1** to improve its activity and pharmacokinetic profiles.[16] One of the major changes was converting the L-leucinyl group of **1** to its D-leucinyl,[16] 2-amino-4-methyl-pentyl,[16] or *N*-alkyl-L-leucinyl[15] derivative, which was well tolerated for N-type Ca^{+2} channel blocking activity. This suggested some flexibility in terms of the electronic and steric volume in the N-terminal region of the molecule. Therefore, we envisioned replacing the leucine moiety of **1** with an alkyl (or aralkyl) group to explore even more active blockers with structural diversity and ease of analoging (Table 1) This modification provided molecules without any amide functional group and with the potential to enhance the oral bioavailability of this series. In this paper, 24 novel antagonists with this 4-piperidinylaniline template are described. Their in vitro as well as in vivo activities and pharmacokinetics are discussed.

Chemistry

The preparation of compound **1** was previously reported.[15] Compounds **2**–**19** were synthesized by the reductive alkylation of **2** with the requisite aldehyde or ketone as outlined in Scheme 1. The synthesis of **2** is demonstrated in Scheme 1; it was a three-step procedure. Reductive alkylation of 4-benzyloxyaniline with *N*-Boc-4-piperidone followed by *N*-alkylation and deprotection with TFA.

Compound **20** was prepared by the reductive alkylation of **20e** (Scheme 2). The synthesis of **20e** began with the protection of the nitrogen of 4-iodoaniline to yield **20a**. Palladium coupling of **20a** followed by hydrogenation of the acetylene adduct (**20b**) provided *N*-Boc-4-(3,3-dimethylbutyl)-aniline (**20c**). Further deprotection of **20c** provided 4-(3,3-dimethylbutyl)-aniline (**20d**). Subsequent reductive alkylation of 4-(3,3-dimethylbutyl)-aniline (**20d**) with *N*-Boc-4-piperidone followed by *N*-alkylation and deprotection with TFA resulted in **20e**. Finally, the reductive alkylation of **20e** provided antagonist **20**. The synthesis of analogues **21**–**24** is illustrated in Scheme 3. The key intermediates **21a**–**24a** were prepared in a similar manner to **2**, except that the appropriately 4-substituted anilines were used as the starting materials instead of 4-benzyloxyaniline. Then, intermediates **21a**–**24a** were individually reductively alkylated with 3,3-dimethylbutyraldehyde to yield antagonists **21**–**24**. The key compound **26** was obtained in the alkylation of **20e** with 4-dimethylaminobenzaldehyde.



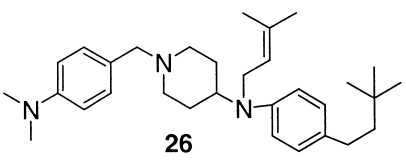
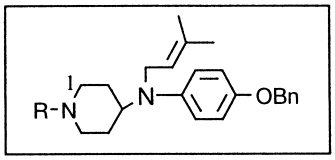


Table 1. Affinities of N-type calcium channel blockers



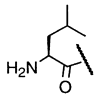
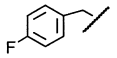
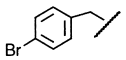
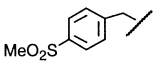
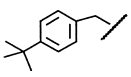
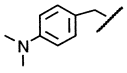
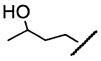
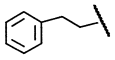
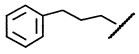
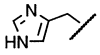
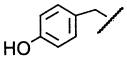
	R	IMR32 IC_{50} (μM)		R	IMR32 IC_{50} (μM)
1		0.67	**10**		0.83
			11		0.48
2	H	4.7	**12**		1.8
3		1.6	**13**		0.38
4		0.60	**14**		0.33
5		3.0	**15**		0.58
6		0.30	**16**		3.9
7		0.50	**17**		3.8
8		0.72	**18**		1.6
9		0.7	**19**		4.8

Table 2. In vivo activities and pharmakodynamic data of N-type calcium channel blockers

	IMR-32 IC_{50} (μM)	Anti-writhing test % protection or ED_{50} (mg/kg) (iv or po) ($n=6$ mice/dose tested)		Volume of distribution Vss (L/kg)	Clearance rate CL (mL/min/kg)
1	0.67	6 (iv)	>60 (po)	26	93
4	0.6	10 (iv)			
6	0.3	Death (iv)			
8	0.72	32% @ 10 (iv)			
14	0.33	5 (iv)	30 (po)	58	52
20	0.9	8% @ 10 (iv)			
25	1.1	14% @	10 (iv)	3.0	31
26	0.7	4 (iv)	12 (po)	5.0	26

Pharmacology

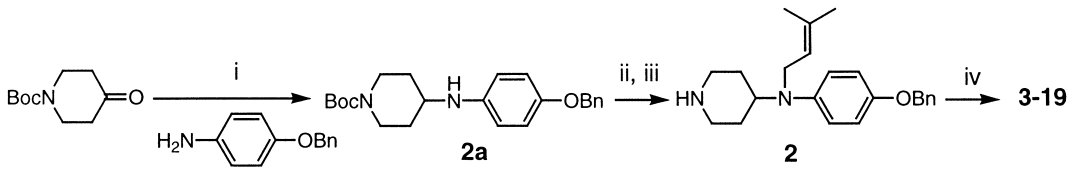
N-type Ca^{+2} channel blocking potencies of the compounds were determined using a fluorescence based Ca^{+2}-flux assay in IMR32 human neuroblastoma cells. PD-151307[14] was run in parallel in each assay as a standard to compare the relative potency of experimental compounds with the initial lead (Tables 1 and 3). The analgesic effects of selected compounds were evaluated in an acetic acid anti-writhing model.[17] Test compounds were administered by intravenous or oral routes approximately 15–30 min prior to administering the 0.6% acetic acid (ip) to the male, CF-1 mice (26 and 30 g). The injection evoked abdominal constrictions, which were counted and recorded for 5 min, beginning 7 min after acetic acid injection. The dose–response relationship for antinociceptive effects during the acetic acid writhing test were assessed by plotting the incidence of abdominal constrictions against dose of the test compound. ED_{50} values were calculated using a four parameter logistic function (Table 2).

Results and Discussion

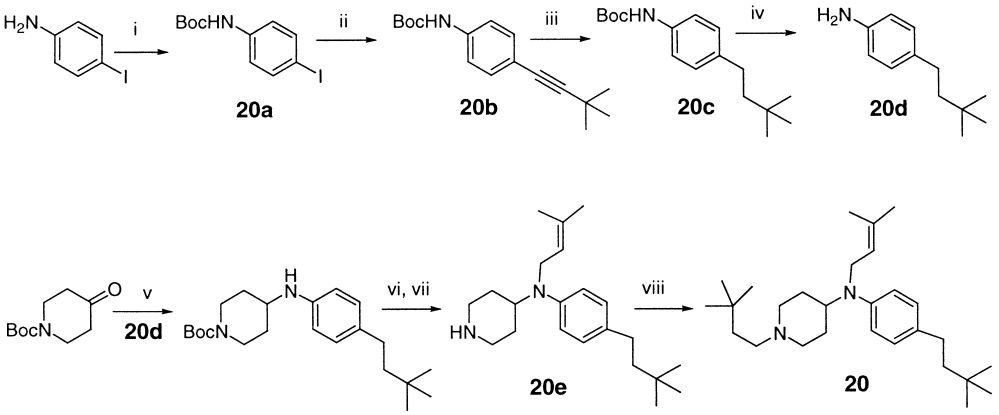
The consideration that the leucine group on the lead **1** might have resulted in poor oral activity prompted us to investigate the leucine replacement in this series. Firstly, we removed the leucine moiety of **1** and prepared **2**, which showed a 7-fold lower potency (**2**, $IC_{50}=4.7$ μM, Table 1). This confirmed our observation that the N^1-substitution is important for blocking N-type Ca^{+2} channels. Secondly, with the suggestion that 2-methylbutyl could mimic the leucine group, we added the 2-methylbutyl group to **2** and restored the activity somewhat (**3**, $IC_{50}=1.6$ μM). Thirdly, we found that the 3,3-dimethylbutyl derivative (**4**) was equipotent to the chemical lead **1** in the IMR32 assay ($IC_{50}=0.6$ μM, Table 1). Furthermore, an in vivo evaluation of compound **4** revealed reasonable analgesic efficacy in the anti-writhing model ($ED_{50}=10$ mg/kg, iv, Table 2). Based on these promising findings, we decided to continue exploring this series for orally active N-Ca^{+2} channel blockers.

Modification of the 3,3-dimethylbutyl group of **4** with a benzyl group provided an ion channel blocker with similar potency (**8**, $IC_{50}=0.7$ μM). An SAR comparison on the alkyl chain length of a set of phenalkyl derivatives (**6–8**) was conducted to optimize the N-Ca^{+2} channel activity of **8**. Among these, the ethylene linker (**6**, $IC_{50}=0.3$ μM) was slightly more preferred than the propylene (**7**, $IC_{50}=0.5$ μM) or the methylene (**8**, $IC_{50}=0.7$ μM) linker (Table 1). Interestingly, the methylene analogue (**8**) showed moderate in vivo efficacy in the anti-writhing model while the ethylene blocker (**6**) displayed lethality for unknown reasons (Table 2). Therefore, the following discovery effort was continued around the benzyl analogue (**8**).

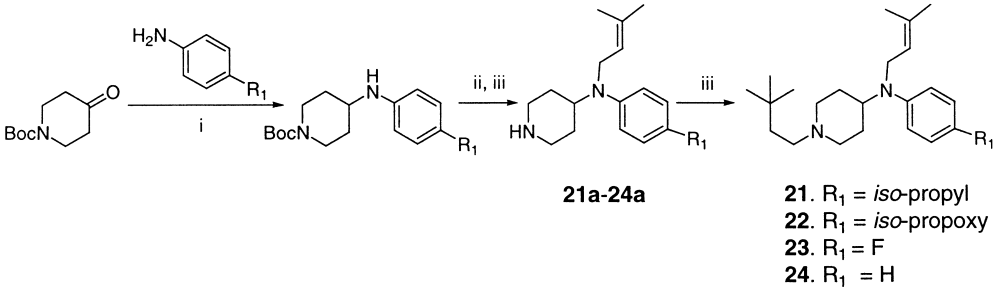
The subsequent synthesis of several substituted benzyl derivatives of **8** was directed toward optimizing N-type Ca^{+2} channel activities. In the *N*-methyl-*N*-benzyl-dipeptidyl Ca^{+2} channel blocker series, it was reported that the substitution effect on the benzyl group significantly influenced the N-Ca^{+2} channel potency.[18] With the goal of improving the activity of this series, here, the SAR of substituted benzyl analogues of **8** was compared among a selected set of analogues. These were hydrogen (**8**), hydroxy (**9**), fluoro (**10**), bromo (**11**), methylsulfonyl (**12**), *t*-butyl (**13**), and *N*,*N*-dimethyl-



Scheme 1. (i) $NaBH(OAc)_3$, CH_2Cl_2; (ii) 4-bromo-2-methyl-2-butene, THF, DIEA; (iii) TFA, CH_2Cl_2; (iv) $NaBH(OAc)_3$, CH_2Cl_2, the requisite aldehyde or ketone.



Scheme 2. (i) $(Boc)_2O$, THF, reflux; (ii) 3,3-dimethyl-1-butyne, $Pd(PPh_3)_2Cl_2$, Cul, TEA, THF; (iii) 20% Pd/C, MeOH, H_2; (iv) TFA, CH_2Cl_2; 9v) $NaBH(OAc)_3$, CH_2Cl_2; (vi) 4-bromo-2-methyl-2-butene, THF, DIEA; (vii) TFA, Ch_2Cl_2; (viii) $NaBH(OAc)_3$, CH_2Cl_2, 3,3-dimethylbutyraldehyde.



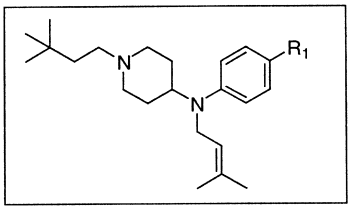
Scheme 3. (i) $NaBH(OAc)_3$, CH_2Cl_2; (ii) 4-bromo-2-methyl-2-butene, THF, DIEA; (iii) TFA, CH_2Cl_2; (iv) $NaBH(OAc)_3$, CH_2Cl_2, 3,3-dimethylbutyraldehyde.

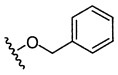
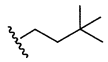
amino (**14**). The potencies of these compounds ranged from 0.33 to 1.8 μM (Table 1). A careful comparison revealed a graded preference for the *N*,*N*-dimethylamino (**14**), *t*-butyl (**13**), and bromo (**11**) over hydrogen (**8**), hydroxy (**9**), fluoro (**10**), or methylsulfonyl (**12**). The observation that the *N*,*N*-dimethylamino (**14**), bromo (**11**), and *t*-butyl (**13**) substituents were more preferred than other substitutions for active blockade is in agreement with our previous observation in the peptidyl compound series.[18] It is worth mentioning that the *N*,*N*-dimethylamino derivative (**14**) was 2-fold more potent than the lead **1** in the IMR32 assay. Remarkably, the in vivo assessment of **14** in the anti-writhing model displayed an enhanced efficacy by po administration ($ED_{50} = 30$ mg/kg) and a similar potency by the iv route ($ED_{50} = 5$ mg/kg) compared to **1** (Table 2). These results clearly revealed that our approach to improve the oral activity of this series was successful.

Replacement of the leucine group of **1** with a benzyl moiety offered compound (**8**) with similar potency. This prompted us to investigate heteroaryl-containing analogues of **8** and targeted N-type Ca^{+2} channel blockers with lower LogP. We compared the benzyl (**8**), 4-*N*,*N*-dimethylaminobenzyl (**14**), 2-pyrrolylmethyl (**15**), 2-furanylmethyl (**16**), 2-imidazolylmethyl (**17**), 2-pyridylmethyl (**18**), and 4-tetrahydropyranyl (**19**) derivatives (Table 1). Their activities ranged from 0.33 to 4.8 μM in the IMR32 assay. The pyrrolylmethyl blocker (**15**) was approximately equipotent to the benzyl analogue (**8**), while antagonists **16**–**19** displayed lower blockade in the IMR32 assay. The 4-*N*,*N*-dimethylaminobenzyl (**14**) was still the most active blocker in this series.

In this study, our goal was to provide an orally active N-type Ca^{+2} channel blocker for analgesic use. And so far, compound **14** showed the most advantageous in vivo profile. However, two in vivo issues were considered: that the *O*-benzyloxy group of **14** could result in fast metabolism via cleavage of the ether linkage and that the phenyl group could be oxidized by P450 liver enzymes. This led us to further investigate the *O*-benzyloxy group of these 4-piperidinyl-(4-benzyloxyaniline) antagonists. Initially, we replaced the *O*-benzyloxy

Table 3. Affinities of N-type calcium channel blockers



	R_1	IMR32 IC_{50} (μM)
4		0.60
20		0.90
21		1.3
22		2.9
23	F	3.9
24	H	7.8

moiety of **4** with a 3,3-dimethylbutyl, *iso*-propyl, *iso*-propoxy, fluoro, or hydrogen group and prepared **20**–**24** (Table 3). These antagonists showed various activities for blocking N-type Ca^{+2} channels. Among these, the 3,3-dimethylbutyl (**20**, $IC_{50}=0.9$ μM) analogue was more active and was approximately equipotent to the benzyloxy lead (**4**), while the *iso*-propyl (**21**, $IC_{50}=1.3$ μM), *iso*-propoxy (**22**, $IC_{50}=2.9$ μM), fluoro (**23**, $IC_{50}=3.9$ μM), or hydrogen (**24**, $IC_{50}=7.8$ μM) group resulted in the decreased activity for N-type Ca^{+2} channel blockade. It appeared that the IMR32 potency correlated with the size and lipophilicity of the R_1 substituent. A larger alkyl group for R_1 was advantageous for blockade, while a small group such as hydrogen for R_1 was not sufficient for antagonizing the N-Ca^{+2} channels.

The previous report indicated that converting the *O*-benzyloxy group of **1** to the 3,3-dimethylbutyl moiety resulted in a N-type Ca^{+2} channel blocker (**25**) with improved pharmacokinetics but approximately 2-fold lower activity ($IC_{50}=1.1$ μM)[16] (Table 2). Here, we discovered that antagonist **14** was efficacious in vivo by both the iv and po routes. Thus, we combined the 4-*N*,*N*-dimethylaminobenzyl group of **14** with the 4-[*N*-(3-methyl-but-2-enyl)-4-(3,3-dimethyl-butyl)-phenylamino]-piperidinyl moiety of **20**/**25** and prepared blocker **26**. Compound **26** showed good activities in the IMR32 assay ($IC_{50}=0.7$ μM) and in the acetic acid anti-writhing model ($ED_{50}=4$ mg/kg, iv) (Table 2). Remarkably, ligand **26** exhibited further enhanced oral activity ($ED_{50}=12$ mg/kg, po) compared to either its parent compound **14** or the lead **1**. This (**26**) is the most orally active N-type Ca^{+2} channel blocker for analgesia found in this series. A time course study of **26** in the anti-writhing model indicated that the CF-1 mice had maximal effect at 120 min after oral dosing at 60 mg/kg. Further evaluation of **26** demonstrated several important and advantageous features: the pharmacokinetic profile of **26** was improved (Versus of 5.9 L/kg and CL of 26 mL/min/kg) and the logPn of **26** was favorable for CNS agent (logPn measured to be 3.20). Overall speaking, antagonist **26** showed the preferred profiles for an analgesic with oral activity.

Conclusion

In summary, a series of 4-piperidinylaniline based N-type calcium channel blockers has been described; they were derived from the early lead **1**. These new structures demonstrated potent in vitro and in vivo activities. From this study, **26** [1-(4-dimethylamino-benzyl)-piperidin-4-yl]-[4-(3,3-dimethyl-butyl)-phenyl]-(3-methyl-but-2-enyl)-amine) was discovered with several important features: potent N-type calcium channel activity ($IC_{50}=0.7$ μM in the IMR32 assay) and good analgesic activity ($ED_{50}=4$ mg/kg, iv in anti-writhing model). Ligand **26** displayed several advantages over the early lead 1: enhanced oral activity ($ED_{50}=12$ mg/kg, po in anti-writhing model), preferred logPn (3.20), and an improved pharmacokinetic profile (Vss = 5 L/kg and CL = 26 mL/min/kg).

Experimental

Chemistry

Melting points were determined in open capillary tubes on a Thomas–Hoover apparatus and are uncorrected. IR spectra were recorded as KBr discs 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. The preparation of compounds **1** and **25** was previously reported.[15,16]

General method for reductive amination for preparing compounds 3–24 and 26

The appropriately 4-substituted piperidine intermediate (0.57 mmol) was dissolved in dichloromethane and treated with the aldehyde (0.57 mmol). The reaction was stirred for 30 min, then cooled to 0 °C in an ice bath and treated with $NaBH(OAc)_3$ (0.18 g, 0.86 mmol). The reaction was allowed to warm to room temperature as

the ice melted and stirred for 18 h at room temperature. The reaction was diluted with EtOAc (125 mL), then washed with saturated bicarbonate (125 mL) and brine (125 mL), dried over Na_2SO_4, and concentrated. The residue was chromatographed on silica gel eluting with 7% MeOH/CH_2Cl_2 to give the product.

4-(4-Benzyloxy-phenylamino)-piperidine-1-carboxylic acid *tert*-butyl ester (2a). 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 stirred 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; ^{1}H NMR ($CDCl_3$) δ 1.24–1.27 (m, 2H), 1.43 (s, 9H), 1.98 (br, 2H, J = 12.0 Hz), 2.85 (t, 2H, J = 11.5 Hz), 3.29–3.31 (m, 2H), 4.00 (br, 2H), 4.95 (s, 2H), 6.54 (d, 2H, J = 8.8 Hz), 6.81 (d, 2H, J = 8.8 Hz), 7.27–7.39 (m, 5H); MS m/z: 383 (M + 1); anal. ($C_{23}H_{30}N_2O_3$) C, H, N.

4-[(4-Benzyloxy-phenyl)-(3-methyl-but-2-enyl)-amino]-piperidine-1-carboxylic acid *tert*-butyl ester (2b). 4-(4-Benzyloxy-phenylamino)-piperidine-1-carboxylic acid *tert*-butyl ester (**2a**, 5.0 g, 13.1 mmol) was dissolved in THF (65 mL), 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, 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. ^{1}H NMR ($CDCl_3$) δ 1.42 (s, 9H), 1.43–1.56 (m, 3H), 1.62 (s, 3H), 1.63 (s, 3H), 1.74 (d, 2H, J = 11.5 Hz), 2.65 (br, 2H), 3.41–3.45 (m, 1H), 3.66 (d, 2H, J = 5.4 Hz), 4.15 (br, 1H), 4.96 (s, 2H), 5.04 (br, 1H), 6.71 (d, 2H, J = 9.0 Hz), 6.84 (d, 2H, J = 9.0 Hz), 7.27–7.40 (m, 5H); MS m/z 451 (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 (2). 4-[(4-Benzyloxy-phenyl)-(3-methyl-but-2-enyl)-amino]-piperidine-1-carboxylic acid *tert*-butyl ester (**2b**, 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, 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 an oil. ^{1}H NMR ($CDCl_3$) δ 1.60 (s, 3H), 1.63 (s, 3H), 1.65–1.75 (m, 2H), 1.86 (d, 2H, J = 11.2 Hz), 2.75 (t, 2H, J = 12.5 Hz), 3.26 (d, 2H, J = 12.7 Hz), 3.41–3.49 (m, 1H), 3.67 (d, 2H, J = 5.4 Hz), 4.62 (br, 1H), 4.97 (s, 2H), 5.01–5.05 (m, 1H), 6.73 (d, 2H, J = 9.3 Hz), 6.84 (d, 2H, J = 9.0 Hz), 7.26–7.40 (m, 5H); MS m/z 351 (M + 1); anal. ($C_{23}H_{30}N_2O_1$.0.58 TFA) C, H, N; HRMS 351.2436 (calcd M + 1 for $C_{23}H_{30}N_2O_1$), 351.2444 (found); HPLC purity: 100%.

(4-Benzyloxy-phenyl)-[1-(3,3-dimethyl-butyl)-piperidin-4-yl]-(3-methyl-but-2-enyl)-amine (4). (4-Benzyloxy-phenyl)-(3-methyl-but-2-enyl)-piperidin-4-yl-amine (**2**, 0.2 g, 0.57 mmol) was dissolved in dichloromethane and treated with that 3,3-dimethylbutyraldehyde (70 μL, 0.57 mmol). The reaction was stirred for 30 min, then cooled to 0 °C in an ice bath and treated with $NaBH(OAc)_3$ (0.18 g, 0.86 mmol). The reaction was allowed to warm to room temperature as the ice melted and stirred for 18 h at room temperature. The reaction was diluted with EtOAc (125 mL), then washed with saturated bicarbonate (125 mL) and brine (125 mL), dried over Na_2SO_4, and concentrated. The residue was chromatographed on silica gel eluting with 7% MeOH/CH_2Cl_2 to give the title compound as an oil. Yield: 54%; ^{1}H NMR ($CDCl_3$, ppm) δ 0.86 (s, 9H), 1.37 (br, 2H), 1.62 (s, 6H), 1.69–1.74 (m, 4H), 1.94 (br, 2H), 2.29 (br, 2H), 2.99 (d, 2H, J = 10.5 Hz), 3.34 (br, 1H), 3.70 (d, 2H, J = 3.9 Hz), 4.96 (s, 2H), 5.03 (br, 1H), 6.70 (d, 2H, J = 8.0 Hz), 6.83 (d, 2H, J = 8.0 Hz), 7.25–7.40 (m, 5H); MS m/z: 435 (M + 1); anal. ($C_{29}H_{42}N_2O_1$) C, H, N.

(4-Benzyloxy-phenyl)-(3-methyl-but-2-enyl)-[1-(3-methyl-butyl)-piperidin-4-yl]-amine (3). This compound was prepared in an analogous manner to that used for **4**, except that isovaleraldehyde was used instead of 3,3-dimethylbutyraldehyde. Yield: 71%; an oil; ^{1}H NMR ($CDCl_3$, ppm) δ 0.86 (d, 6H, J = 6.6 Hz), 1.39 (br, 2H), 1.51–1.58 (m, 1H), 1.62 (s, 6H), 1.75 (br, 4H), 1.99 (br, 2H), 2.34 (br, 2H), 3.01 (d, 2H, J = 9.0 Hz), 3.35 (br, 1H), 3.70 (d, 2H, J = 4.4 Hz), 4.96 (s, 2H), 5.03 (br, 1H), 6.69 (d, 2H, J = 8.8 Hz), 6.83 (d, 2H, J = 9.0 Hz), 7.25–7.40 (m, 5H); MS m/z: 421 (M + 1); anal. ($C_{28}H_{40}N_2O_1$.0.25H_2O) C, H, N.

4-{4-[(4-Benzyloxy-phenyl)-(3-methyl-but-2-enyl)-amino]-piperidin-1-yl}-butan-2-ol (5). This compound was prepared in an analogous manner to that used for **4**, except that 3-hydroxybutyraldehyde was used instead of 3,3-dimethylbutyraldehyde. Yield: 34%; an oil; ^{1}H NMR ($CDCl_3$, ppm) δ 1.13 (d, 3H, J = 6.1 Hz), 1.25–1.40 (br, 3H), 1.59 (s, 6H), 1.67 (br, 4H), 2.16 (br., 1H), 2.34–2.70 (br, 2H), 2.99 (br., 1H), 3.30 (br, 1H), 3.33 (m, 1H), 3.65 (d, 2H, J = 5.4 Hz), 3.90 (m, 1H), 4.93 (s, 2H), 5.01 (br, 1H), 6.68 (d, 2H, J = 8.8 Hz), 6.81 (d, 2H, J = 9.0 Hz), 7.20–7.40 (m, 5H); MS m/z: 423 (M + 1); anal. ($C_{27}H_{38}N_2O_2$.0.25H_2O) C, H, N.

(4-Benzyloxy-phenyl)-(3-methyl-but-2-enyl)-(1-phenethyl-piperidin-4-yl)-amine (6). This compound was prepared in an analogous manner to that used for **4**, except that phenylacetaldehyde was used instead of 3,3-dimethylbutyraldehyde. Yield: 75%; an oil; ^{1}H NMR ($CDCl_3$, ppm) δ 1.63 (s, 6H), 1.78 (br s, 2H), 2.07 (br s, 2H), 2.58 (br s, 2H), 2.80 (br s, 2H), 3.07 (br s, 2H), 3.38 (br s, 2H), 3.71 (s, 2H), 4.96 (s, 2H), 5.05 (s, 1H), 6.70 (d, 2H, J = 8.8 Hz), 6.84 (d, 2H, J = 9.03 Hz), 7.16–7.41 (m, 10 H); MS m/z: 455 (M + 1); anal. ($C_{31}H_{38}N_2O_1$) C, H, N.

(4-Benzyloxy-phenyl)-(3-methyl-but-2-enyl)-[1-(3-phenyl-propyl)-piperidin-4-yl]-amine (7). This compound was prepared in an analogous manner to that used for **4**, except that 3-phenylpropionaldehyde was used instead

of 3,3-dimethylbutyraldehyde. Yield: 59%; an oil; ^{1}H NMR ($CDCl_3$, ppm) δ 1.55 (s, 2H) 1.62–1.80 (m, 6H), 1.93 (t, 3H, J = 11.2 Hz), 2.32 (t, 2H, *J* = 7.6 Hz), 2.59 (t, 2H, *J* = 7.8 Hz), 2.95 (d, 2H, *J* = 11.7 Hz), 3.30–3.35 (m, 1H), 3.70 (d, 2H, *J* = 5.6 Hz), 4.96 (s, 2H), 5.04 (s, 1H), 6.67 (d, 2H, *J* = 9.0 Hz), 6.83 (d, 2H, *J* = 9.0 Hz), 7.15 (d, 3H, *J* = 6.35 Hz), 7.24–7.27 (m, 6H), 7.29–7.40 (m, 4H); MS *m*/*z*: 469 (M + 1); anal. ($C_{32}H_{40}N_2O_1$) C, H, N.

(4-Benzyloxy-phenyl)-(1-benzyl-piperidin-4-yl)-(3-methyl-but-2-enyl)-amine (8). This compound was prepared in an analogous manner to that used for **4**, except that benzaldehyde was used instead of 3,3-dimethylbutyraldehyde. The product was made as the HCl salt. Yield: 90%, an oil; ^{1}H NMR (CD_3OD, ppm) δ 1.63 (s, 3H), 1.63 (s, 3H), 1.63–1.72 (br., 4H), 2.02 (br., 2H), 2.93 (s, 1H), 2.95 (s, 1H), 3.27 (m, 1H), 3.49 (s, 2H), 3.71 (d, 2H, *J* = 4.9 Hz), 4.96 (s, 2H), 5.04 (m, 1H), 6.73 (d, 2H, *J* = 9.3 Hz), 6.84 (d, 2H, *J* = 9.0 Hz), 7.26–7.40 (m, 10 H); MS *m*/*z*: 441 (M + 1 for $C_{30}H_{36}N_2O_1$); anal. ($C_{30}H_{36}N_2O_1.2HCl.0.5H_2O$) C, H, N.

4-{4-[(4-Benzyloxy-phenyl)-(3-methyl-but-2-enyl)-amino]-piperidin-1-ylmethyl}-phenol (9). This compound was prepared in an analogous manner to that used for **4**, except that 4-hydroxybenzaldehyde was used instead of 3,3-dimethylbutyraldehyde. Yield: 31%; ^{1}H NMR ($CDCl_3$, ppm) δ 1.59 (s, 3H), 1.60 (s, 3H), 1.72 (m, 4H), 2.01 (m, 2H), 2.98 (d, 2H, *J* = 11.2 Hz), 3.35–3.42 (m, 3H),), 3.68 (d, 2H, *J* = 4.2 Hz), 4.95 (s, 2H), 5.02 (s, 1H), 6.63 (d, 2H, *J* = 8.3 Hz), 6.67 (d, 2H, *J* = 9.0 Hz), 6.82 (d, 2H, *J* = 9.0 Hz), 7.25–7.40 (m, 5H); MS *m*/*z*: 457 (M + 1); anal. ($C_{30}H_{36}N_2O_2.0.25\ H_2O$) C, H, N.

(4-Benzyloxy-phenyl)-[1-(4-fluoro-benzyl)-piperidin-4-yl]-(3-methyl-but-2-enyl)-amine (10). This compound was prepared in an analogous manner to that used for **4**, except that 4-fluorobenzaldehyde was used instead of 3,3-dimethylbutyraldehyde. Yield: 80%; an oil; ^{1}H NMR ($CDCl_3$, ppm) δ 1.56–1.71 (m, 9H), 2.00 (br., 2H), 2.89 (m, 2H), 3.32–3.46 (m, 5H), 3.69 (br s, 2H), 4.96 (s, 2H), 5.03 (s, 1H), 6.67 (d, 2H, *J* = 8.5 Hz), 6.83 (d, 2H, *J* = 9.0 Hz), 6.94–7.03 (m, 3H), 7.25–7.40 (m, 6H); MS *m*/*z*: 459 (M + 1); anal. ($C_{30}H_{35}N_2O_1F_1$) C, H, N.

(4-Benzyloxy-phenyl)-[1-(4-bromo-benzyl)-piperidin-4-yl]-(3-methyl-but-2-enyl)-amine (11). This compound was prepared in an analogous manner to that used for **4**, except that 4-bromobenzaldehyde was used instead of 3,3-dimethylbutyraldehyde. Yield: 40%; an oil; ^{1}H NMR ($CDCl_3$, ppm) δ 1.63 (s, 6H), 1.60–1.80 (m, 4H), 2.01 (m, 2H), 2.89 (d, 2H, *J* = 11.5 Hz), 3.27 (m,1 H), 3.41 (s, 2H), 3.70 (d, 2H, *J* = 4.6 Hz), 4.96 (s, 2H), 5.03 (m, 1H), 6.68 (d, 2H, *J* = 9.0 Hz), 6.83 (d, 2H, *J* = 9.0 Hz), 7.14–7.46 (m, 9H); MS *m*/*z*: 521 (M + 1); anal. ($C_{30}H_{35}N_2O_1Br_1\cdot H_2O$): C, H, N.

(4-Benzyloxy-phenyl)-[1-(4-methanesulfonyl-benzyl)-piperidin-4-yl]-(3-methyl-but-2-enyl)-amine (12). This compound was prepared in an analogous manner to that used for **4**, except that 4-methylsulfonylbenzaldehyde was used instead of 3,3-dimethylbutyraldehyde. Yield: 55%; an oil; ^{1}H NMR ($CDCl_3$, ppm) δ1.56–1.74 (m, 13 H), 2.05 (b, 1H), 2.98 (br, 1H), 3.02 (s, 3H), 3.35 (br, 1H), 3.56 (br, 1H), 3.71 (br, 1H), 4.96 (s, 2H), 5.05 (br, 1H), 6.71 (br, 1H), 6.83 (d, 2H, *J* = 9.0 Hz), 7.25–7.40 (m, 6H), 7.54 (d, 2H, *J* = .5 Hz), 7.85–7.91 (m, 2H); MS *m*/*z*: 519 (M + 1); HRMS ($C_{31}H_{38}N_2O_1S_1$): calcd 519.2667, found 519.2681, HPLC purity: 95%.

(4-Benzyloxy-phenyl)-[1-(4-*tert*-butyl-benzyl)-piperidin-4-yl]-(3-methyl-but-2-enyl)-amine (13). This compound was prepared in an analogous manner to that used for **4**, except that 4-*tert*-butyl-benzaldehyde was used instead of 3,3-dimethylbutyraldehyde. Yield 76%; ^{1}H NMR ($CDCl_3$, ppm) δ 1.28 (s, 9H), 1.55 (br, 1H), 1.62 (s, 6H), 1.71 (br, 2H), 1.98 (br, 2H), 2.92 (br, 2H), 3.31–3.39 (m, 1H), 3.44 (br, 1H), 3.70 (d, 2H, *J* = 4.9 Hz), 4.95 (s, 2H), 5.03 (br, 1H), 6.67 (d, 2H, *J* = 9.0 Hz), 6.82 (d, 2H, *J* = 9.0 Hz), 7.25–7.39 (m, 5H); MS *m*/*z*: 497 (M + 1); anal. ($C_{34}H_{44}N_2O_1$) C, H, N.

(4-Benzyloxy-phenyl)-[1-(4-dimethylamino-benzyl)-piperidin-4-yl]-(3-methyl-but-2-enyl)-amine (14). This compound was prepared in an analogous manner to that used for **4**, except that 4-dimethylaminobenzaldehyde was used instead of 3,3-dimethylbutyraldehyde. Yield: 18%; ^{1}H NMR ($CDCl_3$, ppm) δ 1.62 (s, 6H), 1.70 (br, 2H), 1.94–1.98 (m, 2H), 2.90 (s, 6H), 2.91–2.94 (m, 1H), 3.30–3.40 (m, 3H), 3.70 (d, 2H, *J* = 3.9 Hz), 4.95 (s, 2H), 5.02 (s, 1H), 6.66 (d, 4H, *J* = 8.5 Hz), 6.82 (d, 2H, *J* = 9.0 Hz), 7.13 (d, 2H, *J* = 8.1 Hz), 7.25–7.40 (m, 5H); MS *m*/*z*: 484 (M + 1); anal. ($C_{32}H_{41}N_3O_1.0.25H_2O$) C, H, N.

(4-Benzyloxy-phenyl)-(3-methyl-but-2-enyl)-[1-(1H-pyrrol-2-ylmethyl)-piperidin-4-yl]-amine (15). This compound was prepared in an analogous manner to that used for **4**, except that pyrrole-2-carboxaldehyde was used instead of 3,3-dimethylbutyraldehyde. The product was converted into the HCl salt. Yield: 40%; ^{1}H NMR (CD_3OD, ppm) δ 1.62 (s, 6H), 1.64–1.68 (m, 1H), 1.76 (d, 2H, *J* = 12.5 Hz), 2.03 (t, 2H, *J* = 11.0 Hz), 2.92 (d, 2H, *J* = 12.0 Hz), 3.31–3.38 (m, 1H), 3.49 (s, 2H), 3.69 (d, 2H, *J* = 5.4 Hz), 4.96 (s, 2H), 5.04 (m, 1H), 5.99 (s, 1H), 6.08 (dd, 1H, *J* = 5.9, 2.7 Hz), 6.68–6.72 (m, 3H), 6.83 (d, 2H, *J* = 9.3 Hz), 7.25–7.40 (m, 5H), 8.60 (br, 1H); MS *m*/*z*: 430 (M + 1 for $C_{28}H_{35}N_3O_1$); anal. ($C_{28}H_{35}N_3O_1.3HCl$) C, H, N.

(4-Benzyloxy-phenyl)-(1-furan-2-ylmethyl-piperidin-4-yl)-(3-methyl-but-2-enyl)-amine (16). This compound was prepared in an analogous manner to that used for **4**, except that furan-2-carboxaldehyde was used instead of 3,3-dimethylbutyraldehyde. Yield: 60%; an oil; ^{1}H NMR ($CDCl_3$, ppm) δ 1.61 (s, 6H), 1.72 (m, 4H), 2.01 (m, 2H), 2.97 (d, 2H, *J* = 11.2 Hz), 3.32 (m, 1H), 3.51 (s, 2H), 3.70 (d, 2H, *J* = 4.9 Hz), 4.96 (s, 2H), 5.02 (s, 1H), 6.16 (br., 1H), 6.28 (br., 1H), 6.68 (d, 2H, *J* = 9.0 Hz), 6.82 (d, 2H, *J* = 9.0 Hz), 7.25–7.80 (m, 6H); MS *m*/*z*: 431 (M + 1); anal. ($C_{28}H_{34}N_2O_2$) C, H, N.

(4-Benzyloxy-phenyl)-[1-(1H-imidazol-4-ylmethyl)-piperidin-4-yl]-(3-methyl-but-2-enyl)-amine (17). This compound was prepared in an analogous manner to that used for **4**, except that 4-formylimidazole was used

instead of 3,3-dimethylbutyraldehyde. Yield: 53%; an oil; ^{1}H NMR ($CDCl_3$, ppm) δ 1.61 (d, 6H, J = 7.8 Hz), 1.65–1.80 (m, 4H), 2.17 (t, 2H, J = 10.7 Hz), 3.00 (d, 2H, J = 11.5 Hz), 3.36 (m, 1H), 3.60 (s, 2H), 3.67 (d, 2H, J = 4.9 Hz), 4.96 (s, 2H), 5.04 (br., 1H), 6.70 (d, 2H, J = 9.0 Hz), 6.83 (d, 2H, J = 9.0 Hz), 6.93 (s, 1H), 7.25–7.40 (m, 5H), 7.57 (s, 1H); MS m/z: 431 (M + 1); anal. ($C_{27}H_{34}N_4O_1.H_2O$) C, H, N.

(4-Benzyloxy-phenyl)-(3-methyl-but-2-enyl)-(1-pyridin-2-ylmethyl-piperidin-4-yl)-amine (18). This compound was prepared in an analogous manner to that used for 4, except that pyridine-2-carboxaldehyde was used instead of 3,3-dimethylbutyraldehyde. Yield: 66%; an oil; ^{1}H NMR ($CDCl_3$, ppm) δ 1.63 (s, 6H), 1.71–1.74 (m, 3H), 2.07–2.13 (m, 2H), 2.94 (d, 2H, J = 12.0 Hz), 3.32–3.40 (m, 1H), 3.61 (s, 2H), 3.71 (d, 2H, J = 5.6 Hz), 4.96 (s, 2H), 5.02–5.05 (s, 1H), 6.68 (dd, 2H, J = 6.8, 2.2 Hz), 6.83 (dd, 2H, J = 6.8, 2.2 Hz), 7.10–7.13 (m, 1H), 7.25–7.40 (m, 5H), 7.61 (dt, 1H, J = 7.6, 2.0 Hz), 8.53 (dd, 1H, J = 4.2, 0.7 Hz); MS m/z: 442 (M + 1); anal. ($C_{29}H_{35}N_3O_1$) C, H, N.

(4-Benzyloxy-phenyl)-(3-methyl-but-2-enyl)-[1-(tetrahydropyran-4-yl)-piperidin-4-yl]-amine (19). This compound was prepared in an analogous manner to that used for **4**, except that tetrahydropyran-4-one was used instead of 3,3-dimethylbutyraldehyde. Yield: 41%; an oil; ^{1}H NMR ($CDCl_3$, ppm) δ 1.56–1.80 (m, 14H), 2.17 (m, 2H), 2.40 (m, 1H), 2.99 (d, 2H, J = 8.3 Hz), 3.33 (t, 3H, J = 11.5 Hz), 3.71 (d, 2H, J = 4.4 Hz), 3.99 (dd, 2H, J = 11.2, 3.9 Hz), 4.96 (s, 2H), 5.03 (s, 1H), 6.68 (d, 2H, J = 9.0 Hz), 6.83 (d, 2H, J = 9.0 Hz), 7.25–7.40 (m, 5H); MS m/z: 435 (M + 1); anal. ($C_{28}H_{38}N_2O_2.0.25H_2O$) C, H, N.

(4-Iodo-phenyl)-carbamic acid *tert*-butyl ester (20a). 4-Iodoaniline (10.0 g, 45.6 mmol) was dissolved in THF (50 mL) in an amber flask, cooled to 0 °C, and treated with *tert*-butoxycarbonyl anhydride. The reaction was heated to 60 °C overnight, then concentrated in vacuo to give 15.5 g of the crude product. ^{1}H NMR ($CDCl_3$, ppm) δ 1.47 (s, 9H), 6.40 (br, 1H), 7.11 (d, 2H, J = 8.3 Hz), 7.53 (d, 2H, J = 8.1 Hz); MS m/z: 318 (M-1 for $C_{11}H_{14}N_1O_2I_1$ in APCI$^-$ spectrum).

[4-(3,3-Dimethyl-but-1-ynyl)-phenyl]-carbamic acid *tert*-butyl ester (20b). (4-Iodo-phenyl)-carbamic acid tert-butyl ester (**20a**, 1.0 g, 3.13 mmol) was dissolved in THF (30 mL) and treated with Et_3N (2.2 mL, 15.7 mmol), 3,3-dimethyl-1-butyne (0.58 mL, 4.70 mmol), bis(triphenyphosphine) palladium (II) chloride (0.218 g, 0.31 mmol), and copper (I) iodide (0.03 g, 0.16 mmol). The reaction was stirred overnight at room temperature, then filtered and concentrated. The residue was chromatographed on silica gel eluting with 8:1 hexanes: EtOAc to give 0.7 g (82%) of the product. ^{1}H NMR ($CDCl_3$, ppm) δ 1.30 (s, 9H), 1.51 (s, 9H), 6.43 (br, 1H), 7.14 (d, 2H, J = 8.7 Hz), 7.57 (d, 2H, J = 8.7 Hz); MS m/z: 272 (M-1 for $C_{17}H_{23}N_1O_2$ in APCI$^-$ spectrum).

[4-(3,3-Dimethyl-butyl)-phenyl]-carbamic acid *tert*-butyl ester (20c). [4-(3,3-Dimethyl-but-1-ynyl)-phenyl]-carbamic acid *tert*-butyl ester (**20b**, 2.50 g, 9.15 mmol) was treated with 1:1 THF:MeOH (50 mL) and Pd/C (0.5 g, 10%), shaken for 15.8 h under an atmosphere of H_2 (51.4 psi). More Pd/C (0.5 g, 20%) was added and the reaction was stirred for an additional 1.13 h, then filtered and concentrated. The crude material was chromatographed on silica gel eluting with 1:9 EtOAc:hexane to give 2.39 g (94%) of the desired product. An oil; ^{1}H NMR ($CDCl_3$, ppm) δ 0.91 (s, 9H), 1.39–1.44 (m, 2H), 1.47 (s, 9H), 2.45–2.49 (m, 2H), 6.35 (br, 1H), 7.06 (d, 2H, J = 8.3 Hz), 7.21 (d, 2H, J = 8.8 Hz); MS m/z: 276 (M-1 for $C_{17}H_{27}NO_2$ in APCI- spectrum).

4-(3,3-Dimethyl-butyl)-phenylamine (20d). [4-(3,3-Dimethyl-butyl)-phenyl]-carbamic acid *tert*-butyl ester (**20c**, 2.39 g, 8.60 mmol) was dissolved in CH_2Cl_2 (30 mL) and treated with TFA (30 mL) and stirred for 30 min. The solution was concentrated down and pumped under high vacuum for 10 min. The reaction was diluted with EtOAc (500 mL), washed three times with saturated bicarbonate solution and once with brine, dried over Na_2SO_4, and concentrated to give the crude product. ^{1}H NMR ($CDCl_3$, ppm) δ 0.93 (s, 9H), 1.41–1.45 (m, 2H), 2.51–2.56 (m, 2H), 7.16–7.21 (m, 4H), 7.76 (br, 2H); MS m/z: 178 (M + 1 for $C_{12}H_{19}N_1$).

[4-(3,3-Dimethyl-butyl)-phenyl]-(3-methyl-but-2-enyl)-piperidin-4-yl-amine (20e). This compound was prepared in an analogous manner to that used for **2**,[15] except that **20d** was used instead of 4-benzyloxyaniline. an oil; ^{1}H NMR ($CDCl_3$, ppm) δ 0.92 (s, 9H), 1.41–1.45 (m, 2H), 1.57–1.67 (m, 8H), 1.80 (d, 2H, J = 12.9 Hz), 2.05 (br, 1H), 2.41–2.46 (m, 2H), 2.68 (td, J = 12.2, 2.2 Hz), 3.16 (d, 2H, J = 12.2 Hz), 3.56–3.64 (m, 1H), 3.77 (d, 2H, J = 5.1 Hz), 5.08 (s, 1H), 6.64 (d, 2H, J = 8.5 Hz), 7.00 (d, 2H, J = 8.5 Hz); MS m/z: 329 (M + 1 for $C_{22}H_{36}N_2$).

[4-(3,3-Dimethyl-butyl)-phenyl]-[1-(3,3-dimethyl-butyl)-piperidin-4-yl]-(3-methyl-but-2-enyl)-amine (20). This compound was prepared in an analogous manner to that used for **4**, except that **20e** was used instead of **2**. The final compound was converted to the HCl salt. Yield: 48%; mp 195 °C (dec.); ^{1}H NMR (CD_3OD, ppm) δ 0.86 (s, 18 H), 0.88 (m, 4H), 1.40–1.60 (m, 4H), 1.50 (s, 3H), 1.59 (s, 3H), 2.62 (m, 2H), 3.14 (br, 4H), 3.70 (br., 1H), 4.17 (m, 2H), 4.28 (d, 2H, J = 7.1 Hz), 5.08 (m, 1H), 6.70 (d, 2H, J = 8.0 Hz), 6.83 (d, 2H, J = 8.0 Hz); MS m/z: 413 (M + 1); anal. ($C_{28}H_{48}N_2.2HCl.0.25H_2O$) C, H, N.

[1-(3,3-Dimethyl-butyl)-piperidin-4-yl]-(4-isopropyl-phenyl)-(3-methyl-but-2-enyl)-amine (21). This compound was prepared in an analogous manner to that used for **4**, except that 4-*iso*-propylaniline was used instead of 4-benzyloxyaniline. The product was made as the HCl salt. Yield: 59%; an oil; ^{1}H NMR ($CDCl_3$, ppm) δ 0.87 (s, 9H), 1.17 (d, 6H, J = 6.8 Hz), 1.39 (br, 2H), 1.47–1.75 (m, 14 H), 1.97 (br, 2H), 2.30 (br, 2H), 2.73–2.82 (m, 1H), 3.02 (br, 2H), 3.52 (br, 1H), 3.76 (d, 2H, J = 4.6 Hz), 5.05 (s, 1H), 6.62 (d, 2H, J = 8.8 Hz), 7.03 (d, 2H, J = 8.5 Hz); MS m/z: 371 (M + 1 for $C_{25}H_{32}N_2$); anal. ($C_{25}H_{42}N_2.2HCl$) C, H, N.

[1-(3,3-Dimethyl-butyl)-piperidin-4-yl]-(4-*iso*-propoxy-phenyl)-(3-methyl-but-2-enyl)-amine (22). This compound was prepared in an analogous manner to that used for **4**, except that 4-*iso*-propoxyaniline was used instead of 4-benzyloxyaniline. Yield: 65%; an oil; ^{1}H NMR ($CDCl_3$, ppm) δ 0.86 (s, 9H), 1.25 (d, 6H, $J=6.1$ Hz), 1.40 (br, 2H), 2.0-1.56 (m, 14 H), 2.26 (br. 2H), 3.01 (d, 2H, $J=12.7$ Hz), 3.35–3.40 (m, 1H), 3.65 (d, 2H, $J=5.4$ Hz), 4.62 (m, 1H), 5.01–5.05 (m, 1H), 6.66 (d, 2H, $J=8.8$ Hz), 6.74 (d, 2H, $J=7.0$ Hz); MS *m*/*z*: 387 (M + 1); anal. ($C_{25}H_{42}N_2O$) C, H, N.

[1-(3,3-Dimethyl-butyl)-piperidin-4-yl]-(4-fluorophenyl)-(3-methyl-but-2-enyl)-amine (23). This compound was prepared in an analogous manner to that used for **4**, except that 4-fluoroaniline was used instead of 4-benzyloxyaniline. The product was made as the HCl salt. Yield: 64%; ^{1}H NMR (CD_3OD, ppm) δ 0.87 (s, 9H), 1.42 (br, 2H), 1.62–1.82 (m, 12 H), 1.99 (br, 2H), 2.38 (br, 2H), 3.01 (br, 2H), 3.40 (br, 2H), 3.72 (br, 2H), 5.00 (s, 1H), 6.63 (br, 2H), 6.87 (t, 2H, $J=8.5$ Hz); MS *m*/*z*: 347 (M + 1 for $C_{22}H_{35}N_2F_1$); anal. ($C_{22}H_{35}N_2F_1.2HCl.0.25H_2O$) C, H, N.

[1-(3,3-Dimethyl-butyl)-piperidin-4-yl]-(phenyl)-(3-methyl-but-2-enyl)-amine (24). This compound was prepared in an analogous manner to that used for **4**, except that aniline was used instead of 4-benzyloxyaniline. Yield: 65%; ^{1}H NMR ($CDCl_3$, ppm) δ 0.87 (s, 9H), 1.38–1.42 (m, 2H), 1.64 (s, 6H), 1.78–1.82 (m, 4H), 2.02–2.08 (m, 2H), 2.36–2.40 (m, 2H), 3.10 (d, 2H, $J=11.5$ Hz), 3.56–3.58 (m, 1H), 3.78 (d, 2H, $J=5.1$ Hz), 5.04 (s, 1H), 6.61–6.68 (m, 3H), 7.14–7.18 (m, 2H); MS *m*/*z*: 329 (M + 1); anal. ($C_{22}H_{36}N_2$) C, H, N.

[1-(4-Dimethylamino-benzyl)-piperidin-4-yl]-[4-(3,3-dimethyl-butyl)-phenyl]-(3-methyl-but-2-enyl)-amine (26). This compound was prepared in an analogous manner to that used for **4**, except that **20e** was used instead of **2** and 4-dimethylaminobenzaldehyde was used instead of 3,3-dimethylbutyraldehyde. Yield: 63%; an oil; ^{1}H NMR ($CDCl_3$, ppm) δ 0.91 (s, 9H), 1.40–1.45 (m, 2H), 1.61–1.72 (m, 10 H), 2.01 (br, 2H), 2.40–2.45 (m, 2H), 2.92–3.00 (m, 8H), 3.43–3.50 (m, 3H), 3.76 (d, 2H, $J=4.6$ Hz), 5.05 (s, 1H), 6.61 (d, 2H, $J=8.5$ Hz), 6.68 (d, 2H, $J=8.5$ Hz), 6.98 (d, 2H, $J=8.5$ Hz), 7.15 (d, 2H, $J=8.5$ Hz); MS *m*/*z*: 462 (M + 1); anal. ($C_{31}H_{47}N_3$) C, H, N.

LogP determination. The protocol (a potentiometric titration method) for this assay has been described earlier.[19]

In vitro pharmacology

(1) IMR-32 assay. The IMR-32 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, 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 $\sim 10^7$ 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, 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. PD-151307[14] was run in parallel as a standard in each assay to compare the relative potencies determined. IC_{50} values of test compounds were calculated by fitting a four-parameter logistic function to the data using the least squares method.

In vivo experiments

(1) 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.[19] 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 are 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.

(2) Pharmacokinetics study. Three Wistar rats received a 5 mg/kg bolus intravenous dose of each compound as a solution and serial plasma samples were collected at various times up to 24 hr postdose. Plasma samples were analyzed using direct protein precipitation with acetonitrile and the compound 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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