Bioorganic & Medicinal Chemistry 17 (2009) 7056–7063

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Comparative molecular field analysis and synthetic validation of a hydroxyamide-propofol binding and functional block of neuronal voltage-dependent sodium channels

Milton L. Brown [a,b,c,*], Hilary A. Eidam [d], Mikell Paige [a,c], Paulianda J. Jones [d], Manoj K. Patel [e]

[a] Departments of Oncology, 3970 Reservoir Road, Georgetown University Medical Center, Washington, DC 20057, United States
[b] Departments of Neuroscience, 3970 Reservoir Road, Georgetown University Medical Center, Washington, DC 20057, United States
[c] Departments of Drug Discovery Program, 3970 Reservoir Road, Georgetown University Medical Center, Washington, DC 20057, United States
[d] Department of Chemistry, University of Virginia, Charlottesville, VA 22904, United States
[e] Department of Anesthesiology, University of Virginia, Charlottesville, VA 22904, United States

ARTICLE INFO

Article history:
Received 22 April 2008
Revised 18 November 2008
Accepted 20 November 2008
Available online 3 December 2008

Keywords:
Amides
Alcohols
Sodium channels
Hippocampal neurons
Propofol

ABSTRACT

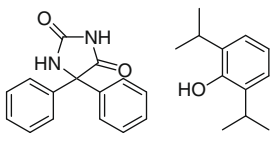
Voltage gated sodium channels represent an important therapeutic target for a number of neurological disorders including epilepsy. Unfortunately, medicinal chemistry strategies for discovering new classes of antagonist for trans-membrane ion channels have been limited to mostly broad screening compound arrays. We have developed new sodium channel antagonist based on a propofol scaffold using the ligand based strategy of comparative molecular field analysis (CoMFA). The resulting CoMFA model was correlated and validated to provide insights into the design of new antagonists and to prioritize synthesis of these new structural analogs (compounds **4** and **5**) that satisfied the steric and electrostatic model. Compounds **4** and **5** were evaluated for [^{3}H]-batrachotoxinin-A-20-α-benzoate ([^{3}H]-BTX-B) displacement yielding IC_{50}'s of 22 and 5.7 μM, respectively. We further examined the potency of these two compounds to inhibit neuronal sodium currents recorded from cultured hippocampal neurons. At a concentration of 50 μM, compounds **4** and **5** tonically inhibited sodium channels currents by 59 ± 7.8% (n = 5) and 70 ± 7.5% (n = 7), respectively. This clearly demonstrates that these compounds functionally antagonize native neuronal sodium channel currents. In summary, we have shown that CoMFA can be effectively used to discover new classes of sodium channel antagonists.

1. Introduction

Voltage-gated sodium (Na) channels play an important role in the generation and conduction of central and peripheral action potentials. They are composed of a pore forming α subunit and auxiliary β subunits that modulate gating properties of the channel.[1] To date nine α isoforms have been cloned together with three β subunits.[2] Clinically important drugs that antagonize sodium channels as a mechanism of their action include anticonvulsants, anti-arrhythmic and anesthetics.

Propofol (2,6-diisopropylphenol, Fig. 1) is a clinically useful intravenous anesthetic used for both the induction and maintenance of general anesthesia. In addition it is also used in the treatment of status epilepticus.[3] It is widely known for its quick anesthetic onset and titratability. The primary mechanism of action of propofol is unknown but is thought to include both ligand gated channels such as GABA and glycine and also voltage gated ion channels including sodium and calcium channels.[4–6] More recent studies suggest that propofol effects on Na channels could play a major role in its mechanism of action. Propofol has been shown to displace [^{3}H]-BTX-B binding from sodium channels in rat synaptoneurosomes[7] and inhibit veratridine-evoked sodium influx in rat synaptoneurosomes.[8] In electrophysiology studies, propofol has been shown to inhibit sodium currents from isolated rat neurohypophysial nerve terminals.[9]

Recently, new propofol analogs and models have been designed to optimize binding to GABA(A) channels and also for solubility.[10,11] Since there is strong evidence for a role of Na channels in the mechanism of action of propofol, we have developed a 3D



Phenytoin Propofol

Figure 1. Structures of propofol and phenytoin.

* Corresponding author. Tel.: +1 202 687 8603.
E-mail address: mb544@georgetown.edu (M.L. Brown).

0968-0896/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2008.11.069

QSAR model for propofol binding to site 2 of the Na channel. We present a general anesthetic/anticonvulsant pharmacophore in the voltage gated sodium channel and validate it with the design and synthesis of two new compounds. Functional antagonism was also assessed on neuronal sodium channels natively expressed in cultured hippocampal neurons.

2. Results and discussion

[^{3}H]-BTX-B displacement measures the affinity of a compound to site 2 on the sodium channel protein and is a useful screening test.[6,22,23] All compounds in our study were tested at 40 μM in duplicate assays. Propofol was also tested as a benchmark and found to inhibit 16.6% at 40 μM. Compound **5** was found to be the most effective sodium channel blocker at 71.4% at 40 μM based on [^{3}H]-BTX-B displacement, with the remaining compounds ranging from 6.8% to 67.1% inhibition (Table 1, structure in Fig. 2). This rank order was predicted by the CoMFA model. The steric map (Fig. 3) shows that a six carbon linker is needed to branch the anesthetic and anticonvulsant binding sites. By aligning each molecule to either the anesthetic or anticonvulsant site, we were able to obtain a CoMFA predicted IC_{50} for [^{3}H]-BTX-B displacement for each (see Table 1). The anticonvulsant site is also termed the hydantoin site based upon previous studies utilizing this CoMFA.[12]

Based upon recent literature reports, we investigated whether propofol was interacting with this hydantoin site or the smaller site, termed as the anesthetic site in this study (Fig. 3). Propofol's predicted IC_{50} for [^{3}H]-BTX-B displacement was 60.3 μM for the anesthetic site and 371.5 μM for the anticonvulsant site, which was consistent with its 16.6% inhibition at 40 μM. Accordingly, compound **2**'s IC_{50} was predicted as 29.5 μM for the anesthetic site and 741.3 μM for the hydantoin site, again falling in line with our predictions based on its experimental value of 24.5% inhibition at 40 μM. Compounds **4** and **5** were only predicted with the propofol core in the anesthetic site. The CoMFA predicted IC_{50}'s for compounds **4** and **5** were 51.3 and 16.2 μM. Our experimentally determined values were 22.0 and 5.7 μM, respectively (Table 1).

With these results in hand, we turned to evaluating the functional effects of compounds **4** and **5** on sodium currents expressed in rat hippocampal neurons. At a concentration of 50 μM compound **5** significantly blocked the Na current by 70 ± 7.5% ($P < 0.05$; $n = 7$). Compound **4** at the same concentration of 50 μM blocked the Na current by 59 ± 7.8% ($P < 0.05$; $n = 5$). Current trace recordings are shown in Figure 4A and B. The effects of both compounds were fully reversible on washout. In agreement with our [^{3}H]-BTX-B data, compound **5** was more effective than compound **4** at blocking the sodium current. Finally, both compounds had a greater neuronal antagonist effect than the intravenous anesthetic propofol, which blocked the sodium current by 43 ± 12% ($n = 3$).

Table 1
CoMFA predictions and 3H-BTX-B displacement

Compound	Alignment site	Predicted BTX IC_{50} (μM)	Observed ^{3}H-BTX-B displacement	
			IC_{50} (μM)	Single concentration tested @ 40 μM
Propofol	Anesthetic	60.3		16.6
Propofol	Hydantoin	371.5		
1	Anesthetic	43.7		6.8
1	Hydantoin	1380.4		
2	Anesthetic	29.5		24.5
2	Hydantoin	741.3		
3	Anesthetic	34.7		55.1
3	Hydantoin	354.8		
4	Both	51.3	22.0	67.1
5	Both	16.2	5.7	71.4

3. Conclusion

We have demonstrated that CoMFA modeling can be an effective medicinal chemistry tool for designing and prioritizing the synthesis of new ion channel antagonists. The CoMFA model was able to accurately predict the [^{3}H]-BTX-B displacement IC_{50} for propofol only when it was placed and aligned to the anesthetic CoMFA site. Using a hydroxyamide motif that interacts with the anticonvulsant site we were able to envision and design a new ligand (compounds **4** and **5**) that linked the two potential pharmacophores. Both compounds **4** and **5**, designed to bridge the two regions had low IC_{50} for [^{3}H]-BTX-B displacement and demonstrated functional antagonism of hippocampal neuronal sodium channel currents.

4. Experimental

4.1. Molecular modeling: conformational analysis

The exact training set for the CoMFA was used directly from our previously reported work.[12] The X-ray coordinates for propofol were utilized in this study.[13] Test compounds **2** and **5** were constructed from the propofol crystal structure by adding the appropriate atoms within the build edit mode of SYBYL (Tripos Inc.) This modified structure was energy-minimized with the Tripos force field,[14] without solvent, using default bond distances and angles and neglecting electrostatics. Minimization was completed by aggregating (using the SYBYL/AGGREGATE module) only the X-ray structure atoms and allowing the modified portion to minimize. For internal consistency with our previous model, we used only the *R*-configuration for **2** and **5**. To determine the low-energy conformations for **2** and **5**, we utilized GRIDSEARCH on rotatable bonds over 360° in one degree increments. The atomic charges for all analogs were calculated using AM1 (MOPAC).

4.2. Molecular alignment

Propofol was utilized as a training molecule and was fit by overlapping the aryl ring atoms of propofol with the phenyl ring of phenytoin in the anticonvulsant pharmacophore (see Fig. 3). A second fit was obtained by fitting the phenyl ring over the C7 of the alkyl side chain in the anesthetic pharmacophore (see Fig. 3). Similarly, the designed hydroxyamides **2** and **5** were aligned such that the OH group was superimposed with N1 and the carboxyamide was aligned with C4 and N3. The *n*-alkyl groups at R_1 in the test set approximated a fully extended conformation following energy minimization, which was arbitrarily selected for this study.

4.3. CoMFA calculations

CoMFA, using default parameters except where noted, was calculated in the QSAR option of SYBYL 6.8 on a Silicon Graphics computer with an Octane II R12000 dual processor. The CoMFA grid spacing was 2.0 Å in the *x*, *y*, and *z* directions, and the grid region was automatically generated by the CoMFA routine to encompass all molecules with an extension of 4.0 Å in each direction. A sp^3 carbon and a charge of +1.0 were used as probes to generate the interaction energies at each lattice point. The default value of 30 kcal/mol was used as the maximum electrostatic and steric energy cutoff.

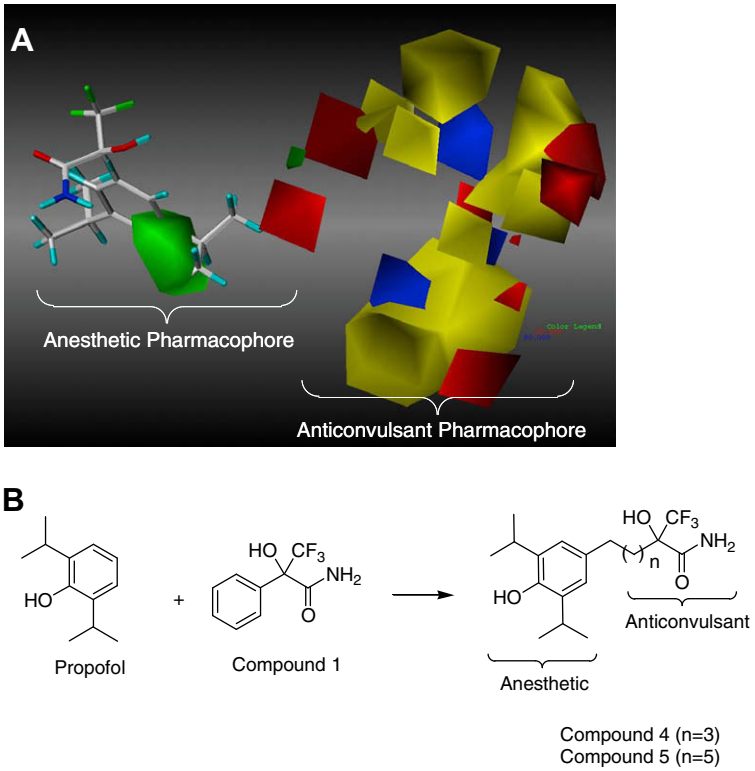


Figure 2. (A) Model of general anesthetic and anticonvulsant pharmacophore; (B) strategy of compound design.

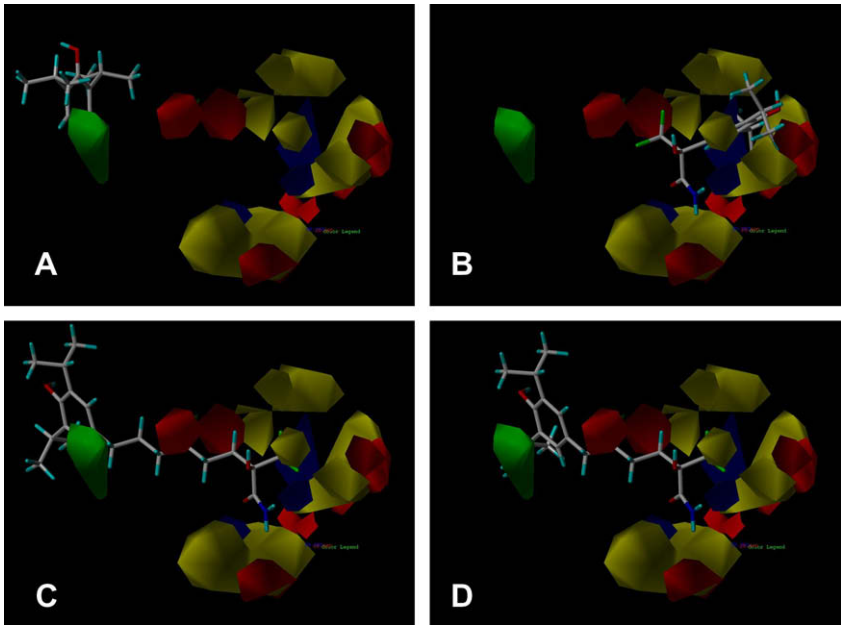


Figure 3. Stereoviews (relaxed) of the electrostatic and steric CoMFA fields for selected compounds. (A) Propofol aligned to the anesthetic site; (B) compound **2** aligned to the hydantoin site; (C) compound **4**; (D) compound **5**.

4.4. Partial least squares (PLS) regression analysis

In this study, we used the final non-cross-validated CoMFA model previously published.[12] The relative steric (0.624) and electrostatic (0.376) contributions to the final model were contoured as the standard deviation multiplied by the coefficient at 80% for favored steric (contoured in green) and favored positive electrostatic (contoured in blue) effects and at 20% for disfavored steric (contoured in yellow) and favored negative electrostatic (contoured in red) effects, as shown in Figure 3. Based on the alignment of our low energy conformers of test set ligands (propofol, and analogs **2** and **5**) to this model, we predicted the sodium channel binding activities (Table 1).

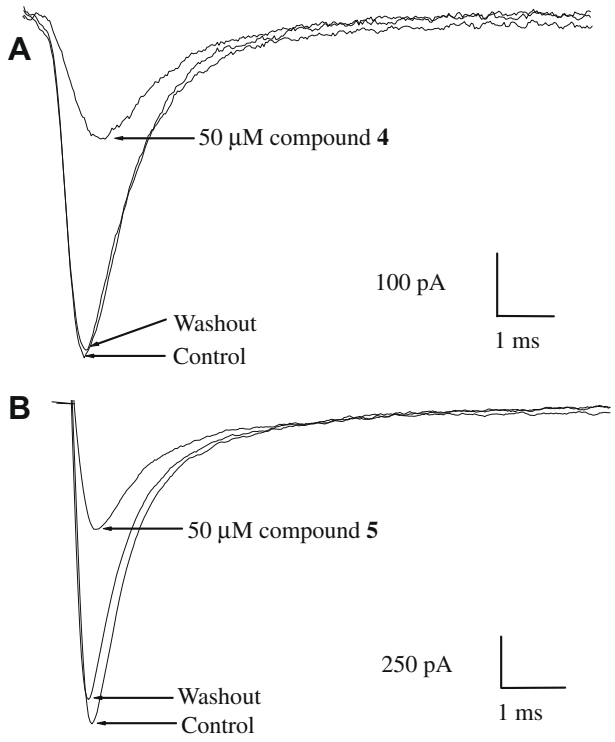


Figure 4. Tonic block of Na current by compounds **4** (A) and **5** (B) at 50 μM in cultured hippocampal neurons. Currents were elicited by a step depolarization to −10 mV for 20 ms from a holding potential of −90 mV. Block of currents was fully reversible on washout.

4.5. Chemistry

The synthesis of compound **1** was previously described in 99% yield (Scheme 1).[15] Compound **2**'s synthesis commenced with bromination of propofol with bromine in carbon tetrachloride in 92% yield.[16] The phenol **7** was then protected with a MOM (methoxymethyl) group using MOMCl and NaH in 96% yield.[17] The trifluoromethyl ketone was obtained from formation of the corresponding Grignard reagent from bromide **8** and adding 2,2,2-trifluoro-1-piperidin-1-yl-ethanone **6** to introduce the fluorine in 99% yield (Scheme 2).[18] Ketone **9** was converted to the trimethylsilyl ether with trimethylsilyl cyanide (TMSCN). Subsequent hydrolysis with 15% HCl generated the cyanohydrin. Conversion of the corresponding cyanohydrin to the hydroxyamide was accomplished under acidic conditions, by saturating with HCl gas and allowing the mixtures to stand at room temperature for 16 h.[19] Ketone **9** was also exposed to Bucherer–Burg conditions to synthesize the hydantoin in 93% yield.[20] The MOM group was then deprotected with the use of $BF_3{\cdot}OEt_2$ in a 3:1 solvent mixture of CH_2Cl_2:Me_2S in 95% yield to yield **3** (Scheme 3).

The synthesis of compound **4** was accomplished by starting with the mono-TBS protection of 1,4-butanediol. Halogenation with I_2, Ph_3P and imidazole yielded alkyl iodide **13a**. Subsequent reaction of **13a** with the Grignard reagent of **8** to introduced the long aliphatic chain. Deprotection of **14a** with TBAF and formation of the alkyl iodide **16a** in the same manner as mentioned above allowed for introduction of the fluorine yielding **17a**. Several unsuccessful attempts were made to form the Grignard reagent from the iodide and bromine (formed with CBr_4, Ph_3P and Hunig's base from **15a**). Finally, a lithium–halogen exchange with *n*-BuLi and addition of **6** allowed for formation of the ketone **17a**. The hydroxyamide **4** was then formed under the above mentioned conditions (Scheme 4). Compound **5** was synthesized in the same manner utilizing 1,6-hexanediol as the starting diol (Scheme 4).

4.6. [^{3}H]-BTX-B displacement

Batrachotoxin is an alkaloidal steroid released through colorless or milky secretions from the granular glands of frogs from the genus *Phyllobates* that binds to site 2 in the NVSC. Inhibition of specifically bound [^{3}H]-BTX-B has served as a facile method to evaluate whether a compound has interaction with site 2 on the sodium channel protein.[7]

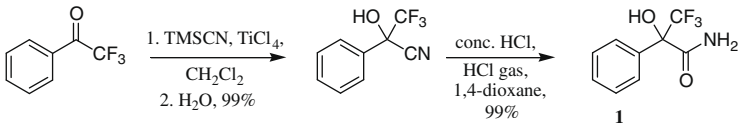
4.7. Cultured hippocampal neurons

Rat hippocampal cultures were prepared according to the method described by Banker et al.[21] Briefly, hippocampi were dissected from 18-day-old rat embryos, dissociated by trypsin, and triturated with a Pasteur pipette. The neurons were plated on coverslips coated with poly-L-lysine in minimal essential medium with 10% horse serum at an approximate density of 25,000/cm^2. Once the neurons had attached to the substrate, they were transferred to a dish containing a glial monolayer and maintained for up to four weeks in serum-free minimal essential medium with N_2 supplement.

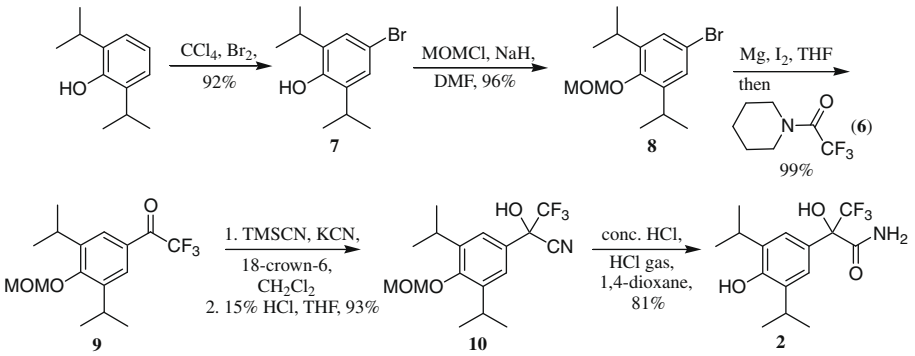
4.8. Electrophysiology studies

Sodium channel currents were recorded from cultured neurons using the whole-cell configuration of the patch clamp recording technique with an Axopatch 200B amplifier (Axon Instruments, Foster City, CA). All voltage protocols were applied using PCLAMP 8 software (Axon, USA) and a Digidata 1322A (Axon, USA). Currents were amplified and low pass filtered (2 kHz) and sampled at 33 kHz. Borosilicate glass pipettes were pulled using a Brown-Flaming puller (model P-87, Sutter Instruments Co, Novato, CA) and heat polished to produce electrode resistance of 1.5–2.0 MΩ when filled with pipette solution containing (in mM) CsF 140, $MgCl_2$ 2, EGTA 1, Na-HEPES 10 (pH adjusted to 7.3 with CsOH). Cultured neurons were superfused with solution containing (in mM) NaCl 30, Choline Cl 110, KCl 3, $CaCl_2$ 1, $CdCl_2$ 0.1, $MgCl_2$ 2, HEPES 10, TEA-Cl 30 (pH adjusted to 7.3 with CsOH). On establishing whole-cell configuration, neurons were held at −90 mV for 5 min to account for equilibrium gating shifts. Currents were elicited from a holding potential of −90 mV with a step to −10 mV for 25 ms. Capacitive and leak currents were corrected for using a P/4 protocol. All experiments were performed at room temperature (20–22 °C).

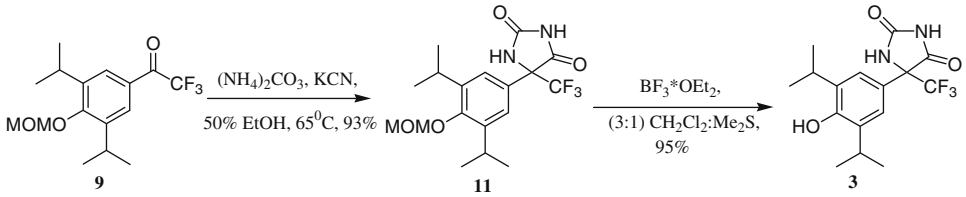
Data analysis was performed using Clampfit software (v8, Axon Instruments, CA, USA) and Origin (v6, Microcal Software, MA, USA). Statistical analyses were performed using the standard one-way ANOVA followed by Tukey's post hoc test (SigmaStat, Jandel). Averaged data are presented as means ± standard error of the mean (S.E.M.). Statistical significance was set at $P < 0.05$.



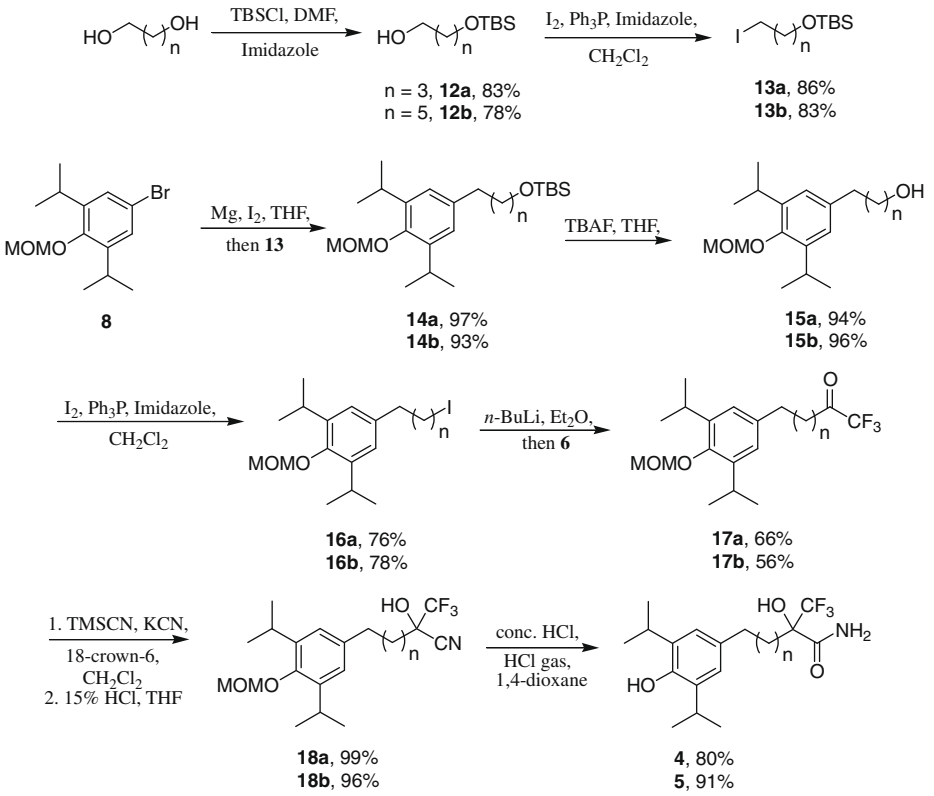
Scheme 1. Synthesis of compound **1**.



Scheme 2. Synthesis of compound **2**.



Scheme 3. Synthesis of compound **3**.



Scheme 4. Synthesis of compounds **4** and **5**.

4.9. Chemistry

All reactions requiring anhydrous conditions were performed in flame-dried glassware under an atmosphere of argon or nitrogen. Melting points were determined with an Electrothermal Mel-Temp melting point apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were measured on a Varian 300 or 500 MHz NMR, unless specified otherwise. Chemical shifts are reported in ppm relative to resonances of the solvent, $CDCl_3$ (unless specified otherwise): 7.25 ppm (s) in the ^{1}H spectra and 77.08 ppm (t) in the ^{13}C spectra. ^{19}F NMR spectra were measured on a Varian 300 MHz instrument, externally referenced with TFA (−76.6 ppm). IR spectra were recorded on a FT-IR Impact 400 D. High-resolution mass spectrometry was performed at the University of Illinois at Urbana-

Champaign School of Chemical Science. Combustion analysis was performed by Atlantic Microlabs, Inc. Norcross, GA. Concentration in vacuo refers to high vacuum (0.35 mmHg). Concentration refers to a rotary evaporator with a water aspirator. Anhydrous THF, diethyl ether, and dichloromethane were purified by pressure filtration through activated alumina. Flash chromatography was performed on silica gel (Merck grade 9385, 230–400 mesh, 60 Å).

4.10. General procedure A: preparation of cyanohydrins from ketones

The ketone (1.0 equiv) was dissolved in a minimal amount of dry CH_2Cl_2. Trimethylsilyl cyanide (TMSCN, 2.2 equiv) and ZnI_2 (1.0 equiv), (or KCN and 18-crown-6, 10 mg each for every 1.0 mmol of ketone) were added. The mixture was stirred at room temperature overnight (16 h). The CH_2Cl_2 was evaporated in vacuo, and a minimal amount of dry THF was added. The mixture was cooled to 0 °C and 15% HCl (5 mL) was added and then stirred at room temperature for 2 h. The solution was combined with H_2O and extracted with Et_2O (3 × 25 mL), dried over $MgSO_4$, filtered, and concentrated to yield the cyanohydrin. The cyanohydrins were used in general procedure C without further purification.

4.11. General procedure B: preparation of α-hydroxyamides from cyanohydrins

The cyanohydrin was dissolved in 1,4-dioxane (2 mL). The mixture was cooled to 0 °C, and previously cooled concd HCl (0.2 mL for every 1 mmol of cyanohydrin) was added. HCl gas was then passed through the reaction mixture for 45 min at 0 °C. The mixture was allowed to stand at room temperature overnight (16 h). The mixture was extracted with EtOAc (3 × 25 mL), dried over $MgSO_4$, filtered, and concentrated to yield the crude α-hydroxyamide. Purification was performed on a flash column (1:1 hexanes/EtOAc), collecting all fractions with a component of $R_f = 0.28$ to yield the pure α-hydroxyamide.

4.12. 4-Bromo-2,6-diisopropyl-phenol (7)

See Ref. 24.

4.13. 5-Bromo-1,3-diisopropyl-2-methoxymethoxy-benzene (8)

To a solution of **7** (1.0 g, 3.9 mmol) in dry DMF (5 mL) was added NaH (172 mg, 4.3 mmol, 60% dispersion) at room temperature. Stirring at this temperature continued until the evolution of hydrogen ceased (30 min). MOMCl (0.35 g, 4.3 mmol) was then added over 5 min and stirring was continued for an additional 2 h. Excess NaH was then destroyed with cautious addition of methanol (5 mL). The reaction mixture was diluted with ether, washed with water, followed by brine. The organic layer was dried over $MgSO_4$, filtered and concentrated. Purification was performed on a flash column (6:1 hexanes/EtOAc), collecting all fractions with a component of $R_f = 0.69$ to yield the product as a white solid. (6.75 g, 96% yield) mp = 34–36 °C; ^{1}H NMR: δ 1.21 (d, J = 6.9 Hz, 12H), 3.31 (m, 2H), 3.61 (s, 3H), 4.90 (s, 2H), 7.19 (s, 2H); ^{13}C NMR: δ 23.7, 26.9, 57.4, 100.4, 118.1, 127.2, 144.3, 151.1; EI-MS: m/z 300.1 ($M+H^+$), 302.1 ($M+2H^+$).

4.14. 1-(3,5-Diisopropyl-4-methoxymethoxy-phenyl)-2,2,2-trifluoro-ethanone (9)

Crushed magnesium (0.94 g, 38.8 mmol), I_2 (2–3 crystals) and a small portion of bromide **8** was heated until the reaction began to occur. The remaining bromide **8** (10.0 g, 33.2 mmol) in dry THF (25 mL) was added dropwise over 30 min. The mixture was refluxed for 2 h and then allowed to cool to room temperature. The mixture was cooled to 0 °C and **6** (5.0 g, 27.7 mmol) in dry THF (5 mL) was added dropwise over 30 min. The mixture was brought to room temperature and allowed to stir for 2 h. The reaction was quenched with satd aq NH_4Cl (5 mL), filtered, dried over $MgSO_4$, filtered and concentrated to yield the crude product. Purification was performed on a flash column (8:1 hexanes/EtOAc), collecting all fractions with a component of $R_f = 0.38$ to yield the pure ketone as a yellow oil (8.9 g, 99% yield). ^{1}H NMR: δ 1.28 (d, J = 7.2 Hz, 12H), 3.56 (m, 2H), 3.62 (s, 3H), 5.03 (s, 2H), 7.92 (s, 2H); ^{13}C NMR: δ 23.2, 26.8, 57.1, 100.5, 116.8 (q, $^1J_{CF}$ = 290.2 Hz), 126.5, 127.0, 143.1, 159.1, 179.2 (q, $^2J_{CF}$ = 37.5 Hz); EI-MS: m/z 318.3.

4.15. 2-(3,5-Diisopropyl-4-methoxymethoxy-phenyl)-3,3,3-trifluoro-2-hydroxy propionitrile (10)

General procedure A was employed with **9** (1.0 g, 3.1 mmol), TMS-CN (0.9 mL, 6.9 mmol), KCN (40 mg) and 18-crown-6 (40 mg). The cyanohydrin was obtained as a yellow oil (1.0 g, 93% yield), and used without further purification.

4.16. 3,3,3-Trifluoro-2-hydroxy-2-(4-hydroxy-3,5-diisopropyl-phenyl)-propionamide (2)

General procedure B was employed with **10** (1.0 g, 2.8 mmol) and concd HCl (0.5 mL). The hydroxyamide was obtained as a white solid (750 mg, 81% yield). mp = 139–142 °C; IR (KBr): 1653 cm^{-1}; ^{1}H NMR: δ 1.26 (d, J = 6.6 Hz, 12H), 3.15 (sept, J = 6.9 Hz, 2H), 4.65 (s, 1H), 5.01 (s, 1H), 5.92 (s, 1H), 6.19 (s, 1H), 7.31 (s, 2H); ^{13}C NMR: δ (CD_3OD, 75 MHz) 173.6, 152.6, 136.3, 127.6, 125.7 ($^1J_{CF}$ = 284 Hz), 122.8, 79.6 ($^2J_{CF}$ = 27.3 Hz), 28.0, 23.3; ^{19}F NMR: δ *−73.6; EI-MS:* 319.3 (m/z); HRMS (EI) calcd for $C_{15}H_{20}F_3NO_3$ 319.1395, found 319.1388. Anal. Calcd for $C_{15}H_{20}F_3NO_3$: C, 56.42; H, 6.31; N, 4.39. Found: C, 56.63; H, 6.26; N, 4.40.

4.17. 5-(3,5-Diisopropyl-4-methoxymethoxy-phenyl)-5-trifluoromethyl-imidazolidine-2,4-dione (11)

To a stirring solution of 50% ethanol (60 mL) were added **9** (4.2 g, 13.2 mmol), KCN (1.7 g, 26 mmol), and $(NH_4)_2CO_3$ (5.0 g, 53 mmol). The solution was warmed to 65 °C for 29 h., using a 3% KOH trap. The precipitate was filtered, and the filtrate was acidified (pH 2) using concd HCl. The resulting solid was filtered, and the filtrate was made basic (pH 8) using 3% KOH. This was concentrated to one-half volume and filtered again. The combined solids were purified by flask chromatography (10:1 CH_2Cl_2/acetone), collecting all fractions containing a component of $R_f = 0.26$ to yield the hydantoin as a white solid (4.7 g, 93% yield). mp = 194–196 °C; ^{1}H NMR: δ *(DMSO-d_6)* 9.89 (s, 1H), 7.55 (s, 2H), 4.90 (s, 2H), 3.49 (s, 3H), 3.29 (sept, J = 6.9 Hz, 2H), 1.15 (d, 12H); ^{13}C NMR: δ (CD_3OD, 500 MHz) 170.3, 158.2, 154.9, 143.7, 142.8, 127.8, 124.6 (q, $^1J_{CF}$ = 118 Hz), 124.0, 123.9, 101.8, 69.5 (q, $^2J_{CF}$ = 17.5 Hz), 57.7, 28.2, 24.1; ESI-MS: m/z 386.7 ($M-H^+$).

4.18. 5-(4-Hydroxy-3,5-diisopropyl-phenyl)-5-trifluoromethyl-imidazolidine-2,4-dione (3)

To a solution of **11** (0.5 g, 1.28 mmol) in 3:1 v/v of CH_2Cl_2:Me_2S (80 mL) at 0 °C was added $BF_3{\cdot}OEt_2$ (0.25 mL, 1.93 mmol). The reaction mixture was stirred at 0 °C for 30 min. The reaction was quenched with satd aq $NaHCO_3$ (5 mL), diluted with EtOAc, washed with satd aq $NaHCO_3$ and brine. The organic layer was dried over $MgSO_4$, filtered and concentrated. Purification was performed on a flash column (2:1 hexanes/EtOAc), collecting all fractions with

a component of R_f = 0.60 to yield the product as a yellow solid (420 mg, 95% yield). mp: 215–217 °C; ^{1}H NMR: δ 7.27 (s, 2H), 6.56 (s, 1H), 5.12 (s, 1H), 5.03 (s, 1H), 3.16 (m, 2H), 1.15 (d, 12H); ^{13}C NMR: δ (CD_3OD, 125 mHz) 170.7, 158.4, 153.4, 136.9,124.3 (q, $^1J_{CF}$ = 270 Hz), 123.1, 122.9, 122.6, 69.7 (q, $^2J_{CF}$ = 35 Hz), 38.6, 28.1, 23.3; ESI-MS: m/z 342.6 (M−H+).

4.19. *tert*-Butyl-(4-iodo-butoxy)-dimethyl-silane (13a)

See Ref. 25.

4.20. *tert*-Butyl-(6-iodo-hexyloxy)-dimethyl-silane (13b)

See Ref. 26.

4.21. General procedure C: Grignard reaction of aromatic bromide and aliphatic iodide

Crushed Mg (1.5 equiv), 2–3 I_2 crystals and a small portion of the bromide was heated until the reaction began to occur. The remaining bromide (1.2 equiv) in dry THF (25 mL) was added dropwise over 30 min. The mixture was refluxed for 2 h. and then allowed to cool to ambient temperature. The mixture was cooled to 0 °C and the iodide (1.0 equiv) and CuI (10 mol %) in dry THF (5 mL) was added dropwise over 30 min. The mixture was brought to ambient temperature and allowed to stir for 2 h. The reaction was quenched with saturated aqueous NH_4Cl (5 mL), filtered, dried over $MgSO_4$, filtered and concentrated to yield the crude product. Purification was performed on a flash column (20:1 hexanes/EtOAc), collecting all fractions with a component of R_f = 0.70 to yield the pure product.

4.22. *tert*-Butyl-[4-(3,5-diisopropyl-4-methoxymethoxy-phenyl)-butoxy]-dimethyl-silane (14a)

General procedure C was employed with magnesium (534 mg, 22.0 mmol), **8** (5.3 g, 17.6 mmol), **13a** (4.6 g, 14.6 mmol) and CuI (279 mg, 1.46 mmol). The aromatic derivative was obtained as a colorless oil (5.3 g, 89% yield). ^{1}H NMR: δ 6.95 (s, 2H), 4.95 (s, 2H), 3.70 (t, J = 6.6 Hz, 2H), 3.66 (s, 3H), 3.38 (septet, J = 6.9 Hz, 2H), 2.62 (t, J = 7.8 Hz, 2H), 1.60–1.75 (m, 4H), 1.27 (d, J = 5.7 Hz, 12H), 0.95 (s, 9H), 0.10 (s, 6H); ^{13}C NMR: δ 149.7, 141.3, 138.7, 123.9, 100.3, 63.0, 57.2, 35.5, 32.5, 27.7, 26.7, 25.9, 23.9, 18.3, -5.3; ESI-MS: (m/z) 426.0 (M+H_2O).

4.23. *tert*-Butyl-[6-(3,5-diisopropyl-4-methoxymethoxy-phenyl)-hexyloxy]-dimethyl-silane (14b)

General procedure C was employed with magnesium (191 mg, 7.8 mmol), **8** (2.0 g, 6.7 mmol), **14b** (1.9 g, 5.6 mmol) and CuI (106 mg, 0.56 mmol). The aromatic derivative was obtained as a colorless oil (2.3 g, 93% yield). ^{1}H NMR: δ 6.96 (s, 2H), 4.97 (s, 2H), 3.64–3.70 (m, 5H), 3.40 (m, 2H), 2.62 (t, J = 7.8 Hz, 2H), 1.43–1.68 (m, 4H), 1.29 (d, J = 5.7 Hz, 12H), 0.96 (s, 9H), 0.12 (s, 6H); ^{13}C NMR: δ 149.7, 141.3, 138.9, 123.9, 100.3, 63.2, 57.1, 35.8, 32.8, 31.6, 29.2, 26.6, 25.9, 25.6, 23.9, 18.3, -5.3; APCI-MS: (m/z) 437.0 (M+H^+).

4.24. General procedure D: deprotection of the TBS group

A solution of TBS-protected alcohol (1.0 equiv) in dry THF (20 mL) was cooled to 0 °C, followed by the addition of TBAF (1.0 M in THF, 1.2 equiv). The reaction mixture was allowed to warm to room temperature and stir for 4 h. The reaction was quenched with satd aq NH_4Cl, extracted with Et_2O, dried over $MgSO_4$, filtered and concentrated. Purification was performed on a flash column (8:1 hexanes/EtOAc), collecting all fractions with a component of R_f = 0.10 to yield the pure alcohol.

4.25. 4-(3,5-Diisopropyl-4-methoxymethoxy-phenyl)-butan-1-ol (15a)

General procedure D was employed with **14a** (1.0 g, 2.45 mmol) and TBAF (1.0 M in THF, 2.94 mL, 2.94 mmol). The alcohol was obtained as a colorless oil (678 mg, 94% yield). ^{1}H NMR: δ 6.92 (s, 2H), 4.92 (s, 2H), 3.66 (t, J = 6.0 Hz, 2H), 3.63 (s, 3H), 3.34 (m, 2H), 2.60 (t, J = 7.5 Hz, 2H), 1.61–1.72 (m, 4H), 1.23 (d, J = 7.2 Hz, 12H); ^{13}C NMR: δ 149.7, 141.3, 138.5, 123.7, 100.2, 62.6, 57.2, 35.6, 32.5, 27.7, 26.6, 23.9; ESI-MS: (m/z) 311.8 (M+H_2O).

4.26. 6-(3,5-Diisopropyl-4-methoxymethoxy-phenyl)-hexan-1-ol (15b)

General procedure D was employed with **14b** (8.8 g, 20.1 mmol) and TBAF (1.0 M in THF, 24.2 mL, 24.2 mmol). The alcohol was obtained as a colorless oil (6.2 g, 96% yield). ^{1}H NMR: δ 6.90 (s, 2H), 4.92 (s, 2H), 3.63 (s, 3H), 3.33 (septet, J = 6.9 Hz, 2H), 2.56 (t, J = 8.1 Hz, 2H), 1.54–1.65 (m, 4H), 1.38–1.45 (m, 4H), 1.29 (d, J = 6.9 Hz, 12H); ^{13}C NMR: δ 149.6, 141.2, 138.8, 123.6, 100.1, 62.5, 57.1, 35.7, 32.5, 31.5, 29.1, 26.5, 25.5, 23.8; EI-MS: (m/z) 322.4.

4.27. General procedure E: halogenation of alcohol to iodide

Imidazole (3.75 equiv) and Ph_3P (1.8 equiv) were added to a solution of alcohol (1.0 equiv) in dry CH_2Cl_2 (120 mL). Iodine (1.75 equiv) in dry CH_2Cl_2 (20 mL) was added dropwise. The mixture was stirred for 30 min, quenched with satd aq $Na_2S_2O_3$ (5 mL). The mixture was then cooled to 0 °C and 30% H_2O_2 was added dropwise to oxidize the unreacted Ph_3P (reaction monitored by the disappearance of Ph_3P by TLC). The mixture was extracted with EtOAc (3 × 40 mL), washed with brine (30 mL), dried over $MgSO_4$, filtered and concentrated. Purification was performed on a flash column (8:1 hexanes/EtOAc), collecting all fractions with a component of R_f = 0.90 to yield the iodide.

4.28. 5-(4-Iodo-butyl)-1,3-diisopropyl-2-methoxymethoxy-benzene (16a)

General procedure E was employed with imidazole (590 mg, 8.6 mmol), Ph_3P (1.1 g, 4.1 mmol), **15a** (680 g, 2.3 mmol) and iodine (1.0 g, 4.0 mmol). The iodide was obtained as a colorless oil (710 mg, 76% yield). ^{1}H NMR: δ 6.91 (s, 2H), 4.93 (s, 2H), 3.64 (s, 3H), 3.35 (m, 2H), 3.23 (m, 2H), 2.60 (t, J = 7.2 Hz, 2H), 1.85–1.94 (m, 2H), 1.71–1.79 (m, 2H), 1.24 (d, J = 6.6 Hz, 12H); ^{13}C NMR: δ 149.9, 141.5, 137.9, 123.7, 100.3, 57.2, 34.6, 33.1, 32.2, 26.6, 23.9, 6.8; EI-MS: (m/z) 404.2.

4.29. 5-(6-Iodo-hexyl)-1,3-diisopropyl-2-methoxymethoxy-benzene (16b)

General procedure E was employed with imidazole (1.2 g, 17.4 mmol), Ph_3P (2.2 g, 8.4 mmol), **15b** (1.5 g, 4.65 mmol) and iodine (2.1 g, 8.1 mmol). The iodide was obtained as a colorless oil (1.6 g, 78% yield). ^{1}H NMR: δ 6.90 (s, 2H), 4.91 (s, 2H), 3.63 (s, 3H), 3.33 (septet, J = 6.9 Hz, 2H), 3.20 (t, J = 7.2 Hz, 2H), 2.56 (t, J = 7.8 Hz, 2H), 1.85 (m, 2H), 1.62 (m, 2H), 1.35–1.48 (m, 4H), 1.23 (d, J = 6.9 Hz); ^{13}C NMR: δ 149.7, 141.3, 138.6, 123.7, 100.2, 57.2, 35.7, 33.4, 31.3, 30.3, 28.2, 26.6, 23.9, 7.1; EI-MS: (m/z) 432.1.

4.30. General procedure F: trifluoromethyl ketone formation

A solution of iodide (1.0 equiv) in dry Et_2O was cooled to −78 °C and *n*-BuLi (1.6 M in hexanes, 2.0 equiv) was added dropwise. The reaction mixture was slowly allowed to warm to 0 °C over 2 h and then cooled back down to −78 °C. A solution of **6** (1.3 equiv) in dry Et_2O (5 mL) was slowly added dropwise over 30 min. The reaction was allowed to warm to 0 °C and then quenched with satd aq NH_4Cl. The Et_2O layer was washed with satd aq NH_4Cl (10 mL), brine (30 mL), dried over $MgSO_4$, filtered and concentrated. Purification was performed on a flash column (20:1 hexanes/EtOAc), collecting all fractions with a component of $R_f = 0.50$ to yield the pure ketone.

4.31. 6-(3,5-Diisopropyl-4-methoxymethoxy-phenyl)-1,1,1-trifluoro-hexan-2-one (17a)

General procedure F was employed with **16a** (708 mg, 1.75 mmol), *n*-BuLi (1.6 M in hexanes, 2.2 mL, 3.6 mmol) and **6** (412 mg, 2.28 mmol). The ketone was obtained as a colorless oil (306 mg, 66% yield). ^{1}H NMR: δ 6.90 (s, 2H), 4.91 (s, 2H), 4.31 (t, J = 5.1 Hz, 2H), 3.62 (s, 2H), 3.32 (septet, J = 6.9 Hz, 2H), 2.55 (t, J = 7.5 Hz, 2H), 1.27–1.37 (m, 4H), 1.22 (d, J = 6.9 Hz, 12H); ^{13}C NMR: δ 167.7 (q, $^2J_{CF}$ = 30 Hz), 149.6, 141.3, 139.0, 129.8 (q, $^1J_{CF}$ = 156 Hz), 123.8, 100.3, 57.3, 35.9, 31.6, 29.5, 29.4, 26.6, 24.0; ^{19}F NMR: δ *−67.82; EI-MS: (m/z)* 374.1.

4.32. 8-(3,5-Diisopropyl-4-methoxymethoxy-phenyl)-1,1,1-trifluoro-octan-2-one (17b)

General procedure F was employed with **16b** (330 mg, 0.76 mmol), *n*-BuLi (1.6 M in hexanes, 1.0 mL, 1.56 mmol) and **6** (109 mg, 0.60 mmol). The ketone was obtained as a colorless oil (172 mg, 56% yield). ^{1}H NMR: δ 6.89 (s, 2H), 4.91 (s, 2H), 3.62 (s, 2H), 3.53 (s, 3H), 3.32 (septet, J = 6.9 Hz, 2H), 2.71 (t, J = 7.2 Hz, 2H), 2.55 (t, J = 8.1 Hz, 2H), 1.64–1.69 (m, 4H), 1.38 (m, 4H), 1.22 (d, J = 6.3 Hz, 12H); ^{13}C NMR: δ 191.6 (q, $^2J_{CF}$ = 36 Hz), 149.7, 141.4, 138.6, 123.7, 116.1 (q, $^1J_{CF}$ = 207 Hz), 100.3, 57.2, 36.3, 35.7, 31.2, 28.9, 28.5, 26.6, 23.9, 22.3; ^{19}F NMR: δ *−79.09; EI-MS: (m/z)* 402.3.

4.33. 6-(3,5-Diisopropyl-4-methoxymethoxy-phenyl)-2-hydroxy-2-trifluoromethyl-hexanenitrile (18a)

General procedure A was employed with **17a** (200 mg, 0.53 mmol), TMSCN (0.18 mL, 1.34 mmol), KCN (5 mg) and 18-crown-6 (5 mg). The cyanohydrin was obtained as a yellow oil (212 mg, 99% yield), and was used without further purification.

4.34. 8-(3,5-Diisopropyl-4-methoxymethoxy-phenyl)-2-hydroxy-2-trifluoromethyl-octanenitrile (18b)

General procedure A was employed with **17b** (23 mg, 0.057 mmol), TMSCN (0.02 mL, 0.14 mmol), KCN (10 mg) and 18-crown-6 (10 mg). The cyanohydrin was obtained as a yellow oil (24 mg, 96% yield), and was used without further purification.

4.35. 2-Hydroxy-6-(4-hydroxy-3,5-diisopropyl-phenyl)-2-trifluoromethyl-hexanoic acid amide (4)

General procedure B was employed with **18a** (212 mg, 0.53 mmol) and concd HCl (4.0 mL). The hydroxyamide was obtained as a orange oil (158 mg, 80% yield). mp = 92–94 °C; ^{1}H NMR: δ 6.84 (s, 2H), 6.09 (d, J = 4.6 Hz, 2H), 4.68 (s, 1H), 4.05 (s, 1H), 3.14 (septet, J = 6.9 Hz, 2H), 2.52 (t, J = 8.4 Hz, 2H), 1.58 (t, J = 3.3 Hz, 2H), 1.37 (t, J = 3.3 Hz, 2H), 1.26 (d, J = 6.6 Hz, 12H); ^{13}C NMR: δ 169.6, 147.9, 134.5, 133.4, 124.0 (q, $^1J_{CF}$ = 171 Hz), 123.3, 96.1 (q, $^2J_{CF}$ = 21 Hz) 35.6, 32.8, 29.0, 28.9, 27.2, 22.8; ^{19}F NMR: δ *−77.32; EI-MS:* 375.4 *m/z*. Anal. Calcd for $C_{19}H_{28}F_3NO_3$: C, 60.79; H, 7.52; N, 3.73. Found: C, 60.80; H, 7.42; N, 3.43.

4.36. 2-Hydroxy-8-(4-hydroxy-3,5-diisopropyl-phenyl)-2-trifluoromethyl-octanoic acid amide (5)

General procedure B was employed with **18b** (24 mg, 0.056 mmol) and concd HCl (2.0 mL). The hydroxyamide was obtained as a white solid (20.5 mg, 91% yield). mp = 105–107 °C; ^{1}H NMR: δ 6.84 (s, 2H), 6.09 (d, J = 4.6 Hz, 2H), 4.68 (s, 1H), 4.05 (s, 1H), 3.14 (septet, J = 6.9 Hz, 2H), 2.52 (t, J = 8.4 Hz, 2H), 1.90 (t, J = 8.4 Hz, 2H), 1.58 (t, J = 3.3 Hz, 2H), 1.37 (t, J = 3.3 Hz, 2H), 1.26 (d, J = 6.6 Hz, 12H); ^{13}C NMR: δ 169.6, 147.9, 134.5, 133.4, 124.0 (q, $^1J_{CF}$ = 171 Hz), 123.3, 35.6, 32.6, 31.7, 29.7, 29.3, 29.1, 27.2, 22.8, 22.1; ^{19}F NMR: δ −77.45; EI-MS: *m/z* 403.2; HRMS (EI) calcd for $C_{21}H_{32}F_3NO_3$ 403.2334, found 403.2328; Anal. Calcd for $C_{21}H_{32}F_3NO_3$: C, 62.51; H, 7.99; N, 3.47. Found: C, 62.61; H, 7.68; N, 3.17.

Acknowledgments

The authors would like to thank NIH Grant RO1 CA105435-01 (M.L.B. & M.K.P.) and the Jeffress Trust Fund (M.L.B.) for financial support. We would like to acknowledge Dr. Jaideep Kapur and Ashley Renick (Dept. Neurology, University of Virginia) for providing the cultured hippocampal neurons. We would also like to thank the Cardiovascular Research Center at the University of Virginia (M.K.P) and the Georgetown Drug Discovery Program (M.L.B.).

References and notes

1. Catterall, W. A. *Neuron* **2000**, *26*, 13–25.
2. Goldin, A. L. *Annu. Rev. Physiol.* **2001**, *63*, 871–894.
3. Prasad, A.; Worrall, B. B.; Bertram, E. H.; Bleck, T. P. *Epilepsia* **2001**, *42*, 380–386.
4. Trapani, Giuseppe; Altomare, Cosimo; Sanna, Enrico; Biggio, Giovanni; Liso, Gaetano *Curr. Med. Chem.* **2000**, *7*, 249–271.
5. Martella, G.; De Persis, C.; Bonsi, P.; Natoli, S.; Cuomo, D.; Bernardi, G.; Calabresi, P.; Pisani, A. *Epilepsia* **2005**, *46*, 624–635.
6. Dong, X.-P.; Xu, T.-L. *Anesthesia Analgesia* **2002**, *95*, 907–914.
7. Ratnakumari, L.; Hemmings, H. C. *Br. J. Pharmacol.* **1996**, *119*, 1498–1504.
8. Ratnakumari, L.; Hemmings, H. C., Jr. *Anesthesiology* **1997**, *86*, 428–439.
9. Ouyang, W.; Wang, G.; Hemmings, H. C. *Mol. Pharmacol.* **2003**, *64*, 373–381.
10. Matthew Krasowski, D.; Hong, X.; Hopfinger, A. J.; Neil Harrison, L. *J. Med. Chem.* **2002**, *45*, 3210–3221.
11. Krasowski, M. D.; Hong, X.; Hopfinger, A. J.; Harrison, N. L.; Cooke, A.; Anderson, A.; Buchanan, K.; Byford, A.; Gemmell, D.; Hamilton, N.; McPhail, P.; Miller, S.; Sundaram, H.; Vijn, P. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 927–930.
12. Brown, M. L.; Van Dyke, C. C.; Brown, G. B.; Brouillette, W. J. *J. Med. Chem.* **1999**, *42*, 1537–1545.
13. Prout, K.; Fail, J.; Jones, R. M.; Warner, R. E.; Emmett, J. C. *J. Chem. Soc. Perkin. Trans. 2: Phys. Org. Chem.* **1988**, *3*, 265–284.
14. Clark, M. D.; Cramer, R. D., III; Opdenbosch, N. V. *J. Comput. Chem.* **1989**, *10*, 982–1012.
15. Choudhury-Mukherjee, I.; Schenck, H. A.; Cechova, S.; Pajewski, T. N.; Kapur, J.; Ellena, J.; Cafiso, D. S.; Brown, M. L. *J. Med. Chem.* **2003**, *46*, 2494–2501.
16. Wheatley, W. B.; Holdrege, C. T. *J. Org. Chem.* **1957**, *23*, 568–571.
17. Chiellini, G.; Nguyen, H.; Yoshihara, H. A.; Scanlan, T. I. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 2607–2611.
18. Nassal, M. *Liebigs Ann. Chem.* **1983**, 1510–1523.
19. Grunewald, G. L.; Brouillette, W. J.; Finney, J. A. *Tetrahedron Lett.* **1980**, *21*, 1219–1220.
20. Ware, E. *Chem. Rev.* **1950**, *46*, 403–470.
21. Banker, G.; Goslin, K. *Nature (London)* **1988**, *336*, 185–186.
22. Brouillette, W. J.; Jestkov, V. P.; Brown, M. L.; Shamim Akhtar, M.; Delorey, T. M.; Brown, G. B. *J. Med. Chem.* **1994**, *37*, 3289–3293.
23. Brown, M. L.; Brown, G. B.; Brouillette, W. J. *J. Med. Chem.* **1997**, *40*, 602–607.
24. Irlapati, N. R.; Baldwin, J. E.; Adlington, R. M.; Pritchard, G. J.; Cowley, A. R. *Tetrahedron* **2006**, *62*, 4603–4614.
25. Nystroem, J. E.; McCanna, T. D.; Helquist, P.; Amouroux, R. *Synthesis* **1988**, 56–58.
26. Suhara, Y.; Oka, S.; Kittaka, A.; Takayama, H.; Waku, K.; Sugiura, T. *Bioorg. Med. Chem.* **2007**, *15*, 854–867.