Novel Inhibitors of Potassium Ion Channels on Human T Lymphocytes

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Received January 6, 1995[⊗]

The in vitro biological characterization of a series of 4-(alkylamino)-1,4-dihydroquinolines is reported. These compounds are novel inhibitors of voltage-activated n-type potassium ion (K^+) channels in human T lymphocytes. This series, identified from random screening, was found to inhibit [^{125}I]charybdotoxin binding to n-type K^+ channels with IC_{50} values ranging from 10^{-6} to 10^{-8} M. These analogs also inhibit whole cell n-type K^+ currents with IC_{50} values from 10^{-5} to 10^{-7} M. The preparation of a series of new 4-(alkylamino)-1,4-dihydroquinolines is described. Structure–activity relationships are discussed. Naphthyl analog **7c**, the best compound prepared, exhibited >100-fold selectivity for inhibition of [^{125}I]charybdotoxin binding to n-type K^+ channels compared with inhibition of [^{3}H]dofetilide binding to cardiac K^+ channels. These compounds represent a potent and selective series of n-type K^+ channel inhibitors that have the potential for further development as anti-inflammatory agents.

Introduction

The most prominent and best characterized ion channel in human T lymphocytes is the voltage-activated n-type potassium ion (K^+) channel.[1,2] The channel is selective for K^+, activates at −40 mV, shows cumulative inactivation, and is blocked by both peptide and nonpeptide antagonists.[1,3,4] Charybdotoxin (ChTX), a 37-amino acid peptide purified from the venom of the scorpion *Leiurus quinquestriatus*,[5] is a potent antagonist of n-type K^+ channels. ChTX is active at low nanomolar concentrations[3,4,6] and, when radiolabeled, binds to human T lymphocytes with characteristics indicative of binding to n-type K^+ channels.[7] Nonpeptide antagonists are significantly less potent (20 μM < IC_{50}'s < 10 mM) and include the nonselective K^+ blockers quinine, 4-aminopyridine, and tetraethylammonium and the Ca^{2+} channel antagonists verapamil and nifedipine.[1,6]

Numerous studies using both peptide and nonpeptide antagonists have indicated that n-type K^+ channels are specifically involved in T lymphocyte proliferation. For example, antagonists of this K^+ conductance inhibit both mitogen- and antigen-stimulated proliferation with similar rank order of potency.[1,6,8–10] Since the production of activated T lymphocytes is essential for initiating and supporting the immune response, T lymphocyte K^+ channels represent a novel therapeutic target for the discovery of anti-inflammatory agents. However, the development of T lymphocyte K^+ channel inhibitors as anti-inflammatory agents will require the discovery of potent and selective nonpeptide antagonists. Reference nonpeptide antagonists of n-type K^+ channels, such as classical K^+ and Ca^{2+} channel blockers, are neither potent nor selective.

In order to identify more potent and selective inhibitors of n-type K^+ channels, high throughput screening of our compound files using [^{125}I]ChTX binding to T lymphocytes was performed. These efforts have resulted in the identification of a novel series of n-type K^+ channel antagonists, the 4-(alkylimino)-1,4-dihydroquinolines. The effects of compounds on both [^{125}I]-ChTX binding and n-type K^+ currents (IKn) as measures of n-type K^+ channel inhibition were examined. In addition, the effects of compounds on [^{3}H]dofetilide binding to cardiac myocytes as a measure of cardiac K^+ channel (Ikr) inhibition and as a determinant of the selectivity of the (alkylimino)-1,4-dihydroquinolines for the lymphocyte versus cardiac K^+ channels were examined.[11,12] This paper describes the structure–activity relationships (SAR) for this series of analogs and the combination of classical medicinal and computational techniques used for new inhibitor design.

Chemistry

The synthesis of (alkylimino)-1,4-dihydroquinolines is illustrated in Scheme 1. The key synthetic precursors, 4-chloroquinolines **12**, were prepared from commercially available anilines according to the well-established route of Price and Roberts.[13] Conversion into the hexyl ether analog **13** was accomplished by treatment of 4-chloroquinoline with hexanol in the presence of base and phase transfer catalyst tris[2-(2-methoxyethoxy)-ethyl]amine, TDA-1. The alkylamine group was installed using a modified procedure of the Surrey method,[14] whereby the 4-chloroquinoline derivative **12** is heated with an excess of alkylamine to furnish 4-(alkylamino)quinoline **14**. Alkylation on the quinoline nitrogen was performed by treatment of the 4-substituted quinolines **13** and **14** with the appropriate alkyl halide or benzylic halide to afford target molecules **6–10**.

The synthesis of 4-benzyl-1-(hexyloxy)naphthalene (**11**) is illustrated in Scheme 2. Commercially available 1-naphthol was first O-alkylated and subsequently acylated through the action of benzoyl chloride/$AlCl_3$ to furnish intermediate **15**. Mild reduction with sodium

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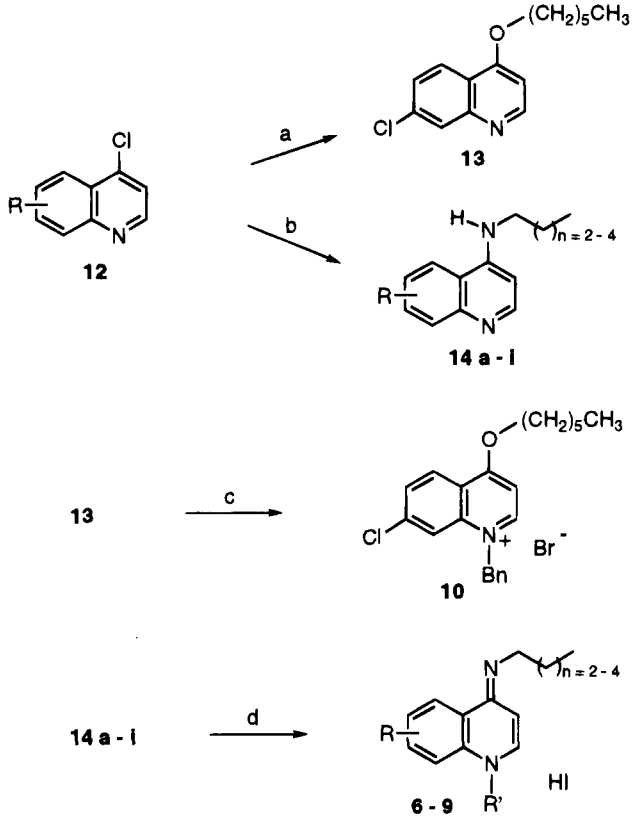
[||] All research prior to manuscript submission was carried out at the Sterling Winthrop Pharmaceuticals Research Division.
[⊗] Abstract published in *Advance ACS Abstracts*, May 1, 1995.

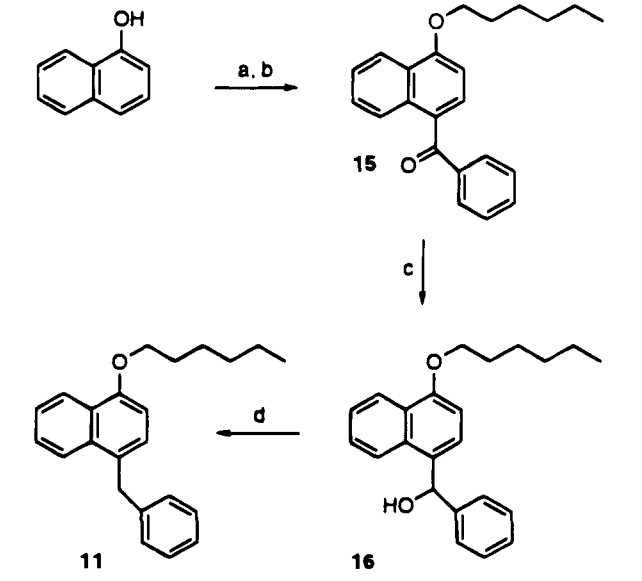
0022-2623/95/1838-1877$09.00/0 © 1995 American Chemical Society

Scheme 1. Synthetic Route to Quinoline Derivatives[a]



[a] Reagents: (a) hexanol, TDA-1, KOH, K_2CO_3; (b) alkylamine, Δ; (c) BnBr, Δ; (d) R'X.

Scheme 2. Synthetic Route to Naphthalene Target **11**[a]



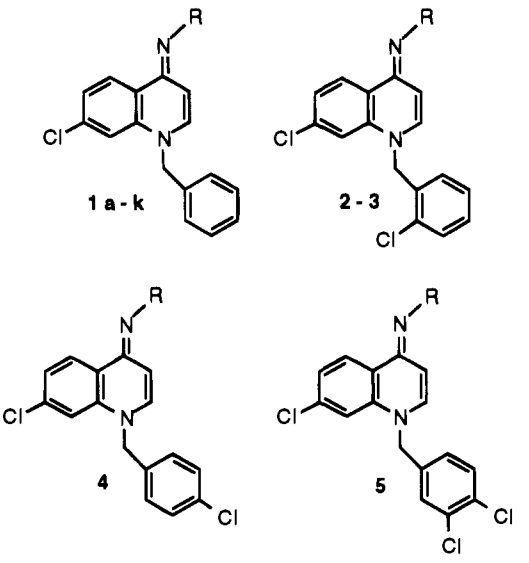
[a] Reagents: (a) 1-iodohexane, K_2CO_3, Δ; (b) BnCOCl, $AlCl_3$; (c) 10% $NaBH_4/Al_2O_3$, 0 °C; (d) $NaBH_4$, CF_3CO_2H.

borohydride on alumina provided the secondary alcohol **16**, which was further reduced with sodium borohydride in the presence of trifluoroacetic acid to target molecule **11**.

Results and Discussion

High throughput screening of our chemical files disclosed the activity of compound **1f**, 1-Benzyl-7-chloro-4-(*n*-hexylimino)-1,4-dihydroquinoline hydrochloride, as one of the most potent and selective compounds from this series.[15] Analog searching for other 4-(alkylimino)-1,4-dihydroquinolines resulted in the identification of a small number of analogs. Activity for these analogs is particularly sensitive to changes at the 4-imino position. The data in Table 1 illustrate that lengthening the alkyl chain results in an increase in primary assay activity from >10 μM for **1a** (no substitution) to 0.032 μM for **1f** ($(CH_2)_5CH_3$). The isobutyl derivative **1i** is equivalent to the butyl derivative, whereas bulky alkyl derivatives such as **1j**,**k** are much less potent. Other variations on the imino nitrogen provided further SAR at this position. The incorporation of an acidic group, **2b**, or permanently charged amine, **2d**, into the alkyl chain abolishes all primary assay activity. Similarly, the direct attachment of a phenyl group (**3a**) to this position exhibited a 60-fold drop in activity compared to a similarly substituted benzyl analog (**3b**). Thus, in general, potency increases with alkyl chain length to an optimum length of six and then decreases with added length, and an increase in bulk of the alkyl chain or the incorporation of hydrophilic substituents was not well tolerated.

Table 1. Affinities for [^{125}I]ChTX, n-Type K^+ Current (IKn), and [^{3}H]Dofetilide



		IC_{50} (nM)		
compd	R	ChTX[a]	IKn[b]	dofetilide[a]
1a	H	>8000		
1b	Me	>5000		*c*
1c	Pr	544	3160	
1d	*n*-Bu	198	2620	*c*
1e	*n*-pentyl	65	250	1300
1f	*n*-hexyl	32	335	820
1g	*n*-heptyl	117	249	2500
1h	*n*-octyl	280	351	2400
1i	*i*-Bu	206		2000
1j	1,5-dimethylhexyl	817	1676	175
1k	1-adamantyl	1170		157
2a	*n*-hexyl	190	216	780
2b	$(CH_2)_2CO_2H$	>10 000		
2c	$(CH_2)_3NHCH_3$	>30 000		
2d	$(CH_2)_3N(CH_3)_3$	>30 000	>10 000	
2e	$(CH_2)_2N(Et)_2$	7680		57
3a	2,4-dichlorophenyl	>30 000	>10 000	>30 000
3b	2,4-dichlorobenzyl	550	259	2380
4	*n*-hexyl	100		3230
5	*n*-hexyl	310	231	>3500

[a] Mean IC_{50}, $n = 2$ determinations. [b] IC_{50} from $n > 9$ data points. [c] 24% at 400 nM.

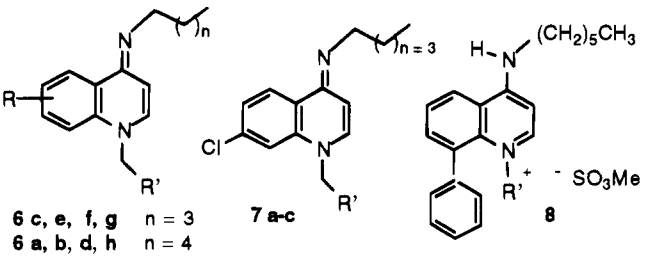
Additional data, including secondary electrophysiological assay (IKn) and cardiac K^+ selectivity [^{3}H]-dofetilide binding, were generated for selected examples from the existing quinolines in our library. The data for IKn was found to correlate closely with the ChTX activity trend, albeit in slightly weaker activity. The [^{3}H]dofetilide binding data illustrate a lack of response toward alkyl chain lengthening, analogs **1e**–**h**, whereas branching of the alkyl chain, analogs **1j**,**k**, resulted in compounds that were >10-fold more potent. Additionally, the incorporation of a permanently charged amine (**2e**) was found to be favorable for [^{3}H]dofetilide binding. Substitution with chlorine in either the 4- or 3,4-positions, analogs **4** and **5**, respectively, was found to greatly diminish dofetilide activity without affecting ChTX binding activity.

Overall, the ChTX and IKn data for this set of analogs were consistent with a region around N-4 binding to a size-restricted hydrophobic pocket. However, the [^{3}H]-dofetilide binding data for this same region were found to be consistent with a bulky hydrophilic pocket. The data from this series show that there are different SAR at the lymphocyte and cardiac K^+ channels. As a result of these two opposing SAR trends, only a modest selectivity favoring lymphocyte K^+ channels versus cardiac K^+ channels was evident. The *n*-pentyl analog **1e** exhibited the most favorable activity profile from the historical series, wherein a 20-fold difference in activity was found.

One of the primary goals of this study was to identify additional SAR that would more strongly support a structure-based mechanism. Our approach was based upon developing pharmacophoric hypotheses for the two separate K^+ channels. This approach identified unexplored conformational space and areas where a divergent SAR between the two channels would exist. The substitutional areas around the quinoline ring positions 6–8 and the pendant benzyl group were initially identified as areas for further investigation. A small number of substituents (OMe, CF_3, and F) at positions 6–8 were investigated, wherein the N-1 and N-4 groups were identical to those for the pre-existing quinolines in Table 1. Analogs **6b**–**h** were found to possess equivalent or decreased binding activity for ChTX and generally poorer selectivity against dofetilide binding (with the exception of **6h**) when compared to the 7-chloro analogs **1e**,**f** or **4** (Table 2). Conformational restriction of the benzyl group into a 1-indanyl analog, **7a**, greatly reduced the ChTX binding with no effect on selectivity. Enlargement of the benzyl aromatic into the 1- and 2-naphthylmethyl analogs **7b**,**c** resulted in a slight loss of ChTX activity for the 1-naphthylmethyl analog and little change on the ChTX binding for the 2-naphthylmethyl analog. However, the 2-positional isomer **7c** was found to be vastly weaker in dofetlide binding. **7c** represents >100-fold selectivity for the lymphocyte K^+ channel over the cardiac K^+ channel.

In an effort to provide further information toward the development of a pharmacophoric hypothesis, analogs were prepared to test the dependence of activity on physical properties. Butylamine analog **9** when compared to hexylamine analog **1f** illustrates that changing structure while holding physical properties essentially constant resulted in a noticeable loss in activity (Table 3). Similarly, structural modification from hexylamine to hexyl ether analog **10** resulted in a noticeable loss in ChTX activity while holding physical properties essentially constant. The further modification of analog **9** physical properties, to the naphthalene ether analog **11**, resulted in a profound loss of activity in the primary assay. The dramatic loss in activity for analog **11** could be a result from the gross change in a physical parameter.

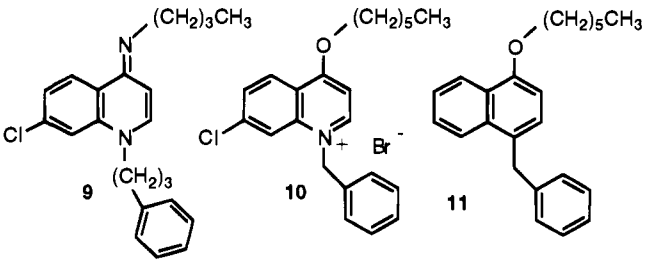
Table 2. Affinities for [^{125}I]ChTX, n-Type K^+ Current (IKn), and [^{3}H]Dofetilide



compd	R	R'	IC$_{50}$ (nM) ChTX[a]	IKn[b]	dofetilide[a]
6a	H	Ph	164	1053	
6b	6-MeO	Ph	47	267	680
6c	6-MeO	*p*-ClPh	275		1990
6d	8-MeO	Ph	475		210
6e	6-CF_3	*p*-ClPh	340	213	140
6f	7-CF_3	*p*-ClPh	239		90
6g	8-F	*p*-ClPh	227		310
6h	7-Cl	*p*-MeOPh	88	329	
7a	7-Cl	1-indanyl	2300		870
7b	7-Cl	1-naphthyl	280	279	900
7c	7-Cl	2-naphthyl	75	434	8000
8		Me	240	602	223

[a] Mean IC$_{50}$, minimum of two determinations. [b] IC$_{50}$ from n > 9 data points.

Table 3. Comparative Physical Properties and [^{125}I]ChTX Affinity



compd	log P[a]	pK_a	ChTX IC$_{50}$ (nM)[b]
1f	3.81	11.52	32
5	4.64	11.53	310
7b	4.01	11.43	280
7c	4.16	11.57	75
8a	3.89	9.58	930
8b	3.73		240
9	3.82	11.37	471
10	3.62	11.17	828
11			>5000

[a] HPLC method of Kraak et al.[16] [b] Mean IC$_{50}$, minimum of two determinations.

Molecular Modeling

Excluded volume maps[17] were generated for both the lymphocyte and cardiac channels to test the hypothesis that selectivity is based on a volume (shape) pharmacophoric model. The ChTX and dofetilide binding data were used to generate these maps. Strongly bound

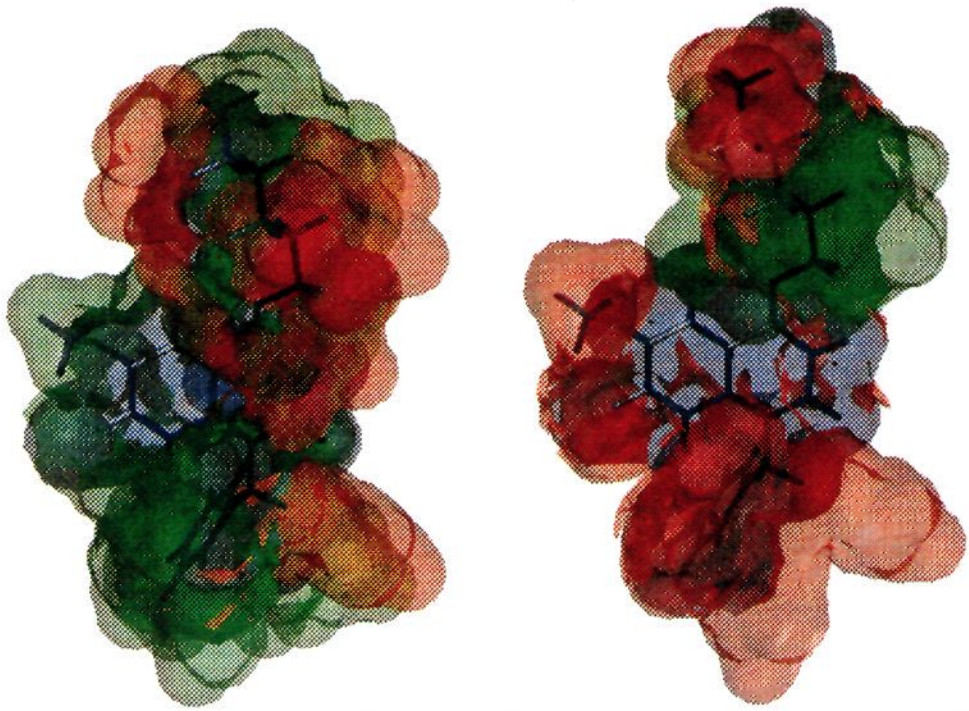


Figure 1. Volume exclusion maps for the lymphocyte (left map) and cardiac channels: green regions, strong binding; red, weak binding; and blue, common volume.

compounds (IC_{50} < 150 nM) of the lymphocyte channel or the cardiac channel were designated as the active set for each respective channel. Weakly bound compounds (IC_{50} > 1000 nM) were designated as the inactive set for each respective channel. The active and inactive volume maps for both the cardiac and lymphocyte channels were created by generating the minimum volume occupied by a low-energy conformation for each of the members of the respective sets. The final volume exclusion maps for the lymphocyte and cardiac channels were generated by superimposing the active and inactive volume maps from their respective channels (Figure 1). The green and red areas represent strong and weak binding regions; the blue areas represent the regions common to both.

The utiltiy of the volume exclusion maps is represented by the following examples. Binding data for the 6-methoxy analogs **6b,c** exhibited a 14- and 7-fold selectivity for the lymphocyte channel, respectively. The 6-position area is green in the lymphocyte volume exclusion map and red in the cardiac volume exclusion map corresponding to a ChTX selective region. For these analogs, the volume exclusion maps illustrate where a divergent SAR exists between the two channels. Analog **11** is an example of a compound that was prepared to test these maps. All low-energy conformations of analog **11** were found to project into the weak binding (red) areas of both the lymphocyte and cardiac volume exclusion maps. Binding data for **11** showed it to be inactive (>5000 μM) in both the lymphocyte and cardiac assays, in agreement with the volume exclusion map prediction. The volume exclusion maps have followed us to visualize where unexplored volume exists and have generated a three-dimensional picture of where the differences in binding occur for lymphocyte and cardiac channels.

Conclusions

A series of 4-(alkylimino)-1,4-dihydroquinolines were identified as inhibitors of [^{125}I]charybdotoxin binding to n-type K^+ channels in human T lymphocytes with IC_{50} values ranging from 10^{-6} to 10^{-8} M and to inhibit whole cell n-type K^+ currents with IC_{50} values from 10^{-5} to 10^{-7} M. These compounds were also evaluated for binding to cardiac K^+ channels via [^{3}H]dofetilide binding assay. A series of new 4-(alkylimino)-1,4-dihydroquinolines was prepared and evaluated for their ability to inhibit binding to both lymphocyte and cardiac K^+ channels. Naphthyl analog **7c**, the best compound prepared, exhibited >100-fold selectivity for inhibition of [^{125}I]carybdotoxin binding to n-type K^+ channels compared with inhibition of [^{3}H]dofetilide binding to cardiac K^+ channels. Additional studies will determine, for this series, if the [^{3}H]dofetilide binding assay correlates into a functional selectivity versus cardiac K^+ channels. These 4-(alkylimino)-1,4-dihydroquinolines represent a potent and selective series of n-type K^+ channel inhibitors that have potential for further development as anti-inflammatory agents.

Experimental Section

General Procedures. All solvents were HPLC grade and used as received with the exception of THF which was freshly distilled from sodium benzophenone ketyl. Melting points were determined using a Thomas Hoover apparatus and are uncorrected. Infrared spectra were run as 1% KBr pellets on a Nicolet 20-SX spectrometer or obtained by Onieda Research Services, Inc., Oneida, NY. Mass spectra were recorded as follows: electron impact spectra were obtained on a Hewlett Packard 5971 GC/MS at 70 eV; desorption chemical ionization spectra were obtained on a Nermag R-10-10C spectrometer; fast atom bombardment spectra were obtained on a Kratos Profile HV-2 spectrometer; high-resolution spectra were obtained by M-Scan, Inc., West Chester, PA. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Unity 300 or General Electric QE 300 spectrometer. Chemical shifts are reported

in parts per million (δ) relative to tetramethylsilane. Elemental analyses were performed by Quantitative Technologies, Inc., Whitehouse, NJ.

Iminoquinolines 1–6a. These compounds were previously prepared as described by Surrey.[14]

7-Chloro-4-(*n*-hexyloxy)quinoline (13). 4,7-Dichloroquinoline (10.0 g, 0.050 mol), K_2CO_3 (6.97 g, 0.050 mol), KOH (11.3 g, 0.20 mol), TDA-1 (1.63, 0.005 mol), and hexanol (7.73g, 0.075 mol) were combined in a round bottom flask equipped with condenser under N_2 and heated to reflux for 55 h. The mixture was cooled, diluted with EtOAc (300 mL), washed with 0.1 M HCl (3 × 200 mL), dried (Na_2SO_4), and concentrated in vacuo. The resulting orange solid was recrystallized with ether/EtOAc to give **13** (6.76 g, 51%): mp 71–73 °C; ^{1}H NMR (DMSO) δ 8.74 (d, J = 5.2 Hz, 1H), 8.13 (d, J = 8.8 Hz, 1H), 7.97 (d, J = 2.1 Hz, 1H), 7.58 (dd, J = 8.9, 2.1 Hz, 1H), 7.04 (d, J = 5.4 Hz, 1H), 4.23 (t, J = 6.4 Hz, 2H), 1.89–1.80 (m, 2H), 1.51–1.44 (m, 2H), 1.37–1.28 (m, 4H), 0.88 (t, J = 7.0 Hz, 3H); MS (CI, CH_4) *m/e* 264 (MH^+). Anal. ($C_{15}H_{18}ClNO$) C, H, N.

General Method A: Preparation of 4-Alkylquinolines 14a–j. A mixture of amine (1.1 equiv) and 4-chloroquinoline **12** (1.0 equiv) was refluxed for 18 h in ethanol (0.5 M). The solution was cooled to room temperature, and the solvents were removed in vacuo. The oily residue was diluted with NaOH (1 N) and extracted with EtOAC (3X). The combined organics were washed with brine, dried over K_2CO_3, and evaporated. Purification was carried out using silica gel and eluting the compounds with EtOAc/Hex.

4-(*n*-Hexylamino)quinoline (14a). Compound **14a** was synthesized according to the method of Surrey,[14] and the ^{1}H NMR and MS spectra were in agreement with its chemical structure.

6-Methoxy-4-(*n*-pentylamino)quinoline (14b). Compound **14b** was synthesized according to general method A: mp 143–146 °C; ^{1}H NMR (CDCl3) δ 8.45 (d, J = 5.3 Hz, 1H), 7.92 (d, J = 9.2 Hz, 1H), 7.30 (dd, J = 9.3, 2.8 Hz, 1H), 7.00 (d, J = 2.6 Hz, 1H), 6.43 (d, J = 5.4 Hz, 1H), 4.93 (bs, 1H), 3.92 (s, 3H), 3.56–3.29 (m, 2H), 1.84–1.74 (m, 2H), 1.49–1.38 (m, 4H), 0.95 (t, J = 7.0 Hz, 3H); MS (CI, CH_4) *m/3* 245 (MH^+). Anal. ($C_{15}H_{20}N_2O$) C, H, N.

6-Methoxy-4-(*n*-hexylamino)quinoline (14c). Compound **14c** was synthesized according to general method A: mp 126–128 °C; ^{1}H NMR ($CDCl_3$) δ 8.46 (d, J = 5.4 Hz, 1H), 7.92 (s, 1H), 7.32 (d, J = 2.6 Hz, 1H), 6.42 (d, J = 5.3 Hz, 1H), 4.8 (bs, 1H), 3.9 (s, 3H), 3.35 (d of t, J = 7.2 Hz, 2H), 1.77 (m, 2H), 1.48 (m, 2H), 1.36 (m, 4H), 0.95 (t, J = 7.2 Hz, 3H); MS (EI) *m/e* 258 (M^+). Anal. ($C_{16}H_{22}N_2O$) C, H, N.

8-Methoxy-4-(*n*-hexylamino)quinoline (14d). Compound **14d** was synthesized according to general method A: mp 146–148 °C; ^{1}H NMR ($CDCl_3$) δ 8.58 (d, J = 5.3 Hz, 1H), 7.35 (d of d, J = 7.2, 7.8 Hz, 1H), 7.26 (d, J = 7.8 Hz, 1H), 6.99 (d, J = 7.2 Hz, 1H), 6.46 (d, J = 5.3 Hz, 1H), 4.9 (bs, 1H), 4.06 (s, 3H), 3.35 (d of t, J = 7.2 Hz, 2H), 1.77 (m, 2H), 1.48 (m, 2H), 1.36 (m, 4H), 0.95 (t, J = 7.2 Hz, 3H); MS (EI) *m/e* 258 (M^+); HRMS (LSIMS) $C_{16}H_{22}N_2O$ (MH^+) requires 259.18104, found 259.18090; Δ = 0.55 ppm. Anal. ($C_{16}H_{22}N_2O \cdot {}^1/_4H_2O$) C, H, N.

6-(Triflouromethyl)-4-(*n*-pentylamino)quinoline (14e). Compound **14e** was synthesized according to general method A: ^{1}H NMR (DMSO) δ 8.75 (s, 1H), 8.49 (d, J = 6.5 Hz, 1H), 8.92 (d, J = 8.5 Hz, 1H), 7.82 (dd, J = 8.5, 1.7 Hz, 1H), 7.59 (t, J = 7.5 Hz, 1H), 6.56 (d, J = 6.5 Hz, 2H), 3.39 (m, 2H), 1.78–1.61 (m, 2H), 1.48–1.29 (m, 4H), 0.88 (t, J = 7.0 Hz, 3H); MS (EI) *m/e* 283.

7-(Triflouromethyl)-4-(*n*-pentylamino)quinoline (14f). Compound **14f** was synthesized according to general method A: ^{1}H NMR (DMSO) δ 8.50–8.47 (m, 2H), 8.06 (s, 1H), 7.65 (dd, J = 8.7, 1.7 Hz, 1H), 7.45 (bs, 1H), 6.54 (d, J = 5.5 Hz, 2H), 3.26 (q, J = 6.5 Hz, 1H), 1.68–1.61 (m, 2H), 1.40–1.29 (m, 4H), 0.87 (t, J = 7.1 Hz, 3H); MS (EI) *m/e* 283.

7-Chloro-4-(*n*-pentylamino)quinoline (14g). Compound **14g** was synthesized according to general method A: mp 119–121 °C; ^{1}H NMR ($CDCl_3$) δ 8.53 (d, J = 5.4 Hz, 1H), 7.96 (s, 1H), 7.64 (d, J = 8.9 Hz, 1H), 7.35 (d, J = 8.9 Hz, 1H), 6.41 (d, J = 5.3 Hz, 1H), 5.0 (bs, 1H), 3.25 (m, 2H), 1.76 (m, 2H), 1.43 (m, 4H), 0.95 (t, J = 7.2 Hz, 3H); MS (CI, CH_4) *m/e* 249 (MH^+). Anal. ($C_{14}H_{17}N_2Cl_1$) C, H, N.

8-Fluoro-4-(*n*-pentylamino)quinoline (14h). Compound **14h** was synthesized according to general method A: mp 103–105 °C; ^{1}H NMR ($CDCl_3$) δ 8.63 (d, J = 5.4 Hz, 1H), 7.52 (m, 1H), 7.31 (m, 2H), 6.48 (d, J = 5.3 Hz, 1H), 5.0 (bs, 1H), 3.25 (m, 2H), 1.76 (m, 2H), 1.43 (m, 4H), 0.95 (t, J = 7.2 Hz, 3H); MS (CI, CH_4) *m/e* 233 (MH^+). Anal. ($C_{14}H_{17}N_2F_1$) C, H, N.

8-Phenyl-4-(*n*-hexylamino)quinoline Hydrochloride (14i). Compound **14i** was synthesized according to general method A: mp 189–191 °C; ^{1}H NMR (DMSO) δ 9.81 (s, 1H), 8.75 (d, J = 5.4 Hz, 1H), 8.24 (d, J = 7.2 Hz, 1H), 7.77 (d of t, J = 6.1, 7.4 Hz, 2H), 7.5 (m, 5H), 6.87 (d, J = 7.2 Hz, 1H), 3.55 (d of t, J = 6.6 Hz, 2H), 1.77 (m, 2H), 1.41 (m, 2H), 1.36 (m, 4H), 0.95 (t, J = 7.2 Hz, 3H); MS (EI) *m/e* 303 (M^+). Anal. ($C_{21}H_{24}N_2 \cdot HCl$) C, H, N.

7-Chloro-4-(*n*-butylamino)quinoline (14j). Compound **14j** was synthesized according to general method A: mp 130–135 °C; ^{1}H NMR (DMSO) δ 8.37 (d, J = 5.4 Hz, 1H), 8.26 (d, J = 9.0 Hz, 1H), 7.76 (dd, J = 2.2 Hz, 1H), 7.43 (dd, J = 9.1, 2.3 Hz, 1H), 7.28 (bs, 1H), 6.44 (d, J = 5.5 Hz, 1H), 3.24 (q, J = 7.0 Hz, 2H), 1.68–1.58 (m, 2H), 1.43–1.36 (m, 2H), 0.92 (t, J = 7.3 Hz, 3H); MS (CI, CH_4) *m/e* 235 (MH^+); HRMS (LSIMS) $C_{13}H_{16}N_2Cl$ (MH^+) requires 235.10020, found 235.10047; Δ = 1.16 ppm.

General Method B: Preparation of 4-(*n*-Hexylimino)-1,4-dihydroquinolines 6–9. A mixture of benzyl hal;ide (1.1 equiv), 4-chloroquinoline **14** (1.0 equiv), and sodium iodide (1.2 equiv) was refluxed for 18 h in acetone (0.5 M). The solution was cooled to room temperature, and the resulting hydroiodide precipitate was collected and washed repeatedly with acetone. The hydroiodide salt was dissolved in hot ethanol (60 mL) and treated with NaOH (35%). The mixture was cooled and the ethanol removed in vacuo. The residue was diluted with H_2O and extracted with EtOAC (3X). The combined organics were washed with brine, dried over K_2CO_3, and evaporated. Purification was carried out by diluting the oil with various ethanolic acids and cooling the solution overnight. The precipitates were collected and washed repeatedly with ethanol.

1-Benzyl-4-(*n*-hexylimino)-1,4-dihydroquinoline (6a). Compound **6a** was synthesized according to the method of Surrey,[14] and the ^{1}H NMR and MS spectra were in agreement with its chemical structure.

1-Benzyl-6-methoxy-4-(*n*-hexylimino)-1,4-dihydroquinoline Hydroiodide (6b). Compound **6b** was synthesized according to general method B: mp 212–215 °C; ^{1}H NMR ($CDCl_3$) δ 8.33 (s, 1H), 8.06 (d, J = 7.4 Hz, 1H), 7.92 (s, 1H), 7.51 (d, J = 9.5 Hz, 1H), 7.31 (m, 4H), 7.11 (m, 2H), 6.48 (d, J = 7.5 Hz, 1H), 5.60 (s, 2H), 4.17 (s, 3H), 3.35 (d of t, J = 7.2 Hz, 2H), 1.77 (m, 2H), 1.48 (m, 2H), 1.36 (m, 4H), 0.95 (t, J = 7.2 Hz, 3H); MS (CI, CH_4) *m/e* 349 (MH^+). Anal. ($C_{23}H_{28}N_2O \cdot HI$) C, H, N.

1-(*p*-Chlorobenzyl)-6-methoxy-4-(*n*-pentylimino)-1,4-dihydroquinoline Hydrochloride (6c). Compound **6c** was synthesized according to general method B: mp 164–167 °C; ^{1}H NMR ($CDCl_3$) δ 8.58 (d, J = 2.6 Hz, 1H), 8.40 (d, J = 7.5 Hz, 1H), 7.44 (d, J = 9.7 Hz, 1H), 7.26–7.20 (m, 3H), 7.06 (d, J = 8.5 Hz, 2H), 6.42 (d, J = 7.4 Hz, 1H), 5.73 (s, 2H), 4.08 (s, 3H), 3.56–3.49 (m, 2H), 1.80–1.76 (m, 2H), 1.39–1.34 (m, 4H), 0.86 (t, J = 7.0 Hz, 3H); MS (CI, CH_4) *m/e* 369 (MH^+); HRMS (LSIMS) $C_{22}H_{25}N_2OCl$ (MH^+) requires 369.17337, found 369.17373; Δ = 0.99 ppm. Anal. ($C_{22}H_{25}N_2OCl \cdot HCl \cdot {}^1/_4H_2O$) C, H, N.

1-Benzyl-8-methoxy-4-(*n*-hexylimino)-1,4-dihydroquinoline Hydrochloride (6d). Compound **6d** was synthesized according to general method B: mp 192–194 °C; ^{1}H NMR ($CDCl_3$) δ 8.73 (s, 1H), 7.82 (d, J = 7.7 Hz, 1H), 7.51 (t, J = 9.5 Hz, 1H), 7.31 (m, 4H), 7.16 (d, J = 8.2 Hz, 1H), 7.01 (d, J = 7.5 Hz, 2H), 6.43 (d, J = 7.7 Hz, 1H), 5.81 (s, 2H), 3.73 (s, 3H), 3.55 (d of t, J = 7.4 Hz, 2H), 1.77 (m, 2H), 1.48 (m, 2H), 1.36 (m, 4H), 0.95 (t, J = 7.2 Hz, 3H); MS (CI, CH_4) *m/e* 349 (MH^+); HRMS (LSIMS) $C_{23}H_{28}N_2O$ (MH^+) requires 349.22799, found 349.22803; Δ = 0.13 ppm.

1-(*p*-Chlorobenzyl)-6-(triflouromethyl)-4-(*n*-pentylimino)-1,4-dihydroquinoline Hydroiodide (6e). Compound

6e was synthesized according to general method B: mp 230–231 °C; ^{1}H NMR (DMSO) δ 9.82 (bs, 1H), 9.09 (s, 1H), 8.89 (d, J = 7.5 Hz, 1H), 8.25 (d, J = 8.6 Hz, 1H), 8.11 (d, J = 8.7 Hz, 1H), 7.45 (d, J = 8.4, 2H), 7.30 (d, J = 8.5, 2H), 7.16 (d, J = 7.5, 1H), 5.89 (s, 2H), 3.68–3.55 (m, 2H), 1.81–1.65 (m, 2H), 1.49–1.29 (m, 4H), 0.92 (t, J = 7.0 Hz, 3H); MS (CI, CH_4) m/e 407 (MH^+). Anal. ($C_{22}H_{22}N_2F_3Cl_1$·HI) C, H, N.

1-(*p*-Chlorobenzyl)-7-(triflouromethyl)-4-(*n*-pentylimino)-1,4-dihydroquinoline Hydroiodide (6f). Compound **6f** was synthesized according to general method B: mp 235–240 °C; ^{1}H NMR (DMSO) δ 9.76 (bs, 1H), 8.92 (d, J = 7.5 Hz, 1H), 8.83 (d, J = 8.8 Hz, 1H), 8.28 (s, 1H), 8.09 (d, J = 8.4 Hz, 1H), 7.45 (d, J = 8.5 Hz, 2H), 7.33 (d, J = 8.5 Hz, 2H), 7.18 (d, J = 7.6 Hz, 1H), 5.98 (s, 2H), 3.60 (bs, 2H), 1.80–1.65 (m, 2H), 1.49–1.25 (m, 4H), 0.89 (t, J = 7.0 Hz, 3H); MS (FAB, NBA) m/e 407 (MH^+). Anal. ($C_{22}H_{22}N_2F_3Cl_1$·HI) C, H, N.

1-(*p*-Chlorobenzyl)-8-flouro-4-(*n*-pentylimino)-1,4-dihydroquinoline Hydrochloride (6g). Compound **6g** was synthesized according to general method B: mp 221–223 °C; ^{1}H NMR (DMSO) δ 8.76 (d, J = 7.5 Hz, 1H), 8.66 (d, J = 8.2 Hz, 1H), 7.81 (m, 1H), 7.70 (m, 1H), 7.38 (d, J = 8.2 Hz, 2H), 7.15 (d, J = 8.2 Hz, 2H), 7.08 (d, J = 7.4 Hz, 1H), 5.86 (bs, 2H), 3.65 (d of t, J = 7.7 Hz, 2H), 1.76 (m, 2H), 1.43 (m, 4H), 0.95 (t, J = 7.2 Hz, 3H); MS (CI, CH_4) m/e 357 (MH^+). Anal. ($C_{21}H_{22}N_2FCl$·HCl) C, H, N.

7-Chloro-1-(*p*-methoxybenzyl)-4-(*n*-hexylimino)-1,4-dihydroquinoline Hydrochloride (6h). Compound **6h** was synthesized according to general method B: mp 239–241 °C; ^{1}H NMR ($CDCl_3$) δ 9.43 (d, J = 9.2 Hz, 1H), 8.15 (d, J = 7.4 Hz, 1H), 7.63 (s, 1H), 7.46 (d, J = 9.0 Hz, 1H), 7.15 (d, J = 8.7 Hz, 2H), 6.91 (d, J = 8.7 Hz, 2H), 6.44 (d, J = 7.6 Hz, 1H), 5.54 (s, 2H), 3.79 (s, 3H), 3.53 (d of t, J = 6.2 Hz, 2H), 1.77 (m, 2H), 1.48 (m, 2H), 1.36 (m, 4H), 0.95 (t, J = 7.2 Hz, 3H); MS (CI, CH_4) m/e 383 (MH^+). Anal. ($C_{23}H_{27}N_2ClO$·HCl·0.5H_2O) C, H, N.

7-Chloro-1-indanyl-4-(*n*-pentylimino)-1,4-dihydroquinoline Hydrochloride (7a). Compound **7a** was synthesized according to general method B: mp 79–80 °C; ^{1}H NMR ($CDCl_3$) δ 8.47 (d, J = 8.4 Hz, 1H), 7.38–7.21 (m, 4H), 7.20–7.13 (m, 2H), 6.58 (d, J = 7.5 Hz, 1H), 5.86 (d, J = 8.4 Hz, 1H), 5.79 (t, J = 6.6 Hz, 1H), 3.27 (t, J = 7.4 Hz, 2H), 3.15–2.96 (m, 2H), 2.75–2.66 (m, 1H), 2.12–2.08 (m, 1H), 1.75–1.65 (m, 2H), 1.45–1.31 (m, 4H), 0.91 (t, J = 6.9 Hz, 3H); MS (CI, CH_4) m/e 365 (MH^+). Anal. ($C_{23}H_{25}ClN_2$·HCl) C, H, N.

7-Chloro-1-(1-methylnaphthyl)-4-(*n*-pentylimino)-1,4-dihydroquinoline Hydrochloride (7b). Compound **7b** was synthesized according to general method B: mp 298–300 °C; ^{1}H NMR (DMSO) δ 8.91 (d, J = 7.5 Hz, 1H), 8.61 (d, J = 8.2 Hz, 1H), 8.15 (d, J = 7.5 Hz, 1H), 8.07 (s, 1H), 8.01 (d, J = 8.2 Hz, 1H), 7.91 (d, J = 8.3 Hz, 1H), 7.81 (d, J = 8.3 Hz, 1H), 7.65 (m, 2H), 7.38 (m, 1H), 7.05 (d, J = 8.2 Hz, 1H), 6.72 (d, J = 7.4 Hz, 1H), 6.35 (bs, 2H), 3.55 (d of t, J = 7.7 Hz, 2H), 1.76 (m, 2H), 1.43 (m, 4H), 0.95 (t, J = 7.2 Hz, 3H); MS (CI, CH_4) m/e 389 (MH^+). Anal. ($C_{25}H_{25}N_2Cl$·HCl) C, H, N.

7-Chloro-1-(2-methylnaphthyl)-4-(*n*-pentylimino)-1,4-dihydroquinoline Hydrobromide (7c). Compound **7c** was synthesized according to general method B: mp 277–279 °C; ^{1}H NMR (DMSO) δ 8.88 (d, J = 7.5 Hz, 1H), 8.65 (d, J = 9.1 Hz, 1H), 8.15 (s, 1H), 7.77–7.97 (m, 5H), 7.51 (m, 2H), 7.41 (d, J = 8.6 Hz, 1H), 7.10 (d, J = 87.6 Hz, 1H), 6.05 (bs, 2H), 3.55 (d of t, J = 7.7 Hz, 2H), 1.76 (m, 2H), 1.43 (m, 4H), 0.95 (t, J = 7.2 Hz, 3H); MS (CI, CH_4) m/e 389 (MH^+). Anal. ($C_{25}H_{25}N_2Cl$·HBr) C, H, N.

4-(*n*-Hexylimino)-8-phenyl-1-methyl-1,4-dihydroquinoline Methanesulfonate (8). Compound **8** was synthesized according to general method B: mp 239–240 °C; ^{1}H NMR ($CDCl_3$) δ 9.60 (m, 1H), 8.80 (m, 1H), 8.26 (d, J = 7.3 Hz, 1H), 7.64 (d, J = 5.1 Hz, 2H), 7.43 (m, 3H), 7.24 (m, 3H), 6.60 (d, J = 7.4 Hz, 1H), 3.51 (d of t, J = 6.2 Hz, 2H), 2.84 (s, 3H), 2.04 (s, 3H), 1.77 (m, 2H), 1.43 (m, 2H), 1.28 (m, 4H), 0.85 (t, J = 7.2 Hz, 3H); MS (CI, CH_4) m/e 319 (MH^+); HRMS (LSIMS) $C_{22}H_{27}N_2$ (MH^+) requires 319.21742, found 319.21745; Δ = 0.07 ppm. Anal. ($C_{23}H_{30}N_2O_3S$·H_2O) C, H, N.

4-(*n*-Butylimino)-7-chloro-1-(3-phenylpropyl)-1,4-dihydroquinoline Hydrochloride (9). Compound **9** was synthesized according to general method B: mp 195–197 °C; ^{1}H NMR (DMSO) δ 10.05 (bs, 1H), 8.89 (d, J = 9.2 Hz, 1H), 8.63 (d, J = 7.6 Hz, 1H), 8.16 (d, J = 1.9 Hz, 1H), 7.77 (dd, J = 9.0, 1.8 Hz, 2H), 7.27–7.13 (m, 5H), 6.88 (d, J = 7.6 Hz, 1H), 4.58 (t, J = 7.5 Hz, 2H), 3.51 (q, J = 69 Hz, 2H), 2.70 (t, J = 7.5 Hz, 2H), 2.12–2.07 (m, 2H), 1.68–1.60 (m, 2H), 1.42–1.35 (m, 2H), 0.92 (t, J = 7.3 Hz, 3H); MS (CI, CH_4) m/e 353 (MH^+); HRMS (LSIMS) $C_{22}H_{25}ClN_2$ (MH^+) requires 353.17845, found 353.17710; Δ = −3.83 ppm.

1-Benzyl-7-chloro-4-(*n*-hexyloxy)-1,4-dihydroquinoline Bromide (10). Compound **10** was synthesized according to general method B: mp 136–138 °C; ^{1}H NMR (DMSO) δ 9.57 (d, J = 7.2 Hz, 1H), 8.48–8.45 (m, 2H), 7.96 (dd, J = 9.0, 1.7 Hz, 1H), 7.80 (d, J = 7.2 Hz, 1H), 7.43–7.31 (m, 4H), 6.19 (s, 2H), 4.62 (t, J = 6.3 Hz, 2H), 1.98–1.90 (m, 2H), 1.55–1.48 (m, 2H), 1.40–1.30 (m, 4H), 0.89 (t, J = 7.0 Hz, 3H); MS (FAB, NBA) m/e 354 (MH^+). Anal. ($C_{22}H_{25}ClNO$·Br) C, H, N.

4-Phenacyl-1-(hexyloxy)naphthalene (15). 1-Naphthol (10.0 g, 0.069 mol), 1-iodohexane (22.0 g, 0.104 mol), and K_2CO_3 were dissolved in DMF (70 mL) and heated to 60 °C under N_2 for 18 h. The reaction mixture was cooled to room temperature, diluted with Et_2O (500 mL), washed successively with H_2O (3 × 500 mL) and NaOH (1 N, 500 mL), dried (Na_2SO_4), and evaporated to dryness. The sample was taken up in hexane (250 mL), washed with H_2O (3 × 400 mL), dried (Na_2SO_4), and evaporated to dryness. The product was purified by flash filtration through silica gel, eluting with hexane, to give 1-(hexyloxy)naphthalene as a yellow oil (11.3 g). The 1-(hexyloxy)naphthalene (1.0 g, 4.38 mmol) was then dissolved in nitrobenzene (5 mL) and added dropwise to a mixture of benzoyl chloride (0.616 g, 4.38 mmol) and $AlCl_3$ (0.584, 4.38 mmol), in nitrobenzene (5 mL), at 0 °C, under N_2. After 18 h at room temperature, the nitrobenzene was removed by steam distillation and the residue was diluted with EtOAc (50 mL), washed with H_2O (50 mL), dried (Na_2SO_4), and concentrated in vacuo. The resulting yellow oil was purified by flash filtration on silica gel with hexane/EtOAc (9:1) as eluent, to give **15** as a yellow oil (263 mg, 18%): ^{1}H NMR ($CDCl_3$) δ 8.36–8.35 (m, 2H), 7.81 (d, J = 8.0 Hz, 1H), 7.58–7.44 (m, 4H), 7.55–7.43 (m, 2H), 6.76 (d, J = 8.0 Hz, 1H), 4.19 (t, J = 7.5 Hz, 2H), 1.97–1.93 (m, 2H), 1.69–1.52 (m, 2H), 1.40–1.37 (m, 4H), 0.94 (t, J = 7.0 Hz, 3H); MS (EI) m/e 332. Anal. ($C_{23}H_{24}O_2$) C, H.

4-(1-Hydroxy-1-phenylmethyl)-1-(hexyloxy)naphthalene (16). To a chilled (0 °C) methanol solution (30 mL) of naphthalene **15** (1.0 g, 3.0 mmol) was slowly added 10% $NaBH_4/Al_2O_3$ (3.4 g, 9.0 mmol) with stirring. The ice bath was removed and the mixtured allowed to return to room temperature overnight. The mixture was filtered, dried (Na_2SO_4), and evaporated. The product was purified by filtration through silica gel with hexane/EtOAc (9:1) as eluent to give **16** (0.74 g, 72%) as a waxy yellow solid: mp 54–55 °C; ^{1}H NMR ($CDCl_3$) δ 8.37–8.32 (m, 1H), 8.06–8.00 (m, 1H), 7.49–7.40 (m, 5H), 7.36–7.25 (m, 3H), 6.77 (d, J = 8.0 Hz, 1H), 6.47 (s, 1H), 4.14 (t, J = 6.4 Hz, 2H), 2.26 (bs, 1H), 1.98–1.89 (m, 2H), 1.63–1.53 (m, 2H), 1.45–1.38 (m, 4H), 0.93 (t, J = 7.0 Hz, 3H); MS (CI, CH_4) m/e 334 (MH^+). Anal. ($C_{23}H_{26}O_2$) C, H.

4-Benzyl-1-(hexyloxy)naphthalene (11). The naphthalene **16** (0.24 g, 0.70 mmol) dissolved in CF_3CO_2H (7 mL) was added dropwise, simultaneously with $NaBH_4$ (0.27 g, 7.0 mmol), to a vigorously stirred solution of CF_3CO_2H under N_2 at 0 °C. After complete addition, stirring was continued for 5 min. The CF_3CO_2H was evaporated and the resulting solid quenched with H_2O (25 mL) and saturated $NaHCO_3$ (25 mL). The mixture was extracted with EtOAc (3 × 20 mL); the combined extracts were washed with H_2O (50 mL), dried (Na_2SO_4), and concentrated in vacuo. The product was p±rified by filtration through silica gel with hexane followed by hexane/EtOAc (9:1) to give a yellow oil. Additional drying at 0.1 Torr gave **11** (0.75 g, 34%) as a waxy yellow solid: mp 66–68 °C; ^{1}H NMR ($CDCl_3$) δ 8.20–8.17 (m, 1H), 7.96–7.93 (m, 1H), 7.50–7.43 (m, 1H), 7.30–7.11 (m, 6H), 6.90 (d, J = 7.8 Hz, 1H), 4.32 (s, 2H), 4.12 (t, J = 6.5 Hz, 2H), 1.90–1.79 (m, 2H), 1.60–1.43 (m, 2H), 1.38–1.29 (m, 4H), 0.88 (t, J = 7.0 Hz, 3H); MS (CI, CH_4) m/e 319 (MH^+); HRMS (LSIMS) $C_{23}H_{26}O$ (M^+) requires 318.19837, found 318.19783; Δ = −1.70 ppm.

[^{125}I]Charybdotoxin Binding Assay. [^{125}I]Charybdotoxin was obtained from New England Nuclear. Charybdotoxin was obtained from Peptides International. All other reagents were obtained from Sigma Chemical Co. (St. Louis, MO). Jurkat T lymphocytes were grown in suspension in RPMI 1640 tissue culture medium supplemented with 10% bovine fetal calf serum, 2 mM glutamine, and 0.5 mg/mL gentamicin. Cells were routinely seeded at 0.25×10^6 cells/mL in 150 cm^2 falcon tissue culture flasks and incubated at 37 °C in an atmosphere of 95% O_2/5% CO_2.

[^{125}I]ChTX binding to T lymphocytes was based on the methodology of Deutsch et al.[7] Jurkat T lymphocytes were resuspended in incubation buffer (NaCl, 5 mM; KCl, 5 mM; sucrose, 320 mM; HEPES, 10 mM; glucose, 6 mM, pH 8.4 with trizma base) to give a cell concentration of 1×10^7 cells/mL. Cells (2×10^6/tube) were incubated in poly(propylene) 1 mL deep well plates in the presence of 25 pM [^{125}I]ChTX test agents for 20 min at 22 °C. Nonspecific binding was determined in the presence of 10 nM ChTX. At the end of the incubation period, samples were filtered through GF/C glass fiber filters that had been presoaked on 0.6% poly(ethylenimine), using a Tomtec Mach II harvester (Orange, CT). Samples were washed with 2×1 mL of ice cold wash buffer (NaCl, 200 mM; 20, mM Hepes, pH 8.0 with trizma base). Radioactivity bound to filters was measured in a β-plate liquid scintillation counter (Wallac, Gaithersburg, MD). Initial experiments showed that the characteristics of [^{125}I]ChTX binding under these conditions are identical to that described by Deutsch et al.[7] for isolated peripheral T lymphocytes and are indicative of [^{125}I]ChTX binding to n-type K^+ channels.

[^{3}H]Dofetilide Binding Assay. [^{3}H]Dofetilide binding to guinea pig cardiac myocytes was determined as previously described.[11]

Electrophysiology. Solutions and recording systems used were previously described.[15] Whole cell recording modes were established using standard techniques.[18] Membrane potential was held at −80 mV, and n-type K^+ channel current was measured by giving 150 ms voltage steps to 30 mV once every minute. Cells were allowed to stabilize for >6 min in all cases. Stability was obtained when currents elicited by more than three consecutive voltage steps were superimposable. Pharmacological inhibition was assessed by obtaining peak outward current values during the voltage step described above. Test agent was then continuously perfused at a fixed concentration for 6 min. Following this period, current amplitudes were measured every minute with the same test pulse as above. Amplitudes for the first pulse following the 6 min exposure period were measured to assess block of closed channels. Amplitudes from the subsequent pulse were used to assess open channel block. Amplitudes in the presence of drug were compared to predrug data to determine the percentage of current blocked. A single drug concentration was tested on each cell (i.e., cumulative dose−response curves were not obtained). Initial experiments showed that the physiological and pharmacological profiles of the currents measured under these conditions are consistent with those previously reported to be n-type K^+ channels.[1,3]

Computational Methods. All compounds were modeled using SYBYL 6.04[19] software. The structures were minimized using default conditions. The lowest energy conformation was determined using the CEDD[20] conformational searching technique followed by minimization. All bonds attached to the 1-position and the heteroatom in the 4-position of the ring system were rotated 360° in 10° increments. A low-energy conformation was defined as any conformation within 3 kcal of the lowest energy conformation. The volume maps were created within SYBYL 6.04 by superimposing the quinoline ring system of the low-energy conformations for all the members of the respective sets.

Supplementary Material Available: Elemental analysis data (1 page). Ordering information is given on any current masthead page.

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JM950010O