Discovery of Novel Non-Peptide CCR1 Receptor Antagonists

Howard P. Ng,[†,1] Karen May,[*,†] John G. Bauman,[†] Ameen Ghannam,[†] Imadul Islam,[†] Meina Liang,[†] Richard Horuk,[‡] Joseph Hesselgesser,[‡] R. Michael Snider,[†] H. Daniel Perez,[‡] and Michael M. Morrissey[†]

Departments of Discovery Research and Immunology, Berlex Biosciences, 15049 San Pablo Avenue, P.O. Box 4099, Richmond, California 94804-0099

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Ligands for the CCR1 receptor (MIP-1α and RANTES) have been implicated in a number of chronic inflammatory diseases, most notably multiple sclerosis and rheumatoid arthritis. Because these ligands share a common receptor, CCR1, we sought to discover antagonists for this receptor as an approach to treating these disorders. A novel series of 4-hydroxypiperidines has been discovered by high throughput screening (HTS) which potently inhibits the binding of MIP-1α and RANTES to the recombinant human CCR1 chemokine receptor. The structure−activity relationships of various segments of this template are described as the initial HTS lead **1** was optimized synthetically to the highly potent receptor antagonist **6s**. This compound has been shown to have at least 200-fold selectivity for inhibition of CCR1 over other human 7-TM receptors, including other chemokine receptors. In addition, data obtained from in vitro functional assays demonstrate the functional antagonism of compound **6s** and structurally related analogues against the CCR1 receptor in a concentration dependent manner. The discovery and optimization of potent and selective CCR1 receptor antagonists represented by compound **6s** potentially represent a novel approach to the treatment of chronic inflammatory diseases.

Introduction

Chemokines are chemotactic cytokines that belong to a large family of chemoattractant molecules involved in the directed migration of immune cells.[2] The physiological role of chemokines in the immune process is to elicit mobilization of immune cells against pathogenic organisms by direct recruitment and activation.

Chemokines are small proteins that are divided into two main classes, based on the position of the first two cysteines. They are the C-X-C and C-C families of chemokines[2] (two smaller branches of this family have been described, the C^3 and CX_3C^4 subfamilies). To date over 40 chemokines have been identified and characterized. The chemokines all act by direct interaction with cell surface receptors, known as chemokine receptors.

Chemokine receptors are members of the superfamily of seven transmembrane domain proteins that signal across the cellular membrane through coupled G proteins.[5] At last count these G-protein coupled receptors (GPCR) number well over 600. Fifteen chemokine receptors have thus far been cloned and identified: five C-X-C, eight C-C, one CX_3C, and one C receptor. They produce their biological effect through a cascade of intracellular events that begin with binding of the chemokines to their respective receptors and lead ultimately to activation and/or directed migration of the cell.

Since the first chemokines were discovered and their receptors cloned, they have been implicated in a number of very important disease states including multiple sclerosis,[6−8] rheumatoid arthritis,[9−11] allograft rejection,[12,13] atherosclerosis,[14] asthma,[15−17] and AIDS.[18] A number of pharmaceutical companies have reported or disclosed significant efforts in discovering chemokine receptor antagonists.[19−24]

Our interest in chemokines was an extension of our interest in the treatment of multiple sclerosis (MS). Multiple sclerosis is a debilitating disease affecting over 200 000 people in the United States. It primarily afflicts young adults, temporarily paralyzing parts of their body with remission of disease usually followed by relapses of greater severity and duration resulting ultimately in permanent disability in many cases. One of the hallmarks of MS disease is the infiltration and activation of peripheral blood leukocytes into the brain. This along with central nervous system immune cell activation lead to active demyelination of the central nervous system. To date no approved orally available small molecule therapy exists for the treatment of MS. However, the possibility of utilizing antagonism of binding of a chemokine to its receptor as a novel treatment for inflammatory diseases such as MS was recently demonstrated.[7]

Studies by Karpus[7] show strong evidence for a role of several chemokines in a mouse experimental autoimmune encephalomyelitis (EAE) model of MS. More specifically, they reported that a CC chemokine macrophage inflammatory protein (MIP-1α) played a key role in the development and progression of rodent EAE disease. Investigators treated the mice with antibodies to MIP-1α and found that both the initial and relapsing paralytic aspects of the disease were significantly reduced. This reduction of clinical disease correlated

* To whom correspondence should be addressed: Berlex Biosciences, Department of Discovery Research, 15049 San Pablo Ave., Richmond, CA 94806. E-mail: karen_may@berlex.com.
† Department of Discovery Research.
‡ Department of Immunology.

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with reduction in infiltration of mononuclear cells into the central nervous system. These results led the authors to conclude that MIP-1α plays an important role in this T cell mediated disease model. In addition Godiska[8] demonstrated that the lesions and spinal fluid isolated from mice from a similar mouse model of EAE contained unregulated levels of mRNA for a number of chemokines, including MIP-1α. The major receptor for MIP-1α, CCR1, therefore represented a potential target for the discovery of a new therapy for MS.

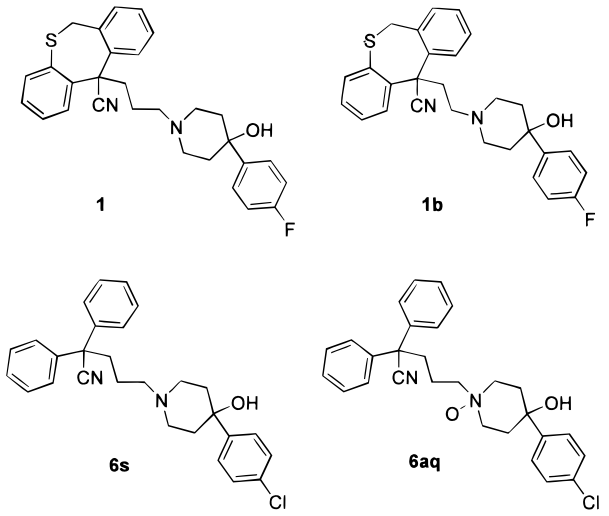
In addition to MS, CCR1 has been implicated in other chronic inflammatory disorders, most notably rheumatoid arthritis (RA). Rheumatoid arthritis is characterized by memory T lymphocyte and monocyte infiltration into synovial tissues, resulting in inflammation, discomfort, and ultimately loss of joint function. Examination of synovial fibroblasts of RA patients revealed that the mRNA and RANTES protein were upregulated.[9,10] In contrast, osteoarthritis tissue does not express RANTES mRNA.[10] Additional evidence implicating RANTES in the pathophysiology of RA was recently reported in a study utilizing anti-RANTES antibodies in an in vivo animal study.[11] In this study, rats from an adjuvant-induced arthritis (AIA) model of RA were treated with antibodies to RANTES, resulting in greatly reduced clinical scores for RA relative to untreated animals. This and other studies suggest that RANTES could play a major role in attracting T cells and monocytes into joints during onset of AIA, thereby making the main receptor for RANTES, CCR1, a candidate for involvement in the inflammatory and destructive processes culminating in rheumatoid arthritis.

Herein, we present the discovery of a family of novel compounds by high throughput screening and their subsequent optimization into potent and selective antagonists of the human CCR1 receptor. The functional antagonism and functional selectivity of compound **6s** has been demonstrated through a number of in vitro experiments and previously reported by this group.[25]

Lead Discovery of Non-Peptide CCR1 Receptor Antagonists by HTS

A stable cell line with the human CCR1 receptor[26] overexpressed in human embryonic kidney (HEK-293) cells was developed and employed in a competitive receptor binding assay with ^{125}I-labeled human MIP-1α.[25] This assay was used to screen approximately 200000 members of the Berlex HTS compound library.[27] The initial screen produced over 250 hits that inhibited binding by at least 50% at an initial compound concentration of 5 μM. These compounds were re-screened in a dose-responsive fashion and their structures verified by analysis (MS, ^{1}H and ^{13}C NMR) and in some cases re-synthesis. Of these compounds, eight showed K_i values under 500 nM and several appeared to belong to the same structural family. The most potent compound from this series is represented by compound **1** (Chart 1) which potently inhibited the binding of ^{125}I-labeled human MIP-1α to the CCR1 receptor (K_i = 44 nM). It is interesting to note that the structurally related analogue **1b** (Chart 1), which differs by one methylene in the chain connecting the dibenzothiepine moiety to the 4-hydroxy-4-phenylpiperidine, was also in the sample collection employed for the CCR1 HTS assay. Compound **1b** was not identified as a potent inhibitor of MIP-1α binding to the CCR1 receptor in the initial screen. Subsequent re-testing of **1b** confirmed that this compound does not significantly inhibit binding of MIP-1α to the CCR1 receptor at 5.0 μM.

Chart 1

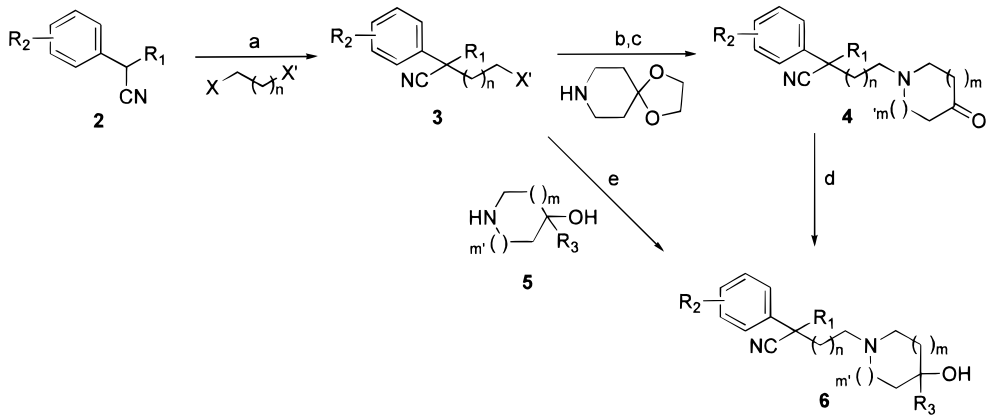


Chemistry

Analogues of compound **1** were synthesized as outlined in Scheme 1. Aryl acetonitriles **2** were deprotonated and reacted with dihaloalkanes to give intermediate **3**. Compound **3** was either reacted with commercially available piperidine **5** or with the protected piperidone (1,4-dioxa-8-azaspiro[4,5]decane) followed by deprotection to piperidone **4** before reacting with Grignard or lithium reagents to give **6s** through **6ao**. In some cases R_1 and R_2 were components of a tricyclic moiety leading to analogues of **6a** through **6r**. [28,29] Analogues of **2** having an ester in place of the cyano group were treated as outlined in Scheme 1 to afford ester analogues **6w** and **6ck**. Ester functional groups were hydrolyzed to the corresponding carboxylic acid **6x** using LiOH while benzyl groups were removed using catalytic hydrogenation with palladium on carbon. The ester **6w** was reduced using LAH to give the alcohol **6y**. Sulfoxides and amine *N*-oxides were prepared by peroxide and/or peracid oxidation of the sulfide or tertiary amine (**6p**,**q**,**aq**). Compounds with quaternary ammonium salts of the piperidine group (**6ar**, **6at**, **6as**) were generated by reaction of **6s**, **6au**, and **8** with methyl iodide, respectively.

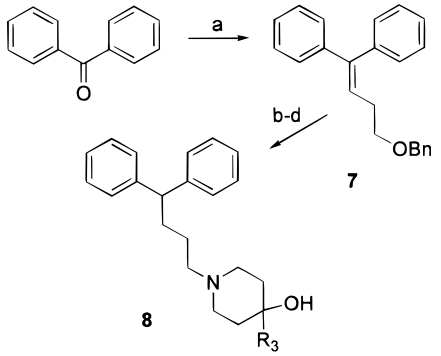
The preparation of the des-nitrile analogue, compound **8**, was accomplished as shown in Scheme 2. Addition of 3-benzyloxypropyltriphenylphosphonium bromide to benzophenone gave intermediate **7**. Subsequent reduction by catalytic hydrogenation both reduced the olefinic group as well as removed the benzyl group. The resulting alcohol was converted to a mesylate and displaced with 4-(4-chlorophenyl)-4-hydroxy piperidine (**5s**) to give **8**. Complete experimental procedures for all compounds described in this paper can be found in the Experimental Section.

Scheme 1[a]



[a] Conditions: (a) NaH, DMF or LDA, THF, 18 h; (b) DMF, 18 h; (c) 6 N HCl, MeOH, 24 h; (d) R_3Li or R_3MgX, THF, 4 h; (e) Cs_2CO_3, DMF, 16 h.

Scheme 2[a]



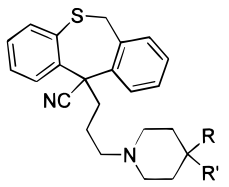
[a] Conditions: (a) $BnO(CH_3)_3PPh_3^+Br^-$, KO^{t}Bu, THF, 70 °C, 6 h; (b) 10% Pd/C, H_2 (50 psi), EtOH; (c) MsCl, Et_3N, CH_2Cl_2; (d) **5**, Cs_2CO_3, DMF, 16 h.

Results and Discussion

Compounds were screened for their potential to competitively inhibit binding of radiolabeled human MIP-1α in cells expressing the CCR1 receptor in a dose range, and these are reported as K_i values.[25] Beginning with compound **1** as the starting point for lead optimization, substituents at the 4-position of the piperidine ring were examined (Table 1). In general, the 4-phenyl group appeared to be very sensitive to changes in regiochemistry, sterics, and electronics. For example, removal of the 4-halogen substituent (**6a**) resulted in a 10-fold loss of potency. While a methyl group introduced at the 2-position (**6b**) did not significantly change the binding affinity of this template, the 3-trifluoromethyl derivative (**6d**) was significantly less potent. This loss of potency can be partially restored by inclusion of a chloro in the 4-position of the phenyl ring (**6d**). Within the group of 4-halophenylpiperidinyl compounds, the 4-bromo (**6e**) and 4-chloro (**6f**) proved to be superior to the 4-fluoro derivative (**1**).

Alternative pharmacophores were explored to potentially replace the 4-hydroxy-4-phenylpiperidine motif because this same structural motif is contained in haloperidol and raised neurotoxicity concerns.[30] Removal of the hydroxyl group proved detrimental to potency (**6g**), and replacement with nitrile or methyl carbonyl (**6h**, **6i**) was not successful for maintaining binding affinity. Alternatives to the 4-phenyl group were also investigated. While the benzyl (**6j**) was only 2-fold less potent than the phenyl analogue, more significant changes such as diphenylhydroxy methyl were not tolerated (**6k**).

Table 1. SAR of the 4-Phenylpiperidine in the Dibenzothiepine Series

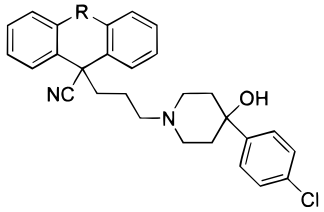


cmpd	R	R′	K_i (nM)[a,b]
1	OH	4-F-Ph	44 ± 19 [c]
6a	OH	Ph	203 ± 28
6b	OH	2-Me-Ph	164 ± 13
6c	OH	3-CF_3-Ph	623 ± 132
6d	OH	3-CF_3, 4-Cl-Ph	224 ± 41
6e	OH	4-Br-Ph	22 ± 3
6f	OH	4-Cl-Ph	24 ± 10
6g	H	Ph	19% @ 3 μM ± 5%
6h	CN	Ph	0% @ 3 μM
6i	$C(O)CH_3$	Ph	0% @ 3 μM
6j	OH	CH_2Ph	405 ± 111
6k	H	$C(Ph)_2OH$	0% @ 3 μM

[a] K_i values are derived from competitive binding on CCR1 with ^{125}I MIP-1α (ref 25). [b] Values represent the mean of $n = 2$ (except where noted). [c] This value is the average of $n = 71 \pm$ SD. Compound **1** was used as a standard for all K_i determinations.

Variations to the tricyclic moiety were also pursued to lower the possibility of cross-reactivity with related CNS receptors.[31] As shown in Table 2, the compound with a single oxygen bridge (**6l**) was less potent than any of the representative two-atom bridged tricyclics (**6m**−**r**). Among the two-atom-bridged compounds tested, the thiomethylene (**6f**) proved optimal. Oxidation of this compound produced two diastereomeric sulfoxides, **6p** and **6q**, which were separated and tested; both exhibited comparable potency to **6f**. Interestingly, substitution of the thiomethylene bridge with isosteric replacements including oxomethylene (**6o**), ethyl (**6m**), ethylene (**6n**), or amide functionality (**6r**) provided potent compounds as well, indicating that a high degree of flexibility is allowed in this part of the template. Finally, the acyclic analogue **6s**, which contains no bridging functionality, demonstrated a similar level of inhibitory potency. With this discovery, the focus of the optimization effort was

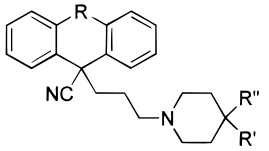
Table 2. SAR of Tricyclic Bridge Atoms



cmpd	R	K_i (nM)[a,b]
6f	CH_2S	24 ± 10
6l	O	234 ± 41
6m	CH_2CH_2	45 ± 1
6n	CH═CH	56 ± 3
6o	CH_2O	34 ± 2
6p	$CH_2S(O)$	54 ± 5
6q	$CH_2S(O)$	71 ± 3
6r	$N(CH_3)C(O)$	32 ± 10
6s	H, H	52 ± 3

[a] K_i values are derived from competitive binding on CCR1 with ^{125}I MIP-1α (ref 25). [b] Values represent the mean of $n = 2$ (except where noted).

Table 3. Comparison of SAR Trends between the Dibenzothiepine Series and the Benzyhydryl Series



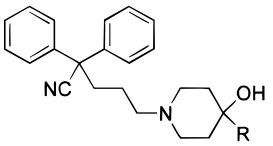
cmpd	R′	R″	R	K_i (nM)[a,b]
1	OH	4-F-Ph	CH_2S	44 ± 19[c]
6a	OH	Ph	CH_2S	203 ± 28
6e	OH	4-Br-Ph	CH_2S	22 ± 3
6f	OH	4-Cl-Ph	CH_2S	24 ± 10
6s	OH	4-Cl-Ph	H, H	52 ± 5[d]
6t	OH	4-F-Ph	H, H	136 ± 13
6u	OH	4-Br-Ph	H, H	29 ± 9
6v	OH	Ph	H, H	1188 ± 252
6w	CO_2Me	Ph	H, H	62% @ 10 μM ± 8%
6x	CO_2H	Ph	H, H	15% @ 10 μM ± 4%
6y	CH_2OH	Ph	H, H	7500[e]
6z	OH	H	H, H	20% @ 10 μM ± 8%

[a] K_i values are derived from competitive binding on CCR1 with ^{125}I MIP-1α (ref 25). [b] Values represent the average of $n = 2$ (except where noted). [c] This value is the average of $n = 71 \pm$ SD. Compound **1** was used as a standard for all K_i determinations.[d] $n = 4 \pm$ SD. [e] $n = 1$.

shifted to the benzylhydryl compound represented by **6s** (Chart 1) because it was equipotent with the dibenzothiepine series represented by **1** (Chart 1) but was both potentially less cross-reactive with CNS receptors and more accessible synthetically.

Analogues were first synthesized to confirm that the SAR discovered in the dibenzothiepine series was applicable to this new series. As shown in Table 3, the same SAR trends for substitution on the 4-phenylpiperidine moiety were observed. Halogen substitution at the 4-position of the phenyl ring proved superior to compounds without this substituent, as demonstrated by the higher binding affinities of **6t**, **6s**, **6u**, **1**, **6f**, and **6e** as compared to **6v** and **6a**. Once again, the chloro (**6s**, **6f**) and bromo (**6u**, **6e**) compounds were more potent than the fluoro (**6t**, **1**). Finally, substitution for the hydroxyl group (**6w**, **6x**, **6y**) or removal of one phenyl group (**6z**) resulted in a large decrease in binding

Table 4. SAR of Substitution at the 4-Position on 4-Hydroxypiperidine



cmpd	R	K_i (nM)[a,b]
6s	4-Cl-Ph	52 ± 5[c]
6v	Ph	1188 ± 252
6aa	2-thienyl	1020 ± 10
6ab	2-pyridyl	906 ± 86
6ac	2-naphthyl	715 ± 28
6ad	3-pyridyl	206 ± 19
6ae	4-diphenyl	30% @ 10 μM ± 4%
6af	*n*-butyl	4010 ± 610
6ag	4-I-Ph	233 ± 22
6ah	3-Cl-Ph	385 ± 23
6ai	3,5-Cl, Cl-Ph	1330 ± 30
6aj	4-MeO-Ph	347 ± 64
6ak	4-CN-Ph	92 ± 30
6al	4-CF_3-Ph	153 ± 8
6am	4-NH_2-Ph	33% @ 10 μM ± 3%
6an	4-Me_2N-Ph	1155 ± 105

[a] K_i values are derived from competitive binding on CCR1 with ^{125}I MIP-1α (ref 25). [b] Values represent the mean of $n = 2$ (except where noted). [c] $n = 4 \pm$ SD.

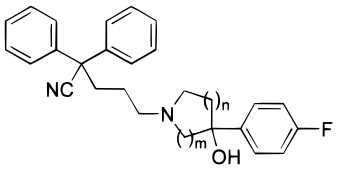
affinity. From these results it was concluded that the SAR discovered in the tricyclic series could be applied to the related acyclic series, and other variations to the piperidine ring were investigated.

More extensive modification of the 4-phenyl group of the piperidine was explored to complement the data in Tables 1 and 3. Other aryl groups (Table 4) appeared to be tolerated in this position, including 2-thienyl (**6aa**), 2-pyridyl (**6ab**), and 2-naphthyl (**6ac**), all of which showed similar binding affinities to the unsubstituted phenyl derivative (**6v**). Interestingly the 3-pyridyl group (**6ad**) was actually more potent than the unsubstituted phenyl group (**6v**). The 4-biphenyl (**6ae**) and the *n*-butyl (**6af**) groups lowered potency.

In addition to the previously discussed 4-halophenyl compounds **6s**, **6t**, and **6u** (Table 3), other analogues of the 4-halophenyl group were investigated. The halogen series was completed with synthesis of the 4-iodophenyl analogue (**6ag**), which showed lower potency. Moving the halogen to the 3-position of the phenyl ring (**6ah**) or bis-halogenation (**6ai**) decreased binding affinities as well, compared to the 4-chlorophenyl (**6s**). Interestingly, when a methoxy group was substituted in the 4-position of the phenyl ring (**6aj**) lower potency than the corresponding 4-chloro compound (**6s**) but higher potency than the unsubstituted ring (**6v**) was observed. This result is somewhat surprising because the methoxy group possesses similar steric and electronic properties to the chloro group. Substitution at the 4-position of the phenyl ring with electron-withdrawing groups such as nitrile (**6ak**) or trifluoromethyl (**6al**) also lowered potency. An amino group in this position (**6am**) eliminated most of the binding affinity, while its dimethyl analogue (**6an**) was less detrimental. Based on these data further optimization of the template was performed with 4-chlorophenyl analogues.

Further synthetic efforts focused on addressing the effect of changing the regiochemistry of substituents on

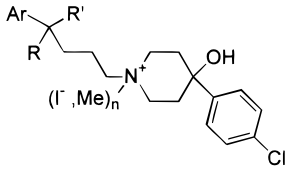
Table 5. SAR of Heterocyclic Ring Position and Size



cmpd	*m*	*n*	K_i (nM)[a,b]
6t	2	1	136 ± 13
6ao	1	2	0% @ 10 μM
6ap	1	1	22% @ 10 μM ± 4%

[a] K_i values are derived from competitive binding on CCR1 with ^{125}I MIP-1α (ref 25). [b] Values represent the mean of $n = 2$ (except where noted).

Table 6. Activity of Quaternary Ammonium Salts vs Nonquaternized Compounds



cmpd	Ar	R	R′	*n*	K_i (nM)[a,b]
6s	Ph	Ph	CN	0	52 ± 5[c]
6ar	Ph	Ph	CN	1	8 ± 2
6au	2-naphthyl	H	CN	0	206 ± 28
6at	2-naphthyl	H	CN	1	22 ± 1
8	Ph	Ph	H	0	116 ± 41
6as	Ph	Ph	H	1	6 ± 1

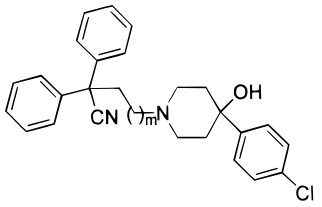
[a] K_i values are derived from competitive binding on CCR1 with ^{125}I MIP-1α (ref 25). [b] Values represent the mean of $n = 2$ (except where noted). [c] $n = 4 \pm$ SD.

the piperidine ring, the size of the ring, and the substitution of the piperidine ring nitrogen itself. Moving the hydroxy and phenyl substituents to the 3-position on the piperidine (**6ao**) or replacement of the piperidine with a pyrrolidine (**6ap**) drastically reduced the binding affinity (Table 5). On the basis of these results, substitution on the 4-position of the piperidine ring (**6t**) appeared optimal. In addition, oxidation of the piperidine nitrogen to the corresponding *N*-oxide (Chart 1, **6aq**) produced a 7-fold loss of potency. This indicated the need for a basic nitrogen at this position.

An interesting trend was observed for the binding affinities of quaternary ammonium salts derived from this series. Although interest in these salts as clinical candidates was limited due to known pharmacokinetic liabilities (i.e., poor oral absorption and rapid clearance in vivo), a surprisingly large number of simple quaternary ammonium salts were found to demonstrate significant inhibitory activity in the original CCR1 HTS screen of our compound library. Consequently, the quaternary ammonium salts (**6ar**, **6at**, **6as**) were synthesized and demonstrated a 5- to 20-fold increase in binding inhibition in comparison to the parent piperidine derivatives **6s**, **6au**, and **8** (Table 6).

The effect of changing the length of the linker between the piperidine and benzhydryl groups was also explored systematically. As shown in Table 7, compound **6s**, with a three-atom linker, was much more potent than compound **6av**, which contains a two-atom linker, and slightly more potent than compounds **6aw** and **6ax**, which contain four and five atoms, respectively. This

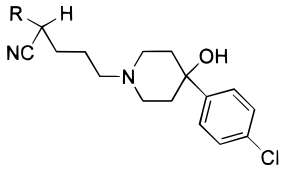
Table 7. SAR of Alkyl Linker Chain Length



cmpd	*m*	K_i (nM)[a,b]
6av	1	4060 ± 800
6s	2	52 ± 5[c]
6aw	3	78 ± 4
6ax	4	99 ± 5

[a] K_i values are derived from competitive binding on CCR1 with ^{125}I MIP-1α (ref 25). [b] Values represent the mean of $n = 2$ (except where noted). [c] $n = 4 \pm$ SD.

Table 8. SAR of Benzhydryl Replacements



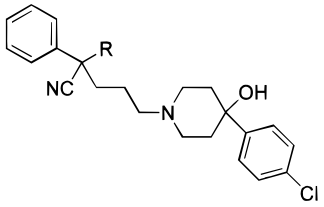
cmpd	R	K_i (nM)[a,b]
6ay	Ph	54 ± 5
6az	3-Br, 4-MeO-Ph	74 ± 8
6bb	2-CN-Ph	98 ± 35
6bc	2,3,4,5,6-F_5-Ph	330 ± 85
6bd	3-OH-Ph	156 ± 50
6be	2-F-Ph	371 ± 41
6bf	4-F-Ph	533 ± 84
6bg	2-Br-Ph	366 ± 37
6bh	4-Cl-Ph	463 ± 90
6bi	2-Cl-Ph	306 ± 93
6bj	2-CF_3-Ph	25% @ 10 μM ± 7%
6bk	2-BnO-Ph	46% @ 10 μM ± 4%
6bl	3-BnO-Ph	0% @ 10 μM
6bm	4-BnO-Ph	3430 ± 80
6bn	3-PhO-Ph	908 ± 89
6bo	2-HO-Ph	790 ± 220
6bp	4-HO-Ph	975 ± 35
6bq	3,4,5-$(MeO)_3$-Ph	497 ± 66
6br	3-NO_2-Ph	510 ± 13
6bs	4-MeO-Ph	359 ± 38
6bt	4-EtO-Ph	1425 ± 65
6bu	2-thienyl	128 ± 36
6bv	3-thienyl	236 ± 53
6au	2-naphthyl	206 ± 29
6bw	1-naphthyl	51% @ 10 μM ± 4%
6bx	2-pyridyl	584 ± 163
6by	3-pyridyl	1000 ± 20
6bz	2-pyrrole(N-Me)	429 ± 231

[a] K_i values are derived from competitive binding on CCR1 with ^{125}I MIP-1α (ref 25). [b] Values represent the mean of $n = 2$ (except where noted).

suggests that the optimal length of the linker is three atoms and corresponds nicely with the results obtained from initial high throughput screening.

With the SAR on the linker and the piperidine established, focus was shifted to chemical optimization of the benzhydryl nitrile moiety to further modify the physicochemical properties of this structural series. As shown in Table 8, removal of one of the two phenyl rings did not result in a drop in potency (**6ay** vs **6s**). Only specific substitutions on the single phenyl ring were tolerated as in **6az**, **6bb**. However the majority of the substituents examined on this ring had a negative effect

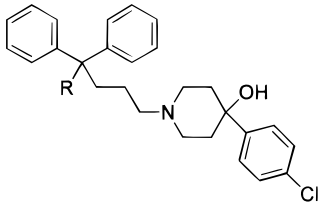
Table 9. SAR of Replacement of Phenyl Ring



cmpd	R	K_i (nM)[a,b]
6s	Ph	52 ± 5[c]
6cc	CO_2Et	33 ± 3
6cd	CO_2Me	53 ± 8
6ce	$CH_2CH_2CH_2Cl$	51 ± 7
6cf	Me	460 ± 60
6cg	Et	93 ± 8
6ch	CH_2CN	125 ± 17
6ci	*c*-hex	400 ± 48
6cj	*n*-piperidyl	130 ± 14
6ay	H	54 ± 5

[a] K_i values are derived from competitive binding on CCR1 with ^{125}I MIP-1α (ref 25). [b] Values represent the mean of $n = 2$ (except where noted). [c] $n = 4 \pm$ SD.

Table 10. SAR of Substitution of Nitrile in Benzhydryl Series



cmpd	R	K_i (nM)[a,b]
6s	CN	52 ± 5[c]
8	H	116 ± 42
6ck	CO_2Me	406 ± 51
6cl	CH_2NH_2	244 ± 81[d]
6cm	$CH_2NHC(O)NH$(4-piperidyl)	9 ± 8[d,e]

[a] K_i values are derived from competitive binding on CCR1 with ^{125}I MIP-1α (ref 25). [b] Values represent the mean of $n = 2$ (except where noted). [c] $n = 4 \pm$ SD. [d] $n = 3 \pm$ SD. [e] The K_i value in our assay is significantly different from the value (5 μM) reported by Takeda for MIP-1α binding (refs 20, 32).

Table 11. Comparison of Binding Assay Data with Functional Assay Data

cmpd	inhibition of ^{125}I-MIP-1α binding to human CCR1 (K_i, nM)[a,b]	inhibition of MIP-1α-induced intracellular calcium mobilization (IC_{50}, μM)[c]
1	44 ± 19[d]	3.0 ± 1.0
6f	24 ± 10	7.0 ± 0.8
6m	45 ± 1	7.7 ± 0.4
6n	56 ± 3	5.3 ± 0.4
6o	34 ± 2	7.7 ± 0.4
6r	32 ± 10	3.3 ± 1.8
6s	52 ± 5[e]	2.5 ± 0.7
6ak	92 ± 30	10.9 ± 1.6
6av	8 ± 2	0.4 ± 0.1
6at	22 ± 1	0.7 ± 0.2
6as	6 ± 1	1.8 ± 0.3
6au	206 ± 28	7.1 ± 0.4
6aw	78 ± 4	1.6 ± 0.6
6ax	89 ± 14	5.6 ± 0.4
6az	74 ± 8	1.9 ± 0.9
6bb	98 ± 35	5.8 ± 1.8
6bd	156 ± 50	9.0 ± 2.8
6bu	128 ± 36	8.4 ± 0.3
6bv	236 ± 53	9.8 ± 3.2
6cc	33 ± 3	7.0 ± 4.2
6ce	51 ± 7	8.3 ± 1.5
6ch	125 ± 17	3.2 ± 1.6
6cj	130 ± 14	4.8 ± 1.1
8	116 ± 41	8.7 ± 1.8

[a] K_i values are derived from competitive binding on CCR1 with ^{125}I MIP-1α (ref 25). [b] Values represent the mean of $n = 2$ (except where noted). [c] Values represent the mean of $n = 2 \pm$ SD. [d] This value is the average of $n = 71 \pm$ SD. Compound **1** was used as a standard for all K_i determinations.

on the binding inhibition of this template (**6bc**, **6bd**, **6be**, **6bf**, **6bg**, **6bh**, **6bi**, **6bj**, **6bk**−**bq**, **6br**, **6bo**, **6bp**). Steric factors also appear to play a role in determining potency as demonstrated by the significant difference in binding affinity between the 4-methoxy (**6bs**) and the 4-ethoxy (**6bt**) analogues.

Additional analogues were synthesized in which both phenyl groups were removed and replaced with other aromatic ring systems. As shown in Table 6, only three analogues, the 2-thienyl (**6bu**), 3-thienyl (**6bv**), and the 2-naphthyl (**6au**) showed comparable binding affinities to the monophenyl compound (**6ay**). Compound **6bu**, with a 2-thienyl group, even had slightly increased potency. The fact that the 2-naphthyl isomer (**6au**) was much better tolerated than the 1-naphthyl (**6bw**) indicates that this portion of the template interacts fairly closely with the CCR1 receptor. The lower binding affinities of the corresponding pyridyl (**6bx** and **6by**) and pyrrole (**6bz**) analogues were also noted.

Replacement of one phenyl ring of the benzhydryl system of **6s** with other groups was also investigated (Table 9). The most effective replacements were found to be the ethyl carboxylate (**6cc**), methyl carboxylate (**6cd**), and followed by the 3-chloropropyl group (**6ce**). Other alkyl groups (**6cf**, **6cg**, and **6ch**) as well as carbocyclic (**6ci**) and heterocyclic groups (represented by **6cj**) were clearly inferior to the phenyl group (**6s**), but several were better than **6ay**, which lacks a substituent at this position.

The SAR of the nitrile functionality was investigated last (Table 10). Removal of the cyano group (**8**) or replacement of the nitrile with an ester (**6ck**) or aminomethyl functionality (**6cl**) resulted in significant loss of potency compared to the cyano-containing **6s**. However, researchers at Takeda Chemical Industries, Ltd., recently disclosed in a published patent application[19] that further elaboration at this position was fruitful. These workers disclosed that compound **6cm**, which is a direct derivative of the aminomethyl derivative **6cl**, inhibited binding of RANTES to CCR1 with an IC_{50} of 6 nM and binding of MIP-1α to CCR1 with an IC_{50} of 5 μM. This compound was synthesized, screened in our binding assay, and confirmed as a highly potent inhibitor of MIP-1α binding to the CCR1 receptor ($K_i = 9$ nM).[32]

Finally, a selected group of analogues described in this paper were characterized in a MIP-1α-induced intracellular calcium mobilization assay using FLIPR (fluorometric imaging plate reader) technology with HEK293 cells that overexpress the human CCR1 receptor (Table 11). These analogues demonstrate functional antagonism against the human CCR1 receptor with an IC_{50} in the sub-micromolar to mid-micromolar range and show no significant agonist activity at the highest concentration tested (5 μM). SAR results from this assay suggest that a positively charged nitrogen plays an important role in functional potency, since the analogues which contain a quaternary ammonium moiety

(**6ar**, **6as**, **6at**) demonstrate the best binding affinity and functional activity.

Conclusion

We have discovered a series of potent and selective non-peptide antagonists of the human CCR1 receptor through HTS and subsequent chemical optimization studies. Upon evaluation of numerous analogues in this series, we have determined that the piperidine and linker subunits are optimal as discovered in the HTS lead **1**. In addition, SAR efforts on the benzhydryl and nitrile components of this template resulted in an increase in inhibitory activity in MIP-1α binding to human CCR1. These compounds have been shown to be both potent and selective in competitive binding assays[25] and demonstrated functional activity in a MIP-1α calcium mobilization assay. These antagonists may represent a novel approach to the treatment of diseases mediated by the CCR1 chemokine receptor.

The most interesting SAR result is the significant increased activity of the quaternary ammonium compounds (**6ar**−**at**) over their nonquaternized analogues (**6s**, **6au**, and **8**). This finding suggests that the binding site favors positively charged species. Although these compounds do not meet the criteria for a drug candidate (i.e., poor oral absorption and rapid clearance in vivo), they do help elucidate the binding site requirements and can influence inhibitor design. Further efforts directed toward additional improvements of potency and selectivity as well as in vivo data will be presented in future publications.

Experimental Section

NMR spectra were obtained on a 300 MHz Varian XL-300 or a 400 MHz Varian UP-400 as noted and were consistent with the assigned structures. Electrospray mass spectra were recorded on a SCIEX API III plus using flow injection. Elemental analyses were performed by Robertson Microlit Laboratories, Madison, NJ, and results were within 0.4% of calculated values except where noted. Silica gel for flash chromatography was E. Merck grade (230−400 mesh). Preparative HPLC was run on a C18 Dynamax column (Ca. #80-240-C8), eluting with a linear gradient of 80:20 to 30:70 (0.1% aqueous TFA:0.1% TFA in MeCN) over 30 min.

11-[3-[4-(4-Fluorophenyl)-4-hydroxy-1-piperidinyl]propyl]-6,11-dihydrodibenzo[*b,e*]thiepin-11-carbonitrile (1). The preparation of **1** was previously described by Sindelar et al.[28] ^{1}H NMR (300 MHz, $CDCl_3$) δ (TMS) 1.75 (m, 5H), 2.14 (m, 2H), 2.51 (m, 4H), 2.80 (m, 3H), 2.08 (m, 1H), 4.55 (d, 1H), 7.04 (t, 2H), 7.16 (m, 3H), 7.28 (m, 3H), 7.46 (m, 2H), 7.84 (m, 2H). Anal. ($C_{29}H_{29}FN_2OS\cdot0.53SiO_2$) C, H, N.

6,11-Dihydro-11-[3-(4-hydroxy-4-phenyl-1-piperidinyl)-propyl]dibenzo[*b,e*]thiepin-11-carbonitrile (6a) was prepared in the same manner as **1** starting from 11-(3-bromopropyl)-6,11-dihydrodibenzo[*b,e*]thiepin-11-carbonitrile (**3a**) (200 mg, 0.56 mmol) and 4-hydroxy-4-phenyl piperidine to afford **6a** as a colorless oil (190 mg, 72% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ (TMS) 1.75 (m, 6H), 2.15 (m, 2H), 2.45 (m, 3H), 2.78 (m, 2H), 3.10 (m, 1H), 4.10 (d, 1H), 4.58 (d, 1H), 7.15 (m, 4H), 7.30 (m, 5H), 7.50 (d, 2H), 7.85 (m, 2H). Anal. ($C_{29}H_{30}N_2OS\cdot0.2SiO_2$) C, H, N.

11-[3-[4-Hydroxy-4-(2-methylphenyl)-1-piperidinyl]propyl]-6,11-dihydrodibenzo[*b,e*]thiepin-11-carbonitrile (6b) was prepared in the same manner as **1** starting with **3a** (358 mg, 1.0 mmol) and 4-(2-methylphenyl)-4-hydroxy piperidine to afford **6b** after flash chromatography as a colorless oil (430 mg, 90% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ (TMS) 1.70 (m, 4H), 1.95 (m, 2H), 2.20 (m, 2H), 2.50 (m, 3H), 2.65 (s, 3H), 2.80 (m, 2H), 3.15 (m, 1H), 4.10 (d, 1H), 4.60 (d, 1H), 7.15 (m, 6H), 7.25 (m, 3H), 7.40 (m, 1H), 7.88 (m, 2H). Anal. ($C_{30}H_{32}N_2OS\cdot0.7SiO_2$) C, H, N.

11-[3-[4-Hydroxy-4-(3-trifluoromethylphenyl)-1-piperidinyl]propyl]-6,11-dihydrodibenzo[*b,e*]thiepin-11-carbonitrile (6c) was prepared in the same manner as **1** starting with **3a** (200 mg, 0.56 mmol) and 4-(3-trifluoromethylphenyl)-4-hydroxy piperidine to afford **6c** after flash chromatography as a colorless oil (180 mg, 61% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ (TMS) 1.70 (m, 6H), 2.20 (m, 2H), 2.50 (m, 5H), 2.80 (m, 3H), 3.05 (m, 1H), 4.15 (d, 1H), 4.55 (d, 1H), 7.15 (m, 3H), 7.30 (m, 3H), 7.50 (m, 2H), 7.70 (m, 1H), 7.90 (m, 3H). Anal. ($C_{30}H_{29}F_3N_2OS\cdot0.36SiO_2$) C, H, N.

11-[3-[4-Hydroxy-4-(3-trifluoromethyl-4-chlorophenyl)-1-piperidinyl]propyl]-6,11-dihydrodibenzo[*b,e*]thiepin-11-carbonitrile, hydrochloride (6d) was prepared in the same manner as **1** starting with **3a** (200 mg, 0.56 mmol) and 4-(3-trifluoromethyl-4-chlorophenyl)-4-hydroxy piperidine to afford **6d** after flash chromatography as a colorless oil (180 mg, 57% yield). The hydrochloride salt was prepared by dissolving the freebase in ethyl acetate and adding an excess of 1 N HCl in ether. The product precipitated as a solid and was isolated by filtration. ^{1}H NMR (300 MHz, $CDCl_3$) δ (TMS) 1.20 (m, 2H), 1.90 (m, 4H), 2.60 (m, 4H), 3.15 (m, 4H), 4.10 (d, 1H), 4.50 (d, 1H), 5.20 (s, 2H), 7.20 (m, 8H), 7.70 (m, 2H), 7.90 (s, 1H). Anal. ($C_{30}H_{38}ClF_3N_2OS\cdot HCl\cdot0.25H_2O$) C, H, N.

11-[3-[4-(4-Bromophenyl)-4-hydroxy-1-piperidinyl]propyl]-6,11-dihydrodibenzo[*b,e*]thiepin-11-carbonitrile (6e) was prepared in the same manner as **1** starting with **3a** (200 mg, 0.56 mmol) and 4-(4-bromophenyl)-4-hydroxy piperidine to afford **6e** as a white solid (220 mg, 73% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ (TMS) 1.70 (m, 6H), 2.10 (m, 2H), 2.45 (m, 3H), 2.75 (m, 2H), 3.08 (m, 1H), 4.10 (d, 1H), 4.55 (d, 1H), 7.13 (m, 4H), 7.25 (m, 2H), 7.38 (d, 2H), 7.43 (d, 2H), 7.88 (m, 2H). Anal. ($C_{29}H_{29}BrN_2OS\cdot0.35SiO_2$) C, H, N.

11-[3-[4-(4-Chlorophenyl)-4-hydroxy-1-piperidinyl]propyl]-6,11-dihydrodibenzo[*b,e*]thiepin-11-carbonitrile (6f) was prepared in the same manner as **1** starting with **3a** and 4-(4-chlorophenyl)-4-hydroxy piperidine to afford **6f** as a yellow solid (184 mg, 6.3% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ (TMS) 1.75 (m, 6H), 2.05 (m, 2H), 2.45 (m, 3H), 2.79 (m, 3H), 3.10 (m, 1H), 4.10 (d, 1H), 4.55 (d, 1H), 7.15 (m, 2H), 7.28 (m, 6H), 7.42 (d, 2H), 7.85 (m, 2H). Anal. ($C_{29}H_{29}ClN_2SO\cdot1.0H_2O$) C, H, N.

6,11-Dihydro-11-[3-(4-phenyl-1-piperidinyl)propyl]-dibenzo[*b,e*]thiepin-11-carbonitrile (6g) was prepared in the same manner as **1** starting with **3a** (200 mg, 0.56 mmol) and 4-phenylpiperidine to afford **6g** as a colorless oil (184 mg, 73% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ (TMS) 1.80 (m, 6H), 2.10 (m, 2H), 2.50 (m, 3H), 2.80 (m, 1H), 3.10 (m, 3H), 4.10 (d, 1H), 4.60 (d, 1H), 7.25 (m, 11H), 7.85 (m, 2H). Anal. ($C_{29}H_{30}N_2S\cdot0.5SiO_2$) C, H, N.

1-[3-(11-Cyano-6,11-dihydrodibenzo[*b,e*]thiepin-11-yl)-propyl]-4-phenyl-4-piperidinecarbonitrile, hydrochloride salt (6h) was prepared in the same manner as **6d** starting with **3a** (200 mg, 0.56 mmol) and 4-cyano-4-phenylpiperidine to afford **6h** as a yellow solid (86 mg, 30% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ (TMS) 1.80 (m, 3H), 2.10 (m, 3H), 3.05 (m, 6H), 3.70 (m, 1H), 4.2 (m, 1H), 4.65 (m, 1H), 7.25 (m, 9H), 7.60 (d, 2H), 7.88 (m, 2H). Anal. ($C_{30}H_{29}N_3S\cdot1.0HCl\cdot0.75H_2O$) C, H, N.

11-[3-(4-Acetyl-4-phenyl-1-piperidinyl)propyl]-6,11-dihydrodibenzo[*b,e*]thiepin-11-carbonitrile, hydrochloride salt (6i) was prepared in the same manner as **6d** starting with **3a** (200 mg, 0.56 mmol) and 4-acetyl-4-phenylpiperidine to afford **6i** as a white solid (156 mg, 52% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ (TMS) 1.95 (s, 3H), 2.20 (m, 2H), 2.70 (m, 5H), 3.05 (m, 3H), 3.50 (m, 2H), 4.10 (d, 1H), 4.55 (d, 1H), 7.30 (m, 11H), 7.80 (m, 2H). Anal. ($C_{31}H_{32}N_2OS\cdot1.6HCl$) C, H, N.

6,11-Dihydro-11-[3-[4-hydroxy-4-(phenylmethyl)-1-piperidinyl]propyl]dibenzo[*b,e*]thiepin-11-carbonitrile (6j) was prepared in the same manner as **1** starting with **3a** (200 mg, 0.56 mmol) and 4-hydroxy-4-phenylmethyl piperidine to afford **6j** as a white foam (200 mg, 43% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ (TMS) 1.50 (d, 2H), 1.75 (m, 4H), 2.25 (m, 2H),

2.45 (m, 2H), 2.70 (m, 2H), 2.80 (s, 3H), 3.05 (m, 1H), 4.10 (d, 1H), 4.55 (d, 1H), 7.25 (m, 11H), 7.85 (m, 2H). Anal. ($C_{30}H_{32}N_2OS \cdot 0.16SiO_2$) C, H, N.

6,11-Dihydro-11-[3-[4-(hydroxydiphenylmethyl)-1-piperidinyl]propyl]dibenzo[*b,e*]thiepin-11-carbonitrile (6k) was prepared in the same manner as **1** starting with **3a** (200 mg, 0.56 mmol) and 4-hydroxydiphenylmethyl piperidine to afford **6k** as a yellow solid (25 mg, 4% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ (TMS) 1.60 (m, 2H), 1.95 (m, 2H), 2.15 (m, 3H), 2.60 (m, 6H), 2.85 (m, 1H), 3.20 (m, 2H), 4.05 (m, 1H), 4.60 (m, 1H), 7.30 (m, 16H), 7.88 (m, 2H). Anal. ($C_{36}H_{36}N_2OS \cdot 1.0H_2O \cdot 1.3HCl$) C, H, N.

9-[3-[4-(4-Chlorophenyl)-4-hydroxy-1-piperidinyl]propyl]-9*H*-xanthene-9-carbonitrile (6l) was prepared from commercially available 9-cyanoxanthene in a manner similar to **1**. ^{1}H NMR (300 MHz, $CDCl_3$) δ (TMS) 1.41 (m, 2H), 2.65 (m, 4H), 2.05 (m, 4H), 2.3 (m, 4H), 2.40 (d, 2H), 7.30 (m, 10H), 7.63 (d, 2H). Anal. ($C_{28}H_{27}ClN_2O_2 \cdot 0.33H_2O$) C, H, N.

5-[3-[4-(4-Chlorophenyl)-4-hydroxy-1-piperidinyl]propyl]-10,11-dihydro-5*H*-dibenzo[*a,d*]cycloheptene-5-carbonitrile (6m) was prepared from commercially available dibenzosuberyl chloride in a manner similar to **1**. ^{1}H NMR (300 MHz, $CDCl_3$) δ (TMS) 1.48 (m, 2H), 1.68 (d, 2H), 2.05 (m, 2H), 2.35 (m, 4H), 2.60 (m, 4H), 3.05 (m, 2H), 3.44 (m, 2H), 7.23 (m, 8H), 7.42 (d, 2H), 8.00 (d, 2H). Anal. ($C_{30}H_{31}ClN_2O \cdot 0.25H_2O$) C, H, N.

5-[3-[4-(4-Chlorophenyl)-4-hydroxy-1-piperidinyl]propyl]-5*H*-dibenzo[*a,d*]cycloheptene-5-carbonitrile (6n) was prepared from commercially available dibenzosuberenol in a manner similar to **1** to give **6n** in a 34% yield from dibenzosubernol. ^{1}H NMR (300 MHz, $CDCl_3$) δ (TMS) 1.38 (m, 2H), 1.65 (m, 2H), 2.04 (m, 2H), 2.35 (m, 6H), 2.64 (d, 2H), 7.00 (s, 2H), 7.38 (m, 10H), 8.04 (d, 2H). Anal. ($C_{30}H_{29}ClN_2O \cdot 0.85H_2O$) C, H, N.

11-[3-[4-(4-Chlorophenyl)-4-hydroxy-1-piperidinyl]propyl]-6,11-dihydrodibenzo[*b,e*]oxepin-11-carbonitrile (6o) was prepared in a manner similar to **1** starting with phenol (1.23 g, 13.1 mmol) to afford **6o** as white solid (130 mg, 2.1% yield over eight steps). ^{1}H NMR (300 MHz, $CDCl_3$) δ (TMS) 1.41 (m, 1H), 1.70 (m, 3H), 2.10 (m, 2H), 2.39 (m, 5H), 2.68 (m, 2H), 2.85 (m, 1H), 5.05 (d, 1H), 5.42 (d, 1H), 7.2 (m, 10H), 7.82 (d, 1H), 8.0 (d, 1H). Anal. ($C_{29}H_{29}ClN_2O_2 \cdot 0.3H_2O$) C, H, N.

11-[3-[4-(4-Chlorophenyl)-4-hydroxy-1-piperidinyl]propyl]-6,11-dihydrodibenzo[*b,e*]thiepin-11-carbonitrile,5-Oxide (6p and 6q). Compound **3a** (186 mg, 0.52 mmol) was dissolved in 30% aqueous hydrogen peroxide (0.43 mL) and acetic acid (3 mL) and stirred for 1 h. The reaction was then poured into water, neutralized with sodium carbonate, and extracted with ethyl acetate. The ethyl acetate was washed with brine, dried over magnesium sulfate, and concentrated in vacuo to give the sulfoxide as an oil (190 mg, 97%). NMR of the oil showed two diastereomers in a 4:6 ratio. ^{1}H NMR (300 MHz, $CDCl_3$) δ (TMS) 2.04 (m, 2H), 2.44 (m, 0.8H), 2.65 (m, 1.2H), 2.84 (m, 2H), 3.3 (m, 4H), 4.28 (m, 1.6H), 4.75 (m, 2.4H), 7.20 (d, 2H), 7.41 (m, 4H), 7.62 (m, 4H), 8.08 (m, 4H). This mixture was treated as described for the preparation of **6f** to afford **6p** and **6q** as a mixture of diastereomers. The diastereomers were separated by flash chromatography on silica gel, eluting with a gradient of 0−5% methanol in ethyl acetate. The first diasteromer to elute (**6p**) was converted to the hydrochloride salt as described for **6d**. The compound was isolated as a white solid (60 mg, 20% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ (TMS) 1.95 (m, 7H), 2.55 (m, 1H), 3.20 (m, 6H), 4.22 (d, 1H), 4.51 (d, 1H), 7.37 (m, 7H), 7.62 (m, 2H), 8.15 (m, 3H). Anal. ($C_{29}H_{29}ClN_2O_2S \cdot 1.6HCl$) C, H, N.

The second diasteromer to elute (**6q**) was isolated also as a colorless oil (90 mg, 35% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ (TMS) 1.78 (m, 5H), 2.04 (m, 2H), 2.38 (m, 4H), 7.65 (m, 4H), 4.65 (d, 1H), 4.84 (d, 1H), 7.35 (m, 7H), 7.56 (m, 2H), 7.98 (m, 3H). Anal. ($C_{29}H_{29}ClN_2SO_2 \cdot 1.36Si_2O$) C, H, N.

11-[3-[4-(4-Chlorophenyl)-4-hydroxy-1-piperidinyl]propyl]-6,11-dihydro-5-methyl-6-oxo-5*H*-dibenzo[*b,e*]azepine-11-carbonitrile (6r). The preparation of the corresponding **2** is described by Akermann et al.[29] Compound **6r** is prepared from **2r** (150 mg, 0.60 mmol) in a manner similar to **1** to afford **6r** after HPLC purification as a white powder (101 mg, 25% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ (TMS) 1.73 (m, 3H), 2.06 (m, 3H), 2.36 (m, 1H), 2.82 (m, 1H), 3.20 (m, 2H), 3.32 (m, 2H), 3.52 (s, 3H), 3.58 (m, 2H), 7.32 (m, 2H), 7.48 (m, 12H), 9.11 (br s, 0.5H), 9.40 (br s, 0.5H). Anal. ($C_{30}H_{30}ClN_3O_2 \cdot 0.55TFA \cdot 0.25C_4H_8O_2 \cdot 0.5H_2O$) C, H, Cl, F, N.

A General Procedure for the Preparation of Target Compounds 6 Using Commercially Available 4-Hydroxy-4-phenylpiperidines. 4-(4-Chlorophenyl)-4-hydroxy-α,α-diphenyl-1-piperidinepentanenitrile (6s). Diphenylacetonitrile (10 g, 52 mmol) and 1,3-dibromopropane (52 g, 259 mmol) were dissolved in anhydrous DMF (125 mL) under nitrogen. The solution was chilled to 5 °C in an ice bath, and sodium hydride (2.48 g, 62.1 mmol, 60% dispersion in oil) was added portionwise. The ice bath was removed, and the mixture was stirred for 18 h at ambient temperature. The reaction was quenched by pouring into water (1 L). The product was extracted into ethyl acetate (3 × 250 mL). The combined organic extract was washed with brine, dried over magnesium sulfate, filtered, and concentrated in vacuo to give an oil. Flash chromatography on silica gel with a gradient of 0−25% methylene chloride in hexanes afforded **3s**, α-(3-bromopropyl)-α-phenyl-benzeneacetonitrile, as a white solid (8.7 g, 51%). ^{1}H NMR (300 MHz, $CDCl_3$) δ (TMS) 2.00 (m, 2H), 2.58 (m, 2H), 3.42 (t, 2H), 7.38 (m, 10H). To a solution of **3s** (200 mg, 0.6 mmol) in anhydrous DMF (6 mL) under nitrogen was added 4-(4-chlorophenyl)-4-hydroxypiperidine, **5s**, (142 mg, 0.67 mmol) and cesium carbonate (300 mg, 0.9 mmol). The resulting solution was stirred for 16 h at ambient temperature. The reaction was quenched with water (30 mL) and extracted with ethyl acetate (3 × 30 mL). Combined organic extract was washed with water and brine, dried over magnesium sulfate, filtered, and evaporated in vacuo to an oil (130 mg). The oil was purified by flash chromatography on silica gel eluting with 0−100% ethyl acetate in methylene chloride to give **6s** as a colorless foam (200 mg, 73%). ^{1}H NMR (300 MHz, $CDCl_3$) δ (TMS) 1.70 (m, 4H), 1.82 (br s, 1H) 2.16 (m, 2H), 2.40 (t, 2H), 2.45 (t, 4H), 2.72 (d, 2H), 7.38 (m, 14H). Anal. ($C_{28}H_{29}ClN_2O \cdot 0.3H_2O$) C, H, N.

A General Procedure for the Preparation of Target Compounds 6 Using Commercially Available Grignard and Lithium Reagents. 4-(4-Fluorophenyl)-4-hydroxy-α,α-diphenyl-1-piperidinepentanenitrile (6t). To a solution of **3s** (6.8 g, 21.6 mmol) in anhydrous DMF (50 mL) under nitrogen was added 1,4-dioxa-8-azaspiro[4.5]decane (7.8 g, 54.5 mmol). The resulting solution was stirred for 18 h at ambient temperature. The reaction was quenched with water and extracted into ethyl acetate. The organic extract was washed with brine, dried over magnesium sulfate, and filtered, and the solvent was removed in vacuo to yield a oil (9.6 g). The oil was dissolved in methanol (35 mL), and 6 N HCl (190 mL) was added. The solution was stirred at ambient temperature. After 24 h the reaction was made basic with aqueous KOH and extracted with ethyl acetate. The organic extract was washed with brine, dried over magnesium sulfate, filtered, and evaporated in vacuo to a solid which was purified by flash chromatography on silica gel eluting with 0−20% ethyl acetate in methylene chloride to give 4-oxo-α,α-diphenyl-1-piperidinepentanenitrile (**4t**) as a white solid (6.9 g, 96%). ^{1}H NMR (300 MHz, $CDCl_3$) δ (TMS) 1.75 (m, 4H), 2.47 (m, 6H), 2.66 (t, 4H), 7.36 (m, 10H). To a 0 °C solution of **4t** (125 mg, 0.36 mmol) in anhydrous THF (3 mL) under nitrogen was added 4-fluorophenyl magnesium bromide (0.22 mL, 2.0 M in diethyl ether, 0.43 mmol). The ice bath was removed, and the solution was stirred for 4 h at ambient temperature. The solution was cooled to 0 °C and quenched with water, acidified with 1 N HCl (1 mL), and then neutralized with 1 N KOH. The solution was buffered to pH 9 with saturated sodium bicarbonate and the product extracted into ethyl acetate (2 × 20 mL). The combined organic extracts were washed with water and brine, dried over magnesium sulfate, filtered, and evaporated in vacuo to a crude product (150 mg). The crude product was purified by

flash chromatography on silica gel eluting with 0−100% ethyl acetate in methylene chloride to give **6t** as a colorless foam (110 mg, 71%). ^{1}H NMR (300 MHz, $CDCl_3$) δ (TMS) 1.78 (d, 4H), 2.05 (m, 2H), 2.48 (m, 6H), 2.75 (d, 2H), 7.04 (t, 2H), 7.42 (m, 12H). Anal. ($C_{28}H_{29}FN_2O\cdot0.2H_2O$) C, H, N.

4-(4-Bromophenyl)-4-hydroxy-α,α-diphenyl-1-piperidinepentanenitrile (6u) was prepared in a manner similar to **6s** starting with **3s** (600 mg, 1.85 mmol) and 4-(4-bromophenyl)-4-hydroxy piperidine to afford **6u** as a white solid (320 mg, 35% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ (TMS) 1.68 (m, 6H), 2.08 (m, 2H), 2.42 (m, 5H), 2.70 (m, 2H), 7.38 (m, 14H). Anal. ($C_{28}H_{29}BrN_2O$) C, H, N.

4-Hydroxy-α,α- 4-triphenyl-1-piperidinepentanenitrile, hydrochloride salt (6v) was prepared in a manner similar to **6s** starting with **3s** (600 mg, 1.85 mmol) and 4-hydroxy-4-phenyl piperidine. The hydrochloride salt was prepared as described in **6d**. The solid was isolated by filtration and dried in vacuo to afford **6v** as a white solid (300 mg, 36% yield). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.78 (d, 2H), 2.32 (t, 2H), 2.52 (s, 2H), 2.64 (t, 2H), 3.20 (m, 3H), 3.35 (m, 3H), 5.48 (s, 1H), 7.42 (m, 15H) 10.17 (br s, 1H). Anal. ($C_{28}H_{30}N_2O\cdot$ 1.0HCl) C, H, N.

1-(4-Cyano-4,4-diphenylbutyl)-4-phenyl-4-piperidinecarboxylic acid, methyl ester, hydrochloride salt (6w) was prepared in a manner similar to **6s** starting with **3s** (250 mg, 0.76 mmol) and 4-phenyl-4-piperidinecarboxylic acid, methyl ester to afford **6w** as a white solid (220 mg, 47% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ (TMS) 1.70 (m, 2H), 2.00 (m, 2H), 2.70 (m, 8H), 3.10 (m, 1H), 3.50 (m, 2H), 3.75, (s, 3H), 7.40 (m, 15H). Anal. ($C_{30}H_{32}N_2O_2\cdot1.3HCl$) C, H, N.

1-(4-Cyano-4,4-diphenylbutyl)-4-phenyl-4-piperidinecarboxylic acid (6x) was prepared by hydrolysis of **6w** with lithium hydroxide followed purification by preparative HPLC to afford **6x** as a white solid (80 mg, 74% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ (TMS) 1.20 (br s, 1H), 1.75 (m, 3H), 1.90 (m, 2H), 2.25 (m, 1H), 2.50 (m, 2H), 2.70 (m, 2H), 2.90 (m, 1H), 3.10 (m, 2H), 355 (d, 1H), 7.35 (m, 15H), 9.20 (br s, 1H). Anal. ($C_{29}H_{30}N_2O_2\cdot1.15TFA$) C, H, N.

4-(Hydroxymethyl)-α,α-4-triphenyl-1-piperidinepentanenitrile (6y) was prepared from **6w** by reduction with LAH to **6y** as a colorless oil (72 mg, 34% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ (TMS) 1.20 (t, 3H), 1.90 (m, 2H), 2.00 (s, 1H), 2.50 (m, 10H), 2.85 (br s, 1H), 3.50 (m, 4H), 4.10 (q, 2H), 7.35 (m, 15H), 11.40 (br s, 1H). Anal. ($C_{29}H_{32}N_2O\cdot0.9HCl\cdot1.0C_4H_8O_2$) C, H, N.

4-Hydroxy-α,α-diphenyl-1-piperidinepentanenitrile (6z) was prepared in a manner similar to **6s** starting with **3s** (200 mg, 0.61 mmol) and 4-hydroxy piperidine to afford **6s** as a white solid (160 mg, 75% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ (TMS) 1.58 (m, 4H), 1.83 (m, 2H), 2.05 (t, 2H), 2.40 (m, 4H), 2.68 (d, 2H), 3.65 (m, 1H), 7.36 (m, 10H). Anal. ($C_{22}H_{26}N_2O\cdot$ $0.4H_2O$) C, H, N.

4-Hydroxy-α,α-diphenyl-4-(2-thienyl)-1-piperidinepentanenitrile (6aa) was prepared in a manner similar to **6t** starting with **4t** (450 mg, 1.35 mmol) and commercially available 2-thienyllithium (1 M in THF) to afford **6aa** after chromatography as a foam (235 mg, 42% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ (TMS) 1.66 (m, 2H), 1.72 (d, 3H), 2.14 (t, 2H), 2.44 (m, 5H), 2.64 (d, 2H), 6.98 (m, 2H), 7.21 (d, 1H), 7.36 (m, 10H). Anal. ($C_{26}H_{28}N_2OS\cdot0.5H_2O$) C, H, N, S.

4-Hydroxy-α,α-diphenyl-4-(2-pyridinyl)-1-piperidinepentanenitrile (6ab) was prepared in a manner similar to **6aj** starting with **4t** (500 mg, 1.5 mmol) and 2-bromopyridine to afford **6ab**, after chromatography as a white solid (288 mg, 47% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ (TMS) 1.64 (m, 4H), 2.02 (t, 2H), 2.45 (m, 6H), 2.78 (d, 2H), 5.24 (s, 1H), 7.2 (m, 1H), 7.34 (m, 11H), 7.71 (t, 1H), 8.52 (d, 1H). Anal. ($C_{27}H_{29}N_3O$) C, H, N.

4-Hydroxy-4-(2-naphthalenyl)-α,α-diphenyl-1-piperidinepentanenitrile, hydrochloride salt (6ac) was prepared in a manner similar to **6aj** starting with **4t** (400 mg, 1.2 mmol), 2-bromonaphthalene, and *n*-butyllithium (1.6 M in hexanes) to afford **6ac** after salt formation as a solid (65 mg, 11% yield). ^{1}H NMR (300 MHz, $CDCl_3$/TFA) δ (TMS) 2.00 (br s, 2H), 2.16 (d, 2H), 2.60 (m, 4H), 3.32 (m, 4H), 3.64 (d, 2H), 3.90 (s, 1H), 7.38 (m, 10H), 7.52, (m, 3H), 7.86 (m, 4H). Anal. ($C_{32}H_{32}N_2O\cdot1.0HCl\cdot0.5H_2O$) C, H, N.

4-Hydroxy-α,α-diphenyl-4-(3-pyridinyl)-1-piperidinepentanenitrile (6ad) was prepared in a manner similar to **6aj** starting with **4t** (500 mg, 1.5 mmol) and 3-bromopyridine to afford **6ad** as a foam (120 mg, 19% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ (TMS) 1.70 (m, 4H), 2.10 (m, 2H), 2.44 (m, 6H), 2.76 (d, 2H), 7.34 (m, 10H), 7.1 (m, 1H), 8.46 (m, 1H), 8.74 (d, 1H). Anal. ($C_{27}H_{29}N_3O\cdot0.1C_4H_8O_2\cdot0.5H_2O$) C, H, N.

4-[1,1′-Biphenyl]-4-yl-4-hydroxy-α,α-diphenyl-1-piperidinepentanenitrile (6ae) was prepared in a manner similar to **6aj** starting with **4t** (500 mg, 1.5 mmol) and 4-bromobiphenyl to afford **6ae** as a solid (50 mg, 8% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ (TMS) 1.70 (m, 4H), 1.96 (m, 2H), 2.46 (m, 6H), 2.76 (m, 2H), 7.38 (m, 13H), 7.59 (m, 6H). ES/MS (M + H) = 487. Anal. ($C_{34}H_{34}N_2O$) C, H, N.

4-Butyl-4-hydroxy-α,α-diphenyl-1-piperidinepentanenitrile, hydrochloride salt (6af) was prepared in a manner similar to **6t** starting with **4t** (500 mg, 1.5 mmol) and butyllithium (2.5 M in hexanes) to afford **6af**, after chromatography and salt formation, as a solid (94 mg, 16% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ (TMS) 0.85 (t, 3H), 1.28 (m, 4H), 1.60 (m, 4H) 1.95 (m, 2H), 2.36 (m, 2H), 2.64 (t, 2H), 3.00 (m, 4H), 3.17 (m, 2H), 7.36 (m, 10H), 11.50 (br s, 1H). Anal. ($C_{26}H_{34}N_2O\cdot1.4HCl\cdot1.0H_2O$) C, H, N.

4-Hydroxy-4-(4-iodophenyl)-α,α-diphenyl-1-piperidinepentanenitrile (6ag) was prepared in a manner similar to **6aj** starting with **4t** (500 mg, 1.5 mmol) and 1,4-diiodobenzene. The crude product was purified by flash chromatography to afford **6ag** as a foam (45 mg, 6% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ (TMS) 1.78 (d, 4H), 2.05 (t, 2H), 2.50 (m, 6H), 2.75 (d, 2H), 7.39 (m, 12H), 7.75 (d, 2H). Anal. ($C_{28}H_{30}IN_2O$) C, H, N.

4-(3-Chlorophenyl)-4-hydroxy-α,α-diphenyl-1-piperidinepentanenitrile, hydrochloride salt (6ah) was prepared in a manner similar to **6aj** starting with **4t** (500 mg, 1.5 mmol) and 3-bromochlorobenzene to give a white solid. The solid was further purified by crystallization from methanol to afford **6ah** as a white solid (180 mg, 24% yield). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.74 (d, 4H), 2.30 (t, 2H), 2.50 (m, 2H), 2.61 (m, 2H), 3.20 (m, 4H), 5.61 (s, 1H), 7.40 (m, 14H), 9.95 (br s, 1H). ES/MS M + H = 445. Anal. ($C_{28}H_{29}ClN_2O\cdot0.85HCl\cdot$ $0.15HBr\cdot1.0H_2O$) C, H, N, Cl, Br.

4-(3,5-Dichlorophenyl)-4-hydroxy-α,α-diphenyl-1-piperidinepentanenitrile (6ai) was prepared in a manner similar to **6aj** starting with **4t** (500 mg, 1.5 mmol) and 3,5-dichlorobromobenzene to afford **6ai** as a white solid (310 mg, 38% yield). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.78 (d, 4H), 2.15 (t, 2H), 2.48 (s, 2H), 2.62 (m, 2H), 3.25 (M, 4H), 5.63 (s, 1H), 7.40 (m, 14H), 10.00 (br s, 1H). Anal. ($C_{28}H_{28}Cl_2N_2O\cdot1.1HCl\cdot$ $1.6H_2O$) C, N, Cl; H: calcd, 5.94; found, 5.26.

A General Procedure for the Preparation of Target Compounds 6 Using Lithium Reagents Prepared by Halogen Exchange. 4-Hydroxy-4-(4-methoxyphenyl)-α,α-diphenyl-1-piperidinepentanenitrile, Hydrochloride Salt (6aj). A solution of 4-bromoanisole (309 mg, 1.65 mmol) in anhydrous ether (5 mL) was chilled to −80 °C under nitrogen. *tert*-Butyllithium (1.95 mL, 1.7 M in pentane, 3.3 mmol) was added over 10 min, and the mixture was stirred for 1 h at −80 °C. A second solution of **4t** (500 mg, 1.5 mmol) in anhydrous THF (5 mL) was prepared and chilled under nitrogen to −70 °C. The litho-anisole solution was added via cannula to the solution of **4t** over 10 min. The mixture was stirred for 30 min at −70 °C and then allowed to come to ambient temperature over 30 min. The reaction was quenched by the addition of 1 N HCl (5 mL). Ether was added, and the product precipitated as the salt. The white solid was isolated by filtration and dried in vacuo to afford **6aj** (620 mg, 81%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.74 (d, 4H), 2.26 (t, 2H), 2.62 (t, 2H), 3.26 (m, 6H), 3.74 (s, 3H), 5.40 (s, 1H), 6.90 (d, 2H), 7.4 (m, 12H), 10.02 (br s, 1H). Anal. ($C_{29}H_{32}N_2O_2\cdot0.7HCl\cdot0.3HBr\cdot1.0H_2O$) C, H, N, Cl, Br, Li.

4-(4-Cyanophenyl)-4-hydroxy-α,α-diphenyl-1-piperidinepentanenitrile (6ak) was prepared in a manner similar to **6aj** starting with **4t** (500 mg, 1.5 mmol) and 4-bromobenzonitrile to afford **6ak** after chromatography as a white solid (98 mg, 15% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ (TMS) 1.70 (d, 6H), 2.10 (m, 2H), 2.44 (m, 4H), 2.76 (d, 2H), 7.36 (m, 10H), 7.64 (s, 4H). Anal. ($C_{29}H_{29}N_3O\cdot0.4H_2O$) C, H, N.

4-Hydroxy-α,α-diphenyl-4-[4-(trifluoromethyl)phenyl]-1-piperidinepentanenitrile, hydrochloride salt (6al) was prepared in a manner similar to **6aj** starting with **4t** (500 mg, 1.5 mmol) and 4-bromobenzotrifluoride. The crude product was purified by flash chromatography, and the hydrochloride salt formed to give **6al** as a white solid (180 mg, 25% yield). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.78 (d, 4H), 2.44 (m, 2H), 2.64 (t, 2H), 3.20, (m, 4H), 3.36 (m, 2H), 5.76 (s, 1H), 7.42 (m, 10H), 7.70 (m, 4H), 10.60 (br s, 1H). Anal. ($C_{29}H_{29}F_3N_2O\cdot1.0HCl$) C, H, N.

4-(4-Aminophenyl)-4-hydroxy-α,α-diphenyl-1-piperidinepentanenitrile (6am) was prepared in a manner similar to **6aj** starting with **4t** (1.0 g, 3.0 mmol) and 4-bromo-*N*,*N*-bistrimethylsilylaniline to afford **6am**, after chromatography, as a solid (445 mg, 35% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ (TMS) 1.70 (m, 4H), 2.06 (t, 2H), 2.41 (m, 6H), 2.68 (d, 2H), 3.65 (br s, 2H), 6.67 (d, 2H), 7.34 (m, 12H). Anal. ($C_{28}H_{31}N_3O\cdot0.1H_2O$) C, H, N.

4-[4-(Dimethylamino)phenyl]-4-hydroxy-α,α-diphenyl-1-piperidinepentanenitrile (6an) was prepared in a manner similar to **6aj** starting with **4t** (500 mg, 1.5 mmol) and *N*,*N*-dimethyl-4-bromoaniline to afford **6an**, after chromatography, as a foam (250 mg, 37% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ (TMS) 1.65 (m, 2H), 1.74 (d, 2H), 2.06 (t, 2H), 2.41 (m, 6H), 2.66 (d, 4H), 2.72 (s, 6H), 6.71 (d, 2H), 7.34 (m, 12H). Anal. ($C_{30}H_{35}N_3O\cdot0.4H_2O$) C, H, N.

3-(4-Fluorophenyl)-α-(1,5-cyclohexadien-1-yl)-3-hydroxy-α-phenyl-1-piperidinehexanenitrile, hydrochloride salt (6ao) was prepared in a manner similar to **6ap** starting from 1-(phenylmethyl)-3-piperidinone (1.1 g, 3.85 mmol) to afford **6ao** freebase as a colorless oil (390 mg, 0.88 mmol). ^{1}H NMR (300 MHz, $CDCl_3$) δ (TMS) 1.68 (m, 6H), 1.95 (m, 2H), 2.10 (d, 2H), 2.45 (m, 4H), 2.60 (d, 1H), 2.85 (m, 1H), 3.84 (br s, 1H), 7.08 (m, 2H), 7.40 (m, 12H). Anal. ($C_{28}H_{29}FN_2O\cdot1.5HCl$) C, H, N.

3-(4-Fluorophenyl)-α-(1,5-cyclohexadien-1-yl)-3-hydroxy-α-phenyl-1-pyrrolidinehexanenitrile, Hydrochloride Salt (6ap). A solution of 1-(phenylmethyl)-3-pyrrolidinone (500 mg, 2.9 mmol) in anhydrous THF (15 mL) was cooled to 0 °C under nitrogen and treated with 4-fluorophenyl magnesium bromide (1.7 mL, 2.0 M in ether, 3.4 mmol). The ice bath was removed and the mixture allowed to stir at ambient temperature for 18 h. The solution was cooled to 0 °C, quenched with water, acidified with 1 N HCl then neutralized with 1 N KOH. The solution was buffered to pH 9 with saturated sodium bicarbonate and the product extracted with ethyl acetate (2 × 50 mL). The combined organic extracts were washed with water and then brine, dried over magnesium sulfate, filtered, and concentrated in vacuo to a brown oil (800 mg). The oil was purified by flash chromatography on silica gel eluting with 0−100% ethyl acetate in methylene chloride to give a clear yellow oil (600 mg). The oil was dissolved in methanol (50 mL), 10% Pd/C (100 mg) was added, and the mixture was shaken under 50 psi H_2 for 16 h at ambient temperature. The mixture was filtered through Celite and the filtrate concentrated in vacuo to give an orange oil (640 mg, quantitative). This material was dissolved in anhydrous DMSO (20 mL) and treated with **3s** (600 mg, 2.2 mmol), followed by diisopropylethylamine (600 mg, 4.4 mmol). The resulting brown solution was heated to 60 °C for 27 h. The mixture was cooled and poured into water (100 mL), and the product was isolated by extraction with ethyl acetate (3 × 50 mL). The combined organic extracts were washed with water and then brine, dried over magnesium sulfate, filtered, and concentrated in vacuo to a brown oil. The product was purified by flash chromatography on silica gel eluting with a 0−40% ethyl acetate in methylene chloride gradient to afford the **6ap** as a brown oil (400 mg, 0.92 mmol). The hydrochloride salt was prepared in a manner previously described. ^{1}H NMR (300 MHz, $CDCl_3$) δ (TMS) 1.68 (m, 2H), 2.22 (m, 2H), 2.53 (m, 6H), 2.95 (m, 1H), 3.10 (m, 1H), 7.02 (m, 2H), 7.35 (m, 12H). Anal. ($C_{27}H_{27}FN_2O\cdot1.3HCl\cdot0.5H_2O$) C, H, N.

4-(4-Chlorophenyl)-4-hydroxy-α,α-diphenyl-1-piperidinepentanenitrile, *N*-oxide (6aq) was prepared from **6s** (200 mg, 0.44 mmol) in 30% aqueous hydrogen peroxide (3 mL) and acetic acid (2 mL) at 110 °C for 1 h. The product was isolated by dilution with acetonitrile and was purified by preparative HPLC to afford **6aq** as a white solid (140 mg, 54% yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ (TMS) 1.95 (d, 2H), 2.12 (m, 2H), 2.60 (m, 4H), 3.70 (m, 2H), 3.88 (m, 4H), 7.38 (m, 12H). Anal. ($C_{28}H_{29}ClN_2O_2\cdot1.15TFA$) C, H, N. LC/MS M + H$^+$ = 461.

A General Procedure for the Preparation of Quaternary Ammonium Salts. 4-(4-Chlorophenyl)-4-hydroxy-1-[5-cyano-5-(1,5-cyclohexadien-1-yl)-5-phenylpentyl]-1-methyl-piperidinium, Iodide (6ar). To a solution of **6s** (400 mg, 0.9 mmol) in methylene chloride (4 mL) was added methyl iodide (0.5 mL, 8.0 mmol). The solution was stirred at ambient temperature for 16 h. Ether was added to the solution to precipitate the product as an oil. The solvent was decanted and the oil redissolved in methylene chloride. Ether was added to this solution dropwise and the product crystallized. The product was isolated by filtration as a yellow solid (270 mg, 51% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ (TMS) 1.98 (m, 2H), 2.17 (m, 4H), 2.70 (m, 2H), 3.14 (s, 3H), 3.44 (m, 2H), 3.82 (m, 4H), 7.40 (m, 14 H). Anal. ($C_{29}H_{32}ClIN_2O\cdot0.8H_2O$) C, H, N, Cl, I. ES/MS *m*/*z* 459.

4-(4-Chlorophenyl)-4-hydroxy-1-[5-(1,5-cyclohexadien-1-yl)-5-phenylpentyl]-1-methyl-piperidinium, iodide (6as) was prepared in a manner similar to **6ar** starting with **8** (90 mg, 0.21 mmol) to afford **6ar** as a white solid after purification by HPLC (40 mg, 33% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ (TMS) 1.70 (m, 4H), 2.21 (m, 4H), 3.00 (s, 3H), 3.30 (m, 2H), 3.48 (m, 3H), 4.05 (t, 1H), 7.15 (m, 2H), 7.30 (m, 8H), 7.39 (m, 2H), 7.57 (m, 2H). Anal. ($C_{28}H_{33}ClNO\cdot1.05TFA\cdot0.8H_2O$) C, H, N, Cl, I.

4-(4-Chlorophenyl)-4-hydroxy-1-[5-cyano-5-(2-naphthalenyl)pentyl]-1-methyl-piperidinium, iodide (6at) was prepared in a manner similar to **6ar** starting with **6au** (200 mg, 0.4 mmol) to afford **6at** as a white solid (120 mg, 50% yield). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.78 (m, 2H), 2.00 (m, 4H), 2.31 (m, 2H), 3.08 (s, 3H), 3.42 (m, 6H), 4.50 (m, 1H), 5.60 (s, 1H), 7.40 (m, 2H), 7.59 (m, 5H), 7.98 (m, 4H). Anal. ($C_{27}H_{30}ClIN_2O\cdot0.33H_2O$) C, H, N, Cl, I.

4-(4-Chlorophenyl)-4-hydroxy-α-(2-naphthalenyl)- 1-piperidinepentanenitrile, hydrochloride salt (6au) was prepared in a manner similar to **6bh** starting with 2-naphthylacetonitrile (840 mg, 5.0 mmol) to afford **25a** as a white solid (610 mg, 26% yield). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.80 (m, 4H), 2.05 (m, 2H), 2.38 (m, 2H), 3.20 (m, 4H), 3.40 (m, 2H), 4.50 (t, 1H), 5.60 (s, 1H), 7.42 (m, 4H), 7.55 (m, 4H), 7.95 (m, 5H), 10.50 (br s, 1H). Anal. ($C_{26}H_{27}ClN_2O\cdot1.0HCl\cdot0.5H_2O$) C, H, Cl, N.

4-(4-Chlorophenyl)-4-hydroxy-α,α-diphenyl-1-piperidinebutanenitrile, hydrochloride salt (6av) was prepared as described in the preparation of **6s** starting with 4-bromo-2,2-diphenyl butyronitrile (200 mg, 0.67 mmol) and 4-(4-chlorophenyl)-4-hydroxypiperidine (155 mg, 0.73 mmol) to afford **6av**, after chromatography and salt formation, as a white solid (65 mg, 20% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ (TMS) 1.75 (br s, 1H), 1.90 (d, 2H), 2.78 (m, 2H), 3.10 (m, 4H), 3.35 (m, 3H), 7.40 (m, 12H). Anal. ($C_{27}H_{27}ClN_2O\cdot1.2HCl$) C, H, N.

4-(4-Chlorophenyl)-4-hydroxy-α,α-diphenyl-1-piperidinehexanenitrile (6aw) was prepared in the same manner as **6s** starting with phenylacetonitrile (600 mg, 3.1 mmol) and 1,4-dibromobutane (3.3 g, 15 mmol) followed by substitution with 4-(4-chlorophenyl)-4-hydroxypiperidine to afford **6aw**, after chromatography, as a white solid (780 mg, 48% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ (TMS) 1.62 (m, 7H), 2.10 (m,

2H), 2.39 (m, 5H), 2.79 (d, 2H), 7.35 (m, 14H). Anal. ($C_{29}H_{31}ClN_2O$) C, H, N.

4-(4-Chlorophenyl)-4-hydroxy-α,α-diphenyl-1-piperidineheptanenitrile, hydrochloride salt (6ax) was prepared in a manner similar to **6s**. Phenylacetonitrile (600 mg, 3.1 mmol) was reacted with 1,5-dibromopentane (3.6 g, 15 mmol) followed by the substitution with 4-(4-chlorophenyl)-4-hydroxypiperidine to afford **6ax**, after chromatography and salt formation, as a white solid (630 mg, 39% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ (TMS) 1.41 (m, 4H), 1.92 (m, 4H), 2.40 (m, 2H), 2.75 (m, 4H), 3.23 (m, 4H), 7.21 (m, 14H). Anal. ($C_{30}H_{33}ClN_2O\cdot1.0HCl\cdot0.5H_2O$) C, H, N.

4-(4-Chlorophenyl)-4-hydroxy-α-phenyl-1-piperidinepentanenitrile, hydrochloric salt (6ay) was prepared in a manner similar to **6bh** starting with phenylacetonitrile (300 mg, 2.56 mmol) to afford **6ay** as a white solid (330 mg, 28% yield). ^{1}H NMR (300 MHz, $CDCl_3$-TFA) δ (TMS) 1.75 (m, 6H), 2.35 (m, 4H), 2.81 (m, 2H), 3.00 (m, 3H), 3.75 (m, 1H), 7.15 (m, 9H), 10.80 (br s, 1H). Anal. ($C_{22}H_{25}ClN_2O\cdot1.2HCl$) C, H, N.

α-(3-Bromo-4-methoxyphenyl)-4-(4-chlorophenyl)-4-hydroxy-1-piperidinepentanenitrile, hydrochloride salt (6az) was prepared in a manner similar to **6bh** starting with 3-bromo-4-methoxyphenylacetonitrile (678 mg, 3.0 mmol) to afford **6az** as a white solid (620 mg, 38% yield). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.77 (m, 4H), 1.90 (m, 2H), 2.35 (m, 2H), 3.17 (m, 4H), 3.39 (m, 2H), 3.85 (s, 3H), 4.29 (m, 1H), 5.59 (s, 1H), 7.17 (d, 1H), 7.44 (m, 5H), 7.65 (d, 1H), 10.32 (br s, 1H). Anal. ($C_{23}H_{26}BrClN_2O_2\cdot1.0HCl\cdot0.65C_4H_{10}O$) C, H, N, Br, Cl.

4-(4-Chlorophenyl)-α-(2-cyanophenyl)-4-hydroxy-1-piperidinepentanenitrile, hydrochloride salt (6bb) was prepared in a manner similar to **6bh** starting with 2-cyanophenylacetonitrile (426 mg, 3.0 mmol) to afford **6bb** as an off-white solid (320 mg, 25% yield). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.83 (m, 4H), 2.03 (m, 2H), 2.34 (m, 2H), 3.25 (m, 4H), 3.38 (m, 2H), 4.58 (m, 1H), 5.58 (s, 1H), 7.45 (m, 4H), 7.60 (m, 1H), 7.74 (m, 1H), 7.95 (m, 1H), 10.38 (br s, 1H). Anal. ($C_{23}H_{24}ClN_3O\cdot1.0HCl$) C, H, N, Cl.

4-(4-Chlorophenyl)-4-hydroxy-α-(2,3,4,5,6-pentafluorophenyl)-1-piperidinepentanenitrile, hydrochloride salt (6bc) was prepared in a manner similar to **6bh** starting with 2,3,4,5,6-pentafluorophenylacetonitrile (830 mg, 4.0 mmol) to afford **6bc** as a white solid (170 mg, 8.6% yield). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.95 (m, 4H), 2.08 (m, 2H), 2.35 (m, 2H), 3.15 (m, 4H), 3.40 (m, 2H), 4.71 (m, 1H), 5.60 (s, 1H), 7.45 (m, 4H), 10.40 (br s, 1H). Anal. ($C_{22}H_{20}ClF_5N_2O\cdot1.0HCl\cdot0.2C_4H_{10}O$) C, H, N, Cl, F.

4-(4-Chlorophenyl)-4-hydroxy-α-(3-hydroxyphenyl)-1-piperidinepentanenitrile, hydrochloride salt (6bd) was prepared from **6bl** (320 mg, 0.67 mmol) by catalytic hydrogenation to afford **6bd** as a white solid (120 mg, 43% yield). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.84 (m, 6H), 2.31 (m, 2H), 3.15 (m, 4H), 3.38 (m, 2H), 4.21 (m, 1H), 5.58 (s, 1H), 6.76 (m, 3H), 7.20 (m, 1H), 7.40 (m, 4H), 9.68 (s, 1H), 10.11 (br s, 1H). Anal. ($C_{22}H_{25}ClN_2O_2\cdot1.0HCl$) C, H, N, Cl.

4-(4-Chlorophenyl)-α-(2-fluorophenyl)-4-hydroxy-1-piperidinepentanenitrile, hydrochloride salt (6be) was prepared in a manner similar to **6bh** starting with 2-fluorophenylacetonitrile (400 mg, 3.0 mmol) to afford **6be** as a white solid (280 mg, 22% yield). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.95 (m, 6H), 2.38 (m, 2H), 3.18 (m, 4H), 3.40 (m, 2H), 4.45 (m, 1H), 5.60 (s, 1H), 7.28 (m, 2H), 7.45 (m, 6H), 10.50 (br s, 1H). Anal. ($C_{22}H_{24}ClFN_2O\cdot1.0HCl$) C, H, N, Cl.

4-(4-Chlorophenyl)-α-(4-fluorophenyl)-4-hydroxy-1-piperidinepentanenitrile, hydrochloride salt (6bf) was prepared in a manner similar to **6bh** starting with 4-fluorophenylacetonitrile (400 mg, 3.0 mmol) to afford **6bf** as a white solid (190 mg, 15% yield). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.95 (m, 6H), 2.38 (m, 2H), 3.18 (m, 4H), 3.40 (m, 2H), 4.38 (m, 1H), 5.60 (s, 1H), 7.25 (m, 2H), 7.45 (m, 6H), 10.45 (br s, 1H). Anal. ($C_{22}H_{24}ClFN_2O\cdot1.0HCl$) C, H, N, Cl.

α-(2-Bromophenyl)-4-(4-chlorophenyl)-4-hydroxy-1-piperidinepentanenitrile, hydroiodide salt (6bg) was prepared in a manner similar to **6bh** starting with 2-bromophenylacetonitrile (840 mg, 4.3 mmol) to afford **6bg** as a white solid (380 mg, 15% yield). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.95 (m, 8H), 3.18 (m, 4H), 3.40 (m, 2H), 4.38 (m, 1H), 5.60 (s, 1H), 7.55 (m, 8H), 9.00 (br s, 1H). Anal. ($C_{22}H_{24}BrClN_2O\cdot1.0HI$) C, H, N, Br, Cl, I.

A General Procedure for the Preparation of Target Compound 6 from Commercially Available Substituted Acetonitriles. α,4-Bis(4-chlorophenyl)-4-hydroxy-1-piperidinepentanenitrile, Hydrochloride Salt (6bh). A solution of LDA (2.0 mL, 1.5 N in cyclohexane, 3.0 mmol) was added by syringe over a 5 min period to a stirred solution (cooled in a dry ice/acetone bath) of 4-chlorobenzylcyanide (0.45 g, 3.0 mmol) and 1-chloro-3-iodopropane (0.32 mL, 3.0 mmol) in anhydrous THF (6.0 mL) under nitrogen. After 5 min the bath was removed, and after warming for 1 h, 4-(4-chlorophenyl)-4-hydroxypiperidine (0.95 g, 4.5 mmol) was added. The mixture was stirred until homogeneous and then heated overnight at 65 °C. The mixture was partitioned between water (50 mL) and ethyl ether (50 mL). The ethyl ether layer was washed successively with 25 mL portions of water, 10% aqueous K_2CO_3, and brine. The ethyl ether solution was dried over $MgSO_4$, filtered, and concentrated in vacuo. Flash chromatography on silica gel (20 g) with a gradient of 0−10% 2-propanol in 4:1 dichloromethane/ethyl acetate afforded 0.51 g of **6bh** as its free base. To prepare the hydrochloride salt, the free base was dissolved in methanol (5 mL) and concentrated HCl (0.15 mL). Ethyl ether (20 mL) was added slowly with stirring. The resulting precipitate was collected by filtration, rinsed with ethyl ether (2 × 5 mL), and dried at 50 °C under vacuum to obtain **6bh** as a white solid (310 mg, 23% yield). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.78 (m, 4H), 1.98 (m, 2H), 2.35 (m, 2H), 3.18 (m, 4H), 3.37 (m, 2H), 4.39 (t, 1H), 7.48 (m, 8H), 10.4 (br s, 1H). Anal. ($C_{22}H_{24}Cl_2N_2O\cdot1.0HCl$) C, H, N, Cl.

α-(2-Chlorophenyl)-4-(4-chlorophenyl)-4-hydroxy-1-piperidinepentanenitrile, hydrochloride salt (6bi) was prepared in a manner similar to **6bh** starting with 2-chlorophenylacetonitrile (455 mg, 3.0 mmol) to afford **6bi** as a white solid (360 mg, 27% yield). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.93 (m, 6H), 2.31 (m, 2H), 3.18 (m, 4H), 3.36 (m, 2H), 4.55 (m, 1H), 5.60 (s, 1H), 7.44 (m, 6H), 7.58 (m, 2H), 10.24 (br s, 1H). Anal. ($C_{22}H_{24}Cl_2N_2O\cdot1.0HCl$) C, H, N, Cl.

4-(4-Chlorophenyl)-4-hydroxy-α-[2-(trifluoromethyl)-phenyl]-1-piperidinepentanenitrile, hydrochloride salt (6bj) was prepared in a manner similar to **6bh** starting with 2-trifluoromethylphenylacetonitrile (370 mg, 2.0 mmol) to afford **6bh** as a white solid (420 mg, 44% yield). ^{1}H NMR (300 MHz, DMSO) δ 1.82 (m, 5H), 2.15 (m, 1H), 2,25 (t, 2H), 3.21 (m, 4H), 3.42 (m, 1H) 4.38 (m, 1H) 5.60 (s, 1H) 7.42 (m, 5H) 7.62 (m, 1H), 7.82 (m, 3H), 10.15 (br s, 1H). Anal. ($C_{23}H_{24}ClF_3N_2O\cdot1.0HCl$) C, H, N, Cl, F.

4-(4-Chlorophenyl)-4-hydroxy-α-[2-(phenylmethoxy)-phenyl]-1-piperidinepentanenitrile (6bk) was prepared in a manner similar to **6bh** starting with 2-(phenylmethoxy)-phenylacetonitrile (4.47 g, 20 mmol) to afford **6bk** as a white solid (830 mg, 9% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ (TMS) 1.6 (m, 6H), 2.01 (m, 4H), 2.18 (m, 4H), 2.72 (t, 2H), 4.35 (t, 1H), 5.10 (s, 2H), 6.98 (m, 2H), 7.40 (m, 11H). Anal. ($C_{29}H_{31}ClN_2O_2$) C, H, N, Cl.

4-(4-Chlorophenyl)-4-hydroxy-α-[3-(phenylmethoxy)-phenyl]-1-piperidinepentanenitrile (6bl) was prepared in a manner similar to **6bh** starting with 3-(phenylmethoxy)-phenylacetonitrile (4.47 g, 20 mmol) to afford **6bl** as a white solid (2.08 g, 20% yield). ^{1}H NMR (300 MHz, DMSO) δ 1.8 (m, 3H), 2.0 (m, 3H) 2.2 (t, 2H), 3.18 (m, 4H), 3.4 (m, 2H), 4.23 (t, 1H), 5.10 (s, 2H), 5.60 (s, 1H), 6.92 (d, 2H), 7.05 (s, 1H), 7.40 (m, 10H), 10.4 (br s, 1H). Anal. ($C_{29}H_{31}ClN_2O_2\cdot1.0HCl$) C, H, N, Cl.

4-(4-Chlorophenyl)-4-hydroxy-α-[4-(phenylmethoxy)-phenyl]-1-piperidinepentanenitrile (6bm) was prepared from **6bp** (147 mg, 0.35 mmol) with benzyl bromide (81 μL) and cesium carbonate (340 mg) in anhydrous DMF (2 mL) to afford **6bm** as a white solid (59 mg, 33% yield). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.83 (m, 6H), 2.33 (m, 2H), 3.14 (m, 4H),

3.40 (m, 2H), 4.23 (m, 1H), 5.10 (s, 2H), 5.58 (s, 1H), 7.06 (d, 1H), 7.35 (m, 11H), 10.27 (br s, 1H). Anal. ($C_{29}H_{31}ClN_2O_2$·1.0HCl) C, H, N, Cl.

4-(4-Chlorophenyl)-4-hydroxy-α-(3-phenoxyphenyl)-1-piperidinepentanenitrile, hydrochloride salt (6bn) was prepared in a manner similar to **6bh** starting with 3-phenoxyphenylacetonitrile (950 mg, 4.5 mmol) to afford **6bn** as a white solid (270 mg, 12% yield). ^{1}H NMR (300 MHz, DMSO) δ 1.82 (m, 6H), 2.40 (t, 2H), 3.15 (m, 4H), 3.40 (m, 2H), 4.32 (t, 1H), 5.58 (s, 1H), 6.95 (m, 1H), 7.08 (m, 3H), 7.19 (m, 2H), 7.40 (m, 7H) 10.29 (br s, 1H). Anal. ($C_{28}H_{29}ClN_2O_2$·1.0HCl) C, H, N, Cl.

4-(4-Chlorophenyl)-4-hydroxy-α-(2-hydroxyphenyl)-1-piperidinepentanenitrile, trifluoroacetic acid salt (6bo) was prepared from **6bk** by (475 mg, 1 mmol) by catalytic hydrogenation using 10% Pd/C and purified by preparative HPLC to afford **6bo** as a white solid (81 mg, 15% yield). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.81 (m, 6H), 2.15 (m, 2H), 3.20 (M, 4H), 3.42 (m, 2H), 4.34, (m, 1H), 5.40 (s, 1H), 6.92 (m, 2H), 7.19 (m, 1H), 7.25 (d, 1H), 7.44 (s, 4H), 9.10 (br s, 1H), 10.08 (s, 1H). Anal. ($C_{22}H_{25}ClN_2O_2$·1.25TFA·0.5H_2O) C, H, N.

4-(4-Chlorophenyl)-4-hydroxy-α-(4-hydroxyphenyl)-1-piperidinepentanenitrile, hydrochloride salt (6bp) was prepared in a manner similar to **6bk** starting with 4-hydroxyphenylacetonitrile (4.93 mg, 37 mmol) which was protected using *tert*-butyldimethylsilyl chloride.[33] The protecting group was removed with TBAF to afford **6bp** as a white solid (450 mg, 3% yield). ^{1}H NMR (300 MHz, DMSO) δ 1.80 (m, 6H), 2.38 (t, 2H), 3.20 (m, 4H), 3.41 (m, 2H), 4.20 (t, 1H), 5.58 (s, 1H), 6.80 (d, 2H), 7.20 (d, 2H), 7.42 (m, 4H), 9.62 (s, 1H), 10.21 (br s, 1H). Anal. ($C_{22}H_{25}ClN_2O_2$·1.0HCl) C, H, N, Cl.

4-(4-Chlorophenyl)-4-hydroxy-α-(3,4,5-trimethoxyphenyl)-1-piperidinepentanenitrile, hydrochloride salt (6bq) was prepared in a manner similar to **6bh** starting with 3,4,5-trimethoxyphenylacetonitrile (1.04 g, 5 mmol) to afford **6bq** as a white solid (290 mg, 12% yield). ^{1}H NMR (300 MHz, DMSO) δ 1.80 (m, 4H), 1.98 (m, 2H), 2.30 (m, 2H), 3.20 (m, 4H), 3.40 (m, 2H), 3.65 (s, 3H), 3.80 (s, 6H), 4.20 (t, 1H), 5.60 (s, 1H), 6.78 (s, 2H) 7.42 (m, 4H), 10.20 (br s, 1H). Anal. ($C_{25}H_{31}ClN_2O_4$·1.0HCl) C, H, N.

4-(4-Chlorophenyl)-4-hydroxy-α-(3-nitrophenyl)-1-piperidinepentanenitrile, hydrochloride salt (6br) was prepared in a manner similar to **6bh** starting with 3-nitrophenylacetonitrile (1.62 mg, 10 mmol) to afford **6br** as a tan solid (250 mg, 6% yield). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.77 (m, 4H), 2.01 (m, 2H), 2.30 (m, 2H), 3.18 (m, 4H), 3.38 (m, 2H), 4.60 (m, 1H), 5.58 (s, 1H), 7.44 (m, 4H), 7.58 (t, 1H), 7.93 (d, 1H), 8.24 (d, 1H), 8.31 (s, 1H), 10.13 (br s, 1H). Anal. ($C_{22}H_{24}ClN_3O_3$·1.0HCl) C, H, N.

4-(4-Chlorophenyl)-4-hydroxy-α-(4-methoxyphenyl)-1-piperidinepentanenitrile, hydrochloride salt (6bs) was prepared from **6bp** (180 mg, 0.47 mmol) and methyl iodide to afford **6bs** as a white solid (40 mg, 19% yield). ^{1}H NMR (300 MHz, DMSO) δ 1.80 (m, 6H), 2.31 (t, 2H), 3.20 (m, 4H), 3.40 (m, 2H), 3.80 (m, 3H), 4.22 (t, 1H), 5.58 (s, 1H) 7.01 (d, 2H) 7.38 (d, 2H) 7.48 (m, 4H), 10.08 (br s, 1H). Anal. ($C_{23}H_{27}ClN_2O_2$·1.0HCl·0.25H_2O) C, H, N, Cl.

4-(4-Chlorophenyl)-4-hydroxy-α-(4-ethoxyphenyl)-1-piperidinepentanenitrile, hydrochloride salt (6bt) was prepared in a manner similar to **6bs** starting with **6bp** (147 mg, 0.35 mmol) and ethyl iodide to afford **6bt** as a white solid (46 mg, 28% yield). ^{1}H NMR (300 MHz, DMSO) δ 1.25 (m, 3H), 1.81 (m, 6H), 2.30 (t, 2H), 3.18 (m, 4H), 4.0 (q, 2H), 4.25 (t, 1H), 5.60 (s, 1H), 6.95 (d, 2H), 7.38 (d, 2H), 7.49 (m, 4H), 10.20 (br s, 1H). Anal. ($C_{24}H_{29}ClN_2O_2$·1.0HCl·0.25H_2O) C, H, N.

4-(4-Chlorophenyl)-4-hydroxy-α-(2-thienyl)-1-piperidinepentanenitrile, hydrochloride salt (6bu) was prepared in a manner similar to **6bh** starting with 2-thienylacetonitrile (370 mg, 3.0 mmol) to afford **6bu** as a tan solid (140 mg, 10% yield). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.80 (m, 4H), 2.0 (m, 2H), 2.38 (m, 2H), 3.20 (m, 4H), 3.40 (m, 2H), 4.75 (t, 1H), 5.40 (s, 1H), 7.05 (m, 1H), 7.20 (d, 1H), 7.45 (m, 4H), 7.58 (d, 2H), 10.40 (br s, 1H). Anal. ($C_{20}H_{23}ClN_2OS$·1.0HCl·0.6$C_4H_{10}O$) C, H, N.

4-(4-Chlorophenyl)-4-hydroxy-α-(3-thienyl)-1-piperidinepentanenitrile, hydrochloride salt (6bv) was prepared in a manner similar to **6bh** starting with 3-thienylacetonitrile (370 mg, 3.0 mmol) to afford **6bv** as a gum (80 mg, 6% yield). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.80 (m, 4H), 1.95 (m, 2H), 2.30 (m, 2H), 3.10 (m, 4H), 3.40 (m, 2H), 4.40 (t, 1H), 5.60 (s, 1H), 7.15 (m, 1H), 7.40 (m, 4H), 7.58 (s, 1H), 7.65 (m, 1H), 10.10 (br s, 1H). Anal. ($C_{20}H_{23}ClN_2OS$·1.0HCl·0.3ether) C, H, N, Cl.

4-(4-Chlorophenyl)-4-hydroxy-α-(1-naphthalenyl)-1-piperidinepentanenitrile (6bw) was prepared in a manner similar to **6bh** starting with 1-naphthalenylacetonitrile (360 mg, 3.0 mmol) to afford **6bw** as a off-white solid (170 mg, 12% yield). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.75 (m, 2H), 2.0 (m, 4H), 2.35 (m, 2H), 3.20 (m, 4H), 3.40 (m, 2H), 5.10 (t, 1H), 5.60 (s, 1H), 7.05 (m, 1H), 7.40 (m, 4H), 7.60 (m, 4H), 8.00 (m, 4H), 8.20 (d, 2H), 10.30 (br s, 1H). Anal. ($C_{26}H_{27}ClN_2O$·1.0HCl) C, H, N, Cl.

4-(4-Chlorophenyl)-4-hydroxy-α-(2-pyridinyl)-1-piperidinepentanenitrile, hydrochloride salt (6bx) was prepared in a manner similar to **6bh** starting with 2-pyridinylacetonitrile (360 mg, 3.0 mmol) to afford **6bx** as an off-white solid (170 mg, 12% yield). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.80 (m, 4H), 2.0 (m, 2H), 2.38 (m, 2H), 3.10 (m, 4H), 3.40 (m, 2H), 4.50 (t, 1H), 7.45 (m, 6H), 7.90 (m, 1H), 8.40 (d, 2H), 10.40 (br s, 1H). Anal. ($C_{21}H_{24}ClN_3O$·1.5HCl) C, H, N.

4-(4-Chlorophenyl)-4-hydroxy-α-(3-pyridinyl)-1-piperidinepentanenitrile, dihydrochloride salt (6by) was prepared in a manner similar to **6bh** starting with 3-pyridinylacetonitrile (360 mg, 3.0 mmol) to afford **6by** as an off-white solid (170 mg, 12% yield). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.80 (m, 4H), 2.0 (m, 2H), 2.40 (m, 2H), 3.20 (m, 4H), 3.40 (m, 2H), 4.60 (t, 1H), 7.45 (m, 4H), 7.80 (m, 1H), 8.30 (d, 1H), 8.70 (d, 1H), 8.90 (d, 1H), 10.80 (br s, 1H). Anal. ($C_{21}H_{24}ClN_3O$·2.0HCl) C, H, N, Cl.

4-(4-Chlorophenyl)-4-hydroxy-α-(1-methyl-1*H*-pyrrol-2-yl)-1-piperidinepentanenitrile, hydrochloride salt (6bz) was prepared in a manner similar to **6bh** starting with (1-methyl-1*H*-pyrrol-2-yl)acetonitrile (360 mg, 3.0 mmol) to afford **6bz** as a off-white solid (170 mg, 12% yield). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.80 (m, 4H), 2.0 (m, 2H), 2.38 (m, 2H), 3.20 (m, 4H), 3.35 (m, 2H), 3.60 (s, 3H), 5.60 (s, 1H), 5.95 (m, 1H), 6.10 (s, 1H), 6.78 (s, 1H), 7.45 (m, 4H), 10.40 (br s, 1H). Anal. ($C_{21}H_{26}ClN_3O$·1.0HCl·1.0^{t}BuOH) C, H, N, Cl.

4-(4-Chlorophenyl)-α-cyano-4-hydroxy-α-phenyl-1-piperidinepentanoic acid, ethyl ester, hydrochloride (6cc) was prepared in the same manner as **6ch** starting with ethyl phenylcyanoacetate (2.0 g, 10.5 mmol) to afford **6cc** as a white solid (2.2 g, 44% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.22 (t, 3H), 2.00 (m, 4H), 2.25 (m, 2H), 2.55 (m, 3H), 3.20 (m, 2H), 3.37 (m, 1H), 3.61 (m, 2H), 4.15 (M, 2H), 7.4 (m, 9H). Anal. ($C_{25}H_{29}ClN_2O_3$·1.0HCl) C, H, N, Cl.

4-(4-Chlorophenyl)-α-cyano-4-hydroxy-α-phenyl-1-piperidinepentanoic Acid, Methyl Ester, Hydrochloride Salt (6cd). To a solution of benzyl cyanide (11.8 g, 100 mmol) and dimethyl carbonate (45.0 g, 500 mmol) in toluene (80 mL) at 0 °C was added sodium methoxide (5.40 g, 54.0 mmol). The resulting slurry was stirred at ambient temperature for 30 min and then warmed to 80 °C for 20 min. As the solution cooled to ambient temperature a precipitate formed. The solid was isolated by filtration to give benzeneacetic acid, α-cyano methyl ester, as its sodium salt. This compound (1.0 g, 5.1 mmol) was treated as described in the preparation of **6bh** to afford **6cd** as a white solid (170 mg, 37% yield). ^{1}H NMR (300 MHz, DMSO) δ 1.78 (m, 4H), 2.25 (m, 3H), 2.50 (m, 2H), 3.20 (m, 4H), 3.40 (br t, 2H), 3.78 (s, 3H), 7.49 (m, 9H). Anal. ($C_{24}H_{27}ClN_2O_3$·1.0HCl) C, H, N.

4-(4-Chlorophenyl)-α-(3-chloropropyl)-4-hydroxy-α-phenyl-1-piperidinepentanenitrile (6ce). A solution of LDA (2.0 mL, 1.5 N in cyclohexane, 3.0 mmol) was added by syringe over a 5 min period to a stirred solution of benzylcyanide (0.35 g, 3.0 mmol) and 1-chloro-3-iodopropane (0.64 mL, 6.0 mmol) in anhydrous THF (12.0 mL) under nitrogen cooled in a dry ice/acetone bath (EXOTHERMIC!). After 5 min the

bath was removed and the mixture was allowed to warm to ambient temperature. The mixture was again cooled in a dry ice/acetone bath and additional LDA (2.0 mL, 1.5 N in cyclohexane, 3.0 mmol) was added by syringe over a 5 min period. After 5 min the bath was removed. After being stirred overnight, the mixture was partitioned between ethyl ether (100 mL) and 1 N HCl (50 mL). The ether layer was washed sequentially with 1 N HCl (50 mL) and brine (25 mL), then dried over magnesium sulfate, filtered, and concentrated in vacuo. The residue (0.8 g) was dissolved in isooctane (20 mL) and concentrated in vacuo. Chromatography on silica gel (15 g) with a gradient of 30−70% methylene chloride in hexane afforded 0.24 g of an oil which was mostly α,α-bis(3-chloropropyl)benzylcyanide. ^{1}H NMR (300 MHz, $CDCl_3$) δ (TMS) 1.59 (m, 2H), 1.96 (m, 2H), 2.14 (m, 4H), 3.48 (t, 4H), 7.4 (m, 4H).

This oil was dissolved in anhydrous DMF (3 mL) and 4-(4-chlorophenyl)-4-hydroxypiperidine (0.56 g, 2.66 mmol) and NaI (27 mg, 0.18 mmol) were added. The mixture was heated overnight at 50 °C then partitioned between ethyl ether (75 mL) and 5% aqueous K_2CO_3 (25 mL). The ethyl ether layer was washed successively with 25 mL portions of water (3×) and brine, then dried over magnesium sulfate, filtered, and concentrated in vacuo. TLC (4:1:1:1 dichloromethane:ethyl acetate:2-propanol:methanol visualized with I_2) showed three components at $R_f = 0.78$, 0.67, and 0.16. The first of these was isolated by chromatography over silica gel (15 g) with a gradient of 0−25% 2-propanol in 4:1 methylene chloride:ethyl acetate to obtain 0.12 g of **6ce** as an oil. This oil was dissolved in methanol (5 mL) and concentrated HCl (0.15 mL), concentrated in vacuo and crystallized from 2-propanol/ethyl ether. The resulting precipitate was collected by filtration, rinsed with ethyl ether (2 × 5 mL), and dried in vacuo to obtain **6ce** as a white solid (70 mg, 4% yield). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.41 (m, 2H), 1.79 (m, 2H), 2.12 (m, 4H), 2. 29 (t, 2H), 3.10 (m, 4H), 3.30 (m, 2H), 3.61 (t, 2H), 5.55 (s, 1H), 7.4 (m, 9H), 10.2 (br s, 1H). Anal. ($C_{25}H_{30}Cl_2N_2O$·1.0HCl) C, H, N, Cl.

4-(4-Chlorophenyl)-4-hydroxy-α-methyl-α-phenyl-1-piperidinepentanenitrile (6cf) was prepared in a manner similar to **6bh** starting with methylphenylacetonitrile (600 mg, 4.6 mmol) to afford **6bf** as a foam (480 mg, 27% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ (TMS) 1.78 (m, 8H), 2.05 (m, 4H), 2.38 (m, 4H), 2.75 (m, 2H), 7.39 (m, 9H). Anal. ($C_{23}H_{27}ClN_2O$) C, H, N.

4-(4-Chlorophenyl)-α-ethyl-4-hydroxy-α-phenyl-1-piperidinepentanenitrile (6cg) was prepared in a manner similar to **6bh** starting with ethylphenylacetonitrile (1.0 g, 6.9 mmol) to afford **6cg** as a foam (430 mg, 16% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ (TMS) 0.95 (t, 3H), 1.35 (m, 1H), 1.78 (m, 3H), 2.10 (m, 6H), 2.37 (m, 4H), 2.70 (m, 2H), 7.38 (m, 9H). Anal. ($C_{24}H_{29}ClN_2O$·0.2H_2O) C, H, N.

2-[3-[4-(4-Chlorophenyl)-4-hydroxy-1-piperidinyl]propyl]-2-phenylbutanedinitrile (6ch). A solution of phenylsuccinylnitrile (1.56 g, 10 mmol) in anhydrous DMF (20 mL) was added dropwise to a 0 °C solution of sodium hydride (420 mg, 10.5 mmol) in anhydrous DMF under nitrogen. The mixture was stirred at 0 °C for 1 h then 3-chloro-1-iodopropane (1.18 mL, 11 mmol) was added, and the mixture was stirred for 16 h at ambient temperature. The reaction mixture was poured into water (150 mL), and the chloropropyl intermediate was extracted into ethyl acetate (3 × 50 mL). The combined organic extracts were washed with water and then brine, dried over magnesium sulfate, filtered, and evaporated in vacuo to an oil which was purified by flash chromatography (10−40% ethyl acetate in hexanes). The purified chloropropyl intermediate, (1.02 g, 44% yield) was dissolved in anhydrous DMF (25 mL) along with diisopropylethylamine (1.13 g, 8.7 mmol) sodium iodide (10 mg) and **5s** (0.92 g, 4.38 mmol). The reaction mixture was heated to 60 °C for 2 h then to 90 °C for 12 h. The reaction was quenched by pouring into water (150 mL) and extracting with ethyl acetate (2 × 100 mL). The combined organic extracts were washed with water and then brine, dried over magnesium sulfate, filtered, and evaporated to an oil which was purified by flash chromatography (0−100% ethyl acetate in methylene chloride) to afford **6ch** as a white solid (250 mg, 14% yield). ^{1}H NMR (300 MHz, DMSO) δ 1.50 (m, 3H), 1.80 (m, 2H), 2.16 (m, 2H), 2.28 (m, 4H), 2.50 (m, 2H), 3.35 (s, 1H), 3.58 (m, 2H) 4.82 (s, 1H), 7.25 (d, 2H), 7.52 (m, 7H). Anal. ($C_{24}H_{26}ClN_3O$) C, H, N.

4-(4-Chlorophenyl)-α-cyclohexyl-4-hydroxy-α-phenyl-1-piperidinepentanenitrile (6ci) was prepared in a manner similar to **6bh** starting with cyclohexylphenylacetonitrile (1.0 g, 5.0 mmol) to afford **6ci** as a foam (160 mg, 7% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ (TMS) 1.20 (m, 8H), 1.75 (m, 7H), 2.20 (m, 9H), 2.69 (t, 2H), 7.35 (m, 9H). Anal. ($C_{28}H_{35}ClN_2O$·0.4H_2O) C, H, N.

4-(4-Chlorophenyl)-4-hydroxy-α-phenyl-α-(1-piperidinyl)-1-piperidinepentanenitrile, dihydrochloride salt (6cj) was prepared in a manner similar to **6bh** starting with 1-(α-cyanobenzyl)piperidine (1.0 g, 5 mmol) to afford **6cj** as a white solid (30 mg, 1.1% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ (TMS) 1.6 (m, 9H), 1.8−2.4 (m, 14H), 3.25 (m, 1H), 4.18 (m, 1H), 7.39 (m, 14H). Anal. ($C_{27}H_{34}ClN_3O$·2.0HCl) C, H, N.

4-(4-Chlorophenyl)-4-hydroxy-α,α-diphenyl-1-piperidinepentanoic acid, methyl ester (6ck) was prepared in a similar manner as **6s** starting with diphenylacetic acid (600 mg, 2.65 mmol) to afford **6ck** as a white solid (210 mg, 17% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ (TMS) 1.32 (m, 2H), 1.65 (d, 4H), 2.08 (m, 2H), 2.38 (m, 6H), 3.70 (s, 3H), 7.30 (m, 12H), 7.41 (m, 2H). Anal. ($C_{29}H_{32}ClNO_3$·0.33SiO_2) C, H, N.

1-(5-Amino-4,4-diphenylpentyl)-4-(4-chlorophenyl)-4-piperidinol, Bistrifluoracetic Acid Salt (6cl). LAH (1.7 g, 45 mmol) was slurried in anhydrous THF (200 mL), and the solution was cooled in an ice bath to 10 °C under nitrogen. $AlCl_3$ (6 g, 45 mmol) was dissolved in anhydrous THF (200 mL) (CAUTION: EXOTHERMIC!). The $AlCl_3$ solution was added to the LAH slurry via an addition funnel over 15 min. Compound **6s** (10 g, 22.5 mmol) was dissolved in anhydrous THF (100 mL). This solution was added to the LAH/$AlCl_3$ mixture at a rate that maintained the internal temperature at >25 °C. The resulting mixture was stirred at ambient temperature for 1 h and then heated to reflux for 8 h. The reaction was cooled to 0 °C and quenched (CAUTION: EXOTHERMIC! H_2 evolution!) with water (4 mL), 15% aqueous NaOH (4 mL), and water (12 mL) then filtered, and the filtrate was concentrated in vacuo to a residue. The residue was dissolved in methylene chloride and washed with $NaHCO_3$ (saturated) and then brine, dried over magnesium sulfate, filtered, and concentrated in vacuo to a foam. The foam was crystallized from 1:15:15, methanol:ether:petroleum ether to afford **7** as a white solid (6.4 g, 63%). The foam was further purified by preparative HPLC to give the TFA salt. ^{1}H NMR (300 MHz, DMSO-d_6) δ (TMS) 1.1 (m, 2H), 1.48 (m, 2H), 1.82 (m, 2H), 2.25 (m, 6H), 2.4 (m, 2H), 3.18 (m, 2H), 7.25 (m, 14H). Anal. ($C_{28}H_{33}$ ClN_2O·2.25TFA·0.5H_2O) C, H, N, Cl, F.

4-(4-Chlorophenyl)-4-hydroxy-1-[5-(1,5-cyclohexadien-1-yl)-5-phenylpentyl]piperidine (8). To a solution of (3-benzyloxypropyl)triphenylphosphonium bromide (4.9 g, 10 mmol) in anhydrous THF (60 mL) under nitrogen was added potassium *tert*-butoxide (1.34 g, 11 mmol). The solution was heated to 70 °C for 3 h and then cooled to ambient temperature. Benzophenone (1.82 g, 10 mmol) was then introduced, and the reaction solution was reheated to 70 °C for 6 h. After cooling to room temperature, the reaction was quenched by pouring into water (200 mL). The product was extracted into ethyl acetate (2 × 200 mL). The combined organic extracts were washed with brine, dried over magnesium sulfate, filtered, and concentrated in vacuo to give a solid which was purified by flash chromatography (ethyl acetate/hexanes) to give an amber oil (2.17 g). This oil was dissolved in ethanol (25 mL), treated with 10% Pd/C (200 mg), and hydrogenated at 50 psi to give 4,4-diphenylbutan-1-ol as an oil (1.31 g). The alcohol was dissolved in methylene chloride (50 mL) with triethylamine (0.41 g, 4.0 mmol) and chilled to −5 °C. Methanesulfonyl chloride (0.46 g, 4.0 mmol) was added dropwise over 10 min. The reaction mixture was stirred at 0 °C for 30 min and then at ambient temperature for 2 h, poured into a separatory funnel, and washed with water (50 mL) and brine (50 mL), then dried over magnesium sulfate, filtered, and

concentrated to an oil (1.32 g). This oil was dissolved in DMF and treated with diisopropyl ethylamine and 4-(4-chlorophenyl)-α-hydroxy piperidine to afford **8** as an offwhite solid (350 mg, 8% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ (TMS) 1.5 (m, 2H), 1.72 (d, 2H), 2.12 (m, 4H), 2.40 (m, 4H), 2.78 (br d, 2H), 6.60 (t, 1H), 7.18 (m, 2H), 7.31 (m, 10H), 7.42 (d, 2H). Anal. ($C_{27}H_{30}ClNO \cdot 0.25H_2O$) C, H, N.

Biological Methods. Radioactivity in binding studies was determined on a Topcount scintillation counter (Packard Instruments). Unlabeled chemokines were from Peprotech (Rocky Hill, NJ). ^{125}I-Labeled chemokines were from NEN Life Science Products.

Chemokine Binding Studies. The binding assays were performed by filtration methods. HEK 293 cells expressing the human CCR1 receptor ($K_D = 1-3$ nM and $B_{max} = (2-3) \times 10^6$ sites/cell) were used as the source of the CCR1 receptor. HEK 293 cells expressing the CCR1 receptor were detached by shaking, washed once in PBS, and resuspended in the assay buffer (130 mM NaCl, 5 mM KCl, 1 mM $MnCl_2$, 50 mM Tris, 30 μg/mL bacitracin, 0.1% BSA, pH 7.4) to about 1.1×10^5 cells/mL. Cells (approximately 8000 cells/assay) were incubated with ^{125}I-MIP-1α, specific activity 2200 Ci/mmol, (approximately 15−20000 cpm/assay), in the presence and absence of varying concentrations of compounds at room temperature for 30−40 min. The reactions were carried out in 96-well v-bottom microtiter plates (Nunc, polypropylene) in a total volume of 100 μL and terminated by harvesting through a GF/B filter plate (Packard) presoaked with 0.3% PEI (Sigma #P-3143) plus 0.5% BSA and washed five times with cold PBS. The radioactivity in each well was determined by scintillation counting in a Topcount (Packard) following addition of 50 μL of scintillation fluid, Scint-20. The nonspecific binding was defined by the binding in the presence of 100 nM of unlabeled MIP-1α. The dose curves for each compound with six concentration points were generated, and IC_{50} values were determined by fitting the data to the log−logit equation (linear) with an EXCEL spreadsheet. The K_i values were then calculated by dividing the IC_{50} by 1.025.

Measurement of Intracellular Calcium Level Using FLIPR (Fluorometric Imaging Plate Reader). HEK-293 cells that express human CCR1 receptor at high levels were plated in the poly-D-lysine coated 96-well black wall plates (Becton Dickinson and Company, Franklin Lakes, NJ) at a density of 80 000 cells/well. After a day in the culture, cells were loaded at 37 °C for 1 h with calcium-sensitive fluorescence dye, Fluo-3, in Hank's buffered salt solution (without phenol red) containing 20 mM Hepes, 3.2 mM $CaCl_2$, 1% fetal bovine serum, 2.5 mM probenecid, 0.04% pluronic acid, and 4 μM Fluo-3. The excess extracellular dye was removed by gently washing four times with Hank's buffered salt solution containing 20 mM Hepes, 0.1% BSA, and 2.5 mM probenecid using Denley cell washer. Cells were pretreated with compounds at various concentrations at 37 °C for 10 min and then stimulated with 30 nM MIP-1α (R&D systems). The change in fluorescence intensity was monitored over a 3 min time period at 37 °C using FLIPR. The dose curves of each compound with six concentrations were generated, and the data was exported to EXCEL file. IC_{50} values were calculated by fitting the data to the log−logit equation (linear) with an EXCEL spreadsheet.

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