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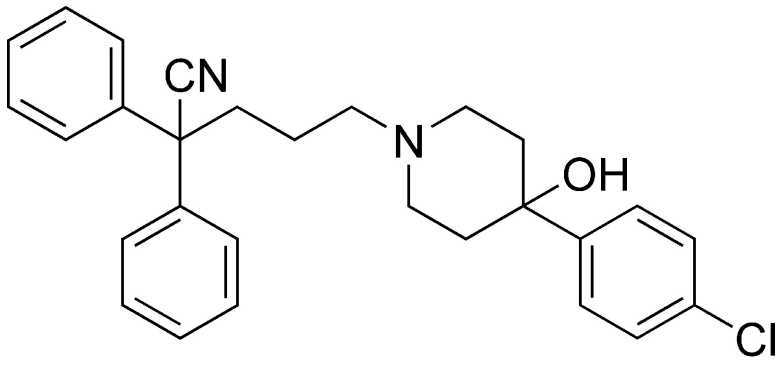

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Synthesis and Structure−Activity Relationship of the First Nonpeptidergic Inverse Agonists for the Human Cytomegalovirus Encoded Chemokine Receptor US28

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Received May 3, 2005

US28 is a human cytomegalovirus (HCMV) encoded G-protein-coupled receptor that signals in a constitutively active manner. Recently, we identified **1** {5-(4-(4-chlorophenyl)-4-hydroxypiperidin-1-yl)-2,2-diphenylpentanenitrile} as the first reported nonpeptidergic inverse agonist for a viral-encoded chemokine receptor. Interestingly, this compound is able to partially inhibit the viral entry of HIV-1. In this study we describe the synthesis of **1** and several of its analogues and unique structure−activity relationships for this first class of small-molecule ligands for the chemokine receptor US28. Moreover, the compounds have been pharmacologically characterized as inverse agonists on US28. By modification of lead structure **1**, it is shown that a 4-phenylpiperidine moiety is essential for affinity and activity. Other structural features of **1** are shown to be of less importance. These compounds define the first SAR of ligands on a viral GPCR (US28) and may therefore serve as important tools to investigate the significance of US28-mediated constitutive activity during viral infection.

Introduction

Human cytomegalovirus (HCMV) is a widespread β-herpesvirus that, like other herpes viruses, persists during the lifetime of the host.[1] Infection of individuals with HCMV is common, reaching a seroprevalence of 50−90% in adults, and is normally without clinical symptoms.[2] However, in immunologically immature or immunocompromised hosts, like premature neonates, AIDS patients, and transplant recipients, the virus can cause serious and even life-threatening disease.[1] Infection with HCMV is furthermore suggested to be associated with vascular diseases such as arterial restenosis, atherosclerosis, and chronic allograft rejection.[3−5]

The genome of human cytomegalovirus encodes four G-protein-coupled receptors (GPCRs), namely, the open reading frames (ORFs) UL33, UL78, US27, and US28.[6] Two of these GPCRs, namely, UL33 and UL78, have counterparts in the genome of both rat CMV (R33 and R78, respectively) and mouse CMV (M33 and M78, respectively), whereas US27 and US28 are specific for HCMV.[7] US28 shows high homology with β mammalian chemokine receptors, binds several CC chemokines with high affinity,[8−10] and is able to sequester CC chemokines from the extracellular environment via endocytosis.[11,12] This feature appears to be a putative strategy of the virus to escape immune surveillance by reducing the immune response to sites of HCMV infection.[13] US28 is able to bind not only CC chemokines but also the membrane-bound CX_3C chemokine CX3CL1/fractalkine, which has been suggested to play a role in the cell to cell transfer of HCMV.[14] Furthermore, upon binding with the CC chemokines CCL2/MCP-1 and CCL5/RANTES, US28 induces migration of vascular smooth muscle cells, which could provide the molecular basis of the role of HCMV in vascular diseases. The migration of smooth muscle cells can also be exploited by HCMV to enhance dissemination of the virus through the body.[15] Similar to other chemokine receptors, such as CCR5 and CXCR4, US28 can act as a coreceptor for HIV-1 entry when coexpressed with CD4.[16] Taken together, US28 is considered as an interesting drug target.

In previous studies we have shown that in transiently transfected COS-7 cells as well as in HCMV-infected cells US28 constitutively activates phospholipase C and the transcription factor NF-κB in an agonist-independent manner.[17,18] Interestingly, the CC chemokines CCL2 and CCL5 do not affect the basal US28 signaling and act as neutral antagonists, whereas the CX_3C chemokine CX3CL1 partially inhibits the constitutive signaling and acts as a partial inverse agonist. Constitutive activity is suggested to be a general characteristic of viral-encoded GPCRs. Besides the HCMV-encoded receptor US28, other viral-encoded receptors also signal in the absence of any ligand, namely, the HHV-8 or Kaposi's sarcoma-associated herpesvirus (KSHV) encoded GPCR ORF74[19] and the M33 gene family members encoded by MCMV, HCMV, and RCMV.[18,20,21] Although the biological relevance of constitutive activity has not been completely elucidated yet, it is believed to play an important role in the pathogenesis of virus infection. This is demonstrated for ORF74, which is a viral oncogene resulting in the development of Kaposi's sarcoma-like lesions in transgenic mice.[22,23]

Inverse agonists are able to inhibit the constitutive activation of GPCRs. The identification of such molecules for viral-encoded GPCRs can be an important tool

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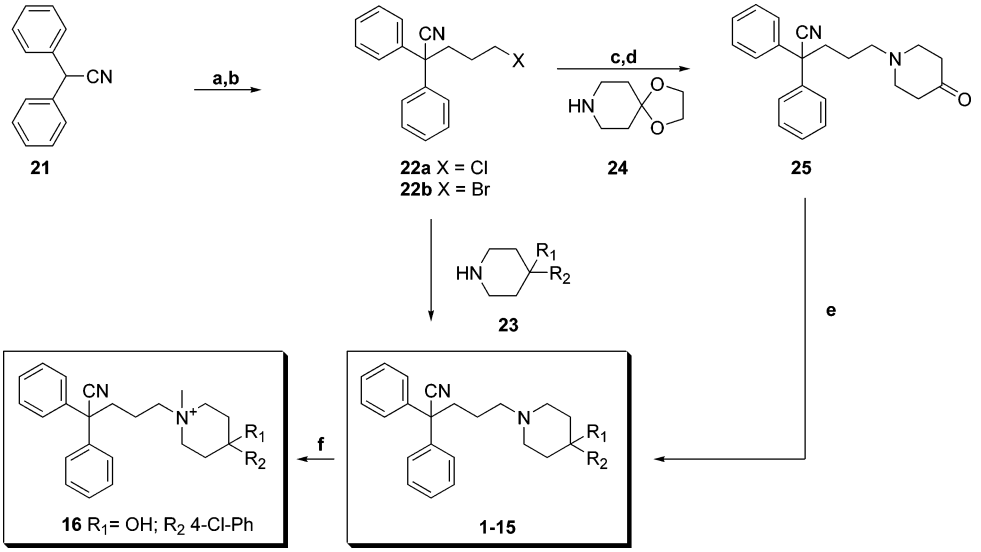
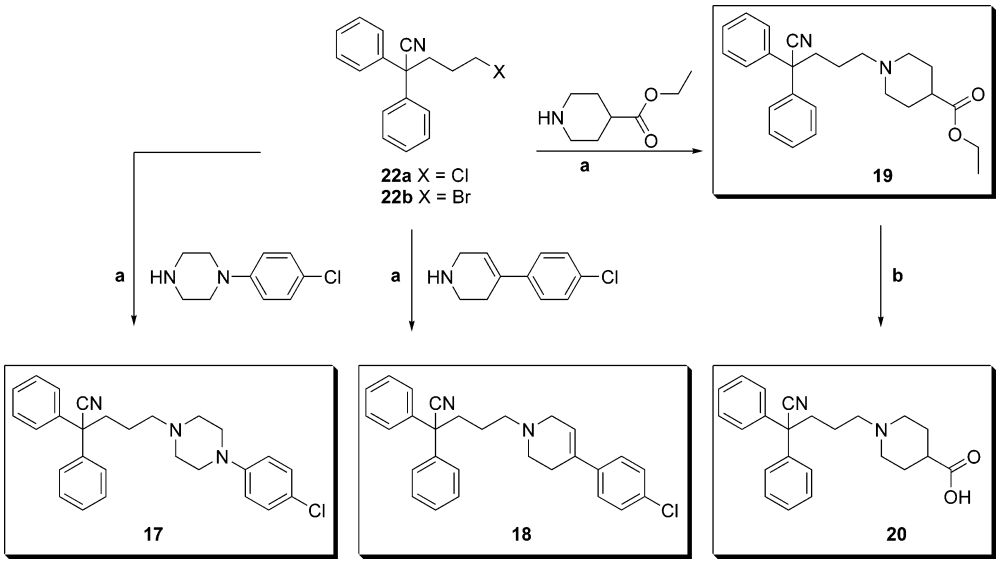
Scheme 1. Synthetic Pathway for **1**−**16**[a]



[a] Reagents and conditions: (a) NaH, DMF, 0 °C; (b) 1,ω-dihalopropane; (c) DMF, K_2CO_3, 90 °C or NaI, Na_2CO_3, CH_3CN, reflux; (d) 6 N HCl, MeOH; (e) R_2MgBr, THF, 0 °C or *n*-BuLi, THF, 0 °C for R_2 = *n*-butyl; (f) MeI, DCM.

Scheme 2. Synthetic Pathway for **17**−**20**[a]



[a] Reagents and conditions: (a) DMF, K_2CO_3, 90 °C or NaI, Na_2CO_3, CH_3CN, reflux; (b) 2 M NaOH, MeOH, reflux.

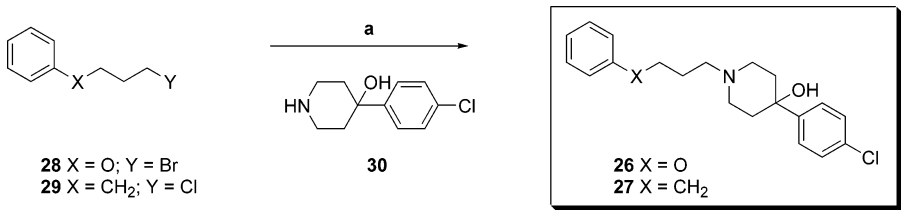
to investigate the significance of constitutive activity and the influence of these receptors in viral infection. Recently, we identified a small nonpeptidergic molecule that acts as a full inverse agonist on US28. Furthermore, this compound is able to inhibit 60% of the US28-mediated HIV entry in cells.[18] This molecule has been previously reported as an antagonist on the human chemokine receptor CCR1[24] and was screened on US28 because of the high sequence homology of this viral receptor with CCR1 (33% identity).[10] In this study the synthesis of VUF2274 and various analogues is reported and we present the first structure−activity relationships for the interaction of these ligands with US28.

Chemistry

For the synthesis of compounds **1**−**20** the following synthetic routes were applied (Schemes 1 and 2). Diphenyl acetonitrile **21** was deprotonated with NaH at 0 °C followed by a reaction with the appropriate 1,ω-dihalopropanes to yield intermediate **22a** or **22b**.[24,25] Compounds **2**, **13**, **17**, and **18** were synthesized by stirring intermediate **22a** in DMF at 90 °C in the presence of the corresponding amine and an excess of K_2CO_3 (method A).[24] The yields after purification were in the range of 15−50%. The reaction conditions were optimized by reacting intermediate **22a** or **22b** with the corresponding piperidine **23** in the presence of NaI, Na_2CO_3, and CH_3CN at reflux temperature (method B)[26] to give the desired products in yields ranging from 23% to 79%.

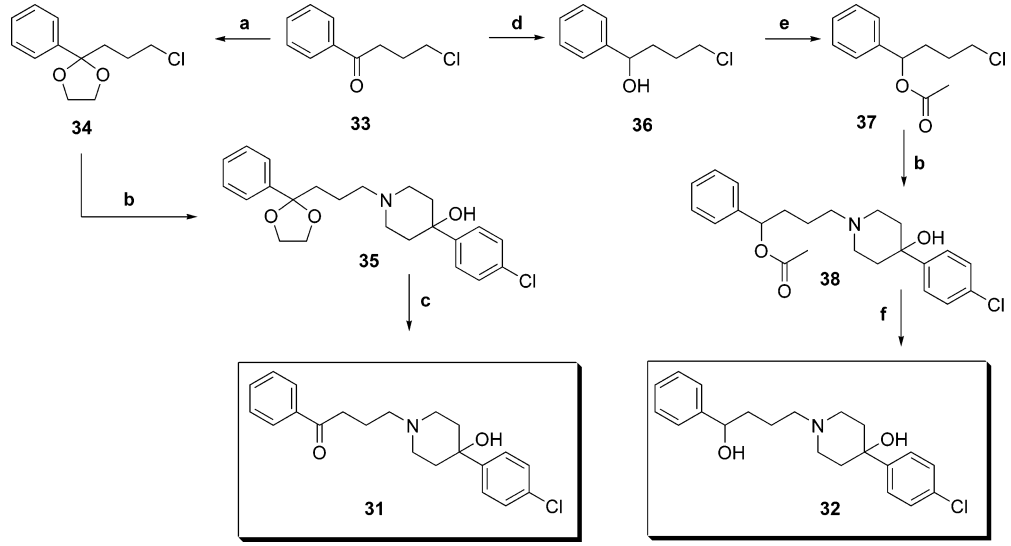
For compounds **5**−**10** the appropriate piperidines were not commercially available. Consequently, **5**−**10** were synthesized according to literature procedures (Scheme 1, method C).[25] Intermediate **22** was reacted with protected piperidone **24**, followed by the deprotection with HCl to give ketone **25**. This was treated with the appropriate Grignard reagents to yield compounds **5**−**9**, whereas the reaction with *n*-BuLi afforded com-

Scheme 3. Synthetic Pathway for **26** and **27**[a]



[a] Reagents and conditions: (a) NaI, Na_2CO_3, CH_3CN, reflux.

Scheme 4. Synthetic Pathway for **31** and **32**[a]



[a] Reagents and conditions: (a) ethylene glycol, *p*-toluenesulfonic acid monohydrate, toluene, reflux; (b) **30**, DMF, K_2CO_3, 90 °C or **30**, NaI, Na_2CO_3, CH_3CN, reflux; (c) HCl, MeOH, reflux; (d) $NaBH_4$, EtOH, 0 °C; (e) acetyl chloride, Et_3N, Et_2O; (f) 10% NaOH, MeOH, reflux.

pound **10**.[25] The yields of products **5**−**10** were moderate to low, ranging from 5% to 42%, because of low yields in the recrystallization step.

4-(Diphenylmethylene)piperidine, which was used for the synthesis of compound **15**, was obtained by acid-catalyzed dehydratation of diphenyl(piperidin-4-yl)-methanol with TFA.[27] The quaternary ammonium salt **16** was synthesized by the methylation of the piperidine nitrogen atom of **1** with methyl iodide.[25]

Target compound **19** was synthesized following method B. The ethyl ester moiety of **19** was hydrolyzed in high yield with 2 M NaOH, resulting in the carboxylic acid derivative **20** (Scheme 2).

Compounds **26** and **27** were prepared via the reaction of intermediates **28** and **29** with 4-chlorophenyl-4-hydroxypiperidine **30** (Scheme 3).

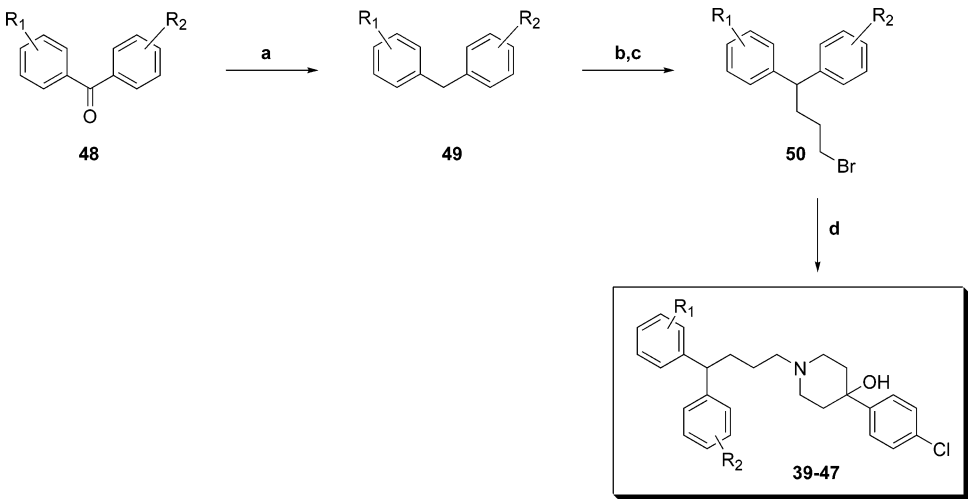
Compounds **31** and **32** were synthesized as depicted in Scheme 4. Butyrophenone derivative **31** was synthesized from commercially available reagents as earlier described.[28] The carbonyl group of **33** was ketalized with ethylene glycol in the presence of *p*-toluenesulfonic acid to afford the protected intermediate **34**. Alkylation of piperidine **30** with **34** and deprotection of the carbonyl group with HCl resulted in product **31**. For the synthesis of compound **32**, 4-chloro-1-phenylbutan-1-one **33** was reduced with $NaBH_4$, resulting in alcohol **36**. Subsequently, the alcohol group was protected with acetyl chloride to give intermediate **37**, which was reacted with 4-(4-chlorophenyl)-4-hydroxypiperidine **30**, resulting in compound **38**. Deprotection with NaOH afforded target compound **32**.

Compounds **39**−**47** were synthesized according to the procedure shown in Scheme 5 (method D). Diphenylmethanes, which were not commercially available, were obtained from benzophenones **48** in the presence of $AlCl_3$ as a Lewis acid and a hydride donor ($NaBH_4$ or *tert*-butylamineborane).[29,30] Intermediates **49** were deprotonated with *n*-BuLi in THF, and the resulting lithium salts reacted with 1,3-dibromopropane to afford bromides **50** in low yields.[31,32] The conversion of 4-methoxydiphenylmethane (**49**, R_1 = 4-OCH_3, R_2 = H) and 3,4-dichlorodiphenylmethane (**49**, R_1 = 3,4-Cl_2, R_2 = H) into the corresponding sodium salts or lithium salts with, respectively, $NaNH_2$ in liquid NH_3 or *n*-BuLi in THF was not successful. However, deprotonation of 3,4-dichlorodiphenylmethane was achieved with LDA in the presence of HMPA. For the deprotonation of 4-methoxydiphenylmethane, the base strength of *n*-BuLi was not sufficient and a mixture of *n*-BuLi and potassium *tert*-butoxide in THF was used. Piperidine **30** was alkylated with intermediate **50** in CH_3CN in the presence of NaI and Na_2CO_3 to give the desired products **39**−**47**.

Pharmacological Results and Discussion

To identify a potential inverse agonist acting on US28, several GPCR-directed ligands were screened for their ability to modulate the basal signaling of this receptor. This resulted in the identification of compound **1** as the first nonpeptidergic inverse agonist acting on US28, as reported in an earlier paper describing US28 pharmacology.[18] It is noted that compound **1** has previously

Scheme 5. Synthetic Pathway for **39**−**47**[a]

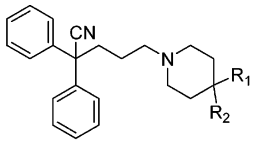


[a] Reagents and conditions: (a) $AlCl_3$, $NaBH_4$, THF, 0 °C or $AlCl_3$ $(CH_3)_3CNH_2.BH_3$, THF, 0 °C; (b) *n*-BuLi, THF, −20 °C or LDA, HMPA, THF, −78 °C or *n*-BuLi, $(CH_3)_3COK$, −100 °C, THF; (c) 1-bromo-3-chloropropane; (d) **30**, NaI, Na_2CO_3, CH_3CN, reflux.

been reported as a potent antagonist for the human chemokine receptor CCR1, but it has no effects on the human chemokine receptors CCR5, CCR2, and CXCR4. Additionally, the selectivity of **1** was tested by screening against a number of human GPCRs and an at least 250-fold selectivity for the CCR1 chemokine receptor was observed. As expected, the only cross-reactivity of compound **1** was demonstrated for several biogenic amine neurotransmitter receptors.[24] Recently, it was also shown that this molecule binds to the human chemokine receptor CCR3 with micromolar affinity.[33] In all, we consider **1** to be a unique and interesting lead structure for the development of ligands directed toward the new class of viral GPCRs and a good tool to study viral GPCRs in a variety of assays with HCMV-infected cells. Thus, compound **1** was used as a starting point for lead optimization, and several analogues were synthesized in order to investigate the first structure−activity relationship for inverse agonism on US28. All the synthesized compounds were evaluated for their potential to dose-dependently displace [^{125}I]CCL5 binding in COS-7 cells expressing US28. The effect on the US28-mediated constitutive inositol phosphate production in transiently expressed COS-7 cells was investigated for a selection of compounds. It is evident from the results in Tables 1−4 that the IC_{50} and EC_{50} values obtained from the binding assay and the functional assay, respectively, correlate well with each other. To determine the specificity of action, the selected compounds were also tested on the constitutively active KSHV-encoded GPCR ORF74. The observed inositol phosphate production in ORF74 expressing cells was not affected up to a concentration of 10 μM (data not shown), indicating that the influence of these compounds on the inositol phosphate production was selective for US28.

To study the importance of the 4-chlorophenyl-4-hydroxypiperidine moiety, various substituted piperidine moieties were synthesized (Table 1). The influence of the substitution pattern of the phenyl ring was investigated by the substitution of the *p*-chloro substituent of **1** for substituents with different electronic and lipophilic properties in compounds **2**−**7**. Interestingly, all the compounds with variations in the para position, including the unsubstituted analogue **2**, were found to be less potent. The introduction of an additional substituent at the meta position (*m*-trifluoro or *m*-chloro substituents in **3** and **6**, respectively) did not result in compounds with a higher affinity. For the human chemokine receptor CCR1, there is a 2-fold improvement in K_i value when *p*-chloro is replaced by a *p*-bromo substituent,[25] but on the viral-encoded receptor US28 the bromo substitution in **4** causes a 2-fold decrease in binding affinity.

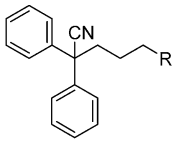
Table 1. Chemical Structures and Pharmacological Properties of Compounds **1**−**14**, **19**, and **20** for the HCMV-Encoded Receptor US28[a]

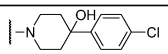
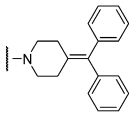
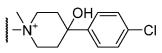
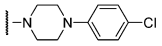
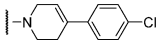


no.	VUF	R_1	R_2	IC_{50} (μM)[b]	EC_{50} (μM)[c]
1	2274	OH	4-Cl-phenyl	9.3 (8.7 -10.0)	3.2 (2.5 – 4.0)
2	5660	OH	phenyl	35.5 (26.9 – 46.8)	28.2 (26.3 – 30.2)
3	5930	OH	3-CF_3-4-Cl-phenyl	45.7 (38.9 – 53.7)[d]	n.d.
4	5931	OH	4-Br-phenyl	19.5 (15.8 – 24.0)[d]	n.d.
5	5753	OH	4-OCH_3-phenyl	72.4 (55.0 – 95.5)	28.8 (25.7 – 32.4)[d]
6	5754	OH	3,4-Cl_2-phenyl	38.0 (28.2 – 51.3)	n.d.
7	5764	OH	4-CH_3-phenyl	60.3 (39.8 – 91.2)	n.d.
8	5786	OH	benzyl	63.1 (50.1 – 79.4)	n.d.
9	5787	OH	methyl	> 100	> 100
10	5765	OH	*n*-butyl	> 100	> 100
11	5744	OH	H	> 100	> 100
12	5929	CN	phenyl	> 100	> 100
13	5662	H	4-Cl-phenyl	10.5 (8.1 – 13.5)	13.8 (13.5 – 14.1)
14	5720	H	C(O)NH_2	>100[d]	> 100[d]
19	5719	H	COOEt	34.7 (26.3 – 45.7)	> 100
20	5718	H	COOH	> 100[d]	> 100

[a] The values are represented as the mean and the interval of the IC_{50} and EC_{50} values of at least three independent experiments, unless otherwise indicated. [b] [^{125}I]CCL5 displacement. [c] Inhibition of [^{3}H]inositol phosphate production. nd = not determined. [d] Result of two independent experiments.

Table 2. Chemical Structures and Pharmacological Properties of Compounds **1** and **15**−**18** for the HCMV-Encoded Receptor US28[a]



no.	VUF	R	IC_{50} (μM)[b]	EC_{50} (μM)[c]
1	2274		9.3 (8.7 -10.0)	3.2 (2.5 – 4.0)
15	5875		> 100	> 100
16	4999		> 100	> 100
17	5658		> 100	> 100
18	5664		> 100	> 100

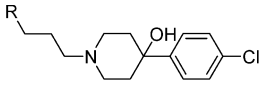
[a] The values are represented as the mean and the interval of the IC_{50} and EC_{50} values of at least three independent experiments, unless otherwise indicated. [b] [^{125}I]CCL5 displacement. [c] Inhibition of [^{3}H]inositol phosphate production.

The binding affinities of the unsubstituted analogue **2** and compound **8**, with a benzyl group at the 4-position of the piperidine ring, were comparable, but both affinities were lower than that of lead compound **1**. Apparently, the introduction of an additional methylene group has no positive effect on the affinity of the compound. Removal of the 4-chlorophenyl group in compound **11** or substitution of this group by a CH_3 group in **9** or *n*-butyl chain in **10** resulted in complete loss of affinity. This suggests that a phenyl ring at the 4-position is of importance.

Next, the role of the 4-hydroxy group was studied in compounds **12** and **13**. It is noted that the 4-chlorophenyl-4-hydroxypiperidine moiety is a structural motif that is also present in, for example, haloperidol. For this antipsychotic drug it is known that the 4-hydroxy group is responsible for the conversion to potentially neurotoxic metabolites.[34,35] In this respect, it is interesting to see that the affinity and activity on US28 did not change significantly after removal of the 4-hydroxy group in **13**. Substitution of the hydroxy group of **2** into a nitrile group in compound **12** resulted in a complete loss of both affinity and activity.

The influence of the piperidine ring was investigated by the synthesis of compounds **15**−**18** (Table 2). Previously, we described that the basic nitrogen atom of the piperidine ring of **1** probably has an important interaction with a glutamic acid residue in transmembrane 7 (Glu^{277}).[18] For this reason, the nitrogen atom at this position was maintained. But substitution of the piperidine ring into a piperazine ring in **17** or tetrahydropyridine moiety in **18** abolished activity. The loss of activity of both compounds might be due to a change in conformation of the 4-chlorophenyl ring through which the interaction of the aromatic moiety with the receptor is lost. Introduction of the bulky 4-(diphenylmethylene)-piperidine moiety of analogue **15** was detrimental for both affinity and potency.

Table 3. Chemical Structures and Pharmacological Properties of Compounds **1**, **26**, **27**, **31**, **32**, and **39** for the HCMV-Encoded Receptor US28[a]



no.	VUF	R	IC_{50} (μM)[b]	EC_{50} (μM)[c]
1	2274		9.3 (8.7 -10.0)	3.2 (2.5 – 4.0)
39	5667		6.5 (6.0 – 6.9)	4.2 (3.1 – 5.6)
26	5714		61.7 (57.5 – 66.1)[d]	n.d
27	5746		14.8 (13.8 – 15.8)	n.d.
31	5666		> 100[c]	n.d.
32	(±) 5715		> 100	> 100

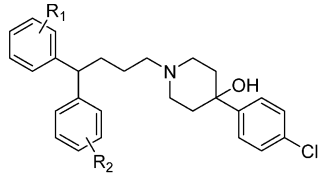
[a] The values are represented as the mean and the interval of the IC_{50} and EC_{50} values of at least three independent experiments, unless otherwise indicated. [b] [^{125}I]CCL5 displacement. [c] Inhibition of [^{3}H]inositol phosphate production. nd = not determined. [d] Result of two experiments.

Methylation of the piperidine nitrogen atom of compound **1** gave quaternary ammonium salt **16**. Interestingly, this variation resulted in complete loss of binding affinity and activity on US28. For the human chemokine receptor CCR1 this modification resulted in a 6.5-fold increase in binding affinity,[25] clearly illustrating selectivity of compound **16** in favor of the CCR1 chemokine receptor. This indicates that there is a difference in structure−activity relationships of these compounds for both receptors, like that shown earlier in this section for compound **4**. Taken together, several analogues were synthesized to optimize the piperidine motif in the molecule, but all the variations in this part of the molecule resulted in a loss of affinity and potency.

Next, the influence of the diphenylacetonitrile moiety was investigated (Tables 3 and 4). The influence of the two phenyl rings was investigated by removal of one of the phenyl rings in compound **27** or replacement by more hydrophilic groups, namely, a carbonyl group in compound **31** and a hydroxy group in compound **32**. Previously, it was suggested that a bulky lipophilic moiety is important at this position,[18] but we observed that removal of a phenyl ring as in **27** resulted in a binding affinity comparable to that of lead compound **1**. In contrast, the more hydrophilic groups in **31** and **32** resulted in loss of effect. Also, the introduction of an isosteric oxygen atom in the butyl chain in compound **26** did not improve affinity.

Interestingly, the nitrile group of compound **1** is not essential in the molecule because its removal, resulting in compound **39**, did not affect the activity of the compound (Table 4). Compounds **40**−**42** and **44**−**47** possess a chiral center as a result of the substitution in one or both of the phenyl rings. The two enantiomers were not separated, and the activity was thus determined for the racemic mixtures (Table 4). Compounds **40**−**42** and **46** were synthesized to determine the effects of the introduction of para substituents with different

Table 4. Chemical Structures and Pharmacological Properties of Compounds **39**−**47** for the HCMV-Encoded Receptor US28[a]



no.	VUF	R_1	R_2	IC_{50} (μM)[b]	EC_{50} (μM)[c]
39	5667	H	H	6.5 (6.0 – 6.9)	4.2 (3.1 – 5.6)
40	(±) 5857	4-OCH_3	H	15.5 (13.2 – 18.2)	n.d
41	(±) 5858	3,4-Cl_2	H	15.8 (14.5 – 17.4)	n.d.
42	(±) 5859	4-Cl	H	17.0 (16.6 – 17.4)	n.d.
43	5860	4-Cl	4-Cl	17.4 (16.2 – 18.6)	n.d.
44	(±) 5861	2-CH_3	H	5.5 (5.0 – 6.0)	9.8 (9.5 – 10.0)[d]
45	(±) 5862	3-CH_3	H	11.5 (8.7 – 15.1)	n.d.
46	(±) 5863	4-CH_3	H	30.9 (27.5 – 34.7)	11.5 (11.0 – 12.0)[d]
47	(±) 5864	4-Cl	4-phenyl	> 100	> 100

[a] The values are represented as the mean and the interval of the IC_{50} and EC_{50} values of at least three independent experiments, unless otherwise indicated. [b] [^{125}I]CCL5 displacement. [c] Inhibition of [^{3}H]inositol phosphate production. nd = not determined. [d] Result of two independent experiments.

electronic and lipohilic properties in the two aromatic rings. The variations in the substitution pattern in the para position did not significantly influence the activity of the compounds. Also, the introduction of an additional *p*-chloro atom (compound **43**) did not increase the affinity of the compound for US28.

The differences in binding affinities between the methyl-substituted analogues **44**−**46** and the unsubstituted analogue **39** reveal a slight preference for *o*-methyl substitution. This could be caused by the restricted conformational freedom of the substructure in compound **44**. *o*-Methyl substitution of a similar aromatic benzhydryl sytem was previously described for a series of histamine H_1 receptor antagonists. It is suggested that the *o*-methyl analogue of diphenhydramine has a markedly reduced antihistamine activity because the methyl group in the ortho position prevents the molecule from adopting the conformation necessary for antihistamine activity.[36]

The influence of a bulky aromatic substituent in the para position was investigated by the synthesis of analogue **47**. The additional *p*-phenyl group resulted in a loss of affinity and potency, and it seems that bulky substituents are not allowed at this position.

Conclusions

This is the very first study of small molecules acting as inverse agonists on US28. We described the synthesis of compound **1** and different analogues, which were evaluated for their binding affinity by displacement of [^{125}I]CCL5. Moreover, a selection of compounds was evaluated for their functionality by measuring the effect on the US28-mediated constitutive inositol phosphate production. This resulted in unique structure−activity relationships for the first small-molecule US28 receptor ligands.

With this study we have acquired important new insights about the first inverse agonists acting on US28. These structural insights will be used to develop new compounds with an improved affinity and potency as well as a better selectivity for this receptor. These small molecules may serve as important tools to investigate the significance of the constitutive activity and the role of US28 during viral infection.

Experimental Section

Chemistry. General Procedures. ^{1}H NMR spectra were recorded on a Bruker AC-200 (200 MHz) spectrometer with tetramethylsilane as internal standard. J. T. Baker silica gel was used for flash chromatography. Mass spectra were recorded on a Finnigan MAT-90 mass spectrometer. Melting points were measured on an Electrothermal IA9200 apparatus and were uncorrected. Elemental analyses were performed by Microanalytisches Labor Pascher, Remagen-Bandorf, Germany, and the results were within ±0.4% of the theoretical values unless otherwise stated. The solvents were dried according to standard procedures. All reactions were performed under an atmosphere of dry nitrogen.

General Method A. 5-(4-(4-Chlorophenyl)piperazin-1-yl)-2,2-diphenypentanenitrile (17). A solution of **22a** (0.51 g, 1.88 mmol), which was synthesized according to literature procedure,[24] 4-chlorophenylpiperazine (0.45 g, 1.67 mmol), and K_2CO_3 (3.53 g, 25.6 mmol) in DMF (25 mL) was stirred overnight at 90 °C. The solvent was removed in vacuo, and the residue was dissolved in water (25 mL) followed by an extraction with EtOAc (3 × 10 mL). The combined organic layers were washed with water (2 × 10 mL), dried over anhydrous $MgSO_4$, and filtered. After evaporation in vacuo, the residue was purified by flash chromatography (0−100% EtOAc in DCM) to give 107 mg (15%) of **17** as a light-yellow solid. Mp: 126.2−126.7 °C. ^{1}H NMR ($CDCl_3$): δ 1.52−1.71 (m, 2H), 2.37−2.51 (m, 8H), 3.11 (m, 4H), 6.80 (d, 2H, J = 9.0 Hz), 7.15−7.40 (m, 12H). MS ESI *m/z*: 431.0 $(M + H)^+$. Anal. ($C_{27}H_{28}ClN_3$) C, H, N.

General Method B. 5-(4-(4-Chlorophenyl)-4-hydroxypiperidin-1-yl)-2,2-diphenylpentanenitrile (1). A solution of **22b** (0.94 g, 3.00 mmol), which was synthesized according to literature procedure,[25] 4-chlorophenyl-4-hydroxypiperidine **30** (0.67 g, 3.15 mmol), NaI (0.50 g, 3.31 mmol), and Na_2CO_3 (0.64 g, 6.04 mmol) in CH_3CN (25 mL) was refluxed overnight. The solvent was removed in vacuo, and the residue was diluted with water (50 mL) followed by an extraction with DCM (3 × 30 mL). The combined organic layers were dried over anhydrous $MgSO_4$, filtered, and evaporated in vacuo. Purification by flash chromatography (0−100% EtOAc in DCM) and crystallization from EtOAc gave 1.04 g (78%) of **1** as a white solid. Mp: 133.6−135.2 °C. ^{1}H NMR ($CDCl_3$): δ 1.55−1.70 (m, 4H), 1.93−2.12 (m, 3H), 2.28−2.50 (m, 6H), 2.61−2.78 (m, 2H), 7.18−7.48 (m, 14H). MS ESI *m/z*: 446.9 $(M + H)^+$. Anal. ($C_{28}H_{29}N_2ClO$·0.21EtOAc) C, H, N.

General Method C. 5-(4-(4-Methoxyphenyl)-4-hydroxypiperidin-1-yl)-2,2-diphenylpentanenitrile (5). A solution of (4-methoxyphenyl)magnesium bromide[37] (7.7 mL, 0.5 M in dry Et_2O, 3.6 mmol) was cooled to 0 °C, and a solution of **25** (1.00 g, 3.00 mmol), which was synthesized according to literature procedure,[25] dissolved in dry THF (15 mL) was added in one portion via a dropping funnel. The reaction mixture was stirred at room temperature for 4 h, and the reaction was quenched with water (30 mL). This solution was acidified with 1 N HCl, stirred for 15 min and basified with K_2CO_3. The product was extracted with EtOAc (3 × 100 mL), and the combined organic layers were washed with water (2 × 100 mL) and brine (100 mL), dried over anhydrous $MgSO_4$, filtered, and evaporated under reduced pressure. Purification by flash chromatography (EtOAc) afforded 780 mg (59%) of **5**. Recrystallization of one batch from Et_2O gave 190 mg (14%) of **5** as white crystals. Mp: 84.7−86.6 °C. ^{1}H NMR ($CDCl_3$): δ 1.69−1.74 (m, 5H), 2.02−2.18 (m, 2H), 2.35−2.50 (m, 6H), 2.68−2.79 (m, 2H), 3.78 (s, 3H), 6.86 (d, 2H, J = 8.9 Hz), 7.24−7.42 (m, 12H). MS ESI *m/z*: 441.4 $(M + H)^+$. Anal. ($C_{29}H_{32}N_2O_2$) C, H, N.

General Method D. 4-(4-Chlorophenyl)-1-(4-phenyl-4-*o*-tolylbutyl)piperidin-4-ol (50). (i) A solution of 2-methylbenzophenone (5.34 g, 27.2 mmol) in dry THF (125 mL) was cooled to 0 °C, and subsequently $AlCl_3$ (10.06 g, 75.4 mmol) and $NaBH_4$ (5.26 g, 139 mmol) were added. The reaction mixture was heated and refluxed for 3 h. Next, the mixture was cooled to 0 °C and diluted carefully by a dropwise addition of water (100 mL). After separation of the organic layer, the water layer was extracted with EtOAc (3 × 100 mL). The combined organic layers were washed with water (3 × 150 mL) and brine (150 mL), dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure. Purification by flash chromatography (hexane) gave 3.81 g (77%) of 2-methyldiphenylmethane as a colorless oil. ^{1}H NMR ($CDCl_3$): δ 2.37 (s, 3H), 4.11 (s, 2H), 7.20−7.46 (m, 9H).

(ii) In a dry atmosphere a solution of 2-methyldiphenylmethane (1.87 g, 10.3 mmol) in dry THF (20 mL) was cooled to −20 °C, and *n*-BuLi (6.40 mL, 1.6 M in hexane, 10.2 mmol) was added. The solution was stirred for 1 h and subsequently added slowly to a solution of 1,3-dibromopropane (2.30 mL, 22.7 mmol) in dry THF (35 mL) at −76 °C. The mixture was allowed to warm to room temperature and was stirred for 30 min. The solvent was evaporated under reduced pressure. Water (25 mL) was added to the residue, and the water layer was extracted with EtOAc (3 × 25 mL). The combined organic layers were washed with water (3 × 50 mL) and brine (50 mL), dried over anhydrous $MgSO_4$, filtered, and evaporated under reduced pressure. The residue was purified by flash chromatography (hexane) to give 0.81 g (26%) of bromide **50** (R_1 = 2-Me; R_2 = H) as a colorless oil. ^{1}H NMR ($CDCl_3$): δ 1.87−2.00 (m, 2H), 2.18−2.30 (m, 2H), 2.34 (s, 3H), 3.44 (t, 2H, J = 6.6 Hz), 4.18 (t, 1H, J = 8.1 Hz), 7.18−7.42 (m, 9H).

(iii) **50** was reacted with 4-chlorophenyl-4-hydroxypiperidine **30** (0.70 g, 3.3 mmol) according to method B to give 834 mg (72%) of **44** as white crystals after recrystallization from EtOAc. Mp: 143.1−144.4 °C. ^{1} H NMR ($CDCl_3$): δ 1.55−1.69 (m, 5H), 2.00−2.12 (m, 4H), 2.25 (s, 3H), 2.33−2.44 (m, 4H), 2.71−2.78 (m, 2H), 4.09 (t, 1H, J = 7.6 Hz), 7.07−7.33 (m, 11H), 7.41 (d, 2H, J = 8.8 Hz). MS ESI *m/z*: 434.4 $(M + H)^+$. Anal. ($C_{28}H_{32}ClNO$) C, H, N, Cl.

5-(4-Hydroxy-4-phenylpiperidin-1-yl)-2,2-diphenylpentanenitrile (2). Following method A using **22a** (0.51 g, 1.90 mmol) and 4-hydroxy-4-phenylpiperidine (0.34 g, 1.93 mmol) gave 319 mg (42%) of **2** as a white solid.[24] Mp: 73.6−74.7 °C. ^{1}H NMR ($CDCl_3$): δ 1.51−1.80 (m, 4H), 2.06−2.19 (m, 3H), 2.30−2.50 (m, 6H), 2.64−2.78 (m, 2H), 7.12−7.51 (m, 15H). MS ESI *m/z*: 411.3 $(M + H)^+$. Anal. ($C_{28}H_{30}N_2O \cdot 0.36H_2O$) C, H, N.

5-(4-(3-Chloro-4-trifluoromethylphenyl)-4-hydroxypiperidin-1-yl)-2,2-diphenylpentanenitrile Hydrochloride (3). Using **22a** (0.65 g, 2.40 mmol) and 4-(4-chloro-3-(trifluoromethyl)phenyl)-4-piperidinol (0.56 g, 2.01 mmol) gave 0.93 g (90%) of the product as a thick oil. This was dissolved in MeOH (5 mL), and subsequently 6 N HCl (0.15 mL) and Et_2O (20 mL) were added dropwise while stirring. The hydrochloride salt was isolated by filtration and recrystallized from Et_2O/MeOH. This gave 666 mg (67%) of **3** as a white solid. Mp: 231.2−234.0 °C (dec). ^{1}H NMR ($CDCl_3$): δ 1.61−1.82 (m, 5H), 2.07−2.26 (m, 2H), 2.31−2.53 (m, 6H), 2.70−2.89 (m, 2H), 7.24−7.46 (m, 13H). MS ESI *m/z*: 513.4 $(M + H)^+$. Anal. ($C_{29}H_{29}Cl_2N_2F_3O$) C, H, N.

5-(4-(4-Bromophenyl)-4-hydroxypiperidin-1-yl)-2,2-diphenylpentanenitrile (4). Following method B using **22a** (0.65 g, 2.39 mmol) and 4-(4-bromophenyl)-4-hydroxypiperidine (0.51 g, 2.01 mmol) gave 819 mg (83%) of **4** as a white solid. Mp: 131.6−132.5 °C. ^{1}H NMR ($CDCl_3$): δ 1.61−1.82 (m, 5H), 2.07−2.26 (m, 2H), 2.31−2.53 (m, 6H), 2.70−2.89 (m, 2H), 7.24−7.46 (m, 14H). MS ESI *m/z*: 490.8 $(M + H)^+$. Anal. ($C_{28}H_{29}N_2BrO \cdot 0.34DCM$) C, H, N.

5-(4-(3,4-Dichlorophenyl)-4-hydroxypiperidin-1-yl)-2,2-diphenylpentanenitrile (6). Following method C using **25** (1.00 g, 3.00 mmol) and (3,4-dichlorophenyl)magnesium bromide[37] (3.3 mL, 1.1 M in dry Et_2O, 3.6 mmol) followed by purification by flash chromatography (0−100% EtOAc in DCM) afforded 944 mg of **6**. Recrystallization from Et_2O gave 212 mg (15%) of **6** as white crystals. Mp: 102.0−103.4 °C. ^{1}H NMR ($CDCl_3$): δ 1.59−1.68 (m, 5H), 2.01−2.12 (m, 2H), 2.28−2.49 (m, 6H), 2.68−2.79 (m, 2H), 7.24−7.41 (m, 12H), 7.59 (d, 1H, J = 2.1 Hz). MS ESI *m/z*: 479.5 $(M + H)^+$. Anal. ($C_{28}H_{28}Cl_2N_2O$) C, H, N, Cl.

5-(4-Hydroxy-4-*p*-tolylpiperidin-1-yl)-2,2-diphenylpentanenitrile Hydrochloride (7). Following method C using **25** (1.00 g, 3.00 mmol) and *p*-tolylmagnesium bromide[37] (3.6 mL, 1.0 M in dry Et_2O, 3.6 mmol) gave 280 mg of a thick oil. The compound was converted to the hydrochloride salt as described for **3**. One batch was recrystallized from Et_2O/MeOH to give 141 mg (10%) of **7** as a white solid. Mp: 225.0−226.3 °C. ^{1}H NMR ($CDCl_3$): δ 1.81−2.03 (m, 5H), 2.30 (s, 3H), 2.62−2.71 (m, 2H), 2.75−2.92 (m, 2H), 3.01−3.22 (m, 6H), 7.13 (d, 2H, J = 8.1 Hz), 7.24−7.45 (m, 12H), 12.15 (br s, 1H). MS ESI *m/z*: 425.4 $(M + H)^+$. Anal. ($C_{29}H_{33}ClN_2O$) C, H, N, Cl.

5-(4-Benzyl-4-hydroxypiperidin-1-yl)-2,2-diphenylpentanenitrile Hydrochloride (8). Following method C using **25** (1.00 g, 3.00 mmol) and benzylmagnesium bromide[37] (3.4 mL, 1.1 M in dry Et_2O, 3.7 mmol) gave 0.147 g of thick oil. The compound was converted to the hydrochloride salt as described for **3**. One batch was recrystallized from Et_2O/MeOH to give 86 mg (5%) of **8** as a white solid. Mp: 121.8−123.8 °C. ^{1}H NMR ($CDCl_3$): δ 1.51−1.65 (m, 3H), 1.90−1.99 (m, 2H), 2.38−2.51 (m, 2H), 2.59−2.65 (m, 2H), 2.80 (s, 2H), 2.89−3.00 (m, 4H), 3.14−3.22 (m, 2H), 7.16−7.38 (m, 15H), 12.02 (br s, 1H). MS ESI *m/z*: 425.5 $(M + H)^+$. Anal. ($C_{29}H_{33}ClN_2O \cdot 1.0H_2O$) C, H, N, Cl.

5-(4-Hydroxy-4-methylpiperidin-1-yl)-2,2-diphenylpentanenitrile Hydrochloride (9). Following method C using **25** (1.00 g, 4.00 mmol) and methylmagnesium iodide[37] (2.8 mL, 1.3 M in dry Et_2O, 3.6 mmol) followed by purification by flash chromatography (0−7% EtOH in DCM) gave 0.50 g of thick oil. The compound was converted to the hydrochloride salt as described for **3**. Recrystallization from Et_2O/MeOH gave 441 mg (42%) of **9** as a white solid. Mp: 192.7−193.5 °C. ^{1}H NMR ($CDCl_3$): δ 1.32 (s, 3H), 1.63−1.69 (m, 3H), 1.88−2.05 (m, 2H), 2.28−2.44 (m, 2H), 2.62 (t, 2H, J = 7.9 Hz), 2.90−3.21 (m, 6H), 7.24−7.43 (m, 10H), 11.88 (br s, 1H). MS ESI *m/z*: 349.4 $(M + H)^+$. Anal. ($C_{23}H_{29}ClN_2O$) C, H, N, Cl.

5-(4-Butyl-4-hydroxypiperidin-1-yl)-2,2-diphenylpentanenitrile Hydrochloride (10). This compound was synthesized as described in the literature[25] and converted to the hydrochloride salt as described for **3**. One batch was recrystallized from Et_2O/MeOH to afford 304 mg (18%) of **10** as a white solid. Mp: 174.2−176.0 °C (dec). ^{1}H NMR ($CDCl_3$): δ 0.80−0.93 (t, 3H, J = 4.6 Hz), 1.18−1.40 (m, 4H), 1.48−1.71 (m, 5H), 1.88−2.08 (m, 2H), 2.28−2.43 (m, 2H), 2.60−70 (m, 2H), 2.89−3.22 (m, 6H), 7.24−7.44 (m, 10H), 12.02 (br s, 1H). MS ESI *m/z*: 391.4 $(M + H)^+$. Anal. ($C_{26}H_{35}ClN_2O$) C, H, N, Cl.

5-(4-Hydroxypiperidin-1-yl)-2,2-diphenylpentanenitrile (11). Following method B using **22b** (0.95 g, 3.01 mmol) and 4-hydroxypiperidine (0.38 g, 3.75 mmol) followed by purification by flash chromatography (10−50% MeOH in EtOAc) afforded 374 mg (37%) of **11** as a white solid.[25] Mp: 114.8−115.8 °C. ^{1}H NMR ($CDCl_3$): δ 1.39−1.68 (m 5H), 1.80−1.91 (m, 2H), 1.95−2.11 (m, 2H), 2.31−2.44 (m, 4H), 2.59−2.71 (m, 2H), 3.59−3.72 (m, 1H), 7.22−7.42 (m, 10H). MS ESI *m/z*: 335.3 $(M + H)^+$. Anal. ($C_{22}H_{26}N_2O$) C, H, N.

1-(4-Cyano-4,4-diphenylbutyl)-4-phenylpiperidine-4-carbonitrile (12). Following method B using **22a** (0.65 g, 2.40 mmol) and 4-phenylpiperidine-4-carbonitrile (0.45 g, 2.00 mmol) followed by crystallization from DCM/Et_2O gave 599 mg (66%) of **12** as a white solid. Mp: 137.5−138.7 °C. ^{1}H NMR ($CDCl_3$): δ 1.59−1.71 (m, 2H), 2.02−2.08 (m, 4H), 2.33−2.49 (m, 6H), 2.83−2.97 (m, 2H), 7.24−7.50 (m, 15H). MS ESI *m/z*: 420.7 $(M + H)^+$. Anal. ($C_{29}H_{29}N_3$) C, H, N.

5-(4-(4-Chlorophenyl)piperidin-1-yl)-2,2-diphenylpentanenitrile (13). A solution of 4-(4-chlorophenyl)-1,2,3,6-tetrahydropyridine hydrochloride (1.00 g, 4.35 mmol) and 5% Pd/C (0.100 g) in EtOH (25 mL) was stirred for 4 h under hydrogen. The solution was filtered and evaporated under reduced pressure. The residue was used without further

purification, dissolved in DMF, and reacted following method A using **22a** (0.63 g, 2.34 mmol). This afforded 954 mg (51%) of **13** as a white solid. Mp: 112.6−113.4 °C. ^{1}H NMR ($CDCl_3$): δ 1.55−1.83 (m, 6H), 1.89−2.08 (m, 2H), 2.35−2.56 (m, 5H), 2.89−3.01 (m, 2H), 7.09−7.43 (m, 14H). MS ESI *m/z*: 430.0 $(M + H)^+$. Anal. ($C_{28}H_{29}ClN_2$) C, H, N.

1-(4-Cyano-4,4-diphenylbutyl)piperidine-4-carboxylic Acid Amide (14). Following method B using **22b** (0.94 g, 3.00 mmol) and piperidine-4-carboxylic acid amide (0.39 g, 3.00 mmol) gave 796 mg (73%) of **14** as an orange oil. ^{1}H NMR ($CDCl_3$): δ 1.61−2.52 (m, 13 H), 2.76−2.99 (m, 2H), 5.57 (br s, 2H), 7.24−7.45 (m, 10H). MS ESI *m/z*: 362.3 $(M + H)^+$. Anal. ($C_{25}H_{27}N_2O_2 \cdot 0.44DCM$) C, H, N.

5-(4-(Diphenylmethylene)piperidin-1-yl)-2,2-diphenylpentanenitrile Hydrochloride (15). Following method B using **22b** (0.92 g, 2.91 mmol) and 4-(diphenylmethylene)-piperidine[27] (0.72 g, 2.86 mmol) followed by purification by flash chromatography (0−25% EtOAc in DCM) gave a thick oil. This was dissolved in a solution of MeOH (5 mL), and subsequently 6 N HCl (0.15 mL) and Et_2O (20 mL) were added dropwise while stirring. The hydrochloride salt was isolated by filtration and recrystallized from Et_2O/MeOH, giving 863 mg (58%) of **15** as a white solid. Mp: 106.8−108.5. ^{1}H NMR (CH_3OH-d_4): δ 1.80−1.99 (m, 2H), 2.54−2.75 (m, 6H), 3.20−3.36 (m, 6H), 7.10−7.47 (m, 20H). MS ESI *m/z*: 483.9 $(M + H)^+$. Anal. ($C_{35}H_{36}Cl_2N_2 \cdot 1.0H_2O$) C, H, N.

4-(4-Chlorophenyl)-1-(4-cyano-4,4-diphenylbutyl)-4-hydroxy-1-methylpiperidinium Iodide (16). Following the same procedure as described in the literature[25] afforded 206 mg (70%) of **16** as a white solid. Mp: 215.8−216.9. ^{1}H NMR ($CDCl_3$/DMSO): δ 1.70−2.00 (m, 4H), 2.06−2.31 (m, 2H), 2.35−2.60 (m, 2H), 3.18 (s, 3H), 3.30−3.50 (m, 2H), 3.52−3.90 (m, 4H), 4.94 (br s, OH), 7.12−7.43 (m, 14H). MS ESI *m/z*: 460.0 $(M + H)^+$. Anal. ($C_{27}H_{32}ClN_2O$) C, H, N.

5-(4-(4-Chlorophenyl)-3,6-dihydro-2*H*-pyridin-1-yl)-2,2-diphenylpentanenitrile (18). Following method A using **22a** (0.81 g, 3.00 mmol) and 4-(4-chlorophenyl)-1,2,3,6-tetrahydropyridine **27** (0.69 g, 3.00 mmol) afforded 400 mg (31%) of **18** as a yellow solid. Mp: 142.1−142.8 °C. ^{1}H NMR ($CDCl_3$): δ 1.56−1.78 (m, 2H), 2.41−2.62 (m, 8H), 3.06 (d, 2H, J = 3.1 Hz), 6.01 (m, 1H), 7.22−7.42 (m, 14H). MS ESI *m/z*: 427.9 $(M + H)^+$. Anal. ($C_{28}H_{27}ClN_2$) C, H, N.

1-(4-Cyano-4,4-diphenylbutyl)piperidine-4-carboxylic Acid Ethyl Ester Hydrochloride (19). Following method B using **22b** (1.26 g, 4.01 mmol) and piperidine-4-carboxylic acid ethyl ester **24** (0.79 g, 5.03 mmol) gave 1.65 g (84%) of **19** as an orange oil. ^{1}H NMR ($CDCl_3$): δ 1.25 (t, 3H, J = 8.0 Hz), 1.45−2.00 (m, 8H), 2.15−2.50 (m, 5H), 2.65−2.80 (m, 2H), 4.10 (q, 2H, J = 7.1), 7.20−7.45 (m, 10H). MS ESI *m/z*: 391.4 $(M + H)^+$. Anal. ($C_{25}H_{30}N_2O_2$) C, H, N.

1-(4-Cyano-4,4-diphenylbutyl)piperidine-4-carboxylic Acid Hydrochloride (20). A solution of **19** (1.41 g, 3.61 mmol) and 2 M NaOH (5 mL) in MeOH (30 mL) was refluxed for 3 h. The reaction mixture was allowed to cool to room temperature, evaporated in vacuo, diluted with water (30 mL), and extracted with Et_2O (1 × 30 mL). The water layer was acidified with 2 N HCl, and 1.23 g (86%) of **20** was isolated by filtration as a white solid. Mp: 231.0−233.1 °C. ^{1}H NMR (DMSO/D_2O): δ 1.40−1.80 (m, 4H), 1.75−2.15 (m, 2H), 2.40−2.65 (m, 3H), 2.70−2.94 (m, 2H), 2.96−3.18 (m, 2H), 3.27−3.39 (m, 2H), 7.29−7.54 (m, 10H). MS ESI *m/z*: 363.8 $(M + H)^+$. Anal. ($C_{23}H_{27}ClN_2O_2 \cdot 0.41H_2O$) C, H, N.

4-(4-Chlorophenyl)-1-(3-phenoxypropyl)piperidin-4-ol (26). Following method B using **28** (0.65 g, 3.00 mmol) and 4-chlorophenyl-4-hydroxypiperidine **30** (0.42 g, 2.00 mmol) gave a mixture of **26** and the quaternary product. The quaternary product was separated from **26** by fractional crystallization in $CHCl_3$. The filtrate was concentrated under reduced pressure and recrystallized from EtOAc to give 203 mg (29%) of **26** as white needles. Mp: 125.8−127.3 °C. ^{1}H NMR ($CDCl_3$/DMSO): δ 1.61−1.82 (m, 3H), 1.88−2.19 (m, 4H), 2.32−2.65 (m, 4H), 2.75−2.90 (m, 2H), 4.03 (t, 2H, J = 6.0 Hz), 6.80−6.95 (m, 3H), 7.15−7.34 (m, 4H), 7.36−7.45 (m, 2H). MS ESI *m/z*: 347.6 $(M + H)^+$. Anal. ($C_{20}H_{24}ClNO_2$) C, H, N.

4-(4-Chlorophenyl)-1-(4-phenylbutyl)piperidin-4-ol (27). Following method B using 1-chloro-4-phenylbutane **29** (0.49 mL, 2.98 mmol) and 4-chlorophenyl-4-hydroxypiperidine **30** (0.70 g, 3.30 mmol) gave 310 mg (35%) of **27** as a white solid.[33] Mp: 112.5−114.0 °C. ^{1}H NMR ($CDCl_3$): δ 1.50−1.72 (m, 7H), 2.00−2.17 (m, 2H), 2.25−2.43 (m, 4H), 2.62 (t, 2H, J = 7.1 Hz), 2.72−2.85 (m, 2H), 7.15−7.31 (m, 7H), 7.42 (d, 2H, J = 8.6 Hz). MS ESI *m/z*: 344.3 $(M + H)^+$. Anal. ($C_{21}H_{26}ClNO$) C, H, N, Cl.

4-(4-(4-Chlorophenyl)-4-hydroxypiperidin-1-yl)-1-phenylbutan-1-one (31). (i) A solution of **33** (6 mL, 37.4 mmol), ethylene glycol (6 mL, 108 mmol), and *p*-toluenesulfonic acid monohydrate (0.50 g, 2.63 mmol) in toluene (400 mL) was refluxed overnight with azeotropic removal of water. The organic layer was washed with 5% $NaHCO_3$ (250 mL) and water (250 mL), dried over anhydrous Na_2SO_4, and evaporated in vacuo to give 9.69 g (98%) of **34** as an orange solid.

(ii) Following method B using **34** (0.72 g, 3.19 mmol) and 4-chlorophenyl-4-hydroxypiperidine **30** (0.57 g, 2.69 mmol) gave 1.281 g of a brown oil. This was dissolved in MeOH (15 mL), and concentrated HCl (1.4 mL) was added. The resulting reaction mixture was refluxed for 2 h, allowed to cool to room temperature, and evaporated in vacuo. The resulting brown oil was dissolved in EtOAc (25 mL) and washed with NH_4OH (5% solution in water, 2 × 15 mL) and water (2 × 15 mL). The organic layer was dried over anhydrous $MgSO_4$, rinsed with hexane, and evaporated in vacuo to give 522 mg (53%) of **31** as a white solid. Mp: 130.4−131.8 °C. ^{1}H NMR ($CDCl_3$): δ 1.67−1.79 (m, 2H), 1.92−2.13 (m, 2H), 2.16−2.32 (m, 2H), 2.98−3.54 (m, 8H), 4.18 (br s, OH), 7.03−7.58 (m, 7H), 7.77 (d, 2H, J = 7.0 Hz). MS ESI *m/z*: 358.2 $(M + H)^+$. Anal. ($C_{21}H_{24}ClNO_2 \cdot 0.24CH_2(CH_2)_4CH_3$) C, H, N.

4-(4-Chlorophenyl)-1-(4-hydroxy-4-phenylbutyl)piperidin-4-ol (32). (i) **33** (3.94 g, 21.64 mmol) was dissolved in EtOH (25 mL), and $NaBH_4$ (0.42 g, 11.10 mmol) was added in small portions at 0 °C. The reaction mixture was allowed to warm to room temperature and stirred for 1 h. After the addition of water (25 mL), the reaction mixture was extracted with Et_2O (3 × 25 mL). The combined organic layers were dried over anhydrous $MgSO_4$, filtered, and evaporated in vacuo to give 3.69 g (93%) of **36** as colorless oil, which was used without further purification.

(ii) Et_3N (8.5 mL, 61.2 mmol) and acetyl chloride (2.6 mL, 30.6 mmol) were added to a solution of **36** in Et_2O (50 mL). The reaction mixture was stirred at room temperature for 1 h, diluted with water (25 mL) and extracted with Et_2O (3 × 25 mL). The combined organic layers were washed with aqueous K_2CO_3 (25 mL), dried over anhydrous $MgSO_4$, and evaporated in vacuo to give 2.51 g (51%) of **37** as brown oil, which was used without further purification.

(iii) Following method B using **37** (0.68 g, 3.00 mmol) and 4-chlorophenyl-4-hydroxypiperidine **30** (0.70 g, 3.32 mmol) gave 1.24 g of **38**. This was dissolved in MeOH (25 mL), and 10% NaOH (5 mL) was added. The resulting reaction mixture was refluxed for 30 min, evaporated in vacuo, and extracted with DCM (3 × 25 mL). The combined organic layers were dried over anhydrous $MgSO_4$, filtered, and evaporated in vacuo. The residue was purified by flash chromatography (0−10% MeOH in EtOAc) to give 449 mg (41%) of **32** as a white solid. Mp: 144.4−145.8 °C. ^{1}H NMR ($CDCl_3$): δ 1.51−2.07 (m, 8H), 2.09−2.31 (m, 2H), 2.40−2.69 (m, 4H), 2.74−2.90 (m, 1H), 2.92−3.10 (m, 1H), 4.59−4.71 (m, 1H), 7.10−7.49 (m 9H). MS ESI *m/z*: 360.9 $(M + H)^+$. Anal. ($C_{21}H_{26}ClN_2O_2$) C, H, N.

4-(4-Chlorophenyl)-1-(4,4-diphenylbutyl)piperidin-4-ol (39). Following method B using 4-chloro-1,1-diphenylbutane (0.82 g, 3.16 mmol), which was synthesized according to literature procedure,[31] and 4-chlorophenyl-4-hydroxypiperidine **30** (0.52 g, 2.34 mmol) gave 329 mg (32%) of **39** as a yellow solid. Mp: 110.1−110.8 °C. ^{1}H NMR ($CDCl_3$): δ 1.39−1.79 (m, 5H), 2.00−2.19 (m, 4H), 2.22−2.49 (m, 4H), 2.67−2.83 (m, 2H), 3.89 (t, 1H, J = 7.8 Hz), 7.14−7.43 (m, 14H). MS ESI *m/z*: 421.2 $(M + H)^+$. Anal. ($C_{27}H_{30}ClNO$) C, H, N.

4-(4-Chlorophenyl)-1-(4-(4-methoxyphenyl)-4-phenylbutyl)piperidin-4-ol (40). (i) Following method D (step i)

starting with 4-methoxybenzophenone (5.75 g, 27.1 mmol) gave 3.18 g (59%) of 4-methoxydiphenylmethane as a colorless oil. ^{1}H NMR ($CDCl_3$): δ 3.87 (s, 3H), 4.06 (s, 2H), 6.97 (d, 2H, J = 8.5 Hz), 7.22−7.41 (m, 7H).

(ii) A solution of potassium *tert*-butoxide (0.90 g, 8.0 mmol) in dry THF (20 mL) was added to a solution of *n*-BuLi (5.00 mL, 1.6 M in hexane, 8.0 mmol) in a dry atmosphere at −100 °C. The reaction mixture was stirred for 5 min, and a solution of 4-methoxydiphenylmethane (1.58 g, 7.97 mmol) in dry THF (15 mL) was added slowly in a period of 15 min. The solution was stirred for 1 h at −95 °C, and 1,3-dibromopropane was added in one portion (4.0 mL, 39.4 mmol). The reaction mixture was allowed to warm to room temperature and was stirred for 1.5 h. The solvent was evaporated under reduced pressure, and the residue was diluted with water (25 mL) and extracted with EtOAc (3 × 25 mL). The combined organic layers were washed with water (3 × 25 mL) and brine (25 mL), dried over anhydrous $MgSO_4$, filtered, and evaporated under reduced pressure (1 mmHg) to afford bromide **50** (R_1 = 4-OCH_3; R_2 = H). The crude product was used without further purification.

(iii) Bromide **50** (R_1 = 4-OCH_3; R_2 = H) was dissolved in CH_3CN, and following method B, purification by flash chromatography (50% DCM in EtOAc) and recrystallization from EtOAc gave 548 mg (15%) of **40** as white crystals. Mp: 119.7−121.0 °C. ^{1}H NMR ($CDCl_3$): δ 1.47−1.69 (m, 5H), 2.00−2.11 (m, 4H), 2.26−2.43 (m, 4H), 2.69−2.75 (m, 2H), 3.74 (s, 3H), 3.84 (t, 1H, J = 7.8 Hz), 6.79 (d, 2H, J = 8.7 Hz), 7.11−7.37 (m, 9H), 7.41 (d, 2H, J = 8.7 Hz). MS ESI *m/z*: 450.4 (M + H)$^+$. Anal. ($C_{28}H_{32}ClNO_2$) C, H, N, Cl.

4-(4-Chlorophenyl)-1-(4-(3,4-dichlorophenyl)-4-phenylbutyl)piperidin-4-ol Hydrochloride (41). (i) A solution of $AlCl_3$ (7.36 g, 55.2 mmol) and *tert*-butylamineborane (9.95 g, 111 mmol) in DCM (150 mL) was stirred for 10 min at 0 °C, and a solution of 3,4-dichlorobenzophenone (4.64 g, 18.4 mmol) in DCM (15 mL) was added. The mixture was stirred for 2 h at 0 °C and overnight at room temperature. A cooled solution of 0.1 N HCl (75 mL) at 0 °C was added carefully followed by extraction with EtOAc (100 mL). The combined organic layers were washed with 0.1 N HCl (2 × 75 mL) and brine (100 mL), dried over anhydrous $MgSO_4$, filtered, and evaporated under reduced pressure. The residue was purified by flash chromatography (hexane) to afford 3.43 g (78%) of 3,4-dichloromethane as a colorless oil. ^{1}H NMR ($CDCl_3$): δ 3.93 (s, 2H), 6.99−7.68 (m, 8H).

(ii) A solution of diisopropylamine (1.22 mL, 8.68 mmol) in dry THF (5 mL) was added to a solution of *n*-BuLi (5.43 mL, 1.6 M in hexane, 8.69 mmol) and stirred for 5 min at −10 °C. The reaction mixture was cooled to −78 °C, and a solution of 3,4-dichlorodiphenylmethane (2.07 g, 8.68 mmol) in dry THF (10 mL) was added slowly in a period of 15 min. The mixture was stirred for 1 h at −10 °C followed by the addition of HMPA (1.5 mL) and 1,3-dibromopropane (4.40 mL, 26.1 mmol) in one portion at −78 °C. The reaction mixture was allowed to warm to room temperature and was stirred for 1 h, and the reaction was quenched with water (25 mL). The solvent was evaporated, and the residue was diluted with water (25 mL) and extracted with EtOAc (3 × 25 mL). The combined organic layers were washed with water (3 × 25 mL) and brine (25 mL), dried over anhydrous $MgSO_4$, filtered, and evaporated under reduced pressure. The residue was purified by flash chromatography (hexane) to afford 4-bromo-1-(3,4-dichlorophenyl)-1-phenylbutane **18** (R_1 = 3,4-Cl_2; R_2 = H) as a colorless oil (0.15 g, 5%). ^{1}H NMR ($CDCl_3$): δ 1.65−1.79 (m, 2H), 2.03−2.14 (m, 2H), 3.32 (t, 2H, J = 6.5 Hz), 3.78 (t, 1H, J = 7.5 Hz), 6.96−7.26 (m, 8H).

(iii) Following method B gave 132 mg (66%) of thick oil. The compound was converted to the hydrochloride salt as described for **3**. Recrystallization from Et_2O/MeOH gave 124 mg (87%) of **41** as a white solid. ^{1}H NMR ($CDCl_3$): δ 1.80−1.86 (m, 4H), 2.01−2.16 (m, 3H), 2.80−3.01 (m, 4H), 3.08−3.46 (m, 4H), 3.87 (t, 1H, J = 7.8 Hz), 7.08−7.46 (m, 12H), 12.23 (br s, 1H). MS ESI *m/z*: 488.4 (M + H)$^+$. Anal. ($C_{27}H_{29}Cl_4NO\cdot 1.0H_2O$) C, H, N, Cl.

4-(4-Chlorophenyl)-1-(4-(4-chlorophenyl)-4-phenylbutyl)piperidin-4-ol (42). Following method D using 4-chlorobenzophenone gave 256 mg (5% overall yield) of **42** as white crystals. Mp: 126.2−127.0 °C. ^{1}H NMR ($CDCl_3$): δ 1.46−1.53 (m, 2H), 1.64−1.69 (m, 3H), 1.97−2.12 (m, 4H), 2.27−2.43 (m, 4H), 2.68−2.74 (m, 2H), 3.86 (t, 1H, J = 7.8 Hz), 7.12−7.43 (m, 13H). MS ESI *m/z*: 454.4 (M + H)$^+$. Anal. ($C_{27}H_{29}Cl_2NO$) C, H, N, Cl.

1-(4,4-Bis(4-chlorophenyl)butyl)-4-(4-chlorophenyl)piperidin-4-ol Hydrochloride (43). Following method D using 4,4′-dichlorobenzophenone gave 291 mg (5% overall yield) of thick oil. The compound was converted to the hydrochloride salt as described for **3**. Recrystallization from Et_2O/MeOH gave 302 mg (97%) of **43** as a white solid. Mp: 185.0−185.5 °C. ^{1}H NMR ($CDCl_3$): δ 1.80−1.86 (m, 5H), 2.00−2.11 (m, 2H), 2.70−2.87 (m, 4H), 3.09−3.22 (m, 4H), 3.85 (t, 1H, J = 7.5 Hz), 7.09 (d, 4H, J = 8.47 Hz), 7.20−7.26 (m, 6H), 7.39 (d, J = 8.6 Hz, 2H), 11.83 (br s, 1H). MS ESI *m/z*: 488.4 (M + H)$^+$. Anal. ($C_{27}H_{29}Cl_4NO$) C, H, N, Cl.

4-(4-Chlorophenyl)-1-(4-phenyl-4-*m*-tolylbutyl)piperidin-4-ol Hydrochloride (45). Following method D using 3-methylbenzophenone gave 294 mg (15% overall yield) of thick oil. The compound was converted to the hydrochloride salt as described for **3**. Recrystallization from Et_2O/MeOH gave 299 mg (93%) of **45** as a white solid. Mp: 143.1−144.4 °C. ^{1}H NMR ($CDCl_3$): δ 1.78−1.84 (m, 4H), 2.01−2.13 (m, 3H), 2.29 (s, 3H), 2.80−2.91 (m, 4H), 3.09−3.29 (m, 4H), 3.88 (t, 1H, J = 7.9 Hz), 7.00−7.33 (m 11H), 7.41 (d, 2H, J = 8.5 Hz), 12.15 (br s, 1H). MS ESI *m/z*: 434.4 (M + H)$^+$. Anal. ($C_{28}H_{33}Cl_2NO\cdot 0.17H_2O$) C, H, N, Cl.

4-(4-Chlorophenyl)-1-(4-phenyl-4-*p*-tolylbutyl)piperidin-4-ol Hydrochloride (46). Following method D using 4-methylbenzophenone gave 468 g (19% overall yield) of thick oil. The compound was converted to the hydrochloride salt as described for **3**. One batch was recrystallized from Et_2O/MeOH to give 304 mg (60%) of **46** as a white solid. Mp: 174.3−176.0 °C. ^{1}H NMR ($CDCl_3$): δ 1.78−1.84 (m, 4H), 1.99−2.12 (m, 2H), 2.26 (s, 3H), 2.69−2.84 (m, 5H), 3.09−3.18 (m, 4H), 3.84 (t, 1H, J = 7.9 Hz), 7.07−7.34 (m, 11H), 7.39 (d, 2H, J = 8.6 Hz), 11.81 (br s, 1H). MS ESI *m/z*: 434.4 (M + H)$^+$. Anal. ($C_{28}H_{32}Cl_2NO\cdot 0.19H_2O$) C, H, N, Cl.

1-(4-Biphenyl-4-y)-4-(4-chlorophenyl)butyl]-4-(4-chlorophenyl)piperidin-4-ol (47). Following method D using 4-chloro-4′-phenylbenzophenone gave the final compound as a white solid. Recrystallization from EtOAc gave 264 mg (9% overall yield) of **47** as white crystals. Mp: 127.8−128.6 °C. ^{1}H NMR ($CDCl_3$): δ 1.50−1.70 (m, 5H), 2.00−2.13 (m, 4H), 2.30−2.46 (m, 4H), 2.72−2.77 (m, 2H), 3.91 (t, 1H, J = 7.7 Hz), 7.16−7.55 (m, 17H). MS ESI *m/z*: 530.5 (M + H)$^+$. Anal. ($C_{33}H_{33}Cl_2NO$) C, H, N, Cl.

Pharmacology. Materials. ATP disodium salt, bovine serum albumin, chloroquine diphosphate, and DEAE-dextran (chloride form) were obtained from Sigma. Cell culture media, penicillin, and streptomycin were obtained from Life Technologies, Inc., and fetal calf serum was purchased from Integro B.V. (Dieren, The Netherlands). *myo*-[2-^{3}H]Inositol (17 Ci/mmol) was obtained from Perkin-Elmer Life Sciences, and Sephadex G-25 gel filtration columns were purchased from ICN Pharmaceuticals Inc. (Costa Mesa, CA). The human chemokine CCL5 (regulated on activation, normal T cell expressed, and secreted) was obtained from Peprotech (Rocky Hill, NJ).

Cell Culture and Transfection. COS-7 cells were grown at 5% CO_2 at 37 °C in Dulbecco's modified Eagle's medium supplemented with 5% fetal calf serum, 2 mM L-glutamine, 50 IU/mL penicillin, and 50 μg/mL streptomycin. Transfection of the COS-7 cells was performed by DEAE-dextran using 2 μg of DNA of each US28 construct pcDEF3-US28 or empty factor per million cells.[17] The total amount of DNA in transfected cells was maintained constant by addition of the empty vector.

[^{125}I]Chemokine Binding Study. Labeling of CCL5 with ^{125}I and binding in COS-7 cells were performed as previously described.[20] Briefly, transfected cells were seeded in 24-well

plates; 48 h after transfection, binding was performed on whole cells for 3 h at 4 °C using 0.3 nM [^{125}I]CCL5 in binding buffer (50 mM Hepes, pH 7.4, 1 mM $CaCl_2$, 5 mM $MgCl_2$, and 0.5% bovine serum albumin) in the presence or absence of varying concentrations of compounds. After incubation, cells were washed four times at 4 °C with binding buffer supplemented with 0.5 M NaCl. Nonspecific binding was determined in the presence of 0.1 μM cold competitor (CCL5).

[^{3}H]Inositol Phoshate Production. Cells were seeded in 24-well plates, and 24 h after transfection they were labeled overnight in inositol-free medium (modified Eagle's medium with Earle's salts) supplemented with 2 mM L-glutamine, L-cysteine, L-leucine, L-methionine, L-arginine, glucose, 0.2% bovine serum albumin, and 2 μCi/ml *myo*-[2-^{3}H]inositol. Subsequently, the labeling medium was aspirated, cells were washed for 10 min with Dulbecco's modified Eagle's medium containing 25 mM HEPES (pH 7.4) and 20 mM LiCl and incubated for 2 h in the same medium in the absence or presence of varying concentrations of compounds. The incubation was stopped by aspiration of the medium and addition of cold 10 mM formic acid. After 90 min of incubation on ice, inositol phosphates were isolated by anion exchange chromatography (Dowex AG1-X8 columns, Bio-Rad) and counted by liquid scintillation.

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Supporting Information Available: Results from elemental analyses of all target compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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