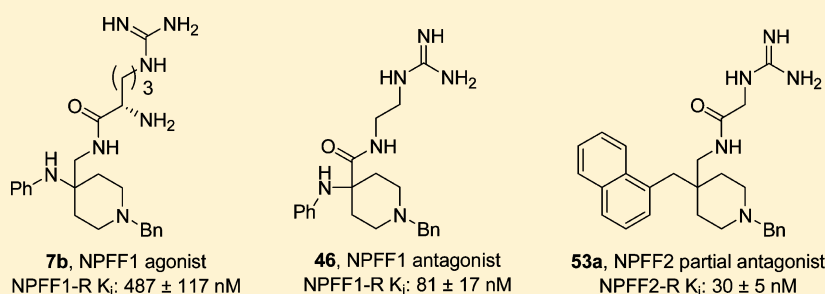# Nonpeptide Small Molecule Agonist and Antagonist Original Leads for Neuropeptide FF1 and FF2 Receptors

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7b, NPFF1 agonist
NPFF1-R K_i: 487 ± 117 nM

46, NPFF1 antagonist
NPFF1-R K_i: 81 ± 17 nM

53a, NPFF2 partial antagonist
NPFF2-R K_i: 30 ± 5 nM

ABSTRACT: Neuropeptide FF1 and FF2 receptors (NPFF1-R and NPFF2-R), and their endogenous ligand NPFF, are one of only several systems responsible for mediating opioid-induced hyperalgesia, tolerance, and dependence. Currently, no small molecules displaying good affinity or selectivity for either subtype have been reported, to decipher the role of NPFF2-R as it relates to opioid-mediated analgesia, for further exploration of NPFF1-R, or for medication development for either subtype. We report the first nonpeptide small molecule scaffold for NPFF1,2-R, the guanidino-piperidines, and SAR studies resulting in the discovery of a NPFF1 agonist (**7b**, K_i = 487 ± 117 nM), a NPFF1 antagonist (**46**, K_i = 81 ± 17 nM), and a NPFF2 partial antagonist (**53a**, K_i = 30 ± 5 nM), which serve as leads for the development of pharmacological probes and potential therapeutic agents. Testing of **46** alone was without effect in the mouse 48 °C warm-water tail-withdrawal test, but pretreatment with **46** prevented NPFF-induced hyperalgesia.

■ INTRODUCTION

The neuropeptide FF (NPFF) system comprises two receptor subtypes, NPFF1 and NPFF2 receptors (NPFF1-R and NPFF2-R), which are $G_{i/o}$ coupled G-protein coupled receptors (GPCRs) with 30−35% homology to neuropeptide Y and orexin receptors,[1] and four endogenous ligands belonging to the Arg-Phe-NH_2 (RFa) peptide family.[2] These peptides include hNPFF (SQAFLFQPQRFa), hNPSF (SLNFEELKDWGPKNVIKMSTPAVNKMPHSFANLPLRFa), and hNPAF (AGEGLSSPFWSLAAPQRFa) (10.7-, 1.2-, and 10.1-fold selective in radioligand competition binding assays for the hNPFF2 subtype, respectively), and hNPVF (VPNLPQRFa) (27.8-fold selective for the hNPFF1 subtype).[2] Both NPFF1-R and NPFF2-R are present in the rat brain; however; only NPFF2-R is found in the spinal cord.[3] Conversely, in humans, the distribution of NPFF1-R is not restricted to the CNS, since NPFF1 mRNA[4] is found in the spinal cord. Talmont and colleagues noted species-related differences in the pharmacological profiles between mouse and human NPFF2-R, further highlighting the consideration that must be taken in the interpretation of preliminary in vitro and in vivo results in mouse models as they translate to human targets.[5]

The opioid-modulating properties (among others) of the NPFF peptide have been chronicled throughout the literature since its discovery in 1985,[6] described as producing either a pro- or antinociceptive effect depending on the route of administration. For example, when administered in rats by intracerebroventricular (icv) injection alone or in combination with morphine or other opioid agonists, NPFF or its stable analog 1DMe (D.YL(N-Me)FQPQRFa) produces an anti-opioid effect,[6−9] however; when given intrathecally (it.), NPFF yields an opioid-like effect and potentiates morphine-induced analgesia in various pain models.[10−13] Thus, a precise role for NPFF1-R and NPFF2-R as anti- or pronociceptive systems in preliminary in vivo models has not been delineated. In rats, the lack of NPFF1-R at the spinal level has formed the basis of the hypothesis that the antinociceptive activity of NPFF observed upon it. administration is a result of its interaction with NPFF2-R, while its pronociceptive activity at the supraspinal (i.e., brain) level is a result of its interaction with NPFF1-R.[2,14] However, the pronociceptive activity of NPFF in the rat brain cannot be exclusively attributed to its activity at the NPFF1-R,
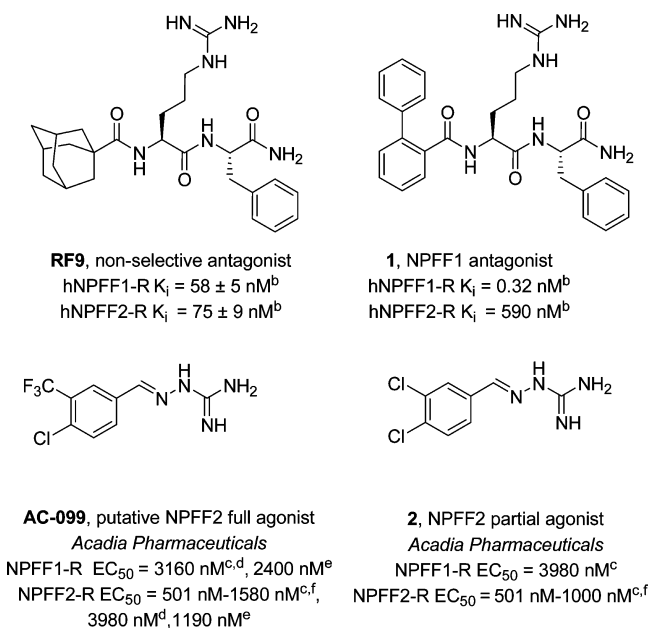
because the activity of the rNPFF peptide (NPAFLFQPQRFa) at rNPFF1,2-R and hence its selectivity has not been reported. Failing the availability of any truly selective ligands for NPFF receptors, gene knockout studies would prove valuable in elucidating the pharmacology of these subtypes.

A limited number of dipeptides and small molecules have been used to try to define the pharmacology of these subtypes, with mixed results. Although a pronociceptive/antiopioid role has been assigned to the NPFF system as a whole based on the in vivo activity of a nonselective dipeptide competitive antagonist RF9 (administered subcutaneously (sc) or intraperitoneally (ip)) (Chart 1)[15,16] in opioid-induced hyperalgesia

Chart 1. Reported Ligands Used for Pharmacological Characterization of NPFF-1 and NPFF-2 Receptors[a]

RF9, non-selective antagonist
hNPFF1-R K_i = 58 ± 5 nM[b]
hNPFF2-R K_i = 75 ± 9 nM[b]

1, NPFF1 antagonist
hNPFF1-R K_i = 0.32 nM[b]
hNPFF2-R K_i = 590 nM[b]

AC-099, putative NPFF2 full agonist
Acadia Pharmaceuticals
NPFF1-R EC_{50} = 3160 nM[c,d], 2400 nM[e]
NPFF2-R EC_{50} = 501 nM-1580 nM[c,f],
3980 nM[d],1190 nM[e]

2, NPFF2 partial agonist
Acadia Pharmaceuticals
NPFF1-R EC_{50} = 3980 nM[c]
NPFF2-R EC_{50} = 501 nM-1000 nM[c,f]

[a]The K_i and EC_{50} values associated with the ligands described are included for discussion purposes only and cannot be directly compared. [b]K_i values were determined by radioligand displacement assays. [c]EC_{50} values were calculated from pEC_{50} values determined in RSAT assay.[20] [d]EC_{50} values were calculated from pEC_{50} values determined in cAMP assay.[20] [e]EC_{50} values were taken directly, as determined in the IP accumulation assay.[22] [f]EC_{50} values were calculated from pEC_{50} values determined in RSAT assay,[19] using hNPFF1-R and hNPFF2-R.

(OIH), tolerance, and dependence assays, conflicting reports exist as to the nociceptive- or opioid-modulating properties of each subtype. NPFF1-R has been classified as a pronociceptive/ antiopioid system using a selective (1843-fold) dipeptide antagonist **1** (administered sc) (Chart 1)[17] and a modestly selective (19.5-fold) small molecule antagonist AC-262620 (administered ip)[14] from Acadia Pharmaceuticals (hNPFF1 K_i = 16.4 ± 10.1 nM, similar in structure to **AC-099** and **2**) based on their activities within in vivo models of pain. Further confirmation of NPFF1-R's pronociceptive role in terms of opioid analgesia could be realized with NPFF1-selective agonists. While being similarly classified as a pronociceptive/ antiopioid construct due to its antiopioid activity at the cellular level using the stable peptide agonist 1DMe,[18] NPFF2-R is also contrarily described as a antinociceptive/pro-opioid system using small molecules of modest affinity, putative full agonist

AC-099 (Chart 1) and partial agonist **2** (Chart 1) (both administered ip) reported by Acadia Pharmaceuticals.[14,19,20] However, it must be noted that 1DMe is not highly selective, exhibiting full agonist activity on both NPFF1-R (EC_{50} = 71 ± 14 nM) and NPFF2-R (EC_{50} = 2.7 ± 0.5 nM),[21] and thus both receptors are likely to contribute to its pharmacological response. Moreover, the modest activity and selectivity of aryliminoguanidines **AC-099** and **2** displayed in unconventional in vitro assays limits their utility in defining NPFF2-R with respect to opioid-mediated analgesia (Chart 1). Additionally, no off-target opioid affinity is reported for these aryliminoguanidines, which calls into question the interpretation of their in vivo analgesic activity.

A single core scaffold, on which modifications can be made to yield a selective agonist or antagonist functional profile, would be of use to help define the pharmacology of NPFF2-R. The functional profiles of the compounds summarized in Chart 1 underscore the need for compounds with NPFF1 agonist or NPFF2 agonist and antagonist efficacy to aid in the exploration of the pharmacology of these subtypes. Other small molecule ligands for NPFF1-R and NPFF2-R, containing a wide range of scaffolds and thus no definitive SAR or pharmacophore, have been reported mainly in the patent literature.[23] However; to our knowledge, no pharmacologically pure and high affinity NPFF1 and NPFF2 agonists and antagonists have been reported (apart from dipeptide NPFF1 antagonist **1**). Moreover, the in vivo activities of these compounds as they pertain to pain modulation, if any, have not been reported.

As summarized above, the majority of these compounds are proprietary and limited in terms of binding affinity, selectivity, and functional profiles necessary to define the pharmacology of these subtypes. Due to the lack of availability of these compounds to the scientific community, a tremendous design space exists to develop novel, selective NPFF1 and NPFF2 ligands of varying functional profiles to aid in the study of these receptors.

SAR of the residues conserved among mouse, rat, bovine and human NPFF (FLFQPQRFa) (K_i 0.21/0.34 nM in the membrane of rat spinal cord or CHO hNPFF2-R)[24,25] indicate that the peptide adheres to a message—address concept,[26] with the C-terminal amide, guanidine of arginine,[7] and aromatic ring of phenylalanine[8] serving as the message component conferring the majority of the peptide's affinity and functional activity. To date, a good number of NPFF ligands reported in the literature contain at least phenyl and guanidine moieties, including dipeptides **RF9** and **1**, and aryliminoguanidines **AC-099** and **2**. Taking these functionalities into consideration, we desired a scaffold on which to accommodate these pharmacophoric elements while at the same time maintaining chemical feasibility. The 4-(phenylamino)piperidine-4-carbonitrile template (Figure 1) seemed to be a reasonable starting point,
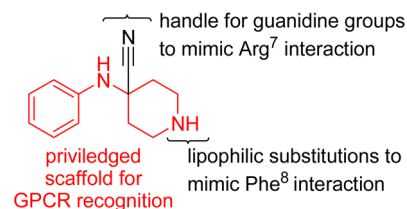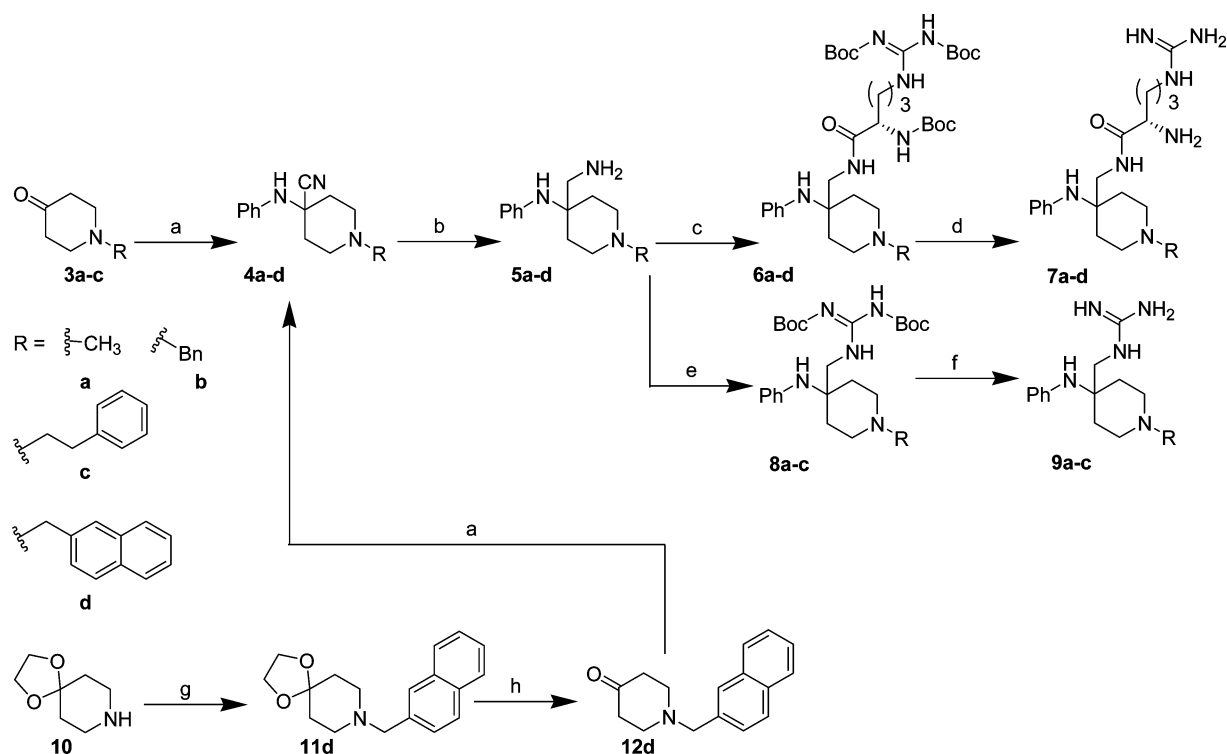

Figure 1. 4-(Phenylamino)piperidine-4-carbonitrile scaffold for designed NPFF ligands.

Scheme 1. Synthesis of 7a–d and 9a–c[a]

[a]Reagents and conditions: (a) aniline, TMSCN, AcOH, 0 °C, 1 h, 70–94%; (b) LiAlH$_4$, diethyl ether (ah), 0 °C, 35–40% (for **5a,5c**) or NH$_3$/MeOH, Raney Ni, 3 atm H$_2$(g), 4 h, rt, 82–90% (for **5b, 5d**); (c) Boc-Arg-(Boc)$_2$-OH, EDCI, HOBt, Et$_3$N, DCM, rt, 24 h, 50–70%; (d) HCl/dioxane, 3 days, rt, 95%; (e) 1,3-bis(*tert*-butoxycarbonyl)-2-methyl-2-thiopseudourea, HgCl$_2$, Et$_3$N, DMF, 0 °C → rt, 10 h, 30%; (f) HCl/dioxane, 4 days, rt, quantitative; (g) 2-bromomethylnaphthalene, K$_2$CO$_3$, KI, 4-methyl-2-pentanone, reflux, 5 h; (h) AcOH, conc. HCl (aq), reflux, 18 h, 40%.

because the 4-anilido piperidine portion is known to be a privileged scaffold for GPCRs, particularly in the field of opioid analgesics (i.e., fentanyl). Moreover, the piperidine nitrogen portion allows for lipophilic substitutions to be made, and the nitrile moiety could be converted to the corresponding amine, to allow for the introduction of guanidine-containing groups. We report herein a series of designed guanidino-piperidines based on this scaffold, which led to the first class of ligands to demonstrate a tractable SAR for modulation of affinity, efficacy, and selectivity at NPFF1-R and NPFF2-R.

RESULTS AND DISCUSSION

Chemistry. Piperidones **3a–c** and **12d** (Scheme 1) bearing various lipophilic substitutions at the nitrogen (either commercially available or prepared via the ketal **10**) were subjected to standard Strecker conditions[27] to generate N-substituted carbonitriles **4a–d** in 70–94% yield. Reduction of the nitriles using either LiAlH$_4$ or hydrogenation afforded the corresponding amines **5a–d** in 35–90% yield, followed by coupling with Boc-Arg-(Boc)$_2$-OH using EDCI/HOBt to give Boc-protected arginine intermediates **6a–d** or HgCl$_2$-assisted guanidation to give protected guanidine intermediates **8a–c** in 30–70% yield. Deprotection with HCl/dioxane afforded guanidines **7a–d** and **9a–c** in excellent yield (95%).

Des-aniline **15** (Scheme 2) was prepared starting with (1-benzyl-4-piperidinyl)methylamine (**13**) and using analogous chemistry to that used for **6a–d** (Scheme 1) in 70% yield, followed by deprotection with HCl/dioxane in excellent yield (95–99%).

Scheme 2. Synthesis of 15[a]
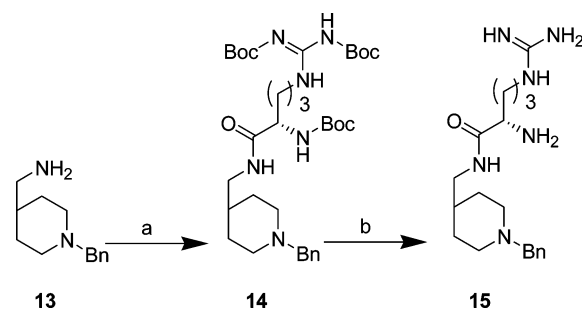
[a]Reagents and conditions: (a) Boc-Arg-(Boc)$_2$-OH, EDCI, HOBt, Et$_3$N, DCM, rt, 24 h, 70%; (b) 4 M HCl/dioxane, rt, 10 h to 4 days (95–99%).

Guanidated glycine **21** (1-carbon aliphatic linker) was synthesized by coupling Boc-Gly-OH with 4-(aminomethyl)-1-benzyl-*N*-phenylpiperidin-4-amine (**5b**) using EDCI/HOBt to give intermediate **16b** in 65% yield, followed by Boc removal with TFA to yield amine **17b** (92%) (Scheme 3). Boc-protected guanidation of amine **17b** with 1,3-bis(*tert*-butox-ycarbonyl)-2-methyl-2-thiopseudourea afforded intermediate **20** in 84% yield, and deprotection with TFA afforded one-carbon linked guanidine **21** in 90% yield. Two carbon homologue **17a** was prepared in a similar manner via EDCI/HOBt coupling of cyanoacetic acid with amine **5b** (Scheme 1) to yield intermediate **16a** in 50% yield, then reduction of the cyano moiety with H$_2$(g)/Raney Ni to amine **18a** (76%). Boc-protected guanidation of amine **18a** afforded intermediate **19a**

Scheme 3. Synthesis of 17a–g and 21[a]
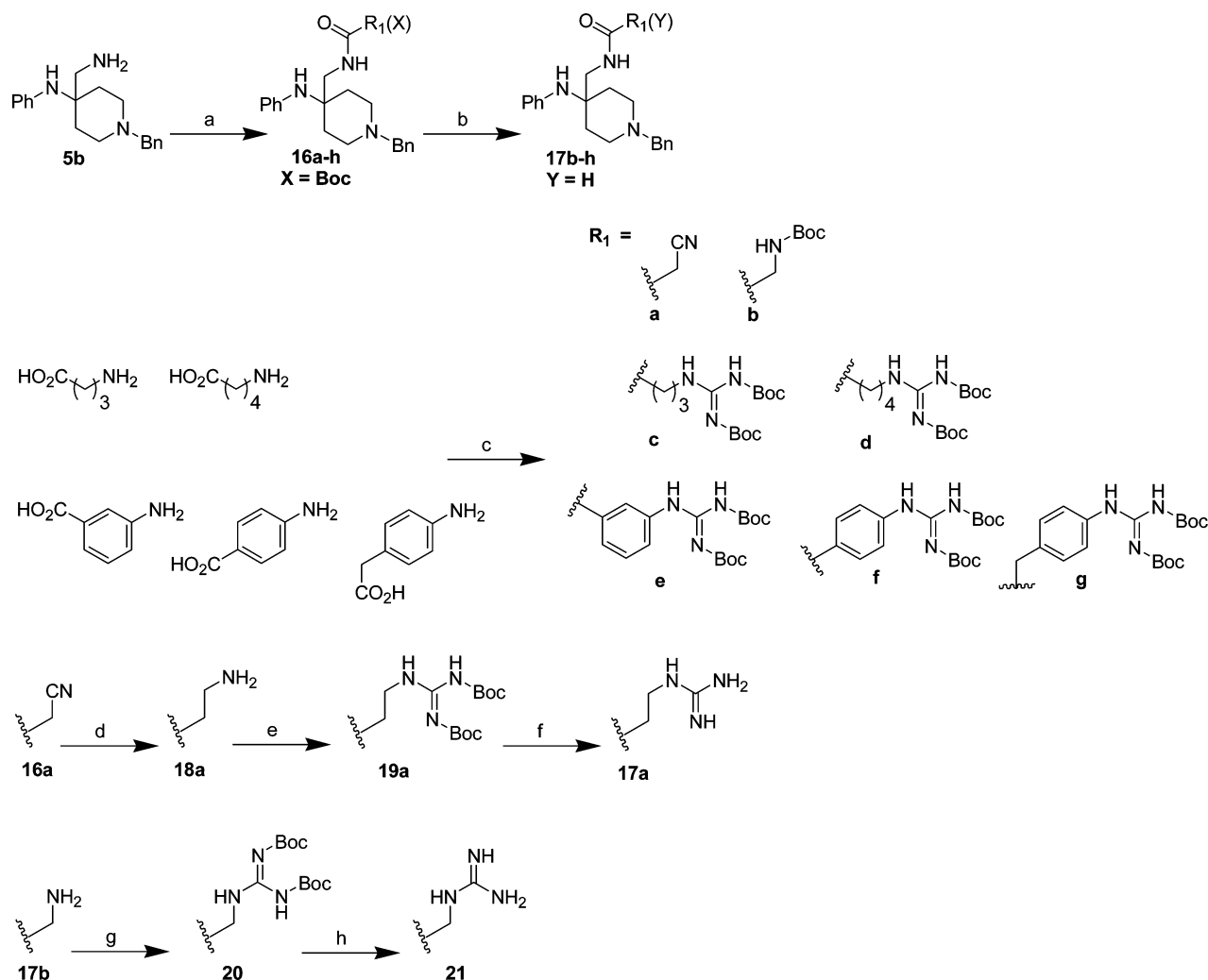
[a]Reagents and conditions: (a) carboxylic acid, EDCI, HOBt, Et$_3$N, DCM, 0 °C → rt, 15 h, 50–84%; (b) TFA, DCM, rt, 2 h, 77–100%; (c) 1,3-bis(*tert*-butoxycarbonyl)-2-methyl-2-thiopseudourea, HgCl$_2$, Et$_3$N, THF/MeOH, rt, 15 h, 16-31%; (d) NH$_3$/MeOH, Raney Ni, 2.4 atm H$_2$(g), 15 h, rt, 76%; (e) 1,3-bis(*tert*-butoxycarbonyl)-2-methyl-2-thiopseudourea, Et$_3$N, DMF(ah), rt, 18 h, 87%; (f) TFA, DCM(ah), rt, 3 h, 77%; (g) 1,3-bis(*tert*-butoxycarbonyl)-2-methyl-2-thiopseudourea, Et$_3$N, DMF(ah), rt, 2 days, 84%; (h) TFA, DCM(ah), rt, 3 h, 90%.

Scheme 4. Synthesis of 26[a]
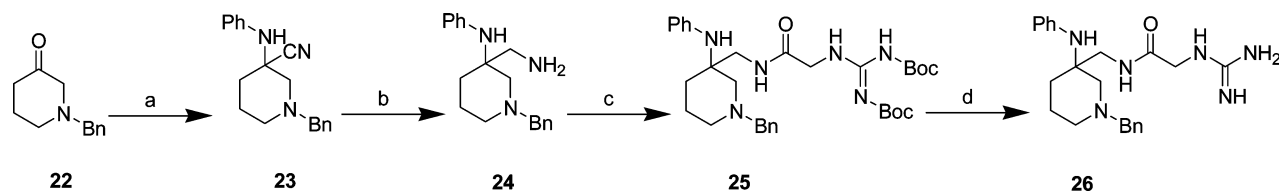
[a]Reagents and conditions: (a) aniline, Ti(O*i*Pr)$_4$, diethylaluminum cyanide, DCE, rt, 24 h, 74%; (b) NH$_3$/MeOH, Raney Ni, 2.7 atm H$_2$(g), 24 h, 78%; (c) 1,3-di-Boc-2-(carboxymethyl)guanidine, HOBt, EDCI, Et$_3$N, DCM, 0 °C → rt, 20 h, 37%; (d) TFA, DCM, rt, 6 h, 65%.

in 87% yield; subsequent deprotection with TFA yielded two-carbon linked guanidine **17a** (77%). Three and four carbon homologues **17c,d** were prepared in a similar manner by first converting 4-aminobutanoic acid and 5-aminopentanoic acid to their corresponding Boc-protected guanidines as described above (17–26%), followed by EDCI/HOBt coupling with amine **5b** to yield intermediates **16c,d** (77–80%). Boc-removal from intermediates **16c,d** with TFA afforded analogs **17c,d**. Phenyl linked guanidines **17e–g** were prepared by converting 3-aminobenzoic acid, 4-aminobenzoic acid, and 4-aminophenyl-

acetic acid to the Boc-protected guanidines as described above (16-31%), followed by EDCI/HOBt coupling with amine **5b** to yield intermediates **16e–g** (66–84%). Boc removal from intermediates **16e–g** with TFA afforded analogs **17e–g** in quantitative yield.

One, three-substituted analog **26** was obtained (Scheme 4) using similar chemistry as shown in Schemes 1 and 2. 1-Benzyl-3-piperidone **22** was coupled to aniline under Strecker conditions to give nitrile **23** in 74% yield, followed by catalytic hydrogenation to amine **24** (78% yield). Boc-protected

Scheme 5. Phenyl (Ring 1) Modification of Lead 21: Synthesis of 30a–d[a]
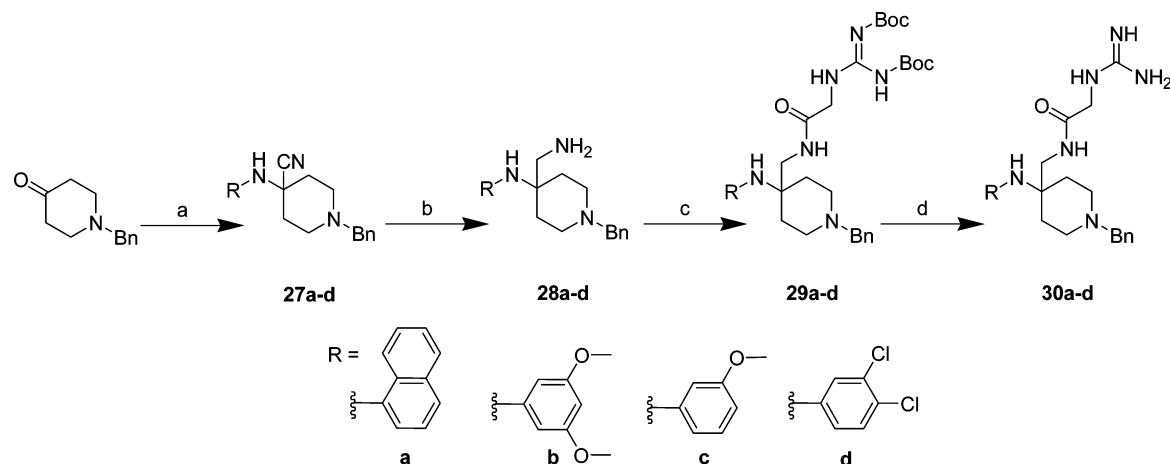
*a*Reagents and conditions: (a) acetic acid, substituted aniline, KCN(aq) or TMSCN, 0 °C → rt, 4 h, 55–77%; (b) NH$_3$/MeOH, Raney Ni, 2.7 atm H$_2$(g), 16–24 h, 89–95%; (c) 1,3-di-Boc-2-(carboxymethyl)guanidine, HOBt, EDCI, Et$_3$N, DCM, 0 °C → rt, 20 h, 33–67%; (d) TFA, DCM, rt, 2–4 h, 53–83%.

Scheme 6. Aniline Amine Modification of Lead 21: Synthesis of 34, 42, and 43[a]
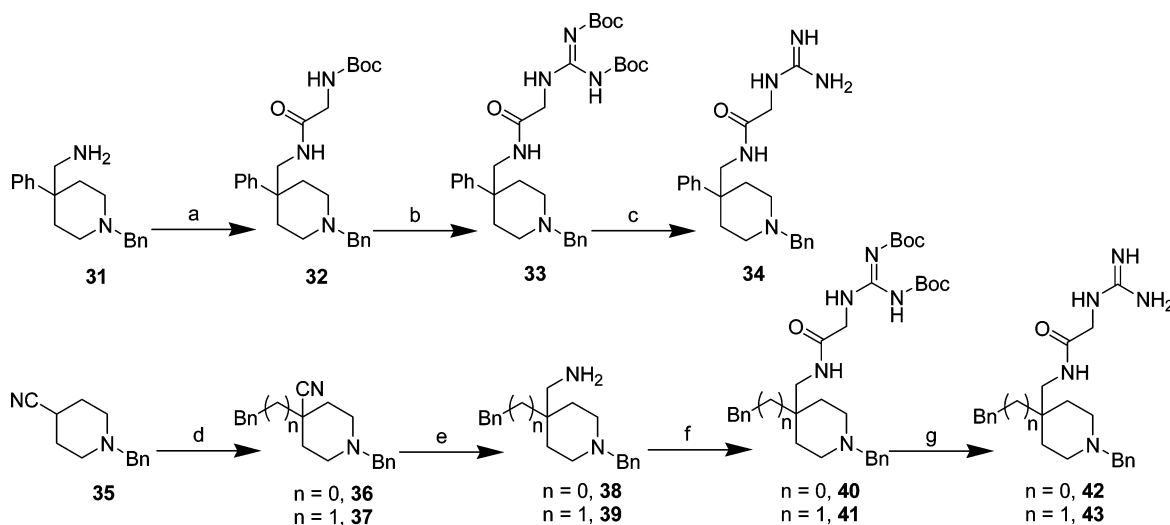
*a*Reagents and conditions: (a) Boc-gly-OH, HOBt, EDCI, Et$_3$N, DCM, 0 °C → rt, 24 h, 78%; (b) TFA, DCM, rt, 1 h then 1,3-bis(*tert*-butoxycarbonyl)-2-methyl-2-thiopseudourea, HgCl$_2$, Et$_3$N, DMF, rt, 1 h, 89%; (c) TFA, DCM, rt, 2 h; (d) benzyl bromide (for 36) or (2-bromoethyl)benzene (for 37), LDA, THF(ah), −70 °C → rt, 2–3 h, 86–89%; (e) NH$_3$/MeOH, Raney Ni, 2.4–2.7 atm H$_2$(g), 24 h, 61–90%; (f) 1,3-di-Boc-2-(carboxymethyl)guanidine, HOBt, EDCI, Et$_3$N, DCM, 0 °C → rt, 22 h, 48% (for 40); for 41 (i) Boc-gly-OH, HOBt, EDCI, Et$_3$N, DCM, 0 °C → rt, 20 h, 91%, (ii) TFA, DCM, rt, 1 h, (iii) 1,3-bis(*tert*-butoxycarbonyl)-2-methyl-2-thiopseudourea, HgCl$_2$, Et$_3$N, DMF, rt, 2 h, 76%; (g) TFA, DCM, rt, 2–3 h, 71–85%.

guanidated glycine 25 was accessed by EDCI/HOBt coupling of amine 24 with 1,3-di-Boc-2-(carboxymethyl)guanidine in 37% yield; subsequent TFA deprotection afforded 1,3-substituted piperidine 26 in 65% yield (Scheme 4).

1-Naphthyl, 3,5-dimethoxyphenyl, 3-methoxyphenyl, and 3,4-dichlorophenyl analogs were prepared by reacting the corresponding substituted aniline with *N*-benzyl piperidone under Strecker conditions to give nitriles 27a–d in 55–77% yield (Scheme 5). Subsequent reduction of the nitriles using catalytic hydrogenation afforded amines 28a–d in excellent yields (89–95%), followed by EDCI/HOBt coupling with 1,3-di-Boc-2-(carboxymethyl)guanidine to give Boc protected guanidines 29a–d in moderate yields (33–67%). Deprotection with TFA afforded guanidines 30a–d in good yield (53–83%).

Analog 34 was synthesized by first coupling commercially available 1-(1-benzyl-4-phenylpiperidin-4-yl)methanamine 31 with Boc-gly-OH using EDCI/HOBt in 78% yield to give intermediate 32, deprotection with TFA and HgCl$_2$-assisted guanidation (33, 89% overall yield), and finally deprotection with TFA (Scheme 6). Methylene 42 and ethylene 43 analogs were prepared by initially generating the carbanion at the 4-position of commercially available 1-benzylpiperidine-4-carbonitrile (35) with LDA, then displacing benzyl bromide (to intermediate 36) or (2-bromoethyl)benzene (to intermediate 37) in 86–89% yield. Catalytic hydrogenation of the cyano moieties of intermediates 36 and 37 afforded the corresponding amines 38 and 39 in 61–90% yield. The Boc-protected guanidated glycines of amines 38 and 39 (intermediates 40 and 41, respectively) were prepared by either coupling with 1,3-di-

Boc-2-(carboxymethyl)guanidine using EDCI/HOBt or employing the sequence above (coupling with Boc-gly-OH, deprotection, HgCl$_2$-assisted Boc guanidation) in reasonable yields. Deprotection with TFA afforded methylene and ethylene analogs 42−43 in 71−85% yield (Scheme 6).

Reverse amide 46 was prepared by coupling commercially available 1-benzyl-4-phenylamino-4-carboxy-piperidine 44 (Scheme 7) with 2-(2-aminoethyl)-1,3-di-Boc-guanidine using EDCI/HOBt to give intermediate 45 in 70% yield, followed by TFA deprotection in 54% yield.

Scheme 7. Methylene Amide Modification of Lead 21: Synthesis of 46[a]
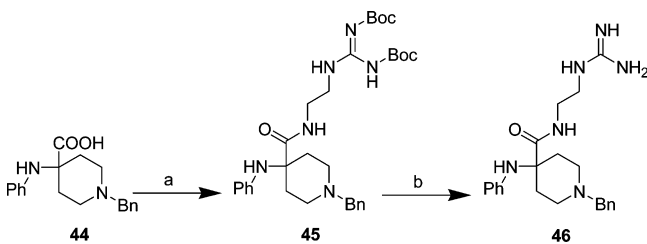
[a]Reagents and conditions: (a) 2-(2-aminoethyl)-1,3-di-Boc-guanidine, HOBt, EDCI, Et$_3$N, 1-methyl-2-pyrrolidone, rt, 24 h, 70%; (b) TFA, DCM, rt, 4 h, 54%.

Various aromatic and nonaromatic groups were explored at the piperidine nitrogen (49b−g, Scheme 8) by initial debenzylation of *tert*-butyl 1-(1-benzyl-4-(phenylamino)-piperidin-4-yl)-10,10-dimethyl-3,8-dioxo-9-oxa-2,5,7-triazaun-decan-6-ylidenecarbamate, 20 (Scheme 8), using catalytic hydrogenation to give intermediate 47 in 68% yield and subsequent displacement of the corresponding substituted bromide or chloride in 27−96% yield to afford intermediates 48b−d,f,g. Intermediate 48e was prepared by coupling 4-(aminomethyl)-1-(naphthalen-2-ylmethyl)-N-phenylpiperidin-4-amine, 5d (Scheme 1), with 1,3-di-Boc-2-(carboxymethyl)-guanidine using EDCI/HOBt in 48% yield. TFA deprotection
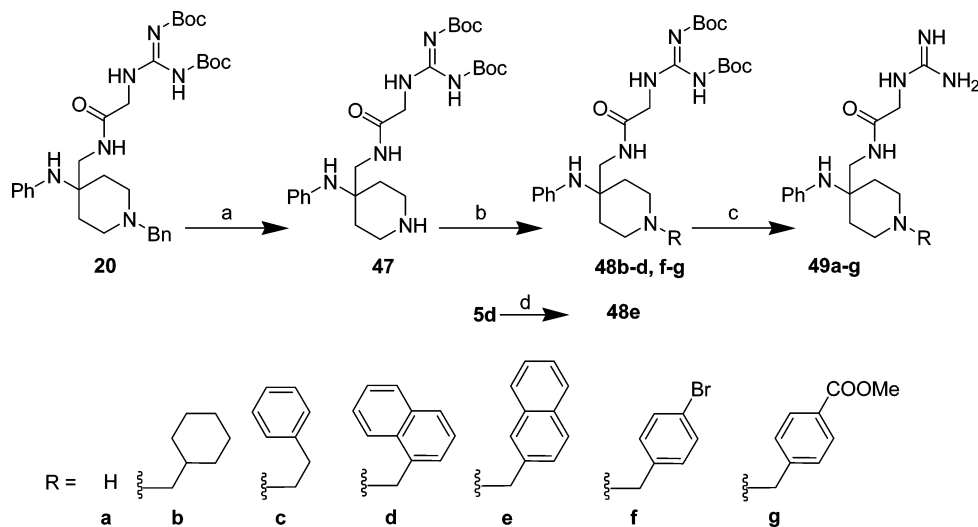
of Boc-protected guanidated glycines 48a−g afforded N-substituted analogs 49a−g in 54−85% yield.

1-Naphthyl (53a), 3,4-dichlorophenyl (53b), 2-naphthyl (53c), and biphenyl (53d) replacements were prepared using the same synthetic sequence as that of benzyl 42 and phenethyl 43 using 1-benzylpiperidine-4-carbonitrile, 35 (Scheme 9). Briefly, displacement of phenyl-substituted halides with the LDA-generated carbanion of carbonitrile 35 gave intermediates 50a−d in 59−91% yield, followed by catalytic hydrogenation of the cyano moieties to yield amines 51a−d (38−93%). The Boc-protected guanidated glycines of amines 51a−d (intermediates 52a−d) were prepared by either coupling with 1,3-di-Boc-2-(carboxymethyl)guanidine using EDCI/HOBt or employing the sequence above (coupling with Boc-gly-OH, deprotection, HgCl$_2$-assisted Boc guanidation) (Scheme 6) in 41−77% yield. Deprotection with TFA afforded 1-naphthyl (53a), 3,4-dichlorophenyl (53b), 2-naphthyl (53c), and biphenyl analogs (53c) in 54−76% yield.

Biology. *Binding Affinity and Functional Efficacy at hNPFF1-R and hNPFF2-R.* The affinities of 7a−d, 9a−c, 15, 17a−g, 21, 26, 30a−d, 34, 42−43, 46, 49a−g, and 53a−d at hNPFF1-R and hNPFF2-R were evaluated by competition experiments using the selective NPFF1 and NPFF2 radio-ligands [125I]YVP (YVPNLPQRFa) and [125I]EYF (EYW-SLAAPQRFa), or [3H]-NPVF and [3H]-EYF, respectively, in membranes of CHO cells stably expressing each receptor (Tables 1−4). Tested compounds were compared with previously reported high affinity peptides 1DMe and NPVF.[21] Selected compounds were tested for off-target affinity at μ-, δ-, and κ-opioid receptors (MOP, DOP, and KOP; Table 5) in competition experiments using the selective radioligands [3H]DAMGO, [3H]DPDPE, and [3H]U-69,593, respectively, in membranes of HEK 293 cells stably expressing each receptor (Table 5) and compared to the standard naloxone. The functional activity of 7b was tested by measuring its capacity to increase the binding of [35S]GTPγS in the membranes of CHO cells stably expressing each receptor (Figure 2). The functional activity of 21, 30a−c, 42, 46, and 53a was assessed by testing each compound's capacity to inhibit forskolin-induced intra-

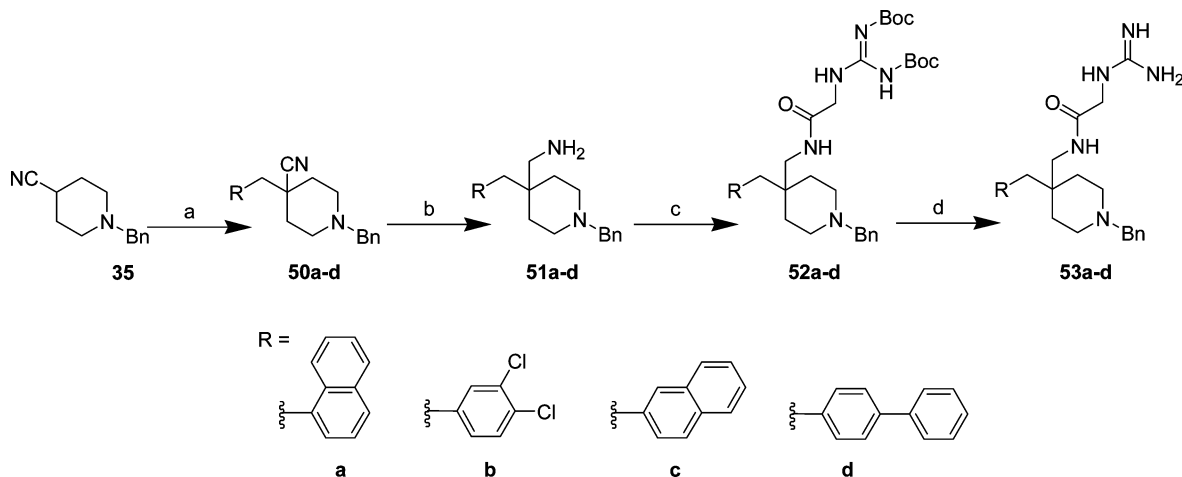## Scheme 8. Phenyl (Ring 2) Modification of Lead 21: Synthesis of 49a−g[a]

[a]Reagents and conditions: (a) Pd/C 10%, 4 atm H$_2$(g), MeOH, 7 days, 68%; (b) RCH$_2$Br(or Cl), DMF or NMP, 0 °C → rt, 33−44 h, 27−96%; (c) TFA, DCM, rt 3−15 h, 54−85%; (d) 1,3-di-Boc-2-(carboxymethyl)guanidine, HOBt, EDCI, Et$_3$N, DMF, 0 °C → rt, 15 h, 48%.

Scheme 9. Phenyl (Ring 1) Modification of NPFF2-Preferring Ligand 42: Synthesis of 53a–d[a]

[a]Reagents and conditions: (a) RCH₂Br(or Cl), LDA, THF(ah), −70 °C → rt, 2−3 h, 59−91%; (b) NH₃/MeOH, Raney Ni, 2.4 atm H₂(g), 6−20 h, 38−93%; (c) 1,3-di-Boc-2-(carboxymethyl)guanidine, HOBt, EDCI, Et₃N, DCM, 0 °C → rt, 15 h, 41−72% (for **52a−c**), for **52d**: (i) Boc-gly-OH, HOBt, EDCI, Et₃N, DCM, 0 °C → rt, 15 h, 77%, (ii) TFA, DCM, rt, 1 h, (iii) 1,3-bis(*tert*-butoxycarbonyl)-2-methyl-2-thiopseudourea, HgCl₂, Et₃N, DMF, rt, 15 h, 80%; (d) TFA, DCM, rt, 3 h, 54−76%.
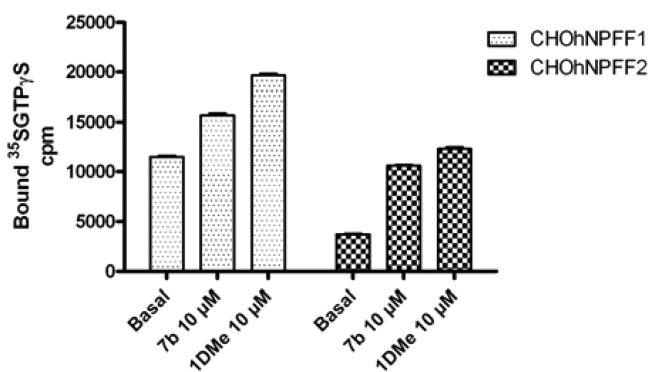

Figure 2. NPFF1-preferring ligand **7b** in the [³⁵S]GTPγS assay in cloned NPFF1-R and NPFF2-R. Compound **7b** (10 μM) increased by 36% and 184% the binding of [³⁵S]GTPγS on membranes of NPFF1-R and NPFF2-R expressing CHO cells, respectively, compared with a 71% and 231% increase by the agonist 1DMe (10 μM). Data represents the mean ± SEM from two-four experiments performed in duplicate.

Figure 3. Ability of the nonselective, low efficacy NPFF1 antagonist **21** to inhibit the effect of NPFF agonists in the forskolin-induced cAMP assay in CHO cells expressing human NPFF1-R (A) or NPFF2-R (B). Increasing doses of **21** were tested either alone or in the presence of 0.1 μM NPVF in CHO hNPFF1 cells or of 0.01 μM 1DMe in CHO hNPFF2 cells. ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$; different from NPVF alone (white bar) (one-way ANOVA followed by Dunnett's multiple comparison test). Data represents the mean ± SEM from two to four experiments performed in duplicate.

cellular cAMP accumulation in CHO cells stably expressing NPFF1-R and NPFF2-R (Figures 3A,B, 6A,B, 7, and 9A–D). The antagonist properties of **21**, **46**, and **53a** were evaluated by measuring its ability to reverse the NPVF- and 1DMe-induced inhibition of cAMP production in NPFF1-R and NPFF2-R, respectively (Figures 3A,B, 6B, and 9C,D), and in the case of **53a** to right-shift the dose−response curve of the aforementioned NPFF agonists in their respective subtypes (Figures 10A,B).

Among compounds bearing either an arginine or guanidine moiety at the 4-position of the piperidine ring and various lipophilic substitutions at the piperidine nitrogen (methyl, benzyl, phenethyl, and 2-naphthalenylmethyl) (**7a−d**, **9a−c**), arginines **7b** and **7d** with benzyl and 2-naphthalenylmethyl substitutions (Scheme 1) yielded affinity below 500 nM, indicating that both arginine and compact aromatic substitutions on the 4-(phenylamino)piperidine scaffold are detrimental for NPFF1,2 affinity (Table 1). While 2-naphthalenylmethyl **7d** gave nonselective affinity at NPFF1

and NPFF2, K_i = 479 ± 39 and 304 ± 14 nM (1.6-fold preference for NPFF2), respectively, we chose to utilize **7b** as the lead compound due to the *N*-benzyl being less bulky and for facile analog synthesis.

This benzyl analog (**7b**) bound to the NPFF1 subtype at K_i = 487 ± 117 nM with 2.2-fold preference over NPFF2. Indeed, the 2-naphthalenylmethyl substitution improved affinity at

Table 1. Binding Affinities of Guanidino-piperidines 7a−d, 9a−c, and 15a,b at NPFF1-R and NPFF2-R[a]

cmpd	hNPFF1-R (K_i, nM)	hNPFF2-R (K_i, nM)	ratio
1DMe	4 ± 0.5	0.8 ± 0.1	
7a	>5K	>5K	
7b	487 ± 117	1052 ± 32	2.2-fold (NPFF1)
7c	2906 ± 576	1694 ± 183	
7d	479 ± 39	304 ± 14	1.6-fold (NPFF2)
9a	>5K	>5K	
9b	1880 ± 53	6616 ± 377	
9c	2965 ± 112	2773 ± 470	
15	>5K	7579 ± 54	

[a]K_i values were determined by using [^{125}I]YVP and [^{125}I]EYF for hNPFF1-R and hNPFF2-R, respectively, and are the mean ± SEM from two to four experiments performed in duplicate.

Table 2. Binding Affinities of Guanidino-piperidines 7b, 17a−g and 21 at NPFF1-R and NPFF2-R[a]

cmpd	hNPFF1-R (K_i, nM)	hNPFF2-R (K_i, nM)	ratio
NPVF	3.66 ± 0.95		
1DMe	3.85 ± 0.42	0.47 ± 0.08	
7b[b]	487 ± 117	1052 ± 32	2.2-fold (NPFF1)
17a	441 ± 14	2907 ± 216	6-fold (NPFF1)
17b	3429 ± 377	3220 ± 53	
17c[b]	2692 ± 121	2682 ± 570	
17d[b]	2454 ± 338	2223 ± 388	
17e[b]	1623 ± 630	993 ± 87	
17f[b]	1966 ± 324	1620 ± 179	
17g[b]	2348 ± 413	2398 ± 288	
21	319 ± 19	405 ± 56	

[a]K_i values were determined by using [^{3}H]NPVF and [^{3}H]EYF for hNPFF1-R and hNPFF2-R, respectively, and are the mean ± SEM from two-four experiments performed in duplicate. [b]K_i values were determined by using [^{125}I]YVP and [^{125}I]EYF for hNPFF1-R and hNPFF2-R, respectively, and are the mean ± SEM from two to four experiments performed in duplicate.

NPFF2 and can be incorporated into future analog development if necessary. To further investigate the moieties of arginine **7b** responsible for NPFF1,2-binding, the aniline was removed (**15**, Scheme 2). This modification resulted in a complete loss of NPFF1 affinity and a 7-fold loss of affinity at NPFF2-R (Table 1), emphasizing the importance of this group on our core scaffold for generating NPFF ligands, as shown in Figure 1.

In the GTPγS assay for functional activity, **7b** exhibits agonist activity at both subtypes when tested at a high concentration of 10 μM (36% stimulation at NPFF1-R and 184% stimulation at NPFF2-R), albeit lower than the effect of full NPFF agonist 1DMe at 10 μM (71% stimulation at NPFF1-R and 231% stimulation at NPFF2-R; Figure 2). Although **7b** has modest affinity and preference for NPFF1-R, this analog serves as a good starting point to develop small molecule NPFF1-selective agonists, a pharmacological profile that is currently lacking in the literature (as shown in Chart 1). From the limited SAR above, we note that preference for either subtype for these arginine-bearing analogs can be modulated in part by substitution at the piperidine nitrogen (**7b** vs **7d**) with 1-C linked aromatic groups. Further substitutions, including 1-C linked cycloalkyl, bicyclic and tricyclic aromatic rings, and heterocyclic rings could be explored to potentially afford NPFF1 or NPFF2 agonists.

SAR of NPFF1 Agonist 7b. From the SAR above, it was determined that disubstitution at the 4-position of the piperidine ring with aniline and a C-terminal linked arginine (linked via a methylene amide) were necessary for affinity at NPFF1,2-R, in addition to compact aromatic substitution at the 1-position. To evaluate the importance of the two positively charged moieties of **7b**, the α-amino and guanidinium groups, the α-amino group was removed in favor of retaining the guanidine moiety present in most reported NPFF1,2 ligands (**17d**) (Scheme 3, Table 2). Additionally, the linker length between the guanidinium and amide groups was explored with aliphatic (one to three carbons, **17a, 17c, 21**) and rigidified aromatic linkers (three to five carbons, **17e−g**) (Scheme 3, Table 2).

Removal of the α-amino group of **7b** (**17d**) yields a 5- and 2-fold loss of affinity at NPFF1-R and NPFF2-R, respectively, indicating the importance of a positively charged moiety near the core piperidine structure. The distance between the positively charged guanidine and the core structure was then investigated by deleting one to three aliphatic linkers (**17c, 17a, 21**). Shortening the linker length by one carbon (**17c**) affords

similar affinity at both subtypes as **17d**, however; two carbon deletion (**17a**) yields similar affinity at NPFF1 as **7b** and 6-fold selectivity. Three carbon deletion (**21**) affords a 1.5- and 2.6-fold increase in affinity at NPFF1-R and NPFF2-R, respectively, compared with lead **7b**, with K_i values of 319 ± 19 and 405 ± 56 nM, greatly simplifying our lead for optimization. Thus, considering **7b** vs **21**, only one positively charged group is needed on this scaffold, projected a short distance from the core piperidine. Substituting an amine (**17b**) in place of the guanidine moiety of **21** results in an 11- and 8-fold loss of affinity at NPFF1-R and NPFF2-R, respectively, suggesting a potential bidentate interaction. Exploring spacers between the guanidinium group and the amide of **7b** with aromatic-linked guanidines (three to five carbons), **17e−g** afford a 3.3−4.8-fold loss of affinity at NPFF1 relative to **7b**, and a 1.5−2.3-fold loss of affinity at NPFF2-R. Such a result indicates that only one aromatic group is tolerable at the 4-position of the piperidine ring, in addition, as seen with the aliphatic linked analogs above, the placement of the guanidine group relative to the core structure is of paramount importance.

When evaluated alone in the forskolin-induced cAMP assay at both subtypes, **21** displays no agonist activity at concentrations up to 1 μM and weak partial agonist activity at concentrations >1 μM (data not shown), in contrast to the effect observed for peptide agonists NPVF and 1DMe at NPFF1-R and NPFF2-R, respectively. In the same assay, guanidated glycine **21** partially reverses the inhibitory effect of 0.1 μM NPVF at NPFF1-R, with an EC$_{50}$ of 21.98 ± 5.08 μM and corresponding E_{max} of 83 ± 2% (Figure 3A). At NPFF2-R, this analog exhibits low agonist activity (20−25% inhibition of cyclase at high concentrations of 10 and 30 μM) and less potently inhibits the effect of 0.01 μM 1DMe compared with its effect on NPVF at NPFF1-R (Figure 3B). We considered this simplified structure (**21**) as our lead for optimization to generate NPFF2 selective small molecule ligands, since compounds of this profile are not available and would aid in the pharmacological classification of this subtype.

Collectively, based on these compounds born from our proposed general scaffold for designed NPFF1,2 ligands (Figure 1), we have noted a 2D pharmacophore for both affinity and modulation of functional activity at both subtypes

(Figure 4). Both subtypes favor substitution at the piperidine nitrogen with methylene-linked aromatic groups such as a phenyl and 2-naphthyl.
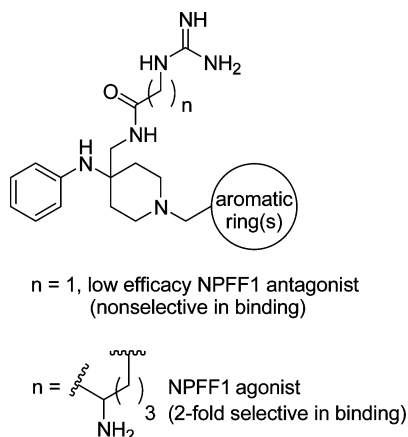

Figure 4. Two-dimensional pharmacophore for affinity and modulation of functional activity at NPFF1-R and NPFF2-R.

Disubstitution at the 4-position of the piperidine ring with an aniline moiety and a methylene amide linked to a positively charged moiety (or moieties) is also necessary for recognition at both subtypes. The nature of the positively charged moiety is crucial for modulation of functional efficacy: while an arginine side chain (**7b**) yields a modestly preferring NPFF1 agonist, a guanidated glycine (**21**) affords a nonselective compound that weakly antagonizes NPFF1-R.

SAR of Low Efficacy NPFF1 Antagonist 21. In our quest to identify NPFF2 selective ligands, we performed SAR analysis of low efficacy NPFF1 antagonist **21** (K_i NPFF1-R = 319 ± 19 nM; K_i NPFF2-R = 405 ± 56 nM), focusing on four areas: (1) the aniline phenyl ring (ring 1), (2) the aniline amine, (3) the methylene amide, and (4) the benzyl ring (ring 2) at the N-1 position (Figure 5).
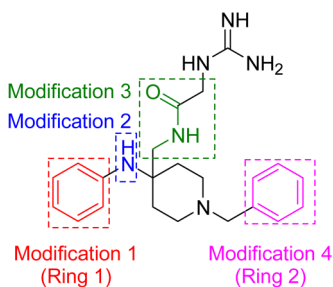

Figure 5. Sites of modification (1−4) for low efficacy NPFF1 antagonist **21**.

To first validate the 4,4-substitution pattern of **21** about the piperidine ring before exploring modifications 1−4 above, the corresponding 3,3-substituted analog was investigated (**26**) (Scheme 4), resulting in a 2.8- and 4.7-fold loss of affinity at NPFF1-R and NPFF2-R (Table 3). Although disubstitution of these pharmacophoric elements at the two-position of the piperidine ring was not explored, we felt a 4,4-substitution pattern was optimal for our novel scaffold.

We next turned our attention to substituting the aniline phenyl ring of lead **21** (modification 1, Scheme 5) with bulky, hydrophobic 1-naphthyl **30a** and 3,4-dichlorophenyl **30d**

Table 3. Binding Affinities of Guanidino-piperidines 26, 30a−d, 34, 42−43, 46, and 49a−g at NPFF1-R and NPFF2-R[a]

cmpd	hNPFF1-R (K_i, nM)	hNPFF2-R (K_i, nM)	ratio
NPVF	3.66 ± 0.95		
1DMe	3.85 ± 0.42	0.47 ± 0.08	
21	319 ± 19	405 ± 56	
26	904 ± 237	1893 ± 490	
30a	94 ± 24	309 ± 55	3.3-fold (NPFF1)
30b	114 ± 17	987 ± 65	8.7-fold (NPFF1)
30c	201 ± 35	884 ± 177	4.4-fold (NPFF1)
30d	191 ± 31	409 ± 39	2.1-fold (NPFF1)
34	511 ± 79	3505 ± 847	6.9-fold (NPFF1)
42	417 ± 74	84 ± 12	5-fold (NPFF2)
43	151 ± 15	465 ± 108	3-fold (NPFF1)
46	81 ± 17	1426 ± 49	17.6-fold (NPFF1)
49a	15085 ± 355	2757 ± 81	
49b	1638 ± 499	298 ± 14	5.5-fold (NPFF2)
49c	1057 ± 150	330 ± 28	3.2-fold (NPFF2)
49d	538 ± 40	129 ± 26	4.2-fold (NPFF2)
49e	141 ± 34	504 ± 95	3.6-fold (NPFF1)
49f	112 ± 11	454 ± 4	4-fold (NPFF1)
49g	340 ± 91	1513 ± 33	4.5-fold (NPFF1)

[a]K_i values were determined by using [³H]NPVF and [³H]EYF for hNPFF1-R and hNPFF2-R, respectively, and are the mean ± SEM from two to four experiments performed in duplicate.

groups, as well as groups containing H-bond acceptor moieties 3,5-dimethoxyphenyl **30b** and 3-methoxyphenyl **30c** (Scheme 5), because the aniline moiety as a whole affects NPFF1,2 binding (**7b** vs **15**). These modifications afforded a 1.6−3.4-fold gain of affinity for NPFF1-R compared with lead **21** (K_i = 94 ± 24 to 201 ± 35 nM), while showing comparable or less affinity at NPFF2-R (Table 3). In particular, 1-naphthyl **30a** and 3,5-dimethoxyphenyl **30b** substitutions were favored at NPFF1-R (K_i = 94 ± 24 and 114 ± 17 nM, respectively), while 3-methoxyphenyl **30c** and 3,4-dichlorophenyl **30d** proved to be half as active at this subtype (K_i = 191 ± 31 to 201 ± 35 nM), indicating that additional hydrophobic interaction(s) accessed by **30a** and **30b** in this region of the scaffold are beneficial for NPFF1 binding only.

Despite showing modest (3.3−8.7-fold) preference for NPFF1-R binding, analogs **30a−c** were assayed alone for functional activity in the forskolin-induced cAMP assay (Figure 6A). No activity was observed at concentrations up to 10 μM; thus, a lack of agonist activity was retained on our scaffold.

Continuing to explore the aniline moiety, the aniline NH (modification 2, Scheme 6) was removed (**34**) and replaced with methylene (**42**) and ethylene (**43**) groups. Removal of the amine (**34**) results in only a modest loss of affinity at NPFF1-R compared with lead **21** (1.6-fold) but a significant loss of affinity at NPFF2-R for this scaffold (8.7-fold) (Table 3), suggesting the necessity of a spacer atom between the phenyl and piperidine rings for binding at NPFF2-R only. Substitution with a methylene moiety (**42**) again yields similar affinity at NPFF1-R, and a 5-fold increase in affinity at NPFF2-R compared with lead **21** (K_i = 84 ± 12 nM), indicating a greater degree of lipophilicity required for NPFF2 binding. Additionally, the spatial arrangement of the phenyl moiety could be important for NPFF2 preference, since switching from a trigonal pyramidal NH (**21**) to an sp³ C (**42**) yields 5-fold preference for NPFF2-R. In the forskolin-induced cAMP assay
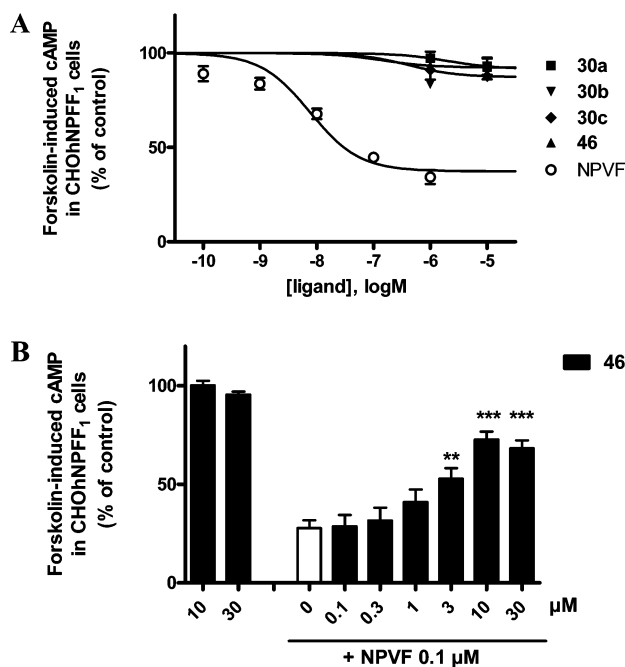
Figure 6. (A) NPFF1-preferring ligands **30a−c** and NPFF1-selective ligand **46** in the forskolin-induced cAMP assay in cloned NPFF1-R, compared with the NPFF1 selective agonist NPVF. (B) Ability of the selective NPFF1 antagonist **46** to reverse the effect of 0.1 μM NPVF in the forskolin-induced cAMP assay in CHO cells expressing hNPFF1-R. Increasing doses of **46** were tested either alone or in the presence of NPVF. ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$; different from NPVF alone (white bar) (one-way ANOVA followed by Dunnett's multiple comparison test). Data represents the mean $\pm$ SEM from two-four experiments performed in duplicate.
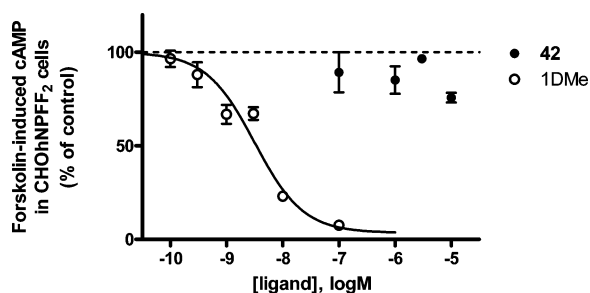

Figure 7. NPFF2-preferring ligand **42** in the forskolin-induced cAMP assay in cloned NPFF2-R, compared with the NPFF agonist 1DMe. Data represents the mean $\pm$ SEM from two to four experiments performed in duplicate.

at NPFF2-R, benzyl **42** alone is inactive at concentrations up to 1 μM (Figure 7), again indicating this scaffold's lack of agonist activity. Extending the phenyl ring by an additional carbon (phenethyl **43**) is not tolerated at NPFF2-R, resulting in a 5.5-fold loss of affinity relative to methylene **42** ($K_i = 465 \pm 108$ nM), instead binding to NPFF1-R with 151 nM affinity. Collectively, mostly NPFF2 binding is affected by substitution of the aniline NH, while NPFF1 binding is affected by substitution of the aniline phenyl ring.

The importance of the methylene amide at the four-position of the piperidine ring of lead **21** was explored by simultaneous removal of the methylene moiety and replacement with a reverse amide (**46**; modification 3, Scheme 7). Additionally, the

linker length between the amide and guanidine moieties was extended by one carbon, in order to retain the same distance between the guanidine and piperidine moieties as lead **21**. This change allowed for a 4-fold improvement at NPFF1 relative to lead **21** ($K_i = 81 \pm 17$ nM) and 17.6-fold selectivity vs NPFF2 (Table 3). Thus, selectivity for NPFF1 is controlled by positioning of the amine and carbonyl groups; in addition, the spatial orientation of the guanidine in relation to the core piperidine scaffold may also contribute to selectivity (connected via a planar carbonyl group (**46**) vs a flexible methylene group (**21**)). In the forskolin-induced cAMP assay at NPFF1-R, reverse amide **46** alone is inactive at concentrations up to 10 μM (Figure 6A,B) and dose-dependently antagonizes the effect of 0.1 μM NPVF at an $EC_{50} = 3.52 \pm 1.42$ μM and $E_{max} = 68 \pm 16\%$ (Figure 6B). In terms of its good affinity and selectivity, reverse amide **46** is the first small molecule, nonpeptide NPFF1-R antagonist known, in contrast to dipeptide NPFF1-R antagonist **1**. Considering its reduced polar surface area and number of metabolically labile amide bonds compared with dipeptide **1**, reverse amide **46** represents a small molecule lead for drug development targeting the NPFF1-R subtype, since dipeptide **1** was recently shown to strongly reduce OIH induced by the opioid agonist fentanyl.[17]

Various methylene-linked aromatic and nonaromatic substitutions of phenyl ring 2, located off of the 1-position of the core piperidine ring, were also explored (**49b−g**; modification 4, Scheme 8). As suggested by N-methyl analogs **7a** and **9a** (Scheme 1, Table 1), removal of the N-substituent (**49a**) resulted in a significant loss of affinity at both subtypes (Table 3). Cyclohexyl **49b**, benzyl **49c**, and 1-naphthyl **49d** provided modest improvement (1.2−3.1-fold) in binding to NPFF2-R compared with lead **21** ($K_i = 298 \pm 14$, 330 ± 28, and 129 ± 26 nM, respectively), with 3.2−5.5-fold preference vs NPFF1-R. Interestingly, replacement of the benzyl group of lead **21** with a cyclohexyl moiety shows the importance of this aromatic ring for NPFF1-R affinity only, since **49b** yields a 5-fold drop in NPFF1-R affinity, while retaining similar affinity at NPFF2-R. Conversely, 2-naphthyl **49e** and 4-bromophenyl **49f** are more active at NPFF1-R, providing 2.3−2.8-fold improvement in binding to NPFF1-R compared with lead **21** ($K_i = 112 \pm 11$ to 141 ± 34 nM), with 3.6−4.5-fold preference vs NPFF2-R. 4-Methyl benzoate analog **49g** yields similar affinity at NPFF1-R as lead **21**, with 4.5-fold preference. In summary, on this scaffold, selectivity for either subtype can be controlled by N-1 substitution: both cycloalkyl (**49b**) and unsubstituted aromatic moieties (**49c**) afford NPFF2-R-preferring compounds, whereas a broad range of substituted aromatic groups favor the NPFF1-R subtype, including both bromo (**49f**) and methyl ester (**49g**) groups. Additionally, selectivity for NPFF2-R vs NPFF1-R can be achieved with a 1- vs 2-naphthyl group (**49d** vs **49e**), indicating that the NPFF2-R pocket is of a more discriminate volume.

*In Vivo Testing of NPFF1-Selective Ligand **46**.* Mice exposed to a 48 °C warm-water bath demonstrated a 7.85 ± 0.51 s latency to tail-withdrawal. NPFF (30 nmol, icv) significantly reduced tail-withdrawal latency in the mouse 48 °C warm-water tail-withdrawal assay over time ($F_{(9,118)} = 5.56$, $p < 0.0001$, one-way ANOVA),[6,8] whereas vehicle (icv) was without effect ($F_{(9,74)} = 0.19$, $p = 0.99$, one-way ANOVA; Figure 8A,B).

Administration of the nonselective NPFF1,2-R antagonist **RF9** (10 nmol, icv) was without effect on the tail-withdrawal latency ($F_{(9,77)} = 1.74$, $p = 0.09$, one-way ANOVA), but a 20
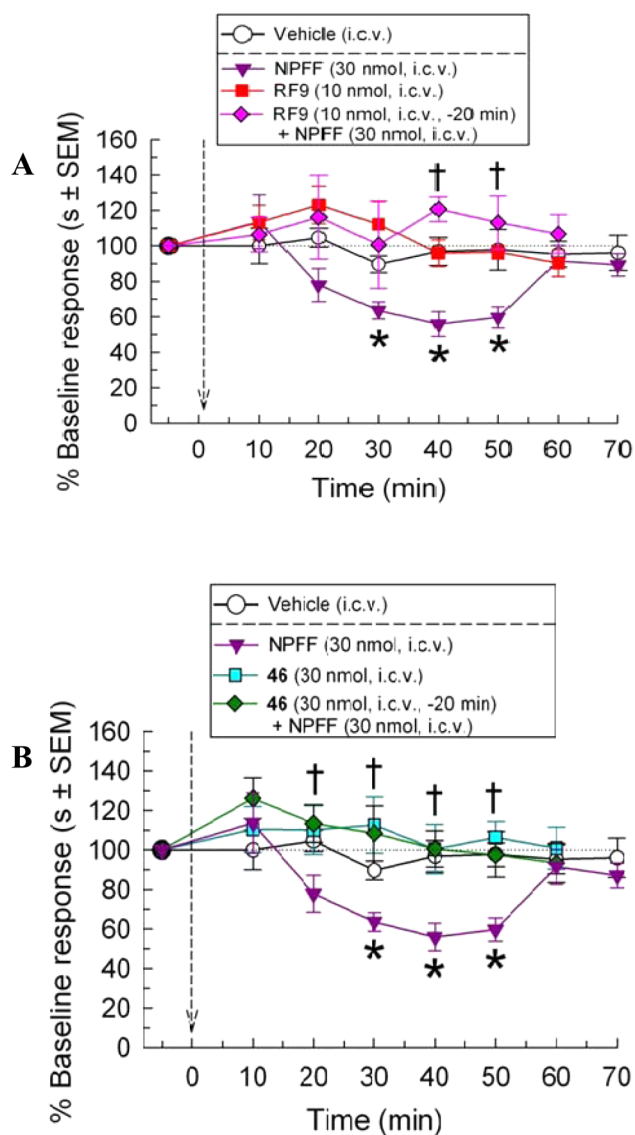
Figure 8. NPFF-induced hyperalgesia is prevented by pretreatment with NPFF-receptor antagonists. Mouse latencies to withdraw their tail from a 48 °C warm-water stimulus were measured repeatedly over 80 min after administration of test compounds. After collection of baseline responses (left of arrow), arrow denotes single administration of vehicle (50% DMSO, icv; white circles), NPFF (30 nmol, icv; purple triangles), RF9 (10 nmol icv, squares in part A) or **46** (30 nmol icv; squares in part B). Additional mice were pretreated 20 min with RF9 (A) or **46** (B) prior to administration of NPFF (diamonds). Points represent $n = 7-12$ mice, with average % baseline response $\pm$ SEM plotted. *$p < 0.05$ compared with baseline response with one-way ANOVA with Tukey HSD *post hoc* test; †$p < 0.05$ compared with NPFF response with two-way ANOVA.

min pretreatment significantly reversed NPFF-mediated hyper-algesia ($F_{(3,188)} = 8.92$, $p < 0.0001$, two-way ANOVA; Figure 8A). Similarly, pretreatment with the NPFF1-R selective antagonist **46** (30 nmol, icv) also significantly prevented the NPFF-induced hyperalgesic effects ($F_{(3,233)} = 9.58$, $p < 0.0001$, two-way ANOVA; Figure 8B), without demonstrating significant differences from either baseline or vehicle-treated responses.

SAR of NPFF2-Preferring Ligand 42. Since substitution at the aniline NH (modification 2) with a methylene group (benzyl **42**) yielded a high affinity NPFF2 ligand ($K_i = 84 \pm 12$

nM) with 5-fold preference, we considered this as our new lead compound to generate NPFF2 ligands and explored the effect of replacing the proximal phenyl ring 1 of this scaffold with bulky aromatic rings as shown in Scheme 9.

Substitution of phenyl ring 1 of lead **42** with a 1-naphthyl group (**53a**) afforded a 2.8-fold increase in affinity at NPFF2-R ($K_i = 30 \pm 5$ nM) relative to lead **42**, with similar preference (Table 4). Substitution with a 3,4-dichlorophenyl moiety (**53b**)

Table 4. Binding Affinities of Guanidino-piperidines 53a–d at NPFF1-R and NPFF2-R[a]

cmpd	hNPFF1-R (K_i, nM)	hNPFF2-R (K_i, nM)	ratio
NPVF	3.66 ± 0.95		
1DMe	3.85 ± 0.42	0.47 ± 0.08	
42	417 ± 74	84 ± 12	5-fold (NPFF2)
53a	112 ± 19	30 ± 5	3.7-fold (NPFF2)
53b	432 ± 14	82 ± 8	5.3-fold (NPFF2)
53c	316 ± 11	205 ± 8	1.5-fold (NPFF2)
53d	296 ± 14	910 ± 107	3-fold (NPFF1)

[a]K_i values were determined by using [³H]NPVF and [³H]EYF for hNPFF1-R and hNPFF2-R, respectively, and are the mean ± SEM from two-four experiments performed in duplicate.

decreased affinity by 2.7-fold at NPFF2 relative to 1-naphthyl **53a**, suggesting a potential $\pi-\pi$ stacking interaction in the NPFF2 pocket. Interestingly, substitution with 2-naphthyl (**53c**) or biphenyl groups (**53d**) results in 6.8- and 30-fold losses of affinity at NPFF2-R relative to 1-naphthyl **53a**, indicating that correct placement of the additional aromatic group on this scaffold is important for maintaining affinity at NPFF2-R.

We note that substitution of phenyl ring 1 on this scaffold improves NPFF2 binding and maintains preference for this subtype, while the same substitutions on the aniline-containing scaffold (**30a**, **30d**, Scheme 5, Table 3) afford compounds with preference toward the NPFF1 subtype. This result suggests different binding conformations for the aniline- vs benzyl-containing scaffolds at NPFF1-R and NPFF2-R, respectively. This observation could be further investigated by installing NPFF2-preferring N-1 substituents (as in **49b–d**) onto the benzyl scaffold.

In the forskolin-induced cAMP assay at NPFF1-R and NPFF2-R, 1-naphthyl **53a** alone exhibits low activity at concentrations up to 10 μM (Figures 9A–D). To evaluate the antagonistic property of naphthyl **53a**, various concentrations were tested in the presence of a fixed concentration of NPVF (0.1 μM, at NPFF1-R) and the stable NPFF peptide agonist 1DMe (0.01 μM, at NPFF2-R) in the cAMP assay (Figures 9C,D). 1-Naphthyl **53a** dose-dependently reversed both NPVF- and 1DMe-inhibition of forskolin-induced cAMP accumulation with EC$_{50}$ values of 2.6 ± 0.69 μM (Figure 9C) and 645 ± 143 nM (Figure 9D), respectively. Corresponding E_{max} values at NPFF1-R and NPFF2-R were 56.2 ± 2.6% and 22 ± 1.5%, respectively.

In addition, a dose–response curve of NPVF and 1DMe in the same assay was performed in the absence or the presence of 10 μM **53a** (Figures 10A,B) at each subtype. 1-Naphthyl **53a** (10 μM) induces a 12-fold loss of functional activity for NPVF at NPFF1-R (EC$_{50}$ = 181 ± 38 nM in the presence of **53a** compared with 15 ± 0.6 nM alone; Figure 10A); similarly, this compound induces a 4-fold loss of functional activity for 1DMe at NPFF2-R (EC$_{50}$ = 11.4 ± 3.2 nM in the presence of **53a**
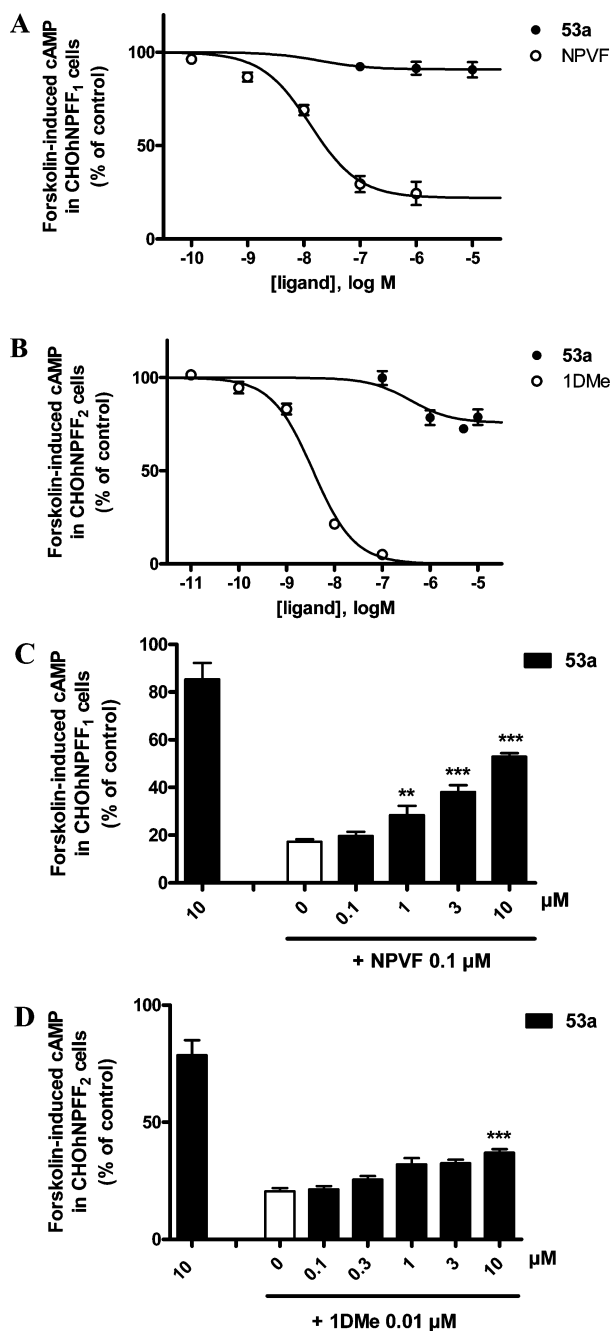
Figure 9. NPFF2-preferring ligand 53a in the forskolin-induced cAMP assay in cloned NPFF1-R (A) and NPFF2-R (B) compared with the NPFF1 and NPFF2 selective agonists NPVF and 1DMe, respectively. Ability of NPFF2-preferring partial antagonist 53a to reverse the effect of 0.1 μM NPVF (C) or 0.01 μM 1DMe (D) in the forskolin-induced cAMP assay in CHO cells expressing hNPFF1-R or hNPFF2-R, respectively. Increasing doses of 53a were tested either alone or in the presence of NPVF or 1DMe. ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$; different from NPVF or 1DMe alone (white bar) (one-way ANOVA followed by Dunnett's multiple comparison test). Data represents the mean ± SEM from two to four experiments performed in duplicate.

Figure 10. Ability of NPFF2-preferring partial antagonist 53a to right-shift the dose−response curve of NPVF (A) or 1DMe (B) in the forskolin-induced cAMP assay in CHO cells expressing hNPFF1-R or hNPFF2-R, respectively. Data represents the mean ± SEM from two to four experiments performed in duplicate.

develop selective small molecule NPFF2 antagonists as pharmacological probes.

Binding Affinity of Selected Compounds at μ-, δ-, and κ-Opioid Receptors. Because no selective, pharmacologically pure, and high affinity NPFF1 and NPFF2 agonists and antagonists are available to the scientific community (save dipeptide NPFF1 antagonist 1) to investigate the opioid-modulating properties of the NPFF1 and NPFF2 subtypes, we tested compounds displaying reasonable affinities and a variety of pharmacological profiles (summarized in Table 6) at both subtypes for off-target affinity at the three opioid subtypes (Table 5).

In general, most compounds possessed modest preference for NPFF1 or NPFF2 vs MOP and KOP (2−12-fold), and most ligands did not bind at DOP with any appreciable affinity (>5K) (Table 5). Notably, the NPFF1-selective antagonist 46 afforded ≥18-fold selectivity at NPFF1 vs MOP/DOP/KOP, while NPFF2-preferring ligands 42 and 53a yielded 2- and 12-fold selectivity, respectively, for NPFF2-R vs MOP and KOP. Similar to compound 46, NPFF1-preferring ligands 30a and 30b were also reasonably selective vs MOP and KOP (7−43-fold).

Such modest preference for NPFF1,2-R vs MOP and KOP is not surprising, given that most of these ligands contain a 4-anilido piperidine, a known scaffold for MOP analgesia (i.e., fentanyl).[28] Additionally, the seemingly requisite guanidine moiety on our scaffold is reminiscent of 5′-guanidinonaltrindole (GNTI),[29] a potent and selective KOP antagonist. These moieties will be taken into account during further SAR exploration of our lead compounds, namely, 7b, 46, and 53a, in order to generate selective NPFF1 and NPFF2 ligands. Additionally, docking of 7b, 46, and 53a into the active state homology models and crystal structures of MOP and KOP (depending on their functional activities at each subtype) could

compared with 2.8 ± 0.3 nM alone; Figure 10B). As a result, 1-naphthyl 53a represents the first small molecule NPFF2 partial antagonist reported in the literature. Although exhibiting modest preference toward the NPFF2 subtype (3.7-fold), this compound's unprecedented high affinity (30 nM) at NPFF2-R bears further SAR investigation, serving as an excellent lead to
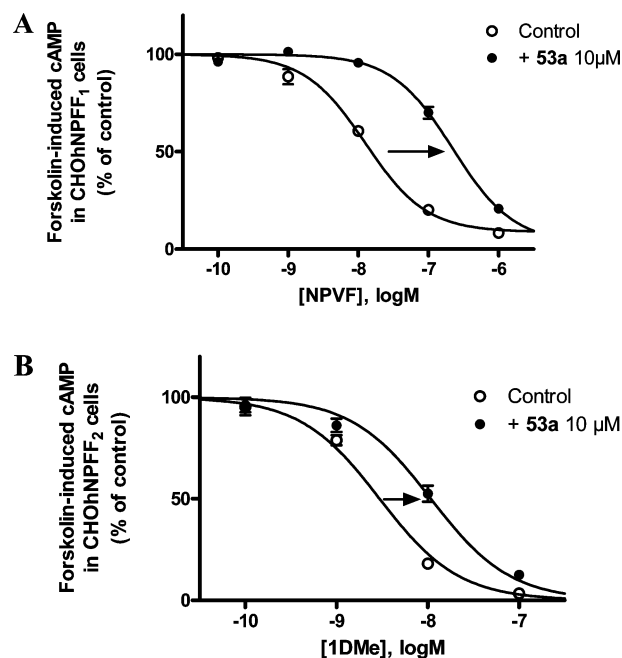
Table 5. Binding Affinities of Selected Guanidino-piperidines at μ-, δ-, and κ-Opioid Receptors[a]

cmpd	hNPFF1-R (K_i, nM)	hNPFF2-R (K_i, nM)	MOP (K_i, nM)	DOP (K_i, nM)	KOP (K_i, nM)
naloxone			3.39 ± 0.08	37.2 ± 1.3	4.83 ± 0.08
RF9	58 ± 5[b]	75 ± 9[b]	>5K[b]	>5K[b]	>5K[b]
7b	487 ± 117	1052 ± 32	>5K	>5K	1233 ± 30
21	319 ± 19	405 ± 56	2022 ± 170	>5K	705 ± 80
30a	94 ± 24	309 ± 55	688 ± 60	>5K	3678 ± 530
30b	114 ± 17	987 ± 65	>5K	>5K	4247 ± 580
42	417 ± 74	84 ± 12	201 ± 10	2486 ± 200	224 ± 20
46	81 ± 17	1426 ± 49	>5K	2607 ± 650	1470 ± 190
53a	112 ± 19	30 ± 5	354 ± 90	1516 ± 330	362 ± 30

[a]K_i values were determined using radioligands [^{3}H]DAMGO, [^{3}H]DPDPE, and [^{3}H]U-69,593 for MOP, DOP, and KOP receptors, respectively, and are the mean ± SEM from three experiments. [b]Data taken from ref 15.

Table 6. Summary of Functional Activities of Selected Guanidino-piperidines on hNPFF1-R and hNPFF2-R Receptors Expressed in CHO Cells

cmpd	cAMP hNPFF1-R		cAMP hNPFF2-R	
	antagonist activity EC_{50},[a] nM	E_{max}, %	antagonist activity EC_{50},[a] nM	E_{max}, %
RF9	4700 ± 1200[b]	79 ± 5[c]	h	h
7b		36[d]		184[d]
21	21980 ± 5080	83 ± 2	g	20−25[e]
30a	>10K[f]	g	g	g
30b	>10K[f]	g	g	g
42	g	g	1K[f]	g
46	3520 ± 1420	68 ± 16	g	g
53a	2600 ± 690	56.2 ± 3	645 ± 143	22 ± 1.5

[a]EC_{50} is the concentration that produces a 50% reversion of the NPVF (0.1 μM) or 1DMe (0.01 μM)-induced inhibition of forksolin-stimulated cAMP accumulation in CHO hNPFF1 and hNPFF2 cells, respectively. [b]Data taken from ref 15. [c]Unpublished observation. [d]% Maximal activation in the [^{35}S]GTPγS assay at 10 μM. [e]% Inhibition of forskolin-stimulated cAMP accumulation at concentrations of 10 and 30 μM. [f]Highest concentration showing no agonist activity when tested alone in the forskolin-induced cAMP assay. [g]Not determined. [h]Not reported.

also shed light on further modifications to be made to this scaffold in order to minimize off-target opioid affinity. While the opioid affinity of these leads is of primary concern, we also recognize the need to assay these compounds for any off-target affinity at neuropeptide Y and orexin receptors due to 30−35% homology with NPFF1,2-R, other receptors within the RFa peptide family (GPR10, GPR54, and GPR103), and the nociceptin opioid receptor (NOPr, an opioid-modulating receptor), as previously done for RF9, the most well characterized NPFF ligand to date.[15]

Collectively, SAR analysis of four regions of nonselective NPFF1 antagonist **21** led to the discovery of three small molecule, NPFF2-preferring ligands, benzyl **42**, 3,4-dichlorophenyl **53b**, and partial antagonist 1-naphthyl **53a**. All analogues displayed good affinity at NPFF2-R, 84 ± 12, 82 ± 8, and 30 ± 5 nM, respectively, and 3.7−5-fold preference vs NPFF1-R. Additionally, SAR analysis of **21** allowed us to generate a 2D pharmacophore for NPFF2-preferring ligands (Figure 11).

We found that substitution of the aniline moiety of nonselective antagonist **21** with compact aromatic groups such as benzyl (**42**), 1-naphthalenylmethyl (**53a**), and 3,4-dichlorophenylmethylene (**53b**) improved affinity and preference for NPFF2, particularly for **53a** (14-fold improvement in binding affinity, 3.7-fold preference vs NPFF1). The methylene amide at the four-position of the piperidine ring proved crucial for maintaining recognition at NPFF2 (**46**). Additionally, lipophilic groups substituted at N-1, including both cycloalkyl (**49b**) and aromatic (**49c,d**) moieties, were preferred at NPFF2, indicating that substitution at the 4- rather than 1-position of the ring may mimic the Phe[8] interaction of the endogenous ligand NPFF. Further SAR studies of NPFF2-preferring lead **53a**, to be done by substituting NPFF2-preferring N-1 substituents (as in **49b−d**), are planned to potentially increase affinity and preference for this subtype.

■ CONCLUSION

Considering the structures of a limited number of NPFF1,2 dipeptides and low affinity small molecules, as well as the endogenous ligand NPFF, ligand-based rational design was employed to construct the first small molecule scaffold for NPFF1,2-R displaying significant improvements in affinity relative to the aryliminoguanidines (Chart 1), as well as a tractable SAR for determining functional activity and preference
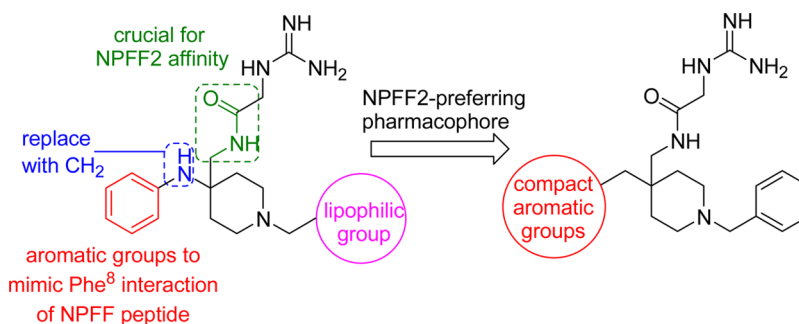
Figure 11. Two-dimensional pharmacophore for NPFF2-preferring ligands from SAR analysis of low efficacy NPFF1 antagonist **21**.

for either subtype, as summarized in our 2D pharmacophore models (Figures 4 and 11). Specifically, the guanidino-piperidine class of NPFF ligands represents the first disclosure of a small molecule scaffold whose SAR results in a wide range of pharmacological profiles at NPFF1,2-R to serve as lead compounds to develop pharmacological tools, namely, a NPFF1 agonist (**7b**, K_i = 487 ± 117 nM), a NPFF1 antagonist (**46**, K_i = 81 ± 17 nM), and a NPFF2 partial antagonist (**53a**, K_i = 30 ± 5 nM). In vivo testing of the novel NPFF1-R antagonist **46** demonstrated antagonism of NPFF-mediated hyperalgesia without significant effect alone on tail-withdrawal latencies (Figure 8B), similar to the effects seen with dipeptide NPFF1,2 antagonist **RF9** (Figure 8A),[15,16] the most established NPFF ligand to date, and dipeptide NPFF1 antagonist **1**.[17] SAR studies of reverse amide **46**, including exploration of this scaffold with NPFF1-preferring moieties (such as in those of **30a,b**, **43**, and **49e,f**), could afford compounds of an improved NPFF1 profile to combat opioid-induced hyperalgesia. The SAR of arginine **7b**, reverse amide **46**, and 1-naphthyl **53a** will be further explored, using the approaches described herein, in order to generate NPFF1 and NPFF2 selective agonists and antagonists to further explore and characterize the pharmacology of these subtypes as they relate to opioid-mediated analgesia.

■ EXPERIMENTAL SECTION

Chemistry. All the solvents and reagents were obtained commercially and used as received unless noted otherwise. Anhydrous diethyl ether was prepared by refluxing with sodium; anhydrous THF was prepared by refluxing with sodium and benzophenone. Flash chromatography was performed with Merck silica gel 60 (230 × 400 mesh). NMR spectra were recorded on either a Bruker AVIII 400 spectrometer or Bruker DRX500. For **7a–7e**, **9a–9c** and **15a,b**, HPLC/MS analyses were recorded on a Waters Alliance LC/MS System, consisting of a Waters ZQ mass detector, photodiode array detector, and an Alliance HPLC system, equipped with an XTerra column (C-18, 2.1 mm × 5 mm). HPLC analysis was done using gradient of $ACN/H_2O/10\%$ HCl (aq). For all remaining final compounds, mass spectra were obtained on a Waters micromass ZQ detector or a Micromass Q-TOF micro hybrid quadrupole/orthogonal high resolution time of flight MS. HPLC analysis was performed on a reverse phase XBridge C18 column (4.6 mm × 75 mm) column, using $40\% H_2O/50\% ACN/10\%$ of 0.2% TFA(aq) as the mobile phase, in gradient conditions at a flow rate of 1 mL/min. Eluted peaks were monitored at 254 nm with a Waters 996 PDA detector. All final compounds tested were confirmed to be of ≥95% purity by the HPLC methods described above.

General Procedure 1: Strecker Synthesis To Generate Carbonitriles. To a solution of N-substituted piperidone (15.9 mmol) in acetic acid (25 mL) was slowly added substituted aniline (17.6 mmol). The mixture was cooled to 5 °C with an ice bath, and a solution of KCN or TMSCN (17.6 mmol) in water (5 mL) was added dropwise. The solution was stirred for 1 h at 0 °C and for 3 h at room temperature and then poured into water. The pH was adjusted to 10 by adding NH_4OH, and the aqueous layer was extracted with methylene chloride (3 × 50 mL). The combined organic extracts were washed with water and brine and dried. The solvent was removed *in vacuo*, and the residue was purified using one of the following methods: flash chromatography using hexanes/EtOAc, trituration with cold ether, or recrystallization.

General Procedure 2: Nitrile Reduction. Method A: Anhydrous ether (500 mL) was added to the nitrile intermediate (0.5 mmol), which was then added dropwise to a solution of $LiAlH_4$ (1.0 mmol) in 1 L of anhydrous ether under nitrogen. The solution was quenched with a few drops of water, decanted, and evaporated. The resulting oil was purified by silica gel chromatography (49:50:1 chloroform−methanol−NH_4OH) affording the amine as a pale yellow solid in 35−

40% yield. **Method B:** The nitrile intermediate (7.21 mmol) was hydrogenated in a solution of methanol saturated with ammonia (210 mL) and Raney Ni (0.4 g) under pressure (2.4−3 atm) for 4−24 h. The catalyst was removed by filtration, and the solvent was evaporated *in vacuo*. The residue was chromatographed on a silica gel column using methylene chloride/methanol as the eluent to afford the amine.

General Procedure 3: Introduction of Arginine Side Chain. A solution of Boc-protected arginine (0.4 mmol) in 10 mL of methylene chloride was stirred at −10 °C for 20 min. Then, triethylamine (0.9 mmol), HOBt (0.4 mmol), and EDCI (0.4 mmol) were added, and the mixture was stirred at −10 °C for 30 min. A solution of amine (0.4 mmol) in 10 mL of methylene chloride was cooled at −10 °C and added dropwise. After stirring overnight at room temperature, water was added to the reaction mixture, followed by 10% NaOH solution to pH 12, and the mixture was extracted 3 times. The organic layer was dried using $MgSO_4$ and evaporated affording the Boc-protected arginine intermediate (50−60% yield).

General Procedure 4: Conversion of Various Amines to Boc-Protected Guanidines. Method A: To a stirred mixture of Boc-protected guanidino carboxylic acid (1.11 mmol), 1-hydroxy-benzotriazole hydrate (1.11 mmol), and triethylamine (1.20 mmol) in methylene chloride (20 mL) at −10 °C was added N-(3-(dimethylamino)propyl)-N′-ethylcarbodiimide hydrochloride (1.11 mmol). After 2 h of stirring at −10 °C, a solution of 4-(aminomethyl)-1-benzyl-N-phenylpiperidin-4-amine (**5b**) (0.85 mmol) in methylene chloride (4 mL) was slowly added, and the reaction mixture was stirred for 1 h at −10 °C and for 15 h at room temperature. The solvent was removed in vacuo, and the residue was chromatographed on a silica gel column using a gradient of petroleum ether/ethyl acetate as the eluent to give the desired material. **Method B:** A mixture of amine (0.57 mmol), 1,3-bis(*tert*-butoxycarbonyl)-2-methyl-2-thiopseudourea (0.68 mmol), and triethylamine (1.42 mmol) in anhydrous DMF (4 mL) was stirred at room temperature for 2 days. The reaction mixture was poured into water and extracted with ethyl acetate (3 × 30 mL). The combined organic layers were washed with brine, dried, and evaporated. The residue was purified by chromatography on a silica gel column using methylene chloride/methanol as the eluent to give the desired material. **Method C:** To a stirred mixture of 1,3-di-Boc-2-(carboxymethyl)guanidine (1.15 mmol), 1-hydroxy-benzotriazole hydrate (1.12 mmol), and triethyl-amine (2.87 mmol) in methylene chloride (10 mL) under argon at 0 °C was added N-(3-(dimethylamino)propyl)-N′-ethylcarbodiimide hydrochloride (1.18 mmol). The reaction mixture was kept at 0 °C for 2 h, and a solution of amine (1.02 mmol) in methylene chloride (10 mL) was slowly added. The reaction mixture was stirred 1 h at 0 °C and then allowed to warm to room temperature. After 15 h of stirring, the solvent was evaporated. Ethyl acetate (50 mL) and water were added. The layers were separated, and the organic layer was washed with brine. The solvent was dried and evaporated. The residue was purified by chromatography on a silica gel column using a gradient of methylene chloride and methanol to give the desired material. **Method D:** (Step I) To a stirred mixture of amine (1.21 mmol), 1-hydroxy-benzotriazole hydrate (1.45 mmol), Boc-gly-OH (1.33 mmol), and triethylamine (3.64 mmol) in methylene chloride (15 mL) at 0 °C under argon was added N-(3-(dimethylamino)propyl)-N′-ethylcarbodiimide hydrochloride (1.33 mmol). The reaction mixture was then allowed to warm to room temperature. After 20 h of stirring, methylene (80 mL) and water (30 mL) were added. The layers were separated, and the organic layer was extracted again with methylene chloride (30 mL). The combined organic layers were washed with brine. The solvent was dried and evaporated. The residue was purified by chromatography on a silica gel column using a gradient of methylene chloride and methanol to yield the Boc-Gly-coupled amine. (Step II) To a solution of the Boc-Gly-coupled amine (0.558 mmol) in anhydrous methylene chloride (6 mL) was added trifluoroacetic acid (3 mL). The reaction mixture was stirred at room temperature under argon for 1 h and evaporated. Methylene chloride (80 mL) and a 10% solution of NaOH (40 mL) were added. The layers were separated, and the organic layer was washed with water and brine. The solvent was dried and evaporated. The residue

was dissolved in anhydrous DMF (7 mL). Triethylamine (1.40 mmol), mercury(II) chloride (0.558 mmol), and 1,3-bis(*tert*-butoxycarbonyl)-2-methyl-2-thiopseudourea (0.670 mmol) were added, and the reaction mixture was stirred at room temperature for 2 h. The reaction mixture was poured into water and extracted with ethyl acetate (3 × 30 mL). The combined organic layers were washed with brine, dried, and evaporated. The residue was purified by chromatography on a silica gel column using a gradient of methylene chloride and methanol as the eluent to provide the title material.

General Procedure 5: Boc Deprotection To Give Final Products. Method A: The Boc-protected guanidine (0.1 mmol) was dissolved in an excess of HCl/dioxane under nitrogen and stirred for 3−4 days after which it was concentrated under reduce pressure to afford the hydrochloride salt of guanidine as colorless solid. **Method B:** To a solution of Boc-protected guanidine (0.3 mmol) in anhydrous methylene chloride (4 mL) was added trifluoroacetic acid (4 mL). The reaction mixture was stirred at room temperature under argon for 3 h. The solvent was removed in vacuo, and the residue was purified by chromatography on a silica gel column using a gradient of methylene chloride and methanol as the eluent to the title material as a tritriflate salt.

General Procedure 6: Synthesis of Various Aromatic Substituted Carbonitriles. 1-Benzylpiperidine-4-carbonitrile (5 mmol) was added to a dry three-necked flash equipped with a low temperature thermometer. Dry THF (60 mL) was added, and the solution was cooled to −70 °C in a dry ice−acetone bath under positive argon pressure. Lithium diisopropylamide (2.0 M solution in heptane/tetrahydrofuran/ethylbenzene, 6.54 mmol) was slowly added while maintaining the temperature below −65 °C, and the resulting mixture was stirred for 30 min at −70 °C. Substituted bromide or chloride (6.54 mmol) was quickly added, and the resulting mixture was then allowed to warm to room temperature, stirred for another 2 h, poured into 40 mL of NH₄Cl saturated solution, and extracted with ethyl acetate (3 × 40 mL). The combined organic extracts were washed with brine and dried. The solvent was removed in vacuo, and the residue was purified by chromatography on a silica gel column using ethyl acetate and hexanes as the eluent to afford the title material.

General Procedure 7: Synthesis of N-Substituted Analogs. A mixture of *tert*-butyl 10,10-dimethyl-3,8-dioxo-1-(4-(phenylamino)-piperidin-4-yl)-9-oxa-2,5,7-triazaundecan-6-ylidenecarbamate (**47**, 0.257 mmol) and potassium carbonate (0.773 mmol) in anhydrous DMF (3 mL) was stirred at 0 °C for 30 min under argon. A solution of substituted bromide or chloride (0.231 mmol) in DMF (1 mL) was added dropwise, and the mixture was stirred at 0 °C for 1 h and then allowed to warm to room temperature. After 3 h of stirring, the reaction mixture was poured into brine (50 mL) and extracted with ethyl acetate (3 × 30 mL). The organic layers were combined, washed with brine, dried, and evaporated. The residue was purified by chromatography on a silica gel column using a gradient of methylene chloride and methanol as the eluent to give the title material.

1-Methyl-4-(phenylamino)piperidine-4-carbonitrile (4a). Prepared according to general procedure 1 to afford the title material in 90% yield. ¹H NMR (600 MHz, CD₃OD): δ 6.72−6.79 (m, 3H), 7.13−7.17 (m, 2H), 3.642 (s, 3H), 2.37−2.40 (m, 4H), 1.92−1.96 (m, 4H). MS (ESI) 216.29 [M + H]⁺.

1-Benzyl-4-(phenylamino)piperidine-4-carbonitrile (4b). Prepared according to general procedure 1 to afford the title material in 94% yield. ¹H NMR (CD₃OD): δ 7.32−7.20 (m, 7H), 6.90−6.87 (m, 3H), 3.67 (s, 1H), 3.53 (s, 2H), 2.79−2.76 (m, 2H), 2.43 (t, *J* = 7.5 Hz, 2H), 2.30−2.27 (m, 2H), 1.89 (t, *J* = 7.8 Hz, 2H). MS (ESI) 292 [M + H]⁺.

1-Phenethyl-4-(phenylamino)piperidine-4-carbonitrile (4c). Prepared according to general procedure 1 to afford the title material in 90% yield. ¹H NMR (400 MHz, CD₃OD): δ 7.31−7.19 (m, 7H), 6.94−6.92 (m, 3H), 3.72 (d, 2H), 3.01−2.42 (m, 4H), 2.42−2.39 (m, 2H), 2.08−2.05 (m, 4H). MS (ESI) 306.2 [M + H]⁺.

1-(Naphthalen-2-ylmethyl)-4-(phenylamino)piperidine-4-carbonitrile (4d). Prepared according to general procedure 1 to afford the title material in 70% yield. ¹H NMR (600 MHz, CDCl₃): δ 7.82−7.80

(m, 4H), 7.52−7.24 (m, 3H), 7.23−7.22 (m, 3H), 6.92−6.90 (m, 2H), 3.70 (s, 2H), 3.69 (s, 1H), 2.93−2.59 (m, 4H), 2.37−2.35 (m, 4H). MS (ESI) 342.5 [M + H]⁺.

4-(Aminomethyl)-1-methyl-N-phenylpiperidin-4-amine (5a). Prepared according to general procedure 2, method A, to afford the title material in 35−40% yield. ¹H NMR (600 MHz, CD₃OD): δ 6.72−6.79 (m, 3H), 7.13−7.17 (m, 2H), 3.642 (s, 3H), 2.86 (s, 2H), 2.37−2.40 (m, 4H), 1.92−1.96 (m, 4H). MS (ESI) 220.2 [M + H]⁺.

4-(Aminomethyl)-1-benzyl-N-phenylpiperidin-4-amine (5b). Prepared according to general procedure 2, method B, to afford the title material in 82% yield. ¹H NMR (CDCl₃): δ 7.34−7.16 (m, 7H), 6.80−6.75 (m, 3H), 3.53 (s, 2H), 2.89 (s, 2H), 2.65−2.62 (m, 2H), 2.33 (t, *J* = 8.1 Hz, 2H), 2.00−1.62 (m, 6H). MS (ESI) *m/z* 296 [M + H]⁺.

4-(Aminomethyl)-1-phenethyl-N-phenylpiperidin-4-amine (5c). Prepared according to general procedure 2, method A, to afford the title material in 35−40% yield. ¹H NMR (600 MHz, CD₃OD): δ 7.30−7.14 (m, 7H), 6.83−6.78 (m, 3H), 3.68−3.67 (d, *J* = 4, 2H), 2.95 (s, 2H), 2.88−2.42 (m, 4H), 2.05−2.02 (m, 2H), 1.82−1.76 (m, 4H). MS (ESI) 310.5 [M + H]⁺.

4-(Aminomethyl)-1-(naphthalen-2-ylmethyl)-N-phenylpiperidin-4-amine (5d). Prepared according to general procedure 2, method B, to afford the title material in 90% yield. ¹H NMR (CDCl₃): δ 7.81−7.71 (m, 4H), 7.48−7.44 (m, 3H), 7.14 (t, *J* = 6.2 Hz, 2H), 6.76−6.71 (m, 3H), 3.64 (s, 2H), 2.86 (s, 2H), 2.64 (d, *J* = 9.1 Hz, 2H), 2.34 (t, *J* = 8.7 Hz, 2H), 1.95 (d, *J* = 10.6 Hz, 2H), 1.75 (br s, 2H), 1.65−1.60 (m, 2H). MS (ESI) *m/z* 346 [M + H]⁺.

2-Amino-5-guanidino-N-((1-methyl-4-(phenylamino)piperidin-4-yl)methyl)pentanamide Hydrochloride (7a). Prepared according to general procedure 3, starting with intermediate **5a**, to afford the corresponding Boc-protected arginine intermediate **6a** in 50−60% yield, which was deprotected using general procedure 5, method A, to afford the title material in 95% yield. ¹H NMR (600 MHz, CD₃OD): δ 7.17−7.14 (m, 3H), 6.95−6.93 (m, 2H), 3.95−3.72 (m, 2H), 3.66−3.55 (m, 1H), 3.41−3.33 (m, 6H), 2.85 (s, 3H), 2.31−1.97 (m, 6H), 1.66−1.64 (2H). ¹³C NMR (600 MHz, CD₃OD): δ 170.8, 169.7, 157.4, 130, 129.5, 126, 72.3, 71.2, 67, 61, 53.2, 53.09, 42.4, 40.7, 24.5. MS (ESI) 376.51 [M + H]⁺.

2-Amino-N-((1-benzyl-4-(phenylamino)piperidin-4-yl)methyl)-5-guanidinopentanamide Hydrochloride (7b). Prepared according to general procedure 3, starting with intermediate **5b**, to afford the corresponding Boc-protected arginine intermediate **6b** in 50−60% yield, which was deprotected using general procedure 5, method A, to afford the title material in 95% yield. ¹H NMR (600 MHz, CD₃OD): δ 7.58−7.47 (m, 7H), 6.97−6.95 (m, 3H), 3.73−3.34 (m, 11 H), 2.30−3.31 (m, 2H), 2.12−2.03 (m, 4H), 1.31−1.28 (s, 2H). ¹³C NMR (600 MHz, CD₃OD): δ 169, 157, 149, 132.5, 132.3, 130.3, 130.2, 124.9, 120, 118.9, 73.4, 72.3, 62.0, 61.4, 54.1, 41.7, 30.7, 25.5. MS (ESI) 452.5 [M + H]⁺.

2-Amino-5-guanidino-N-((1-phenethyl-4-(phenylamino)-piperidin-4-yl)methyl)pentanamide Hydrochloride (7c). Prepared according to general procedure 3, starting with intermediate **5c**, to afford the corresponding Boc-protected arginine intermediate **6c** in 50−60% yield, which was deprotected using general procedure 5, method A, to afford the title material in 95% yield. ¹H NMR (600 MHz, CD₃OD): δ 7.63−7.75 (m, 3H), 7.30−7.23 (m, 7H), 3.72−3.69 (m, 4H), 3.66−3.64 (m, 1H), 3.63−3.57 (m, 2H), 3.56−3.37 (4H), 2.35−2.97 (m, 2H), 2.20−2.00 (m, 4H), 2.0−1.8 (m, 2H), 1.30−1.26 (2H). ¹³C NMR (600 MHz, CD₃OD): δ 170.9, 169.9, 157.4, 130.8, 130.2, 130.06, 129.2, 127.4, 126.6, 72.6, 71.6, 61.3, 58.2, 53.4, 43.6, 41.1, 30.8, 24.8, 24.5. MS (ESI) 466.60 [M + H]⁺.

2-Amino-5-guanidino-N-((1-(naphthalen-2-ylmethyl)-4-(phenylamino)piperidin-4-yl)methyl)pentanamide (7d). Prepared according to general procedure 3, starting with intermediate **5d**, to afford the corresponding Boc-protected arginine intermediate **6d** in 70% yield. This material was deprotected using general procedure 5, method A, to afford the title material in 99% yield. ¹H NMR (600 MHz, CD₃OD), δ 7.81−7.71 (m, 4H), 7.48−7.43 (m, 3H), 7.16−7.13 (m, 3H), 6.79−6.66 (m, 2H), 4.09−4.06 (m, 1H), 3.85−3.83 (m, 2H), 3.66 (s, 2H), 3.63−3.47 (m, 2H), 2.64−2.62 (m, 4H), 1.49 (s, 9H). ¹³C NMR (400 MHz, CD₃OD): δ 172.5, 163.6, 160.9, 155.1, 133.5,

133.0, 129.5, 128.1, 127.9, 127.8, 127.5, 126.1, 125.8, 119.3, 117.5, 63.2, 54.9, 49.3, 44.3, 33.9, 33.5, 25.3.

tert-Butyl (tert-Butoxycarbonylamino)((1-methyl-4-(phenylamino)piperidin-4-yl)methylamino)methylenecarbamate (8a). Prepared according to general procedure 4, method B, also using HgCl$_2$ (1 equiv) to afford the title material in 30% yield. ^{1}H NMR (400 MHz, CD$_3$OD): δ 6.72–6.79 (m, 3H), 7.13–7.17 (m, 2H), 3.642 (s, 2H), 2.27 (s, 3H), 1.9–2.2(m, 4H), 1.657–1.8 (m, 4H), 1.45–1.50 (db, J = 20 Hz, 18H). MS (ESI) 462.80 [M + H]$^+$.

tert-Butyl ((1-Benzyl-4-(phenylamino)piperidin-4-yl)-methylamino)(tert-butoxycarbonylamino)methylenecarbamate (8b). Prepared according to general procedure 4, method B, also using HgCl$_2$ (1 equiv) to afford the title material in 30% yield. ^{1}H NMR (400 MHz, CD$_3$OD): δ 7.33–7.16 (m, 7H), 6.83–6.78 (m, 3H), 3.68 (s, 2H), 3.52 (s, 2H), 2.64–2.61 (m, 2H), 2.32–2.30 (m, 2H), 1.99–1.97 (m, 2H), 1.75–1.70 (m, 2H), 1.53–1.48 (d, J = 20 Hz, 18H). MS (ESI) 538.50 [M + H]$^+$.

tert-Butyl (tert-Butoxycarbonylamino)((1-phenethyl-4-(phenylamino)piperidin-4-yl)methylamino)methylenecarbamate (8c). Prepared according to general procedure 4, method B, also using HgCl$_2$ (1 equiv) to afford the title material in 30% yield. ^{1}H NMR (600 MHz, CD$_3$OD): δ 7.30–7.14 (m, 7H), 6.83–6.78 (m, 3H), 3.68–3.67 (d, J = 4, 2H), 2.95 (s, 2H), 2.88–2.42 (m, 4H), 2.05–2.02 (m, 2H), 1.82–1.76 (m, 4H), 1.51–1.47 (d, J = 16 Hz, 18H). MS (ESI) 552.50 [M + H]$^+$.

1-((1-Methyl-4-(phenylamino)piperidin-4-yl)methyl)guanidine Hydrochloride (9a). Prepared according to general procedure 5, method A, from intermediate **8a** to afford the title material in quantitative yield. ^{1}H NMR (600 MHz, CD$_3$OD): δ 6.72–6.79 (m, 3H), 7.13–7.17 (m, 2H), 3.642 (s, 2H), 2.86 (s, 3H), 2.37–2.40 (m, 4H), 1.92–1.96 (m, 4H). ^{13}C NMR (600 MHz, CD$_3$OD): δ 157.9, 144.5, 129.3, 119.7, 117.6, 52.6, 50.2, 48.9, 48.5, 30.7. MS (ESI) 262.5 [M + H]$^+$.

1-((1-Benzyl-4-(phenylamino)piperidin-4-yl)methyl)guanidine Hydrochloride (9b). Prepared according to general procedure 5, method A, from intermediate **8b** to afford the title material in quantitative yield. ^{1}H NMR (600 MHz, CD$_3$OD): δ 7.59–7.13 (m, 7H), 6.89–6.75 (m, 3H), 3.47 (s, 2H), 3.34 (m, 2H), 2.41–2.38 (m, 4H), 2.06–2.01 (m, 4H). ^{13}C NMR (600 MHz, CD$_3$OD): δ 157.9, 144.5, 134, 131.4, 130.0, 129.1, 129.11, 119.3, 117.1, 60.3, 52.9, 48.4, 48.2, 30.2. MS (ESI) 338.0 [M + H]$^+$.

1-((1-Phenethyl-4-(phenylamino)piperidin-4-yl)methyl)guanidine Hydrochloride (9c). Prepared according to general procedure 5, method A, from intermediate **8c** to afford the title material in quantitative yield. ^{1}H NMR (600 MHz, CD$_3$OD): δ 7.30–7.14 (m, 7H), 6.83–6.78 (m, 3H), 3.68–3.67 (d, J = 4, 2H), 2.95 (s, 2H), 2.88–2.42 (m, 4H), 2.05–2.02 (m, 2H), 1.82–1.76 (m, 4H). ^{13}C NMR (600 MHz, CD$_3$OD): δ 163.7, 145.2, 139.1, 129.35, 128.93, 128.65, 126.35, 119.8, 118.4, 60.6, 54.0, 49.3, 33.6. MS (ESI) 352.5 [M + H]$^+$.

1-(Naphthalen-2-ylmethyl)piperidin-4-one (12d). A solution of 2-bromomethylnaphthalene (5 g, 22.6 mmol), 4-piperidone ethylene ketal (3.23 g, 22.6 mmol), K$_2$CO$_3$ (9.12 g, 55.25 mmol), and KI (117 mg, 0.7 mmol) in methyl isobutyl ketone (300 mL) was heated at reflux for 5 h, cooled, and filtered. The residue was purified using silica gel chromatography (30:70 ethyl acetate–hexane) to afford 1-naphthalen-2-ylmethyl-piperidin-4-one ethylene ketal (**11d**). ^{1}H NMR (600 MHz, CDCl$_3$): δ 7.81–7.75 (m, 4H), 7.52–7.43 (m, 3H), 3.93 (s, 4H), 3.72 (s, 2H), 2.81–2.61 (m, 4H), 1.79–1.77 (m, 4H). MS (ESI) 284.36 [M + H]$^+$. This material was hydrolyzed directly in a mixture of concentrated HCl (40 mL) and acetic acid (210 mL) at reflux for 18 h. The mixture was poured onto ice water, neutralized with 32% NaOH to pH 8, extracted with ethyl acetate, and dried using MgSO$_4$. Evaporation afforded the title material (5g, 40%). ^{1}H NMR (600 MHz, CDCl$_3$): δ 7.83–7.75 (m, 4H), 7.54–7.45 (m, 3H), 3.77 (s, 2H), 2.80–2.78 (m, 4H), 2.48–2.46 (m, 4H). MS (ESI) 240.31 [M + H]$^+$.

2-Amino-N-((1-benzylpiperidin-4-yl)methyl)-5-guanidinopenta-namide Hydrochloride (15). Prepared according to general procedure 3 starting with intermediate **13**, to afford the corresponding Boc-protected arginine intermediate **14** in 70% yield. This material was

deprotected using general procedure 5, method A, to afford the title material in 95–99% yield. ^{1}H NMR (600 MHz, CD$_3$OD): δ 7.62–7.61 (m, 2H), 7.40–7.48 (m, 3H), 4.34 (s, 4H), 3.66 (s, 2H), 3.50–3.47 (m, 2H), 3.31–3.24 (m, 3H), 3.19–3.07 (m, 4H), 1.99–1.91 (6H), 1.73–1.63 (m, 3H). ^{13}C NMR (600 MHz, CD$_3$OD): δ 169, 157.4, 131.4, 130, 129.3, 129.1, 66.9, 60.5, 53, 52.2, 40.64, 34, 28.6, 27, 24.5. MS (ESI) 361.5 [M + H]$^+$.

N-((1-Benzyl-4-(phenylamino)piperidin-4-yl)methyl)-2-cyanoace-tamide (16a). Prepared according to general procedure 4, method A, using cyanoacetic acid (1.3 equiv) to afford 1.26 g (50%) of the title material as a white solid. ^{1}H NMR (500 MHz, DMSO-d_6): δ 7.32–7.27 (m, 5H), 7.21 (t, J = 7.7 Hz, 2H), 6.86 (t, J = 7.2 Hz, 1H), 6.75 (d, J = 7.8 Hz, 2H), 6.68–6.66 (m, 1H), 3.57–3.53 (m, 4H), 3.30 (s, 2H), 2.62–2.60 (m, 2H), 2.34–2.30 (m, 2H), 1.93–1.90 (m, 2H), 1.75–1.71 (m, 2H).

tert-Butyl 2-((1-Benzyl-4-(phenylamino)piperidin-4-yl)-methylamino)-2-oxoethylcarbamate (16b). Prepared according to general procedure 4, method A, using Boc-gly-OH to afford 3.99 g (65%) of the title material as a white solid. ^{1}H NMR (CDCl$_3$): δ 7.30–7.23 (m, 5H), 7.16 (t, J = 7.6 Hz, 2H), 6.79 (t, J = 7.3 Hz, 1H), 6.73 (d, J = 8.0 Hz, 2H), 6.66 (br s, 1H), 5.45 (br s, 1H), 3.72 (d, J = 5.5 Hz, 2H), 3.53 (d, J = 5.4 Hz, 2H), 3.49 (s, 2H), 3.22 (br s, 1H), 2.58–2.55 (m, 2H), 2.31 (t, J = 10.1 Hz, 2H), 1.89–1.86 (m, 2H), 1.73–1.68 (m, 2H), 1.40 (s, 9H). MS (ESI) m/z 453 (M$^+$ + 1).

tert-Butyl 1-(1-Benzyl-4-(phenylamino)piperidin-4-yl)-12,12-di-methyl-3,10-dioxo-11-oxa-2,7,9-triazatridecan-8-ylidenecarbamate (16c). Prepared according to general procedure 4, method A, using 4-(aminomethyl)-1-benzyl-N-phenylpiperidin-4-amine (**5b**) (0.25 g, 0.85 mmol) and 4-(2,3-bis(tert-butoxycarbonyl)guanidino)butanoic acid (0.38 g, 1.11 mmol) to afford 0.41 g (77%) of the title material as a white solid. ^{1}H NMR (CDCl$_3$): δ 11.48 (s, 1H), 8.37 (br s, 1H), 7.39–7.13 (m, 7H), 6.79–6.72 (m, 3H), 6.35 (br s, 1H), 3.55–3.49 (m, 4H), 3.43 (q, J = 4.8 Hz, 2H), 2.57–2.54 (m, 2H), 2.33 (t, J = 7.2 Hz, 2H), 2.21 (t, J = 5.4 Hz, 2H), 1.92–1.34 (m, 24 H). MS (ESI) m/z 623 (M$^+$ + 1). 4-(2,3-Bis(tert-butoxycarbonyl)guanidino)butanoic acid was prepared from 4-aminobutanoic acid according to general procedure 4, method B, also using HgCl$_2$ (1.7 equiv), and matched reported values.[30]

tert-Butyl 1-(1-Benzyl-4-(phenylamino)piperidin-4-yl)-13,13-di-methyl-3,11-dioxo-12-oxa-2,8,10-triazatetradecan-9-ylidenecarba-mate (16d). Prepared according to general procedure 4, method A, using 4-(aminomethyl)-1-benzyl-N-phenylpiperidin-4-amine (**5b**) (0.25 g, 0.85 mmol) and 5-(2,3-bis(tert-butoxycarbonyl)guanidino)-pentanoic acid (0.41 g, 1.11 mmol) to afford 0.43 g (80%) of the title material as a white solid. ^{1}H NMR (CDCl$_3$): δ 11.46 (s, 1H), 8.29 (s, 1H), 7.35–7.14 (m, 7H), 6.79 (t, J = 5.7 Hz, 1H), 6.72–6.70 (m, 2H), 5.96–5.93 (m, 1H), 3.54–3.48 (m, 4H), 3.38 (q, J = 5.1 Hz, 2H), 2.58–2.55 (m, 2H), 2.30 (t, J = 6.9 Hz, 2H), 2.18 (t, J = 5.4 Hz, 2H), 1.86–1.36 (m, 26 H). MS (ESI) m/z 637 (M$^+$ + 1). 5-(2,3-Bis(tert-butoxycarbonyl)guanidino)pentanoic acid was prepared from 5-aminopentanoic acid according to general procedure 4, method B, also using HgCl$_2$ (1.7 equiv), and matched reported values.[31]

tert-Butyl N-[(1Z)-{[3-({[1-Benzyl-4-(phenylamino)piperidin-4-yl]-methyl}carbamoyl)phenyl]amino}({[(tert-butoxy)carbonyl]imino})-methyl]carbamate (16e). Prepared according to general procedure 4, method A, using 4-(aminomethyl)-1-benzyl-N-phenylpiperidin-4-amine (**5b**) and 3-(2,3-bis(tert-butoxycarbonyl)guanidino)benzoic acid (1.67 g, 4.41 mmol) to yield 1.7 g (75% yield) of the title material. ^{1}H NMR (400 MHz, CDCl$_3$): δ 11.65 (s, 1H), 10.45 (s, 1H), 7.86 (s, 2H), 7.54–7.14 (m, 9H), 6.86–6.79 (m, 4H), 3.75 (d, J = 4.9 Hz, 2H), 3.55 (s, 2H), 3.46 (s, 1H), 2.65–2.62 (m, 2H), 2.42–2.38 (m, 2H), 1.97–1.82 (m, 4H), 1.52 (d, J = 27.2 Hz, 18H). MS(ESI) 657.7 [M + H]$^+$. 3-(2,3-Bis(tert-butoxycarbonyl)guanidino)benzoic acid was prepared from 3-aminobenzoic acid according to general procedure 4, method B, also using HgCl$_2$ (1.7 equiv), and matched reported values.[32]

tert-Butyl N-[(1Z)-{[4-({[1-Benzyl-4-(phenylamino)piperidin-4-yl]-methyl}carbamoyl)phenyl]amino}({[(tert-butoxy)carbonyl]imino})-methyl]carbamate (16f). Prepared according to general procedure 4, method A, using 4-(aminomethyl)-1-benzyl-N-phenylpiperidin-4-amine (**5b**) and 4-(2,3-bis(tert-butoxycarbonyl)guanidino)benzoic

acid (1 g, 3.45 mmol) to afford 1.5 g (66%) of the title material. ^{1}H NMR (400 MHz): δ 11.61 (s, 1 H), 10.50 (s, 1 H), 7.73−7.67 (m, 4 H), 7.40−7−28 (m, 5 H), 7.21 (t, J = 7.7, 2 H), 6.88−6.82 (m, 3 H), 6.71 (s, 1 H), 3.75 (d, J = 5.1, 2 H), 3.66 (s, 2 H), 2.73 (s, 2 H), 2.56 (s, 2 H), 2.11−1.83 (m, 4 H), 1.53 (d, J = 13.6, 19 H). MS(ESI) 657.4 [M + H]$^+$. 4-(2,3-Bis(tert-butoxycarbonyl)guanidino)benzoic acid was prepared from was prepared from 4-aminobenzoic acid according to general procedure 4, method B, also using HgCl$_2$ (1.7 equiv), and matched reported values.[33]

tert-Butyl N-[(1Z)-({4-[({[1-Benzyl-4-(phenylamino)piperidin-4-yl]-methyl}carbamoyl)methyl]phenyl}amino)({[(tert-butoxy)carbonyl]-imino})methyl]carbamate (16g). Prepared according to general procedure 4, method A, using 4-(aminomethyl)-1-benzyl-N-phenyl-piperidin-4-amine (5b) (1.08 g, 3.66 mmol) and 4-((2,3-bis(tert-butoxycarbonyl)guanidino)methyl)benzoic acid (1.7 g, 4.49 mmol) to afford the title material in 84% yield. ^{1}H NMR (400 MHz, CDCl$_3$): δ 11.66 (s, 1H), 10.31 (s, 1H), 7.52 (d, J = 7.8 Hz, 1H), 7.28−7.11 (m, 9H), 6.77 (t, J = 7.0 Hz, 1H), 6.59 (d, J = 7.5 Hz, 2H), 5.88 (s, 1H), 3.48 (s, 7H), 2.54−2.51 (m, 2H), 2.30−2.28 (m, 2H), 1.79−1.76 (m, 2H), 1.68−1.63 (m, 2H), 1.53 (d, J = 9.9 Hz, 16H). MS(ESI) 671.7 [M + H]$^+$. 4-((2,3-Bis(tert-butoxycarbonyl)guanidino)methyl)benzoic acid was prepared from 4-aminophenylacetic acid (2.42 g, 16 mmol) according to general procedure 4, method B, also using HgCl$_2$ (1.7 equiv), to afford 1.02 g of target compound (16% yield). ^{1}H NMR (400 MHz, CDCl$_3$): δ 10.31 (s, 2H), 7.53 (d, J = 8.3 Hz, 2H), 7.23 (d, J = 8.2 Hz, 2H), 3.57 (s, 2H), 1.51 (s, 18H). MS(ESI) 394.5 [M + H]$^+$.

N-((1-Benzyl-4-(phenylamino)piperidin-4-yl)methyl)-3-guanidi-nopropanamide Triflate (17a). Prepared according to general procedure 5, method B, using 19a (0.3 g, 0.49 mmol) to afford 0.29 g (77%) of the title material. ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.97 (s, 1H), 8.13−7.97 (m, 1H), 7.50−6.61 (m, 15H), 5.26 (br s, 1H), 4.30 (s, 2H), 3.32−3.11 (m, 8H), 2.45−2.37 (m, 2H), 2.11−1.84 (m, 4H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 170.70, 158.81 (q, J = 34.4 Hz), 156.99, 145.85, 131.32, 129.77, 129.55, 128.83, 128.81, 117.29, 116.25 (q, J = 292.2 Hz), 116.14, 58.91, 53.16, 47.14, 44.49, 37.19, 34.40, 29.17. MS (ESI) m/z 409 (M$^+$ + 1).

2-Amino-N-((1-benzyl-4-(phenylamino)piperidin-4-yl)methyl)-acetamide (17b). Prepared according to general procedure 5, method B, using 16b (5.5 g, 12.15 mmol) using the following modified workup and purification: Methylene chloride (150 mL) and a 10% solution of NaOH (80 mL) were added. The layers were separated, and the organic layer was washed with water and brine. The solvent was dried and evaporated. The residue was purified by chromatography on a silica gel column using a gradient of methylene chloride and methanol saturated with ammonia (10:0 → 8:2) as the eluent to give 4.02 g (92%) of the title material as a white solid. ^{1}H NMR (500 MHz, CDCl$_3$): δ 7.48−7.46 (m, 1H), 7.27−7.21 (m, 5H), 7.14 (t, J = 7.7 Hz, 2H), 6.77 (t, J = 7.2 Hz, 1H), 6.72 (d, J = 7.7 Hz, 2H), 3.53 (d, J = 5.7 Hz, 2H), 3.47 (s, 2H), 3.28 (s, 2H), 2.57−2.55 (m, 2H), 2.30 (t, J = 9.7 Hz, 2H), 1.88−1.85 (m, 2H), 1.73−1.68 (m, 2H), 1.62 (br s, 2H). ^{13}C NMR (125 MHz, CDCl$_3$): δ 173.15, 145.53, 138.28, 129.37, 129.19, 128.33, 127.18, 119.31, 117.62, 63.12, 54.51, 49.12, 45.60, 44.92, 33.79. MS (ESI) m/z 353 (M$^+$ + 1).

N-((1-Benzyl-4-(phenylamino)piperidin-4-yl)methyl)-4-guanidi-nobutanamide (17c). Prepared according to general procedure 5, method B, (no purification) using 16c (0.19 g, 0.30 mmol). ^{1}H NMR (DMSO-d_6): δ 10.12−10.10 (m, 1H), 7.99−7.89 (m, 2H), 7.50−7.07 (m, 10H), 6.81−6.63 (m, 3H), 4.37−4.30 (m, 2H), 3.32−3.05 (m, 8H), 2.23−1.66 (m, 8H). ^{13}C NMR (DMSO-d_6): δ 172.18, 158.93, 158.58, 156.99, 145.74, 131.20, 129.63, 129.42, 128.74, 128.69, 117.50, 117.47, 117.27, 116.15, 114.58, 114.56, 58.88, 53.09, 47.09, 44.48, 31.83, 29.18, 24.60. MS (ESI) m/z 423 (M$^+$ + 1).

N-((1-Benzyl-4-(phenylamino)piperidin-4-yl)methyl)-5-guanidi-nopentanamide (17d). Prepared according to general procedure 5, method B, (no purification) using 16d (0.20 g, 0.31 mmol). ^{1}H NMR (CD$_3$OD): δ 7.51−7.45 (m, 5H), 7.21−7.16 (m, 3H), 6.88−6.76 (m, 2H), 4.32 (s, 2H), 3.49−3.17 (m, 7H), 2.29−2.26 (m, 5H), 1.99−1.94 (m, 2H), 1.64−1.57 (m, 4H). ^{13}C NMR (CD$_3$OD): δ 174.79, 157.28, 145.09, 130.94, 129.81, 128.93, 128.91, 128.81, 118.57, 116.79, 60.14,

53.23, 45.04, 40.68, 34.74, 29.87, 27.99, 22.31. HRMS (ESI) calcd for C$_{25}$H$_{37}$N$_6$O [M + H]$^+$ 437.3029, found 437.3044.

N-((1-Benzyl-4-(phenylamino)piperidin-4-yl)methyl)-3-guanidi-nobenzamide (17e). Prepared according to general procedure 5, method B, (no purification) using 16e (0.1 g, 0.152 mmol). ^{1}H NMR (500 MHz, DMSO-d_6): δ 10.33 (s, 1H), 9.70 (s, 1H), 8.59 (s, 1H), 7.78−7.73 (m, 5H), 7.51−7.39 (m, 6H), 7.19−7.05 (m, 2H), 6.89 (d, J = 7.9 Hz, 2H), 6.66 (t, J = 7.1 Hz, 1H), 4.32 (d, J = 3.6 Hz, 2H), 3.55 (d, J = 5.7 Hz, 1H), 3.38−3.08 (m, 4H), 2.20 (d, J = 14.5 Hz, 2H), 2.04−1.90 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6): δ 166.76, 156.64, 146.39, 136.33, 136.07, 131.74, 130.17, 130.10, 129.96, 129.31, 129.22, 127.70, 125.74, 123.77, 117.78, 116.64, 59.42, 54.07, 47.65, 45.61, 29.77. HRMS (ESI) calcd for C$_{27}$H$_{33}$N$_6$O 457.2716, found 457.2708 [M + H]$^+$.

N-{[1-Benzyl-4-(phenylamino)piperidin-4-yl]methyl}-4-carbami-midamidobenzamide (17f). Prepared according to general procedure 5, method B, (no purification) using 16f (0.1 g, 0.152 mmol). ^{1}H NMR (500 MHz, DMSO-d_6): δ 10.36−10.31 (m, 1H), 9.65 (s, 1H), 8.54 (s, 1H), 7.93−7.92 (m, 2H), 7.79 (s, 4H), 7.58−7.41 (m, 4H), 7.32−7.31 (m, 2H), 7.14−7.09 (m, 2H), 6.89−6.82 (m, 2H), 6.66 (t, J = 6.3 Hz, 1H), 4.32 (s, 2H), 3.55−3.54 (m, 2H), 3.25−3.23 (m, 2H), 3.18−3.13 (m, 2 H), 2.20−2.17 (m, 2H), 1.96 (t, J = 13.4 Hz, 2H). ^{13}C NMR (126 MHz, DMSO-d_6): δ 166.64, 156.27, 146.39, 139.04, 131.89, 131.75, 130.16, 129.98, 129.31, 129.24, 123.31, 118.24, 117.71, 116.57, 115.86, 59.40, 54.01, 47.67, 45.58, 29.69. HRMS (ESI) calcd for C$_{27}$H$_{33}$N$_6$O 457.2716, found 457.2697 [M + H]$^+$.

N-((1-Benzyl-4-(phenylamino)piperidin-4-yl)methyl)-2-(4-guanidinophenyl)acetamide (17g). Prepared according to general procedure 5, method B, (no purification) using 16g (0.19 g, 0.283 mmol). ^{1}H NMR (500 MHz, DMSO-d_6): δ 10.02 (s, 1H), 9.89 (s, 1H), 8.17 (s, 1H), 7.65−7.42 (m, 9H), 7.30 (d, J = 7.9 Hz, 2H), 7.15 (d, J = 7.8 Hz, 2H), 7.09 (t, J = 7.5 Hz, 2H), 6.81 (d, J = 7.8 Hz, 2H), 6.64 (t, J = 7.2 Hz, 1H), 4.32 (d, J = 2.3 Hz, 2H), 3.47 (s, 2H), 3.33 (d, J = 5.8 Hz, 2H), 3.25−3.22 (m, 2H), 3.15−3.09 (m, 2H), 2.15−2.12 (m, 2H), 1.87 (t, J = 13.3 Hz, 2H). ^{13}C NMR (126 MHz, DMSO-d_6): δ 156.56, 146.34, 134.89, 134.22, 131.66, 130.74, 129.89, 129.20, 124.54, 118.96, 117.62, 116.52, 116.45, 116.00, 59.31, 55.01, 53.47, 49.01, 47.53, 41.88, 29.70. HRMS (ESI) calcd for C$_{28}$H$_{35}$N$_6$O 471.2872 [M + H]$^+$, found 471.2856.

3-Amino-N-((1-benzyl-4-(phenylamino)piperidin-4-yl)methyl)-propanamide (18a). Prepared according to general procedure 2, method B, using 16a (0.5 g, 1.38 mmol). The residue was purified by chromatography on a silica gel column using a gradient of methylene chloride and methanol saturated with ammonia (9:1 → 8:2) as the eluent to give 0.38 g (76%) of the title material as a white solid. ^{1}H NMR (CDCl$_3$): δ 7.30−7.23 (m, 5H), 7.16 (t, J = 8.0 Hz, 2H), 7.07−7.05 (m, 1H), 6.78 (t, J = 7.3 Hz, 1H), 6.73 (d, J = 7.8 Hz, 2H), 3.55 (d, J = 5.5 Hz, 2H), 3.49 (s, 2H), 2.94 (t, J = 5.7 Hz, 2H), 2.58−2.55 (m, 2H), 2.34−2.27 (m, 4H), 1.90−1.87 (m, 2H), 1.79−1.69 (m, 2H), 1.65 (br s, 2H). MS (ESI) m/z 367 (M$^+$ + 1).

tert-Butyl 1-(1-Benzyl-4-(phenylamino)piperidin-4-yl)-11,11-di-methyl-3,9-dioxo-10-oxa-2,6,8-triazadodecan-7-ylidenecarbamate (19a). Prepared according to general procedure 4, method B, using 18a (0.32 g, 0.88 mmol) to afford 0.47 g (87%) of the title material as a white solid. ^{1}H NMR (500 MHz, CDCl$_3$): δ 11.47 (s, 1H), 8.77−8.75 (m, 1H), 7.31−7.23 (m, 5H), 7.18 (t, J = 7.6 Hz, 2H), 6.81 (t, J = 7.2 Hz, 1H), 6.74 (d, J = 8.0 Hz, 2H), 6.15−6.13 (m, 1H), 3.74−3.70 (m, 2H), 3.57 (d, J = 5.8 Hz, 2H), 3.51 (s, 2H), 2.60−2.57 (m, 2H), 2.44 (t, J = 6.0 Hz, 2H), 2.35−2.31 (m, 2H), 1.89−1.87 (m, 2H), 1.76−1.72 (m, 2H), 1.50 (s, 9H), 1.46 (s, 9H). MS (ESI) m/z 609 (M$^+$ + 1).

tert-Butyl 1-(1-Benzyl-4-(phenylamino)piperidin-4-yl)-10,10-di-methyl-3,8-dioxo-9-oxa-2,5,7-triazaundecan-6-ylidenecarbamate (20). Prepared according to general procedure 4, method B, using 17b (0.2 g, 0.57 mmol) to afford 0.285 g (84%) of the title material as a white solid. ^{1}H NMR (500 MHz, CDCl$_3$): δ 11.36 (s, 1H), 8.87 (s, 1H), 7.30−7.25 (m, 5H), 7.16 (s, 2H), 6.79 (s, 1H), 6.72 (s, 2H), 6.54 (s, 1H), 4.00 (s, 2H), 3.56 (s, 2H), 3.51 (s, 2H), 3.50 (br s, 1H), 2.57 (s, 2H), 2.37 (s, 2H), 1.90−1.88 (m, 2H), 1.76−1.74 (m, 2H), 1.49 (s, 9H), 1.47 (s, 9H). MS (ESI) m/z 595 (M$^+$ + 1).

N-((1-Benzyl-4-(phenylamino)piperidin-4-yl)methyl)-2-guanidinoacetamide tritriflate (21). Prepared according to general procedure 5, method B, using **20** (0.18 g, 0.3 mmol) to afford 0.2 g (90%) of the title material as a white solid. ¹H NMR (500 MHz, CD₃OD): δ 7.49−7.42 (m, 5H), 7.12−7.09 (m, 2H), 6.90−6.69 (m, 3H), 4.27 (s, 2H), 3.91 (s, 2H), 3.48−3.29 (m, 6H), 2.26−1.99 (m, 4H). ¹³C NMR (125 MHz, CD₃OD): δ 170.14, 163.18, 159.37, 146.64, 132.33, 131.10, 130.34, 130.23, 130.16, 119.86, 118.25 (q, J = 282.7 Hz), 118.15, 61.47, 54.60, 49.01, 46.59, 44.74, 31.21. MS (ESI) *m/z* 395 (M⁺ + 1).

1-Benzyl-3-(phenylamino)piperidine-3-carbonitrile (23). To a mixture of 1-benzyl-3-piperidone (2 g, 1.06 mmol) and aniline (0.96 mL, 1.06 mmol) in 1,2-dichloroethane (40 mL) was added titanium isopropoxide (4 mL, 1.37 mmol). The reaction mixture was stirred at room temperature for 21 h and then diethylaluminum cyanide (1 M in toluene, 21 mL, 2.11 mmol) was added. After 3 h of stirring, ethyl acetate (100 mL) and a solution of saturated sodium bicarbonate were added. The mixture was stirred for 10 min and filtered through a pad of Celite. The Celite was washed with water and ethyl acetate, and the layers of the filtrate were separated. The organic layer was washed with brine, dried, and evaporated. The residue was purified by chromatography on a silica gel column using ethyl acetate and hexanes (4:6) as the eluent to give 2.36 g (74%) of the title material as a white solid. ¹H NMR (CDCl₃): δ 7.41−7.32 (m, 5H), 7.26 (t, J = 8.0 Hz, 2H), 6.96 (d, J = 7.7 Hz, 2H), 6.90 (t, J = 7.4 Hz, 1H), 4.35 (s, 1H), 3.62 (s, 2H), 2.88−2.80 (m, 2H), 2.60 (s, 1H), 2.40 (s, 1H), 2.23 (s, 1H), 1.91 (s, 1H), 1.67 (s, 2H). MS (ESI) *m/z* 292 (M⁺ + 1).

3-(Aminomethyl)-1-benzyl-N-phenylpiperidin-3-amine (24). Prepared according to general procedure 2, method B, to afford 1.34 g (78%) of the title material as a yellow oil. ¹H NMR (500 MHz, CDCl₃): δ 7.38−7.21 (m, 7H), 6.80 (br s, 3H), 4.25 (br s, 1H), 3.62−3.53 (m, 2H), 3.23 (br s, 2H), 2.91−2.68 (m, 3H), 2.02 (s, 3H), 1.58−1.15 (m, 4H). MS (ESI) *m/z* 296 (M⁺ + 1).

tert-Butyl 1-(1-Benzyl-3-(phenylamino)piperidin-3-yl)-10,10-dimethyl-3,8-dioxo-9-oxa-2,5,7-triazaundecan-6-ylidenecarbamate (25). Prepared according to general procedure 4, method C, to afford 0.23 g (37%) of the title material as a white solid. ¹H NMR (500 MHz, CDCl₃): δ 11.36 (s, 1H), 8.94 (s, 1H), 7.38−7.28 (m, 5H), 7.19 (t, J = 7.6 Hz, 2H), 6.82 (t, J = 7.2 Hz, 1H), 6.73 (d, J = 7.7 Hz, 2H), 6.55−6.53 (m, 1H), 4.50 (br s, 1H), 4.05−4.04 (m, 2H), 3.59−3.42 (m, 4H), 2.79−2.78 (m, 1H), 2.70−2.68 (m, 1H), 2.06−1.98 (m, 4H), 1.63−1.40 (m, 21H). MS (ESI) *m/z* 595 (M⁺ + 1).

N-((1-Benzyl-3-(phenylamino)piperidin-3-yl)methyl)-2-guanidinoacetamide Tritriflate (26). Prepared according to general procedure 5, method B, to afford 0.16 g (65%) of the title material as a white solid. ¹H NMR (400 MHz, DMSO-d₆): δ 9.26 (br s, 1H), 8.26 (s, 1H), 7.90 (s, 1H), 7.57−7.17 (m, 8H), 7.00 (s, 2H), 6.68−6.65 (m, 3H), 5.24 (br s, 1H), 4.32−4.17 (m, 2H), 3.88 (s, 2H), 3.50−2.95 (m, 6H), 2.00−1.59 (m, 4H). ¹³C NMR (125 MHz, DMSO-d₆): δ 168.15, 158.65 (q, J = 31.3 Hz), 157.81, 145.12, 131.49, 129.53, 129.14, 128.73, 128.72, 118.46, 117.23, 117.15 (q, J = 297.2 Hz), 59.81, 55.93, 55.02, 52.47, 43.68, 43.38, 28.62, 18.28. MS (ESI) *m/z* 395 (M⁺ + 1).

1-Benzyl-4-(naphthalen-1-ylamino)piperidine-4-carbonitrile (27a). Prepared according to general procedure 1, using *N*-benzylpiperidone, 1-naphthylamine, and KCN to afford 3.2 g (59%) of the target material as a thick oil. ¹H NMR (CDCl₃): δ 7.91−7.84 (m, 2H), 7.52−7.27 (m, 10H), 4.24 (s, 1H), 3.58 (s, 2H), 2.85−2.82 (m, 1H), 2.77−2.74 (m, 1H), 2.57−2.43 (m, 4H), 2.14−2.09 (m, 2H). MS (ESI) *m/z* 342 (M⁺ + 1).

1-Benzyl-4-(3,5-dimethoxyphenylamino)piperidine-4-carbonitrile (27b). Prepared according to general procedure 1, using *N*-benzylpiperidone, 3,5-dimethoxyaniline, and TMSCN to afford 6.44 g (69%) of the title material as a white solid. ¹H NMR (500 MHz, DMSO-d₆): δ 7.34−7.29 (m, 5H), 6.11−6.07 (m, 3H), 3.78 (s, 6H), 3.58 (s, 2H), 2.83−2.81 (m, 2H), 2.49 (t, J = 10.6 Hz, 2H), 2.38 (d, J = 12.7 Hz, 2H), 1.96−1.94 (m, 2H). MS (ESI) *m/z* 352 (M⁺ + 1).

1-Benzyl-4-(3-methoxyphenylamino)piperidine-4-carbonitrile (27c). Prepared according to general procedure 1, using *N*-benzylpiperidone, *m*-anisidine, and TMSCN to afford 4.26 g (63%) of the title material as a white solid. ¹H NMR (DMSO-d₆): δ 7.31−

7.25 (m, 5H), 7.07 (t, J = 8.0 Hz, 1H), 6.44 (d, J = 8.0 Hz, 1H), 6.40 (s, 1H), 6.32 (d, J = 8.0 Hz, 1H), 6.06 (s, 1H), 3.68 (s, 3H), 3.51 (s, 2H), 2.76−2.74 (m, 2H), 2.29−2.27 (m, 4H), 1.85−1.80 (m, 2H). MS (ESI) *m/z* 322 (M⁺ + 1).

1-Benzyl-4-(3,4-dichlorophenylamino)piperidine-4-carbonitrile (27d). Prepared according to general procedure 1, using *N*-benzylpiperidone, 3,4-dichloroaniline, and TMSCN to afford 7.31 g (77%) of the title material as a light brown solid. ¹H NMR (DMSO-d₆): δ 7.39−7.24 (m, 6H), 7.01 (d, J = 2.3 Hz, 1H), 6.83 (dd, J = 8.8, 2.4 Hz, 1H), 6.56 (s, 1H), 3.50 (s, 2H), 2.76−2.73 (m, 2H), 2.32−2.29 (m, 4H), 1.85−1.82 (m, 2H). MS (ESI) *m/z* 360 (M⁺ + 1).

4-(Aminomethyl)-1-benzyl-N-(naphthalen-1-yl)piperidin-4-amine (28a). Prepared from **27a** (3 g, 8.7 mmol) according to procedure 2, method B, to afford 2.7 g (89%) of the title material as a light brown solid. ¹H NMR (CDCl₃): δ 7.95−7.93 (m, 1H), 7.86−7.83 (m, 1H), 7.52−7.50 (m, 2H), 7.35−7.30 (m, 7H), 6.87−6.85 (m, 1H), 4.24 (br s, 1H), 3.52 (s, 2H), 3.06 (s, 2H), 2.70−2.67 (m, 2H), 2.39 (t, J = 10.7 Hz, 2H), 2.25−2.18 (m, 2H), 1.79−1.73 (m, 2H), 1.45 (br s, 2H). MS (ESI) *m/z* 346 (M⁺ + 1).

4-(Aminomethyl)-1-benzyl-N-(3,5-dimethoxyphenyl)piperidin-4-amine (28b). Prepared from **27b** (2.89 g, 8.24 mmol) according to general procedure 2, method B, to afford 2.8 g (97%) of the title material as a greenish oil. ¹H NMR (CDCl₃, 50 °C): δ 7.31−7.22 (m, 5H), 5.97−5.93 (m, 3H), 3.73 (s, 6H), 3.51 (s, 2H), 2.92 (br s, 2H), 2.59−2.53 (m, 3H), 2.35 (br s, 2H), 2.15−1.69 (m, 6H). MS (ESI) *m/z* 356 (M⁺ + 1).

4-(Aminomethyl)-1-benzyl-N-(3-methoxyphenyl)piperidin-4-amine (28c). Prepared from **27c** (2.3 g, 7.16 mmol) according to general procedure 2, method B, to afford 2.2 g (95%) of the title material as a brown oil. ¹H NMR (500 MHz, DMSO-d₆): δ 7.29−7.22 (m, 5H), 6.92 (t, J = 7.8 Hz, 1H), 6.34−6.31 (m, 2H), 6.14 (d, J = 6.3 Hz, 1H), 4.82 (s, 1H), 3.65 (s, 3H), 3.18 (s, 2H), 2.70−2.75 (s, 2H), 2.50−2.28 (m, 6H), 1.91−1.89 (m, 2H), 1.55−1.53 (m, 2H). MS (ESI) *m/z* 326 (M⁺ + 1).

4-(Aminomethyl)-1-benzyl-N-(3,4-dichlorophenyl)piperidin-4-amine (28d). Prepared from **27d** (3 g, 8.3 mmol) according to general procedure 2, method B, to afford 2.8 g (95%) of the title material as a thick colorless oil. ¹H NMR (CDCl₃): δ 7.31−7.24 (m, 5H), 7.16 (d, J = 8.7 Hz, 1H), 6.81 (d, J = 2.6 Hz, 1H), 6.55 (dd, J = 8.7, 2.6 Hz, 1H), 3.55−3.43 (m, 3H), 2.86 (s, 2H), 2.63−2.60 (m, 2H), 2.26 (t, J = 10.4 Hz, 2H), 1.94 (d, J = 13.6 Hz, 2H), 1.67−1.60 (m, 2H), 1.44 (s, 2H). MS (ESI) *m/z* 364 (M⁺ + 1).

tert-Butyl 1-(1-Benzyl-4-(naphthalen-1-ylamino)piperidin-4-yl)-10,10-dimethyl-3,8-dioxo-9-oxa-2,5,7-triazaundecan-6-ylidenecarbamate (29a). Prepared from **28a** (0.35 g, 1.02 mmol) according to general procedure 4, method C, to afford 0.33 g (50%) of the title material as a white solid. ¹H NMR (500 MHz, CDCl₃): δ 11.24 (s, 1H), 8.79 (s, 1H), 7.90−7.88 (m, 1H), 7.82−7.81 (m, 1H), 7.51−7.47 (m, 2H), 7.31−7.25 (m, 7H), 6.86−6.84 (m, 1H), 6.46−6.45 (m, 1H), 3.96 (d, J = 5.1 Hz, 2H), 3.72 (d, J = 5.6 Hz, 2H), 3.51 (s, 2H), 2.64−2.62 (m, 2H), 2.44−2.40 (m, 2H), 2.17−2.14 (m, 2H), 1.89−1.85 (m, 2H), 1.46 (m, 18H). MS (ESI) *m/z* 645 (M⁺ + 1).

tert-Butyl 1-(1-Benzyl-4-(3,5-dimethoxyphenylamino)piperidin-4-yl)-10,10-dimethyl-3,8-dioxo-9-oxa-2,5,7-triazaundecan-6-ylidenecarbamate (29b). Prepared from **28b** (0.39 g, 1.10 mmol) according to general procedure 4, method C, to afford 0.34 g (48%) of the title material as a white solid. ¹H NMR (500 MHz, CDCl₃): δ 11.36 (s, 1H), 8.85 (s, 1H), 7.31−7.25 (m, 5H), 6.42 (s, 1H), 5.94−5.89 (m, 3H), 3.99 (d, J = 5.3 Hz, 2H), 3.76 (s, 6H), 3.59 (d, J = 5.6 Hz, 2H), 3.52 (s, 2H), 2.59−2.57 (m, 2H), 2.37 (t, J = 9.3 Hz, 2H), 1.92 (d, J = 13.5 Hz, 2H), 1.78−1.74 (m, 2H), 1.51 (s, 9H), 1.49 (s, 9H). MS (ESI) *m/z* 655 (M⁺ + 1).

tert-Butyl 1-(1-Benzyl-4-(3-methoxyphenylamino)piperidin-4-yl)-10,10-dimethyl-3,8-dioxo-9-oxa-2,5,7-triazaundecan-6-ylidenecarbamate (29c). Prepared from **28c** (0.39 g, 1.10 mmol) according to general procedure 4, method C, to afford 0.46 g (67%) of the title material as a white solid. ¹H NMR (500 MHz, CDCl₃): δ 11.36 (s, 1H), 8.85 (s, 1H), 7.31−7.25 (m, 5H), 7.07 (t, J = 8.0 Hz, 1H), 6.48 (s, 1H), 6.36−6.32 (m, 2H), 6.29 (s, 1H), 3.99 (d, J = 5.0 Hz, 2H), 3.77 (s, 3H), 3.58 (d, J = 5.3 Hz, 2H), 3.52 (br s, 1H), 3.51 (s, 2H), 2.58−2.56 (m, 2H), 2.37 (t, J = 9.5 Hz, 2H), 1.91 (d, J = 13.0 Hz, 2H),

1.77−1.73 (m, 2H), 1.51 (s, 9H), 1.48 (s, 9H). MS (ESI) *m/z* 625 (M+ + 1).

tert-Butyl 1-(1-Benzyl-4-(3,4-dichlorophenylamino)piperidin-4-yl)-10,10-dimethyl-3,8-dioxo-9-oxa-2,5,7-triazaundecan-6-ylidenecarbamate (29d). Prepared from **28d** (0.4 g, 1.10 mmol) according to general procedure 4, method C, to afford 0.24 g (33%) of the title material as a white solid. ¹H NMR (CDCl₃): δ 11.31 (s, 1H), 8.82 (s, 1H), 7.30−7.24 (m, 5H), 7.17 (d, *J* = 8.7 Hz, 1H), 6.80 (d, *J* = 2.6 Hz, 1H), 6.66−6.63 (m, 1H), 6.56 (dd, *J* = 8.7, 2.6 Hz, 1H), 3.97 (d, *J* = 5.5 Hz, 2H), 3.62 (s, 1H), 3.52 (d, *J* = 5.7 Hz, 2H), 3.50 (s, 2H), 2.57−2.55 (m, 2H), 2.35−2.30 (m, 2H), 1.90−1.86 (m, 2H), 1.77−1.71 (m, 2H), 1.49 (s, 9H), 1.46 (s, 9H). MS (ESI) *m/z* 663 (M+ + 1).

N-((1-Benzyl-4-(naphthalen-1-ylamino)piperidin-4-yl)methyl)-2-guanidinoacetamide Tritriflate (30a). Prepared from **29a** (0.2 g, 0.31 mmol) according to general procedure 5, method B, to afford 0.18 g (74%) of the title material as a white solid. ¹H NMR (400 MHz, DMSO-*d₆*): δ 10.35 (s, 1H), 8.29−8.16 (m, 2H), 7.91−7.22 (m, 14H), 6.90 (d, *J* = 7.23 Hz, 1H), 5.33 (s, 1H), 4.40−4.27 (m, 2H), 3.96−3.74 (m, 2H), 3.55−3.53 (m, 2H), 3.25−3.07 (m, 4H), 2.52−2.49 (m, 2H), 2.03−1.97 (m, 2H). ¹³C NMR (125 MHz, DMSO-*d₆*): δ 167.98, 158.95 (q, *J* = 31.9 Hz), 157.74, 140.42, 134.39, 131.37, 129.64, 129.52, 128.72, 128.05, 126.10, 125.72, 125.34, 124.48, 118.26, 117.74, 115.89, 115.63 (q, *J* = 526.6 Hz), 58.80, 53.60, 47.18, 44.28, 43.36, 28.94. MS (ESI) *m/z* 445 (M+ + 1).

N-((1-Benzyl-4-(3,5-dimethoxyphenylamino)piperidin-4-yl)methyl)-2-guanidinoacetamide Tritriflate (30b). Prepared from **29b** (0.17 g, 0.26 mmol) according to general procedure 5, method B, to afford 0.17 g (83%) of the title material as a white solid. ¹H NMR (500 MHz, DMSO-*d₆*): δ 10.10 (s, 1H), 8.24 (s, 1H), 7.75 (s, 1H), 7.50−7.43 (m, 9H), 5.97 (s, 2H), 5.81 (s, 1H), 5.46 (s, 1H), 4.30 (s, 2H), 3.83−3.09 (m, 14H), 2.12−1.88 (m, 4H). ¹³C NMR (125 MHz, DMSO-*d₆*): δ 167.89, 160.89, 158.88 (q, *J* = 31.0 Hz), 157.60, 147.54, 131.29, 129.80, 129.52, 128.78, 116.11 (q, *J* = 296.3 Hz), 94.27, 89.50, 58.86, 54.77, 53.15, 47.09, 44.48, 43.34, 29.18. MS (ESI) *m/z* 455 (M+ + 1).

N-((1-Benzyl-4-(3-methoxyphenylamino)piperidin-4-yl)methyl)-2-guanidinoacetamide Tritriflate (30c). Prepared from **29c** (0.2 g, 0.32 mmol) according to general procedure 5, method B, to afford 0.13 g (53%) of the title material as a white solid. ¹H NMR (400 MHz, DMSO-*d₆*): δ 10.24 (s, 1H), 8.21 (s, 1H), 7.80 (s, 1H), 7.51−7.18 (m, 9H), 6.98 (t, *J* = 7.9 Hz, 1H), 6.40−6.37 (m, 2H), 6.21 (d, *J* = 7.8 Hz, 1H), 5.39 (br s, 1H), 4.30 (s, 2H), 3.85−3.83 (m, 2H), 3.67 (s, 3H), 3.36−3.08 (m, 6H), 2.14−1.88 (m, 4H). ¹³C NMR (125 MHz, DMSO-*d₆*): δ 167.93, 160.05, 158.95 (q, *J* = 31.9 Hz), 157.71, 147.20, 131.32, 131.10, 129.83, 129.54, 128.80, 117.09 (q, *J* = 297.0 Hz), 108.51, 102.65, 101.81, 58.87, 54.68, 53.18, 47.11, 44.60, 43.36, 29.20. MS (ESI) *m/z* 425 (M+ + 1).

N-((1-Benzyl-4-(3,4-dichlorophenylamino)piperidin-4-yl)methyl)-2-guanidinoacetamide Tritriflate (30d). Prepared from **29d** (0.22 g, 0.33 mmol) according to general procedure 5, method B, to afford 0.19 g (72%) of the title material as a white solid. ¹H NMR (500 MHz, DMSO-*d₆*): δ 10.13 (s, 1H), 8.25 (s, 1H), 7.70 (s, 1H), 7.49−7.13 (m, 10H), 6.98 (s, 1H), 6.77−6.75 (m, 1H), 5.90 (s, 1H), 4.29 (s, 2H), 3.83 (s, 2H), 3.59−3.04 (m, 6H), 2.14−1.88 (m, 4H). ¹³C NMR (125 MHz, DMSO-*d₆*): δ 167.85, 158.57 (q, *J* = 31.2 Hz), 157.52, 146.16, 131.24, 130.97, 130.27, 129.74, 129.50, 128.76, 117.65, 117.13 (q, *J* = 297.3 Hz), 116.34, 115.10, 58.82, 53.25, 46.96, 44.00, 43.33, 28.96. MS (ESI) *m/z* 463 (M+ + 1).

tert-Butyl 2-((1-Benzyl-4-phenylpiperidin-4-yl)methylamino)-2-oxoethylcarbamate (32). Prepared according to general procedure 4, method D (step I), using 1-(1-benzyl-4-phenylpiperidin-4-yl)-methanamine (**31**, 0.75 g, 2.66 mmol) to afford 0.9 g (78%) of the title material as a white solid. ¹H NMR (CDCl₃): δ 7.39−7.35 (m, 2H), 7.31−7.23 (m, 8H), 5.73 (br s, 1H), 5.11 (br s, 1H), 3.65 (d, *J* = 5.8 Hz, 2H), 3.45 (s, 4H), 2.61−2.59 (m, 2H), 2.34 (br s, 2H), 2.34−2.10 (m, 2H), 1.93−1.89 (m, 2H), 1.42 (s, 9H). MS (ESI) *m/z* 438 (M+ + 1).

tert-Butyl 1-(1-Benzyl-4-phenylpiperidin-4-yl)-10,10-dimethyl-3,8-dioxo-9-oxa-2,5,7-triazaundecan-6-ylidenecarbamate (33). Prepared according to general procedure 4, method D (step II), from intermediate **32** (0.3 g, 0.685 mmol) to afford 0.35 g (89%) of the title

material as a white solid. ¹H NMR (CDCl₃): δ 11.36 (s, 1H), 8.74−8.72 (m, 1H), 7.36−7.22 (m, 10H), 5.74−5.73 (m, 1H), 3.89 (d, *J* = 5.3 Hz, 2H), 3.47−3.46 (m, 4H), 2.61−2.59 (m, 2H), 2.36 (br s, 2H), 2.14−2.12 (m, 2H), 1.94−1.91 (m, 2H), 1.50 (s, 9H), 1.47 (s, 9H). MS (ESI) *m/z* 580 (M+ + 1).

N-((1-Benzyl-4-phenylpiperidin-4-yl)methyl)-2-guanidinoacetamide Ditriflate (34). Prepared according to general procedure 5, method B, from intermediate **33** (0.12 g, 0.207 mmol) to afford the title material as a white solid. ¹H NMR (500 MHz, CD₃OD): δ 7.41−7.25 (m, 10H), 4.03 (s, 2H), 3.85 (s, 2H), 3.37−3.23 (m, 4H), 2.79 (br s, 2H), 2.39−2.37 (m, 2H), 2.15−2.11 (m, 2H). ¹³C NMR (125 MHz, CD₃OD): δ 169.96, 163.14 (q, *J* = 34.2 Hz), 159.32, 142.36, 132.09, 131.93, 130.48, 130.19, 129.98, 128.11, 127.87, 118.12 (q, *J* = 290.9 Hz), 61.84, 50.34, 44.70, 42.28, 31.13. MS (ESI) *m/z* 380 (M+ + 1).

1,4-Dibenzylpiperidine-4-carbonitrile (36). Prepared according to general procedure 6 using benzyl bromide to afford 1.29 g (89%) of the title material as a white solid. ¹H NMR (500 MHz, CDCl₃): δ 7.39−7.28 (m, 10H), 3.56 (s, 2H), 2.91−2.89 (m, 4H), 2.34 (t, *J* = 12.1 Hz, 2H), 1.88 (d, *J* = 13.2 Hz, 2H), 1.73−1.67 (m, 2H). MS (ESI) *m/z* 291 (M+ + 1).

1-Benzyl-4-phenethylpiperidine-4-carbonitrile (37). Prepared according to general procedure 6 using (2-bromoethyl)benzene to afford 1.25 g (86%) of the title material as a colorless oil. ¹H NMR (CDCl₃): δ 7.35−7.20 (m, 10H), 3.55 (s, 2H), 2.89−2.81 (m, 4H), 2.37 (t, *J* = 12 Hz, 2H), 1.96 (d, *J* = 13.1 Hz, 2H), 1.88−1.83 (m, 2H), 1.64−1.57 (m, 2H). MS (ESI) *m/z* 305 (M+ + 1).

4-(Aminomethyl)-1-benzyl-N-phenylpiperidin-4-amine (38). Prepared from **36** (0.53 g, 1.82 mmol) using general procedure 2, method B, to afford 0.33 g (61%) of the title material as a colorless oil. ¹H NMR (500 MHz, CDCl₃): δ 7.35−7.17 (m, 10H), 3.56 (s, 2H), 2.69−2.45 (m, 8H), 1.56−1.47 (m, 4H), 1.18 (br s, 2H). MS (ESI) *m/z* 295 (M+ + 1).

(1-Benzyl-4-phenethylpiperidin-4-yl)methanamine (39). Prepared from **37** (0.436 g, 1.43 mmol) using general procedure 2, method B, to afford 0.4 g (90%) of the title material as a colorless oil. ¹H NMR (CDCl₃): δ 7.36−7.23 (m, 10H), 3.55 (s, 2H), 2.69 (s, 2H), 2.57−2.43 (m, 6H), 1.70−1.66 (m, 2H), 1.56−1.54 (m, 4H), 1.18 (br s, 2H). MS (ESI) *m/z* 309 (M+ + 1).

tert-Butyl 1-(1-Benzyl-4-(phenylamino)piperidin-4-yl)-10,10-dimethyl-3,8-dioxo-9-oxa-2,5,7-triazaundecan-6-ylidenecarbamate (40). Prepared from **38** (0.32 g, 1.08 mmol) according to general procedure 4, method C, to afford 0.31 g (48%) of the title material as a white solid. ¹H NMR (500 MHz, CDCl₃): δ 11.45 (s, 1H), 8.89 (s, 1H), 7.33−7.20 (m, 7H), 7.10 (d, *J* = 7.1 Hz, 2H), 6.89−6.88 (m, 1H), 4.04 (d, *J* = 5.5 Hz, 2H), 3.54 (s, 2H), 3.20 (d, *J* = 5.4 Hz, 2H), 2.63−2.58 (m, 4H), 2.43−2.41 (m, 2H), 1.52−1.43 (m, 22H). MS (ESI) *m/z* 594 (M+ + 1).

tert-Butyl 1-(1-Benzyl-4-phenethylpiperidin-4-yl)-10,10-dimethyl-3,8-dioxo-9-oxa-2,5,7-triazaundecan-6-ylidenecarbamate (41). Prepared from **39** (0.374 g, 1.21 mmol) according to general procedure 4, method D, to afford 0.258 g (76%) of the title material as a white solid. ¹H NMR (500 MHz, CDCl₃): δ 11.41 (s, 1H), 8.93 (s, 1H), 7.34−7.14 (m, 10H), 6.97 (s, 1H), 4.06−4.05 (m, 2H), 3.55 (s, 2H), 3.33−3.32 (m, 2H), 2.56−2.50 (m, 6H), 1.57−1.55 (m, 6H), 1.51 (s, 9H), 1.41 (s, 9H). MS (ESI) *m/z* 608 (M+ + 1).

N-((1,4-Dibenzylpiperidin-4-yl)methyl)-2-guanidinoacetamide Ditriflate (42). Prepared from **40** (0.13 g, 0.22 mmol) according to general procedure 5, method B, to afford 0.096 g (71%) of the title material as a white solid. ¹H NMR (500 MHz, DMSO-*d₆*): δ 9.89−9.48 (m, 1H), 8.29−8.15 (m, 1H), 7.79−7.71 (m, 1H), 7.52−7.13 (m, 14H), 4.38−4.27 (m, 2H), 3.98−3.88 (m, 2H), 3.28−3.11 (m, 4H), 2.93−2.82 (m, 2H), 2.54−2.50 (m, 2H), 1.64−1.44 (m, 4H). ¹³C NMR (125 MHz, DMSO-*d₆*): δ 168.23, 168.02, 158.60 (q, *J* = 31.2 Hz), 137.00, 136.52, 131.17, 130.91, 130.59, 130.04, 129.83, 129.50, 128.90, 128.80, 128.15, 127.95, 126.42, 126.32, 116.16 (q, *J* = 297.5 Hz), 58.88, 58.63, 47.44, 47.08, 45.55, 43.52, 41.12, 36.38, 35.44, 35.31, 28.32, 28.16. MS (ESI) *m/z* 394 (M+ + 1).

N-((1-Benzyl-4-phenethylpiperidin-4-yl)methyl)-2-guanidinoacetamide Ditriflate (43). Prepared from **41** (0.1 g, 0.197 mmol)

according to general procedure 5, method B, to afford 0.11 g (85%) of the title material as a white solid. ^{1}H NMR (500 MHz, CD$_3$OD): δ 7.54−7.52 (m, 5H), 7.29−7.18 (m, 5H), 4.37−4.36 (m, 2H), 4.01 (m, 2H), 3.52−3.18 (m, 6H), 2.67−2.63 (m, 2H), 1.81−1.60 (m, 6H). ^{13}C NMR (125 MHz, 45 °C, CD$_3$OD): δ 170.25, 163.06 (q, J = 34.5 Hz), 159.49, 143.32, 132.19, 131.02, 130.37, 130.18, 129.37, 129.33, 126.79, 118.25 (q, J = 289.2 Hz), 61.29, 49.62, 44.96, 44.86, 41.94, 35.98, 30.66, 30.15. MS (ESI) m/z 408 (M$^+$ + 1).

tert-Butyl 1-(1-Benzyl-4-(phenylamino)piperidin-4-yl)-10,10-di-methyl-1,8-dioxo-9-oxa-2,5,7-triazaundecan-6-ylidenecarbamate (**45**). To a stirred mixture of 1-benzyl-4-phenylamino-4-carboxy-piperidine (**44**, 0.3 g, 0.9 mmol), 1-hydroxy-benzotriazole hydrate (0.2 g, 1.46 mmol), 2-(2-aminoethyl)-1,3-di-Boc-guanidine (0.34 g, 1.1 mmol), and triethylamine (0.6 mL, 4.5 mmol) in 1-methyl-2-pyrrolidone (15 mL) was added N-(3-(dimethylamino)propyl)-N′-ethylcarbodiimide hydrochloride (0.22 g, 1.1 mmol). The reaction mixture was stirred at room temperature for 24 h. The mixture was then poured into 80 mL of water, extracted with ethyl acetate (3 × 30 mL), washed with saturated aqueous NaCl, and dried. The solvent was removed in vacuo, and the residue was chromatographed on a silica gel column using a gradient of methylene chloride and methanol (10:0 → 9.5:0.5) as the eluent to give 0.37 g (70%) of *tert*-butyl 1-(1-benzyl-4-(phenylamino)piperidin-4-yl)-10,10-dimethyl-1,8-dioxo-9-oxa-2,5,7-triazaundecan-6-ylidenecarbamate as a white solid. ^{1}H NMR (500 MHz, CDCl$_3$): δ 11.19 (s, 1H), 8.42 (s, 1H), 7.77 (s, 1H), 7.32−7.25 (m, 5H), 7.14 (t, J = 7.4 Hz, 2H), 6.75 (t, J = 7.2 Hz, 1H), 6.54 (d, J = 7.8 Hz, 2H), 3.99 (s, 1H), 3.55−3.43 (m, 6H), 2.74 (d, J = 11.6 Hz, 2H), 2.33 (t, J = 11.2 Hz, 2H), 2.16 (t, J = 11.6 Hz, 2H), 1.90 (d, J = 13.2 Hz, 2H), 1.53 (s, 9H), 1.49 (s, 9H). MS (ESI) m/z 595 (M$^+$ + 1).

1-Benzyl-N-(2-guanidinoethyl)-4-(phenylamino)piperidine-4-car-boxamide Tritriflate (**46**). Prepared from **45** (0.12 g, 0.20 mmol) according to general procedure 5, method B, to afford 0.08 g (54%) of the title material as a white solid. ^{1}H NMR (500 MHz, DMSO-d_6): δ 10.40 (s, 1H), 8.28−7.91 (m, 2H), 7.50−7.07 (m, 12H), 6.63−6.55 (m, 3H), 6.06 (s, 1H), 4.29 (s, 2H), 3.36−3.10 (m, 8H), 2.25−1.95 (m, 4H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 174.46, 158.77 (q, J = 31.4 Hz), 157.32, 144.44, 131.30, 129.53, 128.79, 128.71, 117.52, 117.12 (q, J = 296.6 Hz), 115.02, 114.52, 59.02, 55.95, 46.73, 40.19, 38.00, 28.07. MS (ESI) m/z 395 (M$^+$ + 1).

tert-Butyl 10,10-Dimethyl-3,8-dioxo-1-(4-(phenylamino)-piperidin-4-yl)-9-oxa-2,5,7-triazaundecan-6-ylidenecarbamate (**47**). Intermediate **20** (1 g, 1.68 mmol) was dissolved in methanol (65 mL) and hydrogenated over 10% Pd/C (0.4 g) under pressure (4 bar) for 7 days. The catalyst was removed by filtration, and the solvent was evaporated in vacuo. The residue was purified by chromatography on a silica gel column using a gradient of methylene chloride and methanol saturated with ammonia (10:0 → 9:1) as the eluent to give 0.57 g (68%) of the title material as a white solid. ^{1}H NMR (CDCl$_3$): δ 11.34 (br s, 1H), 8.83 (br s, 1H), 7.15 (t, J = 7.6 Hz, 2H), 6.79 (t, J = 7.4 Hz, 1H), 6.72 (d, J = 7.8 Hz, 2H), 6.53−6.55 (m, 1H), 3.98 (d, J = 5.2 Hz, 2H), 3.55 (d, J = 5.6 Hz, 2H), 3.42 (br s, 1H), 2.89−2.87 (m, 4H), 1.86−1.82 (m, 2H), 1.68−1.63 (m, 3H), 1.49 (s, 9H), 1.47 (s, 9H). MS (ESI) m/z 505 (M$^+$ + 1).

tert-Butyl 1-(1-(Cyclohexylmethyl)-4-(phenylamino)piperidin-4-yl)-10,10-dimethyl-3,8-dioxo-9-oxa-2,5,7-triazaundecan-6-ylidene-carbamate (**48b**). Prepared according to general procedure 7 using intermediate **47** (0.13 g, 0.257 mmol) and cyclohexylmethyl bromide (0.055 g, 0.308 mmol) in anhydrous NMP (5 mL) to afford 0.04 g (27%) of the title material as a white solid. ^{1}H NMR (CDCl$_3$): δ 11.31 (br s, 1H), 8.84 (br s, 1H), 7.10 (t, J = 7.6 Hz, 2H), 6.76−6.69 (m, 4H), 3.97 (d, J = 4.9 Hz, 2H), 3.50 (d, J = 5.3 Hz, 2H), 2.67 (s, 2H), 2.48 (s, 2H), 2.24 (s, 2H), 1.91−1.59 (m, 10H), 1.45 (s, 9H), 1.43 (s, 9H), 1.21−1.08 (m, 4H), 0.89−0.84 (m, 2H). MS (ESI) m/z 601 (M$^+$ + 1).

tert-Butyl 10,10-Dimethyl-3,8-dioxo-1-(1-phenethyl-4-(phenylamino)piperidin-4-yl)-9-oxa-2,5,7-triazaundecan-6-ylidene-carbamate (**48c**). Prepared according to general procedure 7 using intermediate **47** (0.15 g, 0.297 mmol) and 2-(bromoethyl)benzene (0.055 g, 0.297 mmol) to afford 0.13 g (72%) of the title material as a white solid. ^{1}H NMR (CDCl$_3$): δ 11.35 (s, 1H), 8.85 (s, 1H), 7.29−7.25 (m, 2H), 7.19−7.14 (m, 5H), 6.79 (t, J = 7.2 Hz, 1H), 6.73 (d, J =

7.8 Hz, 2H), 6.62−6.60 (m, 1H), 3.99 (d, J = 5.1 Hz, 2H), 3.56 (d, J = 5.3 Hz, 2H), 3.56 (br s, 1H), 2.84−2.80 (m, 2H), 2.67−2.64 (m, 4H), 2.53−2.52 (m, 2H), 1.97−1.94 (m, 2H), 1.85−1.80 (m, 2H), 1.49 (s, 9H), 1.48 (s, 9H). MS (ESI) m/z 609 (M$^+$ + 1).

tert-Butyl 10,10-Dimethyl-1-(1-(naphthalen-1-ylmethyl)-4-(phenylamino)piperidin-4-yl)-3,8-dioxo-9-oxa-2,5,7-triazaundecan-6-ylidenecarbamate (**48d**). Prepared according to general procedure 7 using intermediate **47** (0.13 g, 0.257 mmol) and 1-(chloromethyl)-naphthalene (0.041 g, 0.231 mmol) to afford 0.096 g (65%) of the title material as a white solid. ^{1}H NMR (CDCl$_3$): δ 11.37 (s, 1H), 8.86−8.84 (m, 1H), 8.28 (d, J = 8.0 Hz, 1H), 7.83 (t, J = 7.2 Hz, 1H), 7.76 (d, J = 7.2 Hz, 1H), 7.51−7.47 (m, 2H), 7.40−7.37 (m, 2H), 7.16 (t, J = 7.7 Hz, 2H), 6.79 (t, J = 7.3 Hz, 1H), 6.73 (d, J = 7.9 Hz, 2H), 6.56−6.53 (m, 1H), 4.00 (d, J = 5.2 Hz, 2H), 3.90 (s, 2H), 3.55 (d, J = 5.4 Hz, 2H), 3.43 (br s, 1H), 2.64−2.61 (m, 2H), 2.43 (t, J = 9.5 Hz, 2H), 1.89−1.85 (m, 2H), 1.73−1.68 (m, 2H), 1.49 (s, 9H), 1.47 (s, 9H). MS (ESI) m/z 645 (M$^+$ + 1).

tert-Butyl 10,10-Dimethyl-1-(1-(naphthalen-2-ylmethyl)-4-(phenylamino)piperidin-4-yl)-3,8-dioxo-9-oxa-2,5,7-triazaundecan-6-ylidenecarbamate (**48e**). Prepared according to general procedure 4, method C, using **5d** (0.3 g, 0.87 mmol) to afford 0.31 g (48%) of the title material as a yellow solid. ^{1}H NMR (500 MHz, CDCl$_3$): δ 11.30 (s, 1H), 8.80 (s, 1H), 7.77−7.67 (m, 4H), 7.43−7.39 (m, 3H), 7.11 (t, J = 7.4 Hz, 2H), 6.74 (t, J = 7.2 Hz, 1H), 6.68 (d, J = 7.7 Hz, 2H), 6.54 (s, 1H), 3.95 (br s, 3H), 3.62−3.51 (m, 4H), 2.56−2.55 (m, 2H), 2.38−2.37 (m, 2H), 1.87−1.84 (m, 2H), 1.74−1.72 (m, 2H), 1.44 (s, 9H), 1.40 (s, 9H). MS (ESI) m/z 645 (M$^+$ + 1).

tert-Butyl 1-(1-(4-Bromobenzyl)-4-(phenylamino)piperidin-4-yl)-10,10-dimethyl-3,8-dioxo-9-oxa-2,5,7-triazaundecan-6-ylidenecar-bamate (**48f**). Prepared according to general procedure 7 using intermediate **47** (0.13 g, 0.257 mmol) and 4-bromobenzyl bromide (0.058 g, 0.231 mmol) to afford 0.12 g (69%) of the title material as a white solid. ^{1}H NMR (CDCl$_3$): δ 11.33 (s, 1H), 8.82 (s, 1H), 7.40 (d, J = 8.2 Hz, 2H), 7.18−7.12 (m, 4H), 6.78 (t, J = 7.1 Hz, 1H), 6.70 (d, J = 8.0 Hz, 2H), 6.51−6.50 (m, 1H), 3.98 (d, J = 5.2 Hz, 2H), 3.54 (d, J = 5.3 Hz, 2H), 3.44 (s, 2H), 3.42 (br s, 1H), 2.53−2.51 (m, 2H), 2.37−2.33 (m, 2H), 1.88−1.85 (m, 2H), 1.75−1.70 (m, 2H), 1.48 (s, 9H), 1.45 (s, 9H). MS (ESI) m/z 673 (M$^+$ + 1) for ^{79}Br, 675 (M$^+$ + 1) for ^{81}Br.

Methyl 4-((4-(6-(tert-Butoxycarbonylamino)-10,10-dimethyl-3,8-dioxo-9-oxa-2,5,7-triazaundec-6-enyl)-4-(phenylamino)piperidin-1-yl)methyl)benzoate (**48g**). Prepared according to general procedure 7 using intermediate **47** (0.13 g, 0.257 mmol) and methyl 4-(bromomethyl)benzoate (0.053 g, 0.231 mmol) to afford 0.14 g (96%) of the title material as a white solid. ^{1}H NMR (500 MHz, CDCl$_3$): δ 11.33 (s, 1H), 8.82 (s, 1H), 7.96 (d, J = 8.1 Hz, 2H), 7.37 (d, J = 8.1 Hz, 2H), 7.14 (t, J = 7.7 Hz, 2H), 6.78 (t, J = 7.2 Hz, 1H), 6.70 (d, J = 7.8 Hz, 2H), 6.52−6.50 (m, 1H), 3.98 (d, J = 5.3 Hz, 2H), 3.89 (s, 3H), 3.55−3.46 (m, 5H), 2.54−2.52 (m, 2H), 2.39−2.36 (m, 2H), 1.89−1.86 (m, 2H), 1.76−1.72 (m, 2H), 1.48 (s, 9H), 1.45 (s, 9H). MS (ESI) m/z 653 (M$^+$ + 1).

2-Guanidino-N-((4-(phenylamino)piperidin-4-yl)methyl)-acetamide Tritriflate (**49a**). Prepared according to general procedure 5, method B, from **47** (0.1 g, 0.198 mmol) to afford 0.11 g (85%) of the title material as a brown solid. ^{1}H NMR (DMSO-d_6): δ 10.85 (br s, 2H), 8.74−8.63 (m, 2H), 8.18 (s, 1H), 7.74 (s, 1H), 7.41 (br s, 3H), 7.10 (t, J = 6 Hz, 2H), 6.85 (d, J = 6.2 Hz, 2H), 6.66−6.64 (m, 1H), 3.86−3.85 (m, 2H), 3.42−3.41 (m, 2H), 3.12−3.03 (m, 4H), 2.07−2.06 (m, 2H), 1.80−1.75 (m, 2H). ^{13}C NMR (DMSO-d_6): δ 168.01, 158.70 (q, J = 34.2 Hz), 157.60, 145.43, 128.87, 117.59, 116.33, 116.23 (q, J = 290.6 Hz), 53.58, 44.46, 43.42, 30.65, 28.66. MS (ESI) m/z 305 (M$^+$ + 1).

N-((1-(Cyclohexylmethyl)-4-(phenylamino)piperidin-4-yl)methyl)-2-guanidinoacetamide Tritriflate (**49b**). Prepared according to general procedure 5, method B, from **48b** (0.04 g, 0.066 mmol) to afford 0.04 g (81%) of the title material as a yellow solid. ^{1}H NMR (400 MHz, CD$_3$OD): δ 7.14 (t, J = 4 Hz, 2H), 6.90−6.88 (m, 2H), 6.73 (t, J = 4 Hz, 1H), 3.97 (s, 2H), 3.53 (s, 2H), 3.45−3.42 (m, 2H), 3.26−3.20 (m, 2H), 2.95 (d, J = 8 Hz, 2H), 2.30−2.26 (m, 2H), 2.16−2.09 (m, 3H), 1.84−1.70 (m, 7H), 1.38−1.28 (m, 3H). MS (ESI) m/z 401 (M$^+$ + 1).

2-Guanidino-N-((1-phenethyl-4-(phenylamino)piperidin-4-yl)methyl)acetamide Tritriflate (49c). Prepared according to general procedure 5, method B, from **48c** (0.12 g, 0.197 mmol) to afford 0.076 g (51%) of the title material as a yellow solid. ^{1}H NMR (400 MHz, CD$_3$OD): δ 7.35–7.24 (m, 5H), 7.16 (t, J = 7.6 Hz, 2H), 6.89 (d, J = 8.0 Hz, 2H), 6.75 (t, J = 7.2 Hz, 1H), 3.96 (s, 2H), 3.56–3.51 (m, 4H), 3.35–3.31 (m, 4H), 3.10–3.05 (m, 2H), 2.35–2.31 (m, 2H), 2.12–2.01 (m, 2H). ^{13}C NMR (125 MHz, CD$_3$OD): δ 170.15, 163.05 (q, J = 37.0 Hz), 159.37, 146.68, 137.65, 130.19, 129.93, 129.79, 128.21, 119.85, 118.26 (q, J = 290.9 Hz), 118.12, 59.02, 54.58, 46.61, 44.72, 43.00, 31.39, 30.62. MS (ESI) m/z 409 (M$^+$ + 1).

2-Guanidino-N-((1-(naphthalen-1-ylmethyl)-4-(phenylamino)-piperidin-4-yl)methyl)acetamide Tritriflate (49d). Prepared according to general procedure 5, method B, from **48d** (0.09 g, 0.139 mmol) to afford 0.07 g (65%) of the title material as a white solid. ^{1}H NMR (500 MHz, CD$_3$OD): δ 8.15 (d, J = 8.3 Hz, 1H), 7.92 (d, J = 8.2 Hz, 1H), 7.87 (d, J = 8.0 Hz, 1H), 7.66 (d, J = 6.8 Hz, 1H), 7.57–7.46 (m, 3H), 7.09–7.06 (m, 2H), 6.80–6.65 (m, 3H), 4.71 (s, 2H), 3.84 (s, 2H), 3.40–3.25 (m, 6H), 2.17–1.92 (m, 4H). ^{13}C NMR (125 MHz, CD$_3$OD): δ 170.05, 163.07 (q, J = 34.4 Hz), 159.33, 146.63, 135.33, 133.46, 132.45, 132.13, 130.16, 130.07, 128.50, 127.53, 126.32, 124.23, 120.36, 119.83, 118.15 (q, J = 291.0 Hz), 118.09, 57.85, 54.50, 49.56, 46.47, 44.66, 31.17. MS (ESI) m/z 445 (M$^+$ + 1).

2-Guanidino-N-((1-(naphthalen-2-ylmethyl)-4-(phenylamino)-piperidin-4-yl)methyl)acetamide Tritriflate (49e). Prepared according to general procedure 5, method B, from **48e** (0.1 g, 0.15 mmol) to afford 0.08 g (66%) of the title material as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.44 (s, 1H), 8.19 (s, 1H), 8.07 (s, 1H), 7.97–7.78 (m, 4H), 7.66–7.17 (m, 8H), 7.08–7.05 (t, J = 7.4 Hz, 2H), 6.80 (d, J = 8.0 Hz, 2H), 6.61 (t, J = 7.0 Hz, 1H), 5.32 (br s, 1H), 4.46 (s, 2H), 3.84–3.83 (m, 2H), 3.58–3.16 (m, 6H), 2.14–2.11 (m, 2H), 1.98–1.92 (m, 2H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 167.86, 158.65 (q, J = 31.6 Hz), 157.69, 145.90, 133.07, 132.53, 131.11, 128.81, 128.37, 128.08, 127.99, 127.71, 127.42, 127.07, 126.72, 117.32, 117.13 (q, J = 295.4 Hz), 116.19, 59.02, 53.19, 47.28, 44.74, 43.38, 29.21. MS (ESI) m/z 445 (M$^+$ + 1).

N-((1-(4-Bromobenzyl)-4-(phenylamino)piperidin-4-yl)methyl)-2-guanidinoacetamide Tritriflate (49f). Prepared according to general procedure 5, method B, from **48f** (0.1 g, 0.148 mmol) to afford 0.066 g (54%) of the title material as a white solid. ^{1}H NMR (500 MHz, CD$_3$OD): δ 7.50 (d, J = 8.1 Hz, 2H), 7.32 (d, J = 8.1 Hz, 2H), 7.10 (t, J = 7.4 Hz, 2H), 6.81 (d, J = 8.1 Hz, 2H), 6.68 (t, J = 7.1 Hz, 1H), 3.92 (s, 2H), 3.84 (s, 2H), 3.51 (s, 2H), 2.89–2.81 (m, 4H), 2.11–2.09 (m, 2H), 1.86–1.84 (m, 2H). ^{13}C NMR (125 MHz, CD$_3$OD): δ 170.00, 163.18 (q, J = 35.8 Hz), 159.33, 146.87, 134.00, 133.36, 132.83, 130.03, 123.64, 119.60, 118.26, 118.09 (q, J = 290.7 Hz), 61.81, 54.99, 49.46, 46.62, 44.78, 32.36. MS (ESI) m/z 473 (M$^+$ + 1) for ^{79}Br, 475 (M$^+$ + 1) for ^{81}Br.

Methyl 4-((4-((2-Guanidinoacetamido)methyl)-4-(phenylamino)-piperidin-1-yl)methyl)benzoate Tritriflate (49g). Prepared according to general procedure 5, method B, from **48g** (0.13 g, 0.199 mmol) to afford 0.09 g (56%) of the title material as a white solid. ^{1}H NMR (500 MHz, CD$_3$OD): δ 8.10 (d, J = 8.0 Hz, 2H), 7.67 (d, J = 8.1 Hz, 2H), 7.16 (t, J = 7.8 Hz, 2H), 6.87 (d, J = 7.8 Hz, 2H), 6.76 (s, 1H), 4.42 (s, 2H), 3.94 (s, 5H), 3.54 (s, 2H), 3.36–3.33 (m, 4H), 2.30–2.06 (m, 4H). ^{13}C NMR (125 MHz, CD$_3$OD): δ 170.14, 167.70, 163.09 (q, J = 35.6 Hz), 159.38, 146.64, 135.40, 132.89, 132.58, 131.16, 130.18, 119.90, 118.29 (q, J = 288.8 Hz), 118.15, 60.77, 54.61, 52.89, 49.34, 46.61, 44.72, 31.22. MS (ESI) m/z 453 (M$^+$ + 1).

1-Benzyl-4-(naphthalen-1-ylmethyl)piperidine-4-carbonitrile (50a). Prepared according to general procedure 6 using 1-benzylpiperidine-4-carbonitrile (**35**, 0.9 g, 4.49 mmol) and 1-(chloromethyl)naphthalene (1 g, 5.84 mmol) to afford 1.4 g (91%) of the title material as a white solid. ^{1}H NMR (CDCl$_3$): δ 8.11 (d, J = 8.2 Hz, 1H), 7.89 (d, J = 7.8 Hz, 1H), 7.83 (d, J = 8.1 Hz, 1H), 7.58–7.47 (m, 4H), 7.36–7.27 (m, 5H), 3.53 (s, 2H), 3.40 (s, 2H), 2.87 (d, J = 12.1 Hz, 2H), 2.32 (t, J = 10.0 Hz, 2H), 1.88–1.78 (m, 4H). MS (ESI) m/z 341 (M$^+$ + 1).

1-Benzyl-4-(3,4-dichlorobenzyl)piperidine-4-carbonitrile (50b). Prepared according to general procedure 6 using 1-benzylpiperidine-4-carbonitrile (**35**, 0.9 g, 4.49 mmol) and 3,4-dichlorobenzyl bromide (1.4 g, 5.84 mmol) to afford 0.98 g (61%) of the title material as a white solid. ^{1}H NMR (DMSO-d_6): δ 7.59 (d, J = 8.2 Hz, 1H), 7.55 (s, 1H), 7.32–7.23 (m, 6H), 3.46 (s, 2H), 2.91 (s, 2H), 2.78 (d, J = 11.9 Hz, 2H), 2.07 (t, J = 11.0 Hz, 2H), 1.72–1.61 (m, 4H). MS (ESI) m/z 359 [M + H]$^+$.

1-Benzyl-4-(naphthalen-2-ylmethyl)piperidine-4-carbonitrile (50c). Prepared according to general procedure 6 using 1-benzylpiperidine-4-carbonitrile (**35**, 0.7 g, 3.49 mmol) and 2-(bromomethyl)naphthalene (1 g, 4.54 mmol) to afford 0.85 g (71%) of the title material as a white solid. ^{1}H NMR (CDCl$_3$): δ 7.86–7.83 (m, 3H), 7.75 (s, 1H), 7.53–7.43 (m, 3H), 7.34–7.27 (m, 5H), 3.54 (s, 2H), 3.04 (s, 2H), 2.89 (d, J = 12.3 Hz, 2H), 2.33 (t, J = 12.2 Hz, 2H), 1.90 (d, J = 12.4 Hz, 2H), 1.74 (td, J = 12.5, 2.9 Hz, 2H). MS (ESI) m/z 341 [M + H]$^+$.

1-Benzyl-4-(biphenyl-4-ylmethyl)piperidine-4-carbonitrile (50d). Prepared according to general procedure 6 using 1-benzylpiperidine-4-carbonitrile (**35**, 0.7 g, 3.49 mmol) and 4-(bromomethyl)biphenyl (1.12 g, 4.54 mmol) to afford 0.76 g (59%) of the title material as a white solid. ^{1}H NMR (500 MHz, CDCl$_3$): δ 7.59–7.55 (m, 4H), 7.44 (t, J = 7.5 Hz, 2H), 7.35–7.26 (m, 8H), 3.53 (s, 2H), 2.89–2.86 (m, 4H), 2.31 (d, J = 10.6 Hz, 2H), 1.88 (d, J = 12.4 Hz, 2H), 1.71–1.66 (m, 2H). MS (ESI) m/z 367 (M$^+$ + 1).

(1-Benzyl-4-(naphthalen-1-ylmethyl)piperidin-4-yl)methanamine (51a). Prepared according to general procedure 2, method B, from **50a** (1.3 g, 3.81 mmol) at 2.38 atm for 15 h. The catalyst was removed by filtration, and the solvent was evaporated *in vacuo*. The residue was purified by chromatography on a silica gel column using a gradient of methylene chloride and methanol (10:0 → 8:2) as the eluent to give 0.49 g (38%) of the title material as a white solid. ^{1}H NMR (CDCl$_3$): δ 8.29 (d, J = 8.2 Hz, 1H), 7.88 (d, J = 8.1 Hz, 1H), 7.77 (d, J = 8.0 Hz, 1H), 7.55–7.43 (m, 3H), 7.38–7.27 (m, 6H), 3.52 (s, 2H), 3.17 (s, 2H), 2.72 (br s, 2H), 2.65–2.62 (m, 2H), 2.30 (t, J = 9.8 Hz, 2H), 1.70–1.64 (m, 4H), 1.57–1.54 (m, 2H). MS (ESI) m/z 345 (M$^+$ + 1).

(1-Benzyl-4-(3,4-dichlorobenzyl)piperidin-4-yl)methanamine (51b). Prepared according to general procedure 2, method B, from **50b** (0.9 g, 2.5 mmol) to afford 0.85 g (93%) of the title material as a white solid. ^{1}H NMR (DMSO-d_6): δ 7.49–7.43 (m, 2H), 7.29–7.15 (m, 6H), 3.43 (s, 2H), 3.17 (s, 2H), 2.60–2.26 (m, 8H), 1.31 (s, 4H). MS (ESI) m/z 363 (M$^+$ + 1), 365 (M$^+$ + 1).

(1-Benzyl-4-(naphthalen-2-ylmethyl)piperidin-4-yl)methanamine (51c). Prepared according to general procedure 2, method B, from **50c** (0.8 g, 2.35 mmol) to afford 0.62 g (77%) of the title material as a colorless oil. ^{1}H NMR (CDCl$_3$): δ 7.84–7.76 (m, 3H), 7.62 (s, 1H), 7.50–7.44 (m, 2H), 7.37–7.27 (m, 6H), 3.57 (s, 2H), 2.85 (s, 2H), 2.63–2.58 (m, 4H), 2.48–2.46 (m, 2H), 1.63–1.50 (m, 4H), 1.21 (br s, 2H). MS (ESI) m/z 345 [M + H]$^+$.

(1-Benzyl-4-(biphenyl-4-ylmethyl)piperidin-4-yl)methanamine (51d). Prepared according to general procedure 2, method B, from **50d** (0.69 g, 1.88 mmol) to afford 0.67 g (96%) of the title material as a colorless oil. ^{1}H NMR (CDCl$_3$): δ 7.60 (d, J = 7.5 Hz, 2H), 7.52 (d, J = 8.0 Hz, 2H), 7.45 (t, J = 7.4 Hz, 2H), 7.35–7.32 (m, 4H), 7.29–7.23 (m, 3H), 3.56 (s, 2H), 2.72 (s, 2H), 2.60–2.57 (m, 4H), 2.47–2.46 (m, 2H), 1.59–1.52 (m, 4H), 1.32 (br s, 2H). MS (ESI) m/z 371 (M$^+$ + 1).

tert-Butyl 1-(1-Benzyl-4-(naphthalen-1-ylmethyl)piperidin-4-yl)-10,10-dimethyl-3,8-dioxo-9-oxa-2,5,7-triazaundecan-6-ylidenecarbamate (52a). Prepared according to general procedure 4, method C, from **51a** (0.35 g, 1.02 mmol). The residue was purified by chromatography on a silica gel column using a gradient of methylene chloride and methanol (10:0 → 97:3) to give 0.27 g (41%) of the title material as a white solid. ^{1}H NMR (CDCl$_3$): δ 11.45 (s, 1H), 8.87 (s, 1H), 8.07 (d, J = 8.0 Hz, 1H), 7.83 (d, J = 7.4 Hz, 1H), 7.73 (d, J = 8.1 Hz, 1H), 7.49–7.42 (m, 2H), 7.39 (t, J = 7.2 Hz, 1H), 7.27–7.22 (m, 6H), 6.91–6.89 (m, 1H), 3.98 (d, J = 5.4 Hz, 2H), 3.48 (s, 2H), 3.35 (d, J = 5.6 Hz, 2H), 3.09 (s, 2H), 2.62–2.59 (m, 2H), 2.31 (t, J = 10.3 Hz, 2H), 1.70–1.65 (m, 2H), 1.51–1.47 (m, 11H), 1.40 (s, 9H). MS (ESI) m/z 644 (M$^+$ + 1).

(tert-Butyl 1-(1-Benzyl-4-(3,4-dichlorobenzyl)piperidin-4-yl)-10,10-dimethyl-3,8-dioxo-9-oxa-2,5,7-triazaundecan-6-ylidenecarbamate (52b). Prepared according to general procedure 4, method C,

from **51b** (0.37 g, 1.02 mmol) to afford 0.33 g (49%) of the title material as a white solid. ^{1}H NMR (CDCl$_3$): δ 11.42 (s, 1H), 8.92 (s, 1H), 7.33−7.19 (m, 8H), 6.94 (d, J = 8.1 Hz, 1H), 4.05 (d, J = 5.8 Hz, 2H), 3.50 (s, 2H), 3.15 (d, J = 5.4 Hz, 2H), 2.57−2.52 (m, 4H), 2.39−2.37 (m, 2H), 1.50−1.40 (m, 22H). MS (ESI) m/z 662 (M$^+$ + 1), 664 (M$^+$ + 1).

tert-Butyl 1-(1-Benzyl-4-(naphthalen-2-ylmethyl)piperidin-4-yl)-10,10-dimethyl-3,8-dioxo-9-oxa-2,5,7-triazaundecan-6-ylidenecarbamate (52c). Prepared according to general procedure 4, method C, from **51c** (0.35 g, 1.02 mmol) to afford 0.38 g (72%) of the title material as a white solid. ^{1}H NMR (CDCl$_3$): δ 11.47 (s, 1H), 8.97−8.95 (m, 1H), 7.80−7.72 (m, 3H), 7.53 (s, 1H), 7.46−7.42 (m, 2H), 7.31−7.19 (m, 7H), 4.08 (d, J = 5.6 Hz, 2H), 3.52 (s, 2H), 3.21 (d, J = 5.6 Hz, 2H), 2.94 (s, 1H), 2.88 (s, 1H), 2.78 (s, 2H), 2.60−2.58 (m, 2H), 2.43−2.40 (m, 2H), 1.63−1.56 (m, 2H), 1.51 (s, 9H), 1.40 (s, 9H). MS (ESI) m/z 644 (M$^+$ + 1).

(tert-Butyl 1-(1-Benzyl-4-(biphenyl-4-ylmethyl)piperidin-4-yl)-10,10-dimethyl-3,8-dioxo-9-oxa-2,5,7-triazaundecan-6-ylidenecarbamate (52d). Prepared according to general procedure 4, method D, from **51d** (0.413 g, 1.11 mmol) to afford 0.223 g (80%) of the title material as a white solid. ^{1}H NMR (500 MHz, CDCl$_3$): δ 11.47 (s, 1H), 8.95 (s, 1H), 7.59 (d, J = 7.3 Hz, 2H), 7.51 (d, J = 8.0 Hz, 2H), 7.44 (t, J = 7.5 Hz, 2H), 7.36−7.31 (m, 5H), 7.28−7.25 (m, 1H), 7.18 (d, J = 8.0 Hz, 2H), 7.12 (br s, 1H), 4.09 (d, J = 6.0 Hz, 2H), 3.57 (s, 2H), 3.22 (d, J = 5.7 Hz, 2H), 2.68 (s, 2H), 2.61 (s, 2H), 2.46 (s, 2H), 1.64−1.60 (m, 4H), 1.52 (s, 9H), 1.44 (s, 9H). MS (ESI) m/z 670 (M$^+$ + 1).

N-((1-Benzyl-4-(naphthalen-1-ylmethyl)piperidin-4-yl)methyl)-2-guanidinoacetamide Tritriflate (53a). Prepared according to general procedure 5, method B, from **52a** (0.12 g, 0.189 mmol). The residue was purified by chromatography on a silica gel column using column using a gradient of methylene chloride and methanol (95:5 → 8:2) as the eluent to give 0.07 g (54%) of the title material as a white solid. ^{1}H NMR (500 MHz, CD$_3$OD): δ 8.03 (d, J = 8.3 Hz, 1H), 7.81 (d, J = 8.0 Hz, 1H), 7.74 (t, J = 8.1 Hz, 1H), 7.48−7.27 (m, 9H), 4.27−4.13 (m, 2H), 4.08−3.84 (m, 2H), 3.59 (s, 2H), 3.35−3.02 (m, 6H), 1.88−1.61 (m, 4H). ^{13}C NMR (125 MHz, CD$_3$OD): δ 170.65, 163.05 (q, J = 34.2 Hz), 159.53, 135.52, 134.74, 134.49, 133.79, 132.13, 131.09, 131.00, 130.21, 129.86, 128.69, 127.06, 126.57, 126.15, 125.34, 118.24 (q, J = 292.9 Hz), 61.40, 49.47, 44.84, 44.13, 40.42, 38.22, 29.86. MS (ESI) m/z 444 (M$^+$ + 1).

N-((1-Benzyl-4-(3,4-dichlorobenzyl)piperidin-4-yl)methyl)-2-guanidinoacetamide Ditriflate (53b). Prepared according to general procedure 5, method B, from **52b** (0.1 g, 0.151 mmol) to afford 0.07 g (72%) of the title material as a white solid. ^{1}H NMR (500 MHz, CD$_3$OD): δ 7.57−7.41 (m, 7H), 7.22−7.13 (m, 1H), 4.39−4.29 (m, 2H), 4.07−4.00 (m, 2H), 3.46−3.14 (m, 6H), 2.91−2.67 (m, 2H), 1.88−1.85 (m, 2H), 1.69−1.68 (m, 2H). ^{13}C NMR (125 MHz, 45 °C, CD$_3$OD): δ 170.49, 163.06 (q, J = 34.4 Hz), 159.56, 138.37, 133.71, 133.10, 132.18, 131.82, 131.75, 131.24, 131.08, 130.33, 130.23, 118.56 (q, J = 280.0 Hz), 61.43, 49.45, 44.90, 44.14, 42.65, 36.89, 29.81. MS (ESI) m/z 462 (M$^+$ + 1) 464 (M$^+$ + 1).

N-((1-Benzyl-4-(naphthalen-2-ylmethyl)piperidin-4-yl)methyl)-2-guanidinoacetamide Ditriflate (53c). Prepared according to general procedure 5, method B, from **52c** (0.13 g, 0.202 mmol) to afford 0.10 g (75%) of the title material as a white solid. ^{1}H NMR (500 MHz, CD$_3$OD): δ 7.85−7.69 (m, 4H), 7.52−7.32 (m, 8H), 4.42−4.26 (m, 2H), 4.11−3.99 (m, 2H), 3.52−3.27 (m, 6H), 3.09−2.86 (m, 2H), 1.85−1.76 (m, 4H). MS (ESI) m/z 444 (M$^+$ + 1).

N-((1-Benzyl-4-(biphenyl-4-ylmethyl)piperidin-4-yl)methyl)-2-guanidinoacetamide Ditriflate (53d). Prepared according to general procedure 5, method B, from **52d** (0.1 g, 0.149 mmol) to afford 0.08 g (76%) of the title material as a white solid. ^{1}H NMR (500 MHz, CD$_3$OD): δ 7.62−7.57 (m, 4H), 7.51−7.43 (m, 6H), 7.36−7.34 (m, 2H), 7.28−7.27 (m, 2H), 4.40−4.29 (m, 2H), 4.09−4.00 (m, 2H), 3.50−3.20 (m, 6H), 2.96−2.73 (m, 2H), 1.78−1.70 (m, 4H). ^{13}C NMR (125 MHz, 45 °C, CD$_3$OD): δ 170.41, 163.02 (q, J = 32.4 Hz), 159.53, 141.89, 140.86, 136.46, 132.34, 132.16, 131.05, 130.32, 130.20, 129.78, 128.24, 127.75, 127.74, 118.56 (q, J = 290.0 Hz), 61.38, 49.45, 44.92, 44.91, 42.81, 36.91, 29.93. MS (ESI) m/z 470 (M$^+$ + 1).

Biology. NPFF1 and NPFF2 Receptor Binding Studies. Compounds **7a−d**, **9a−c**, and **15a,b** were tested against [^{125}I]YVP and [^{125}I]EYF for NPFF1 and NPFF2 affinity, respectively, as described in Mollereau et al.[21] The affinities of **17a−g**, **21**, **30a−d**, **34**, **42−43**, **46**, **49a−g**, and **53a−c** on NPFF1 and NPFF2 receptors were evaluated by competition experiments using the selective NPFF1 and NPFF2 radioligands [^{3}H]-NPVF and [^{3}H]-EYF, respectively, in membranes of CHO cells stably expressing each receptor. For membrane preparation, CHO cells expressing human NPFF receptors were harvested in phosphate buffer saline (PBS), frozen at least for 1 h at −70 °C, and then homogenized in 50 mM Tris-HCl, pH 7.4, in a Potter Elvehjem tissue grinder. The nuclear pellet was discarded by centrifugation at 1000g for 15 min at 4 °C, and the membrane fraction was collected upon centrifugation of the supernatant at 100000g for 30 min at 4 °C. Membranes were aliquoted and stored at −80 °C in Tris 50 mM, pH 7.4, and the protein concentration was determined by the Lowry method. [^{3}H]-NPVF and [^{3}H]-EYF were custom-made by RC TRITEC AG (Teufen, Switzerland) by hydrogenation of the unsaturated peptide precursors with 99% tritium gas. Tritiated products were purified (>98%) by HPLC and dissolved in ethanol to obtain 1 mCi/mL (37 MBq/mL). Binding of [^{3}H]-NPVF and [^{3}H]-EYF was measured by rapid filtration. Membranes (5−15 μg of protein) were incubated in polypropylene tubes in a final volume of 500 μL containing 50 mM Tris-HCl, pH 7.4, 0.1% bovine serum albumin, 60 mM NaCl (for NPFF2 receptors only), the radioligand at 0.5−1 nM, and compounds to be tested at the desired concentration. The nonspecific binding was determined in the presence of 1 μM YVPNLPQRFa (for hNPFF1 receptor) and 1 μM EYWSLAAPQRFa (for hNPFF2 receptor). After 1 h incubation at 25 °C, samples were rapidly filtered on Whatman GF/B filters preincubated in 0.3% polyethylenimine. The filters were rinsed three times with 4 mL of ice cold buffer containing 0.1% bovine serum albumin, and the bound radioactivity was counted in a liquid scintillation spectrophotometric counter (50% efficiency, Packard).

cAMP Assay at NPFF1 and NPFF2 Receptors. Recombinant cells (200000) were seeded in glass tubes and incubated overnight as usual. The culture medium was then removed and replaced by fresh medium (200 μL) containing 0.1 μM adenine and 0.6 μCi of [^{3}H]adenine (26 Ci/mmole, Amersham, France). After 1 h incubation at 37 °C in the incubator under 5% CO$_2$ atmosphere, cells were rinsed two times with 400 μL of HEPES-buffered Krebs−Ringer saline (KRH, 124 mM NaCl, 5 mM KCl, 1.25 mM MgSO$_4$, 1.5 mM CaCl$_2$, 1.25 mM KH$_2$PO$_4$, 25 mM HEPES, 8 mM glucose, 0.5 mg/mL bovine serum albumin; pH 7.4). Prewarmed KRH (150 μL) was added to each tube, and the reaction was initiated by the addition of 50 μL of KRH containing 20 μM forskolin (Sigma, France), 0.4 mM IBMX (3-isobutyl-1-methylxanthine; Sigma, France), 0.4 mM Ro-20 1724 (4-(3-butoxy-4methoxyphenyl)methyl-2-imidazolidone; Fisher, France), and the ligand to be tested. To evaluate the antagonist activity, the compound was tested in the presence of the NPFF agonists NPVF and 1DMe for hNPFF-1 and hNPFF-2 receptors, respectively. After 10 min at 37 °C, the reaction was stopped by addition of 20 μL of HCl (2.2 N) with rapid mixing. The [^{3}H]cAMP content of each tube was isolated by chromatographic procedures on acid alumina columns as described by Alvarez and Daniels[34] and counted in a liquid scintillation analyzer (Packard).

[^{35}S]GTPγS Binding Assay at NPFF1 and NPFF2 Receptors. The assay buffer consisted of 20 mM HEPES, pH 7.4, 20 mM NaCl, 3 mM MgCl$_2$, and 0.1% BSA. Aliquots (50 μL, equivalent to 1.5 μg and to 0.7 μg protein, for NPFF$_1$ and NPFF$_2$, respectively) were incubated in polypropylene tubes at 30 °C for 60 min in 500 μL of buffer with 5 μg of saponin, 0.1 μM (NPFF1) or 1 μM (NPFF2) GDP, the ligand to be tested, and 0.066 nM [^{35}S]GTPγS. Reaction was stopped by rapid vacuum filtration through GF/B Whatman glass fiber filters, preincubated in the buffer at room temperature for 1 h, and washed three times with 4 mL of ice-cold buffer. Membrane-bound radioactivity retained on the filters was determined by liquid scintillation spectrophotometry (94% efficiency, Packard counter) after overnight extraction of the filters in 4 mL of Ready Protein scintillation fluid (Beckman).

Opioid Receptor (μ, δ, κ) Binding Studies. *Reagents.* Buffer reagents were purchased from Sigma-Aldrich (St. Louis, MO). All radioligands and MicroScint were purchased from PerkinElmer (Waltham, MA). Nonlabeled controls were purchased from Tocris Bioscience (Minneapolis, MN). Membrane preparation and all assay dilutions were made using a Tris-HCl buffer (50 mM Tris-HCl), pH 7.4.

Cell Culture. HEK293 cells stably transfected with human opioid receptor subtypes μ, δ, or κ were used to perform the opioid receptor binding assays. These cells were maintained at 37 °C and 5% CO_2 in a DMEM nutrient mixture supplemented with 2 mM L-glutamine, 10% fetal bovine serum, 1% penicillin—streptomycin, and G418 antibiotic solutions. When the cells reached 90% confluency, the media was removed, and the cells were washed with cold PBS, pH 7.4. The cells were lysed and scraped in cold Tris-HCl, pH 7.4, and then centrifuged at 5200g for 10 min at 4 °C. The supernatant was discarded ,and the pellet was resuspended in the same buffer and homogenized via Sonic Dismembrator model 100 (Fisher Scientific, Pittsburgh, PA) for 30 s and then centrifuged at 1000g for 10 min at 4 °C. The supernatant was saved, and the pellet underwent the suspension and homogenization process two more times with the same conditions. The supernatants were combined and centrifuged at 23300g for 40 min at 4 °C. The pellet was resuspended in cold Tris-HCl buffer, aliquoted into 2 mL vials, and stored at −80 °C. The total protein concentration was determined using a Pierce BCA protein assay kit (Thermo Scientific, Rockford, IL) using manufacturer's instructions.

Radioligand Binding Assays. For each assay, nonspecific binding was determined using 10 μM of a positive control [U-69,593 (κ), DPDPE (δ), or DAMGO (μ)], and total binding was ascertained with 0.1% DMSO in Tris-HCl buffer. Each test well contained 50 μL of its respective radioligand (U-69,593,[phenyl-3,4-^{3}H] (κ), DAMGO, [tyrosyl-3,5-^{3}H(N)] (μ) or enkephalin(DPDPE),[tyrosyl-3,5-^{3}H(N)] (δ)), 50 μL of compound, control, or vehicle, and 100 μL of cell membrane. The assays were incubated for 60 min at room temperature. The reaction was terminated via rapid filtration with cold Tris-HCl buffer through a UniFilter GF/B 96-well plate presoaked with 0.3% BSA. When the filters were dry, 50 μL of MicroScint-20 was applied to each filter, and the plates were read on a TopCount NXT HTS microplate scintillation counter (PerkinElmer, Waltham, MA) where the counts per minute (CPM) were recorded.

The K_d for ligands for each receptor was established through a membrane evaluation and saturation binding experiment. For the membrane evaluation experiment, 1−15 μg of membrane was incubated with 0.6 nM of its respective radioligand. Total, specific, and nonspecific binding were used to calculate the % binding of the nonlabeled control to receptor. The membrane concentration exhibiting good % binding (>90%), and total binding with high signal (thousands of CPM) was used as the optimal membrane concentration for the assay. For the saturation assay, the optimal membrane concentration and 0.25−10 nM of its respective radioligand were incubated with 10 μM of a nonlabeled positive control or 0.1% DMSO in buffer. Data was analyzed by a nonlinear curve fit model using GraphPad Prizm 5.04 software (GraphPad, La Jolla, CA), and the K_d value was calculated.

The competitive binding assay was performed used the optimal concentration of membrane with a radioligand concentration $\leq K_d$ and 12 concentrations of each compound ranging from 0.00001 to 1000 μM. Each compound was tested in triplicate. The assays were performed as stated above. The K_i values were calculated by a nonlinear curve fit model using GraphPad Prizm 5.0 software.

In Vivo Characterization. *Animals.* Adult (8−11 weeks old) male C57BL/6J were obtained from the Jackson Laboratory, Bar Harbor, Maine, USA. All mice were housed four to a cage in a temperature- and humidity-controlled room at the Torrey Pines Institute for Molecular Studies (Port Saint Lucie, Florida, USA) vivarium on a 12:12-h light/dark cycle (lights off at 19:00 h) with free access to food and water except during experimental sessions. All procedures were preapproved and carried out in accordance with the Institutional Animal Care and Use Committees at the Torrey Pines Institute for Molecular Studies as specified by the 2008 National Institutes of

Health Guide for the Care and Use of Laboratory Animals. Consistent with these guidelines, ongoing statistical testing of data collected was used to minimize the number of animals used, within the constraints of necessary statistical power.

Injection Techniques. Intracerebroventricular (icv) injections were made directly into the lateral ventricle according to the modified method of Haley and McCormick.[35] The volume of all icv injections was 5 μL, using a 10-μL Hamilton microliter syringe. The mouse was lightly anesthetized with isoflurane, an incision was made in the scalp, and the injection was made 2 mm lateral and 2 mm caudal to bregma at a depth of 3 mm.

Hyperalgesic Testing: The 48 °C Warm-Water Tail-With-drawal Assay. The nociceptive stimulus was 48 °C water, with the latency to withdraw the tail taken as the end point.[36,37] The water temperature of 48 °C was selected for this work to ensure a moderate tail-withdrawal response, with a measurable decrease in withdrawal time possible but also a significant temperature for hyperalgesic testing. After determining control latencies, mice received a single icv dose of vehicle (50% DMSO) or novel ligand pretreatment as discussed above. The dose of RF9 used in this study (10 nmol, icv) was selected because it was previously reported effective in antagonizing the hyperalgesic response of NPFF.[38] The dose of compound **46** (30 nmol, icv) was selected on the basis of similar affinity for NPFF1-R as RF9 (75 nM vs the reported 58 nM).[15] Animals showing an initial baseline latency fewer than 4 s or more than 15 s were removed from the study; two mice were so excluded. Mice administered the test compound were tested for analgesia every 10 min for 80 min postinjection. If the mouse failed to display a tail-flick in 30 s, the tail was removed from the water to minimize tissue damage. Experimentally induced increases in tail-withdrawal latencies provide a measure of antinociceptive effect, whereas decreases in tail-withdrawal latency indicate hyperalgesic effects.[39−41]

Data Analysis. All tail-withdrawal latency data for hyperalgesic testing are reported as percent baseline response to control for each animal's baseline latency, ±SEM. Percent baseline response is calculated by the following equation: % baseline response = 100(test latency − baseline latency). Responses to treatment over time were analyzed by one- or two-way ANOVA followed by Tukey's *post hoc* test as appropriate, with factors of treatment and time as required.

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Notes
The authors declare no competing financial interest.

■ ABBREVIATIONS

NPFF, neuropeptide FF; 1DMe, (D.YL(N-Me)FQPQRFa); RF9, n-adamantanecarbonyl-Arg-Phe-NH$_2$; AC-099, 1-(3-bromo-4,5-dimethoxybenzylideneamino)guanidine hydrochlor-

ide; EDCI, *N*-(3-(dimethylamino)propyl)-*N*′-ethylcarbodiimide hydrochloride; HOBt, 1-hydroxybenzotriazole hydrate; [^{35}S]GTPγS, guanosine-5′-*O*-(3-[^{35}S]thio)triphosphate; cAMP, cyclic adenosine monophosphate; YVP, YVPNLPQRFa; EYF, EYWSLAAPQRFa (EYW-NPSF); NPVF, VPNLPQRFa; CHO, Chinese hamster ovary; ANOVA, analysis of variance; HEK, human embryonic kidney; DAMGO, (D-Ala^2,MePhe^4,Gly-ol^5)enkephalin; DPDPE, [D-Pen^2,D-Pen^5]-enkephalin; U69,593, (5α,7α,8β)-(−)-*N*-methyl-*N*-[7-(1-pyrrolidinyl)-1-oxaspiro[4,5]dec-8-yl]benzeneacetamide; TMSCN, trimethylsilyl cyanide; DMF, dimethylformamide; THF, tetrahydrofuran; NMP, *N*-methyl-2-pyrrolidone; HEPES, 4-(2-hydroxyethyl)-1-piperazine-1-ethanesulfonic acid; DMEM, Dulbecco's Modified Eagle's medium; Boc, *tert*-butyloxycarbonyl; TFA, trifluoroacetic acid; DCM, dichloromethane; LDA, lithium diisopropylamide; icv, intracerebroventricular; it., intrathecal; OIH, opioid-induced hyperalgesia

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