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Dihydropyridine neuropeptide Y Y_1 receptor antagonists 2: bioisosteric urea replacements

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Abstract—Structure–activity studies around the urea linkage in BMS-193885 (**4a**) identified the cyanoguanidine moiety as an effective urea replacement in a series of dihydropyridine NPY Y_1 receptor antagonists. In comparison to urea **4a** ($K_i = 3.3$ nM), cyanoguanidine **20** (BMS-205749) displayed similar binding potency at the Y_1 receptor ($K_i = 5.1$ nM) and full functional antagonism ($K_b = 2.6$ nM) in SK-N-MC cells. Cyanoguanidine **20** also demonstrated improved permeability properties in Caco-2 cells in comparison to urea **4a** (43 vs 19 nm/s).

1. Introduction

Neuropeptide Y (NPY) is a 36-amino acid peptide, which was first isolated in 1982 from porcine brain.[1] The peptide is a member of a larger peptide family which also includes peptide YY (PYY), pancreatic peptide (PP), and a non-mammalian fish pancreatic peptide (PY).[2] NPY is very highly conserved in a wide variety of animal, reptile, and fish species and is found in many central and peripheral sympathetic neurons. NPY is the most abundant peptide observed in mammalian brain and has long been implicated in the regulation of feeding behavior and energy homeostasis.[3] To date, NPY remains the most potent orexigenic agent known.

Currently, five different NPY receptor subtypes have been cloned and characterized (Y_1, Y_2, Y_4, Y_5, and y_6).[4] Although recent evidence suggests the Y_5 receptor is closely associated pharmacologically with the regulation of food intake, it is generally believed that both Y_1 and Y_5 receptors are involved in the feeding response to NPY.[5,6]

Over the past several years, a number of specific and selective, small molecule Y_1 and Y_5 receptor antagonists have been identified and their inhibitory effects on feeding described (Fig. 1).[7] With respect to the involvement of Y_1 receptor antagonists in feeding, BIBP 3226 was the first potent, non-peptidic agent reported with inhibitory activity on feeding, although the effect remains controversial.[8,9] A more potent and less controversial analogue, BIBO 3304, was later reported to attenuate feeding in rats when centrally dosed.[10] More recently, several new Y_1 antagonists (LY357897,[11] J-104870,[12] and J-115814[13]) have appeared which demonstrate inhibitory effects on feeding in rodents and support the involvement of the Y_1 receptor on food intake.

We recently reported structure–activity studies around a series of novel dihydropyridine NPY Y_1 receptor antagonists which ultimately led to the discovery of BMS-193885 (**4a**) (Fig. 1).[14] Urea **4a** is a potent and selective Y_1 receptor antagonist ($K_i = 3.3$ nM) and displays anorectic activity in several animal models of feeding after intraperitoneal (ip) dosing. Although **4a** was found to have satisfactory brain penetrant properties, its bioavailability after oral administration in rats was negligible ($F_{po} = 0.1\%$). Since **4a** displayed low permeability in Caco-2 cells ($P_c = 19$ nm/s) but showed good stability after incubation in rat liver microsomes, it was postulated that its lack of oral exposure was most likely due to poor intestinal absorption. This was further corroborated by ip and intraportal (ipt) dosing

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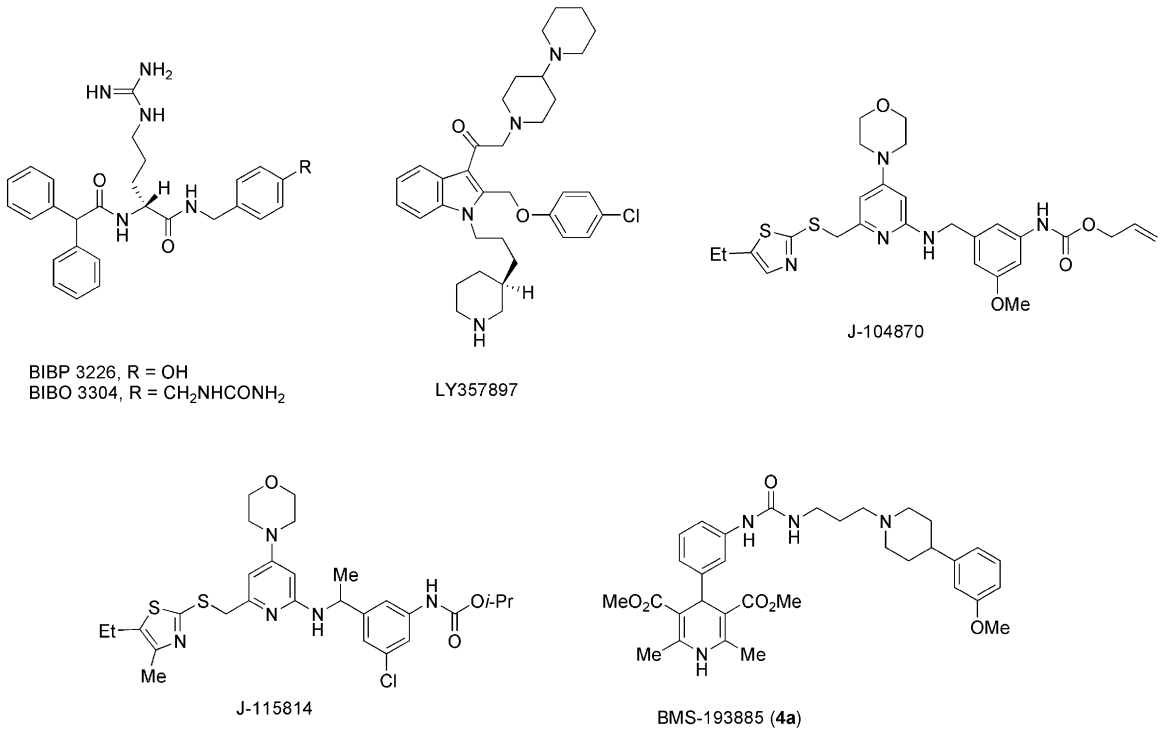
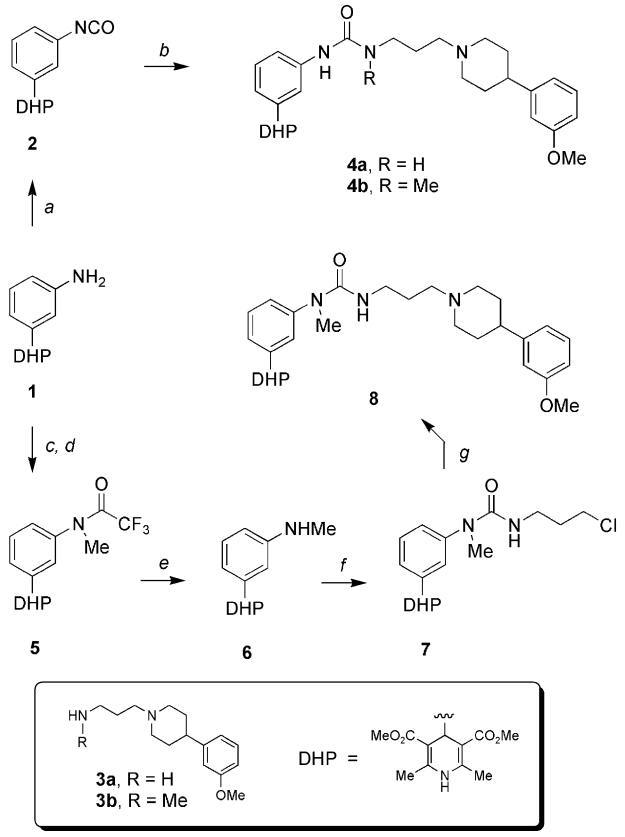


Figure 1. Known NPY Y_1 receptor antagonists.



Scheme 1. Synthesis of ureas **4** and **8**: (a) $COCl_2$, THF (100%); (b) **3a** or **3b**, CH_2Cl_2 (60% for **4a**, 67% for **4b**); (c) $(CF_3CO)_2O$, pyridine, CH_2Cl_2 (74%); (d) NaH, MeI, DMF (90%); (e) NaOH, EtOH (86%); (f) $Cl(CH_2)_3NCO$, CH_2Cl_2 (75%); (g) 4-(3-methoxyphenyl)piperidine, NaI, K_2CO_3, MeCN (46%).

studies in rats which showed **4a** to be completely bioavailable by these routes of administration ($F_{ip} = 100\%$, $F_{ipt} = 100\%$).

The physiochemical profile of **4a** did not adequately explain its poor permeability characteristics. Although the crystalline free base of **4a** is poorly soluble in water ($\sim$2 μg/mL), the L-lactate salt was found to have excellent solubility (3 mg/mL in H_2O). Except for its marginally high molecular weight ($M_r = 591$) and number of rotatable bonds (12), the physicochemical properties of **4a**[15] do not excessively violate the guidelines established by either Lipinski[16] or Veber and Kopple[17] for oral absorption. Structure–activity studies around the chemotype showed both the dihydropyridine and arylpiperidine portions of the molecule were important for activity, suggesting that reducing M_r and/or the number of rotatable bonds would be difficult to achieve. Nevertheless, we felt that improvements could be made on the general chemotype to increase intestinal permeability and thus enhance oral bioavailability. This report will describe some of our efforts to find suitable replacement functionality for the urea linker portion of BMS-193885 (**4a**).

2. Results and discussion

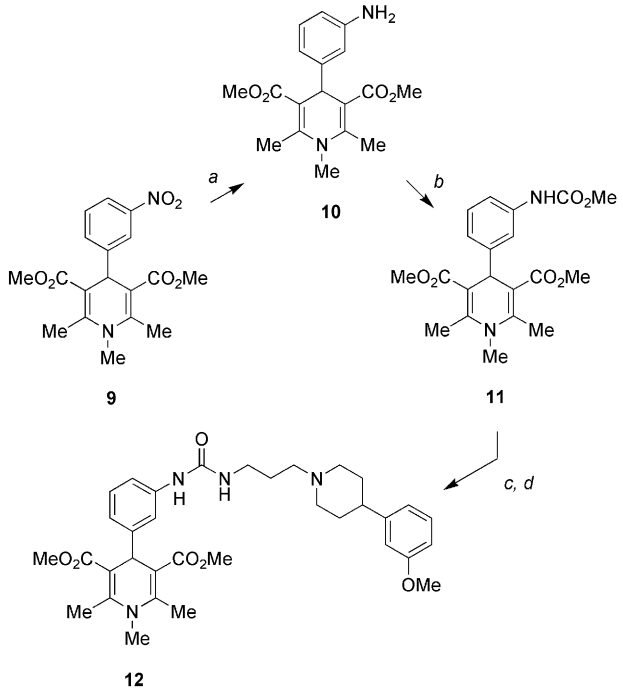
We first examined the influence of the urea NH bonds on both NPY binding affinity and Caco-2 cell permeability. The preparation of urea derivatives **4a**, **4b**, and **8** are shown in Scheme 1. Dihydropyridine aniline **1**[18] was treated with excess phosgene in THF to furnish isocyanate

2. Isocyanate **2** was then condensed in CH_2Cl_2 with either aminopropylpiperidine **3a** to give parent urea **4a** (BMS-193885), or *N*-(methylamino)propylpiperidine **3b** to give the corresponding *N*-methyl derivative **4b**.

The isomeric methylated urea **8** was also prepared from aniline **1** (Scheme 1). Treatment of **1** with trifluoroacetic anhydride and pyridine in CH_2Cl_2 gave the corresponding trifluoroacetamide which was subsequently methylated (NaH/MeI in DMF) to afford *N*-methyl trifluoroacetamide **5**. The amide was hydrolyzed by refluxing in NaOH/EtOH to yield *N*-methylaniline **6**. Condensation of **6** with 3-chloropropyl isocyanate in CH_2Cl_2 gave urea **7** which, after reaction with 4-(3-methoxyphenyl)piperidine in MeCN, furnished the desired *N*-methyl urea **8**.

To complete the methylation studies around the chemotype, *N*-methyl dihydropyridine **12** was also prepared. As outlined in Scheme 2, *N*-methyl dihydropyridine **9**[19] was reduced to aniline **10** by hydrogenolysis using sulfided platinum on carbon, and the resulting aniline was converted to urethane **11** by treatment with $ClCO_2Me$ in pyridine. The urethane was subsequently reacted with *B*-chlorocatecholborane under the conditions described by Alper[20] to give an intermediate isocyanate. The isocyanate was immediately condensed with **3a** to furnish the desired *N*-methyl derivative **12**.

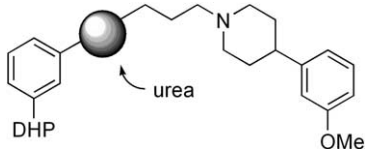
All of the compounds were examined in a competition binding affinity assay using ^{125}I-PYY as the radioligand in SK-N-MC cell membranes which endogenously express the human Y_1 receptor. Additionally, Caco-2 cell permeability coefficients for these compounds were determined to assess the effect of the selective removal of the urea and dihydropyridine NH bonds on intestinal absorption.[21] It is thought that amide and amide-like NH bonds are a major contributor to poor membrane permeability, due to the desolvation energy required for a molecule to move from an extracellular aqueous environment into a hydrophobic cell membrane.[22]

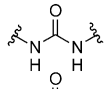
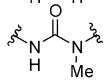
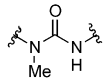
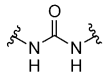


Scheme 2. Synthesis of 1-methyldihydropyridine **12**: (a) H_2, Pt(S)/C, MeOH, $CHCl_3$ (100%); (b) $ClCO_2Me$, pyridine (74%); (c) *B*-chlorocatecholborane, Et_3N, THF (100%); (d) **3a**, CH_2Cl_2 (64%).

The Y_1 binding and Caco-2 cell results for ureas **4a**, **4b**, **8**, and **12** are summarized in Table 1. In comparison to the unsubstituted parent **4a**, methylation of the *distal* NH in **4b** enhanced Caco-2 cell permeability ($P_c = 68$ nm/s) but reduced Y_1 affinity ($K_i = 520$ nM) suggesting the removal of one of the hydrogen bond donors and potential hydration sites had a positive effect on permeability but negative effect on binding. Interestingly, methylation of the *proximal* NH in **8** further enhanced Caco-2 cell permeability ($P_c = 96$ nm/s) but resulted in a derivative devoid of Y_1 activity ($K_i = > 1000$ nM). Although it appears that removal of the *proximal* NH has a profound effect on permeability, it is also clear that this NH is critical for Y_1 receptor recognition. Similarly, the *N*-methyl dihydropyridine derivative **12** was less potent than **4a** in Y_1 binding ($K_i = 540$ nM). However, methylation of the dihydropyridine ring NH had little effect on Caco-2 cell permeability ($P_c = 25$ nm/s) suggesting the urea NH's play a more influential role in solvation and intestinal permeability than the former.[23]

Table 1. NPY Y_1 binding affinities and Caco-2 cell permeabilities for ureas **4**, **8**, and **12**



Compd	Urea	NPY Y_1 (K_i, nM)[a]	Caco-2 cell permeability (P_c, nm/s)[b]
4a		3.3 (±0.17)[c]	19
4b (*proximal* NH)		520	68
8 (*distal* NH)		> 1000	96
12		540	25

[a] ^{125}I-PYY binding in SK-N-MC cell membranes. Unless otherwise indicated, the K_i's were obtained from a single experiment ($n = 1$) with each point being run in duplicate. Standard reference agent BIBN 3226 (Fig. 1) displayed a $K_i = 15$ nM in this assay.

[b] Caco-2 cell permeability's determined in Biocoat monolayers. The P_c's were obtained by averaging the results from two experiments. For comparison purposes, methoxyinulin and propranolol, the negative and positive controls, displayed P_c's of < 10 and > 100 nm/s in this assay, respectively.

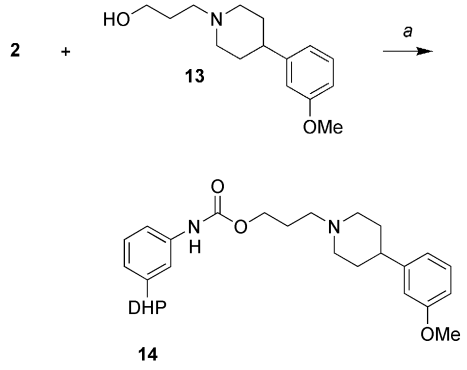
[c] The standard error was generated from three curves, each data point was obtained from the average of six determinations.

Knowing the *proximal* NH was required for potent Y_1 binding affinity, we next prepared a series of urea replacement derivatives (Table 2) which incorporated this key structural element. The synthesis of urethane **14** was effected by the condensation isocyanate **2** with hydroxypropylpiperidine **13** (Scheme 3).

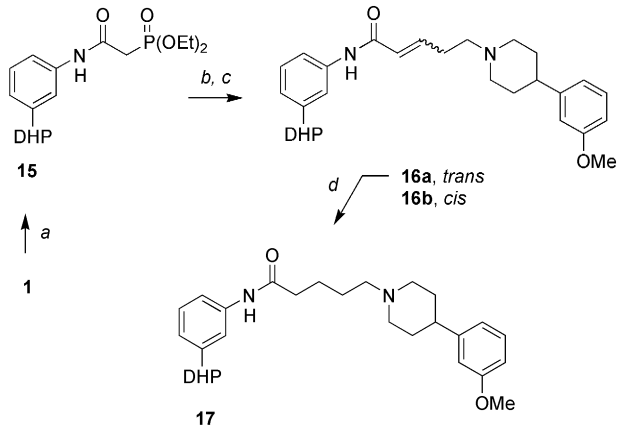
The preparation of amides **16** and **17** are shown in Scheme 4. Aniline **1** was treated with diethyl phosphonoacetic acid, 2-mercaptothiazoline, DCC, and DMAP using the procedure of Nagoa[24] to give the amido phosphonate **15**. After anion formation with NaH in THF, **15** was condensed with a β-amino aldehyde intermediate [prepared from 4-(3-methoxyphenyl)piperidine and acrolein using the conditions of Markó[25]] to furnish a mixture of *cis* and *trans* α,β-unsaturated amides **16a** and **16b**. The saturated amide derivative **17** was obtained by reduction of **16a** with H_2 over Pd/C in MeOH.

Cyanoguanidine **21**, thiourea **22**, and nitroethylene **24** were prepared as shown in Scheme 5. After converting aniline **1** to the corresponding isothiocyanate **18** by treatment with thiocarbonyl diimidazole (TCDI) in DMF, condensation with amine **3a** yielded thiourea **22**. Condensation of **18** with sodium cyanamide afforded the thiolate intermediate **19** which was subsequently reacted with amine **3a** using $HgCl_2$ under the conditions by Tilley[26] to give the corresponding cyanoguanidine **20**. Interestingly, we observed the monohydrochloride salt of **20** slowly decomposed on storage at room temperature. The decomposition product was subsequently identified as the corresponding guanylurea derivative **21**. The monohydrochloride salt of **20** was obviously exposed to small amounts of moisture since hydrolysis of cyanoguanidines to guanylureas has been observed by others in low pH environments.[26,27] We subsequently found that salt formation with less acidic acids (e.g., maleic acid) afforded stable salt forms. Lastly, the nitroethylene derivative **24** was prepared from aniline **1** using the method of Young.[28] Reaction of **1** with bis (thiomethyl)nitroethylene gave intermediate **23** which was subsequently condensed with amine **3a** to afford **24**.

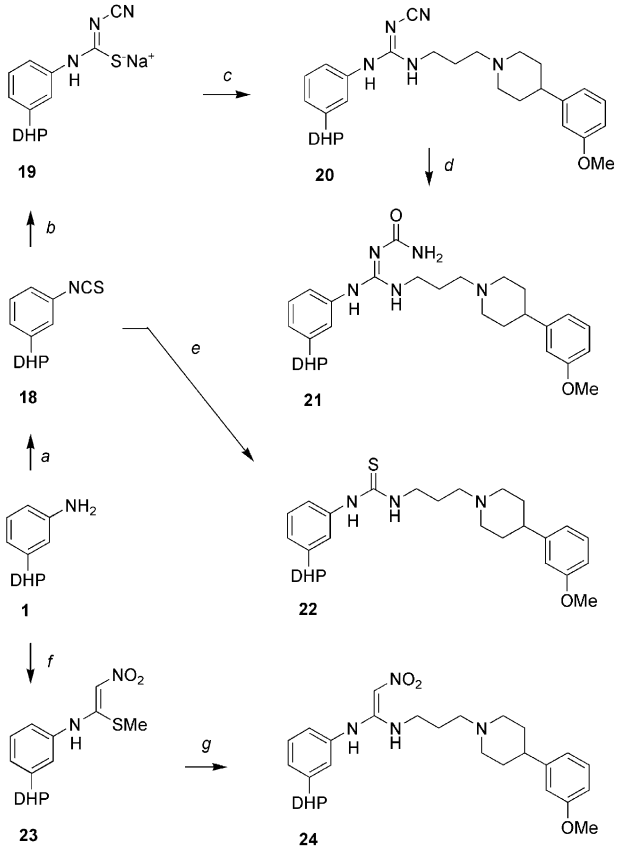
The Y_1 binding results for urea replacements **14**, **16**, **17**, **20**, **21**, **22**, and **24** are reported in Table 2. Urethane **14** (K_i = 480 nM) and the α,β-unsubstituted amide derivatives **16a** and **16b** (K_i's = > 1000 nM) were considerably less potent than urea **4a** suggesting conformational factors and possibly the *distal* urea NH contribute to binding recognition at the Y_1 receptor. Interestingly, the fully reduced amide **17** showed substantial Y_1 binding potency (K_i = 110 nM) in comparison to either urethane **14** or **16a** and **16b**, but was still 30-fold less potent than urea **4a**, again suggesting the importance of the *distal* NH. Whether these results are due to electronic or conformational factors is not known at this time.



Scheme 3. Synthesis of urethane **14**: (a) CH_2Cl_2 (61%).



Scheme 4. Synthesis of amides **16** and **17**: (a) $(EtO)_2POCH_2CO_2H$, DMAP, 2-mercaptothiazoline, DCC, CH_2Cl_2 (80%); (b) NaH, THF; (c) acrolein, DBU, 4-(3-methoxyphenyl)piperidine, THF, (15% for **16a**, 5% for **16b**); (d) H_2, 10% Pd/C, MeOH (60%).



Scheme 5. Synthesis of urea replacements **20**, **21**, **22**, and **24**: (a) TCDI, DMF (75%); (b) NaNHCN, EtOH (100%); (c) **3a**, $HgCl_2$, THF (58%); (d) **20**·HCl, storage at rt; (e) **3a**, $CHCl_3$ (100%); (f) $(MeS)_2CCHNO_2$, MeCN (77%); (g) **3a**, CH_2Cl_2 (66%).

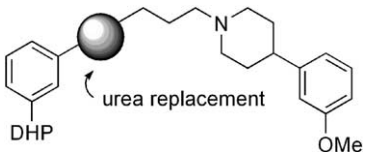
Both the cyanoguanidine **20** ($K_i = 5.1$ nM) and thiourea **22** ($K_i = 12$ nM) derivatives demonstrated potent binding affinity at the Y_1 receptor. These results are not surprising considering that the bioequivalence of urea, thiourea, and cyanoguanidine functionalities has been reported previously in the H_2 receptor antagonist area.[27a,28,29] Both guanylurea **21** and nitroethylene **24** were found to be devoid of Y_1 activity (K_i's = > 1000 nM). The latter results may possibly be explained by the increased polar nature of the functionality in comparison to urea **4a**, cyanoguanidine **20**, and thiourea **22**.[28]

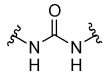
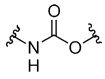
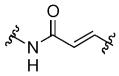
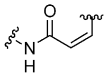
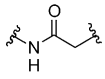
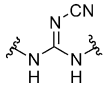
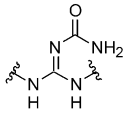
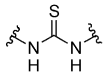
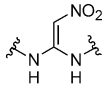
We lastly examined a series of heterocycles as urea replacements. As shown in Scheme 6 squaric acid derivative **26** was prepared by treatment of **1** with 3,4-diethoxy-3-cyclobutene-1,2-dione in DMF to give the intermediate **25**, followed by reaction with **3a** in DMF. Thiadiazole oxide **28** was prepared in a similar manner from **1** using the conditions of Karady.[30] Reaction of **1** with 4,5-diethoxythiadiazole oxide and $AlMe_3$ in CH_2Cl_2 furnished intermediate **27**, which was subsequently treated with **3a** to give thiadiazole oxide **28** after chromatography.

Amino-substituted 1,3,4-oxadiazole derivatives **31a** and **31b** were prepared by condensation of isocyanate **2** in CH_2Cl_2 with either hydrazide **29a** or **29b** to furnish the respective intermediate acylsemicarbazides **30a** and **30b** (Scheme 7). After heating in $POCl_3$, the intermediate acylsemicarbazides were converted to the respective aminooxadiazoles **31a** and **31b**.

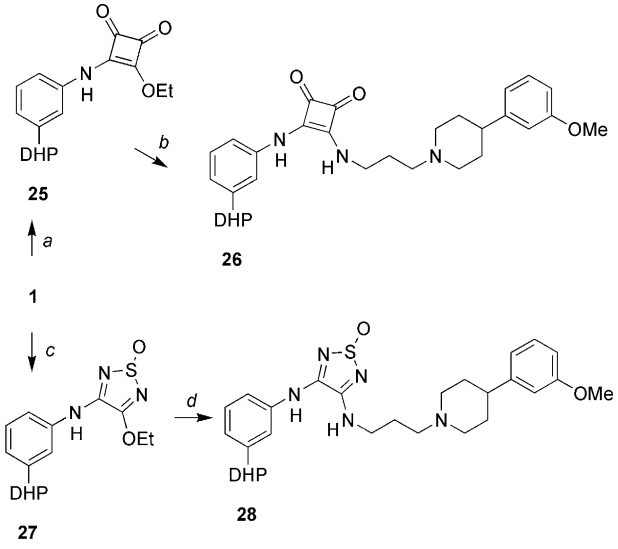
Lastly, imidazolone **34** was prepared according to the route outlined in Scheme 8. Using the procedure of Reddy,[31] aniline **1** was treated with cyanogen bromide to give **32** in quantitative yield. Condensation of cyanamide **32** with ethyl glycinate **33** afforded the 4-oxo imidazolone

Table 2. NPY Y_1 binding affinities for urea replacements **14**, **16**, **17**, **20**, **21**, **22**, and **24**

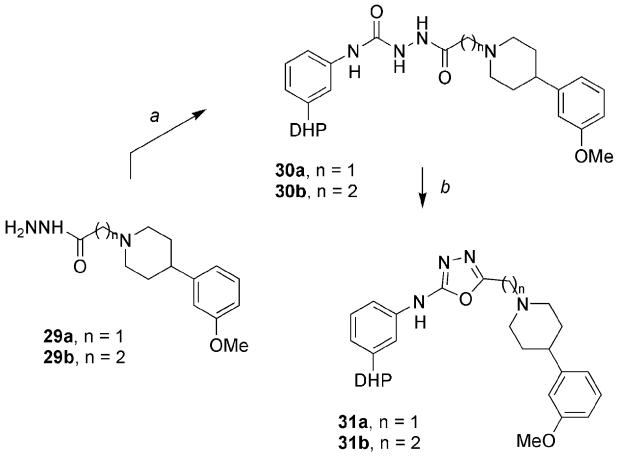


Compd	Urea replacement	*n*	NPY Y_1 (K_i, nM)[a]
4a		3	3.3 (±0.17)[b]
14		3	480[c]
16a		2	> 1000[c]
16b		2	> 1000
17		2	110
20		3	5.1[c]
21		3	> 1000
22		3	12
24		3	> 1000

[a] ^{125}I-PYY binding in SK-N-MC cell membranes. Unless otherwise indicated, the K_i's were obtained from a single experiment ($n = 1$) with each point being run in duplicate. Standard reference agent BIBN 3226 (Fig. 1) displayed a $K_i = 15$ nM in this assay.
[b] The standard error was generated from three curves, each data point was obtained from the average of six determinations.
[c] The K_i's were obtained from two experiments ($n = 2$) being run in duplicate.



Scheme 6. Synthesis of heterocycles **26** and **28**: (a) 3,4-diethoxy-3-cyclobuten-1,2-dione, DMF (52%); (b) **3a**, DMF (54%); (c) Me_3Al, CH_2Cl_2, 4,5-diethoxythiadiazole oxide (48%); (d) **3a**, MeCN (55%).

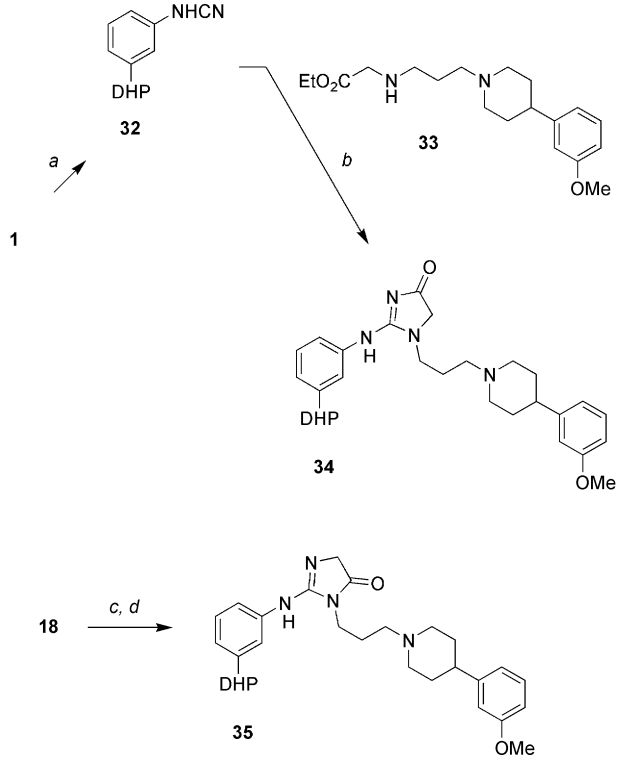


Scheme 7. Synthesis of oxadiazoles **31**: (a) **2**, CH_2Cl_2 (26% for **30a**, 29% for **30b**); (b) $POCl_3$ (8% for **31a**, 30% for **31b**).

34. The isomeric 5-oxo derivative **35** was prepared in a similar manner by condensing isothiocyanate **18** with the sodium salt of propanamine **3a**. The resulting thiolate was then treated with ethyl glycinate and $HgCl_2$ in THF to give **35**.[32]

The Y_1 binding results for these heterocyclic replacement analogues are reported in Table 3. The two known urea replacements, squaric acid **26** ($K_i = 21$ nM) and thiadiazole oxide **28** ($K_i = 24$ nM), demonstrated good binding affinity at the Y_1 receptor, although both were still less than 10-fold as active compared to parent urea **4a**. The oxadiazole derivatives **31a** and **31b** showed moderate affinity for the receptor (K_i's = 99 and 160 nM, respectively), suggesting that the 2-amino-1,3,4-oxadiazole moiety can partially function as a urea replacement in this series of compounds, and that no significant difference was observed for either the two- or three-carbon tether. Both of the conformationally restricted, aminoimidazolone derivatives **34** and **35** were devoid of Y_1 binding affinity (K_i's = > 1000 nM).

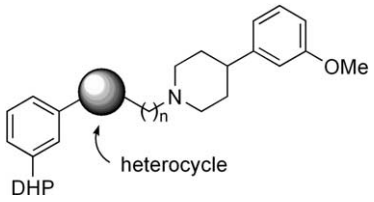
Cyanoguanidine **20** was selected for further investigation based on its potent binding affinity relative to the parent urea ($K_i = 5.1$ nM vs 3.3 nM for **4a**). Scatchard analysis of **20** based upon the effect of ^{125}I-PYY binding in SK-N-MC cell membranes, suggested the binding inhibition is competitive.[33] Thus, cyanoguanidine **20** at a concentration of 10 nM reduced PYY binding affinity (K_d of 6.5 vs 1.5 nM), without statistically affecting its binding capacity (B_{max} of 4.9 ± 0.9 vs 3.5 ± 1.1 pmol/mg protein)]. Functional studies in SK-N-MC cells expressing the human Y_1 receptor found that **20** antagonized the NPY-mediated inhibition of forskolin-stimulated *c*AMP accumulation in a competitive manner ($K_b = 2.6 \pm 0.8$ nM), indicating the compound is a full functional antagonist at the Y_1 receptor.[34] In additional competition binding experiments, **20** showed no affinity (K_i's > 1000 nM) for other cloned human NPY receptor subtypes (Y_2, Y_4, and Y_5). In comparison to the urea parent, cyanoguanidine **20** showed improved permeability characteristics in Caco-2 cells ($P_c = 43$ nm/s vs 19 nm/s for **4a**). Although this represents only a moderate enhancement in Caco-2 permeability, the cyanoguanidine group functions very effectively as a urea replacement in this series dihydropyridine Y_1 receptor antagonists.[35]

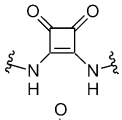
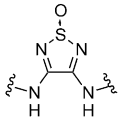
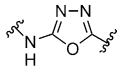
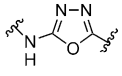
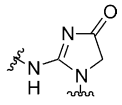
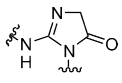


Scheme 8. Synthesis of imidazolones **34** and **35**: (a) $NaHCO_3$, BrCN, EtOH (100%); (b) Na_2CO_3, MeCN (3%); (c) **3a**, Na, EtOH; (d) ethyl glycinate, $HgCl_2$, THF (5%).

In summary, we prepared series of dihydropyridine derivatives which incorporated both classical and non-classical urea replacement groups. Out of a series of 12 targeted functionalities, the cyanoguanidine moiety was identified as the most effective urea replacement. In comparison to the starting urea **4a**, cyanoguanidine **20** (BMS-205749) displayed similar binding potency at the Y_1 receptor and showed improved permeability

Table 3. NPY Y_1 binding affinities for heterocycles **26**, **28**, **31**, **34**, and **35**



Compd	Heterocycle	*n*	NPY Y_1 (K_i, nM)[a]
26		3	21[b]
28		3	24[b]
31a[c]		1	99[b]
31b[c]		2	160[b]
34		3	> 1000
35		3	> 1000[b]

[a] ^{125}I-PYY binding in SK-N-MC cell membranes. Unless otherwise indicated, the K_i's were obtained from a single experiment ($n = 1$) with each point being run in duplicate. Standard reference agent BIBN 3226 (Fig. 1) displayed a $K_i = 15$ nM in this assay.

[b] The K_i's were obtained from two experiments ($n = 2$) being run in duplicate.

[c] The precursor acylsemicarbazides **30a** and **30b** displayed K_i's of 210 and 170 nM, respectively, in the Y_1 binding assay.

properties in Caco-2 cells. Additional examples of other cyanoguanidine-linked, dihydropyridine Y_1 receptor antagonists will be reported in the future.

3. Experimental

3.1. General

Melting points were determined using a Thomas-Hoover capillary melting apparatus and are uncorrected. Elemental analyses were performed at Robertson Microlabs, Madison, NJ, USA, and are within ±0.4% of calculated values. Unless otherwise indicated, ^{1}H NMR spectra were recorded at 300 MHz and ^{13}C spectra at 75.5 MHz on a Bruker AC 300 spectrometer in the indicated solvents. High field NMR spectra were recorded on a Bruker Avance 500 spectrometer at 500 and 125 MHz for ^{1}H and ^{13}C, respectively, in the indicated solvents. IR spectra were recorded on a Perkin-Elmer Model 1800 FT spectrophotometer. High resolution mass spectrometry (HRMS) was performed using a Finnigan MAT 900 spectrometer. Reverse-phase HPLC purifications were performed using a YMC, Inc., 20×250 mm, 5-μm particle size, C18 S5 column: 35–100% B at a flow rate of 20 mL/min for 20 min and a gradient time of 20 min. Mobile phase: B = (10% MeOH, 90% H_2O, 0.1% TFA); A = (90% MeOH, 10% H_2O, 0.1% TFA). A Shimadzu SPD-10A detector at 220 nm was used for peak detection. Starting dihydropyridines **1**[18] and **9**[19] and 4-(3-methoxyphenyl)piperidine[37] were prepared by literature accounts.

3.1.1. 1,4-Dihydro-4-(3-isocyanatophenyl)-2,6-dimethyl-3,5-pyridinedicarboxylic acid, dimethyl ester (2). A solution of **1** (27 g, 85 mmol) in anhydrous THF (10 L) was refluxed under N_2 for 30 min to dissolve all solids. After cooling, the resulting solution was added in dropwise fashion to 180 mL of a stirred phosgene solution (1.92 M in toluene) at 0 °C under N_2. The resulting mixture was stirred an additional 30 min while allowing to warm to room temperature. After sparging with N_2 for 16 h to remove any residual phosgene, the volatiles were removed in vacuo to furnish 31.4 g of the carbamoyl chloride intermediate as a cream solid. After standing for 2 days at room temperature, gradual elimination of HCl afforded 28 g of **2** as a cream solid (the isocyanate was still contaminated with small amounts of the carbamoyl chloride intermediate): IR (KBr) 2272 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.12 (m, 2H), 6.97 (s, 1H), 6.86 (t, 1H, J = 7.5 Hz), 5.80 (s, 1H), 4.99 (s, 1H), 3.66 (s, 6H), 2.36 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 168.0, 149.4, 144.7, 133.2, 129.2, 125.5, 124.2, 122.9, 103.6, 51.3, 39.5, 19.8.

3.1.2. 4-(3-Methoxyphenyl)piperidine-1-propanamine (3a). A solution of 4-(3-methoxyphenyl)piperidine (26.2 g, 137 mmol) and acrylonitrile (10.8 mL, 164 mmol) in MeCN (150 mL) was refluxed 4 h and then the solvent was removed in vacuo to yield the β-aminonitrile intermediate as an amber oil. The oil subsequently underwent a rapid, exothermic crystallization to form a white solid (32.3 g, 96% yield): mp 90–94 °C; IR (KBr) 2246 cm^{-1}. This material (31.3 g, 128 mmol) was taken up in MeOH (850 mL) and 30% aq NH_4OH (150 mL) containing Raney Ni and then hydrogenated for 1 h at 50 psi H_2 in a Parr apparatus. The catalyst was removed by filtration through Celite and the filtrate concentrated in vacuo. The residue was taken up in CH_2Cl_2, dried over Na_2SO_4, and then the volatiles removed in vacuo to yield a light amber oil. Bulb-to-bulb distillation of the crude material furnished 26.6 g of **3a** as a colorless oil (84% yield): mp 156–157 °C (maleic acid salt); ^{1}H NMR ($CDCl_3$) δ 7.19 (t, 1H, J = 7.5 Hz), 6.79 (d, 1H, J = 7.5 Hz), 6.76 (s, 1H), 6.70 (d, 1H, J = 7.5 Hz), 3.76 (s, 3H), 3.03 (d, 2H, J = 11.4 Hz), 2.73 (t, 2H, J = 6.9 Hz), 2.46 (m, 1H), 2.40 (t, 2H, J = 7.2 Hz), 1.99 (m, 2H), 1.88 (s, 2H), 1.80 (m, 4H), 1.65 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 159.7, 148.2, 129.5, 119.4, 112.7, 111.4, 57.0, 55.3, 54.6, 42.9, 41.0, 33.6, 30.6. Anal. calcd for $C_{15}H_{24}N_2O·0.5H_2O$; C, 70.00; H, 9.79; N, 10.89. Found: C, 70.14; H, 10.01; N, 10.75.

3.1.3. *N*-Methyl-[4-(3-methoxyphenyl)piperidine-1-propanamine (3b). A solution of **3a** (1.75 g, 7.06 mmol) and 80 mL of ethyl formate was refluxed overnight (16 h). After the volatiles were removed in vacuo, and the crude formamide was taken up in anhydrous THF (40 mL) and 24 mmol of $Me_2S·BH_3$ (12 mL of a 2.0 M solution in THF) was added. The solution was stirred 16 h at room temperature and then MeOH was carefully added. After removing the volatiles in vacuo, the residue was taken up in 25 mL of 1 N HCl and refluxed 2 h. The solution was then made basic with saturated aq Na_2CO_3 and extracted with CH_2Cl_2. The solvent was removed in vacuo to give 1.4 g of crude **3b** as a colorless oil (~72% yield). ^{1}H NMR analysis indicated some *N*,*N*-dimethyl propanamine impurity was also present.

3.1.4. 1,4-Dihydro-[3-[[3-[4-(3-methoxyphenyl)-1-piperidinyl]propyl]amino]carbonyl]amino]phenyl]-2,6-dimethyl-3,5-pyridinedicarboxylic acid, dimethyl ester, L-lactate salt (4a). A solution of isocyanate **2** (374 mg, 1.1 mmol) in dry CH_2Cl_2 (20 mL) was added in a dropwise fashion over 20 min to a stirred solution of the propanamine **3a** (340 mg, 1.35 mmol) in anhydrous CH_2Cl_2 (20 mL). The reaction mixture was stirred for 1 h, and was then rinsed with water (2×50 mL), and dried (Na_2SO_4). After filtration, the solvent was removed in vacuo to afford a crude residue of 590 mg. The residue was taken up in MeCN (5 mL) and combined with a solution of L-lactic acid (90 mg, 1.0 mmol) in MeCN (5 mL), heated, and then allowed to stand overnight. The resulting white precipitate was collected by filtration, rinsed with a minimum of MeCN, and recrystallized from MeCN to furnish **4a** as a fluffy white solid (440 mg, 60% yield): mp 125–126 °C; ^{1}H NMR (DMSO-d_6) δ 8.87 (s, 1H), 8.52 (s, 1H), 7.24 (d,1H, J = 8.1 Hz), 7.20 (t, 1H, J = 8.0 Hz), 7.10 (s, 1H), 7.02 (t, 1H, J = 7.8 Hz), 6.78 (m, 3H), 6.65 (d, 1H, J = 7.5 Hz), 6.28 (m, 1H), 4.84 (s, 1H), 3.95 (q, 1H, J = 6.9 Hz), 3.73 (s, 3H), 3.55 (s, 6H), 3.10 (m, 4H), 2.50 (m, 3H), 2.25 (s, 6H), 2.18 (m, 2H), 1.67 (m, 6H), 1.21 (d, 3H, J = 6.9 Hz); ^{13}C NMR (DMSO-d_6) δ 176.9, 167.4, 159.3, 155.3, 148.1, 147.5, 145.6, 140.4, 129.3, 128.2, 119.7, 118.8, 116.4, 115.4,

112.4, 111.4, 101.3, 66.0, 55.2, 54.9, 53.3, 50.6, 41.3, 38.4, 37.2, 32.2, 26.6, 20.7, 18.2. Anal. calcd for $C_{33}H_{42}N_4O_6 \cdot C_3H_6O_3 \cdot 0.5H_2O$: C, 62.68; H, 7.16; N, 8.12. Found: C, 62.45; H, 7.23; N, 8.21.

3.1.5. 1,4-Dihydro-[3-[[3-[4-(3-methoxyphenyl)-1-piperidinyl]propyl]methylamino]-carbonyl]amino]phenyl]-2,6-dimethyl-3,5-pyridinedicarboxylic acid, dimethyl ester, hydrochloride salt (4b). In a manner similar to that above for **4a**, a solution of **2** (1.40 g, 40.9 mmol) and **3b** (40 mmol) was stirred at room temperature in CH_2Cl_2 (40 mL) for 2 h. After concentration in vacuo, the residue was purified by flash chromatography (SiO_2, MeOH/CH_2Cl_2) to furnish 1.05 g (65%) of **4b** as a white solid. The free base was then converted to the hydrochloride salt by treatment with ethereal HCl in CH_2Cl_2 and isolated as a white solid: mp decomposed (>130 °C); ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.4 (br s, 1H), 8.91 (s, 1H), 8.24 (s, 1H), 7.25 (m, 3H), 7.05 (t, 1H, J = 8.0 Hz), 6.80 (m, 3H), 6.72 (d, 1H, J = 7.5 Hz), 4.87 (s, 1H), 3.70 (s, 3H), 3.57 (m, 2H), 3.55 (s, 6H), 3.40 (t, 2H, J = 6.5 Hz), 3.33 (s, 6H), 3.03 (m, 4H), 2.97 (s, 3H), 2.78 (m, 1H), 2.26 (s, 6H), and 2.01 (m, 6H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 167.1, 159.3, 155.6, 147.7, 145.7, 145.5, 140.0, 129.5, 127.6, 120.7, 119.0, 118.5, 118.2, 112.5, 111.6, 101.3, 54.9, 53.6, 51.8, 50.5, 40.0, 38.6, 38.4, 34.2, 29.7, 21.9, 18.1. Anal. calcd for $C_{34}H_{44}N_4O_6 \cdot HCl \cdot H_2O$: C, 61.95; H, 7.19; N, 8.50. Found: C, 62.18; H, 7.26; N, 8.44.

3.1.6. 1,4-Dihydro-2,6-dimethyl-4-[3-[methyl(trifluoroacetyl)amino]phenyl]-3,5-pyridinedicarboxylic acid, dimethyl ester (5). A solution of **1** (10.0 g, 31.6 mmol) in CH_2Cl_2 (500 mL) containing pyridine (2.8 mL, 35 mmol) was cooled to 0 °C. Trifluoroacetic anhydride (4.7 mL, 33 mmol) was then added dropwise, and the resulting mixture was stirred at room temperature for 2 h, resulting in a thick suspension. A white solid was collected by filtration and dried in an Abderhalden apparatus under reduced pressure for 2 h to afford the intermediate trifluoroacetamide (9.58 g, 74%). To a solution of this material (2.06 g, 5.0 mmol) in DMF (20 mL) under N_2 was added NaH (60% mineral oil dispersion, 240 mg, 6.0 mmol). This mixture was stirred for 10 min, followed by the addition of MeI (0.60 mL, 10 mmol). The reaction mixture was stirred for 1 h, and then was quenched with H_2O (100 mL). A precipitate readily formed, which was collected by filtration and rinsed with H_2O. The resulting solid was taken up in CH_2Cl_2, dried (Na_2SO_4), and concentrated in vacuo to furnish 1.91 g (90%) of **5** as a light amber solid: mp 184–186 °C; ^{1}H NMR (DMSO-d_6) δ 8.92 (s, 1H), 7.34 (t, 1H, J = 7.8 Hz), 7.19 (m, 2H), 7.09 (s, 1H), 4.87 (s, 1H), 3.52 (s, 6H), 3.25 (s, 3H), 2.26 (s, 6H); ^{13}C NMR (DMSO-d_6) δ 197.3, 168.9, 156.9, 151.3, 147.8, 141.4, 131.0, 129.3, 127.8, 126.5, 102.9, 52.3, 41.2, 40.4, 19.8. Anal. calcd for $C_{20}H_{21}F_3N_2O_5 \cdot 0.2H_2O$: C, 55.87; H, 5.02; N, 6.52. Found: C, 55.84; H, 4.96; N, 6.24.

3.1.7. 1,4-Dihydro-2,6-dimethyl-4-[3-(methylamino)phenyl]-3,5-pyridinedicarboxylic acid, dimethyl ester (6). A solution of **5** (1.67 g, 3.92 mmol) in EtOH (30 mL) and 1 N NaOH (30 mL) was heated to reflux, and then was allowed to cool to room temperature and extracted with CH_2Cl_2. The organic extract was dried (Na_2SO_4), and the solvent was removed in vacuo to afford **6** as a pale-yellow solid (1.11 g, 86%): mp 182–184 °C; ^{1}H NMR (DMSO-d_6) δ 8.78 (s, 1H), 6.91 (t, 1H, J = 7.8 Hz), 6.33 (m, 2H), 6.26 (d, 1H, J = 8.1 Hz), 5.45 (q, 1H, J = 5.1 Hz), 4.82 (s, 1H), 3.56 (s, 6H), 2.60 (d, 3H, J = 5.1 Hz), 2.25 (s, 6H); ^{13}C NMR (DMSO-d_6) δ 169.3, 151.4, 149.9, 147.0, 130.3, 116.2, 112.6, 110.7, 103.3, 52.3, 40.0, 31.4, 19.9. HRMS calcd for $C_{18}H_{22}O_4NaN_2$ (M + Na): 353.148. Found: 353.148.

3.1.8. 4-[3-[3-(Chloropropyl)amino]carbonyl]methylamino]phenyl]-1,4-dihydro-2,6-dimethyl-3,5-pyridinedicarboxylic acid, dimethyl ester (7). A solution of **6** (1.18 g, 3.58 mmol) and 3-chloropropyl isocyanate (0.4 mL, 4 mmol) in CH_2Cl_2 (100 mL) was refluxed 2 h. After concentration in vacuo, the solid residue was purified by chromatography (SiO_2, MeOH/CH_2Cl_2) to afford 1.20 g (75%) of **7** as an off-white solid: mp 172–174 °C; ^{1}H NMR (DMSO-d_6) δ 7.22 (m, 2H), 7.11 (s, 1H), 7.00 (d, 1H, J = 7.3 Hz), 5.87 (s, 1H), 4.99 (s, 1H), 4.53 (t, 2H, J = 5.8 Hz), 3.65 (s, 6H), 3.51 (t, 2H, J = 6.4 Hz), 3.28 (q, 2H, J = 6.4 Hz), 3.23 (s, 3H), 2.34 (s, 6H), 1.93 (p, 2H, J = 6.4 Hz). Anal. calcd for $C_{22}H_{28}ClN_3O_5 \cdot 0.7H_2O$: C, 57.13; H, 6.41; N, 9.09. Found: C, 57.08; H, 6.14; N, 9.09.

3.1.9. 1,4-Dihydro-[3-[[3-[4-(3-methoxyphenyl)-1-piperidinyl]propyl]amino]carbonyl]methylamino]phenyl]-2,6-dimethyl-3,5-pyridinedicarboxylic acid, dimethyl ester, hydrochloride salt (8). A mixture of **7** (1.00 g, 2.22 mmol), 4-(3-methoxyphenyl)piperidine (420 mg, 2.2 mmol), K_2CO_3 (0.35 g, 2.5 mmol), and NaI (25 mg) in MeCN (50 mL) was refluxed overnight. The mixture was then partitioned between CH_2Cl_2 and H_2O and the organic extract dried (Na_2SO_4), filtered, and concentrated in vacuo. The residue was purified by flash chromatography (SiO_2, MeOH/CH_2Cl_2) to give 610 mg (46%) of **8**. The free base was converted to the hydrochloride salt by treatment with ethereal HCl in CH_2Cl_2 and isolated as a white solid: mp 60–70 °C (sintered); ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.5 (br s, 1H), 9.07 (s, 1H), 7.23 (p, 2H, J = 7.5 Hz), 7.04 (d, 1H, J = 8.0 Hz), 6.98 (m, 2H), 6.80 (m, 3H), 6.23 (br t, 1H), 4.88 (s, 1H), 3.74 (s, 3H), 3.56 (s, 6H), 3.52 (m, 2H), 3.43 (s, 6H), 3.12 (s, 3H), 3.10 (m, 2H), 3.01 (m, 4H), 2.79 (m, 1H), 2.28 (s, 6H), 2.08 (m, 2H), 1.95 (m, 2H), and 1.86 (m, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 167.3, 159.3, 156.7, 148.7, 145.9, 145.8, 143.5, 129.5, 128.7, 124.2, 124.0, 123.7, 118.5, 112.6, 111.6, 101.1, 54.9, 53.8, 51.7, 50.6, 38.9, 38.7, 38.3, 37.5, 37.0, 29.6, 24.0, 18.1. Anal. calcd for $C_{34}H_{44}N_4O_6 \cdot HCl \cdot 3.0H_2O$: C, 57.74; H, 7.39; N, 8.06. Found: C, 59.11; H, 7.31; N, 7.96.

3.1.10. 4-(3-Aminophenyl)-1,4-dihydro-1,2,6-trimethyl-3,5-pyridinedicarboxylic acid, dimethyl ester (10). A suspension of **9** (9.26 g, 25.7 mmol), 5% Pt on sulfided carbon (1.0 g) in a mixture of 2:1 MeOH/$CHCl_3$ (200 mL) was shaken under 65 psi of H_2 on a Parr Hydrogenator overnight (17 h). The mixture was filtered through Celite and concentrated in vacuo to yield a solid residue After trituration in a mixture of hot

i-PrOH/EtOH, 8.48 g (100%) of **10** was obtained as a colorless, white solid: mp 213–214 °C; ^{1}H NMR (DMSO-d_6) δ 6.79 (t, 1H, J = 7.4 Hz), 6.26 (m, 2H), 6.19 (d, 1H, J = 7.6 Hz), 4.90 (s, 1H), 4.86 (br s, 1H), 3.60 (s, 6H), 3.15 (s, 3H), and 2.39 (s, 6H); ^{13}C NMR (DMSO-d_6) δ 167.7, 149.6, 148.4, 146.1, 128.6, 114.0, 112.2, 111.8, 104.2, 51.0, 37.3, 16.0. Anal. calcd for $C_{18}H_{22}N_2O_4 \cdot 0.33H_2O$: C, 64.28; H, 6.79; N, 8.33. Found: C, 64.29; H, 6.80; N, 8.07.

3.1.11. 1,4-Dihydro-4-[3-[(methoxycarbonyl)amino]phenyl]-1,2,6-trimethyl-3,5-pyridinedicarboxylic acid, dimethyl ester (11). To a solution of **10** (8.25 g, 25.0 mmol) in pyridine (50 mL) and CH_2Cl_2 (50 mL) was slowly added 2.90 g (27.1 mmol) of methyl chloroformate. After stirring an additional 3 h at room temperature, the mixture was poured into water (500 mL) and extracted with CH_2Cl_2. The combined organic portions were washed successively with H_2O, 0.1 N HCl, H_2O, and brine and then dried ($MgSO_4$) and concentrated. The residue was purified by flash chromatography (SiO_2, EtOAc/hexanes) to give 7.20 g (74%) of **11** as a white solid: mp 163–165 °C; ^{1}H NMR (DMSO-d_6) δ 9.53 (s, 1H), 7.26 (d, 1H, J = 8.1 Hz), 7.17 (s, 1H), 7.08 (t, 1H, J = 7.8 Hz), 6.70 (d, 1H, J = 7.7 Hz), 4.99 (s, 1H), 3.62 (s, 3H), 3.61 (s, 6H), 3.17 (s, 3H), and 2.41 (s, 6H); ^{13}C NMR (DMSO-d_6) δ 167.6, 154.0, 150.3, 146.1, 139.2, 128.6, 120.5, 116.4, 115.9, 104.0, 51.6, 51.3, 37.4, 16.0. Anal. calcd for $C_{20}H_{24}N_2O_6$: C, 61.85; H, 6.23; N, 7.21. Found: C, 61.88; H, 6.10; N, 7.16.

3.1.12. 1,4-Dihydro-[3-[[3-[4-(3-methoxyphenyl)-1-piperidinyl]propyl]amino]carbonyl]amino]phenyl] - 1,2,6 - trimethyl-3,5-pyridinedicarboxylic acid, dimethyl ester, hydrochloride salt (12). Using the conditions of Alper,[20] *B*-chlorocatecholborane (2.16 g, 14.0 mmol) was added under N_2 in one portion to a solution of **11** (3.90 g, 10.1 mmol) and Et_3N (1.60 g, 15.8 mmol) in THF (80 mL). The resulting mixture was heated with a heat gun for 5 min to effect dissolution and then the volatiles were removed in vacuo. The residue was taken up in CH_2Cl_2 and washed successively with 1 N HCl, 1 N NaOH, brine, and dried (Na_2SO_4). Filtration and concentration in vacuo furnished 4.21 g of the intermediate isocyanate as a gray solid [IR (KBr) 2272 cm^{-1}]. The material was then added to a solution of **3a** (2.60 g, 10.5 mmol) in CH_2Cl_2 (30 mL) and stirred 3 h at room temperature. The solution was washed with 1 N HCl, H_2O, 0.5 N NaOH, H_2O, brine, and dried ($MgSO_4$). After filtration, the solution was concentrated and the residue purified by flash chromatography (SiO_2, EtOAc/hexanes) to afford 3.86 g (64%) of **12** as a yellow solid. The solid was converted to the hydrochloride salt by treatment with ethereal HCl and then triturated overnight in acetone/hexane to furnish the salt as a creamy white solid: mp 154–160 °C; ^{1}H NMR (DMSO-d_6) δ 10.42 (s, 1H), 7.28 (d, 1H, J = 8.1 Hz), 7.22 (t, 1H, J = 7.5 Hz), 7.07 (s, 1H), 7.01 (t, 1H, J = 7.9 Hz), 6.80 (m, 3H), 6.64 (br s, 1H), 6.61 (t, 1H, J = 8.0 Hz), 4.96 (s, 1H), 3.71 (s, 3H), 3.60 (s, 6H), 3.51 (m, 2H), 3.18 (s, 3H), 3.05 (m, 6H), 2.76 (m, 1H), 2.41 (s, 6H), 2.06 (m, 2H), and 1.94 (m, 4H); ^{13}C NMR (DMSO-d_6) δ 167.6, 159.5, 155.7, 150.2, 146.0, 145.9, 140.6, 139.9, 129.7, 128.5, 119.1, 118.7, 115.6, 115.3, 112.7, 111.8, 104.1, 55.0, 54.1, 52.0, 51.1, 39.8, 37.4, 36.5, 34.0, 29.8, 24.6, 16.1. Anal. calcd for $C_{34}H_{44}N_4O_6 \cdot HCl \cdot 0.4H_2O$: C, 62.95; H, 7.12; N, 8.64. Found: C, 62.95; H, 7.26; N, 8.45.

3.1.13. *3.1.13.N*-3-(Hydroxypropyl)-4-(3-methoxyphenyl)piperidine (13). A mixture of 4-(3-methoxyphenyl)piperidine (2.0 g, 10.4 mmol), 3-bromopropanol (1.1 mL, 12 mmol), K_2CO_3 (1.66 g, 12.0 mmol) and MeCN (50 mL) was stirred at room temperature for 3 days. The solvent was removed in vacuo and the residue partitioned between CH_2Cl_2 and H_2O. The phases were separated and the organic portion dried (Na_2SO_4) and concentrated. The crude material was purified by flash chromatography (SiO_2, MeOH/CH_2Cl_2) to give 810 mg (31%) of **13** as a clear oil: ^{1}H NMR ($CDCl_3$) δ 7.24 (m, 1H), 6.80 (d, 1H, J = 7.7 Hz), 6.73 (m, 2H), 3.81 (t, 2H, J = 5.5 Hz), 3.73 (s, 3H), 3.20 (m, 2H), 2.66 (t, 2H, J = 5.7 Hz), 2.50 (m, 1H), 2.07 (m, 2H), 1.79 (m, 6H); ^{13}C NMR ($CDCl_3$) δ 159.7, 147.6, 129.4, 119.2, 112.6, 111.5, 64.6, 59.1, 55.2, 54.5, 43.5, 33.3, 27.2. Anal. calcd for $C_{15}H_{23}NO_2 \cdot 0.4H_2O$: C, 70.22; H, 9.35; N, 5.46. Found: C, 70.18; H, 9.46; N, 5.36.

3.1.14. 1,4-Dihydro-[3-[[3-[4-(3-methoxyphenyl)-1-piperidinyl]propyloxy]carbonyl]amino]phenyl]-2,6-dimethyl-3,5-pyridinedicarboxylic acid, dimethyl ester, hydrochloride salt (14). A solution of **2** (656 mg, 1.92 mmol) and **13** (600 mg, 2.41 mmol) in CH_2Cl_2 (50 mL) was stirred at room temperature for 2 h and then concentrated in vacuo. The crude material was purified by flash chromatography (SiO_2, MeOH/CH_2Cl_2). The resulting free base was converted to the hydrochloride salt by treatment with ethereal HCl in CH_2Cl_2 to give 680 mg (61%) of **14** as a white solid: mp 120–132 °C (sintered); ^{1}H NMR (DMSO-d_6) δ 10.71 (br s, 1H), 9.63 (s, 1H), 9.00 (s, 1H), 7.23 (m, 3H), 7.13 (m, 1H), 6.83 (m, 4H), 4.88 (s, 1H), 4.17 (t, 2H, J = 6.1 Hz), 3.76 (s, 3H), 3.57 (m, 2H), 3.38 (s, 6H), 3.08 (m, 4H), 2.81 (m, 1H), 2.28 (s, 6H), 2.00 (m, 6H); ^{13}C NMR (DMSO-d_6) δ 167.5, 159.4, 153.3, 148.3, 145.8, 138.6, 129.7, 128.3, 121.2, 118.7, 117.2, 116.0, 112.7, 111.8, 101.2, 61.5, 55.0, 53.3, 52.1, 50.7, 38.4, 29.8, 23.4, 18.2. Anal. calcd for $C_{33}H_{41}N_3O \cdot HCl \cdot H_2O$: C, 61.34; H, 6.86; N, 6.50. Found: C, 61.29; H, 6.85; N, 6.39.

3.1.15. 1,4-Dihydro-4-[3-[[(diethylphosphonato)methyl]-carbonyl]amino]phenyl] - 2,6 - dimethyl - 3,5 - pyridinedicarboxylic acid, dimethyl ester (15). Using the procedure of Nagao,[24] a solution of diethyl phosphonoacetic acid (1.96 g, 10.0 mmol) in CH_2Cl_2 (30 mL) was treated sequentially with 4-(dimethylamino)pyridine (DMAP) (50 mg), 2-mercaptothiazoline (1.19 g, 10.0 mmol), and 1,3-dicyclohexylcarodiimide (DCC) (1.19 g, 10.0 mmol) at 25 °C. The reaction was stirred for 2 h followed by the addition of aniline **1** (3.16 g, 10.0 mmol). The mixture was then stirred an additional 90 min, followed by filtration through Celite. The filtrate was concentrated in vacuo and the resulting residue was purified by flash chromatography (SiO_2, EtOAc/MeOH) providing **15** (80%) as a gold resin: ^{1}H NMR ($CDCl_3$) δ 8.64 (s, 1H), 7.35 (s, 1H), 7.28 (d, 1H, J = 7.6 Hz), 7.09 (t, 1H, J = 7.6 Hz), 6.98 (d, 1H, J = 7.6 Hz), 6.30 (s, 1H), 4.94 (s, 1H),

4.10 (m, 4H), 3.58 (s, 6H), 2.90 (d, 2H, $J=20.7$ Hz), 2.24 (s, 6H), 1.28 (t, 6H, $J=7.1$ Hz); ^{13}C NMR ($CDCl_3$) δ 167.9, 161.8, 148.2, 144.7, 137.5, 128.3, 123.9, 118.9, 117.8, 103.1, 62.9, 50.8, 39.0, 36.1 (d, $J=129$ Hz), 19.2, 16.2. Anal. calcd for $C_{23}H_{31}N_2O_8P$: C, 55.87; H, 6.32; N, 5.67. Found: C, 55.51; H, 6.36; N, 5.57.

3.1.16. 1,4-Dihydro-4-[3-[[4-[4-(3-methoxyphenyl)-1-piperidinyl]-*E*-but-1-ene-1-yl]carbonyl]amino]phenyl]-2,6-dimethyl - 3,5 - pyridinedicarboxylic acid, dimethyl ester, hydrochloride salt (16a) and 1,4-dihydro-4-[3-[[4-[4-(3-methoxyphenyl)-1-piperidinyl]-*Z*-but-1-ene-1-yl]carbonyl]amino]phenyl] - 2,6 - dimethyl - 3,5 - pyridinedicarboxylic acid, dimethyl ester, hydrochloride salt (16b). Phosphonate **15** (988 mg, 2.00 mmol) in THF (5 mL) was treated with NaH (240 mg of a 60% suspension in mineral oil, 6.0 mmol) and stirred for 30 min. In a separate flask, acrolein (133 μL, 2.00 mmol) was added dropwise to a 0 °C solution of 4-(3-methoxyphenyl)piperidine (392 mg, 2.00 mmol), DBU (3.0 μL, 20 μmol) and THF (5.0 mL). This mixture was stirred for 20 min. and then added to the above phosphonate solution. The resulting mixture was stirred for 2 h at 25 °C. The reaction was then quenched with H_2O (10 mL) and the product extracted with ether/THF (5:1). The residue was concentrated in vacuo and purified by flash chromatography (SiO_2, EtOAc/MeOH) to afford 172 mg (15%) of **16a** and 57 mg (5%) of **16b** as clear oils. Both were converted to their hydrochloride salts by treatment with ethereal HCl.

16a. Mp 155 °C; ^{1}H NMR (DMSO-d_6) δ 10.74 (br s, 1H), 10.12 (s, 1H), 9.01 (s, 1H), 7.51 (d, 1H, $J=8.1$ Hz), 7.46 (s, 1H), 7.25 (t, 1H, $J=8.4$ Hz), 7.13 (t, 1H, $J=8.1$ Hz), 6.82 (m, 4H), 6.72 (d of t, 1H, $J=6.6$ and 15.4 Hz), 6.26 (d, 1H, $J=15.4$ Hz), 4.88 (s, 1H), 3.74 (s, 3H), 3.60 (m, 2H), 3.56 (s, 6H), 3.22 (m, 2H), 3.03 (m, 2H), 2.76 (m, 3H), 2.27 (s, 6H), 2.0 (m, 4H); ^{13}C NMR (DMSO-d_6) δ 167.4, 162.9, 159.3, 148.1, 145.9, 145.8, 138.8, 138.7, 129.6, 128.3, 126.9, 122.1, 118.6, 118.1, 117.1, 112.7, 111.7, 101.2, 54.9, 54.2, 51.8, 50.6, 38.7, 38.4, 29.7, 26.0, 18.2. Anal. calcd for $C_{34}H_{41}N_3O_6{\cdot}HCl{\cdot}H_2O$: C, 63.60; H, 6.91; N, 6.54. Found: C, 63.49; H, 6.86; N, 6.35.

16b. Mp indistinct; ^{1}H NMR (DMSO-d_6) δ 10.58 (br s, 1H), 10.01 (s, 1H), 8.97 (s, 1H), 7.43 (d, 1H, $J=7.8$ Hz), 7.36 (s, 1H), 7.25 (t, 1H, $J=7.8$ Hz), 7.11 (t, 1H, $J=7.8$ Hz), 6.80 (m, 4H), 6.07 (m, 1H), 5.80 (m, 1H), 4.87 (s, 1H), 3.74 (s, 3H), 3.70 (m, 2H), 3.55 (s, 6H), 3.20 (m, 2H), 2.97 (m, 2H), 2.77 (m, 2H), 2.26 (m, 6H), 1.99 (m, 4H); ^{13}C NMR (DMSO-d_6) δ 168.2, 167.4, 159.4, 148.3, 145.8, 145.7, 138.9, 134.8, 129.6, 128.2, 122.0, 121.7, 118.6, 117.9, 116.9, 112.7, 111.7, 101.2, 57.1, 54.9, 51.3, 50.7, 39.9, 39.7, 38.4, 29.7, 18.2. Anal. calcd for $C_{34}H_{41}N_3O_6{\cdot}HCl{\cdot}2.0H_2O$: C, 61.86; H, 7.03; N, 6.37; H_2O, 5.52. Found: C, 62.15; H, 6.83; N, 6.00; H_2O, 5.60.

3.1.17. 1,4-Dihydro-4-[3-[[4-[4-(3-methoxyphenyl)-1-piperidinyl]butyl]carbonyl]amino]phenyl] - 2,6 - dimethyl - 3,5-pyridinedicarboxylic acid, dimethyl ester (17). To a solution of **16a** (200 mg, 0.34 mmol) in MeOH (2.0 mL) was added 10% Pd on carbon (10 mg). The mixture was stirred under 1 atm of H_2 for 12 h. The mixture was then filtered through Celite and the filtrate concentrated in vacuo to provide 122 mg (60%) of **17** as a colorless white solid: mp 115–120 °C; ^{1}H NMR ($CDCl_3$) δ 9.16 (s, 1H), 7.59 (d, 1H, $J=8.0$ Hz), 7.50 (s, 1H), 7.19 (t, 1H, $J=7.7$ Hz), 7.08 (t, 1H, $J=8.0$ Hz), 6.98 (m, 2H), 6.75 (m, 3H), 4.97 (s, 1H), 3.75 (s, 3H), 3.60 (s, 6H), 3.47 (m, 2H), 2.88 (m, 2H), 2.67 (m, 3H), 2.36 (m, 4H), 2.30 (s, 6H), 1.94 (m, 2H), 1.80 (m, 2H), 1.68 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 170.9, 168.2, 159.7, 148.3, 145.2, 144.5, 138.6, 138.5, 129.7, 128.0, 123.2, 118.8, 117.7, 112.8, 111.8, 102.9, 55.1, 53.0, 52.9, 50.8, 40.2, 38.9, 36.1, 29.9, 23.4, 22.5, 19.2. HRMS calcd for $C_{34}H_{44}O_6N_3$ (M + H): 590.3230. Found: 590.3239.

3.1.18. 1,4-Dihydro-4-[3-[isothiocyanato]phenyl]-2,6-dimethyl-3,5-pyridinedicarboxylic acid, dimethyl ester (18). Aniline **1** (19.0 g, 60.0 mmol) in DMF (250 mL) was added via addition funnel to a solution of thiocarbonyldiimidazole (TCDI) (11.8 g, 60.0 mmol) in DMF (100 mL) over 2 h. The reaction was stirred an additional 30 min and then H_2O was added slowly, resulting in a copious white precipitate. The precipitate was collected by filtration, washed with H_2O, and air dried to afford 16.1 g (75%) of **18** as a white solid: mp 163–166 °C; IR (KBr) 2122, 2086 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 8.98 (s, 1H), 7.31 (t, 1H, $J=7.7$ Hz), 7.17 (m, 2H), 7.06 (s, 1H), 4.88 (s, 1H), 3.55 (s, 6H), 2.27 (s, 6H); ^{13}C NMR (DMSO-d_6) δ 167.1, 149.8, 146.3, 133.2, 129.7, 126.8, 123.9, 123.8, 100.9, 50.8, 38.6, 18.2. Anal. calcd for $C_{18}H_{18}N_2O_4S{\cdot}0.5H_2O$: C, 58.84; H, 5.21; N, 7.63; S, 8.73. Found: C, 58.89; H, 5.04; N, 8.01; S, 8.73.

3.1.19. 1,4-Dihydro-4-[3-[(cyanamido)(thiolate)methimino]phenyl]-2,6-dimethyl-3,5-pyridinedicarboxylic acid, dimethyl ester, sodium salt (19). Cyanamide (2.52 g, 60 mmol) in dry EtOH (50 mL) was added to a solution of sodium metal (1.38 g, 60 mmol) in EtOH (150 mL). Isothiocyanate **18** (17.9 g, 50 mmol) in EtOH (150 mL) was then added to the sodium cyanamide solution via an addition funnel. After the addition was complete, the reaction was brought to a brief boil, and then allowed to stir for 1 h at room temperature. The mixture was then concentrated and the residue triturated with EtOAc to provide 22.6 g (100%) of **19** as a gray solid: ^{1}H NMR (DMSO-d_6) δ 9.05 (s, 1H), 7.51 (s, 1H), 7.35 (d, 1H, $J=8.0$ Hz), 6.98 (t, 1H, $J=8.0$ Hz), 6.65 (d, 1H, $J=8.0$ Hz), 4.84 (s, 1H), 3.57 (s, 6H), 2.27 (s, 6H); ^{13}C NMR (DMSO-d_6) δ 186.5, 167.4, 147.4, 145.7, 140.1, 127.4, 121.2, 119.8, 118.7, 117.6, 101.2, 50.6, 38.0, 18.1; IR (KBr) 2159 cm^{-1}. HRMS calcd for $C_{19}H_{19}N_4O_4S$: 399.1127. Found: 399.1111.

3.1.20. 1,4-Dihydro-4-[3-[[3-[4-(3-methoxyphenyl)-1-piperidinyl]propyl]amino](cyanoimino)methyl]amino]phenyl] - 2,6-dimethyl-3,5-pyridine dicarboxylic acid, dimethyl ester, hydrochloride (20). $HgCl_2$ (10.9 g, 40.2 mmol) was added in 10 portions over 20 min to a solution of **19** (16.9 g, 42.3 mmol) and **3a** (14.9 g, 60.1 mmol) in THF (500 mL). The resulting brown suspension was stirred an additional 30 min, followed by the addition of H_2O (50 mL). The thick brown material was then filtered

through Celite, dried (K_2CO_3), and concentrated in vacuo. The crude product was purified by flash chromatography (SiO_2, MeOH/CH_2Cl_2) to provide 14.4 g (58%) of **20** as a colorless solid. This material was converted to the hydrochloride salt by treatment with ethereal HCl: mp 135–140 °C; IR (KBr) 2173 cm^{-1}; ^1H NMR (DMSO-d_6) δ 10.65 (br s, 1H), 9.14 (s, 1H), 9.08 (s, 1H), 7.40 (t, 1H, $J = 5.3$ Hz), 7.23 (m, 2H), 7.03 (m, 2H), 6.92 (d, 1H, $J = 7.6$ Hz), 6.81 (m, 3H), 4.90 (s, 1H), 3.74 (s, 3H), 3.57 (s, 6H), 3.52 (m, 2H), 3.31 (m, 2H), 3.03 (m, 4H), 2.79 (m, 1H), 2.29 (s, 6H), 1.97 (m, 6H); ^{13}C NMR (DMSO-d_6) δ 167.3, 159.4, 157.9, 148.5, 146.1, 145.9, 137.4, 129.6, 128.4, 123.3, 121.8, 120.9, 118.6, 117.2, 112.6, 111.7, 101.0, 54.9, 53.6, 51.9, 50.7, 38.2, 29.6, 23.6, 18.2. Anal. calcd for $C_{34}H_{42}N_6O_5 \cdot HCl \cdot 2.0H_2O$: C, 59.44; H, 6.89; N, 12.27. Found: C, 59.33; H, 6.47; N, 12.05.

3.1.21. 4-[3-[(Carbamoylimino)[3-[4-(3-methoxyphenyl)-1-piperidinyl]propyl]amino]methyl)amino]phenyl]-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylic acid dimethyl ester, trifluoroacetic acid salt (21). The hydrolysis product was obtained after storage of **20**·HCl in a closed container for several weeks at room temperature. A small portion of **21** was purified by reverse-phase HPLC and isolated as a white solid: mp 89–93 °C; ^1H NMR ($CDCl_3$) δ 7.18–7.08 (m, 4H), 6.91–6.90 (m, 1H), 6.77–6.69 (m, 4H), 4.97 (s, 1H), 3.75 (s, 3H), 3.58 (s, 6H), 3.34 (m, 2H), 2.94–2.92 (m, 2H), 2.55–2.27 (m, 9H), 1.96 (t, 2H, $J = 12.0$ Hz), 1.72–1.59 (m, 6H), 1.22 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 168.01, 159.57, 147.68, 145.04, 129.36, 129.23, 129.00, 124.33, 122.44, 119.19, 112.81, 111.13, 102.98, 102.69, 55.05, 54.07, 52.99, 52.32, 50.90, 42.47, 39.69, 39.66, 39.19, 32.76, 31.82, 29.66, 29.59, 29.26, 28.26, 26.46, 22.58, 19.20, 19.12, 14.02. HRMS calcd for $C_{34}H_{45}N_6O_6$ (M + H): 633.3300. Found: 633.3420.

3.1.22. 1,4-Dihydro-[3-[[3-[4-(3-methoxyphenyl)-1-piperidinyl]propyl]amino]thiocarbonyl]amino]phenyl]-2,6-dimethyl-3,5-pyridinedicarboxylic acid, dimethyl ester (22). A mixture of **18** (600 mg, 1.68 mmol) and **3a** (420 mg, 1.69 mmol) in $CHCl_3$ (4 mL) was stirred at room temperature 4 h. The volatiles were removed in vacuo to yield 1.01 g (99%) of **22** as a pale-yellow foam: ^1H NMR (DMSO-d_6) δ 9.45 (s, 1H), 8.92 (s, 1H), 7.66 (br s, 1H), 7.10 (m, 4H), 6.86 (d, 1H, $J = 7.5$ Hz), 6.80 (m, 3H), 4.87 (s, 1H), 3.72 (s, 3H), 3.54 (s, 6H), 3.47 (m, 2H), 3.95 (br s, 2H), 2.34 br s, 2H), 2.55 (s, 6H), 1.94 (m, 2H), 1.80 (m, 7H); ^{13}C NMR (DMSO-d_6) δ 180.0, 167.3, 159.3, 148.3, 147.9, 145.9, 129.2, 128.1, 122.7, 120.5, 118.9, 112.4, 111.3, 101.2, 55.8, 54.9, 53.7, 50.7, 42.6, 41.9, 38.2, 32.8, 25.7, 18.2. Anal. calcd for $C_{33}H_{42}N_4SO_5 \cdot 0.47H_2O$: C, 64.42; H, 7.03; N, 9.11. Found: C, 64.41; H, 7.16; N, 8.76.

3.1.23. 1,4-Dihydro-4-[3-[1-(thiomethyl)-2-nitroethylene]amino]phenyl]-2,6-dimethyl-3,5-pyridinedicarboxylic acid, dimethyl ester (23). Aniline **1** (1.89 g, 5.89 mmol) and bis(thiomethyl)nitroethylene (0.99 g, 6.00 mmol) were refluxed in MeCN (15 mL) for 7 h. The reaction was then concentrated and the residue purified by flash chromatography (SiO_2, MeOH/CH_2Cl_2) to furnish 2.0 g (77%) of **23** as a yellow solid: mp 189–191 °C; ^1H NMR (DMSO-d_6) δ 11.61 (s, 1H), 8.98 (s, 1H), 7.32 (d of d, 1H, $J = 4.3$, 7.9 Hz), 7.12 (m, 3H), 6.77 (s, 1H), 4.92 (s, 1H), 3.57 (s, 6H), 2.46 (s, 3H), 2.29 (s, 6H); ^{13}C NMR (DMSO-d_6) δ 167.3, 163.5, 149.0, 146.2, 136.5, 129.1, 126.5, 124.7, 123.9, 107.6, 101.2, 50.9, 38.6, 18.3, 14.5. Anal. calcd for $C_{20}H_{23}N_3O_6S \cdot 0.5H_2O$: C, 54.29; H, 5.47; N, 9.50; S, 7.25. Found: C, 54.13; H, 5.21; N, 9.29; S, 7.40.

3.1.24. 1,4-Dihydro-4-[3-[[3-[4-(3-methoxyphenyl)-1-piperidinyl]propyl]amino]-2-(nitro)ethylen-1-yl]amino]phenyl]-2,6-dimethyl-3,5-pyridinedicarboxylic acid, dimethyl ester, hydrochloride (24). A solution of **23** (868 mg, 2.00 mmol) and **3a** (744 mg, 3.00 mmol) in MeOH (20 mL) was refluxed for 90 min. The resulting solution was cooled to room temperature and concentrated in vacuo. The crude material was purified by flash chromatography (SiO_2, MeOH/EtOAc) to afford 835 mg, (66%) of **24**. The product was converted to its hydrochloride salt by treatment with ethereal HCl: mp 174–184 °C; ^1H NMR (DMSO-d_6) δ 10.81 (br s, 1H), 9.08 (s, 1H), 7.27 (m, 3H), 7.06 (m, 2H), 6.81 (m, 3H), 5.75 (s, 1H), 4.86 (s, 1H), 3.89 (m, 2H), 3.37 (s, 3H), 3.57 (m, 2H), 3.54 (s, 6H), 3.09 (m, 4H), 2.80 (m, 1H), 2.27 (s, 6H), 2.11 (m, 4H), 1.94 (m, 2H); ^{13}C NMR (DMSO-d_6) δ 167.4, 159.5, 155.3, 149.6, 148.0, 146.2, 146.0, 129.7, 129.4, 125.7, 122.1, 118.8, 116.2, 112.8, 111.8, 101.3, 98.2, 65.0, 55.1, 53.6, 52.1, 50.9, 38.8, 29.8, 23.4, 18.3, 15.3. Anal. calcd for $C_{34}H_{43}N_5O_7 \cdot HCl \cdot 2.6H_2O$: C, 56.96; H, 6.91; N, 9.77. Found: C, 56.66; H, 6.51; N, 9.47.

3.1.25. 4-[3-[(2-Ethoxy-3,4-dioxo-1-cyclobuten-1-yl)amino]phenyl]-1,4-dihydro-2,6-dimethyl-3,5-pyridinedicarboxylic acid, dimethyl ester (25). A mixture of **1** (950 mg, 3.01 mmol) and 3,4-diethoxy-3-cyclobutene-1,2-dione (527 mg, 3.09 mmol) in DMF (6 mL) was heated to 100 °C for 16 h under N_2. After addition of H_2O, the phases were separated and the aqueous portion extracted with EtOAc (3×15 mL). After drying the combined organic extracts (Na_2SO_4) and concentration in vacuo, 690 mg (52%) of **25** was isolated as off-white, crystalline solid: mp 249–252 °C; ^1H NMR (DMSO-d_6) δ 10.66 (br s, 1H), 8.92 (b. s, 1H), 7.21–7.16 (m, 3H), 6.91–6.87 (m, 1H), 4.89 (s, 1H), 4.75 (q, 2H, $J = 7.1$ Hz), 3.55 (s, 6H), 2.27 (s, 6H), 1.41 (t, 3H, $J = 7.1$ Hz); ^{13}C NMR (DMSO-d_6) δ 196.9, 184.2, 167.2, 148.7, 145.9, 137.7, 128.4, 122.7, 118.1, 117.1, 100.9, 69.3, 50.5, 18.1, 15.5. Anal. calcd for $C_{23}H_{24}N_2O_7 \cdot 0.4H_2O$: C, 61.71; H, 5.58; N, 6.26. Found: C, 61.74; H, 5.48; N, 6.37.

3.1.26. 1,4-Dihydro-4-[3-[2-[3-[4-(3-methoxyphenyl)-1-piperidinyl]propyl]amino]-3,4-dioxo-1-cyclobuten-1-yl]amino]phenyl]-2,3-dimethyl-3,5-pyridindicarboxylic acid, dimethyl ester (26). A mixture of **25** (54.7 mg, 0.124 mmol) and **3a** (41.0 mg, 0.165 mmol) was heated in DMF (2 mL) at 100 °C for 16 h. After addition of H_2O and cooling to room temperature, the phases were separated and the aqueous portion extracted with EtOAc (3×15 mL). The combined organic portions were dried (Na_2SO_4) and then concentrated in vacuo. The resulting crude material was purified by chromatography (SiO_2, MeOH/CH_2Cl_2) to give 43 mg (54%) of **26** as a white foam: ^1H NMR (DMSO-d_6) δ 9.86 (br s,

1H), 8.93 (br s, 1H), 7.95 (br s, 1H), 7.30 (d, 1H, $J=7.7$ Hz), 7.18–7.13 (m, 4H), 6.83–6.74 (m, 4H), 4.88 (s, 1H), 3.72 (s, 3H), 3.66–3.63 (m, 2H), 3.55 (s, 6H), 3.06 (m, 2H), 2.6–2.4 (m, 2H), 2.26 (s, 6H), 1.9–1.7 (m, 8H); ^{13}C NMR (DMSO-d_6) δ 167.4, 159.3, 146.0, 139.0, 129.4, 118.8, 112.5, 101.1, 54.9, 50.7, 18.3.[38] Anal. calcd for $C_{36}H_{42}N_4O7{\cdot}2.10H_2O$: C, 63.53; H, 6.84; N, 8.23. Found: C, 63.48; H, 6.85; N, 8.58.

3.1.27. (±)-1,4-Dihydro-4-[3-[4-(ethoxy)-1,2,5-thiadiaz-3-ole-1-oxide]amino]phenyl]-2,6-dimethyl-3,5-pyridinedicarboxylic acid, dimethyl ester (27). $AlMe_3$ (1.1 mL of a 2.0 M soln in hexanes; 2.2 mmol) was added to **1** (316 mg, 1.00 mmol) in CH_2Cl_2 (10 mL) at 0 °C. The mixture was stirred for 30 min at 0 °C, followed by the addition of 4,5-diethoxythiadiazole oxide (285 mg, 1.50 mmol). The reaction was then brought to reflux for 18 h, cooled to 0 °C, and quenched by the slow addition of sat NH_4Cl. The crude product was extracted with CH_2Cl_2, dried (Na_2SO_4), and concentrated in vacuo. The residue was purified by flash chromatography (SiO_2, MeOH/CH_2Cl_2) to afford 220 mg (48%) of **27** as a yellow solid: mp 191–197 °C; ^{1}H NMR (DMSO-d_6) δ 10.30 (s, 1H), 8.94 (s, 1H), 7.84 (s, 1H), 7.64 (d, 1H, $J=8.0$ Hz), 7.24 (t, 1H, $J=8.0$ Hz), 6.94 (d, 1H, $J=8.0$ Hz), 4.93 (s, 1H), 4.55 (m, 2H), 3.56 (s, 6H), 2.27 (s, 6H), 1.45 (t, 3H, $J=7.1$ Hz); ^{13}C NMR (DMSO-d_6) δ 167.3, 164.0, 152.6, 148.3, 145.9, 137.7, 128.4, 123.4, 119.5, 118.3, 101.0, 68.9, 50.7, 38.4, 18.2, 13.8. Anal. calcd for $C_{21}H_{24}N_4O_6S{\cdot}0.5H_2O$: C, 53.72; H, 5.37; N, 11.93. Found C, 53.94; H, 5.25; N, 12.17.

3.1.28. (±)-1,4-Dihydro-4-[3-[3-[3-[4-(3-methoxyphenyl)-1-piperidinyl]propyl]amino]-1,2,5-thiadiaz-2-ole-1-oxide]amino]phenyl]-2,6-dimethyl-3,5-pyridinedicarboxylic acid, dimethyl ester (28). A solution of **27** (263 mg, 0.572 mmol) and **3a** (149 mg, 0.601 mmol) in MeCN (3 mL) was stirred at room temperature for 18 h. After concentration in vacuo, the residue was purified by flash chromatography (SiO_2, MeOH/CH_2Cl_2) to give 110 mg (55%) of **28** as a yellow solid: mp 128–134 °C. ^{1}H NMR ($CDCl_3$) δ 7.45 (br s, 1H), 7.11 (t, 1H, $J=7.8$ Hz), 7.00 (d, 1H, $J=7.8$ Hz), 6.68 (m, 6H), 6.36 (br s, 1H), 4.97 (s, 1H), 3.70 (s, 3H), 3.54 (s, 6H), 3.29 (m, 1H), 3.19 (m, 1H), 2.98 (m, 2H), 2.42 (m, 3H), 2.26 (s, 3H), 2.19 (s, 3H), 2.08 (m, 2H), 1.75 (m, 6H); ^{13}C NMR ($CDCl_3$) δ 168.1, 159.7, 159.2, 153.5, 148.2, 147.4, 145.5, 145.4, 137.7, 129.5, 128.7, 124.4, 119.9, 119.2, 118.1, 112.9, 111.4, 103.1, 102.9, 55.8, 55.2, 54.2, 54.1, 51.2, 51.1, 42.6, 42.2, 38.8, 32.9, 25.1, 19.7, 19.5. Anal. calcd for $C_{34}H_{42}N_6O_6S{\cdot}1.9H_2O$: C, 58.58; H, 6.62; N, 12.05. Found: C, 58.97; H, 6.32; N, 11.65. HRMS calcd for $C_{34}H_{43}N_6O_6S$ (M + H): 663.2965. Found: 663.2976.

3.1.29. 1-[4-(3-Methoxypheny)-1-piperidinyl]acetic acid hydrazide (29a). A mixture of 4-(3-methoxyphenyl)piperidine (7.64 g, 40.0 mmol), methyl bromoacetate (6.24 g, 40.8 mmol), K_2CO_3 (6.44 g, 46.7 mmol) and MeCN (125 mL) was refluxed overnight under N_2. After cooling to room temperature, the volatiles were removed in vacuo and the residue taken up in H_2O and extracted with CH_2Cl_2. The combined organic extracts were then washed with H_2O, brine, dried ($MgSO_4$), filtered and concentrated. The crude material was subjected to flash chromatography (SiO_2, EtOAc) to afford 7.90 g (74%) the intermediate amino ester as a yellow oil. A small portion of this material was converted to the maleic acid salt for characterization (mp 103–104 °C). A solution of the free base (7.00 g, 26.6 mmol), $NH_2NH_2{\cdot}H_2O$ (2.66 g (83.1 mmol) and EtOH (75 mL) was refluxed for 24 h. After cooling, the solution was concentrated and the crude material purified by flash chromatography (SiO_2: ammoniated MeOH/EtOAc) to afford 6.45 g (92%) of **29a** as a colorless solid: mp 82–83 °C; ^{1}H NMR (CDCl3) δ 8.22 (br s, 1H), 7.22 (m, 1H), 6.74 (m, 3H), 3.86 (br s, 2H), 3.79 (s, 3H), 3.09 (s, 2H), 2.91 (m, 2H), 2.46 (m, 1H), 2.25 (m, 2H), and 1.45 (m, 4H); ^{13}C NMR (CDCl3) δ 170.9, 159.8, 147.6, 129.5, 119.2, 112.8, 111.4, 61.1, 55.2, 55.0, 42.0, and 33.6. Anal. calcd for $C_{14}H_{21}N_3O_2$: C, 63.85; H, 8.04; N, 15.96. Found: C, 63.70; H, 7.96; N, 15.69.

3.1.30. 2-[4-(3-Methoxypheny)-1-piperidinyl]propionic acid hydrazide (29b). A solution of 4-(3-methoxyphenyl)piperidine (5.85 g, 30.6 mmol) and methyl acrylate (8.9 mL) was stirred at room temperature for 2.5 h. The volatiles were removed in vacuo and the residue filtered through a plug of SiO_2 to furnish 7.83 g (93% yield) of the intermediate amino ester as a very pale yellow oil. A solution of this material (7.20 g, 26.0 mmol), $NH_2NH_2{\cdot}H_2O$ (3.33 g, 104 mmol) and EtOH (130 mL) was refluxed for 24 h. After cooling, the solution was concentrated, and the crude material was purified by flash chromatography (SiO_2: ammoniated MeOH/EtOAc) to give 4.10 g (57%) of **29b** as a clear oil: ^{1}H NMR (DMSO-d_6) δ 9.00 (br s, 1H), 7.19 (t, 1H, $J=7.8$ Hz), 6.75 (m, 3H), 4.14 (m, 2H), 3.72 (s, 3H), 2.92 (m, 2H), 2.50 (t, 2H, $J=7.1$Hz), 2.45 (m, 1H), 2.20 (t, 2H, $J=7.1$ Hz), 1.97 (m, 2H), and 1.66 (m, 4H).

3.2. General procedure for the preparation of acylsemicarbazides 30a and 30b

To a solution of hydrazide **29** (22.8 mmol) in 65 mL of CH_2Cl_2, was added isocyanate **2** (23.0 mmol). The resulting solution was stirred overnight at room temperature, washed with H_2O, brine, and dried ($MgSO_4$). After filtration and concentration in vacuo, the residue was purified by flash chromatography (SiO_2, MeOH/CH_2Cl_2).

3.2.1. 1,4-Dihydro-4-[3-[[2-[2-[4-(3-methoxyphenyl)-1-piperidinyl]-1-oxoethyl]hydrazino]carbonyl]amino]phenyl-2,6-dimethyl-3,5-pyridinedicarboxylic acid, dimethyl ester (30a). This compound was obtained as a clear foam (26% yield): mp indistinct; ^{1}H NMR (DMSO-d_6) δ 9.51 (br s, 1H), 8.88 (br s, 1H), 8.66 (br s, 1H), 7.88 (br s, 1H), 7.21 (m, 3H), 7.07 (t, 1H, $J=7.7$ Hz), 6.76 (m, 4H), 4.86 (s, 1H), 3.73 (s, 3H), 3.55 (s, 6H), 3.34 (s, 2H), 3.02 (m, 2H), 2.45 (m, 1H), 2.25 (s, 6H), 2.19 (m, 2H), 1.71 (m, 4H); ^{13}C NMR (DMSO-d_6) δ 169.4, 167.4, 159.3, 155.2, 148.2, 148.0, 145.6, 139.3, 129.3, 128.3, 120.6, 118.9, 117.1, 116.1, 112.5, 111.3, 101.3, 60.3, 54.9, 53.8, 50.6, 38.4, 32.9, and 18.2. Anal. calcd for $C_{32}H_{39}N_5O_7{\cdot}0.81H_2O$: C, 61.96; H, 6.60; N, 11.29. Found: C, 61.96; H, 6.38, N, 11.23.

3.2.2. 1,4-Dihydro-4-[3-[[2-[3-[4-(3-methoxyphenyl)-1-piperidinyl]-1-oxopropyl]hydrazino]carbonyl]amino]phenyl-2,6-dimethyl-3,5-pyridinedicarboxylic acid, dimethyl ester (30b). This compound was obtained as a creamy white foam (29% yield): mp indistinct; ^{1}H NMR ($CDCl_3$) δ 8.29 (br s, 1H), 8.16 (br s, 1H), 7.20 (m, 4H), 7.07 (t, 1H, $J=7.7$ Hz), 6.97 (d, 1H, $J=7.9$ Hz), 6.74 (m, 4H), 4.97 (s, 1H), 3.78 (s, 3H), 3.59 (s, 6H), 3.01 (m, 2H), 2.60 (m, 2H), 2.43 (m, 3H), 2.20 (s, 6H), 2.03 (m, 2H), and 1.78 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 171.6, 168.4, 159.7, 155.3, 148.4, 147.6, 145.4, 138.6, 129.5, 128.5, 122.8, 119.4, 118.8, 117.8, 112.9, 111.4, 103.1, 55.2, 53.6, 51.0, 42.4, 39.0, 33.1, 31.0, and 19.1. Anal. calcd for $C_{33}H_{41}N_5O_7{\cdot}0.50H_2O$: C, 63.05; H, 6.73; N, 11.14. Found: C, 63.04; H, 6.66; N, 11.12.

3.3. General procedure for the preparation of oxadiazoles 31a and 31b

A solution of the acylsemicarbazide **30** (3.5 mmol) and $POCl_3$ (19 mL) was heated on a steam bath until the reaction was judged complete by TLC analysis (45 min to 2 h). The dark solution was cooled to room temperature, diluted with CH_2Cl_2 (100 mL) and then poured into 300 mL of a stirred ice–water mixture. The mixture was allowed to warm to room temperature and then extracted with 1:1 mixture of CH_2Cl_2 and MeOH. The combined organic extracts were washed with H_2O, brine, and dried ($MgSO_4$). The volatiles were removed in vacuo and the residue purified by flash chromatography (SiO_2, EtOAc/MeOH):

3.3.1. 1,4-Dihydro-4-[3-[5-[4-(3-methoxyphenyl)-1-piperidinyl]methyl]-1,3,4-oxadiazol-2-yl]amino]phenyl]-2,6-dimethyl-3,5-pyridinedicarboxylic acid, dimethyl ester, hydrochloride salt (31a). The compound was isolated as an orange-yellow solid (8% yield): mp indistinct; ^{1}H NMR (DMSO-d_6) δ 11.2 (br s, 1H), 9.00 (br s, 1H), 7.38 (m, 1H), 7.19 (m, 3H), 6.76 (m, 4H), 4.90 (s, 1H), 3.68 (s, 3H), 3.64 (s, 2H), 3.56 (s, 6H), 2.95 (m, 2H), 2.40 (m,1H), 2.27 (s, 6H), 2.19 (m, 2H), 1.72 (m, 4H). Anal. calcd for $C_{33}H_{37}N_5O_6{\cdot}2HCl{\cdot}1.60H_2O$: C, 56.56; H, 6.06; N, 9.99. Found: C, 56.56; H, 6.06; N, 9.77.

3.3.2. 1,4-Dihydro-4-[3-[5-[2-[4-(3-methoxyphenyl)-1-piperidinyl]ethyl]-1,3,4-oxadiazol-2-yl]amino]phenyl]-2,6-dimethyl-3,5-pyridinedicarboxylic acid, dimethyl ester, hydrochloride salt (31b). The compound was obtained as a yellow solid (30% yield): mp 75–80 °C (sintered); ^{13}C NMR (DMSO-d_6) δ 167.3, 160.0, 159.3, 156.3, 148.4, 145.8, 145.7, 138.4, 129.5, 128.6, 120.3, 118.6, 115.9, 114.6, 112.6, 111.6, 101.1, 54.9, 52.6, 51.9, 51.8, 50.6, 38.2, 29.6, 20.0, and 18.1. HRMS calcd for $C_{33}H_{40}N_5O_6$ (M + H): 602.2979. Found: 602.2993.

3.3.3. 4-[3-(Cyanoamino)phenyl]-1,4-dihydro-2,6-dimethyl-3,5-pyridinedicarboxylic acid, dimethyl ester (32). To a suspension of finely powdered dihydropyridine **1** (2.66 g, 8.51 mmol) in EtOH (55 mL) at 0 °C was added an excess of cyanogen bromide (1.80 g, 17.0 mmol) in a single portion, followed by the addition of solid Na_2CO_3 (2.00 g, 23.8 mmol). The mixture was allowed to warm to room temperature and stir overnight (16 h). The crude reaction mixture was purified by column chromatography (SiO_2, EtOAc/hexane) to give 2.90 g (100%) of **32** as a pale yellow solid: mp 199–201 °C; IR (KBr) 2229 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 10.5 (br s. 1H), 9.0 (s, 1H), 7.2 (t, 1H, $J=8.0$ Hz), 6.8 (m, 2H), 6.7 (d, 1H, $J=9.2$ Hz), 4.9 (s, 1H), 3.5 (s, 6H), 2.2 (s, 6H); ^{13}C NMR (DMSO-d_6) δ 167.3, 149.4, 146.0, 138.6, 129.5, 121.2, 113.7, 112.8, 112.3, 101.1, 50.8, 38.5, 18.2.

3.3.4. *3.3.4.N*-[4-(3-Methoxyphenyl)-1-piperidinyl]propyl]glycine, ethyl ester (33). A solution of ethyl bromoacetate (1.32 mL, 12.0 mmol) in MeCN (50 mL) was slowly added over a period of 1 h to a mixture of **3a** (3.00 g, 12.1 mmol) and K_2CO_3 (8.25 g, 60.0 mmol) in MeCN (100 mL). After stirring for an additional 1 h at room temperature, the mixture was filtered through Celite and the filtrate concentrated in vacuo. The material was purified by flash chromatography (SiO_2, MeOH/CH_2Cl_2) to furnish 1.27 g (32%) of **33** as an oil: ^{1}H NMR ($CDCl_3$) δ 7.20 (m, 2H), 6.74 (m, 3H), 4.18 (q, 2H, $J=7.2$ Hz), 3.78 (s, 3H), 3.40 (s, 2H), 3.11 (m, 2H), 2.68 (t, 2H, $J=6.8$ Hz), 2.49 (m, 5H), 2.07 (m, 2H), 1.83 (M, 5H), 1.26 (t, 3H, $J=7.2$ Hz); ^{1}H NMR ($CDCl_3$) δ 172.5, 159.5, 147.8, 129.4, 119.3, 112.7, 111.4, 60.8, 57.2, 55.1, 54.3, 50.9, 48.4, 42.6, 33.1, 26.9, 14.2.

3.3.5. 1,4-Dihydro-4-[3-[4,5-dihydro-1-[3-[4-(3-methoxyphenyl)-1-piperidinyl]propyl]-4-oxo-1*H*-imidazol-2-yl]-aminophenyl]-2,6-dimethyl-3,5-pyridinedicarboxylic acid, dimethyl ester, trifluoroacetic acid salt (34). A mixture of **32** (508 mg, 1.49 mmol), **33** (500 mg, 1.50 mmol) and Na_2CO_3 (126 mg, 1.50 mmol) in MeCN (25 mL) was heated at 85 °C for 48 h in a sealed tube. After cooling, the reaction mixture was concentrated in vacuo and then prepurified by column chromatography (SiO_2, MeOH/THF/EtOAc). Purification by reverse-phase preparative HPLC afforded 38.9 mg (3%) of **34** as a clear foam: ^{1}H NMR (500 MHz, MeOD) δ 1.86–2.03 (m, 4H), 2.08–2.18 (m, 2H), 2.19 (s, 6H), 3.0–3.15 (m, 3H), 3.15–3.25 (m, 2H), 3.50 (s, 6H), 3.58–3.68 (m, 2H), 3.67 (s, 3H), 3.68–3.70 (m, 2H), 4.91, (s, H), 6.54–7.38 (m, 8H), 8.0 (s, 1H), 8.42 (s, 1). HRMS calcd for $C_{35}H_{43}N_5O_6$ (M + H): 630.3292. Found: 630.3288.

3.3.6. 1,4-Dihydro-4-[3-[4,5-dihydro-1-[3-[4-(3-methoxyphenyl)-1-piperidinyl]propyl]-5-oxo-1H-imidazol-2-yl]-aminophenyl]-2,6-dimethyl-3,5-pyridinedicarboxylic acid, dimethyl ester, trifluoroacetic acid salt (35). Sodium metal (46 mg, 2.0 mmol) was dissolved in abs EtOH (8 mL) and **3a** (347 mg, 1.40 mmol) was added. After being stirred for 10 min, a suspension of **18** (644 mg, 1.88 mmol) in abs EtOH (12 mL) was added, and the reaction was briefly heated (2 min, heat gun) until the suspension dissolved. The solution was then stirred an additional 1 h at room temperature and concentrated in vacuo. The resulting thiolate was taken up in THF (10 mL) and glycine ethyl ester (247 mg, 2.4 mmol) added. After cooling to 0 °C, $HgCl_2$ (542 mg, 2 mmol) was added and the reaction mixture was stirred for 18 h at room temperature. H_2O (1.5 mL) was added and the solution filtered through Celite and rinsed with EtOH. The material was purified by reverse-phase preparative HPLC to isolate 68.7 mg (5%) of **35** as a clear foam:

^{1}H NMR (500 MHz, MeOD) δ 1.95–2.11 (m, 4H), 2.18–2.27 (m, 2H), 2.32 (s, 6H), 3.09–3.19 (m, 2H), 3.20-3.28 (m, 1H), 3.28–3.34 (m, 2H), 3.62–3.68 (m, 2H), 3.62 (s, 6H), 3.81 (s, 3H), 3.87–3.95 (m, 2H), 4.19 (s, 2H), 5.05 (s, 1H), 6.78–7.51 (m, 8H), 8.0 (s, 1H), 8.55 (s, 1H). HRMS calcd for $C_{35}H_{43}N_5O_6$ (M + H): 630.3292. Found: 630.3276.

3.4. NPY Y_1 receptor binding experiments

Membranes were harvested from SK-N-MC cells which endogenously express the human Y_1 receptor. Adherent cells were washed twice with cold (4 °C) phosphate buffered saline pH 7.4, without Ca^{2+} and Mg^{2+}. Cells were then lifted with PBS-based enzyme free cell dissociation buffer, and pelleted by centrifugation. After the cells were lysed in ice-cold hypotonic buffer (50 mM HEPES, 5 mM $MgCl_2$) using a Dounce homogenizer, they were pelleted by centrifugation (18,000 RPM, SS-34 rotor, 15 min, 4 °C). The pellets were resuspended, homogenized in ice-cold hypotonic buffer, and again centrifuged. The final membrane pellet was resuspended using a 27 G needle into a small volume of cold (4 °C) hypotonic buffer (approximately 100 μL per T175 flask, 10–20 mg/mL protein concentration). Protein concentration was measured by the Bradford method using Bio-Rad Reagent with a BSA standard curve.[39] Membranes were held on ice for up to 2 h or flash frozen in liquid N_2 and stored at −80 °C.

Membrane solutions were diluted to 0.15 μg/μL in binding buffer (20 mM HEPES, 137 mM NaCl, 5.4 mM KH_2PO_4, 1.26 mM $CaCl_2$ 0.81 mM $MgSO_4$) supplemented with 1 mg/mL bacitracin, 0.3% BSA, and 10 μg/mL aprotinin. Test compounds (or PYY for the control curve) were diluted to 0.1–1.0 μM in DMSO from 10 mM stock solutions. Each of these five concentrations were then diluted 1:100 into supplemented binding buffer, of which 20 μL was added to polypropylene assay tubes containing 30 μL supplemented binding buffer to yield a final concentration of 0.1% DMSO. For non-specific binding, PYY was diluted in 1% DMSO and Millipore H_2O and then added to assay tubes containing 30 μL supplemented binding buffer. Duplicate samples were prepared by mixing ^{125}I PYY (50 μL, 0.1 nM final concentration), test compounds or competing peptide (PYY) (50 μL, 0.1 nM to 1.0 μM final concentration) or supplemented binding buffer (50 μL 0.4% DMSO for total binding) and finally membrane suspension (100 μL) for a total volume of 200 μL. Samples were incubated in a 25 °C (±2°) shaking water bath for 1 h. Incubation was terminated by filtration through Whatman GF/B Filters (pre-soaked in 1% polyethyleneimine for at least 2 h. followed by three 5-mL washes of ice cold wash buffer (50 mM Tris, pH 7.4). Samples were then counted for ^{125}I in a Packard Cobra Auto-Gamma Counter. Non-specific binding was defined in the presence of 1 μM PYY. Binding data were analyzed by non-linear regression using ExcelFit or KaleidaGraph software. The K_d of ^{125}I PYY at the Y_1 receptor was 0.35 nM and the maximal binding capacity 0.16 pmol/mg protein. K_i's were calculated based on the Cheng–Prusoff equation.[40]

3.5. NPY Y_1 functional studies

SK-N-MC cells were seeded on 48-well cell culture plates (Polyfiltronics catalogue #PF-150-SCC9; ordered from VWR) at a density of 5×10^4–1×10^5 cells/mL and incubated at 37 °C under 5% CO_2 overnight. The medium was then aspirated and changed to fresh medium containing 1 mM IBMX (to inhibit degradation of adenylate cyclase) and 10 μM forskolin (for stimulating adenylate cyclase) and varying concentrations of NPY with or without the test compounds. The cell plate was incubated for 10 min at 37 °C. At the end of the incubation, *c*-AMP production was terminated by medium aspiration followed by addition of 1 mL 0.1 N HCl. The plates were subsequently incubated for at least one h at room temperature before starting radioimmunoassay. *c*-AMP levels were measured using a RIA kit from Amersham (RPA 509) according to the manufacturer's instruction. The *c*-AMP levels were calculated as fmol *c*-AMP/well based on a standard curve. K_b's were calculated based on Schild-plot analysis. The NPY-induced inhibition of forskolin-stimulated *c*-AMP was measured in absence, and in the presence, of three concentrations of the test compounds, and dose-ratios were calculated from IC_{50} values, and plotted according to Schild.[34]

3.6. Caco-2 cell permeability studies

The Caco-2 cell permeability coefficients (P_c's) for **4a**, **4b**, **8**, **12**, and **20** were determined in Biocoat monolayers using the procedure previously described by Chong.[41]

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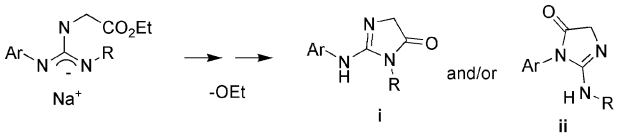

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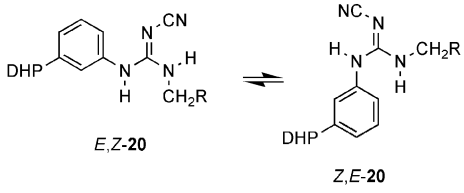

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