Discovery of *N*-(3-Fluorophenyl)-1-[(4-([(3*S*)-3-methyl-1-piperazinyl]methyl)phenyl)acetyl]-4-piperidinamine (GSK962040), the First Small Molecule Motilin Receptor Agonist Clinical Candidate

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N-(3-Fluorophenyl)-1-[(4-([(3*S*)-3-methyl-1-piperazinyl]methyl)phenyl)acetyl]-4-piperidinamine **12** (GSK962040) is a novel small molecule motilin receptor agonist. It possesses excellent activity at the recombinant human motilin receptor and also at the native rabbit motilin receptor where its agonist activity results in potentiation of the amplitude of neuronal-mediated contractions of isolated gastric antrum tissue. Compound **12** also possesses highly promising pharmacokinetic profiles in both rat and dog, and these results, in combination with further profiling in human native tissue and an in vivo model of gastrointestinal transit in the rabbit, have led to its selection as a candidate for further development.

The treatment of gastric and upper intestinal stasis offers an opportunity for the relief of symptoms associated with conditions such as diabetic gastroparesis[1] and functional dyspepsia[2] and also for improvement in recovery times of patients who are subject to enteral feeding in a critical care setting.[3] The antibiotic erythromycin **1** (Figure 1) possesses gastroprokinetic activity and is often used "off-label" in such circumstances.[1] However, there is a significant requirement for an alternative because of the safety profile of erythromycin (potential for drug/drug interactions and cardiac liabilities) as well as the need to avoid the development of antibiotic resistance through chronic usage. Erythromycin exerts its gastroprokinetic effects through agonism of the motilin receptor[4] (formerly known as GPR38[a]) for which the endogenous ligand is the 22-amino acid peptide hormone motilin.[5] The motilin receptor (MTL-R) is located primarily in the gastrointestinal tract on the enteric nerves, smooth muscle and gastric vagal nerve terminals with the highest levels present in the stomach and duodenum.[5−7]

Motilin is thought to act as a "housekeeper", promoting the migrating motor complex that sweeps the GI tract in the fasted state.[8] However, it has also been demonstrated that motilin[9] and other motilin receptor agonists such as erythromycin[10] also promote gastric emptying and propulsion of GI tract contents in an anal direction in the fed state.

Extensive progress in modifying erythromycin has been made by a number of researchers giving rise to a class of motilin receptor agonists known as the motilides.[11] Despite demonstrating improved gastric emptying in healthy volunteers, some of these motilides[12,13] have suffered difficulties in the clinic.[11,14]

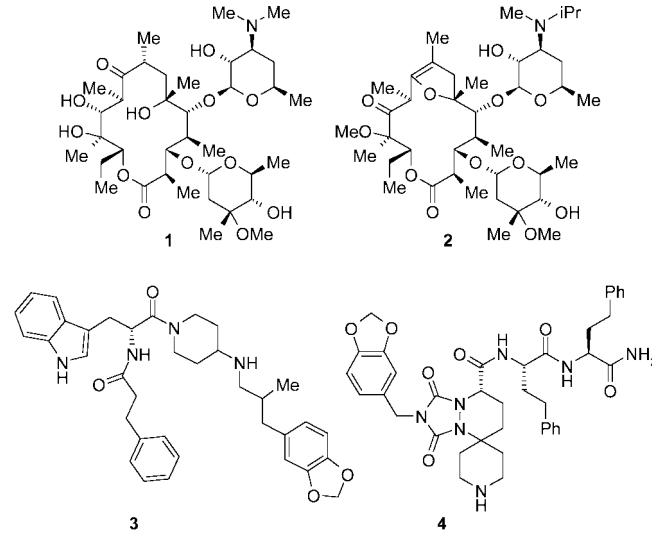

Figure 1. Published motilin receptor agonists.

However, two members of the motilide class including mitemcinal (GM-611) **2**[15] are currently in clinical trials.[14d] Non-motilide agonists **3**[16] and **4**[17] (Figure 1) have also been disclosed, but at present there is no further information on the progress of these molecules that possess relatively high molecular weights (MW 594 and 723 for **3** and **4**, respectively).

At GlaxoSmithKline we embarked on a research program to identify novel small molecule rather than motilide agonists of the motilin receptor. We have previously reported on the results of our high throughput screening approach and the subsequent identification of an initial lead molecule **5**.[18] Further optimization resulted in the discovery of amide **6**, which possessed very promising in vitro and in vivo profiles (Figure 2).[19]

In this paper we report on further optimization of **6** that resulted in the discovery of a series of 4-(piperazinylmethyl)phenylacetamides of which compound **12** (GSK962040)[20] possessed a profile suitable for further development.

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[a] Abbreviations: CLi, intrinsic clearance; CYP, cytochrome P 450; DDI, drug/drug interaction; EFS, electrical field stimulation; FLIPR, fluorometric imaging plate reader; GI, gastrointestinal; GPR38, G-protein-coupled receptor 38; MTL-R, motilin receptor; PK, pharmacokinetic; TDI, time-dependent inhibition.

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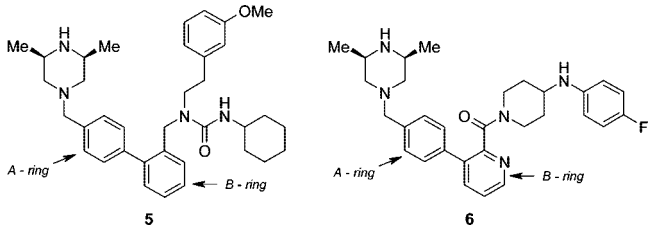


Figure 2. Motilin receptor agonist lead compounds **5** and **6**.

As previously described,[19] the overall profile of **6** was very promising as summarized in Table 1.[21] However, on further assessment of the compound as a potential candidate for preclinical development we discovered that it showed an increased potential for drug/drug interactions (DDI) in the clinic due to time-dependent inhibition (TDI) of CYP3A4 in an in vitro isolated enzyme assay as shown in Table 2. In our hands, erythromycin showed similar levels of TDI although the initial IC_{50} values were considerably lower than for **6**. While these data did not preclude further development, we rapidly undertook a further assessment of the key features of **6** in order to identify alternatives with a reduced potential for DDI.

Since inhibition of CYP3A4 is driven by size and lipophilicity, we targeted analogues of **6** with reduced molecular weight and cLogP. We had previously shown that the benzylpiperazine moiety of our lead series was crucial for motilin receptor agonist activity.[18] However, replacement of the B-ring of the biphenyl motif present in the original lead series (see Figure 2) to give, for example, the phenylpyridyl motif present in **6** was well tolerated and resulted in a much improved CYP inhibition profile.[19] Therefore, we examined removal of the B-ring in compound **6** and removal of one of the piperazine methyl groups to give a series of 4-(piperazinylmethyl)phenylpropionamides and -acetamides (Figure 3).

Chemistry

The ethylene linked analogues **7**−**9** were synthesized according to Scheme 1. Hydrogenation of 4-formylcinnamic acid gave a 1:1 mixture of 4-methyl- and 4-hydroxymethylphenylpropionic acids **18**. The acid mixture was coupled with the required piperidine, and oxidation with manganese dioxide furnished the required aldehyde intermediates **20a** and **20b**. Reductive amination with the appropriate protected piperazine followed by deprotection gave **7**−**9**. The first phenylacetamide to be synthesized **10** was prepared according to Scheme 2. 4-Formylphenylacetic acid was coupled with the required piperidine using standard conditions to give **21**, which was subject to reductive amination with Boc-protected *cis*-2,6-dimethylpiperazine. A final acidic deprotection step then afforded **10**.

Compounds **11**−**13** and **16** were synthesized as shown in Scheme 3. The key protected 4-(piperazinylmethyl)phenylacetic acid intermediates **25a/b** and **27** could be synthesized from either 4-(bromomethyl)phenyl acetic acid or 4-bromobenzaldehyde using standard methods as shown. Reaction with the appropriate 4-(substituted phenyl)piperidinamine right-hand sides under carbodiimide coupling conditions, either in solution phase or through use of polymer supported reagent, furnished the protected final products. Standard Boc- and Cbz-deprotection conditions gave the target compounds.

α-Substituted analogues **14** and **15** were prepared according to Scheme 4. Bromide **28** was prepared from 4-bromobenzaldehyde and 1-Boc-*cis*-2,6-dimethylpiperazine using standard reductive amination conditions. Palladium mediated coupling with diethyl malonate as previously described gave **29**, which was methylated, hydrolyzed, and decarboxylated to give acid **30**. Coupling with the appropriate piperidinamine and deprotection then gave **14**. Bromide **28** was also coupled with methyl isobutyrate to give **31**, which was converted to **15** using conditions similar to those described above.

Results and Discussion

Replacement of the pyridyl B-ring of **6** with a two-carbon linker **7** resulted in ∼12-fold drop in potency, and replacement of the dimethylpiperazine warhead with the (2*S*)-methylpiperazine **8** offered no improvement. However, these changes in combination with a terminal 3-fluoro substituent gave compound **9**, which possessed sufficient potency for further evaluation and we were pleased to observe a much improved TDI profile compared to **6** (Table 3).

Further reduction in molecular weight in the dimethylpiperazine series was achieved by reducing the linker size to one carbon as in **10**. The TDI profile of **10** was very encouraging (2.7-fold decrease in CYP3A4 (DEF) IC_{50}), and we went on to prepare the monomethyl analogues **11** and **12**.

We were gratified to observe an increase in agonist potency at the motilin receptor for both compounds as well as acceptable TDI profiles (<2-fold decrease in CYP3A4 (DEF) IC_{50}). Of the two compounds, **12** was preferred because its initial IC_{50} values at CYP3A4 were significantly higher than our preferred threshold of 10 μM. The corresponding (2*R*)-methyl analogue **13** of compound **12** was also synthesized but showed slightly reduced agonist potency at the motilin receptor. We also prepared analogues of **10** whereby the linker was substituted with methyl groups in an attempt to improve potency in the series **14** and **15**; however, this was unsuccessful and the CYP profiles were also unacceptable. Following further SAR exploration around the terminal phenyl ring of **12**, we found that replacement of the 3-fluoro substituent with a 3-cyano group, **16**, resulted in a further improvement in potency together with acceptable CYP inhibition and TDI profiles.

Compounds **12** and **16** were assessed for their prokinetic-like activity in native rabbit gastric antrum tissue whereby their ability to potentiate electrical-field-stimulated (EFS) cholinergic contractions of the tissue was assessed[24] (Table 4). Both compounds were less potent than [Nle^{13}]motilin (a more stable analogue of motilin),[24] as expected from their activity compared with human motilin in the recombinant assay (Table 3), and they also showed partial responses with respect to efficacy when compared to [Nle^{13}]motilin in this tissue.[24] The cyano analogue **16** gave a response similar to that of erythromycin **1** but at a 3-fold lower concentration of 1 μM, and the fluoro analogue **12** gave a lower maximal response than **1** but at the same concentration of 3 μM. With these promising yet different profiles in hand, compounds **12** and **16** were assessed further to determine their in vivo pharmacokinetic properties in the rat (Table 4).

We found that the presence of the more polar cyano group was detrimental to the oral pharmacokinetics of compound **16**. In the male Sprague−Dawley rat, **16** showed low and variable oral exposure whereas the fluoro analogue **12** gave higher and more consistent levels. Following determination of its intravenous pharmacokinetics, the oral bioavailability (F_{po}) of **12** was found to be 48 ± 13%. This highly promising result led us to determine the pharmacokinetic profile of **12** in the male beagle dog, and we were pleased to find that its oral bioavailability was 51 ± 16%.

Compound **12** has also been assessed for its inhibition of the other major human CYP isoforms, and it was found to possess

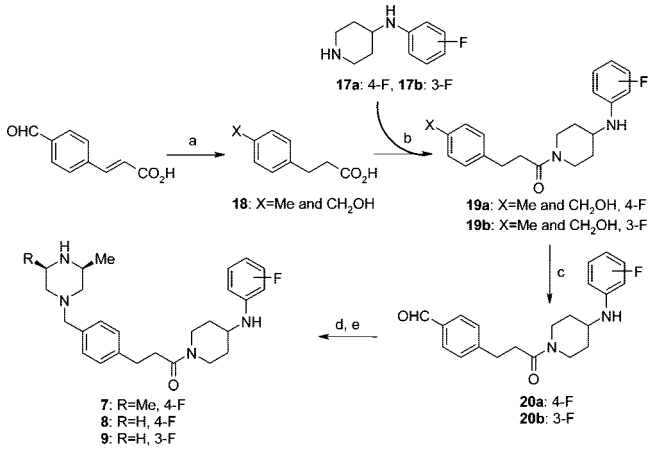
Table 1. Profile of Lead Amide **6**

hMTL-R pEC_{50}	CYP IC_{50} (μM)	CL_i (mL·min^{-1}·g^{-1}) human, rat, dog	F_{po} (%) rat, dog	rabbit native tissue potentiation	hGhrelinR pEC_{50}	hERG (binding) pIC_{50}	MW	cLogP	log *D* (pH 7.4)	aqueous solubility (mg·mL^{-1})
8.4	all >23	2.6, 2.8, <0.5	13, 58	343% at 3 μM	7.0	<4.5	501	4.1	0.7	≥1 (HCl salt)

Table 2. hMTL-R FLIPR Potency[22] and CYP3A4 (DEF) Time Dependent Inhibition (TDI) Data[23] for Lead **6** and Erythromycin **1**

compd	hMTL-R pEC_{50}	CYP 3A4 (DEF) initial IC_{50} (μM), 0−5 min	CYP 3A4 (DEF) final IC_{50} (μM), 25−30 min	fold decrease
1	7.3	1.9	0.19	10
6	8.4	20	1.4	14

Scheme 1. Synthesis of Phenylpropionamides **7**−**9**[a]



[a] Reagents: (a) H_2, 10% Pd/C, EtOH; (b) 4-(4-fluorophenyl)piperidinamine **17a** or 4-(3-fluorophenyl)piperidinamine **17b**, EDCI·HCl, HOBt, DMF, room temp; (c) MnO_2, DCM, room temp; (d) 1-Boc-*cis*-2,6-dimethylpiperazine or 1-Cbz-(2*S*)-methylpiperazine, $NaBH(OAc)_3$, 1,2-DCE, room temp; (e) TFA/DCM, room temp, or H_2, 10% Pd/C, MeOH.

a favorable profile (1A2, 2C19, 2C9 IC_{50} > 100 μM, 2D6 IC_{50} = 34 μM). Furthermore, there were no TDI liabilities at any of these isoforms or at CYP3A4 with 7BQ as the substrate. Selectivity at the closely related human ghrelin acceptor was high (pEC_{50} < 6.0), and there were no liabilities at the hERG channel (binding assay pIC_{50} = 4.8). In vitro plasma protein binding levels were acceptable (human 83%, rat 63%), and solubility in water and a range of simulated gastrointestinal fluids was high (HCl salt, >1 mg/mL).

Additionally, the duration of action of **12** in the rabbit gastric antrum native tissue assay has been determined. This will be reported in full elsewhere,[25] but in summary, **12** shows a long lasting effect ($T_{1/2}$ = 46.9 ± 5.0 min at 3 μM) when compared with the short-lived effect of [Nle^{13}]motilin ($T_{1/2}$ = 11.4 ± 1.5 min at 0.3 μM). Its duration of action is also longer than that of erythromycin **1** ($T_{1/2}$ = 24.0 ± 5.6 min at 3 μM), which is used successfully in the clinic to improve gastric emptying when dosed repeatedly at a low level. Therefore, these data may indicate a low potential for **12** to cause tachyphylaxis when

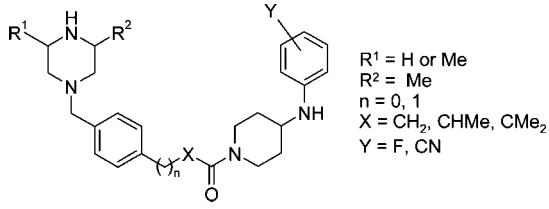
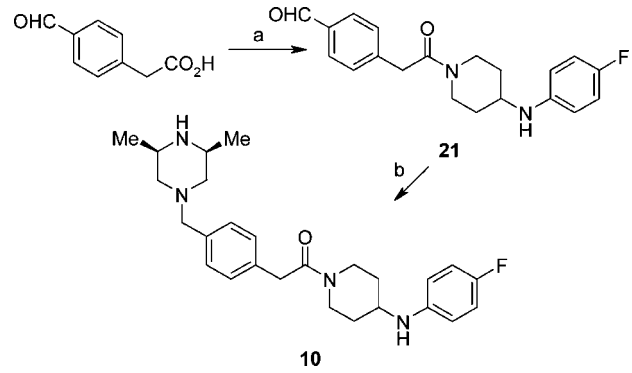


Figure 3. 4-(Piperazinylmethyl)phenylpropionamide and -acetamide targets.

Scheme 2. Synthesis of Phenylacetamide **10**[a]



[a] Reagents: (a) 4-(4-fluorophenyl)piperidinamine **17a**, EDCI·HCl, HOBt, DMF, room temp; (b) (i) 1-Boc-*cis*-2,6-dimethylpiperazine, $NaBH(OAc)_3$, 1,2-DCE, room temp, (ii) TFA/DCM, room temp.

dosed appropriately in vivo.[24] General selectivity, efficacy in human native stomach tissue, and prokinetic activity in a rabbit model of whole gut transit have also been determined for **12**, and these data will also be reported in full elsewhere.[25]

Conclusion

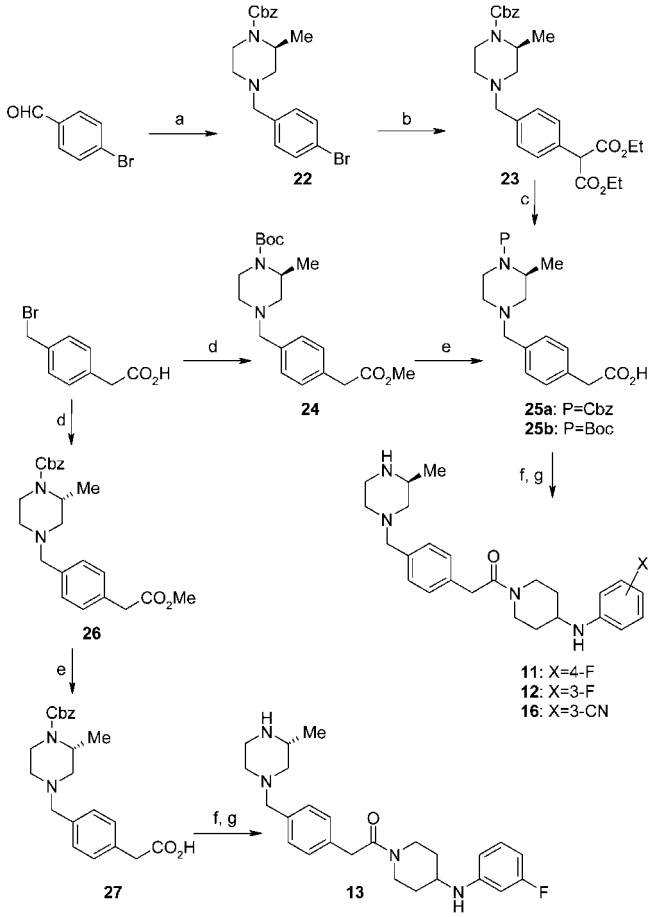
In summary, compound **12** demonstrated a highly favorable overall profile and has been selected as a candidate for further development. Thus, compound **12** represents a novel and selective small molecule motilin receptor agonist (molecular weight, 424) developed via an extensive SAR program based on activity at the recombinant human motilin receptor and is the first such compound to progress to phase I clinical trials for potential use as a therapeutic agent for conditions associated with delayed gastric emptying.

Experimental Section

Chemistry. All anhydrous solvents were purchased from Romil Ltd. Other solvents were purchased from Fisher Scientific. Commercially available reagents were used as received. All reactions were followed by TLC analysis (TLC plates GF254, Merck) or LCMS (liquid chromatography−mass spectrometry). ^{1}H NMR spectra were recorded on Bruker AVANCE 400 or DPX250 spectrometers and are referenced to TMS (tetramethylsilane) at 0 ppm. Column chromatography was performed on prepacked silica gel columns (Isolute SPE Flash Si II or Biotage Flash+) using Flashmaster II or Biotage Horizon automated chromatography systems. Electrospray mass spectra (ESI-MS) and purity were analyzed using an LCMS system as described in further detail in the Supporting Information. The UV detection was an averaged signal from wavelength of 210 to 350 nm, and mass spectra were recorded using alternate-scan positive and negative electrospray ionization. Mass directed autoprep (MDAP) purification of samples was carried out using the system and methods described in the Supporting Information.

***N*-(4-Fluorophenyl)-4-piperidinamine 17a.** A solution of 1,1-dimethylethyl 4-oxo-1-piperidinecarboxylate (1 g, 5 mmol), 4-fluoroaniline (0.56 g, 5 mmol), and acetic acid (0.26 mL, 5 mmol) in 1,2-DCE (30 mL) was stirred at room temperature for 24 h. Sodium tri(acetoxy)borohydride (1.48 g, 7 mmol) was then added, and stirring continued for 24 h. The reaction mixture was washed with water, dried ($MgSO_4$), and then concentrated in vacuo to give 1,1-dimethylethyl 4-[(4-fluorophenyl)amino]-1-piperidinecarboxylate as

Scheme 3. Synthesis of Phenylacetamides **11**−**13** and **16**[a]



[a] Reagents: (a) 1-Cbz-(2*S*)-methylpiperazine, $NaBH(OAc)_3$, 1,2-DCE; (b) diethyl malonate, $Pd(OAc)_2$, 2-(di-*tert*-butylphosphino)-2′-methylbiphenyl, K_3PO_4, 1,4-dioxane, reflux; (c) (i) 2 M NaOH, 1,4-dioxane, room temp, then 2 M HCl, (ii) PhMe, reflux; (d) (i) TMS-Cl, MeOH, room temp, (ii) 1-Boc-(2*S*)-methylpiperazine or 1-Cbz-(2*R*)-methylpiperazine, $^{i}Pr_2NEt$, DMF, 0 °C to room temp; (e) 2 M NaOH, THF, room temp. (f) For **12**, **13**, and **16**: 4-(3-fluorophenyl)piperidinamine **17b** or 4-(3-cyanophenyl)piperidinamine **17c**, EDCI·HCl, HOBt, Et_3N, DMF, room temp. For **11**: 4-(4-fluorophenyl)piperidinamine **17a**, polymer supported carbodiimide, HOBt, DMF/DCM, room temp. (g) P = Boc: TFA/DCM, 0 °C to room temp, or 2 M HCl, dioxane 50 °C. P = Cbz: H_2, 10% Pd/C or Pd black, MeOH.

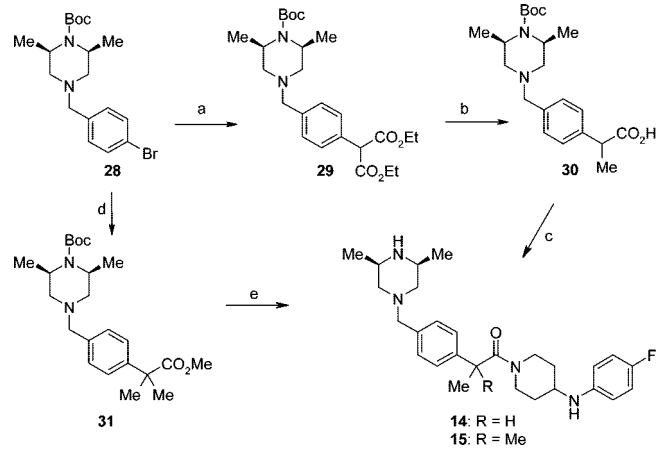
a crude solid (1.6 g). δ_H ($CDCl_3$, 250 MHz) 6.88 (2H, t, *J* 8.8 Hz), 6.54 (2H, dd, *J* 8.8, 4.4 Hz), 4.04 (2H, m), 3.35 (1H, m), 2.91 (2H, m), 2.02 (2H, m), 1.46 (9H, s), 1.30 (2H, m).

A solution of 1,1-dimethylethyl 4-[(4-fluorophenyl)amino]-1-piperidinecarboxylate (1.6 g) in 2 M HCl (5 mL) and 1,4-dioxane (20 mL) was heated at 60 °C for 24 h. On cooling, the solution was diluted with water, basified with 2 M NaOH solution, and extracted with EtOAc (×3). The combined organics were dried ($MgSO_4$) and concentrated in vacuo to give the title compound as a yellow oil (0.71 g, 73% over two steps). δ_H ($CDCl_3$, 250 MHz) 6.88 (2H, t, *J* 8.8 Hz), 6.54 (2H, dd, *J* 8.8, 4.4 Hz), 3.30 (1H, m), 3.20 (2H, m), 2.70 (2H, m), 2.05 (2H, m), 1.62 (2H, br), 1.29 (2H, m).

***N*-(3-Fluorophenyl)-4-piperidinamine 17b. 17b** was prepared as for **17a**. δ_H ($CDCl_3$, 400 MHz) 7.07 (1H, dt, *J* 6.8, 8.0 Hz), 6.33 (3H, m), 3.83 (1H, br s), 3.33 (1H, br s), 3.12 (2H, m), 2.71 (2H, m), 2.04 (2H, m), 1.30 (2H, m).

***N*-(3-Cyanophenyl)-4-piperidinamine 17c.** A mixture of BINAP (560 mg, 0.9 mmol), palladium acetate (135 mg, 0.6 mmol), and cesium carbonate (2.932 g, 9 mmol) in 1,4-dioxane (10 mL) was sonicated for 50 min. 1,1-Dimethylethyl 4-amino-1-piperidinecarboxylate (1.2 g, 6 mmol) and 3-bromobenzonitrile (1.638 g, 9 mmol) were added, and the mixture was heated to 105 °C

Scheme 4. Synthesis of α-Substituted Phenylacetamides **14** and **15**[a]



[a] Reagents: (a) diethyl malonate, $Pd(OAc)_2$, 2-(di-*tert*-butylphosphino)-2′-methylbiphenyl, K_3PO_4, 1,4-dioxane, reflux; (b) (i) NaH, MeI, DMF, 0 °C to room temp, (ii) 2 M NaOH, 1,4-dioxane, 80 °C; (c) (i) 4-(4-fluorophenyl)piperidinamine **17a**, polymer supported carbodiimide, HOBt, DMF/DCM, room temp, (ii) TFA/DCM, room temp; (d) $^{i}PrCO_2Me$, $Pd(dba)_2$, $P^{t}Bu_3$, $LiNCy_2$, PhMe, room temp; (e) (i) LiOH, 1,4-dioxane/H_2O, room temp, (ii) 4-(4-fluorophenyl)piperidinamine **17a**, polymer supported carbodiimide, HOBt, DMF/DCM, room temp, (iii) 4 M HCl/1,4-dioxane, room temp.

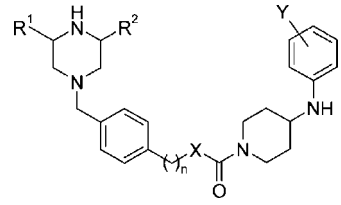
overnight under an argon atmosphere. When the mixture was cooled, the solvent was removed in vacuo and the residue partitioned between water (100 mL) and EtOAc (100 mL). The organic layer was separated, dried, and concentrated and the crude product purified by column chromatography. Elution with a 0−50% Et_2O/petroleum ether gradient gave 1,1-dimethylethyl 4-[(3-cyanophenyl)amino]-1-piperidinecarboxylate as a white solid (1.49 g, 83%). δ_H ($CDCl_3$, 250 MHz) 7.22 (1H, t, *J* 7.6 Hz), 6.95 (1H, m), 6.77 (2H, m), 4.07 (2H, m), 3.77 (1H, m), 3.41 (1H, m), 2.93 (2H, m), 2.03 (2H, m), 1.47 (9H, s), 1.34 (2H, m). MS (ES^+): 302 (MH^+).

A solution of 1,1-dimethylethyl 4-[(3-cyanophenyl)amino]-1-piperidinecarboxylate (750 mg, 2.43 mmol) in DCM (30 mL) was cooled in an ice bath, and TFA (6 mL) was added. The reaction mixture was then stirred at room temperature for 1 h. The solvent was removed in vacuo and the residue loaded onto an Isolute SCX cartridge. Elution with MeOH (100 mL) followed by 2 M NH_3 in MeOH (100 mL) followed by removal of solvent gave **17c** as a white solid (613 mg, 122% yield, 76% pure, contains residual DCM and MeOH). δ_H ($CDCl_3$, 250 MHz) 7.21 (1H, t, *J* 7.6 Hz)), 6.93 (1H, m), 6.77 (2H, m), 3.78 (1H, m), 3.35 (1H, m), 3.14 (2H, m), 2.73 (2H, m), 2.06 (2H, m), 1.34 (2H, m). MS (ES^+): 202 (MH^+).

1-[3-(4-[(3*R*,5*S*)-3,5-Dimethyl-1-piperazinyl]methyl)phenyl)propanoyl]-*N*-(4-fluorophenyl)-4-piperidinamine 7. (2*E*)-3-(4-Formylphenyl)-2-propenoic acid (2.55 g) was dissolved in EtOH (250 mL) and hydrogenated at atmospheric pressure with 10% Pd/C (0.8 g) as catalyst. After 5 h the reaction mixture was filtered and concentrated to give an ∼1:1 mixture of 3-[4-(hydroxymethyl)phenyl]propanoic acid and 3-(4-methylphenyl)propanoic acid **18** (2.43 g, 98%). LCMS: hydroxymethyl product, (ES^+) 163 ($MH^+ - H_2O$), (ES^-) 179 ($M - H^+$), retention time 1.55 min; methyl product, (ES^+) 147 ($MH^+ - H_2O$), (ES^-) 163 ($M - H^+$), retention time 2.43 min.

Acid mixture **18** (500 mg), *N*-[3-(dimethylamino)propyl]-*N*′-ethylcarbodiimide hydrochloride (723 mg, 3.78 mmol), and 1-hydroxybenzotriazole (578 mg, 3.78 mmol) in DMF (10 mL) were treated with *N*-(4-fluorophenyl)-4-piperidinamine **17a** (561 mg, 2.9 mmol), and the mixture was stirred at room temperature for 2 h. The DMF was removed in vacuo, and EtOAc and water were added. The aqueous layer was extracted with EtOAc, and the combined organics were washed with saturated aqueous $NaHCO_3$ solution

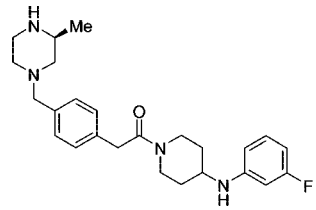
Table 3. hMTL-R FLIPR Potency[22] for Compounds **7**−**16**, Human Motilin, and Erythromycin **1** and CYP3A4 and Time Dependent Inhibition (TDI) Data[23] for Compounds **7**−**16**



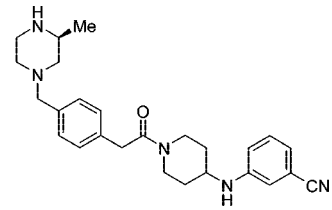
compd	R^1	R^2	*n*	X	Y	hMTL-R pEC_{50}	hMTL-R IA[a]	MW	cLogP	CYP 3A4 IC_{50} (μM)[b] DEF	CYP 3A4 IC_{50} (μM)[b] 7BQ	CYP 3A4 (DEF) TDI fold decrease
7	(*R*)-Me	(*S*)-Me	1	CH_2	4-F	7.3	0.9	452	3.6	nd	nd	nd
8	H	(*S*)-Me	1	CH_2	4-F	7.2	1.2	438	3.1	nd	nd	nd
9	H	(*S*)-Me	1	CH_2	3-F	7.6	1.0	438	3.1	10	18	3.2
10	(*R*)-Me	(*S*)-Me	0	CH_2	4-F	7.4	0.9	438	3.8	41	59	2.7
11	H	(*S*)-Me	0	CH_2	4-F	8.0	0.8	424	3.3	4.9	14	0.4
12	H	(*S*)-Me	0	CH_2	3-F	7.9	0.9	424	3.3	26	69	1.4
13	H	(*R*)-Me	0	CH_2	3-F	7.7	0.7	424	3.3	29	69	nd
14	(*R*)-Me	(*S*)-Me	0	CHMe	4-F	7.5	1.1	452	4.1	8.7	nd	7.6
15	(*R*)-Me	(*S*)-Me	0	CMe_2	4-F	7.5	0.7	466	4.5	4.7	nd	6.1
16	H	(*S*)-Me	0	CH_2	3-CN	8.4	1.1	431	3.0	36	58	2.0
h-motilin						10.4	1.0	2699				
1						7.3	1.3	733				

[a] IA: intrinsic activity relative to motilin (1.0). [b] nd: not determined.

Table 4. Prokinetic-like Activity in Isolated Rabbit Gastric Antrum of **12**, **16**, Erythromycin **1**, and [Nle[13]]Motilin[24] and Rat Pharmacokinetic Profiles of **12** and **16**



12



16

compd	% potentiation of EFS-evoked contractions (n = 4−8)[a] concn 3 μM	concn 1 μM	concn 0.3 μM	concn 0.1 μM	CL_i (mL·min^{-1}·g^{-1}), human, rat	rat PK 5 mg·kg^{-1} po (n = 3) C_{max} (μM)	T_{max} (h)	AUC(0−t)/dose (min·kg·L^{-1})
12	248 ± 47	108 ± 21	53 ± 30	nd	0.6, ≤1.0	0.4 ± 0.1	2−3	12 ± 3
16	nd	462 ± 99	218 ± 86	98 ± 16	0.5, <0.5	0.2 ± 0.2	4−6	2.8 ± 2.6
1	490 ± 17	189 ± 71	69 ± 32	14 ± 6				
[Nle[13]]motilin	nd	nd	740 ± 151	721 ± 185				

[a] nd: not determined.

and brine, then dried (Na_2SO_4) and concentrated to give a crude mixture of [4-(3-(4-[(4-fluorophenyl)amino]-1-piperidinyl)-3-oxopropyl)phenyl]methanol and *N*-(4-fluorophenyl)-1-[3-(4-methylphenyl)propanoyl]-4-piperidinamine **19a** (1.1 g). LCMS: hydroxymethyl product, (ES^+) 357 (MH^+), retention time 2.08 min; methyl product, (ES^+) 341 (MH^+), retention time 2.94 min.

Mixture **19a** (1.1 g) was dissolved in DCM (20 mL) and treated with manganese dioxide (2 g). After the mixture was stirred overnight, further manganese dioxide was added (5 g), and stirring continued for 30 min. The mixture was filtered, concentrated, and purified by column chromatography (10−90% EtOAc/pentane) to give **20a** as a yellow gum (282 mg, 28% over two steps). δ_H ($CDCl_3$, 400 MHz) 9.96 (1H, s), 7.80 (2H, d, *J* 8.0 Hz), 7.39 (2H, d, *J* 8.0 Hz), 6.86 (2H, t, *J* 9.0 Hz), 6.53 (2H, dd, *J* 9.2, 4.4 Hz), 4.49 (1H, br d), 3.80 (1H, br d), 3.41 (2H, m), 3.11 (1H, m), 3.06 (2H, t, *J* 7.8 Hz), 2.84 (1H, m), 2.68 (2H, t, *J* 7.8 Hz), 2.05 (2H, m), 1.25 (2H, m). LCMS: (ES^+) 355 (MH^+), retention time 2.43 min, >98% pure.

To a mixture of **20a** (100 mg, 0.282 mmol) and 1,1-dimethylethyl (2*R*,6*S*)-2,6-dimethyl-1-piperazinecarboxylate (61 mg, 0.283 mmol) in 1,2-DCE (5 mL) was added sodium tri(acetoxy)borohydride (90 mg, 0.423 mmol), and the mixture was stirred at room temperature overnight. Saturated aqueous $NaHCO_3$ solution was added, and the mixture was stirred for 15 min and then extracted with EtOAc. The combined extracts were dried (Na_2SO_4) and concentrated in vacuo to give the crude product which was purified by column chromatography, eluting with 0−100% EtOAc/pentane to give 1,1-dimethylethyl (2*R*,6*S*)-4-([4-(3-(4-[(4-fluorophenyl)amino]-1-piperidinyl)-3-oxopropyl)phenyl]methyl)-2,6-dimethyl-1-piperazinecarboxylate. δ_H ($CDCl_3$, 400 MHz) 7.29 (2H, d, *J* 7.8 Hz), 7.17 (2H, d, *J* 7.8 Hz), 6.87 (2H, t, *J* 8.8 Hz), 6.53 (2H, dd, *J* 9.2, 4.4 Hz), 4.52 (1H, br d), 4.05 (2H, m), 3.80 (1H, br d), 3.45 (2H, s), 3.39 (2H, m), 3.10 (1H, m), 2.96 (2H, m), 2.84 (1H, m), 2.60 (4H, m), 2.08 (4H, m), 1.46 (9H, s), 1.29 (6H, d, *J* 6.8 Hz), 1.22 (2H, m). LCMS: (ES^+) 553 (MH^+), retention time 2.39 min, >98% pure. This whole sample was dissolved in 2:1 DCM/TFA (3 mL) and stirred for 1.5 h. The mixture was concentrated and eluted through an Isolute SCX cartridge (2 M NH_3 in MeOH) to give **7** (100 mg, 78% over two steps). δ_H ($CDCl_3$, 400 MHz) 7.23 (2H, d, *J* 8.0 Hz), 7.17 (2H, d, *J* 8.0 Hz), 6.88 (2H, t, *J* 8.8 Hz), 6.53 (2H, m, *J* 8.8, 4.4 Hz), 4.52 (1H, m), 3.79 (1H, m), 3.42 (4H, m), 3.09 (1H, m), 2.94 (4H, m), 2.83 (1H, m), 2.75 (2H, m), 2.63 (2H, m), 2.03 (2H, m), 1.59 (2H, t, *J* 10.6 Hz), 1.29 (1H, m), 1.17 (1H, m), 1.02 (6H, d, *J* 6.4 Hz). LCMS: (ES^+) 453 (MH^+), retention time 1.50 min, >98% pure. Free base **7** (100 mg) was dissolved in DCM

and treated with 1 M HCl in Et_2O (243 μL) to give the hydrochloride salt (98 mg).

***N*-(4-Fluorophenyl)-1-[3-(4-([(3*S*)-3-methyl-1-piperazinyl]methyl)phenyl)propanoyl]-4-piperidinamine 8.** To a mixture of **20a** (100 mg, 0.282 mmol) and phenylmethyl (2*S*)-2-methyl-1-piperazinecarboxylate (66 mg, 0.281 mmol) in 1,2-DCE (5 mL) was added sodium tri(acetoxy)borohydride (90 mg, 0.423 mmol), and the mixture was stirred at room temperature overnight. Saturated aqueous $NaHCO_3$ solution was added, and the mixture was stirred for 15 min and then extracted with EtOAc. The combined extracts were dried (Na_2SO_4) and concentrated in vacuo to give the crude product which was purified by column chromatography, eluting with 0−100% EtOAc/pentane to give phenylmethyl (2*S*)-4-([4-(3-(4-[(4-fluorophenyl)amino]-1-piperidinyl)-3-oxopropyl)phenyl]methyl)-2-methyl-1-piperazinecarboxylate. δ_H ($CDCl_3$, 400 MHz) 7.27−7.35 (5H, m), 7.24 (2H, d, *J* 8.0 Hz), 7.16 (2H, d, *J* 8.0 Hz), 6.87 (2H, t, *J* 8.8 Hz), 6.52 (2H, m), 5.12 (2H, 2 × AB d), 4.51 (1H, br d), 4.27 (1H, br s), 3.88 (1H, br d), 3.79 (1H, br d), 3.48 (1H, d, *J* 13.2 Hz), 3.39 (2H, m), 3.18 (1H, m), 3.09 (1H, m), 2.96 (2H, m), 2.83 (1H, m), 2.75 (1H, br d), 2.65−2.58 (3H, m), 2.13 (1H, m), 2.05−1.97 (4H, m), 1.27 (3H, d, *J* 6.8 Hz), 1.23 (2H, m). LCMS: (ES^+) 573 (MH^+), retention time 2.21 min, 95% pure.

This material was hydrogenated in MeOH (5 mL) with 10% Pd/C catalyst (20 mg) for 2.5 h. The mixture was filtered, concentrated, and purified by MDAP to give **8** (69 mg, 56% over two steps). δ_H ($CDCl_3$, 400 MHz) 7.24 (2H, d, *J* 8.0 Hz), 7.17 (2H, d, *J* 8.0 Hz), 6.88 (2H, t, *J* 8.8 Hz), 6.53 (2H, m), 4.51 (1H, m), 3.79 (1H, m), 3.43 (4H, m), 3.09 (1H, m), 2.73−2.98 (8H, m), 2.63 (2H, t, *J* 7.8 Hz), 1.96−2.06 (4H, m), 1.66 (1H, t, *J* 10.4 Hz), 1.27 (1H, m), 1.15 (1H, m), 1.00 (3H, d, *J* 6.4 Hz). LCMS: (ES^+) 439 (MH^+), retention time 1.46 min, >98% pure. Free base **8** (69 mg) was treated with 1 M HCl in Et_2O (174 μL) to give the hydrochloride salt (69 mg).

***N*-(3-Fluorophenyl)-1-[3-(4-([(3*S*)-3-methyl-1-piperazinyl]methyl)phenyl)propanoyl]-4-piperidinamine 9.** Compound **9** was prepared from 3-[4-(hydroxymethyl)phenyl]propanoic acid and 3-(4-methylphenyl)propanoic acid **18**, *N*-(3-fluorophenyl)-4-piperidinamine **17b**, and phenylmethyl (2*S*)-2-methyl-1-piperazinecarboxylate using the same procedure as that described for compound **8**. δ_H ($CDCl_3$, 400 MHz) 7.24 (2H, d, *J* 8.0 Hz), 7.17 (2H, d, *J* 8.0 Hz), 7.08 (1H, m), 6.36 (2H, m), 6.27 (1H, m), 4.52 (1H, br d), 3.79 (1H, br d), 3.46 (2H, s), 3.43 (1H, m), 3.09 (1H, m), 2.73−2.98 (8H, m), 2.63 (2H, m), 2.01 (3H, m), 1.83 (1H, br s), 1.65 (1H, t, *J* 10.6 Hz), 1.30 (1H, m), 1.16 (1H, m), 1.00 (3H, d, *J* 6.4 Hz). LCMS: (ES^+) 439 (MH^+), retention time 1.76 min, >98% pure.

1-[(4-([(3*R*,5*S*)-3,5-Dimethyl-1-piperazinyl]methyl)phenyl)acetyl]-*N*-(4-fluorophenyl)-4-piperidinamine 10. A mixture of (4-formylphenyl)acetic acid (87 mg, 0.53 mmol), *N*-(4-fluorophenyl)-4-piperidinamine **17a** (102 mg, 0.53 mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (123 mg, 0.64 mmol), and 1-hydroxybenzotriazole (98 mg, 0.64 mmol) in DMF (2 mL) was stirred at room temperature overnight. The DMF was removed in vacuo, and then EtOAc and water were added. The product was extracted into EtOAc, and the combined organic layers were washed with saturated aqueous $NaHCO_3$ solution (×2) and brine and then dried (Na_2SO_4). The solvent was removed in vacuo and the resulting residue was purified by column chromatography, eluting with an EtOAc/petroleum ether gradient to afford **21** (149 mg, 83%). δ_H ($CDCl_3$, 400 MHz) 10.0 (1H, s), 7.86 (2H, d, *J* 8.0 Hz), 7.43 (2H, d, *J* 8.0 Hz), 6.86 (2H, m), 6.57 (2H, m), 4.51 (1H, m), 3.85 (1H, m), 3.83 (2H, s), 3.37 (2H, m), 3.16 (1H, m), 2.90 (1H, m), 2.03 (2H, m), 1.29 (1H, m), 1.12 (1H, m). LCMS: (ES^+) 341 (MH^+), (ES^-) 339 ($M - H^+$), retention time 2.17 min, >98% pure.

A mixture of **21** (149 mg, 0.438 mmol) and 1,1-dimethylethyl (2*R*,6*S*)-2,6-dimethyl-1-piperazinecarboxylate (94 mg, 0.438 mmol) in 1,2-DCE (3 mL) was stirred for 5 min at room temperature. Sodium tri(acetoxy)borohydride (139 mg, 0.66 mmol) was added, and the mixture was stirred for 3 h. Then saturated aqueous $NaHCO_3$ solution was added. The mixture was stirred for 15 min and then extracted with EtOAc. The combined extracts were dried (Na_2SO_4) and concentrated in vacuo to give the crude product, which was purified by column chromatography. Elution with 20−90% EtOAc/pentane gave 1,1-dimethylethyl (2*R*,6*S*)-4-([4-(2-(4-[(4-fluorophenyl)amino]-1-piperidinyl)-2-oxoethyl)phenyl]methyl)-2,6-dimethyl-1-piperazine carboxylate (143 mg, 61%). δ_H ($CDCl_3$, 400 MHz) 1.06 (1H, m), 1.27 (6H, d, *J* 6.4 Hz), 1.30 (1H, m), 1.46 (9H, s), 1.93 (1H, m), 2.05 (1H, m), 2.12 (2H, dd, *J* 11.2, 4.4 Hz), 2.59 (2H, d, *J* 11.2 Hz), 2.88 (1H, m), 3.13 (1H, m), 3.29 (1H, br s), 3.38 (1H, m), 3.45 (2H, s), 3.74 (2H, s), 3.86 (1H, m), 4.07 (2H, m), 4.50 (1H, m), 6.50 (2H, m), 6.87 (2H, t, *J* 8.8 Hz), 7.20 (2H, d, *J* 8.0 Hz), 7.31 (2H, d, *J* 8.0 Hz). LCMS: (ES^+) 539 (MH^+), retention time 1.85 min, >98% pure.

This whole sample was dissolved in 2:1 DCM/TFA and stirred for 1 h. The mixture was concentrated and eluted through an Isolute SCX cartridge (2 M NH_3 in MeOH) to give free base **10**. δ_H ($CDCl_3$, 400 MHz) 1.01 (6H, d, *J* 6.4 Hz), 1.06 (1H, m), 1.29 (1H, m), 1.62 (2H, t, *J* 10.4 Hz), 1.93 (1H, m), 2.04 (1H, m), 2.74 (2H, d, *J* 10.4 Hz), 2.83−2.94 (3H, m), 3.12 (1H, m), 3.36 (2H, m), 3.46 (2H, s), 3.73 (2H, s), 3.86 (1H, m), 4.51 (1H, m), 6.55 (2H, m), 6.86 (2H, t, *J* 8.8 Hz), 7.20 (2H, d, *J* 8.0 Hz), 7.26 (2H, d, *J* 8.0 Hz). LCMS: (ES^+) 439 (MH^+), retention time 1.20 min, >98% pure. Free base **10** was converted to the dihydrochloride salt (104 mg, 77% from Boc-protected material).

***N*-(4-Fluorophenyl)-1-[(4-([(3*S*)-3-methyl-1-piperazinyl]methyl)phenyl)acetyl]-4-piperidinamine 11.** A mixture of 4-bromobenzaldehyde (1.19 g, 6.42 mmol), phenylmethyl (2*S*)-2-methyl-1-piperazinecarboxylate (1.505 g, 6.42 mmol), and sodium triacetoxy borohydride (2.04 g, 9.63 mmol) in 1,2-DCE (15 mL) was stirred at room temperature overnight. Saturated aqueous $NaHCO_3$ solution was added and the mixture stirred for 30 min and then extracted with EtOAc. The extracts were dried (Na_2SO_4) and concentrated. Column chromatography (0−30% EtOAc in pentane) gave **22** (2.18 g, 84%). δ_H ($CDCl_3$, 400 MHz) 7.43 (2H, d, *J* 8.4 Hz), 7.34 (5H, m), 7.21 (2H, d, *J* 8.4 Hz), 5.13 (2H, AB 2 × d), 4.28 (1H, br s), 3.90 (1H, m), 3.47 (1H, d, *J* 13.6 Hz), 3.34 (1H, d, *J* 13.6 Hz), 3.18 (1H, td, *J* 12.8, 3.2 Hz), 2.74 (1H, m), 2.57 (1H, dd, *J* 11.2, 3.6 Hz), 2.03 (1H, m), 1.26 (3H, m). LCMS: (ES^+) 403/405 (MH^+), retention time 2.39 min, >98% pure.

A mixture of **22** (1.62 g, 4 mmol), diethyl malonate (0.73 mL, 4.8 mmol), palladium(II) acetate (27 mg, 0.12 mmol), potassium phosphate (1.95 g, 2.3 mmol), and 2-(di-*tert*-butylphosphino)-2′-methylbiphenyl (83 mg, 0.264 mmol) in 1,4-dioxane (20 mL) was refluxed under argon for ∼20 h. The mixture was filtered through Celite and concentrated. Column chromatography (0−40% EtOAc/hexane) gave **23** as a clear oil (1.165 g, 60%). δ_H ($CDCl_3$, 400 MHz) 7.35 (9H, m), 5.12 (2H, AB 2 × d), 4.60 (1H, s), 4.10−4.31 (5H, m), 3.89 (1H, m), 3.51 (1H, d, *J* 13.4 Hz), 3.41 (1H, d, *J* 13.4 Hz), 3.19 (1H, td, *J* 12.8, 3.2 Hz), 2.76 (1H, m), 2.61 (1H, m), 2.15 (1H, dd, *J* 11.2, 4.0 Hz), 2.02 (1H, td, *J* 11.6, 3.2 Hz), 1.26 (9H, m). MS: (ES^+) 483 (MH^+).

A mixture of **23** (761 mg, 1.58 mmol), 2 M NaOH solution (6 mL), and 1,4-dioxane (6 mL) was stirred at room temperature for 2 h. The solvents were removed, and the residue was dissolved in water and the pH adjusted to 4 with 2 M HCl. The product was extracted with EtOAc, and the combined extracts were dried and concentrated. The product was refluxed in toluene (∼20 mL) for 2 h and the solvent was evaporated to give **25a** as a yellow foam (505 mg, 84%). δ_H ($CDCl_3$, 400 MHz) 7.34 (5H, m), 7.28 (2H, d, J 8.0 Hz), 7.26 (2H, d, J 8.0 Hz), 5.13 (2H, AB 2 × d), 4.28 (1H, m), 3.90 (1H, m), 3.63 (2H, s), 3.52 (1H, d, *J* 13.0 Hz), 3.41 (1H, d, *J* 13.0 Hz), 3.19 (1H, td, *J* 12.8, 3.2 Hz), 2.80 (1H, m), 2.63 (1H, m), 2.16 (1H, dd, *J* 11.6, 4.0 Hz), 2.03 (1H, td, *J* 11.6, 3.2 Hz), 1.27 (3H, d, *J* 6.8 Hz). MS: (ES^+) 383 (MH^+), (ES^-) 381 ($M - H^+$).

A mixture of **25a** (115 mg, 0.3 mmol), polymer-supported carbodiimide (270 mg, 1.7 mmol/g, 0.45 mmol), and 1-hydroxybenzotriazole (55 mg, 0.36 mmol) in 2:1 DMF/DCM (3 mL) was treated with *N*-(4-fluorophenyl)-4-piperidinamine **17a** (58 mg, 0.3 mmol) and stirred overnight. Scavenger resins (PS-trisamine, PS-isocyanate, and Si-carbonate) together with DCM (∼3 mL) were added. The mixture was stirred for ∼2 h and then filtered and

concentrated. Column chromatography (0−60% EtOAc/pentane) gave phenylmethyl (2*S*)-4-([4-(2-(4-[(4-fluorophenyl)amino]-1-piperidinyl)-2-oxoethyl)phenyl]methyl)-2-methyl-1-piperazinecarboxylate (148 mg, 88%). δ_H ($CDCl_3$, 400 MHz) 7.34 (5H, m), 7.27 (2H, d, J 8.0 Hz), 7.19 (2H, d, J 8.0 Hz), 6.85 (2H, t, *J* 8.8 Hz), 6.50 (2H, dd, *J* 9.2, 4.4 Hz), 5.13 (2H, AB 2 × d), 4.50 (1H, m), 4.27 (1H, br s), 3.90 (2H, m), 3.73 (2H, s), 3.49 (1H, d, *J* 13.2 Hz), 3.38 (1H, d, *J* 13.2 Hz), 3.37 (1H, m), 3.14 (2H, m), 2.87 (1H, m), 2.75 (1H, m), 2.58 (1H, m), 2.14 (1H, dd, *J* 11.2, 3.6 Hz), 2.01 (2H, m), 1.93 (1H, m), 1.30 (1H, m), 1.25 (3H, m), 1.07 (1H, m). LCMS: (ES^+) 559 (MH^+), retention time 2.12 min, >95% pure.

This whole sample was hydrogenated in MeOH (∼5 mL) with 10% Pd/C catalyst (∼20 mg) for 2 h. Column chromatography (0−20% MeOH/DCM) gave **11** (19 mg, 17%). δ_H ($CDCl_3$, 400 MHz) 7.27 (2H, d, J 8.0 Hz), 7.20 (2H, d, J 8.0 Hz), 6.85 (2H, t, *J* 9.2 Hz), 6.50 (2H, dd, *J* 9.2, 4.4 Hz), 4.51 (1H, m), 3.86 (1H, m), 3.73 (2H, s), 3.47 (2H, s), 3.38 (1H, m), 3.12 (1H, m), 2.83−3.00 (4H, m), 2.75 (2H, m), 2.27 (1H, br s), 2.03 (2H, m), 1.94 (1H, m), 1.70 (1H, m), 1.30 (2H, m), 1.06 (1H, m), 1.02 (3H, d, *J* 6.4 Hz). LCMS: (ES^+) 425 (MH^+), retention time 1.42 min, >95% pure. Free base **11** (19 mg) was treated with 1.1 equiv of 1 M HCl in Et_2O to give the hydrochloride salt (25 mg).

***N*-(3-Fluorophenyl)-1-[(4-([(3*S*)-3-methyl-1-piperazinyl]methyl)phenyl)acetyl]-4-piperidinamine 12.** Compound **12** was initially prepared as for compound **11** described above using *N*-(3-fluorophenyl)-4-piperidinamine **17b** instead of *N*-(4-fluorophenyl)-4-piperidinamine **17a**. Alternatively, a larger scale preparation was used as follows:

To a solution of 4-(bromomethyl)phenylacetic acid (20 g, 87.3 mmol) in MeOH (200 mL) was added trimethylsilyl chloride (2 mL), and the mixture was stirred for 2 h. The solvent was removed in vacuo and the residue was twice redissolved in MeOH (200 mL) and reconcentrated to give methyl 4-(bromomethyl)phenylacetate (21.08 g, 99%). δ_H ($CDCl_3$, 250 MHz) 7.36 (2H, d, *J* 8.0 Hz), 7.26 (2H, d, *J* 8.0 Hz), 4.49 (2H, s), 3.69 (3H, s), 3.62 (2H, s). LCMS: (ES^+) 243/245 (MH^+), 183/185 $(M - CO_2Me)^+$ retention time 2.84 min, >98% pure.

To a solution of methyl 4-(bromomethyl)phenylacetate (20.8 g, 85.6 mmol) and diisopropylethylamine (16.4 mL, 94.1 mmol) in dry DMF (100 mL) was added a solution of 1,1-dimethylethyl (2*S*)-2-methyl-1-piperazinecarboxylate (18.8 g, 94.1 mmol) in dry DMF (75 mL) with cooling in an ice bath. The mixture was warmed to room temperature and stirred for 15 min. The solvent was removed in vacuo and the residue partitioned between EtOAc and 2 M NaOH solution (400 mL, 1:1). The organic phase was washed with water (200 mL) and brine (200 mL), and the combined aqueous washings were back-extracted with EtOAc (200 mL). The EtOAc extracts were combined, dried ($MgSO_4$), and concentrated in vacuo to give the crude product which was purified by column chromatography. Elution with 20−25% EtOAc/hexane gave **24** as a colorless oil (29.3 g, 95%). δ_H ($CDCl_3$, 250 MHz) 7.29 (2H, d, *J* 8.0 Hz), 7.22 (2H, d, *J* 8.0 Hz), 4.18 (1H, m), 3.79 (1H, m), 3.69 (3H, s), 3.62 (2H, s), 3.51 (1H, d, *J* 13.2 Hz), 3.39 (1H, d, *J* 13.2 Hz), 3.10 (1H, td, *J* 12.8, 3.5 Hz), 2.75 (1H, m), 2.59 (1H, m), 2.12 (1H, dd, *J* 11.1, 3.9 Hz), 1.99 (1H, m), 1.45 (9H, s), 1.24 (3H, d, *J* 6.8 Hz). LCMS: (ES^+) 363 (MH^+), retention time 1.78 min, 90% pure.

To a solution of **24** (29.3 g, 80.9 mmol) in THF (200 mL) was added 2 M NaOH solution (100 mL), and the two-phase reaction mixture was stirred at room temperature for 3 h. The mixture was concentrated in vacuo to remove the THF, and the aqueous solution was extracted with EtOAc (2 × 100 mL). The aqueous phase was acidified to pH 6 with concentrated HCl and extracted with DCM (3 × 300 mL). The combined organics were washed with brine (×2), dried and concentrated in vacuo to give **25b** as a colorless foam (27.8 g, 99%). δ_H ($CDCl_3$, 400 MHz) 7.27 (2H, d, *J*), 7.23 (2H, d), 4.20 (1H, m), 3.81 (1H, m), 3.62 (2H, s), 3.59 (1H, d, *J* 8.3 Hz), 3.49 (1H, d, *J* 8.3 Hz), 3.15 (1H, m), 2.88 (1H, m), 2.69 (1H, m), 2.20 (1H, dd, *J* 11.2, 4.4 Hz), 2.05 (1H, m), 1.45 (9H, s), 1.25 (3H, d, *J* 6.8 Hz). LCMS: (ES^+) 349 (MH^+), (ES^-) 347 ($M - H^+$), retention time 1.53 min, 94% pure.

A mixture of **25b** (27.8 g, 79.8 mmol), *N*-[3-(dimethylamino)-propyl]-*N*′-ethylcarbodiimide hydrochloride (22.9 g, 119.7 mmol), 1-hydroxybenzotriazole hydrate (16.2 g, 119.7 mmol), triethylamine (45 mL, 319.1 mmol), and *N*-(3-fluorophenyl)-4-piperidinamine **17b** hydrochloride salt (18.4 g, 79.8 mmol) in dry DMF (400 mL) was stirred at room temperature overnight. The solvent was removed in vacuo, and the residue was redissolved in DCM (300 mL) and washed with 2 M NaOH (2 × 200 mL), water (200 mL), and brine (200 mL). All aqueous washings were combined and back-extracted with DCM (2 × 100 mL). The combined organics were dried and concentrated to give a solid which was purified by column chromatography (silica prewashed with 50% EtOAc/hexane). Elution with 70% EtOAc/hexane gave 1,1-dimethylethyl (2*S*)-4-([4-(2-(4-[(3-fluorophenyl)amino]-1-piperidinyl)-2-oxoethyl)phenyl-]methyl)-2-methyl-1-piperazinecarboxylate as a white solid (34.95 g, 84%). δ_H ($CDCl_3$, 400 MHz) 7.28 (2H, d, *J* 8.0 Hz), 7.19 (2H, d, *J* 8.0 Hz), 7.07 (1H, dt, *J* 8.0, 6.4 Hz), 6.23−6.40 (3H, m), 4.52 (1H, m), 4.17 (1H, m), 3.78−3.88 (2H, m), 3.74 (2H, s), 3.60 (1H, d, *J* 7.6 Hz), 3.40 (1H, d, *J* 13.4 Hz), 3.43 (1H, m), 3.38 (1H, d, *J* 13.4 Hz), 3.06−3.17 (2H, m), 2.89 (1H, m), 2.74 (1H, m), 2.57 (1H, m), 1.94−2.13 (4H, m), 1.45 (9H, s), 1.32 (1H, m), 1.21 (3H, d, *J* 6.4 Hz), 1.09 (1H, m). LCMS: (ES^+) 525 (MH^+), retention time 2.18 min, 95% pure.

A solution of 1,1-dimethylethyl (2*S*)-4-([4-(2-(4-[(3-fluorophenyl)amino]-1-piperidinyl)-2-oxoethyl)phenyl]methyl)-2-methyl-1-piperazinecarboxylate (83.01 g, 0.158 mol) in DCM (900 mL) was split into two portions and each cooled to 0 °C and treated with TFA (100 mL). After being stirred for 0.5 h at 0 °C, the mixtures were warmed to room temperature and stirred for 3.25 h. The solvent was removed in vacuo, and the residues were combined and partitioned between DCM and 2 M NaOH solution. The aqueous phase was re-extracted with DCM (×2), and the combined organics were then washed with 2 M NaOH and brine. The organics were dried and concentrated in vacuo to give an off-white solid. The NaOH phase was re-extracted with DCM, which was dried and concentrated to give a further batch of white foam. The two batches were combined to give **12** as an off-white solid (66.94 g, 100%). δ_H ($CDCl_3$, 400 MHz) 7.26 (2H, d, *J* 8.0 Hz), 7.20 (2H, d, *J* 8.0 Hz), 7.06 (1H, q, *J* 8.0 Hz), 6.36 (1H, m), 6.31 (1H, m), 6.25 (1H, m), 4.52 (1H, m), 3.86 (1H, m), 3.73 (2H, s), 3.66 (1H, m), 3.48 (2H, s), 3.41 (1H, m), 2.84−3.16 (6H, m), 2.76 (2H, d, *J* 11.2 Hz), 2.03−2.13 (2H, m), 1.94 (1H, m), 1.78 (1H, t, *J* 10.8 Hz), 1.34 (1H, m), 1.07 (4H, m). LCMS: (ES^+) 425 (MH^+), retention time 1.64 min, 98% pure, mp 144 °C (onset by DSC).

A batch of free base **12** (66.22 g, 0.156 mol) was dissolved in EtOAc (1.7 L) at 45−50 °C to give a homogeneous pale-yellow solution which was then cooled to room temperature and flushed with argon. Then 1 M HCl in Et_2O (156 mL, 0.156 mol) was added with vigorous stirring, and after 15 min, the resultant creamy-white precipitate was collected by filtration under a blanket of argon. This was washed with further EtOAc (0.8 L) and partially dried on the filter under a blanket of argon for 15 min The solid was further dried at 80 °C in vacuo to give the hydrochloride salt of **12** (58.95 g, 82%). δ_H ($CDCl_3$, 400 MHz) 9.70 (1H, br), 7.32 (2H, m), 7.24 (2H, d, *J* 8.0 Hz), 7.08 (1H, q, *J* 8.0 Hz), 6.38 (2H, m), 6.31 (1H, m), 4.52 (1H, m), 3.86 (1H, m), 3.74 (4H, m), 3.51 (1H, br s), 3.41 (2H, m), 3.24 (1H, br s), 3.16 (1H, m), 2.99 (2H, m), 2.87 (2H, m), 2.64 (1H, br s), 2.02 (2H, m), 1.45 (3H, d, *J* 6.8 Hz), 1.34 (1H, m), 1.12 (1H, m). LCMS: (ES^+) 425 (MH^+), retention time 1.61 min, >98% pure.

***N*-(3-Fluorophenyl)-1-[(4-([(3*R*)-3-methyl-1-piperazinyl]methyl)phenyl)acetyl]-4-piperidinamine 13.** A mixture of methyl 4-(bromomethyl)phenylacetate (243 mg, 1 mmol) (see compound **12** for synthesis), diisopropylethylamine (174 μL, 1 mmol), and 1,1-dimethylethyl (2*R*)-2-methyl-1-piperazinecarboxylate (234 mg, 1 mmol) in DMF was stirred at room temperature for 1 h and then allowed to stand overnight. The solvent was removed in vacuo. Then the residue was diluted with water (20 mL) and extracted with EtOAc (2 × 20 mL). The combined organic extracts were dried ($MgSO_4$) and concentrated in vacuo to give **26** as a yellow oil (383 mg, 97%). δ_H ($CDCl_3$, 250 MHz) 7.21−7.40 (9H, m), 5.13

(2H, AB 2 × d) 4.29 (1H, m), 3.91 (1H, m), 3.70 (3H, s), 3.62 (2H, s), 3.43 (2H, m), 3.20 (1H, m), 2.78 (1H, m), 2.62 (1H, m), 2.05−2.18 (2H, m), 1.29 (3H, d, *J* 6.3 Hz). MS: (ES^+) 397 (MH^+).

A solution of **26** (380 mg, 0.96 mmol) in THF (4 mL) and 2 M NaOH (1 mL) was stirred at room temperature overnight. Water (10 mL) was added and the solution washed with EtOAc (20 mL). The aqueous phase was adjusted to pH 6 and extracted with EtOAc (2 × 20 mL). The combined extracts were washed with brine, dried ($MgSO_4$), and concentrated in vacuo to give **27** as a colorless gum (255 mg, 69%). δ_H ($CDCl_3$, 250 MHz) 7.20−7.40 (9H, m), 5.12 (2H, AB 2 × d) 4.29 (1H, m), 3.91 (1H, d, *J* 13.5 Hz), 3.61 (2H, s), 3.51 (2H, AB, 2 × d), 3.20 (1H, m), 2.85 (1H, d, *J* 10.8 Hz), 2.69 (1H, d, *J* 11.5 Hz), 2.20 (1H, m), 2.10 (1H, m), 1.29 (3H, m).

A mixture of **27** (100 mg, 0.261 mmol), *N*-[3-(dimethylamino)-propyl]-*N*′-ethylcarbodiimide hydrochloride (75 mg, 0.392 mmol), 1-hydroxybenzotriazole (53 mg, 0.392 mmol), triethylamine (110 μL, 0.784 mmol), and *N*-(3-fluorophenyl)-4-piperidinamine **17b** hydrochloride salt (60 mg, 0.261 mmol) in DMF (2 mL) was stirred at room temperature for 3 days. The solvent was removed in vacuo, and the residue was purified by column chromatography. Elution with 0−10% MeOH/DCM gave phenylmethyl (2*R*)-4-([4-(2-(4-[(3-fluorophenyl)amino]-1-piperidinyl)-2-oxoethyl)phenyl]methyl)-2-methyl-1-piperazine carboxylate as a colorless oil (136 mg, 93%). δ_H ($CDCl_3$, 400 MHz) 7.31−7.38 (5H, m), 7.28 (2H, d, *J* 8.0 Hz), 7.19 (2H, d, *J* 8.0 Hz), 7.07 (1H, m), 6.23−6.39 (3H, m), 5.13 (2H, AB 2 × d), 4.52 (1H, m), 4.27 (1H, br s), 3.88 (2H, m), 3.74 (2H, s), 3.62 (1H, br s), 3.50 (1H, d, *J* 13.2 Hz), 3.42 (1H, m), 3.38 (1H, d, *J* 13.2 Hz), 3.16 (2H, m), 2.88 (1H, m), 2.76 (1H, d, *J* 10.8 Hz), 2.59 (1H, d, *J* 10.8 Hz), 1.94−2.15 (4H, m), 1.32 (1H, m), 1.26 (3H, m), 1.08 (1H, m). LCMS: (ES^+) 559 (MH^+), retention time 2.26 min, 91% pure.

A solution of phenylmethyl (2*R*)-4-([4-(2-(4-[(3-fluorophenyl)-amino]-1-piperidinyl)-2-oxoethyl) phenyl]methyl)-2-methyl-1-piperazinecarboxylate (136 mg, 0.24 mmol) was hydrogenated in MeOH (5 mL) with palladium black catalyst (68 mg) for 0.75 h. The reaction mixture was concentrated in vacuo to give a pale-yellow crude oil which was purified by column chromatography. Elution with 0−10% (2 M NH_3 in MeOH)/DCM gave **13** as a colorless oil (38 mg, 37%). δ_H ($CDCl_3$, 400 MHz) 7.27 (2H, d, *J* 8.0 Hz), 7.20 (2H, d, *J* 8.0 Hz), 7.07 (1H, q, *J* 8.0 Hz), 6.37 (1H, m), 6.31 (1H, m), 6.25 (1H, m), 4.52 (1H, m), 3.86 (1H, m), 3.74 (2H, s), 3.62 (1H, m), 3.47 (2H, s), 3.42 (1H, m), 3.13 (1H, m), 2.90 (4H, m), 2.75 (2H, m), 2.00 (4H, m), 1.69 (1H, t, *J* 10.4 Hz), 1.30 (1H, m), 1.06 (1H, m), 1.02 (3H, d, *J* 6.4 Hz). LCMS: (ES^+) 425 (MH^+), retention time 1.64 min, >98% pure. Free base **13** (38 mg) was treated with 1.1 equiv of 1 M HCl in Et_2O to give the hydrochloride salt (41 mg).

1-[2-(4-([(3*R*,5*S*)-3,5-Dimethyl-1-piperazinyl]methyl)phenyl)propanoyl]-*N*-(4-fluorophenyl)-4-piperidinamine 14. Malonate **29** was prepared in the same manner as **23** using 1,1-dimethylethyl (2*R*,6*S*)-2,6-dimethyl-1-piperazinecarboxylate instead of phenylmethyl (2*S*)-2-methyl-1-piperazinecarboxylate in the first step (see compound **11** for details). LCMS: (ES^+) 463 (MH^+), retention time 2.62 min, 87% pure.

A solution of **29** (538 mg, 1.16 mmol) in DMF (10 mL) was added dropwise to sodium hydride (61 mg, 60% w/w in oil, 1.51 mmol) in DMF (2 mL) at 0 °C under argon. After the mixture was stirred for 10 min, methyl iodide (0.144 mL, 2.32 mmol) was added and the reaction mixture allowed to warm to room temperature over 1 h. Ammonium chloride solution was added and the mixture extracted with EtOAc. The extracts were washed with saturated aqueous $NaHCO_3$ solution and water, then dried (Na_2SO_4) and concentrated in vacuo. Column chromatography, eluting with 0−10% EtOAc/hexane, gave diethyl (4-[((3*S*)-4-([(1,1-dimethylethyl)oxy]carbonyl)-3-methyl-1-piperazinyl)methyl]phenyl)propanedioate as a clear gum (356 mg, 65%). LCMS: (ES^+) 477 (MH^+), retention time 2.72 min, >95% pure.

A solution of diethyl (4-[((3*S*)-4-([(1,1-dimethylethyl)oxy]carbonyl)-3-methyl-1-piperazinyl)methyl]-phenyl)propanedioate (356 mg, 0.748 mmol) in 2 M NaOH solution (3 mL) and 1,4-dioxane (3 mL) was stirred at room temperature for 1 h and then at 80 °C for 3 h. The solvents were removed in vacuo, water was added, and the mixture was adjusted to pH 4 with 2 M HCl. The product was extracted into EtOAc and the extracts were washed with brine and then dried (Na_2SO_4) and concentrated to give **30** (256 mg, 91%). δ_H ($CDCl_3$, 400 MHz) 7.32 (2H, d, *J* 8.0 Hz), 7.27 (2H, d, *J* 8.0 Hz), 4.10 (2H, m), 3.74 (1H, q, *J* 7.2 Hz), 3.49 (2H, br s), 2.64 (2H, m), 2.14 (2H, m), 1.52 (3H, d, *J* 7.2 Hz), 1.46 (9H, s), 1.30 (6H, d, *J* 6.8 Hz). LCMS: (ES^+) 377, (ES^-) 375 ($M - H^+$), retention time 1.93 min, >95% pure.

A mixture of **30** (100 mg, 0.27 mmol), polymer-supported carbodiimide (310 mg, 1.3 mmol/g, 0.40 mmol), and 1-hydroxybenzotriazole (50 mg, 0.324 mmol) in 2:1 DMF/DCM (3 mL) was treated with *N*-(4-fluorophenyl)-4-piperidinamine **17a** (51 mg, 0.26 mmol) and the mixture stirred overnight. Scavenger resins (PS-trisamine, PS-isocyanate, and Si-carbonate) together with DCM (∼3 mL) were added. The mixture was stirred for ∼3 h and then filtered and concentrated to give 1,1-dimethylethyl (2*R*,6*S*)-4-([4-(2-(4-[(4-fluorophenyl)amino]-1-piperidinyl)-1-methyl-2-oxoethyl)phenyl]methyl)-2,6-dimethyl-1-piperazinecarboxylate. LCMS: (ES^+) 553 (MH^+), retention time 2.47 min, >98% pure.

This whole sample was dissolved in DCM (2 mL) and TFA (1 mL) and then stirred for 1.5 h. The solvents were removed in vacuo and the residue re-evaporated from toluene and ether. Purification using an Isolute SCX cartridge, eluting with MeOH followed by 2 M NH_3 in MeOH, gave **14** as ∼1:1 mixture of diastereoisomers. δ_H ($CDCl_3$, 400 MHz) 7.26 (2H, m), 7.18 (2H, m), 6.83 (2H, m), 6.50 (0.5H, m), 6.44 (0.5H, m), 4.61 (0.5H, br d), 4.43 (0.5H, m), 3.87 (2H, m), 3.46 (2H, s), 3.31 (2H, m), 3.05 (1H, m), 2.91 (3H, m), 2.73 (3H, m), 2.00 (1H, m), 1.84 (0.5H, br d), 1.72 (0.5H, br d), 1.59 (2H, m), 1.44 (3H, d, *J* 8.8 Hz), 1.31 (1H, m), 1.14 (0.5H, m), 0.97−1.03 (6H, m), 0.45 (0.5H, m). LCMS: (ES^+) 453 (MH^+), retention time 1.53 min, >98% pure. Free base **14** was dissolved in DCM and treated with 1.1 equiv of 1 M HCl in ether to give the hydrochloride salt of the title compound (122 mg, 94% from acid **30**). LCMS: (ES^+) 453 (MH^+), retention time 1.55 min, >98% pure.

1-[2-(4-([(3*R*,5*S*)-3,5-Dimethyl-1-piperazinyl]methyl)phenyl)-2-methylpropanoyl]-*N*-(4-fluorophenyl)-4-piperidinamine 15. Bromobenzene **28** was prepared in the same manner as **22** using 1,1-dimethylethyl (2*R*,6*S*)-2,6-dimethyl-1-piperazinecarboxylate instead of phenylmethyl (2*S*)-2-methyl-1-piperazinecarboxylate (see compound **11** for details). δ_H ($CDCl_3$, 250 MHz) 7.44 (2H, d, *J* 8.0 Hz), 7.25 (2H, d, *J* 8.0 Hz), 4.09 (2H, m), 3.42 (2H, s), 2.57 (2H, d, *J* 11.0 Hz), 2.12 (2H, dd, *J* 11.3, 4.5 Hz), 1.46 (9H, s), 1.28 (6H, d, 7.8 Hz). LCMS: (ES^+) 383/385 (MH^+), retention time 3.10 min, >95% pure.

A solution of methyl 2-methylpropanoate (188 μL, 1.64 mmol) in toluene (3 mL) was added to lithium dicyclohexylamide (362 mg, 1.93 mmol) under glovebox conditions. The suspension was stirred for 10 min and then added to a mixture of **30** (570 mg, 1.49 mmol) and bis(dibenzylideneacetone) palladium(0) (43 mg, 0.074 mmol). Tri-(*tert*-butyl)phosphine (18 μL, 0.074 mmol) was added and the reaction mixture stirred at room temperature overnight. The solvent was removed in vacuo, and column chromatography, eluting with 0−90% EtOAc/petroleum ether, gave **31** as a yellow oil (395 mg, 66%). δ_H ($CDCl_3$, 250 MHz) 7.29 (4H, m), 4.07 (2H, m), 3.66 (3H, s), 3.46 (2H, s), 2.61 (2H, m), 2.12 (2H, dd, *J* 11.5, 4.3 Hz), 1.58 (6H, s), 1.46 (9H, s), 1.29 (6H, d, *J* 6.5 Hz). MS: (ES^+) 405 (MH^+).

A mixture of **31** (395 mg, 0.978 mmol) and lithium hydroxide monohydrate (82 mg, 1.95 mmol) in water (5 mL) and 1,4-dioxane (10 mL) was stirred at room temperature for 3 days. The solvents were removed in vacuo, and the residue was dissolved in water. The solution was washed with ether, acidified to pH 4 with 1 M HCl and then extracted with DCM (×2). The combined organics were dried (Na_2SO_4) and concentrated in vacuo to give 2-(4-[((3*R*,5*S*)-4-([(1,1-dimethylethyl)oxy]carbonyl)-3,5-dimethyl-1-piperazinyl)methyl]-phenyl)-2-methylpropanoic acid as a yellow foam (316 mg, 83%). δ_H ($CDCl_3$, 250 MHz) 7.35 (4H, m), 4.11 (2H, br), 3.71 (2H, br), 2.67 (2H, br), 2.17 (2H, br), 1.61 (6H, s), 1.46 (9H, s), 1.33 (6H, br d). MS: (ES^+) 391 (MH^+), (ES^-) 389 ($M - H^+$).

A mixture of 2-(4-[((3*R*,5*S*)-4-([(1,1-dimethylethyl)oxy]-carbonyl)-3,5-dimethyl-1-piperazinyl)methyl]phenyl)-2-methylpropanoic acid (100 mg, 0.256 mmol), polymer-supported carbodiimide (296 mg, 1.3 mmol/g, 0.385 mmol), and 1-hydroxybenzotriazole (18 mg, 0.128 mmol) in 1:4 DMF/DCM (5 mL) was treated with *N*-(4-fluorophenyl)-4-piperidinamine **17a** (50 mg, 0.256 mmol), and the mixture was stirred overnight. Scavenger resins (PS-trisamine, PS-isocyanate, and Si-carbonate) were added, and the mixture was shaken for 2 h and then filtered and concentrated to give crude 1,1-dimethylethyl (2*R*,6*S*)-4-([4-(2-(4-[(4-fluorophenyl)-amino]-1-piperidinyl)-1,1-dimethyl-2-oxoethyl)phenyl]methyl)-2,6-dimethyl-1-piperazinecarboxylate (contains DMF, ∼12% by mass by ^{1}H NMR). δ_H ($CDCl_3$, 400 MHz) 7.32 (2H, d, *J* 8.4 Hz), 7.18 (2H, d, *J* 8.4 Hz), 6.83 (2H, t, *J* 8.8 Hz), 6.45 (2H, dd, *J* 9.2, 4.4 Hz), 4.51 (1H, br), 4.06 (2H, m), 3.45 (2H, s), 3.26 (2H, m), 2.75 (2H, br), 2.58 (2H, d, *J* 11.2 Hz), 2.12 (2H, dd, *J* 11.2, 4.4 Hz), 1.69 (1H, br), 1.54 (6H, s), 1.46 (9H, s), 1.26 (6H, d, *J* 6.8 Hz). Some piperidine signals were very broad and merged into baseline. MS: (ES) 567 (MH^+). This whole sample was treated with 4 M HCl in 1,4-dioxane (2 mL) for 1 h. The solvents were removed in vacuo to give a colorless solid which was dried under vacuum to give the dihydrochloride salt of **15** (115 mg, 83% from acid). LCMS: (ES^+) 467 (MH^+), retention time 1.66 min, >98% pure.

3-((1-[(4-([(3*S*)-3-Methyl-1-piperazinyl]methyl)phenyl)acetyl]-4-piperidinyl)amino)benzonitrile 16. Compound **16** was prepared by methods similar to those used for the synthesis of compounds **11** and **12** using *N*-(3-cyanophenyl)-4-piperidinamine **17c** as required. δ_H ($CDCl_3$, 400 MHz), 7.28 (2H, m), 7.20 (3H, m), 6.94 (1H, d, *J* 7.6 Hz), 6.74 (2H, m), 4.54 (1H, m), 3.88 (1H, m), 3.80 (1H, m), 3.74 (2H, s), 3.47 (2H, s), 3.14 (1H, m), 2.89 (4H, m), 2.74 (2H, br d), 1.92−2.06 (3H, m), 1.66 (1H, m), 1.33 (1H, m), 1.06 (1H, m), 1.00 (3H, d, *J* 6.4 Hz). LCMS: (ES^+) 432 (MH^+), retention time 1.59 min, >98% pure. The free base of **16** was converted to the hydrochloride salt as for previous compounds. LCMS: (ES^+) 432 (MH^+), retention time 1.59 min, >98% pure.

Motilin Receptor Agonist FLIPR Assay. The potency and efficacy of target compounds at the human motilin receptor were studied using a fluorometric imaging plate reader (FLIPR) and Chinese hamster ovary (CHO-K1) cells which stably express the human motilin receptor. Briefly, the human motilin receptor was cloned (PCR from human genomic DNA) into pCDNA3.1 (Invitrogen) in the vector, subcloned into pENTR/D-TOPO (using the pENTR D-TOPO directional cloning kit) and then recombined into pCIN1GW vector (LR clonase gateway reaction kit) to give the pCIN1 motilin receptor. CHO-K1 cells (ATCC No. CCl-61) were then stably transfected with the pCIN1 motilin receptor plasmid. These cells were grown as a monolayer in DMEM/HamF-12 supplemented with 10% v/v of FBS, 2 mM GlutaMAX, and 1 mg/mL Geneticin. Stimulation of this cell line with motilin causes intracellular signaling leading to an increase in intracellular calcium which was measured using calcium sensitive fluorescent dyes and quantified using a FLIPR. Briefly, cells were seeded onto 384-well black-walled, clear-bottom microtiter plates (Greiner) (10 000 cells/well) and incubated for 24 h. On the day of assay, media were aspirated from cell plates using a cell washer (leaving 10 μL of media). Cells were immediately loaded with loading buffer (Tyrodes (Elga water + 145 mM NaCl + 5 mM KCl + 20 mM HEPES + 10 mM glucose + 1 mM $MgCl_2$) + 1.5 mM $CaCl_2$ + 0.714 mg/mL Probenicid (predissolved in 1 M NaOH) + 0.5 mM brilliant black + 2.5 μM Fluo 4 dye) and incubated at 37.5 °C for 1 h. Master compound plates were prepared in 100% DMSO. A top concentration of 3 mM was used (giving 12 μM final concentration in assay), and this was serially diluted 1 in 4. Then 1 μL from the master plate was transferred to a daughter plate, to which 50 μL of compound dilution buffer (Tyrodes + 1 mg/mL BSA + 1.5 mM $CaCl_2$) was added. An amount of 10 μL from the compound plates was then added immediately to cell plates using a FLIPR 3 calcium imaging instrument, and changes in fluorescence were measured over a 1 min time frame. Maximum change in fluorescence over baseline was used to determine agonist response, and concentration response curves were constructed using a four-parameter logistic equation. The intrinsic activity of target compounds was calculated by using the maximum asymptote of its concentration−response curve relative to the maximum asymptote of the motilin concentration−response curve.

Table 5

enzyme	CYP3A4
incubation/assay temperature (°C)	37
substrate	DEF
final substrate concentration (equivalent to K_m) (μM)	1
final enzyme concentration (mg/mL)	0.1
final volume of incubation mix (μL)	220
volume of test compound (μL)	5
volume of cofactor (μL)	25
buffer	50 mM K_3PO_4 at pH 7
excitation wavelength (nm)	485
emission wavelength (nm)	530

CYP 3A4 Time Dependent Inhibition (TDI) Assay. Inhibition was determined by quantifying the production of fluorescent metabolite following incubation of CYP3A4 specific profluorescent probe substrate diethoxyfluorescein (DEF), with heterologously expressed CYP3A4 in *E. coli* (Cypex) and the test compound or positive control (troleandomycin). A NADPH-regenerating system (cofactor) was prepared as follows: 7.8 mg/mL glucose 6-phosphate (27.65 mM), 1.7 mg/mL NADP (2.22 mM), and glucose 6-phosphate dehydrogenase at 6 enzyme units/mL were made up in 2% w/v sodium bicarbonate solution. Incubation mixtures containing enzyme, probe substrate, and 50 mM potassium phosphate buffer (at pH 7.4) were prepared, and 220 μL was added to each well of a 96-well plate. An amount of 5 μL of the serially diluted test compounds was added, and the plate was incubated at 37 °C for 10 min. To start the reaction, 25 μL of cofactor was added. The production of fluorescence was then measured every minute over a 30 min time frame at 37 °C. A summary of the assay conditions is given in Table 5.

A seven point compound concentration curve was used to determine IC_{50} values in addition to no-compound controls and the positive control. Thus, the control rate of fluorescent metabolite production was established from no-compound control incubations (uninhibited). The extent of inhibition at each test compound concentration (0.1, 0.33, 1.0, 3.3, 10, 33, and 100 μM) was calculated as a percentage of the uninhibited control rate (assigned as 100%), and the IC_{50} value was determined from these results. An IC_{50} was determined for each 5 min interval (0−5, 5−10, 10−15, 15−20, 20−25, and 25−30 min). The ratio of the first and last IC_{50} gave a fold change (IC_{50}(initial)/IC_{50}(final)), and if this was >2, the compound was denoted as displaying TDI.

Rat Pharmacokinetic Studies. The pharmacokinetics and oral bioavailability of the HCl salt of compound **12** were investigated in the male Sprague−Dawley rat ($n = 3$). The study was carried out on 2 study days with a period of 2 days between each study day. On study day 1, compound **12** was dissolved in 0.9% (w/v) saline at a target concentration of 0.2 mg of free base/mL. Compound **12** was administered as a 1 h intravenous infusion at 5 (mL/kg)/h to three rats to achieve a target dose of 1 mg of free base/kg. Serial blood samples were taken from each rat up to 12 h after the start of the infusion. On study day 2, compound **12** was suspended in 1% (w/v) methylcellulose at a target concentration of 1 mg of free base/mL. Three rats received an oral gavage dose of compound **12** administered at 5 mL/kg to achieve a target dose of 5 mg of free base/kg. Serial blood samples were taken from each rat up to 12 h after dosing. Diluted blood samples were analyzed for compound **12** by LC/MS/MS (LLQ was 5 ng/mL, 0.012 μM).

The systemic exposure of the HCl salt of compound **16** following oral suspension administration was investigated the male Sprague−Dawley rat. Compound **16** was dosed to three rats orally by gavage at a target dose of 5 mg of free base/kg. Compound **16** was prepared on the day of dosing in 1% (w/v) aqueous methylcellulose at a concentration of 1 mg of free base/mL and administered at 5 mL/kg. Serial blood samples were taken from each rat up to 8 h after

dose administration. Diluted blood samples were analyzed for parent compound by LC/MS/MS.

Dog Pharmacokinetic Studies. The pharmacokinetics and oral bioavailability of the HCl salt of compound **12** were investigated in the male beagle dog ($n = 3$). The study was carried out on 2 study days with a period of 7 days between each study day. On study day 1, compound **12** was dissolved in 0.9% (w/v) saline at a concentration of 0.4 mg of free base/mL. Compound **12** was administered as a 1 h intravenous infusion at 5 (mL/kg)/h to three dogs to achieve a target dose of 2 mg of free base/kg. Serial blood samples were taken from each dog up to 30 h after the start of the infusion. On study day 2, compound **12** was suspended in 1% (w/v) methylcellulose at a concentration of 1 mg of free base/mL. The same three dogs each received an oral gavage dose of compound **12** administered at 5 mL/kg to achieve a target dose of 5 mg of free base/kg. Serial blood samples were taken from each dog up to 30 h after dosing. Diluted blood samples were analyzed for compound **12** by LC/MS/MS (LLQ was 5 ng/mL, 0.012 μM).

Supporting Information Available: Details of the liquid chromatography−mass spectrometry (LCMS) procedure and hardware used for the determination of purity; LCMS chromatograms and spectra for key compounds **12** and **16**; details of the procedure and hardware for mass directed autoprep (MDAP) purification. This material is available free of charge via the Internet at http://pubs.acs.org.

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(21) The potency data quoted for compound **6** (and all other compounds) in this paper are those obtained using the recombinant human motilin receptor expressed in a CHO cell line (see Experimental Section for details), whereas that given previously[19] was obtained using a HEK293 cell line. There is a shift in potency between the two assay formats, but the rank order of potency remains consistent; see ref 19 for a comparison of potency data for standard compounds. In addition, the CYP inhibition data given in this paper were obtained using enzyme sourced from Cypex whereas those given previously were obtained using enzyme sourced from Gentest; hence, there is some variation in the data quoted in this publication and in ref 19.

(22) pEC_{50} data quoted are the mean of at least three values with SEM $\leq$ 0.3 in all cases except for compounds **7** and **8**, where $n = 2$.

(23) See Experimental Section for the method used to assess time dependent inhibition (TDI) of CYP3A4.

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