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Discovery of a Selective Small-Molecule Melanocortin-4 Receptor Agonist with Efficacy in a Pilot Study of Sexual Dysfunction in Humans

Mark I. Lansdell,* David Hepworth, Andrew Calabrese, Alan D. Brown, Julian Blagg,† Denise J. Burring, Peter Wilson, David Fradet, T. Bruce Brown, Faye Quinton, Neela Mistry, Kim Tang, Natalie Mount, Peter Stacey, Nick Edmunds, Cathryn Adams, Samantha Gaboardi, Stevie Neal-Morgan, Chris Wayman, Susan Cole, Joanne Phipps, Mark Lewis, Hugh Verrier, Val Gillon, Neil Feeder, Anne Heatherington, Stefan Sultana, Scott Haughie, Steven W. Martin, Maria Sudworth, and Sarah Tweedy

Pfizer Global Research and Development, Sandwich Laboratories, Ramsgate Road, Sandwich, Kent CT13 9NJ, U.K.
†*Current address: Cancer Research UK Centre for Cancer Therapeutics, The Institute of Cancer Research, Haddow Laboratories, 15 Cotswold Road, Sutton SM2 5NG, UK.*

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The relevance of the melanocortin system to sexual activity is well established, and nonselective peptide agonists of the melanocortin receptors have shown evidence of efficacy in human sexual dysfunction. The role of the MC4 receptor subtype has received particular scrutiny, but the sufficiency of its selective activation in potentiating sexual response has remained uncertain owing to conflicting data from studies in preclinical species. We describe here the discovery of a novel series of small-molecule MC4 receptor agonists derived from library hit **2**. The addition of methyl substituents at C3 and C5 of the 4-phenylpiperidin-4-ol ring was found to be markedly potency-enhancing, enabling the combination of low nanomolar potencies with full rule-of-five compliance. In general, the series shows only micromolar activity at other melanocortin receptors. Our preferred compound **40a** provided significant systemic exposure in humans on both sublingual and oral administration and was safe and well tolerated up to the maximum tested dose. In a pilot clinical study of male erectile dysfunction, the highest dose of **40a** tested (200 mg) provided a similar level of efficacy to sildenafil.

Introduction

The melanocortin-4 (MC4)[a] receptor is a member of a family of five G-protein coupled receptors (GPCRs) activated by the melanocortin peptides α-, *β*-, and *γ*-melanocyte stimulating hormone (MSH) and adrenocorticotropin hormone (ACTH).[1−4] The MSH and ACTH peptides are products derived from post-translational processing of a single pro-opiomelanocortin (POMC) precursor[5,6] and share a common His-Phe-Arg-Trp motif considered crucial to binding. Uniquely among seven-transmembrane GPCRs, the melanocortin receptors are also subject to regulation by two endogenous inverse agonists, namely agouti and agouti-related protein (AGRP).[2,7] The MC1 receptor is expressed mainly on cutaneous melanocytes and is involved in skin and hair pigmentation.[1,2,4] The MC2 receptor is activated only by ACTH, differentiating it pharmacologically from the other melanocortin receptors, and expression is localized in the adrenal cortex, where it controls steroidoneogenesis. In contrast, both the MC3 and MC4 receptors are extensively expressed in the central nervous system (CNS), where they have been shown to have major roles in energy homeostasis and feeding behavior, and they are present only to a much lesser extent in the periphery. The MC5 receptor is widely distributed in peripheral tissues but has only very limited expression in the CNS, and, beyond control of sebaceous secretion and immunoregulation, its function is not well established. These disparate expression patterns are reflected by the wide range of disease pathologies in which the melanocortin receptors are considered to have relevance.[3]

The involvement of the melanocortin system in regulating sexual response was first indicated several decades ago after observation of the effects of administration of melanocortin peptides in male rabbits[8] and female rats.[9] More recently, studies designed to reveal the relative importance of the melanocortin receptor subtypes in promoting erectile activity in male rodents have reached conflicting conclusions. The use of a selective peptidic MC4 receptor antagonist to inhibit the action of α-MSH and ACTH failed to block penile erection

*To whom correspondence should be addressed. Phone: +44 1304 645037. Fax: +44 1304 651819. E-mail: mark.i.lansdell@googlemail.com.

[a] Abbreviations: MC, melanocortin; GPCR, G-protein coupled receptor; ACTH, adrenocorticotropin hormone; MSH, melanocyte stimulating hormone; POMC, pro-opiomelanocortin; AGRP, agouti-related protein; CNS, central nervous system; BBB, blood−brain barrier; MED, male erectile dysfunction; PDE, phosphodiesterase; FSAD, female sexual arousal disorder; DMF, *N*,*N*-dimethylformamide; TFA, trifluoroacetic acid; THF, tetrahydrofuran; NMP, *N*-methyl pyrrolidinone; KHMDS, potassium bis(trimethylsilyl)amide; PMB, *para*-methoxybenzyl; ACE-Cl, 1-chloroethylchloroformate; NMR, nuclear magnetic resonance; BINAP, 2,2′-bis(diphenylphosphino)-1,1′-binaphthalene; CHO, Chinese hamster ovary; SAR, structure−activity relationship; hERG, human ether-à-go-go related gene; SEM, standard error of the mean; HEK, human embryonic kidney cells; HLM, human liver microsomes; RLM, rat liver microsomes; DLM, dog liver microsomes; MDCK, Madin−Darby canine kidney cells; MDR, multidrug resistance protein; CSF, cerebrospinal fluid; AUC, area under the curve; VSS, visual sexual stimulation; MTBE, methyl *tert*-butyl ether; SPA, scintillation proximity assay.

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Ac-Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val-NH_2

α-melanocyte stimulating hormone (α-MSH)

Ac-Ser-Tyr-Ser-Nle-Glu-His-DPhe-Arg-Trp-Gly-Lys-Pro-Val-NH_2

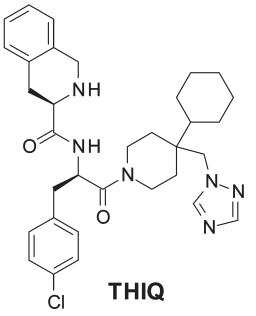
melanotan I (MT-I)

Ac-Nle-c[Asp-His-DPhe-Arg-Trp-Lys]-NH_2

melanotan II (MT-II)

Ac-Nle-c[Asp-His-DPhe-Arg-Trp-Lys]-OH

bremelanotide (PT-141)



THIQ

Figure 1. Structures of some important melanocortin receptor agonists.

even though other melanocortin-induced behavioral effects such as grooming, stretching, and yawning were abolished.[10,11] In contrast, observation of the effects of the selective nonpeptide MC4-receptor agonist THIQ (Figure 1) on wild-type versus MC4 receptor knockout mice,[12] and in rats with and without competing peptide antagonists,[13] has implicated the MC4 receptor in promoting erectile activity.

This unresolved discrepancy in preclinical species notwithstanding, the therapeutic potential of melanocortin receptor activation for the treatment of human sexual dysfunction[4,14−16] had already been demonstrated by the fortuitous discovery[17,18] of the pro-erectile side effect of melanotan II (MT-II), a cyclic peptide analogue of α-MSH exhibiting agonist activity at the MC1, MC3, MC4, and MC5 receptors and developed originally to enhance skin pigmentation.[19,20] Subsequent trials confirmed the efficacy of subcutaneously administered MT-II in producing erections in men both with psychogenic[21] and organic[22] erectile dysfunction. This efficacy has been attributed to a centrally mediated effect on the basis of a long latency to MT-II-induced erections and an absence of erectogenic activity observed on subcutaneous administration of melanotan I (MT-I), a related potential tanning agent which lacks penetration of the blood−brain barrier (BBB).[23] Further development was focused on the intranasal delivery of bremelanotide (PT-141),[24,25] an active metabolite of MT-II, for which efficacy has been demonstrated both in male erectile dysfunction (MED)[26,27] (including PDE5-inhibitor nonresponders[28,29]) and in female sexual arousal disorder (FSAD).[30,31] Despite these very encouraging results, the development of intranasal bremelanotide for sexual dysfunction has since been halted prior to phase III due to concerns regarding increases in blood pressure.[32]

In contrast to the clinical efficacy observed with the nonselective agonists MT-II and PT-141, an absence of clinical

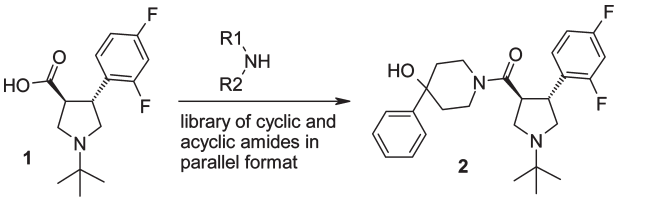
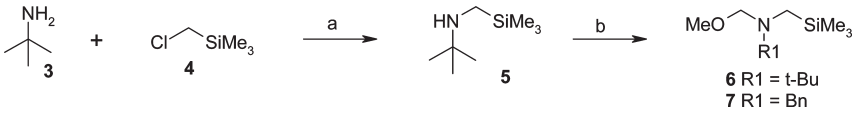


Figure 2. Identification of compound **2** as a lead from a library of diverse carboxamides.

erectogenic activity has recently been reported for MK-0493,[33] which is described as a selective small-molecule MC4 receptor agonist. Unfortunately, detailed descriptions of the pharmacological and pharmacokinetic profiles of MK-0493 are not available, precluding deeper analysis of the features which may be necessary to elicit clinical efficacy via activation of the melanocortin system. This situation prompts us to report here in detail our own efforts toward the design and study of a small molecule with selectivity for MC4.

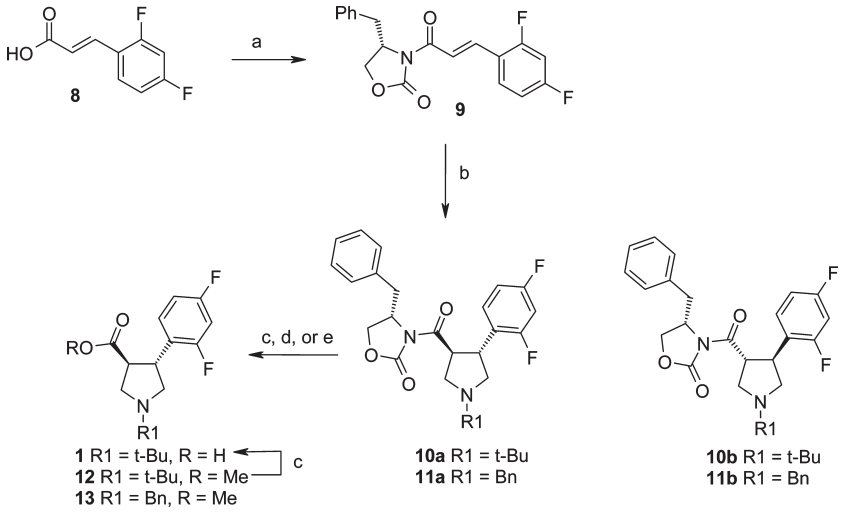
In view of the anticipated challenges of designing an orally available, CNS-penetrant small molecule capable of agonizing a peptidic GPCR, we were attracted by the *trans*-4-phenylpyrrolidine-3-carboxamide template first disclosed by Goulet and co-workers[34] (and subsequently also explored by others[35−39]) due to its combination of relatively low basicity, relatively high rigidity, and nonpeptide-like features compared to most other reported MC4 receptor agonist chemotypes.[40,41] Synthesis and screening of a library of diverse amides based on template **1** provided very few compounds with sufficient potency to represent attractive leads aside from a simple 4-phenylpiperidin-4-ol **2** (Figure 2). During optimization of an earlier phenylalanine-based MC4 receptor agonist chemotype also containing a 4-phenylpiperidin-4-ol subunit,

Scheme 1[a]



[a] Reagents and conditions: (a) 200 °C; (b) CH_2O, MeOH, K_2CO_3.

Scheme 2[a]



[a] Reagents and conditions: (a) (i) $(COCl)_2$, DMF, CH_2Cl_2, (ii) (*S*)-(−)-4-benzyl-2-oxazolidinone, LiCl, CH_2Cl_2; (b) **6** or **7**, TFA, CH_2Cl_2; (c) formation of **1** from **10a** or **12**: LiOH, H_2O, THF; (d) formation of **12** from **10a**: NaOMe, dimethyl carbonate, CH_2Cl_2; (e) formation of **13** from **11a**: MeOH, $Sm(OTf)_3$.

we had observed[42] the beneficial effects on potency of introducing small flanking substituents at C3 and C5 of the piperidine ring, and thus we chose to begin our follow-up of lead **2** by examining the addition of a methyl group at these positions.

Chemistry

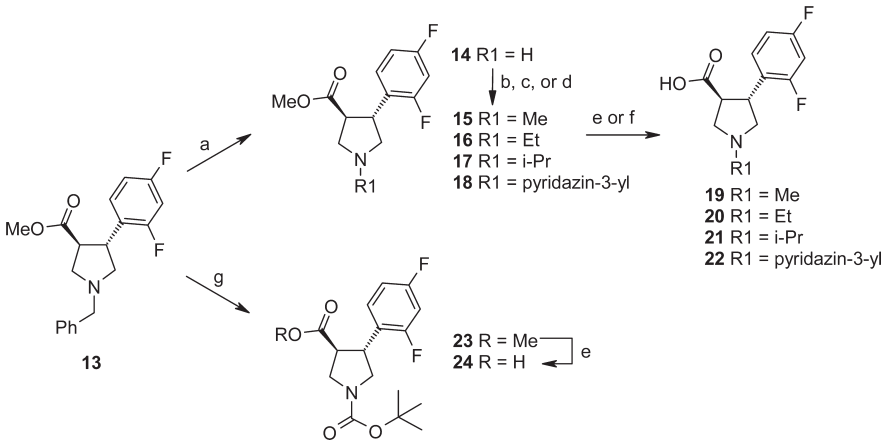
Enantiopure pyrrolidine **1** was prepared by a slightly modified version of literature [3 + 2] cycloaddition routes.[34,39,43] Thus, reaction of chloromethyltrimethylsilane **4** with an excess of *tert*-butylamine **3** in a sealed bomb at high temperature provided secondary amine **5** (Scheme 1), the excess **4** being readily removed by washing with water. Azomethine ylide precursor **6** was then obtained by reaction of **5** with formaldehyde and methanol in the presence of potassium carbonate. In our hands, the grade of potassium carbonate (325 mesh) proved crucial to the success of the reaction, and we found it most convenient to isolate **6** as a 4:1 mixture with unreacted **5** because purification procedures led to significant loss of **6**. The presence of **5** did not influence subsequent cycloaddition reactions.

The dipolarophile **9** was prepared by conversion of 2,4-difluorocinnamic acid **8** to the corresponding acid chloride and subsequent reaction with the Evans auxiliary (*S*)-(−)-4-benzyl-2-oxazolidinone in the presence of lithium chloride (Scheme 2). Exposure of a solution of **6** and **9** to trifluoroacetic acid provided 3,4-*trans* disubstituted pyrrolidine cycloadducts **10a**/**b** with negligible diastereoselectivity. However, separation was readily achieved via flash column chromatography, thereby enabling selective access to both **1** and *ent*-**1**. On multigram scales, we found that **10a** could be obtained selectively in good yield and high purity from the crude mixture of cycloadducts **10a**/**b** by crystallization from ethyl acetate and pentane. Removal of the chiral auxiliary was initially achieved by exposure of **10a** to lithium hydroxide followed by neutralization with a stoichiometric quantity of aqueous hydrochloric acid, providing carboxylic acid **1**, which we isolated as an amorphous foam and used without complete removal of the residual lithium chloride. On larger scales, a convenient alternative was to use a two-step process involving generation of the methyl ester **12** by cleavage of the oxazolidinone auxiliary with sodium methoxide in the presence of dimethyl carbonate,[44] followed by hydrolysis with lithium hydroxide and acidification to provide the hydrochloride salt of **1**, which could be crystallized from isopropyl alcohol.

To access different pyrrolidine *N*-substituents, a similar route was employed using the commercially available *N*-benzyl azomethine ylide precursor **7**, leading to the formation of cycloadducts **11a**/**b**, again readily separable by flash chromatography. The only substantial difference in the route was that the chiral auxiliary was removed from the cycloadduct by reaction with methanol in the presence of samarium triflate,[45] forming ester **13**. This key intermediate allowed two alternative paths forward, which were chosen according to convenience or the demands of other substituents elsewhere in the target compounds. Hydrogenolysis of **13** provided the secondary amine **14** (Scheme 3), which was then alkylated to provide tertiary amines **15**−**17** or heteroarylated to **18**, and these esters in turn were hydrolyzed to acids **19**−**22**. Alternatively, **13** was subjected to transfer hydrogenation in the presence of di-*tert*-butyl dicarbonate to generate carbamate **23**, which similarly was hydrolyzed to the corresponding acid **24**.

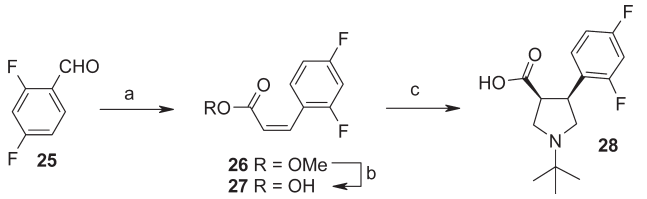
A racemic *cis*-3,4-disubstituted pyrrolidine **28** was obtained by cycloaddition of **6** with *Z*-olefin **27**, itself prepared via a Still−Gennari modification[46] of the Horner−Emmons olefination

Scheme 3[a]



[a] Reagents and conditions: (a) $Pd(OH)_2/C$, 50 psi H_2, EtOH; (b) CH_2O (for **15**), or acetone (for **17**), $NaBH(OAc)_3$, AcOH, CH_2Cl_2; (c) EtOTs, K_2CO_3, MeCN, 70 °C (for **16**); (d) 3-chloropyridazine, *i*-Pr_2NEt, NMP, 120 °C; (e) LiOH, THF, then $HCl_{(aq)}$ (for **19**, **21**, **22**, and **24**); (f) $HCl_{(conc)}$ (for **20**); (g) $Pd(OH)_2/C$, 1-methylcyclohexa-1,4-diene, $(Boc)_2O$, EtOH.

Scheme 4[a]



[a] Reagents and conditions: (a) $(CF_3CH_2O)_2P(O)CH_2CO_2Me$, KHMDS, 18-crown-6, THF, −78 °C; (b) LiOH, H_2O, THF; (c) **6**, TFA, CH_2Cl_2.

(Scheme 4). Use of the acrylic acid as the dipolarophile avoided issues of stereochemical lability which we had encountered during base-mediated hydrolysis of the 3,4-*cis* isomer of methyl ester **12**.

Synthesis of 3,5-dimethyl-4-arylpiperidin-4-ols[47] and 3,5-dimethyl-4-alkylpiperidin-4-ols began with dimethylation of dimethyl 3-oxopentanedioate **29**, the use of 325-mesh potassium carbonate once again proving crucial for successful reaction (Scheme 5). The double Mannich product **31**, obtained on exposure of **30** to benzylamine and formaldehyde in methanol, was decarboxylated to generate a 6:1 mixture of meso **33a** and racemic **33b**. The ratio could be improved to 24:1 favoring the meso-isomer by epimerization with sodium methoxide in methanol, and the mixture was readily separated by chromatography on silica. Addition of Grignard reagents or aryl and alkyl lithium species with subsequent hydrogenolytic benzyl removal provided piperidines **39**. It was found that some aryl groups (e.g., *para*-chlorophenyl) were insufficiently stable to hydrogenolysis, and therefore an alternative *N*-PMB protection was employed (**32**), leading to a similar 24:1 ratio of stereoisomers **34a**/**b**. The PMB protection was also advantageous for larger scale work because **34a** could be purified by crystallization. Removal of the PMB group occurred rapidly in the presence of 1-chloroethylchloroformate, and this process was found to be significantly more facile than for benzyl deprotection under the same conditions. Similarly, *N*-Boc protection was found to be preferable for the addition of some nucleophiles to the piperidinone, and therefore **33a** was debenzylated in the presence of di-*tert*-butyl dicarbonate to provide **35**.

Finally, the secondary amines **39** were then coupled to carboxylic acids **1**, *ent*-**1**, and **19**−**22** to provide test compounds **40**−**45** or **49** (Scheme 6), generally after conversion to their hydrochloride salts. Alternatively, coupling to carboxylic acid **24** produced **46**, which was *N*-deprotected and capped by subsequent *N*-alkylation or heteroarylation, followed again by conversion to the hydrochloride salts. The products **40**−**45** and **47**−**49** exist as mixtures of amide rotamers partly resolved on the NMR time-scale, leading to very complex spectra. However, the full relative and absolute stereochemistry of a representative compound, **40a**, was confirmed by X-ray crystallography (Figure 3).[48]

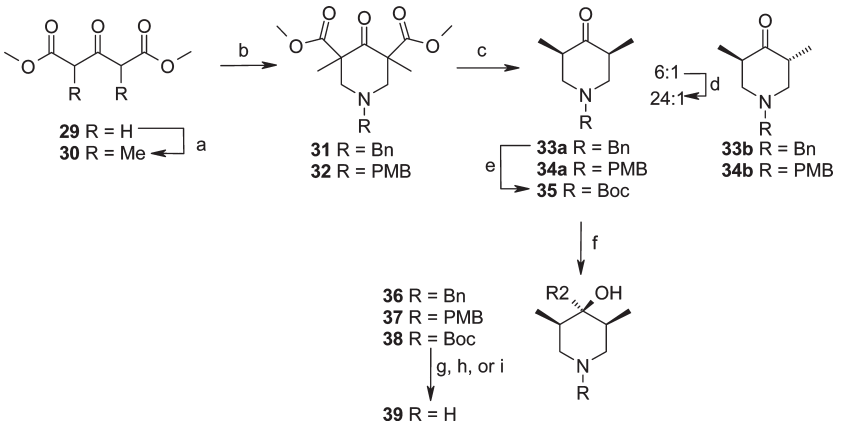
Results and Discussion

The structure−activity relationship (SAR) of our compounds at the MC4 receptor is shown in Table 1. Addition of methyl substituents in a *cis*-relationship at C3 and C5 of the piperidine ring was found to provide a marked increase in potency at the MC4 receptor (compare compounds **2** and **40a**). Comparison of the potencies for enantiomeric pair **40a**/**44** and racemic *cis*-diastereomer **45** clearly established that attractive levels of activity reside only in the *trans*-3*S*,4*R*-pyrrolidine, and subsequent efforts were focused on this stereoisomer.

Exploration of the R2 group revealed that various small substituents are broadly tolerated on the phenyl ring (**40a**−**k**), although no significant potency advantage was found versus unsubstituted **40a**. Notably, the least successful substituent of these, nitrile, is also the most polar (**40f**/**h**). Replacement of the phenyl ring by a 2-pyridyl (**40l**) occurred with only minor reduction in activity, but moving to a 3-pyridyl or 5-pyrimidinyl led to a progressively more pronounced loss of potency (**40 m**/**n**). Replacement of the piperidine's aromatic substituent by small alkyl groups at C4 was also briefly examined (**40o**−**q**) in an effort to temper lipophilicity, but the extent of potency loss outweighed the modest gain in physicochemical properties (vide infra).

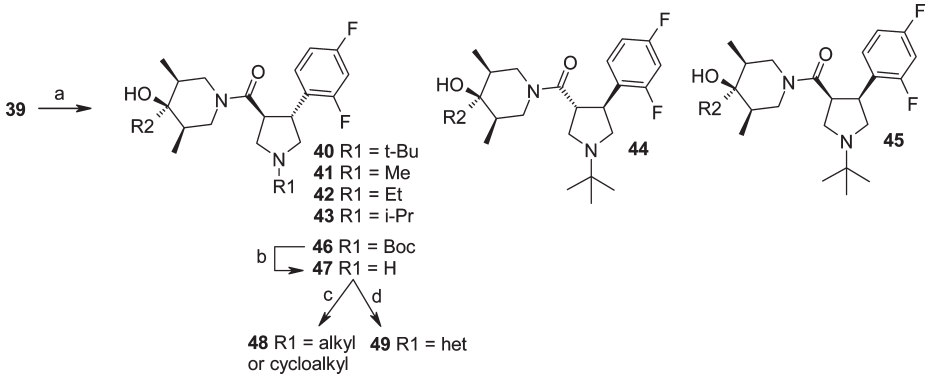
The pyrrolidine *N*-substituent was also subjected to systematic variation. The SAR in progressing from one- to four-carbon alkyl groups was found to be rather flat, for example *N*-ethyl **42a** is essentially equipotent with *N*-butyl isomers **48c**−**e**, and yet *tert*-butyl remained the optimum alkyl group throughout this study, with only *n*-propyl, cyclopropyl, and cyclobutyl substituents maintaining potency within 5-fold. Introduction of polarity into the alkyl chain by means of an

Scheme 5[a]



[a] Reagents and conditions: (a) MeI, K_2CO_3, THF, 60 °C; (b) benzylamine, $HCl_{(aq)}$, MeOH, CH_2O; (c) 1 M $HCl_{(aq)}$, reflux; (d) NaOMe, MeOH; (e) $Pd(OH)_2/C$, H_2, EtOH, Boc_2O; (f) R2Li, or R2MgBr, THF; (g) from **36**: Pd/C, dihydrotoluene, EtOH, or $Pd(OH)_2/C$, H_2, EtOH, or Pd/C, H_2, MeOH; (h) from **37**: ACE-Cl, Et_3N, CH_2Cl_2, then MeOH reflux; (i) from **38**: 4 M HCl in dioxane, CH_2Cl_2.

Scheme 6[a]



[a] Reagents and conditions: (a) **1**, *ent*-**1**, **19**−**22**, or **24**, 1-propylphosphonic acid cyclic anhydride, Et_3N, CH_2Cl_2; (b) 4 M HCl in dioxane, CH_2Cl_2; (c) aldehydes or ketones, $NaBH(OAc)_3$, CH_2Cl_2; (d) Het-Br or Het-Cl, Et_3N, EtOH, reflux; or Het-Cl, Et_3N, DMF, 80−100 °C; or Het-Br, sodium 2-methylpropan-2-olate, $Pd_2(dba)_3$, (±)-BINAP, PhMe, reflux.

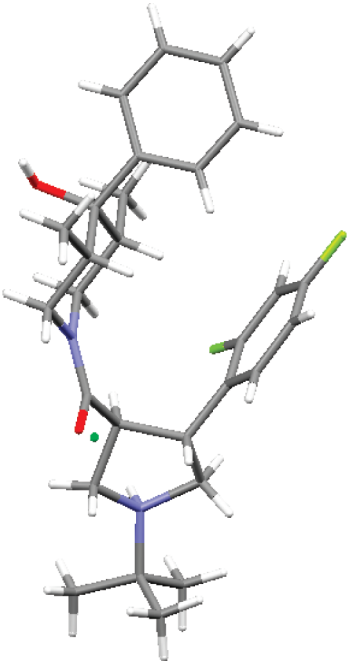


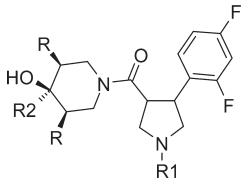
Figure 3. X-ray crystal structure of compound **40a**.

ether function diminished potency significantly (**48g**), whereas within a cyclic framework the ether was well tolerated (**48h**). The absence of a pyrrolidine *N*-substituent altogether led to a more dramatic loss of potency (**47a**−**d**), although these compounds nonetheless maintained superiority to the original lead **2**.

We also capped the pyrrolidine nitrogen with six-membered heterocycles, which can be organized into two groups on potency terms: those that maintained or even slightly enhanced potency versus a *tert*-butyl substituent (**49a**−**c**), and those that suffered a 10- to 20-fold loss (**49j**−**l**). The results obtained with these heterocyclic members demonstrate that a strongly basic center is not essential for achieving high potency and full efficacy at the MC4 receptor. Of particular note is the impact that switching to a heterocycle can have on the tolerance for otherwise less ideal R1 substituents, as demonstrated by **49e**−**h** in comparison with **40l**/**o**−**q**. Alongside the crucial 3,5-dimethyl substituents on the piperidine ring, the use of a heterocycle at R1 was the only markedly potency-enhancing SAR feature that we encountered during this study.

To assess further the potential of this series, a selection of compounds was screened for selectivity over activity at other melanocortin receptors and the hERG potassium ion channel.

Table 1. Functional Activity of α-MSH, MT-II, **2**, **40**−**45**, and **47**−**49** at the MC4 Receptor



compd	R2	R1	R	pyrrolidine stereochemistry	MC4 EC_{50} (nM)[a,b]	E_{max} (%)[b]
α-MSH					2.2 ± 0.2	99 ± 1
MT-II					0.11 ± 0.05	109 ± 3
2	Ph	*t*-Bu	H	3*S*,4*R*	1166 ± 211	87 ± 6
40a	Ph	*t*-Bu	Me	3*S*,4*R*	12 ± 1	97 ± 2
40b	4-MeO-Ph	*t*-Bu	Me	3*S*,4*R*	53 ± 14	100 ± 0.3
40c	4-Cl-Ph	*t*-Bu	Me	3*S*,4*R*	30 ± 4	95 ± 3
40d	4-F-Ph	*t*-Bu	Me	3*S*,4*R*	18 ± 3	97 ± 1
40e	4-CF_3-Ph	*t*-Bu	Me	3*S*,4*R*	108 ± 11	83 ± 8
40f	4-CN-Ph	*t*-Bu	Me	3*S*,4*R*	347 ± 117[c]	100 ± 0
40g	3-F-Ph	*t*-Bu	Me	3*S*,4*R*	6 ± 0.7	90 ± 3
40h	3-CN-Ph	*t*-Bu	Me	3*S*,4*R*	644 ± 414	97 ± 4
40i	2,6-diF-Ph	*t*-Bu	Me	3*S*,4*R*	45 ± 8	92 ± 4
40j	2,4-diF-Ph	*t*-Bu	Me	3*S*,4*R*	62 ± 28	95 ± 5
40k	3,4-diF-Ph	*t*-Bu	Me	3*S*,4*R*	15 ± 3	93 ± 3
40l	pyridin-2-yl	*t*-Bu	Me	3*S*,4*R*	46 ± 16	100 ± 2
40m	pyridin-3-yl	*t*-Bu	Me	3*S*,4*R*	3630 ± 1630	95 ± 5
40n	pyrimidin-5-yl	*t*-Bu	Me	3*S*,4*R*	11100 ± 2900[c]	97 ± 3
40o	*n*-Pr	*t*-Bu	Me	3*S*,4*R*	126 ± 18	89 ± 3
40p	*i*-Pr	*t*-Bu	Me	3*S*,4*R*	626 ± 112[c]	100 ± 0
40q	*c*-Pr	*t*-Bu	Me	3*S*,4*R*	962 ± 205	101 ± 2
41a	Ph	Me	Me	3*S*,4*R*	717 ± 241	93 ± 3
41b	4-Cl-Ph	Me	Me	3*S*,4*R*	130 ± 21	77 ± 2
41c	4-F-Ph	Me	Me	3*S*,4*R*	365 ± 75	86 ± 4
42a	Ph	Et	Me	3*S*,4*R*	85 ± 11[c]	100 ± 0
42b	4-F-Ph	Et	Me	3*S*,4*R*	147 ± 12[c]	100 ± 0
42c	3-F-Ph	Et	Me	3*S*,4*R*	61 ± 9	79 ± 4
42d	2,4-diF-Ph	Et	Me	3*S*,4*R*	51 ± 7[c]	81 ± 2
42e	3,4-diF-Ph	Et	Me	3*S*,4*R*	69 ± 12[c]	92 ± 8
43a	Ph	*i*-Pr	Me	3*S*,4*R*	69 ± 17	90 ± 4
43b	4-Cl-Ph	*i*-Pr	Me	3*S*,4*R*	91 ± 9	100 ± 0
43c	4-F-Ph	*i*-Pr	Me	3*S*,4*R*	52 ± 2[c]	100 ± 0
43d	3-F-Ph	*i*-Pr	Me	3*S*,4*R*	40 ± 5	86 ± 4
43e	2,4-diF-Ph	*i*-Pr	Me	3*S*,4*R*	220 ± 124	88 ± 5
43f	3,4-diF-Ph	*i*-Pr	Me	3*S*,4*R*	257 ± 216	100 ± 0.5
44	Ph	*t*-Bu	Me	3*R*,4*S*	> 10000[c]	
45	Ph	*t*-Bu	Me	3*RS*,4*RS*	3900 ± 1940	86 ± 0.5
47a	Ph	H	Me	3*S*,4*R*	462 ± 115	90 ± 4
47b	4-F-Ph	H	Me	3*S*,4*R*	255 ± 59	84 ± 5
47c	3-F-Ph	H	Me	3*S*,4*R*	159 ± 65	79 ± 3
47d	3,4-diF-Ph	H	Me	3*S*,4*R*	288 ± 50[c]	108 ± 15
48a	Ph	*n*-Pr	Me	3*S*,4*R*	19 ± 5	93 ± 7
48b	Ph	*c*-Pr	Me	3*S*,4*R*	55 ± 20	
48c	Ph	*n*-Bu	Me	3*S*,4*R*	105 ± 74	100 ± 0
48d	Ph	*i*-Bu	Me	3*S*,4*R*	73 ± 38	100 ± 1
48e	Ph	*c*-PrCH_2	Me	3*S*,4*R*	81 ± 23[c]	100 ± 0
48f	Ph	*c*-Bu	Me	3*S*,4*R*	32 ± 9	100 ± 0.3
48g	Ph	$(CH_2)_2OMe$	Me	3*S*,4*R*	374 ± 106	78 ± 4
48h	Ph	THP-4-yl	Me	3*S*,4*R*	18 ± 5	82 ± 4
49a	Ph	pyridin-2-yl	Me	3*S*,4*R*	15 ± 8	100 ± 1
49b	Ph	pyridin-3-yl	Me	3*S*,4*R*	5 ± 0.6	90 ± 2
49c	Ph	pyridazin-3-yl	Me	3*S*,4*R*	6 ± 3	92 ± 4
49d	4-F-Ph	pyridazin-3-yl	Me	3*S*,4*R*	4 ± 0.8[c]	97 ± 7
49e	pyridin-2-yl	pyridazin-3-yl	Me	3*S*,4*R*	7 ± 7	94 ± 7
49f	*n*-Pr	pyridazin-3-yl	Me	3*S*,4*R*	21 ± 4	95 ± 4
49g	*i*-Pr	pyridazin-3-yl	Me	3*S*,4*R*	89 ± 8	89 ± 7
49h	*c*-Pr	pyridazin-3-yl	Me	3*S*,4*R*	82 ± 20	98 ± 6
49i	Ph	pyrimidin-4-yl	Me	3*S*,4*R*	4 ± 1	88 ± 4
49j	Ph	pyrimidin-5-yl	Me	3*S*,4*R*	117 ± 21[c]	73 ± 2

Table 1. Continued

compd	R2	R1	R	pyrrolidine stereochemistry	MC4 EC_{50} (nM)[a,b]	E_{max} (%)[b]
49k	Ph	pyrimidin-2-yl	Me	3*S*,4*R*	243 ± 37	75 ± 4
49l	Ph	pyrazin-2-yl	Me	3*S*,4*R*	111 ± 19	87 ± 4

[a] Human recombinant MC4 receptor expressed in CHO cells, β-lactamase reporter gene format, with the maximal response defined by MT-II (see the Supporting Information for full details). [b] Except where noted, values are mean of $n \geq 3$ experiments ± SEM. [c] $n = 2$.

Table 2. Activities of Selected Compounds at the MC1, MC3, and MC5 Receptors, the hERG Potassium Ion Channel, and in Binding at the MC4 Receptor

compd	R2/R1	MC3 EC_{50} (μM) [E_{max} (%)][a,f]	MC1 EC_{50} (μM) [E_{max} (%)][b,f]	MC5 EC_{50} (μM) [E_{max} (%)][c,f]	hERG K_i (μM)[d,f]	MC4 binding K_i (nM)[e,f]
α-MSH		0.0027 ± 0.0004 [99 ± 4]	0.0009 ± 0.0001 [96 ± 2]	0.36 ± 0.07 [105 ± 4]	NT	21 ± 2
MT-II		0.0054 ± 0.0008 [87 ± 7]	0.0001 ± 0.00002 [98 ± 6]	0.069 ± 0.016 [61 ± 12]	NT	NT
40a	Ph/*t*-Bu	1.16 ± 0.35 [99 ± 5]	1.02 ± 0.30 [103 ± 6]	1.98 ± 0.20 [100 ± 5]	> 10.0	27 ± 4[g]
40c	4-Cl-Ph/*t*-Bu	3.68 ± 2.89 [75 ± 21][g]	> 5.88	5.19 ± 3.00 [107 ± 5]	> 10.0[h]	48 ± 12
40d	4-F-Ph/*t*-Bu	3.05 ± 1.25 [79 ± 3]	1.12 ± 0.36 [99 ± 3]	3.60 ± 1.22 [116 ± 14]	7.91 ± 0.34[g]	36 ± 4
40g	3-F-Ph/*t*-Bu	0.57 ± 0.09 [81 ± 4][g]	> 6.47	1.80 [74][h]	NT	97 ± 22
40i	2,6-diF-Ph/*t*-Bu	4.57 ± 2.79 [76 ± 34]	> 5.01	> 5.81	5.00 ± 0.7[g]	99 ± 14
40j	2,4-di-F-Ph/*t*-Bu	10.7 ± 5.1	6.57 ± 2.00 [93 ± 12][g]	3.50 [84][h]	NT	176 ± 24
40l	pyridin-2-yl/*t*-Bu	> 33.3	1.17 ± 0.10 [97 ± 10]	17.8 [100][h]	5.84[h]	350 ± 66
40o	*n*-Pr/*t*-Bu	> 24.7	3.61 ± 0.26 [95 ± 9]	NT	NT	844 ± 4[g]
41a	Ph/Me	> 19.1	> 7.81	> 20.0[h]	NT	1070 ± 598[g]
41c	4-F-Ph/Me	> 33.4	> 6.15	15.0 [100][h]	NT	346 ± 66[g]
42c	3-F-Ph/Et	> 13.5	> 8.25	6.41 [92][h]	NT	355 ± 62
43a	Ph/*i*-Pr	11.6 ± 7.44 [72 ± 5]	0.75 ± 0.26 [100 ± 8]	3.64 ± 1.33 [103 ± 21]	> 7.88[h]	114 ± 15
47a	Ph/H	> 25.2	> 8.74	> 20.0[g]	6.65 ± 1.19[g]	264 ± 94[g]
48b	Ph/*c*-Pr	> 33.3[h]	> 4.91	NT	> 9.17[h]	290 ± 106
48c	Ph/*n*-Bu	> 5.72[h]	1.10 ± 0.16 [72 ± 7]	1.43 [72][h]	NT	233 ± 30
48f	Ph/*c*-Bu	0.50 ± 0.06 [81 ± 1][g]	0.35 ± 0.06 [79 ± 5][g]	NT	5.87 ± 0.28[g]	62 ± 3
48h	Ph/THP-4-yl	> 14.5	> 11.9	> 20.0[h]	> 5.27[g]	36 ± 5
49a	Ph/pyridin-2-yl	0.071 ± 0.006 [78 ± 6][g]	> 16.7	0.96 [29][h]	4.65 ± 0.36[g]	79 ± 2
49b	Ph/pyridin-3-yl	0.065 ± 0.024 [90 ± 1][g]	> 8.22	NT	5.07 ± 0.71[g]	50 ± 5
49c	Ph/pyridazin-3-yl	0.49 ± 0.21 [89 ± 6]	> 3.07	> 20.0	> 10.0[g]	10 ± 3
49e	Pyridin-2-yl/pyridazin-3-yl	3.66 ± 2.50 [75 ± 8]	1.85 ± 0.38 [67 ± 3]	NT	NT	52 ± 4
49f	*n*-Pr/pyridazin-3-yl	7.48 ± 6.40 [79 ± 14]	3.90 ± 0.55 [104 ± 6][g]	NT	> 10.0[g]	56 ± 4
49h	*c*-Pr/pyridazin-3-yl	4.13 ± 0.5 [42 ± 6][g]	> 16.7	NT	> 10.0[g]	172 ± 29
49i	Ph/pyrimidin-4-yl	0.60 ± 0.28 [75 ± 4]	> 9.00	> 14.0	> 10.0[g]	64 ± 19
49k	Ph/pyrimidin-2-yl	> 26.7	> 10.00	> 11.1[h]	NT	300 ± 41[g]

[a] Human recombinant MC3 receptor expressed in CHO cells, measuring adenylyl cyclase activation. [b] Human recombinant MC1 receptor expressed in HEK-293 cells, measuring adenylyl cyclase activation. [c] Human recombinant MC5 receptor expressed in CHO-cells, β-lactamase reporter gene format. [d] Competitive binding versus [^{3}H]-dofetilide at the hERG product expressed in HEK-293 cells. [e] Competitive binding versus [^{125}I]-MT-II at the human recombinant MC4 receptor expressed in CHO cells. [f] Except where noted, values are the mean of ≥3 experiments ± SEM. [g] $n = 2$. [h] $n = 1$.

As can be seen from Table 2, compounds in the R1 = alkyl or H series (**40**−**43**, **47**, **48**) were found in general to have only relatively weak activity at the MC1, MC3, and MC5 receptors, providing good selectivity margins in many cases. Among the R1 = alkyl subset, the presence of a cyclobutyl substituent at R1 (**48f**) stands out as providing the highest absolute potency at both the MC3 and MC1 receptors, although, given the high MC4 receptor potency of this compound, significant selectivity still exists.

In contrast, the presence of a heteroaryl substituent at R1 was found to confer high potency at the MC3 receptor in some instances, especially the pyridyls **49a** and **49b**. However, it is clear that the R2 substituent can be varied to selectively attenuate MC3 receptor activity in preference to MC4 activity (compare **49c** with **49e**/**f**, Tables 1 and 2). Interestingly, the heterocycles which were found to be less active at the MC4 receptor were also correspondingly weaker at MC3 (e.g., **49k**). No significant activity at the MC1 and MC5 receptors was observed within the heteroaryl subset.

Only weak micromolar activity was encountered at the hERG potassium ion channel across the whole series, regardless of basicity and despite the high lipophilicities. Finally, all compounds were confirmed as binders of the MC4 receptor (Table 2), their affinities very approximately tracking with the agonist potencies.

A further selection of compounds was assessed for metabolic stability in human liver microsomes (HLM), and representative examples are shown in Table 3. The presence of a metabolically inert substituent at the 4-position of the R2 aryl ring consistently provided increased microsomal stability (compare **40c**−**e** versus **40a**, or **40g** versus **40d**/**k**, and similarly **42f** versus **42d** and **43b**/**d**/**f** versus **43a**), suggesting this as a location of oxidation on the unsubstituted parents (vide infra). Replacement of the aryl ring by a small alkyl chain was also beneficial to metabolic stability (**40q** versus **40a**), although here the reduction in lipophilicity may also play a role alongside the removal of a potentially labile aryl site.

Complete removal of the pyrrolidine *N*-substituent conferred high metabolic stability (**47b**/**d**), whereas variation of the R1 alkyl group isomers and homologues showed little benefit to be derived from minimization of the lipophilicity in this region, and all changes explored were actually detrimental compared to *tert*-butyl (compare for example compound **40c** versus **43b**, and compound **40k** versus **42f** and **43f**). The marked deterioration in microsomal stability for the cyclopropyl analogue **48b** may result in part from its significantly increased

logD, presumably driven by diminished pyrrolidine basicity. The placement of polarity directly within the R2 alkyl group was also unsuccessful in improving metabolic stability (**48g**/**h** versus **40a**).

The *N*-heteroaryl subseries showed very poor HLM stability (e.g., **49c**), and even the use of a 4-fluoro substituent on the aryl ring (**49d**) or reduction in logD (**49e**/**f**) provided no improvement. Nonetheless, the high MC4 receptor potency of these compounds remained attractive to us, and the examples shown above ultimately became the progenitors of a promising neutral/weakly basic series that will be described in more detail elsewhere.

On the basis of the combined considerations of potency at the MC4 receptor, selectivity over the MC1/3/5 receptors and the hERG ion channel, and in vitro metabolic stability, we selected compounds **40a** and **40d** for further evaluation owing to the superior balance of their profiles across these various parameters. Table 4 shows additional in vitro data that were generated prior to in vivo profiling in preclinical species. Both compounds showed somewhat higher metabolic vulnerability in rat liver microsomes (RLM) than in HLM. In dog liver microsomes (DLM), however, **40d** has high stability, whereas **40a** is metabolized very rapidly. Unsurprisingly, both compounds are similar to each other in their levels of plasma protein binding across the three species, the slightly higher figures for **40d** no doubt reflecting its slightly higher lipophilicity. Both compounds showed a slight preference for partitioning into blood versus plasma across all three species. Their good flux across MDR1-overexpressing MDCK monolayers, with minor (**40a**) or negligible (**40d**) efflux ratios, provided encouragement that CNS penetration would be high.

Table 3. Experimentally Measured logD and Human Liver Microsome Stability

compd	R2/R1	$\log D_{(pH7.4)}$	HLM Cl_{int} (μL/min/mg protein)[a]
40a	Ph/*t*-Bu	2.8	45
40c	4-Cl-Ph/*t*-Bu	3.9	16
40d	4-F-Ph/*t*-Bu	3.1	28
40e	4-CF_3-Ph/*t*-Bu	4.1	< 7
40g	3-F-Ph/*t*-Bu	3.3	52
40k	3,4-diF-Ph/*t*-Bu	3.5	22
40q	*c*-Pr/*t*-Bu	2.2	14
42c	3-F-Ph/Et	2.9	73
42f	3,4-diF/Et	3.2	28
43a	Ph/*i*-Pr	3.2	128
43b	4-Cl-Ph/*i*-Pr	3.5	34
43d	3-F-Ph/*i*-Pr	3.2	70
43f	3,4-diF-Ph/*i*-Pr	3.3	28
47b	4-F-Ph/H	1.8	13
47d	3,4-diF-Ph/H	2.0	7
48b	Ph/*c*-Pr	> 4	342
48g	Ph/$(CH_2)_2$OMe	3.1	84
48h	Ph/THP-4-yl	3.1	151
49c	Ph/pyridazin-3-yl	3.3	243
49d	4-F-Ph/pyridazin-3-yl	3.70	310
49e	pyridin-2-yl/pyridazin-3-yl	3.0	> 440
49f	*n*-Pr/pyridazin-3-yl	2.8	> 440

[a] The volume of in vitro incubation cleared of test compound per unit time, normalized to 1 mg of human liver microsomal protein.

The results from rat and dog pharmacokinetic studies with **40a** and **40d** are shown in Table 5. In keeping with the rapid in vitro metabolism of **40a** in rat and dog liver microsomes, **40a** was found to be subject to a high rate of metabolism by both species in vivo, with oral bioavailability being limited by the high first-pass extraction. The high volumes of distribution measured for **40a** and **40d** are consistent with the physicochemistry of a lipophilic base (measured pK_a 8.95 and 8.74, respectively) and provide for moderate half-lives in both rat and dog. As anticipated by the good MDCK-MDR1 flux values, **40a** and **40d** partitioned freely into the cerebrospinal fluid (CSF) of the rat, suggesting unimpaired blood−brain barrier penetration.

Analysis of rat urine following iv administration revealed that < 3% of the dose was renally excreted as the parent compound. Furthermore, in an isolated perfused rat liver experiment, < 1% of **40a** was eliminated unchanged in the bile. The identification of metabolites generated from **40a** in vitro was carried out after incubation in human, rat, and dog hepatocyte preparations. In human hepatocytes, the major metabolites detected were a mono-oxidized species consistent with oxidation of the *tert*-butyl substituent (**50**) and *N*-dealkylated product **47a** (Figure 4). Several less abundant metabolites were also identified, including at least two species resulting from oxidation localized within the phenylpiperidine moiety (**51**) and an M + 34 species (**52**) presumed to be a dihydrodiol. Metabolites **50**−**52** and **47a** were all major metabolites in dog hepatocytes, whereas in rat **50**, **47a**, and several mono-oxidized species **51** were predominant. A pyrrolidine *N*-oxide was also detected as a major component in all hepatocyte experiments but was likewise found in buffer control, so is not necessarily enzymatic in origin.

In comparison with **40a**, the improved rat and dog liver microsome stability of **40d** translated accordingly to lower

Table 4. In Vitro Pharmacokinetic Measurements for Compounds **40a** and **40d**

compd	RLM Cl_{int} (μL/min/mg protein)[a]	DLM Cl_{int} (μL/min/mg protein)[a]	human plasma f_u[b]	rat plasma f_u[b]	dog plasma f_u[b]	human/rat/dog blood:plasma ratio	MDCK-MDR1 AB/BA P_{app} ($\times 10^6$ cm·s^{-1})[c]
40a	60	242	0.13	0.07	0.15	1.1/1.1/1.1	18/41
40d	47	< 8	0.07	0.04	0.09	1.7/1.3/1.6	22/26

[a] The volume of in vitro incubation cleared of test compound per unit time, normalized to 1 mg of rat or dog liver microsomal protein. [b] The free fraction of test compound in human, rat, or dog plasma. [c] The apparent permeability of the test compound across cell monolayers as measured in the apical to basolateral (AB) and basolateral to apical (BA) directions.

Table 5. In Vivo Rat and Dog Pharmacokinetic Measurements for Compounds **40a** and **40d**

compd	rat[a] Cl_{plasma} (mL/min/kg)	rat[a] Vd_{plasma} (L/kg)	rat[a] $T_{1/2}$ (h)	rat[b] CSF:free plasma	rat[c] *F* (%)	dog[d] Cl_{plasma} (mL/min/kg)	dog[d] Vd_{plasma} (L/kg)	dog[d] $T_{1/2}$ (h)	dog[e] *F* (%)
40a	89	19	2.4	0.9	20	46	17	4.2	1
40d	34	11	3.9	0.8	74	12	11	10.4	28

[a] **40a/d** 1 mg/kg iv. [b] iv infusion to steady state. [c] **40a** 1 mg/kg po, **40d** 0.1 mg/kg po. [d] **40a** 0.5 mg/kg iv, **40d** 0.05 mg/kg iv. [e] **40a** 0.5 mg/kg po, **40d** 0.1 mg/kg po.

in vivo plasma clearances in these species, leading to significantly enhanced oral bioavailabilities (Table 5). Metabolite identification after incubation in liver microsomes revealed a similar array of metabolites to those observed with **40a** across all three species, with the exception that in this case two further oxidized products, corresponding to **53** and **54**, were detected in HLM and DLM (Figure 5).

The broad off-target profiles of **40a** and **40d** were assessed in the CEREP Bioprint wide ligand screening panel and were both found to be similar and generally very clean, with the most potent binding activities being at the σ receptor (**40a** K_i = 330 nM; **40d** not tested), the sodium ion channel (**40a** K_i = 690 nM; **40d** K_i = 520 nM), and the muscarinic M2 receptor (**40a** K_i = 730 nM; **40d** K_i = 1200 nM). Thus, judged by the results of the preclinical pharmacokinetic studies, **40d** appears to potentially offer advantages over **40a**. However, although the data are presented here side-by-side, in reality the chronology of compound synthesis meant that **40a** progressed through our screen sequence far in advance of **40d**. Consequently, the potential utility of **40a** for testing the mechanism in humans was considered in isolation from much of the data shown herein for **40d**, with the latter eventually being positioned as a possible back-up to **40a**.

Because **40a** suffers essentially liver-blood-flow clearance in rat and dog, we were concerned that, on oral dosing in humans, the compound's otherwise attractive profile could

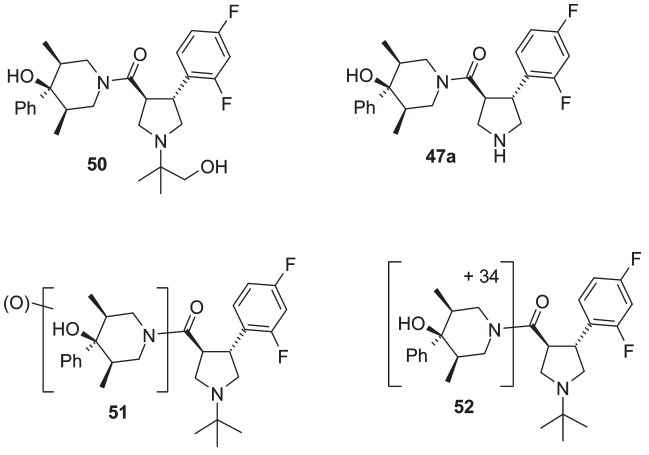


Figure 4. Metabolites of **40a** identified from incubation with human, rat, and dog liver microsomes.

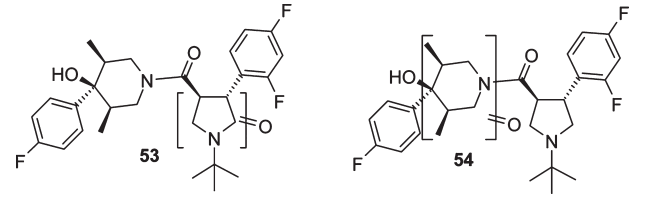


Figure 5. Additional oxidized species identified from **40d** in human and dog liver microsomes.

be compromised by very low bioavailability owing to extensive first-pass extraction. To mitigate this risk, we chose to examine alternative routes of administration, specifically intranasal and sublingual, deemed to be appropriate for on-demand dosing in sexual health indications. In dog pharmacokinetic studies, both intranasal and sublingual dosing of **40a** produced significant improvement in AUC and bioavailability over oral administration (Table 6). Nonetheless, it was considered important to be able to demonstrate higher C_{max} levels than that achieved by the 0.14 mg/kg doses (equating to ~1 nM unbound). The prospects for achieving this via the intranasal route seemed poor owing to limitations in the maximum solution volume which can be administered, whereas sublingual dosing offered greater flexibility. Two higher sublingual doses were therefore studied, and we were pleased to observe dose-dependent, approximately proportional increases in C_{max} and AUC.

With our increased confidence that adequate bioavailability in humans would be enabled by use of sublingual dosing should oral bioavailability prove poor, compound **40a**, designated internally as PF-00446687,[49] was progressed through the remaining preclinical in vitro and in vivo toxicology studies with satisfactory outcomes. In phase I trials in healthy male volunteers, **40a** was found to be safe and generally well tolerated via both oral and sublingual administration up to the maximum tested dose. The most common treatment-related systemic adverse event was restlessness (6.3%). Stretching and yawning were other events recorded which could be indicative of centrally mediated effects. Nausea (6.1%) was predominantly associated with sublingual dosing, and its lack of prevalence is notable when compared with the human data from MT-II and PT-141, for which nausea is dose-limiting.[22,23] Sublingual dosing of **40a** also gave rise to reports of application-site anesthesia, irritation, and hypothesia. There were no serious adverse events and no discontinuations.

Following sublingual doses ranging from 3 to 195 mg, peak plasma concentrations were achieved within 4 h at the lower doses and up to 8 h at the higher doses. Systemic exposures increased supraproportionally with dose (C_{max} increasing 162-fold for a 65-fold increase in dose across the entire range), and the estimated mean terminal half-life was 12 h for all doses. In the event, oral dosing provided very similar exposures at the comparable dose levels (32.5 and 195 mg). Peak plasma concentrations were uniformly found at 4 h post oral dose, with a terminal half-life again of 12 h. Reflecting the preclinical pharmacokinetics in rat (vide supra), unchanged **40a** excreted in urine was $<2.5\%$ of the 195 mg doses and similar for both routes of administration. The mean peak concentration from the 195 mg oral dose was 30 ng/mL (compared to 31 ng/mL from the 195 mg sublingual dose), equating to 8.3 nM unbound.

The effect of **40a** at oral doses of up to 200 mg in men with moderate to severe MED was assessed versus 100 mg of sildenafil[50,51] by the penile plethysmography technique in a pilot placebo-controlled trial utilizing a randomized, double-blind,

Table 6. Comparison of Oral, Intranasal, and Sublingual Dog Pharmacokinetic Parameters for **40a**

dose (mg/kg)	route	C_{max} (ng/mL)	AUC_{inf} (ng.h/ml)	T_{max} (h)	$T_{1/2}$ (h)	F (%)
0.5	oral	0.9	1.8	1.0	1.9	1
0.14	intranasal	2.6	12	1.6	3.3	24
0.14	sublingual	2.6	17	0.7	3.7	35
0.67	sublingual	15	64	0.3	3.6	26
2.04	sublingual	41	146	0.3	4	20

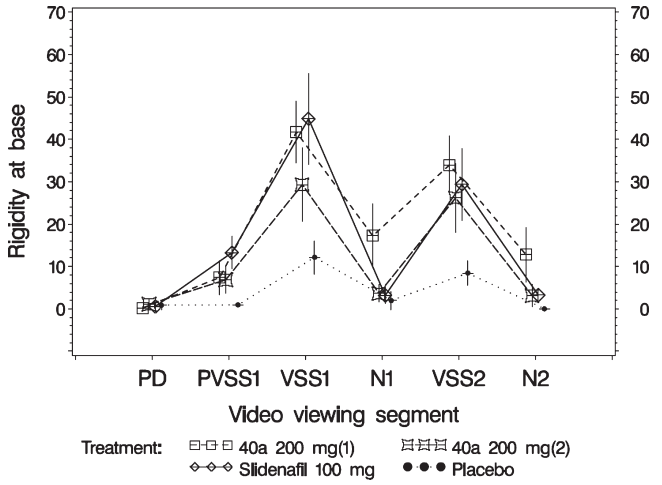


Figure 6. Mean and 80% confidence interval of rigidity response (%) by treatment over viewing segments (PD = predose; PVSS1 = previsual sexual stimulation period 1; VSS1 = visual sexual stimulation period 1; N1 = neutral period 1; VSS2 = visual sexual stimulation period 2; N2 = neutral period 2). The two doses of **40a** were separated by a minimum 7-day washout between each study period.

crossover design.[52] Subjects were shown sequences of visual sexual stimulation (VSS) and neutral (non-VSS) material beginning 2 h postdose. The 200 mg dose of **40a** (n = 15) provided similar efficacy to sildenafil and was superior to placebo for both base-rigidity (Figure 6) and questionnaire (data not shown) end-points during periods of VSS. However, below 200 mg (n = 24), only a weak dose−response relationship (not considered clinically meaningful) was observed.

Conclusion

In summary, we have discovered a novel series of small-molecule MC4 receptor agonists based on initial library hit **2**. The addition of methyl substituents at C3 and C5 of the 4-phenylpiperidin-4-ol unit provided significant potency enhancement and allowed us to achieve potent agonism with compounds which remained fully rule-of-five compliant,[53] a major challenge when targeting a peptidic GPCR.[54,55] Exploration of the SAR at the pyrrolidine *N*-substituent revealed *tert*-butyl to provide the optimum balance of potency and metabolic stability compared to other alkyl substituents. Use of a six-membered heterocycle at this position can be advantageous for potency, especially with regard to allowing greater flexibility in the substituents at C4 of the piperidine, but in some cases significant MC3 receptor activity is encountered, and in all cases shown herein high metabolic vulnerability was a major flaw. With regard to variation at C4 of the piperidine ring, efforts to diminish metabolic vulnerability by introduction of polarity or reduction in lipophilic bulk did not lead to overall superiority because of the counterbalancing detrimental impact on MC4 receptor potency. However, the presence of a lipophilic substituent in the para-position of the aryl ring enhanced in vitro metabolic stability in several cases. The translation of this effect to a reduction in in vivo clearance and a corresponding increase in oral bioavailability was demonstrated with **40d**. Compound **40a** was deemed to provide the best opportunity for most rapidly testing the MC4-selective hypothesis in the clinic, although alternative routes of administration were explored in an effort to mitigate the risk that oral dosing would provide inadequate bioavailability. Ultimately, in contrast to the observed dog pharmacokinetics, exposure after oral dosing of **40a** in humans compared favorably with that achieved by sublingual administration. At the highest dose tested (200 mg), compound **40a** has shown a level of efficacy that is similar to sildenafil and differentiated from placebo in a study of erectile activity in patients with moderate to severe MED, although lower doses were not efficacious. The mean peak unbound plasma concentration achieved at the highest dose is approximately equal to the EC_{50} of **40a** measured at the MC4 receptor in vitro and markedly lower than its potency at other melanocortin receptors, thus providing support for the hypothesis that MC4 receptor activation is indeed sufficient to elicit erectogenic effects in humans.

Experimental Section

All commercially available chemical reagents were used as received without further purification unless otherwise noted. Proton NMR data were recorded at 400 MHz on a Varian Mercury 400BB or a Varian Inova 400 instrument. Mass spectroscopic data were recorded using a Thermo Scientific Finnigan aQa APCI instrument, a Thermo Scientific Navigator ESI instrument, a Waters ZQ APCI LCMS instrument, or a Waters ZQ ESI LCMS instrument. Elemental analyses were performed by Exeter Analytical (UK) Ltd., and optical rotations were measured by Warwick Analytical Service. All test compounds were confirmed to be of ≥95% purity by either elemental analysis or HPLC (see Supporting Information).

The syntheses of compounds **40a**, **40c**−**d**, **40f**−**g**, **40i**−**l**, **40o**−**q**, **41b**−**c**, **42c**, **43a**−**c**, **43e**−**f**, **45**, **47a**−**d**, **48a**−**h**, **49a**−**c**, **49e**, and **49i**−**l** have been described previously.[49] Compounds **40a**, **40d**, **40k**, and **45** serve as representative procedures for the final-step synthesis of the remainder of the compounds.

Method A. (3*R*,4*R*,5*S*)-1-{[(3*S*,4*R*)-1-*tert*-Butyl-4-(2,4-difluorophenyl)pyrrolidin-3-yl]carbonyl}-3,5-dimethyl-4-phenylpiperidin-4-ol Hydrochloride (40a). (3*S*,4*R*)-1-*tert*-Butyl-4-(2,4-difluorophenyl)pyrrolidine-3-carboxylic acid[49] (**1**) (26.0 g, 92 mmol) and (3*R*,4*s*,5*S*)-3,5-dimethyl-4-phenylpiperidin-4-ol[49] (**39**, R2 = Ph) (17.4 g, 85 mmol) were suspended in dichloromethane (1000 mL). Triethylamine (14.2 mL, 102 mmol) was added, and the mixture was cooled to 0 °C with stirring under nitrogen. 1-Propylphosphonic acid cyclic anhydride (50% in ethyl acetate, 54.5 mL, 92 mmol) was added dropwise, maintaining the temperature below 5 °C. The mixture was then allowed to warm to room temperature with continuous stirring, and after 1 h acetic acid (5 mL) was added to remove the last traces of the (3*R*,4*s*,5*S*)-3,5-dimethyl-4-phenylpiperidin-4-ol. The reaction mixture was stirred for a further 1 h at room temperature. A 10% aqueous potassium carbonate solution (500 mL) was added, and the mixture was stirred vigorously at room temperature for 2 h. The organic layer was separated and then stirred with 10% aqueous potassium carbonate solution (500 mL) for 1 h. The dichloromethane layer was then separated, washed with water (3 × 300 mL), dried over sodium sulfate, and filtered. A solution of 4 M hydrogen chloride in dioxane (50 mL) was then added to the dichloromethane solution. The solvent was then evaporated to give the crude hydrochloride as a white powder. Acetone (500 mL) was added to the crude hydrochloride, and the mixture was boiled for 30 min and then allowed to cool to room temperature. The hydrochloride salt was filtered off and washed with acetone (5 × 100 mL). Recrystallization of the product from isopropyl alcohol gave analytically pure hydrochloride (39.5 g). ^{1}H NMR (400 MHz, CD_3OD) δ (rotamers) 1.28−1.59 (4d, 6H), 0.64−1.70 (m, 10H), 1.97−12.80 (m, 2H), 3.18 (t, 1H), 3.43−3.62 (m, 2H), 3.70−3.98 (m, 4H), 4.02−4.15 (m, 1H), 4.36 (dd, 1H), 7.02−7.40 (m, 7H), 7.60−7.78 (2q, 1H). MS (APCI) m/z 471 $[MH]^+$. $[\alpha]_D^{25}$ −51.9 (3.00 mg/mL, MeOH).

Method B. (3*R*,4*R*,5*S*)-1-{[(3*S*,4*R*)-1-*tert*-Butyl-4-(2,4-difluorophenyl)pyrrolidin-3-yl]carbonyl}-4-(4-fluorophenyl)-3,5-dimethylpiperidin-4-ol Hydrochloride (40d). 1-Propylphosphonic acid cyclic

anhydride (50 wt % solution in ethyl acetate, 0.67 mL, 2.0 mmol) was added dropwise to a mixture of (3*R*,4*s*,5*S*)-4-(4-fluorophenyl)-3,5-dimethylpiperidin-4-ol[49] (**39**, R2 = 4-F-Ph) (267 mg, 1.2 mmol), (3*S*,4*R*)-1-*tert*-butyl-4-(2,4-difluorophenyl)pyrrolidine-3-carboxylic acid[49] (**1**) (450 mg, 1.4 mmol), and triethylamine (0.48 mL, 3.6 mmol) in dichloromethane (25 mL) at 0 °C under nitrogen. On completion of addition, the resulting homogeneous solution was stirred for 6 h at room temperature. The solution was washed with 10% aqueous potassium carbonate solution (3 × 20 mL) and then dried over sodium sulfate and filtered. The solvent was removed in vacuo, and the crude product was purified by column chromatography (reverse phase C-18, 40 g Redisep cartridge, utilizing an ISCO Companion autopurification system. Mobile phase gradient over 20 min: MeCN/H_2O/TFA(5%/95%/0.1%) 95%:MeCN (100%) 5% eluting to MeCN/H_2O/TFA-(5%/95%/0.1%) 5%:MeCN(100%) 95%). The purified product was then dissolved in 1,4-dioxane (100 mL), and a 4 M solution of hydrogen chloride in dioxane (20 mL) was added. The solution was then concentrated to dryness, and the residue was redissolved in a solution of 4 M hydrogen chloride in dioxane (100 mL) and concentrated to dryness once more. The residue was then dried in vacuo at 50 °C to give the product hydrochloride (391 mg) as a white amorphous solid. ^{1}H NMR (CD_3OD 400 MHz) δ (rotamers) 0.31−0.57 (3d, 6H), 0.83−2.08 (3m, 2H), 1.55 (s, 9H), 1.60−2.07 (3m, 2H), 2.68−3.20 (2m, 2H), 3.20−4.12 (m, 5H), 4.29−4.35 (m, 1H), 6.95−7.19 (m, 5H), 7.38−7.85 (m, 2H). MS (APCI) *m*/*z* 489 $[MH]^+$. $[\alpha]_D^{25}$ −42.7 (3.10 mg/mL, MeOH).

Method C. (3*R*,4*R*,5*S*)-1-{[(3*S*,4*R*)-1-*tert*-Butyl-4-(2,4-difluorophenyl)pyrrolidin-3-yl]carbonyl}-4-(3,4-difluorophenyl)-3,5-dimethylpiperidin-4-ol Hydrochloride (40k). A solution of (3*S*,4*R*)-1-*tert*-butyl-4-(2,4-difluorophenyl)pyrrolidine-3-carboxylic acid[49] (**1**) (159 mg, 0.49 mmol), (3*R*,4*s*,5*S*)-4-(3,4-difluorophenyl)-3,5-dimethylpiperidin-4-ol[49] (**39**, R2 = 3,4-diF-Ph) (100 mg, 0.41 mmol), 1-propylphosphonic acid cyclic anhydride (50% in ethyl acetate, 244 μL, 0.41 mmol), and triethylamine (120 μL, 0.41 mmol) in dichloromethane (2.5 mL) was stirred for 3 days at room temperature. The reaction was then diluted with dichloromethane (20 mL) and partitioned with 10% aqueous potassium carbonate solution (20 mL). The phases were separated, and the organic phase was washed with brine, dried over magnesium sulfate, filtered, and concentrated in vacuo. Purification by column chromatography on silica gel using dichloromethane: methanol:0.88 ammonia (99:1:0.1 to 96:4:0.4) as eluent gave the free base as a colorless oil 117 mg. The oil was dissolved in dichloromethane (1 mL) and treated with 2 M hydrogen chloride in diethyl ether (3 mL). The solvent was then removed in vacuo, and the residue was azeotroped with diethyl ether to afford the title compound as a white solid, 108 mg. ^{1}H NMR (400 MHz, CD_3OD) δ (rotamers) 0.29−0.60 (4d, 6H), 1.49 (s, 9H), 1.58−2.08 (m, 2H), 2.75−3.19 (2t, 2H), 3.38−3.64 (m, 2H), 3.70−3.99 (m, 4H), 4.01−4.17 (m, 1H), 4.36 (dd, 1H), 7.02−7.37 (m, 5H), 7.58−7.73 (m, 1H). MS (APCI) *m*/*z* 507 $[MH]^+$. $[\alpha]_D^{25}$ −34.89 (2.30 mg/mL, MeOH).

Method D. (3*R*,4*S*,5*S*)-1-{[(3*RS*,4*RS*)-1-*tert*-Butyl-4-(2,4-difluorophenyl)pyrrolidin-3-yl]carbonyl}-3,5-dimethyl-4-phenylpiperidin-4-ol (45). To a cooled solution of (3*RS*,4*RS*)-1-*tert*-butyl-4-(2,4-difluorophenyl)pyrrolidine-3-carboxylic acid[49] (**28**) (500 mg, 1.76 mmol) in dichloromethane (20 mL) was added a catalytic amount of *N*,*N*-dimethylformamide followed by oxalyl chloride (309 μL, 3.53 mmol). The reaction mixture was stirred for 2 h at room temperature, and the solvent was then removed in vacuo. The residual white powder was azeotroped with dichloromethane (2 × 10 mL). The white powder was then redissolved in dichloromethane (10 mL), and the resulting solution was added dropwise to a solution of (3*R*,4*s*,5*S*)-3,5-dimethyl-4-phenylpiperidin-4-ol[49] (**39**, R2 = Ph) (362 mg, 1.76 mmol) and triethylamine (246 μL, 1.76 mmol) in dichloromethane (10 mL) over 10 min at room temperature. The resulting mixture was stirred for 24 h at room temperature, diluted with dichloromethane (10 mL), and partitioned with saturated aqueous sodium hydrogen carbonate solution (2 × 30 mL). The phases were separated, and the organic phase was washed with brine (30 mL), dried over magnesium sulfate, filtered, and concentrated in vacuo to give a crude residue. Purification by column chromatography on silica gel using dichloromethane:methanol:0.88 ammonia (99:1:0.1 to 98:2:0.2) gave the desired product as a white foam, 497 mg. ^{1}H NMR (400 MHz, CD_3OD) δ (rotamers) 0.43−0.55 (m, 6H), 0.75−0.79 (m, 1H), 1.25 (s, 9H), 1.87−1.97 (m, 1H), 2.16−2.66 (m, 2H), 3.09 (t, 2H), 3.18−3.30 (m, 2H), 3.41−3.61 (m, 2H), 3.80−4.17 (m, 3H), 6.91−7.09 (m, 3H), 7.28−7.35 (m, 3H), 7.47 (q, 1H). MS (APCI) *m*/*z* 471 $[MH]^+$.

1-{[(3*S*,4*R*)-1-*tert*-Butyl-4-(2,4-difluorophenyl)pyrrolidin-3-yl]carbonyl}-4-phenylpiperidin-4-ol (2). (3*S*,4*R*)-1-*tert*-Butyl-4-(2,4-difluorophenyl)pyrrolidine-3-carboxylic acid[49] (**1**) (670 mg, 2.3 mmol) and 4-phenylpiperidin-4-ol (350 mg, 2.0 mmol) were suspended in dichloromethane (50 mL). Triethylamine (0.61 mL, 4.4 mmol) was added, and the mixture was cooled to 0 °C with stirring under nitrogen. 1-Propylphosphonic acid cyclic anhydride (50% in ethyl acetate, 1.46 mL, 2.3 mmol) was added dropwise while the reaction temperature was maintained below 5 °C. The mixture was allowed to warm to room temperature with continuous stirring and was then stirred for 1 h at room temperature. The mixture was then washed with 10% aqueous potassium carbonate solution (3 × 50 mL), dried over sodium sulfate, filtered, and then concentrated in vacuo. The crude residue was purified by column chromatography on silica gel eluting from 100% dichloromethane to dichloromethane:methanol:0.880 ammonia (90:10:0.5) to give the title compound as a white amorphous solid (690 mg, 68%). ^{1}H NMR (400 MHz, CD_3OD) δ 1.25 (s, 9H), 1.87−1.97 (m, 2H), 2.16−2.66 (m, 2H), 3.09 (t, 2H), 3.18−3.30 (m, 2H), 3.41−3.61 (m, 2H), 3.80−4.17 (m, 4H), 6.91−7.09 (m, 3H), 7.28−7.35 (m, 4H), 7.47 (q, 1H). MS (APCI) *m*/*z* 443 $[MH]^+$. $[\alpha]_D^{25}$ −21.6 (1.61 mg/mL, MeOH).

(3*R*,4*R*,5*S*)-1-{[(3*S*,4*R*)-1-*tert*-Butyl-4-(2,4-difluorophenyl)pyrrolidin-3-yl]carbonyl}-4-(4-methoxyphenyl)-3,5-dimethylpiperidin-4-ol Hydrochloride (40b). Prepared according method B from (3*S*,4*R*)-1-*tert*-butyl-4-(2,4-difluorophenyl)pyrrolidine-3-carboxylic acid[49] (**1**) and (3*R*,4*s*,5*S*)-4-(4-methoxyphenyl)-3,5-dimethylpiperidin-4-ol[49] (**39**, R2 = 4-MeO-Ph). ^{1}H NMR (400 MHz, CD_3OD) δ (rotamers) 0.31−0.59 (3d, 6H), 1.58 (s, 9H), 0.79−1.99 (m, 4H), 1.60−2.07 (m, 3H), 2.68−3.20 (m, 2H), 3.21−4.12 (m, 5H), 4.29 (m, 2H), 6.81−7.29 (m, 5H), 7.60−7.74 (m, 2H). MS (APCI) *m*/*z* 501 $[MH]^+$. $[\alpha]_D^{25}$ −18.53 (1.11 mg/mL, MeOH).

(3*R*,4*R*,5*S*)-1-{[(3*S*,4*R*)-1-*tert*-Butyl-4-(2,4-difluorophenyl)pyrrolidin-3-yl]carbonyl}-3,5-dimethyl-4-[4-(trifluoromethyl)phenyl]-piperidin-4-ol Hydrochloride (40e). Prepared according to method C from (3*S*,4*R*)-1-*tert*-butyl-4-(2,4-difluorophenyl)pyrrolidine-3-carboxylic acid[49] (**1**) and (3*R*,4*s*,5*S*)-3,5-dimethyl-4,4-(trifluoromethyl)phenyl-piperidin-4-ol[49] (**39**, R2 = 4-CF_3-Ph). ^{1}H NMR (400 MHz, CD_3OD) δ (rotamers) 0.29−0.58 (4d, 6H), 1.50 (s, 9H), 1.60−2.15 (m, 2H), 2.70−2.81 (m, 1H), 3.11−3.21 (m, 1H), 3.38−3.72 (m, 3H), 3.79−4.15 (m, 4H), 4.36 (d, 1H), 7.02−7.24 (m, 3H), 7.58−7.70 (m, 4H). MS (APCI) *m*/*z* 539 $[MH]^+$. $[\alpha]_D^{25}$ −49.53 (2.15 mg/mL, MeOH).

(3*R*,4*s*,5*S*)-4-(3-Bromophenyl)-1-(4-methoxybenzyl)-3,5-dimethylpiperidin-4-ol (37, R2 = 3-Br-Ph). A solution of 3-bromoiodobenzene (4.6 g, 25 mmol) in anhydrous diethyl ether (200 mL) was cooled to −78 °C under nitrogen. *n*-Butyl lithium (2.5 M in hexanes, 15.2 mL, 20 mmol) was added dropwise while the reaction temperature was maintained below −65 °C. The mixture was stirred at −78 °C for 2 h and then allowed to warm to room temperature. The resulting solution of 3-bromophenyl lithium was then added dropwise to a solution of (3*R*,5*S*)-1-(4-methoxybenzyl)-3,5-dimethylpiperidin-4-one[49] (**34a**) (5.0 g, 20 mmol) in diethyl ether (25 mL) at −78 °C. The mixture was stirred at −78 °C for a further 2 h and then allowed to warm to room temperature. The mixture was quenched with saturated aqueous ammonium chloride (50 mL). The organic phase was separated, washed with water (3 × 50 mL), dried over sodium sulfate, and then concentrated in vacuo. Recrystallization of the

residue from cyclohexane/ethyl acetate afforded the pure product as light-yellow needles (6.5 g, 56%). ^{1}H NMR (400 MHz, CD_3OD) δ 0.48 (d, 6H), 2.19−2.27 (m, 2H), 2.31−2.39 (m, 2H), 2.79−2.83 (m, 4H), 3.65 (s, 3H), 7.15−7.20 (d, 2H), 7.26−7.35 (d, 2H), 7.46−7.51 (m, 1H), 7.94−8.25 (m, 3H). MS (APCI) m/z 405 $[MH]^+$.

3-[(3*R*,4*s*,5*S*)-4-Hydroxy-1-(4-methoxybenzyl)-3,5-dimethylpiperidin-4-yl]benzonitrile (37, R2 = 3-CN-Ph). A solution of (3*R*,4*s*,5*S*)-4-(3-bromophenyl)-1-(4-methoxybenzyl)-3,5-dimethylpiperidin-4-ol (**37**, R2 = 3-Br-Ph) (3.50 g, 8 mmol), potassium cyanide (1.05 g, 16 mmol), tetrakis(triphenylphosphine)palladium(0) (0.462 g, 0.4 mmol), and copper iodide (1.52 g, 8 mmol) in propionitrile (30 mL) was heated at reflux for 1 h. The mixture was cooled to room temperature, diluted with ethyl acetate (30 mL), and filtered through celite. The filtrate was washed with water (3 × 50 mL), brine (1 × 50 mL), dried over sodium sulfate, and filtered. Concentration in vacuo gave a residue which was purified by column chromatography on silica gel using ethyl acetate:hexane (3:97 to 15:85, gradient elution) to afford the title compound as a yellow solid (2.51 g, 59%). ^{1}H NMR (400 MHz, CD_3OD) δ 0.51 (d, 6H), 2.25−2.31 (m, 2H), 2.42−2.53 (m, 2H), 2.79−2.83 (m, 4H), 3.61 (s, 3H), 7.22 (d, 2H), 7.25−7.35, (d, 2H) 7.66−7.79 (m, 1H), 8.12−8.35 (m, 3H). MS (APCI) m/z 351 $[MH]^+$.

3-[(3*R*,4*s*,5*S*)-4-Hydroxy-3,5-dimethylpiperidin-4-yl]benzonitrile (39, R2 = 3-CN-Ph). 1-Chloroethylchloroformate (1.50 mL, 14 mmol) was added dropwise to a stirred solution of 3-[(3*R*,4*s*,5*S*)-4-hydroxy-1-(4-methoxybenzyl)-3,5-dimethylpiperidin-4-yl]-benzonitrile (**37**, R2 = 3-CN-Ph) (2.50 g, 7.1 mmol) and triethylamine (2.0 mL, 14 mmol) in dichloromethane (50 mL) at −15 °C. The mixture was then stirred for 30 min at −15 °C. The solvent was removed in vacuo, and the crude residue was heated in methanol (100 mL) at reflux for 3 h. After cooling to room temperature, the solvent was removed in vacuo, and the residue was dissolved in dichloromethane (100 mL). Potassium carbonate (325 mesh, 5 g) was added, and the mixture was stirred at room temperature for 1 h. The mixture was then filtered, and the filtrate was concentrated in vacuo. The crude residue was recrystallized from acetonitrile to afford the pure product as fine white needles (1.23 g, 61%). ^{1}H NMR (400 MHz, CD_3OD) δ 0.60 (d, 6H), 2.25−2.45 (m, 2H), 3.1−3.36 (m, 4H), 7.62−7.75 (m, 1H), 8.14−8.28 (m, 3H). MS (APCI) m/z 231 $[MH]^+$.

3-[(3*R*,4*R*,5*S*)-1-{[(3*S*,4*R*)-1-*tert*-Butyl-4-(2,4-difluorophenyl)-pyrrolidin-3-yl]carbonyl}-4-hydroxy-3,5-dimethylpiperidin-4-yl]-benzonitrile Hydrochloride (40h). Prepared according to method B from (3*S*,4*R*)-1-*tert*-butyl-4-(2,4-difluorophenyl)pyrrolidine-3-carboxylic acid[49] (**1**) and 3-[(3*R*,4*s*,5*S*)-4-hydroxy-3,5-dimethylpiperidin-4-yl]benzonitrile (**39**, R2 = 3-CN-Ph). ^{1}H NMR (400 MHz, CD_3OD) δ (rotamers), 0.36−0.57 (m, 6H), 0.83−2.08 (m, 2H), 1.55 (s, 9H), 1.60−2.07 (m, 2H), 2.68−3.20 (m, 2H), 3.20−4.12 (m, 7H), 4.29−4.32 (m, 1H), 6.95−7.19 (m, 5H), 7.78−7.99 (m, 2H). MS (APCI) m/z 496 $[MH]^+$. $[\alpha]_D^{25}$ −40.53 (2.10 mg/mL, MeOH).

(3*R*,4*R*,5*S*)-1-{[(3*S*,4*R*)-1-*tert*-Butyl-4-(2,4-difluorophenyl)pyrrolidin-3-yl]carbonyl}-3,5-dimethyl-4-pyridin-3-ylpiperidin-4-ol Dihydrochloride (40m). Prepared according to method C from (3*S*,4*R*)-1-*tert*-butyl-(2,4-difluorophenyl)pyrrolidine-3-carboxylic acid[49] (**1**) and (3*R*,4*s*,5*S*)-3,5-dimethyl-4-pyridin-3-ylpiperidin-4-ol[49] (**39**, R2 = pyridin-3-yl). ^{1}H NMR (400 MHz, CD_3OD) δ (rotamers) 0.37−0.61 (4d, 6H), 0.70−0.99 (m, 1H), 1.50 (s, 9H), 1.80−1.98 (m, 1H), 2.09−2.29 (m, 1H), 2.63−2.81 (m, 2H), 3.19 (q, 1H), 3.45−3.57 (m, 1H), 3.59−3.70 (m, 1H), 3.82−4.17 (m, 4H), 4.38−4.45 (m, 1H), 7.01−7.13 (m, 2H), 7.60−7.83 (m, 1H), 8.09−8.17 (m, 1H), 8.79 (dd, 1H), 8.82−8.92 (m, 1H). MS (ESI) m/z = 472 $[MH]^+$. $[\alpha]_D^{25}$ −35.95 (4.20 mg/mL, MeOH).

***tert*-Butyl (3*R*,4*s*,5*S*)-4-Hydroxy-3,5-dimethyl-4-pyrimidin-5-ylpiperidine-1-carboxylate (38, R2 = pyrimidin-5-yl).** *tert*-Butyl (3*R*,5*S*)-3,5-dimethyl-4-oxopiperidine-1-carboxylate[49] (**35**) (560 mg, 2.46 mmol) and 5-bromopyrimidine (1.17 g, 7.33 mmol) were dissolved in anhydrous THF (5 mL) with stirring, and the resulting solution was cooled to −78 °C. *n*-Butyl lithium (2.5 M in hexanes) (1.08 mL, 2.7 mmol) was then added dropwise over 25 min. The reaction mixture was then allowed to warm to ambient temperature over 16 h. The solvents were removed in vacuo, and the residual orange oil was dissolved in EtOAc (25 mL) and partitioned with water (25 mL). The organic layer was separated, washed with brine (25 mL), and then dried over anhydrous $MgSO_4$ before being filtered and concentrated in vacuo to give the crude product as an orange oil. Purification by cartridge chromatography (Redisep 20 g cartridge) eluting with CH_2Cl_2:MeOH:NH_3 98:2:0.2 gave the title compound as a yellow oil (252 mg, 33%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 0.62 (d, 6H), 1.49 (s, 9H), 1.90 (s, 1H), 2.08 (bs, 2H), 2.82 (bs, 2H), 3.90−4.10 (m, 2H), 8.76−8.79 (m, 2H), 9.15 (s, 1H). MS (APCI) m/z 308 $[MH]^+$.

(3*R*,4*s*,5*S*)-3,5-Dimethyl-4-pyrimidin-5-ylpiperidin-4-ol Hydrochloride (39, R2 = pyrimidin-5-yl). To a room temperature solution of *tert*-butyl (3*R*,4*s*,5*S*)-4-hydroxy-3,5-dimethyl-4-pyrimidin-5-ylpiperidine-1-carboxylate (**38**, R2 = pyrimidin-5-yl) (251 mg, 0.82 mmol) in CH_2Cl_2 (1 mL) was added HCl in dioxane (4 M solution, 5 mL, 20 mmol), and the reaction mixture was stirred at room temperature for 16 h. The solvents were evaporated in vacuo, and the residue was azeotroped with CH_2Cl_2 (2 × 20 mL) to yield the title compound as a white solid (182 mg, 80%). ^{1}H NMR (400 MHz, CD_3OD) δ 1.70 (d, 6H), 2.36−2.40 (m, 2H), 3.15 (t, 2H), 3.24 (dd, 2H), 8.82−9.10 (m, 2H), 9.17 (s, 1H). MS (APCI) m/z 208 $[MH]^+$.

(3*R*,4*R*,5*S*)-1-{[(3*S*,4*R*)-1-*tert*-Butyl-4-(2,4-difluorophenyl)-pyrrolidin-3-yl]carbonyl}-3,5-dimethyl-4-pyrimidin-5-ylpiperidin-4-ol Dihydrochloride (40n). Prepared according to method C from (3*S*,4*R*)-1-*tert*-butyl-4-(2,4−difluorophenyl)pyrrolidine-3-carboxylic acid[49] (**1**) and (3*R*,4*s*,5*S*)-3,5-dimethyl-4-pyrimidin-5-ylpiperidin-4-ol hydrochloride (**39**, R2 = pyrimidin-5-yl). ^{1}H NMR (400 MHz, CD_3OD) δ (rotamers) 0.36−0.64 (4d, 6H), 1.51 (s, 9H), 1.70−2.21 (m, 1H), 2.65−2.81 (m, 1H), 3.18 (q, 1H), 3.42−3.72 (m, 3H), 3.80−4.16 (m, 4H), 4.36−4.42 (m, 1H), 7.02−7.20 (m, 2H), 7.58−7.76 (m, 1H), 8.70 (bs, 1H), 8.96 (d, 1H), 9.16−9.21 (m, 1H). MS (APCI) m/z 473 $[MH]^+$. $[\alpha]_D^{25}$ −46.8 (2.1 mg/mL, MeOH).

(3*R*,4*R*,5*S*)-1-{[(3*S*,4*R*)-4-(2,4-Difluorophenyl)-1-methylpyrrolidin-3-yl]carbonyl}-3,5-dimethyl-4-phenylpiperidin-4-ol Hydrochloride (41a). Prepared according to method C from (3*S*,4*R*)-4-(2,4-difluorophenyl)-1-methylpyrrolidine-3-carboxylic acid[49] (**19**) and (3*R*,4*s*,5*S*)-3,5-dimethyl-4-phenylpiperidin-4-ol[49] (**39**, R2 = Ph). ^{1}H NMR (400 MHz, CD_3OD) δ (rotamers) 0.21−0.58 (4d, 6H), 0.90−1.05 (m, 1H), 1.71−2.07 (m, 1H), 2.75−2.81 (m, 1H), 3.10 (s, 3H), 3.11−3.19 (m, 1H), 3.32−3.38 (m, 1H), 3.43−3.79 (m, 2H), 3.85−4.20 (m, 4H), 4.36 (d, 1H), 7.05−7.39 (m, 7H), 7.58−7.76 (m, 1H). MS (ESI) m/z = 429 $[MH]^+$. $[\alpha]_D^{25}$ −49.56 (4.10 mg/mL, MeOH).

(3*R*,4*R*,5*S*)-1-{[(3*S*,4*R*)-4-(2,4-Difluorophenyl)-1-ethylpyrrolidin-3-yl]carbonyl}-3,5-dimethyl-4-phenylpiperidin-4-ol Hydrochloride (42a). Prepared according to method C from (3*S*,4*R*)-4-(2,4-difluorophenyl)-1-ethylpyrrolidine-3-carboxylic acid[49] (**20**) and (3*R*,4*s*,5*S*)-3,5-dimethyl-4-phenylpiperidin-4-ol[49] (**39**, R2 = Ph). ^{1}H NMR (400 MHz, CD_3OD) δ (rotamers) 0.23−0.56 (4d, 6H), 0.61−1.01 (m, 1H), 1.28−1.48 (m, 3H), 1.68−2.04 (m, 1H), 2.71−2.86 (m, 1H), 3.10−3.22 (m, 1H), 3.35−3.50 (m, 3H), 3.57−4.24 (m, 6H), 4.31−4.47 (m, 1H), 7.01−7.73 (m, 8H). MS (ESI) m/z = 443 $[MH]^+$. $[\alpha]_D^{25}$ −49.06 (1.60 mg/mL, MeOH).

(3*R*,4*R*,5*S*)-1-{[(3*S*,4*R*)-4-(2,4-Difluorophenyl)-1-ethylpyrrolidin-3-yl]carbonyl}-4-(4-fluorophenyl)-3,5-dimethylpiperidin-4-ol Hydrochloride (42b). Prepared according to method B from (3*S*,4*R*)-4-(2,4-difluorophenyl)-1-ethylpyrrolidine-3-carboxylic acid[49] (**20**) and (3*R*,4*s*,5*S*)-4-(4-fluorophenyl)-3,5-dimethylpiperidin-4-ol[49] (**39**, R2 = 4-F-Ph). ^{1}H NMR (400 MHz, CD_3OD) δ (rotamers) 0.30−0.59 (3d, 6H), 0.89−2.01 (m, 2H), 1.51−1.60 (m, 4H), 1.54−2.09 (m, 2H), 2.61−2.81 (m, 1H), 3.16−4.26 (m, 8H), 4.39−4.53 (m, 1H), 6.80−7.27 (m, 4H), 7.39−7.46 (m, 2H),

7.81−7.97 (m, 1H). MS (APCI) m/z 461 $[MH]^+$. $[\alpha]_D^{25}$ −21.07 (1.45 mg/mL, MeOH).

(3*R*,4*R*,5*S*)-4-(2,4-Difluorophenyl)-1-{[(3*S*,4*R*)-4-(2,4-difluorophenyl)-1-ethylpyrrolidin-3-yl]carbonyl}-3,5-dimethylpiperidin-4-ol Hydrochloride (42d). Prepared according to method B from (3*S*,4*R*)-4-(2,4-difluorophenyl)-1-ethylpyrrolidine-3-carboxylic acid[49] (**20**) and (3*R*,4*s*,5*S*)-4-(2,4-difluorophenyl)-3,5-dimethylpiperidin-4-ol[49] (**39**, R2 = 2,4-diF-Ph). ^{1}H NMR (400 MHz, CD_3OD) δ (rotamers), 0.32−0.59 (m, 6H), 0.85−1.98 (m, 2H), 1.47−1.59 (m, 4H), 1.55−2.05 (m, 2H), 2.55−2.61 (m, 2H), 3.11−4.23 (m, 7H), 4.31−4.48 (m, 1H), 6.85−7.20 (m, 2H), 7.35−7.42 (m, 2H), 7.76−7.99 (m, 2H). MS (APCI) m/z 479 $[MH]^+$. $[\alpha]_D^{25}$ −29.53 (1.83 mg/mL, MeOH).

(3*R*,4*R*,5*S*)-4-(3,4-Difluorophenyl)-1-{[(3*S*,4*R*)-4-(2,4-difluorophenyl)-1-ethylpyrrolidin-3-yl]carbonyl}-3,5-dimethylpiperidin-4-ol Hydrochloride (42e). Prepared according to method C from (3*S*,4*R*)-4-(2,4-difluorophenyl)-1-ethylpyrrolidine-3-carboxylic acid[49] (**20**) and (3*R*,4*s*,5*S*)-4-(3,4-difluorophenyl)-3,5-dimethylpiperidin-4-ol[49] (**39**, R2 = 3,4-diF-Ph). ^{1}H NMR (400 MHz, CD_3OD) δ (rotamers) 0.26−0.57 (4d, 6H), 0.62−0.92 (m, 1H), 1.39−1.46 (m, 3H), 1.69−2.00 (m, 1H), 2.69−2.82 (m, 1H), 3.13 (t, 1H), 3.35−4.14 (m, 8H), 4.33 (d, 1H), 6.78−7.34 (m, 5H), 7.55−7.72 (m, 1H). MS (APCI) m/z = 479 $[MH]^+$. $[\alpha]_D^{25}$ −56.17 (3.00 mg/mL, MeOH).

(3*R*,4*R*,5*S*)-1-{[(3*S*,4*R*)-4-(2,4-Difluorophenyl)-1-isopropylpyrrolidin-3-yl]carbonyl}-4-(3-fluorophenyl)-3,5-dimethylpiperidin-4-ol Hydrochloride (43d). Prepared according to method B from (3*S*,4*R*)-4-(2,4-difluorophenyl)-1-isopropylpyrrolidine-3-carboxylic acid[49] (**21**) and (3*R*,4*s*,5*S*)-4-(3-fluorophenyl)-3,5-dimethylpiperidin-4-ol[49] (**39**, R2 = 3-F-Ph). ^{1}H NMR (400 MHz, CD_3OD) δ (rotamers), 0.34−0.52 (3d, 6H), 0.79−1.20 (m, 2H), 1.39−1.61 (m, 6H), 1.49−2.30 (m, 2H), 2.65−2.82 (m, 1H), 3.19−4.21 (m, 8H), 4.35−4.42 (m, 1H), 6.78−7.18 (m, 5H), 7.28−7.75 (m, 2H). MS (APCI) m/z 475 $[MH]^+$. $[\alpha]_D^{25}$ −31.53 (1.05 mg/mL, MeOH).

(3*R*,4*S*,5*S*)-1-{[(3*R*,4*S*)-1-*tert*-Butyl-4-(2,4-difluorophenyl)pyrrolidin-3-yl]carbonyl}-3,5-dimethyl-4-phenylpiperidin-4-ol (44). Prepared from (3*R*,4*S*)-1-*tert*-butyl-4-(2,4-difluorophenyl)pyrrolidine-3-carboxylic acid (*ent*-**1**) and (3*R*,4*s*,5*S*)-3,5-dimethyl-4-phenylpiperidin-4-ol[49] (**39**, R2 = Ph) according to method D. ^{1}H NMR (400 MHz, CD_3OD) δ (rotamers), 0.43−0.55 (m, 6H), 0.75−0.79 (m, 1H), 1.25 (s, 9H), 1.87−1.97 (m, 1H), 2.16−2.66 (m, 2H), 3.09 (t, 2H), 3.18−3.30 (m, 2H), 3.41−3.61 (m, 3H), 3.80−4.17 (m, 3H), 6.91−7.09 (m, 3H), 7.28−7.35 (m, 3H), 7.47 (q, 1H). MS (APCI) m/z 471 $[MH]^+$.

Methyl (3*S*,4*R*)-4-(2,4-Difluorophenyl)-1-pyridazin-3-ylpyrrolidine-3-carboxylate (18). To a room temperature solution of methyl (3*S*,4*R*)-4-(2,4-difluorophenyl)pyrrolidine-3-carboxylate[49] (**14**) (1.00 g, 4.14 mmol) in anhydrous NMP (10 mL) was added *N*,*N*-diisopropylethylamine (2.15 mL, 12.42 mmol) and 3-chloropyridazine (2.37 g, 20.7 mmol). The resulting solution was then warmed to 120 °C under nitrogen with stirring for 4 h. The reaction mixture was then cooled to room temperature and water (250 mL) and MTBE (150 mL) were added. The aqueous layer was separated and extracted with MTBE (150 mL). The organic fractions were combined and washed with brine (150 mL) and then dried over anhydrous $MgSO_4$ (s), filtered, and evaporated in vacuo to give a crude red oil. Purification by column chromatography on silica gel eluting with EtOAc:pentane 20:80 to EtOAc 100% (gradient elution) gave the title compound as an orange oil (581 mg, 44%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 3.54 (q, 1H), 3.67 (s, 3H), 3.76−3.85 (m, 1H), 3.93−3.98 (m, 1H), 4.00−4.06 (m, 1H), 4.12−4.24 (m, 2H), 6.79−6.88 (m, 2H), 7.28−7.31 (m, 2H), 7.81−7.84 (m, 1H), 8.65−8.66 (m, 1H). MS (APCI) m/z = 320 $[MH]^+$.

(3*S*,4*R*)-4-(2,4-Difluorophenyl)-1-pyridazin-3-ylpyrrolidine-3-carboxylic Acid Hydrochloride (22). To a room temperature solution of methyl (3*S*,4*R*)-4-(2,4-difluorophenyl)-1-pyridazin-3-ylpyrrolidin-3-yl-carboxylate (**18**) (576 mg, 1.8 mmol) in tetrahydrofuran (10 mL) was added dropwise a freshly prepared solution of lithium hydroxide (129 mg, 5.4 mmol) in water (3 mL). The resulting solution was then stirred at room temperature for 5 h. The solvents were removed in vacuo, and the residual orange oil was dissolved in water (10 mL) and HCl (1N aq solution) (5.4 mL) was added to give an orange precipitate which had a gel-like consistency. The solution was filtered, and the residual gel was dissolved in EtOAc:MeOH (1:1) (200 mL). The solvents were then evaporated in vacuo to provide an orange solid. The filtrate was extracted with EtOAc:MeOH (95:5) (2 × 60 mL), and the combined organic fractions were dried over anhydrous $MgSO_4$ (s), filtered, combined with the previously collected orange solid, and concentrated in vacuo to give an orange solid (561 mg, 97%). ^{1}H NMR (400 MHz, CD_3OD) δ = 3.63 (q, 1H), 3.72 (t, 1H), 3.99 (t, 1H), 4.09−4.12 (m, 4H), 7.01 (t, 2H), 7.52 (q, 1H), 7.75 (d, 1H), 7.86 (dd, 1H), 8.55 (s, 1H). MS (APCI) m/z = 306 $[MH]^+$. $[\alpha]_D^{25}$ −2.14 (2.80 mg/mL, MeOH).

(3*R*,4*R*,5*S*)-1-{[(3*S*,4*R*)-4-(2,4-Difluorophenyl)-1-pyridazin-3-ylpyrrolidin-3-yl]carbonyl}-4-(4-fluorophenyl)-3,5-dimethylpiperidin-4-ol Hydrochloride (49d). (3*R*,4*R*,5*S*)-1-{[(3*S*,4*R*)-4-(2,4-Difluorophenyl)pyrrolidin-3-yl]carbonyl}-4-(4-fluorophenyl)-3,5-dimethylpiperidin-4-ol hydrochloride[49] (**47b**) (250 mg, 0.53 mmol) and 3-chloropyridazine (305 mg, 2.65 mmol) were treated as for compound **18** above to give the title compound as an off-white solid (24 mg, 6%). ^{1}H NMR (400 MHz, CD_3OD) δ (rotamers) 0.40−0.61 (4d, 6H), 0.72−0.81 (m, 1H), 1.61−1.69 (m, 1H), 2.70−2.80 (m, 1H), 3.10 (2t, 1H), 3.71 (2 × dd, 1H), 3.81−4.01 (m, 2H), 4.10−4.38 (m, 5H), 6.95−7.20 (m, 5H), 7.36 (q, 1H), 7.49−7.66 (m, 1H), 7.71−7.75 (m, 1H), 7.87 (dd, 1H), 8.52−8.57 (m, 1H). MS (ESI) m/z = 511 $[MH]^+$. $[\alpha]_D^{25}$ −30.85 (2.35 mg/mL, MeOH).

(3R,4*R*,5*S*)-1-{[(3*S*,4*R*)-4-(2,4-Difluorophenyl)-1-pyridazin-3-ylpyrrolidin-3-yl]carbonyl}-3,5-dimethyl-4-propylpiperidin-4-ol Hydrochloride (49f). Prepared according to method C from (3*S*,4*R*)-4-(2,4-difluorophenyl)-1-pyridazin-3-ylpyrrolidin-3-yl-carboxylic acid hydrochloride (**22**) and (3*R*,4*s*,5*S*)-3,5-dimethyl-4-propylpiperidin-4-ol[49] (**39**, R2 = *n*-Pr). ^{1}H NMR (400 MHz, CD_3OD) δ (rotamers) 0.41−0.49 (m, 1H), 0.67−0.87 (4d, 6H), 0.87−0.96 (m, 4H), 1.16−1.71 (m, 4H), 2.58−2.67 (m, 1H), 2.96 (2t, 1H), 3.51 (2 × dd, 1H), 3.82−3.98 (m, 2H), 4.01−4.10 (m, 2H), 4.13−3.20 (m, 3H), 7.00−7.11 (m, 2H), 7.44−7.60 (m, 1H), 7.70−7.74 (m, 1H), 7.87 (dd, 1H), 8.53−8.55 (m, 1H). MS (APCI) m/z = 459 $[MH]^+$. $[\alpha]_D^{25}$ −7.30 (3.05 mg/mL, MeOH).

(3*R*,4*R*,5*S*)-1-{[(3*S*,4*R*)-4-(2,4-Difluorophenyl)-1-pyridazin-3-ylpyrrolidin-3-yl]carbonyl}-4-isopropyl-3,5-dimethylpiperidin-4-ol Hydrochloride (49g). Prepared according to method C from (3*S*,4*R*)-4-(2,4-difluorophenyl)-1-pyridazin-3-ylpyrrolidin-3-yl-carboxylic acid hydrochloride (**22**) and (3*R*,4*s*,5*S*)-4-isopropyl-3,5-dimethylpiperidin-4-ol[49] (**39**, R2 = *i*-Pr). ^{1}H NMR (400 MHz, CD_3OD) δ (rotamers) 0.58−0.67 (m, 1H), 0.72−1.01 (6d, 12H), 1.38−1.86 (m, 1H), 1.93−2.13 (m, 1H), 2.64−2,74 (m, 1H), 2.86−3.13 (2t, 1H), 3.35−3.51 (m, 1H), 3.78−3.90 (m, 1H), 3.93−4.02 (m, 1H), 4.02−4.21 (m, 4H), 6.99−7.11 (m, 2H), 7.44−7.61 (m, 1H), 7.69−7.73 (m, 1H), 7.86 (dd, 1H), 8.53−8.55 (m, 1H). MS (APCI) m/z = 459 $[MH]^+$. $[\alpha]_D^{25}$ −7.80 (2.05 mg/mL, MeOH).

(3*R*,4*R*,5*S*)-4-Cyclopropyl-1-{[(3*S*,4*R*)-4-(2,4-difluorophenyl)-1-pyridazin-3-ylpyrrolidin-3-yl]carbonyl}-3,5-dimethylpiperidin-4-ol Hydrochloride (49h). Prepared according to method C from (3*S*,4*R*)-4-(2,4-difluorophenyl)-1-pyridazin-3-ylpyrrolidin-3-yl-carboxylic acid hydrochloride (**22**) and (3*R*,4*s*,5*S*)-4-cyclopropyl-3,5-dimethylpiperidin-4-ol[49] (**39**, R2 = *c*-Pr). ^{1}H NMR (400 MHz, CD_3OD) δ (rotamers) 0.26−0.39 (m, 4H), 0.43 (q, 1H), 0.47−0.56 (m, 1H), 0.81−1.00 (4d, 6H), 1.31−1.80 (m, 1H), 2.56−2.65 (m, 1H), 2.94 (2t, 1H), 3.51 (2 × dd, 1H), 3.79−3.97 (m, 2H), 4.05−4.21 (m, 5H), 7.00−7.21 (m, 2H), 7.53 (2q, 1H), 7.71−7.75 (m, 1H), 7.87 (dd, 1H), 8.52−8.57 (m, 1H). MS (ESI) m/z = 457 $[MH]^+$. $[\alpha]_D^{25}$ −3.88 (2.90 mg/mL, MeOH).

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Supporting Information Available: Elemental and HPLC analyses for test compounds, X-ray crystallographic data for compounds **10a** and **40a**, proof of absolute stereochemistry of compound **11a** (via X-ray crystallography of a derivative), in vitro biological assay protocols, in vitro and in vivo pharmacokinetic protocols. This material is available free of charge via the Internet at http://pubs.acs.org.

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