4-Acyl-1-(4-aminoalkoxyphenyl)-2-ketopiperazines as a Novel Class of Non-Brain-Penetrant Histamine H_3 Receptor Antagonists

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A series of ketopiperazines were prepared and evaluated for their activity as histamine H_3 antagonists. From investigation of the tertiary basic center in the aminopropyloxyphenyl template, the 2(*R*)-methylpyrrolidine was identified as the most potent amine. In the more rigid piperidineoxyphenyl template the *N*-cyclobutyl group was the most potent amine. The 4-fluorobenzyol, 4-cyanobenzoyl, and 2,4-difluorobenzoyl groups provided good pharmacokinetic profiles for the various amides. The PSA and log *D* values of these compounds suggested low brain penetration. The compounds had very high selectivity over other receptors and did not inhibit hepatic cytochrome P450, indicating low drug−drug interaction potential. Compound **22i** was identified as the best compound of this series based on its overall profile of high potency, selectivity, low brain penetration, lack of CYP450 inhibition, high oral bioavailability, and pharmacokinetic properties.

Allergic rhinitis, also known as "hay fever", affects at least 10–25% of the world's population and has shown a steady increase in prevalence during the past 40 years.[1] There are two types of allergic rhinitis: seasonal and perennial. The symptoms of seasonal allergic rhinitis include at the early stage nasal itching, irritation, and sneezing, and at the late stage rhinorrhea and nasal congestion. The symptoms of perennial allergic rhinitis are similar; however, nasal congestion may be more pronounced. Either type of allergic rhinitis may also cause other symptoms such as irritation of the throat and/or eyes, epiphora, and edema around the eyes.[2] Allergic rhinitis and other allergic conditions are associated with the release of histamine from various cell types but particularly mast cells. The physiological effects of histamine are mediated by four major G-protein-coupled receptors, termed H_1, H_2, H_3, and H_4, which differ in their expression, signal transduction, and histamine-binding characteristics.[3] H_1 receptors are widely distributed throughout the central nervous system (CNS) and periphery and play a critical role in regulating inflammatory responses and CNS activity, such as wakefulness. H_1 antagonists, also known as H_1 blockers or antihistamines, are the most commonly used first-line medications for allergic rhinitis.[4] H_2 receptors regulate gastric acid secretion, and H_2 antagonists are used clinically to treat excess acid production and gastric ulceration.[5] The third receptor subtype (H_3) is a presynaptic autoreceptor that controls the synthesis and release of histamine as well as other neurotransmitters such as acetylcholine, dopamine, GABA, glutamate, 5-HT, and noradrenaline.[6] Consequently many applications have been proposed for H_3 receptor–ligands, particularly in the CNS, where centrally acting H_3 antagonists may provide novel therapies for neurological disorders such as epilepsy, Parkinson's disease, Alzheimer's disease, attention-deficit hyperactivity disorder, sleep disturbances, cognition, schizophrenia, and obesity.[7,8] The more recently identified fourth receptor subtype (H_4) appears to be restricted to cells of the immune and inflammatory systems; a physiological role for this receptor remains to be identified.[9]

H_1 receptor antagonists are effective in treating all the symptoms of allergic rhinitis, apart from the nasal congestion. Hence, they are often used in combination with α-adrenergic agonist decongestants such as pseudoephedrine. However, the use of α-adrenergic agonists is limited because of their potential to produce hypertension, agitation, and insomnia.

Histamine H_3 receptors are expressed widely on both CNS and peripheral nerve endings and mediate the inhibition of neurotransmitter release.[6,10] Activation of H_3 receptor by histamine modulates outflow to resistance and capacitance vessels, causing vasodilatation in rats,[11] guinea pigs,[12] and cats.[13] In vitro electrical stimulation of peripheral sympathetic nerves in isolated human saphenous vein[14,15] and porcine nasal mucosa[16] results in an increase in noradrenaline release and smooth muscle contraction, which can be inhibited by histamine H_3 receptor agonists such as (*R*)-α-methylhistamine. In addition, activation of H_3 receptors in isolated human nasal turbinate mucosa inhibits sympathetic vasoconstriction.[17] The same report also described high distribution of H_3 receptors in the human nasal mucosa. It is thought that activation of the H_3 receptor on the presynaptic terminals of sympathetic neurones reduces noradrenaline release, and this may contribute, together with the activation of the postsynaptic H_1 receptors, to the nasal blockage caused by histamine release. Consistent with this hypothesis, combination treatment of H_1 and H_3 antagonists has been shown to reverse the effects of mast cell activation on nasal airway resistance and nasal cavity volume in a cat model of nasal congestion in vivo.[18,19] Further evidence for the contribution of H_3 receptors to histamine-induced blockage of the nasal airway in normal healthy human volunteers was provided recently by acoustic rhinometry.[20] Scientists at the

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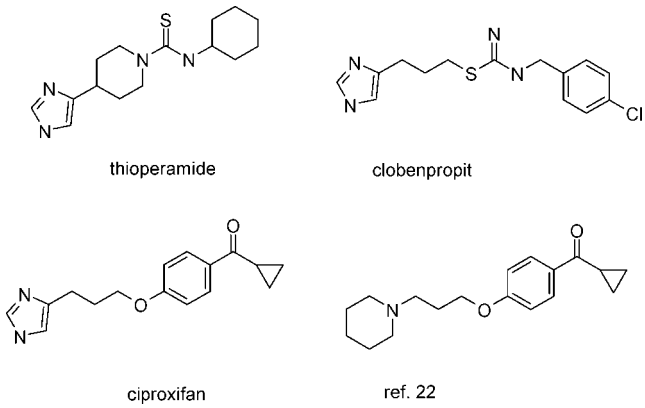
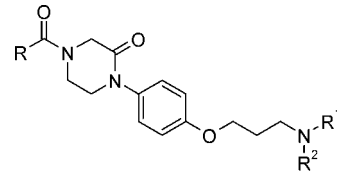
Chart 1



Schering-Plough Research Institute have published the first dual H_1H_3 receptor antagonist based on the first-generation H_1 antagonist chlorpheniramine and a 4-substituted imidazole.[21] In principle there are two ways of targeting dual H_1H_3 pharmacology, either by using a combination of two individual selective antagonists or by identifying a molecule that exhibits antagonism at both receptors. We have examined both strategies, and in this paper we are reporting our efforts in identifying an H_3 antagonist suitable for oral administration for the potential treatment of allergic rhinitis.

Several compounds are known to be potent histamine H_3 receptor antagonists. Most of the early H_3 antagonists were imidazole derivatives, such as thioperamide, ciproxifan, or clobenpropit (Chart 1). The design of H_3 receptor antagonists devoid of an imidazole ring, as a means of providing compounds with improved pharmacokinetic properties and avoiding the possibility of drug−drug interactions, leads to analogues of ciproxifan where the imidazole ring was replaced by piperidine.[22] During the past 3 years a large number of reports were published by both pharmaceutical research groups in industry and academia describing various classes of H_3 receptor antagonists including the Johnson and Johnson,[23–31] Hoffmann-La Roche,[32] Novo Nordisk,[33,34] Eli Lilly,[35] Schering Plough,[36,37] Abbott,[38,39] and the universities of Parma,[40,41] Krakow,[42] Amsterdam,[43] and Lodz;[44] however, all these compounds were designed for the treatment of CNS diseases.

The target profile for this project was a nonimidazole, human H_3 antagonist ($pK_i \geq 9$), selectivity over H_1 (>100-fold to ensure that the H_1 effects from the fixed dose combination are entirely due to the admixed H_1 antagonist), low brain penetration to avoid CNS H_3 effects (low lipophilicity, high polar surface area, rat brain/blood ratio of less than 0.5), high oral bioavailability (molecular weight less than 500, rat $F > 30\%$, dog $F > 30\%$), low clearance (rat and dog Cl < $^1/_3$ liver blood flow), moderate half-life (rat $t_{1/2} = 2$ h, dog $t_{1/2} = 4$ h), no CYP450 liability ($IC_{50} > 10\ \mu M$ on human isoforms), and no hERG liability (IC_{50} > 30-fold the predicted human free C_{max}). A basic amine connected through an alkoxy chain to a lipophilic group is a feature shared by several classes of nonimidazole H_3 antagonists,[22,26–30,45–51] and that we wished to retain in our analogues. The lipophilic group is often a biaryl moiety,[45,46,48–52] which it was our intention to replace with a group of higher polar surface area in order to keep our H_3 antagonists out of the brain, thus avoiding any off-target activity. We envisaged replacing one of the aryl rings of the biphenyl group with an N-linked ketopiperazine, which would be a planar ring like the replaced aryl ring, and acylating the remaining nitrogen to provide target compounds **1**. Optimization of the properties of **1** would require variation of the acyl group of the amide, and the basic center.



1

Chemistry

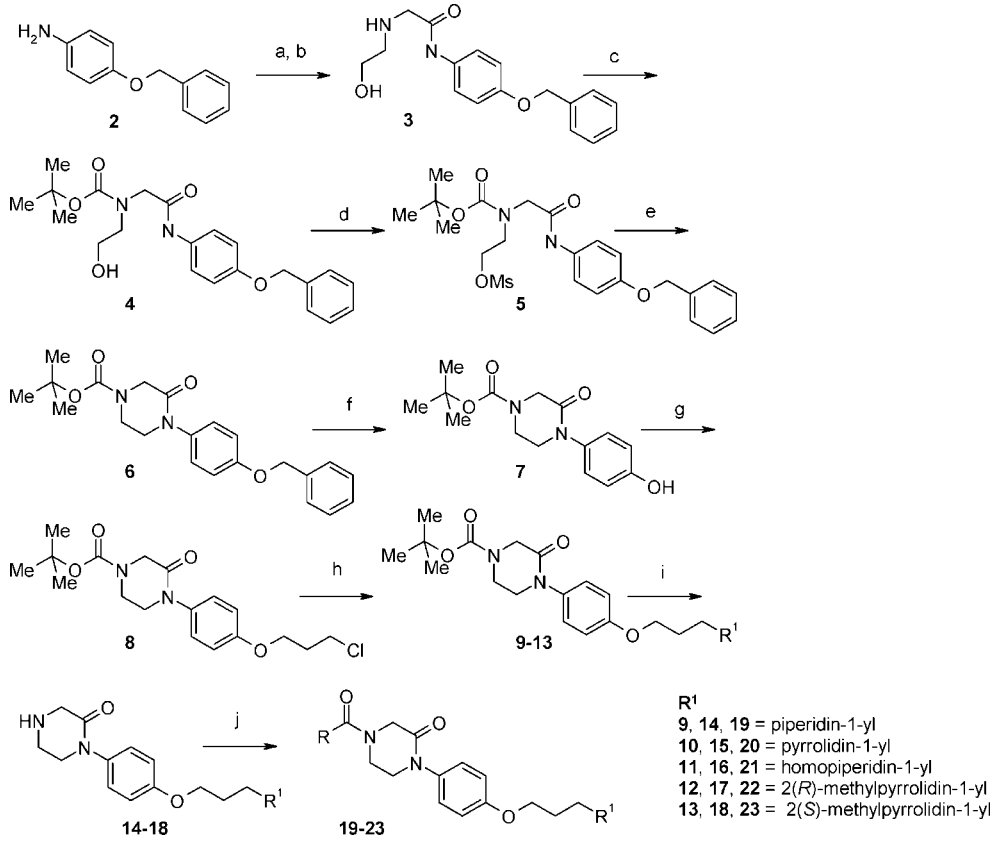
The synthesis of analogues of **1** is outlined in Scheme 1. Treatment of 4-benzyloxyaniline **2** with chloroacetyl chloride, followed by reaction with ethanolamine gave amide **3**. The amino group of **3** was converted to the BOC derivative **4**. The hydroxyl group was reacted with mesyl chloride to the methanesulfonate **5** and then cyclized with sodium hydride to the protected ketopiperazine **6**. Hydrogenolysis of **6** gave the phenol **7**, which was reacted with 1-bromo-3-chloropropane to give the chloride **8**. Reaction of **8** with piperidine, pyrrolidine, homopiperidine, 2(*R*)-methylpyrrolidine, or 2(*S*)-methylpyrrolidine gave the corresponding amines **9–13**. Cleavage of the BOC protecting group provided the free amines **14–18**, which were acylated with a variety of acids to give the corresponding amides **19–23**. In addition to these molecules possessing the flexible aminopropyloxy chain, analogues where the chain is fixed in a ring were also prepared in the hope of improving oral absorption. The synthesis of the more rigid analogues is outlined in Scheme 2. Reaction of 1-iodo-4-fluorobenzene (**24**) with 1-BOC-4-hydroxypiperidine (**25**) in the presence of sodium hydride in 1-methyl-2-pyrrolidinone gave **26**, which was then reacted with 4-benzyloxycarbonylpiperazin-2-one (**27**) in the presence of *N*,*N*′-dimethylethylenediamine and copper(I) iodide to give **28**.[53] The BOC protecting group was removed selectively under acid conditions to give the piperidine **29**, which was reductively alkylated to tertiary amines **30–32** using sodium triacetoxyborohydride and the appropriate ketone (acetone, cyclobutanone, or cyclopentanone). The protected amines were hydrogenolyzed to give the secondary amines **33–35** and finally acylated with a number of acids to provide the corresponding amides **36–38**. All carboxylic acids used were commercially available except for **41**, which was prepared from the commercially available acid chloride **39** and azetidine, followed by ester hydrolysis of the resulting amide **40** (Scheme 3).

Results and Discussion

Amides **19–23** and **36–38** were evaluated in vitro in membranes from CHO cells transfected with cloned human histamine H_3 receptors for their ability to reduce histamine stimulated GTP-γ-S binding as determined by scintillation proximity detection. Selected compounds were also assessed for their ability to bind to native H_3 receptor preparations from rat or guinea pig cortex. The affinity of compounds was also evaluated at recombinant human histamine H_1 receptor and adrenergic α_{1A} and α_{1B} receptors in intact CHO cells by means of plate-based calcium imaging. Inhibition of agonist-induced cellular calcium mobilization was monitored using the calcium sensing dye Fluo-4AM[a] in a FLIPR instrument.[54] Antihistamines are regarded

[a] Abbreviations: Fluo-4AM, 4-(6-acetoxymethoxy-2,7-difluoro-3-oxo-9-xanthenyl)-4′-methyl-2,2′-(ethylenedioxy)dianiline-*N*,*N*,*N*′,*N*′-tetraacetic acid tetrakis(acetoxymethyl) ester; GTP-γ-S, guanosine 5′-[γ-thio]triphosphate; TBTU = (*O*-benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate.

Scheme 1[a]

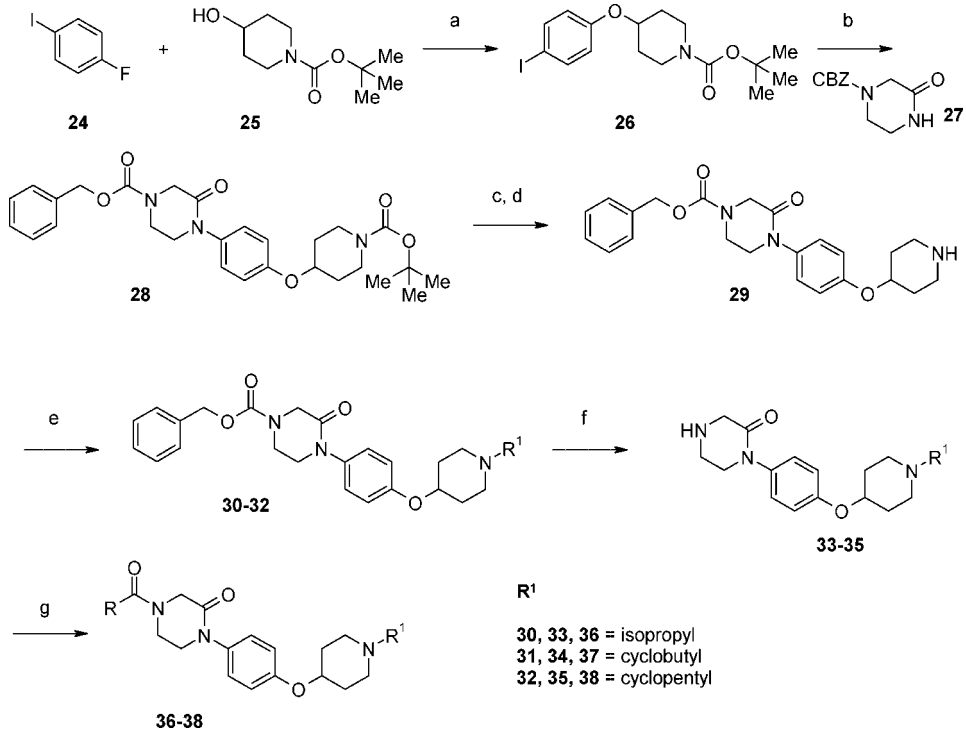


[a] Reagents and conditions: (a) chloroacetyl chloride, K_2CO_3, THF; (b) ethanolamine 60°C; (c) BOC_2O, (d) MsCl, Et_3N, DCM; (e) NaH; (f) H_2, 10% Pd/C, EtOH; (g) 1-bromo-3-chloropropane, K_2CO_3, 2-butanone; (h) amine piperidine, pyrrolidine, homopiperidine, 2(*R*)-methylpyrrolidine, or 2(*S*)-methylpyrrolidine; (i) TFA, DCM; (j) acylation, RCO_2H, Et_3N, TBTU, DMF; RCOCl, Et_3N, DCM; or RCO_2H, HOBT, resin bound DCC, Et_3N.

as a class of compounds with a potential for inducing electrophysiological effects that may occasionally trigger life-threatening cardiac arrhythmias in humans.[55] The binding affinity of the test compounds for the human ether-a-go-go gene (hERG) was assessed in ^{3}H-dofetilide radioligand competition assay in CHO cell membranes, and the data are summarized in Table 1. The optimal cyclic amine groups reported from various H_3 antagonists of different chemical series were piperidine and pyrrolidine. In the present study piperidines **19a**−**j** (see Chart 2 for structure information) were found to be potent antagonists with pK_i values ranging from 8.4 to 9.2.

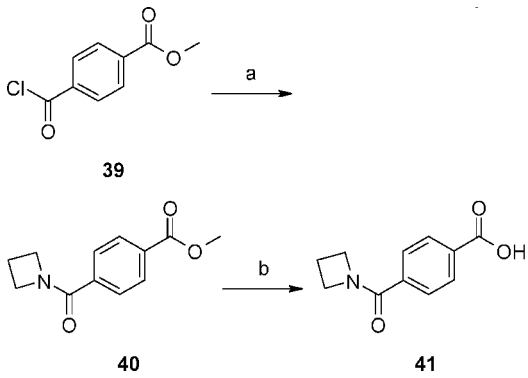
Heteroaromatic amides such **19k** and **19l** and alkyl amides such as **19m** and **19n** were found to possess pK_i values in the range 8.1–8.6. The para-regioisomeric sulfone **19f** was equipotent to the meta isomer **19g**. The *p*-azetidine amide **19j** and toluamide **19b** were the most potent analogues from this subgroup; however, **19b** might be metabolically unstable, whereas the molecular weight of the **19j** parent was >500, and it had a measured log *D* of −0.95 and possessed too many hydrogen bond acceptors, making it an unlikely candidate for good oral absorption.[56,57] The next best compounds were the fluoride and nitrile (**19d** and **19e**). The fluoride **19d** was selective for H_3 against H_1, hERG, α_{1A}, and α_{1B}. The selectivity of **19d** for H_3 over H_1 receptors was confirmed in the guinea pig ileum (H_3, 8.4, $n = 2$; H_1, <6.0, $n = 2$). Several second-generation H_1 antagonists are substrates and modulators of hepatic cytochrome P450, in particular the subtype CYP3A4. It is essential to avoid any drug−drug interactions that might lead to CYP450 activation and reduced safety in the clinic. The inhibitory effects of selected antagonists for the CYP450 system are summarized in Table 2. Clearly **19d** had no activity against 1A2, 2C19, 2C9, 2D6, and 3A4 (IC_{50} >100 μM). Furthermore, **19d** had low brain/plasma ratio (0.3 and 0.4) (Table 3), making this compound suitable for progressing to in vivo pharmacokinetic studies. Finally the pharmacokinetic properties of **19d** were investigated in rat and dog, and the data are summarized in Table 4, indicating high oral bioavailability in both species. The pyrrolidine analogues **20** had lower potencies than the piperidine analogues **19** except for **20d** and **20h**. The latter compound had potency below the cutoff value of 9 and was therefore rejected. The former compound was further tested in the guinea pig ileum where it showed selectivity for H_3 over H_1 receptors (H_3, 8.9, $n = 2$; H_1, <6.0, $n = 2$). Its pharmacokinetic properties however, were disappointing with rat oral bioavailability of 12% (Table 4). The homopiperidine analogues **21** had lower in vitro potencies than the piperidine analogues **19**. The introduction of a methyl group at the C2 position of a pyrrolidine ring was reported by the Abbott group to impart high in vitro potency at human and rat H_3 receptors.[51,58] We have examined both the (*R*)- and (*S*)- enantiomers (**22** and **23**) and confirmed the higher potency of 2(*R*)-methylpyrrolidines **22**. The analogue **22d** was a potent H_3 antagonist and selective for H_3 against H_1, hERG, α_{1A}, and α_{1B}. Racemic **22d** was tested in the guinea pig ileum where it showed selectivity over H_1 receptors (H_3, 8.7; H_1, <6.6; $n = 2$). Analogue **22e** was also a potent H_3 antagonist and selective for H_3 against H_1, hERG, α_{1A}, and α_{1B}. Its pharmacokinetic properties, however, were disappointing with oral bioavailability of 7% (Table 4). The low bioavailability might be due to a combination of its low log *D* value of −0.59 and high PSA of 77 Å^2.[59] Analogues **22h** and **22i** had identical H_3 potency and almost identical selectivity profile. In addition, **22i** had a low brain/blood ratio (0.2 and 0.3, Table 3). The

Scheme 2[a]



[a] Reagents and conditions: (a) NaH, 1-methyl-2-pyrrolidinone, 81°C; (b) CuI, K_3PO_4, *N*,*N*′-dimethylethylenediamine, dioxane, 100°C; (c) TFA, dichloromethane; (d) NaOH; (e) acetone, cyclobutanone, or cyclopentanone, AcOH, $NaB(OAc)_3H$, DCM; (f) H_2, Pd/C, EtOH; (g) acylation, RCO_2H, Et_3N, TBTU, DMF.

Scheme 3[a]



[a] Reagents and conditions: (a) azetidine hydrochloride, Et_3N, DCM; (b) 2 M NaOH, MeOH, H_2O.

pharmacokinetic properties of **22i** were investigated in rat and dog, and the data are summarized in Table 4, indicating high oral bioavailability in both species (48% for the rat and 100% for the dog).

The Abbott group has recently published on some very lipophilic H_3 antagonists, analogues of **22i** possessing a naphthalene core substituted with a 4-cyanophenyl ring, in order to enhance CNS penetration.[60] This is exactly the reverse of our own strategy, where we have used a polar core with a ketopiperazine ring and an amide linker in order to keep out of the CNS. The SAR observed with both series is remarkably similar and fits the typical H_3 pharmacophore of a basic amine connected through an alkoxy group to a core.

It is expected that molecules with fewer rotatable bonds have better pharmacokinetic properties, and for this reason analogues having the aminopropyloxy chain locked in a piperidine ring were prepared. The isopropyl piperidines **36** were generally less potent than the analogues **19** or had pK_i below 9, apart from **36e**, which had a low log *D* value of −0.64. The cyclobutylpiperidines **37** were either equipotent or more potent than the analogues **19**. The most potent analogue (pK_i 9.5) was the azetidine amide (**37j**), which as expected from its high PSA (73 Å^2) had a low brain/blood ratio of 0.1; however, it had high molecular weight (>500), high number of hydrogen bond acceptors, and a low log *D* value of −0.29, properties that predict low oral bioavailabity, and this was confirmed in rats (8%). The sulfone **37f** had high molecular weight, high number of H-bond acceptors, and low log *D* value, properties similar to those of the amide **37j**, and not unexpectedly low oral exposure as measured by the AUC (67 h·ng/mL). From the remaining compounds **37** that had potencies higher than their analogues of structure **19**, the chloride **37c** had a log *D* of 1.52 and a brain/blood ratio of 0.4. The fluoride **37d** was a potent H_3 antagonist with good selectivity for H_3 against H_1, hERG, α_{1a}, and α_{1b} and an acceptable log *D* value of 0.90. The selectivity for H_3 against H_1 was confirmed in other species such as rat (H_3, 8.0, H_1, <5.3) and guinea pig cortex (H_3, 8.3, H_1, <5.6). The rat bioavailability of 35%, and half-life of 2 h, makes this compound one of the preferred ones in this subgroup. The nitrile **37e**, however, had a selectivity profile similar to that of **37d** against H_1, hERG, α_{1A}, and α_{1B}. It had an acceptable log *D* of 0.38, a low brain/blood ratio of 0.2, good rat and dog bioavailability (42% and 69%, respectively), low or no activity against CYP450 (Table 2), and no time-dependent inhibition of CYP450. The pharmacokinetic half-life of **37e** was, however, low (1.3 and 1.4 h in rats and 2.3 h in the dog). Finally, the cyclopentylpiperidine analogues **38** had no advantage over the cyclobutyl analogues **37** and were not investigated any further.

The above studies identified **22i** and **37d** as the two compounds fulfilling the selection criteria set at the outset of this project. Further selectivity for **22i** was examined versus a

Table 1. Antagonist Potency of Target Compounds at the Human H_3 Receptor, Determined by a Functional GTPγ[S] Assay, H_1, hERG, α_{1A}, α_{1B}, log *D* and PSA

compd	H_3 pK_i ± SEM (*n*)[a]	H_1 pK_i ± SEM (*n*)	hERG pIC$_{50}$ (*n*)	α_{1A} pK_i (*n*)	α_{1B} pK_i (*n*)	log *D*	PSA (Å^2)
19a·HCl	8.4 ± 0.2 (5)	5.9 ± 0.1 (3)	4.2 (1)	<5.6 (4)	<5.6 (4)	−0.09	53
19b·HCl	9.1 ± 0.1 (5)	5.7 ± 0.1 (3)	4.5 (1)	<5.6 (4)	<5.8(4)	0.48	53
19c·HCl	8.7 ± 0.1 (3)	5.7 ± 0.1 (4)	ND[b]	ND[b]	ND[b]	ND[b]	53
19d	8.7 ± 0.1 (13)	<5.7 (13)	<4.5 (1)	<5.6 (4)	<5.8 (4)	0.11	53
19d·HCO_2H	8.8 ± 0.1 (4)	<5.7 (4)	4.3 (1)	ND[b]	ND[b]	0.12	53
19e·HCl	8.8 ± 0.1 (3)	5.7 ± 0.1 (3)	ND[b]	ND[b]	ND[b]	−0.38	77
19f	8.6 ± 0.1 (4)	<5.7 (4)	<4.2 (2)	ND[b]	ND[b]	−1.19	87
19g·HCO_2H	8.5 ± 0.1 (6)	<5.5 (7)	<4.2 (1)	ND[b]	ND[b]	−1.22	87
19h·HCO_2H	8.7 ± 0.0 (4)	<5.7 (4)	4.4 (1)	ND[b]	ND[b]	0.20	53
19i·HCO_2H	8.6 ± 0.1 (4)	<5.7 (4)	<4.2 (1)	ND[b]	ND[b]	0.10	53
19j·HCO_2H	9.2 ± 0.1 (5)	<5.6 (7)	<4.2 (1)	ND[b]	ND[b]	−0.95	73
19k. HCO_2H	8.1 ± 0.1 (4)	<5.6 (4)	<4.2 (1)	ND[b]	ND[b]	−1.60	66
19l·HCO_2H	8.4 ± 0.1 (6)	<5.5 (9)	<4.2 (1)	ND[b]	ND[b]	−1.10	83
19m·HCl	8.5 ± 0.1 (6)	<5.7 (6)	<4.2 (2)	<5.4 (4)	<5.6 (4)	−0.02	53
19n·HCl	8.6 ± 0.0 (9)	<5.6 (4)	ND[b]	ND[b]	ND[b]	ND[b]	53
20d·HCO_2H	9.1 ± 0.1 (4)	<5.6 (4)	5.2 (1)	ND[b]	ND[b]	0.14	53
20e·HCO_2H	8.2 ± 0.1 (4)	<5.6 (4)	<4.8 (1)	ND[b]	ND[b]	−1.07	77
20h	8.7 ± 0.2 (6)	<5.7 (6)	5.1 (1)	ND[b]	ND[b]	−0.38	53
20i	8.4 ± 0.1 (4)	<5.7 (4)	<4.8 (1)	ND[b]	ND[b]	−0.45	53
21d·HCO_2H	8.4 ± 0.1 (4)	<5.6 (4)	4.9 (1)	ND[b]	ND[b]	−0.47	53
21e·HCO_2H	8.5 ± 0.1 (4)	<5.6 (4)	4.9(1)	ND[b]	ND[b]	−0.39	77
21h·HCO_2H	8.3 ± 0.1 (4)	<5.6 (4)	4.9 (1)	ND[b]	ND[b]	0.24	53
21i·HCO_2H	8.1 ± 0.0 (4)	<5.6 (4)	4.3 (1)	ND[b]	ND[b]	0.15	53
22d·HCO_2H	9.2 ± 0.1 (8)	<5.7 (8)	4.7 (1)	<5.8 (1)	<5.8(2)	−0.13	53
22e·HCO_2H	9.3 ± 0.1 (8)	<5.6 (9)	<4.2 (1)	<5.7 (3)	<6.1 (2)	−0.59	77
22h·HCO_2H	9.2 ± 0.1 (8)	<5.6 (9)	6.3 (1)	<5 (1)	<5.8 (2)	−0.02	53
22i·HCO_2H	9.2 ± 0.1 (15)	<5.6 (15)	5.8 (2)	<5.7 (3)	<6.1 (2)	−0.08	53
23d·HCO_2H	8.5 ± 0.1 (4)	<5.7 (4)	4.6 (1)	ND[b]	ND[b]	−0.18	53
23e·HCO_2H	8.8 ± 0.1 (4)	<5.7 (4)	<4.2 (1)	ND[b]	ND[b]	−0.60	77
23h·HCO_2H	8.3 ± 0.0 (4)	<5.7 (4)	4.9 (1)	ND[b]	ND[b]	0.09	53
23i·HCO_2H	8.5 ± 0.1 (4)	<5.7 (4)	4.5 (1)	ND[b]	ND[b]	−0.13	53
36c·HCl	8.6 ± 0.1 (3)	<5.5 (2)	ND[b]	ND[b]	ND[b]	ND[b]	53
36d·HCl	8.4 ± 0.1 (3)	<5.6 (2)	ND[b]	ND[b]	ND[b]	ND[b]	53
36e·HCO_2H	9.1 ± 0.4 (6)	<5.5 (6)	<4.2 (1)	ND[b]	ND[b]	−0.64	77
36h·HCO_2H	8.7 ± 0.1 (4)	<5.6 (4)	<4.2 (1)	ND[b]	ND[b]	0.06	53
36i·HCO_2H	8.4 ± 0.1 (4)	<5.6 (4)	<4.2 (1)	ND[b]	ND[b]	−0.05	53
37a·HCl	8.5 ± 0.2 (6)	<5.6 (6)	<4.2 (2)	<5.4 (4)	<5.6 (4)	0.73	53
37b·HCl	9.0 ± 0.1 (6)	<5.7 (6)	<4.2 (1)	<5.4 (2)	<5.7 (2)	1.30	53
37c·HCl	9.2 ± 0.1 (6)	<5.7 (5)	4.5 (1)	<5.4 (4)	<5.6(4)	1.51	53
37d·HCl	9.2 ± 0.1 (8)	<5.6 (6)	<4.5 (2)	<5.5 (2)	<5.5 (2)	0.90	53
37e·HCO_2H	9.0 ± 0.1 (8)	<5.6 (8)	5.03 (1)	<5.7(2)	<6.1(2)	0.38	77
37f·HCO_2H	8.9 ± 0.1 (4)	<5.6 (4)	<4.2 (1)	ND[b]	ND[b]	−0.38	87
37h·HCO_2H	9.1 ± 0.2 (4)	<5.8 (4)	<4.2 (1)	<5.7 (2)	<6.1 (2)	0.99	53
37i·HCO_2H	8.8 ± 0.1 (6)	<5.6 (6)	<4.2 (2)	ND[b]	ND[b]	0.95	53
37j·HCO_2H	9.5 ± 0.0 (4)	<5.6 (4)	<4.2 (1)	ND[b]	ND[b]	−0.29	73
38c·HCO_2H	8.5 ± 0.2 (4)	<5.8 (4)	5.4 (1)	ND[b]	ND[b]	1.52	53
38d·HCO_2H	8.6 ± 0.0 (4)	<5.6 (6)	4.7 (1)	ND[b]	ND[b]	0.88	53
38e·HCO_2H	9.1 ± 0.1 (4)	<5.6 (4)	<4.2 (1)	ND[b]	ND[b]	0.37	77
38f·HCO_2H	9.1 ± 0.1 (4)	<5.6 (6)	<4.2 (1)	ND[b]	ND[b]	−0.46	87
38g·HCO_2H	8.0 ± 0.1 (4)	<5.6 (6)	<4.2 (1)	ND[b]	ND[b]	−0.41	87
38h·HCO_2H	8.6 ± 0.1 (4)	<5.6 (6)	5.1 (1)	ND[b]	ND[b]	1.03	53
38i·HCO_2H	8.4 ± 0.1 (4)	<5.6 (6)	4.6 (1)	ND[b]	ND[b]	0.96	53

[a] Negative log of the antagonist dissociation constant obtained from concentration−response curves of antagonist inhibition of a response to a fixed concentration of agonist in functional assays and calculated by application of a modified form of the Cheng−Prusoff equation.[62] [b] ND = not determined.

panel of 50 other monoamine and hormone receptors, ion channels, and neurotransmitter uptake sites and found to be inactive in all apart from PDE4B with pIC$_{50}$ of 6.15. Moreover, human serum albumin binding (HSA) for **22i** was 36%. Compound **22i** was chosen as a candidate for further progression. Compound **37d**, being a piperidinyloxy restrained analogue, was retained as a backup compound.

Conclusion

A novel series of potent ketopiperazine human H_3 receptor antagonists have been synthesized. The most potent ketopiperazine template was the 2(*R*)-methylpyrrolidine (**22**). Other potent templates include the piperidine (**19**) and the constrained cyclobutylpiperidine (**37**). The new compounds had very high selectivity over other receptors and low brain penetration. The absence of hepatic CYP450 inhibition indicated low drug−drug interaction potential. Optimization of the pharmacokinetic properties of the new antagonists was achieved by variation of the amide group. The 4-fluorobenzoyl, 4-cyanobenzoyl, and 2,4-difluorobenzoyl groups provided better pharmacokinetic profiles for the various amides. The optimal overall profile of compound **22i** (high potency, very good selectivity, low brain penetration, lack of CYP450 liability, high oral bioavailability) merits further investigation for the potential use in the treatment of allergic rhinitis.

Experimental Section

Organic solutions were dried over anhydrous Na_2SO_4 or $MgSO_4$. TLC was performed on Merck 0.25 mm Kieselgel 60 F_{254} plates. Products were visualized under UV light and/or by staining with

Chart 2

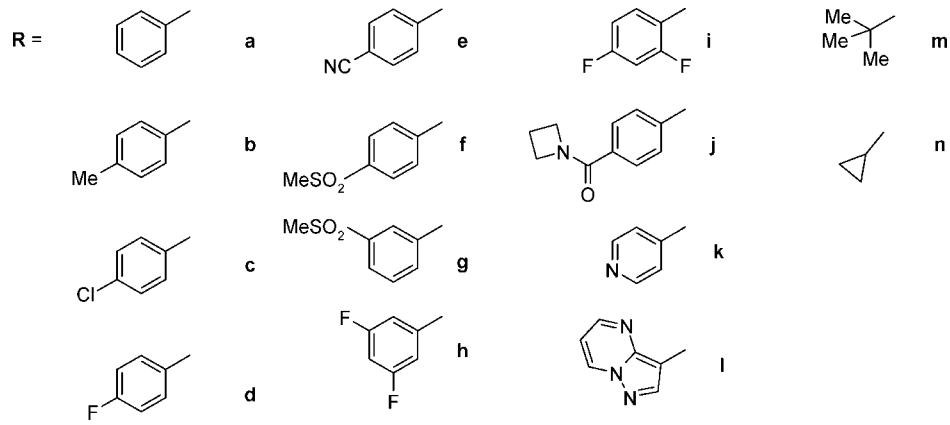


Table 2. In Vitro IC_{50} Concentration for **19d**, **19e**, **22i**, and **37e** against Human Cytochrome P450 Enzymes Expressed in *E. coli*, Assessed by the Effect on the *O*-Dealkylase Metabolic Rate for Specific Profluorescent Probe Substrates[a]

	enzyme isoform and substrate						
compd	1A2, ER (μM)	2C19, BMC (μM)	2C9, FCA (μM)	2D6, MMMC (μM)	3A4, DEF (μM)	3A4, 7BQ (μM)	3A4, PPR (μM)
19d	>100	>100	>100	>100	100	100	ND
19e	>100	>100	>100	77	ND	ND	>100
22i	>100	>100	>100	>100	>100	100	>100
37e	>100	>100	>100	41	>100	>100	ND

[a] ER = ethoxyresorufin, incubated at 0.5 μM; BMC = 3-butyryl-7-methoxycoumarin, incubated at 10 μM; FCA = 7-methoxy-4-trifluoromehylcoumarin-3-acetic acid, incubated at 50 μM; MMMC = 4-methylaminomethyl-7-methoxycoumarin, incubated at 10 μM; DEF = diethoxyfluorescein, incubated at 1 μM; 7BQ = 7-benzloxyquinolone, incubated at 10 μM; PPR = 7-{3-(4-phenylpiperazin-1-ylmethyl)benzyl} resorufin, incubated at 2 μM; ND = not determined.

Table 3. Rat Brain/Blood Ratio Determined at 5 min after a 1 mg/kg Intravenous Dose of **19d**, **22i**, **37c**, **37e**, and **37j**

19d	**19m**	**22i**	**37c**	**37e**	**37j**
0.4 0.3	0.1	0.2 0.3	0.4	0.2	0.1

aqueous $KMnO_4$ solution. LCMS analysis was conducted on a Supelcosil LCABZ+PLUS column (3.3 cm × 4.6 mm id), eluting with 0.1% formic acid and 0.01 M ammonium acetate in water (solvent A) and 0.05% formic acid and 5% water in acetonitrile (solvent B), using the following elution gradient 0.0−0.7 min 0% B, 0.7−4.2 min 100% B, 4.2–5.3 min 0% B, 5.3–5.5 min 0% B at a flow rate of 3 mL min^{-1}. The mass spectra were recorded on a Fisons VG Platform spectrometer using electrospray positive and negative mode (ES+ve and ES−ve). This method was the default, unless otherwise stated. In an alternative method (method B), LCMS was conducted on a Waters Atlantis column (50 mm × 4.6 mm id), eluting with 0.05% formic acid in water (solvent A) and 0.05% formic acid in acetonitrile (solvent B), using the following elution gradient 0.0–4.0 min 0−100% B at a flow rate of 1 mL min^{-1}. The mass spectra were recorded on a Waters ZQ platform using electrospray positive and negative mode (ES+ve and ES−ve). In yet another method (method C), LCMS was conducted on a Waters Atlantis column (50 mm × 4.6 mm id), eluting with 0.05% formic acid in water (solvent A) and 0.05% formic acid in acetonitrile (solvent B), using the following elution gradient 0.0–1.5 min 0−100% B at a flow rate of 1 mL min^{-1}. The mass spectra were recorded on a Waters ZQ platform using electrospray positive and negative mode (ES+ve and ES−ve). Column chromatography was performed on Merck Kieselgel 60 (article 9385) or Biotage prepacked silica gel cartridges containing KP-Sil run on a flash 12i chromatography module or on Flashmaster 2. The Flashmaster 2 is an automated multiuser flash chromatography system, available from Argonaut Technologies Ltd., which utilizes disposable, normal phase, SPE cartridges (2−100 g). Mass-directed autopreparative HPLC (MDAP) was conducted on a Waters FractionLynx system comprising a Waters 600 pump with extended pump heads, Waters 2700 autosampler, Waters 996 diode array, and Gilson 202 fraction collector on a 10 cm × 2.54 cm id ABZ+ column, eluting with 0.1% formic acid in water (solvent A) and 0.1% formic acid in acetonitrile (solvent B), using an appropriate elution gradient over 15 min at a flow rate of 20 mL min^{-1} and detecting at 200−320 nm at room temperature. Mass spectra were recorded on Micromass ZMD mass spectrometer using electrospray positive and negative modes, alternate scans. The software used was MassLynx 3.5 with OpenLynx and FractionLynx options. ^{1}H NMR spectra were recorded at 400 MHz unless otherwise stated. The chemical shifts are expressed in ppm relative to tetramethylsilane. High-resolution positive ion mass spectra were acquired on a Micromass Q-Tof 2 hybrid quadrupole time-of-flight mass spectrometer. Chiral HPLC was conducted on Chiralpak AD (25 cm × 0.46 cm) column, eluting with ethanol−heptane−TFA (25:75:0.1) at a flow rate of 1 mL min^{-1} for 60 min at room temperature, detecting at 215 nm. Optical rotations were measured with an Optical Activity AA100 digital polarimeter.

***N*^{2}-(2-Hydroxyethyl)-*N*^{1}-{4-[(phenylmethyl)oxy]phenyl}glycinamide (3).** 4-(Benzyloxy)aniline hydrochloride (**2**) (10 g, 42.5 mmol) was stirred in dry tetrahydrofuran (100 mL) and cooled in an ice/water bath to ∼5 °C. A solution of potassium carbonate (16.15 g, 117 mmol) in water (60 mL) was added to the above mixture, followed by the dropwise addition of chloroacetyl chloride (4.22 mL, 53 mmol) over 30 min The mixture was allowed to warm to room temperature and the organic phase separated. The organic phase was cooled to ∼5 °C in an ice/water bath and 2-aminoethanol (9.0 g, 150 mmol) added. The mixture was allowed to warm to room temperature and heated at 60 °C for 2 h, left overnight at room temperature, and heated at 60 °C for a further 2 h. The reaction mixture was partitioned between EtOAc and water. The organic phase was separated, washed with water and brine, dried, and concentrated. The residue was recrystallized from chloroform to give **3** (8.1 g, 64%). ES+ve *m/z* 301 $(M + H)^+$; ^{1}H NMR δ ($CDCl_3$) 9.17 (1H, s), 7.50 (2H, d, J = 9 Hz), 7.45–7.30 (5H, m), 6.95 (2H, d, J = 9 Hz), 5.05 (2H, s), 3.76 (2H, t, J = 5 Hz), 3.42 (2H, s), 2.85 (2H, t, J = 5 Hz).

1,1-Dimethylethyl (2-Hydroxyethyl)[2-oxo-2-({4-[(phenylmethyl)oxy]phenyl}amino)ethyl]carbamate (4). Bis(1,1-dimethylethyl) dicarbonate (14.5 g, 67.5 mmol) and 4-dimethylaminopyridine (150 mg, 1.2 mmol) were added to a solution of **3** (8.1 g, 27 mmol) in DCM (200 mL). The mixture was stirred for 30 min,

Table 4. Pharmacokinetic Parameters for Compounds **19d**, **20d**, **22e**, **22i**, **37d**, **37e**, and **37f**

compd	MW	species	dose (mg/kg) (route)	C_{max} (ng/mL)	t_{max} (h)	AUC (h·ng/mL)	Cl ((mL/min)/kg	V (L/kg)	$t_{1/2}$ (h)	F (%)
19d	439.6	rat	3.0 (po)	118	3.00	614				47
		rat	3.0 (po)	155	2.00	805				61
		rat	1.0 (iv)	300	0.08	437	37	2.4	1.5	
19d·HCO_2H	485.6	dog	1.0 (iv)	530	0.08	1570	9.0	2.9	4.3	
		dog	2.0 (po)	400	0.75	3090				86
20d·HCO_2H	471.5	rat	3.0 (po)	40.0	7.00	355				12
		rat	1.0 (iv)	342	0.08	536	31	2.0	1.3	
		rat	1.0 (iv)	386	0.08	557	30	1.9	1.2	
22e·HCO_2H	492.6	rat	3.0 (po)	52.0	7.00	441				7
		rat	1.0 (iv)	729	0.08	1190	13	1.6	1.8	
22i·HCO_2H	503.5	rat	3.0 (po)	46.0	4.00	375				
		rat	1.0 (iv)	291	0.08	682	24	2.1	1.3	
		rat	3.0 (po)	109	2.00	753				48
		rat	1.0 (iv)	235	0.08	529	31	2.0	1.1	
		dog	2.0 (po)	734	4.00	4800				100
		dog	1.0 (iv)	414	0.08	2160	6.5	2.3	4.3	
		dog	2.0 (po)	734	4.00	4800				100
		dog	1.0 (iv)	414	0.08	2160	6.5	2.3	4.3	
37d·HCl	451.6	rat	2.9 (po)	91.0	2.00	760				35
		rat	1.0 (iv)	337	0.08	837	18	2.5	2.0	
		rat	1.0 (iv)	262	0.08	680	23	2.9	2.0	
37e·HCO_2H	504.6	rat	3.0 (po)	149	1.00	1150				42
		rat	1.0 (iv)	305	0.08	577	29	2.1	1.3	
		rat	1.0 (iv)	350	0.08	601	27	2.2	1.4	
		dog	2.0 (po)	294	1.00	1540				69
		dog	1.0 (iv)	419	0.08	1100	15	2.3	2.3	
37f·HCO_2H	557.7	rat	3.0 (po)	10.0	4.00	67				

and aqueous saturated sodium bicarbonate was added (150 mL). The organic phase was separated, dried, and evaporated. The residue was dissolved in methanol (150 mL), and potassium carbonate (14.5 g, 105 mmol) was added. The mixture was heated on a steam bath for 15 min, allowed to cool to room temperature, and left standing for 18 h. The reaction mixture was evaporated and partitioned between DCM and water. The organic phase was separated, washed with brine, dried, and evaporated. The residue was preabsorbed onto flash silica and purified by flash chromatography (100 g silica cartridge, from 0% to 100% EtOAc−cyclohexane gradient over 30 min) to give **4** (5.0 g, 46%). ES+ve *m/z* 401 $(M + H)^+$.

1,1-Dimethylethyl 3-Oxo-4-{4-[(phenylmethyl)oxy]phenyl}-1-piperazinecarboxylate (6). Methanesulfonyl chloride (2.5 mL, 32 mmol) was added to a solution of **4** (4.99 g, 9.93 mmol) in dry DCM (50 mL) and triethylamine (20 mL) under nitrogen. The mixture was stirred for 30 min and then partitioned between dichloromethane and aqueous saturated sodium bicarbonate. The organic layer was separated and concentrated in vacuo. The residue was dissolved in dry DMF (20 mL), and sodium hydride (60% oil dispersion, 0.6 g, 15 mmol) was added. The mixture was stirred for 30 min, quenched with aqueous saturated sodium bicarbonate, and extracted with EtOAc. The organic phases were combined, dried, and evaporated. The residue was purified by flash chromatography (100 g silica cartridge, from 0% to 100% EtOAc−cyclohexane gradient over 40 min) to give **6** (4.1 g). ES+ve *m/z* 383 $(M + H)^+$; ^{1}H NMR δ ($CDCl_3$) 7.45–7.32 (5H, m), 7.19 (2H, d, $J = 9$ Hz), 7.01 (2H, d, $J = 9$ Hz), 5.08 (2H, s), 4.25 (2H, s), 3.80–3.75 (2H, m), 3.71–3.68 (2H, m), 1.50 (9H, s).

1,1-Dimethylethyl 4-(4-Hydroxyphenyl)-3-oxo-1-piperazinecarboxylate (7). A solution of **6** (3.1 g, 8.1 mmol) in EtOH (25 mL) and EtOAc (25 mL) was hydrogenated over 10% Pd/C (1 g) for 2 h. The catalyst was removed by filtration through Celite and washed with EtOH. The combined filtrate and washings were concentrated to give **7** (1.92 g, 81%). ES+ve *m/z* 293 $(M + H)^+$; ^{1}H NMR δ ($CDCl_3$) 6.98 (2H, d, $J = 9$ Hz), 6.64 (2H, d, $J = 9$ Hz), 4.26 (2H, s), 3.80–3.75 (2H, m), 3.68–3.64 (2H, m), 1.51 (9H, s).

1,1-Dimethylethyl 4-{4-[(3-Chloropropyl)oxy]phenyl}-3-oxo-1-piperazinecarboxylate (8) and 1,1-Dimethylethyl 4-{4-[(3-Bromopropyl)oxy]phenyl}-3-oxo-1-piperazinecarboxylate (8′). To a solution of **7** (5.0 g, 17 mmol) in 2-butanone (200 mL) was added potassium carbonate (4.7 g, 34 mmol) followed by 1-bromo-3-chloropropane (4.0 g, 25.5 mmol). The mixture was heated at 90 °C under nitrogen for 72 h and cooled to room temperature, and water (200 mL) was added. The organic phase was separated, and the aqueous layer was extracted twice with DCM. The organic phases were combined, dried, and evaporated. The residue was purified by flash chromatography (100 g silica cartridge, from 0% to 100% EtOAc−cyclohexane gradient over 60 min) to give a mixture of **8** and **8′** (6.2 g, 9:1). ES+ve *m/z* 369/371 (chloride) and 413/415 (bromide) $(M + H)^+$; ^{1}H NMR δ ($CDCl_3$) 7.19 (2H, d, $J = 9$ Hz), 6.94 (2H, d, $J = 9$ Hz), 4.25 (2H, s), 4.12 (2H, t, $J = 6$ Hz), 3.80–3.74 (2H, m), 3.74 (1.8H, t, $J = 6$ Hz), 3.71–3.67 (2H, m), 3.60 (0.2H, t, $J = 7$ Hz), 2.28–2.21 (2H, m), 1.50 (9H, s).

1,1-Dimethylethyl3-Oxo-4-(4-{[3-(1-piperidinyl)propyl]oxy}phenyl)-1-piperazinecarboxylate (9). To a solution of **8** and **8′** (1.28 g, 3.45 mmol) in acetone (50 mL) was added sodium iodide (0.517 g, 3.45 mmol) and piperidine (2 mL). The resultant mixture was heated to 60 °C and stirred for 16 h and then at room temperature for 5 days. More sodium iodide (0.517 g, 3.45 mmol) and piperidine (2 mL) were added, and the reaction mixture was stirred at 60 °C for a further 5 h. The reaction mixture was allowed to cool to room temperature and concentrated in vacuo. The residue was partitioned between EtOAc and saturated sodium bicarbonate solution. The aqueous portion was extracted with EtOAc, and the combined organic solutions were washed with water, brine, dried (Na_2SO_4), and evaporated. The residue was purified by Biotage column chromatography (90 g cartridge) and eluted with DCM–EtOH−aqueous NH_3 (150:8:1) to give **9** (345 mg, 25%). ES+ve *m/z* 418 $(M + H)^+$; ^{1}H NMR δ ($CDCl_3$) 7.17 (2H, d, $J = 9$ Hz), 6.92 (2H, d, $J = 9$ Hz), 4.24 (2H, s), 4.01 (2H, d, $J = 6$ Hz), 3.80–3.75 (2H, m), 3.72–3.66 (2H, m), 2.54–2.40 (6H, m), 2.04–1.95 (2H, m), 1.65–1.57 (4H, m), 1.50 (9H, s), 1.5–1.40 (2H, m).

1-(4-{[3-(1-Piperidinyl)propyl]oxy}phenyl)-2-piperazinone Trifluoroacetate (14). A solution of **9** (0.345 g, 0.826 mmol) in DCM−trifluoroacetic acid (20 mL, 4:1) was stirred at room temperature for 45 min. The solvents were then removed in vacuo to leave **14** (0.745 g). ES+ve *m/z* 318 $(M + H)^+$; ^{1}H NMR δ (CD_3OD) 7.26 (2H, d, $J = 9$ Hz), 7.01 (2H, d, $J = 9$ Hz), 4.12 (2H, t, $J = 6$ Hz), 3.99 (2H, s), 3.93–3.88 (2H, m), 3.69–3.64 (2H,

m), 3.63–3.55 (2H, m), 3.33–3.27 (2H, m), 3.00–2.92 (2H, m), 2.27–2.18 (2H, m), 2.01–1.93 (2H, m), 1.90–1.70 (3H, m), 1.6–1.5 (1H, m).

1-(4-{[3-(Hexahydro-1*H*-azepin-1-yl)propyl]oxy}phenyl)-2-piperazinone (16). A solution of **11** (0.9 g, 2 mmol) in DCM (10 mL) was treated with a mixture of trifluoroacetic acid−DCM (10 mL, 2:5). The reaction mixture was stirred at room temperature for 18 h. The solvent was removed in vacuo. The residue was dissolved in methanol, and the solution was applied to an SCX-2 cartridge (70 g). The cartridge was washed with methanol, and the product eluted with 10% 0.880 NH_3 in methanol. The appropriate ammoniacal fractions were combined and concentrated to give **16** (0.67 g, 97%). ES+ve *m/z* 332 $(M + H)^+$; ^{1}H NMR δ ($CDCl_3$) 7.18 (2H, d, $J = 9$ Hz), 6.93 (2H, d, $J = 9$ Hz), 4.01 (2H, t, $J = 6$ Hz), 3.70 (2H, s), 3.65 (2H, t, $J = 5$ Hz), 3.21 (2H, t, $J = 5$ Hz), 2.68–2.62 (6H, m), 2.08–1.90 (2H, m), 1.80–1.58 (8H, m).

1-[4-({3-[(2*R*)-2-Methyl-1-pyrrolidinyl]propyl}oxy)phenyl]-2-piperazinone (17). 2-(*R*)-Methylpyrrolidine was prepared according to the method of Ku, Cowart, and Sharma.[61] ES+ve *m/z* 318 $(M + H)^+$; ^{1}H NMR δ ($CDCl_3$) 7.18 (2H, d, $J = 9$ Hz), 6.92 (2H, d, $J = 9$ Hz), 4.05–4.00 (2H, m), 3.75 (2H, s), 3.65 (2H, t, $J = 5$ Hz), 3.24–3.15 (3H, m), 3.01–2.94 (1H, m), 2.33–1.65 (9H, m), 1.48–1.38 (1H, m), 1.10 (3H, d $J = 6$ Hz).

1,1-Dimethylethyl 4-[(4-Iodophenyl)oxy]-1-piperidinecarboxylate (26). Sodium hydride (60% oil dispersion, 2.04 g, 52 mmol) was added to a solution of *N*-BOC-4-hydroxypiperidine (**25**) (10 g, 50 mmol) in 1-methyl-2-pyrrolidinone (50 mL) under nitrogen at room temperature. 1-Iodo-4-fluorobenzene (11.1 g, 50 mmol) was added at room temperature, and then the mixture was heated to 81 °C overnight. The mixture was allowed to cool to room temperature and partitioned between water and EtOAc. The aqueous phase was extracted with more EtOAc, and the combined organic solutions were washed with brine, dried, and concentrated. The residue was dissolved in DCM and purified by chromatography on Flashmaster II, eluting with EtOAc−cyclohexane (from 0% to 100% over 60 min) to give **26** (11.6 g, 57%). ES+ve *m/z* 304 $(MH - BOC)^+$; ^{1}H NMR δ ($CDCl_3$) 7.56 (2H, d, $J = 9$ Hz), 6.70 (2H, d, $J = 9$ Hz), 4.46–4.40 (1H, m), 3.72–3.64 (2H, m), 3.38–3.30 (2H, m), 1.95–1.86 (2H, m), 1.79–1.69 (2H, m), 1.58 (9H, s).

Phenylmethyl 4-{4-[(1-{[(1,1-Dimethylethyl)oxy]carbonyl}-4-piperidinyl)oxy]phenyl}-3-oxo-1-piperazinecarboxylate (28). A mixture of **26** (10.9 g, 27 mmol), 4-benzyloxycarbonylpiperazin-2-one (**27**) (7.6 g, 32 mmol), tripotassium phosphate (6.79 g, 32 mmol), *N*,*N*′-dimethylethylenediamine (0.29 mL, 2.7 mmol), and copper(I) iodide (257 mg, 1.35 mmol) in dioxane (200 mL) was heated under nitrogen to 100 °C for 24 h. The mixture was allowed to cool to room temperature and partitioned between water and EtOAc. The aqueous solution was extracted with more EtOAc, and the combined organic solutions were washed with brine, dried, and concentrated. The residue was dissolved in DCM and purified by chromatography on Biotage (400 g), eluting with EtOAc−cyclohexane (2:3 to 1:1) to give **28** (7.36 g, 53%). ^{1}H NMR δ ($CDCl_3$) 7.40–7.33 (5H, m), 7.17 (2H, d, $J = 9$ Hz), 6.93 (2H, d, $J = 9$ Hz), 5.20 (2H, s), 4.40–4.43 (1H, m), 4.33 (2H, s), 3.89–3.84 (2H, m), 3.74–3.64 (4H, m), 3.39–3.31 (2H, m), 1.95–1.87 (2H, m), 1.80–1.70 (2H, m), 1.48 (9H, s).

Phenylmethyl 3-Oxo-4-[4-(4-piperidinyloxy)phenyl]-1-piperazinecarboxylate (29). A solution of **28** (7.413 g, 145 mmol) in DCM−TFA (4:1, 200 mL) was stirred at room temperature for 1 h and then concentrated under reduced pressure. The residue was diluted with water and basified with NaOH to give a white suspension. The solid was collected by filtration, and the aqueous filtrate was extracted with EtOAc. The organic solution was washed with water and brine, dried, and evaporated to give **29** (812 mg). The aqueous solution was basified with more NaOH to pH 13 and extracted with EtOAc. The organic solution was washed with water and brine, dried, and evaporated to give additional **29** (2.7 g). The solid (3.5 g) was dissolved in a mixture of DCM and MeOH (80 mL) and applied to an aminopropyl cartridge (70 g), eluting with MeOH. The appropriate fractions were combined and concentrated to give a further quantity of **29** (2.28 g). ES+ve *m/z* 410 $(M + H)^+$; ^{1}H NMR δ (CD_3OD) 7.42–7.28 (5H, m), 7.19 (2H, d, $J = 8$ Hz), 6.98 (2H, d, $J = 8$ Hz), 5.18 (2H, s), 4.51–4.43 (1H, m), 4.25 (2H, m), 3.90–3.80 (2H, m), 3.75–3.68 (2H, m), 3.10–3.02 (2H, m), 2.75–2.67 (2H, m), 2.05–1.95 (2H, m), 1.70–1.60 (2H, m).

Phenylmethyl 4-(4-{[1-(1-Methylethyl)-4-piperidinyl]oxy}phenyl)-3-oxo-1-piperazinecarboxylate (30). A suspension of **29** (545 mg, 1.33 mmol) in acetone (10 mL), DCM (5 mL), and AcOH (1 mL) was treated portionwise with sodium triacetoxyborohydride (0.57 g, 2.66 mmol), and the mixture was stirred at room temperature under nitrogen overnight. A further portion of sodium triacetoxyborohydride (0.57 g, 2.66 mmol) was added, and the mixture was stirred for another day. The reaction mixture was partitioned between EtOAc and aqueous $NaHCO_3$. The organic solution was washed with $NaHCO_3$ and brine, dried, and concentrated to give **30** (576 mg, 96%). ES+ve *m/z* 452 $(M + H)^+$, ^{1}H NMR δ ($CDCl_3$) 7.40–7.32 (5H, m), 7.15 (2H, d, $J = 9$ Hz), 6.92 (2H, d, $J = 9$ Hz), 5.20 (2H, s), 4.32 (2H, s), 4.32–4.27 (1H, m), 3.85 (2H, br t, $J = 6$ Hz), 3.74–3.67 (2H, m), 2.83–2.74 (3H, m), 2.47–2.38 (2H, m), 2.08–2.00 (2H, m), 1.90–1.78 (2H, m), 1.08 (6H, d, $J = 6$ Hz).

1-(4-{[1-(1-Methylethyl)-4-piperidinyl]oxy}phenyl)-2-piperazinone (33). A solution of **30** (1.168 g, 2.586 mmol) in EtOH (20 mL) and EtOAc (5 mL) was hydrogenated over 10% Pd/C (200 mg) for 2 h. More catalyst (200 mg) was added, and the mixture was stirred under hydrogen for a further 1.5 h. The catalyst was removed by filtration, and the filtrate was hydrogenated over a fresh batch of catalyst (200 mg) overnight. The catalyst was removed by filtration through a Celite cartridge (10 g) and washed with EtOH. The combined filtrate and washings were concentrated to give **33** (732 mg, 89%). ES+ve *m/z* 318 $(M + H)^+$; ^{1}H NMR δ (CD_3OD) 7.19 (2H, d, $J = 9$ Hz), 6.98 (2H, d, $J = 9$ Hz), 4.44–4.37 (1H, m), 3.65 (2H, br t, $J = 5$ Hz), 3.55–3.51 (2H, m), 3.15 (2H, t, $J = 5$ Hz), 2.88–2.73 (3H, m), 2.54–2.46 (2H, m), 2.08–1.99 (2H, m), 1.84–1.74 (2H, m), 1.10 (6H, d, $J = 6.5$ Hz).

Generic Method for Acylation of 14 in Array Format Using Resin Bound DCC. Polymer bound DCC (Nova; 1.8 mmol/g, 853 mg, 1.5 mmol) was suspended in enough DCM to mobilize on stirring in Radley's tube on a carousel reaction station. The carboxylic acid (**a**, **e**) (0.77 mmol, 2 equiv) was added to the resin after 10 min and diluted with DMF to form a solution. The reaction mixture was then treated with *N*-hydroxybenztriazole monohydrate (104 mg, 0.77 mmol, 2 equiv), followed by a mixture of **14** (150 mg, 0.38 mmol) and triethylamine (0.1 mL, 0.8 mmol) in DCM and stirred at room temperature for 3 days. The resin was removed by filtration and washed with DCM, and the filtrate was acidified with acetic acid. The solution was then applied to a 10 g SCX ion exchange cartridge, eluting sequentially with MeOH, water and 10% aqueous ammonia in MeOH. The ammoniacal fractions were evaporated, and the residue was dissolved in DCM and purified by chromatography on silica, eluting with (5% aqueous ammonia in MeOH)−DCM (1:19). Appropriate fractions were combined and evaporated under reduced pressure to give the product, which was then dissolved in MeOH and treated with 1 equiv of 1 M HCl in diethyl ether. The solvent was removed by evaporation, and the residue was triturated in diethyl ether to give the hydrochloride salts of the products.

Generic Method for the Acylation of 14 Using Acid Chlorides. A solution of **14** (130 mg, 0.41 mmol) in DCM (5 mL) was treated with diethylaminomethyl polystyrene (3.2 mmol/g, 194 mg, 0.62 mmol) and the appropriate acid chloride (**a**, **m**, **n**) (0.62 mmol) and stirred for 30 min. The mixture was diluted with MeOH and passed down a SCX-2 cartridge, eluting with MeOH and then with 10% aqueous ammonia in MeOH. The ammoniacal fractions were combined and evaporated under reduced pressure to give the product, which was then dissolved in MeOH and treated with 1 equiv of 1 M HCl in diethyl ether. The solvent was removed by evaporation, and the residue was triturated in diethyl ether to give the hydrochloride salts of the products.

Generic Method for Acylation of Ketopiperazines 14–18 and 33–35 in Array Format Using TBTU. A solution of the appropriate amine (0.1 mmol) in DMF (0.5 mL) and triethylamine

(0.14 mL, 1 mmol) was treated with the appropriate carboxylic acid (0.11 mmol) and finally (*O*-benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate (TBTU) (42 mg, 0.13 mmol). The mixture was stirred overnight at room temperature and then applied to a 10 g SCX-2 cartridge, eluting with MeOH, followed by 10% aqueous ammonia in MeOH. The ammoniacal fractions were combined and evaporated under reduced pressure to give the free base of the amides **19–23** and **36–38** in Table 1. All the analogues in Table 1 shown as formate salts were the product of a further purification by MDAP. The appropriate fractions possessing the expected mass ion were evaporated under reduced pressure or under a constant stream of nitrogen gas.

The arylamides in Table 1 were obtained as mixtures of rotamers that produced broad NMR spectra. A selection of NMR solutions was heated, and the spectra of these compounds were recorded at elevated temperatures as indicated. The spectra of these compounds sharpened up, confirming the presence of rotamers.

4-[(4-Fluorophenyl)carbonyl]-1-(4-{[3-(1-piperidinyl)propyl]-oxy}phenyl)-2-piperazinone Formate (19d). ES+ve *m*/*z* 440 (M + H)$^+$; ^{1}H NMR δ (CD_3OD) 8.38 (1H, s), 7.60 (2H, dd, J = 9,6 Hz), 7.26 (2H, d, J = 9 Hz), 7.24 (2H, t, J = 9 Hz), 6.99 (2H, d, J = 9 Hz), 4.51–4.30 (2H, m), 4.11 (2H, t, J = 6 Hz), 4.09–3.82 (2H, m), 3.82–3.74 (2H, m), 3.64–3.06 (6H, s), 2.28–2.18 (2H, m), 1.96–1.81 (4H, m), 1.78–1.60 (2H, m); HRMS ES+ve *m*/*z* found 440.2354, calcd for $C_{25}H_{31}FN_3O_3$ 440.2349.

4-[(2,4-Difluorophenyl)carbonyl]-1-[4-({3-[(2*R*)-2-methyl-1-pyrrolidinyl]propyl}oxy)phenyl]-2-piperazinone formate (22i). $[\alpha]_D^{22}$ –26 (*c* 0.88 in MeOH); ES+ve *m*/*z* 458 (M + H)$^+$; chiral HPLC t_R = 42.2 min, 100%; ^{1}H NMR δ (CD_3OD) 8.40 (1H, s), 7.64–7.50 (1H, m), 7.27 (2H, d, J = 9 Hz), 7.19–7.10 (2H, m), 7.01 (2H, d, J = 9 Hz), 4.49 (1.2H, s), 4.16 (0.8H, s), 4.13 (2.8H, m), 3.85–3.64 (4.2H, m), 3.60–3.48 (2H, m), 3.27–3.13 (2H, m), 2.39–2.28 (1H, m), 2.28–2.16 (2H, m), 2.16–2.03 (2H, m), 1.82–1.71 (1H, m), 1.45 (3H, d, J = 6.5 Hz); HRMS ES+ve *m*/*z* found 458.2250, calcd for $C_{25}H_{30}N_3O_3F_2$ 458.2255.

1-{4-[(1-Cyclobutyl-4-piperidinyl)oxy]phenyl}-4-[(4-fluorophenyl)carbonyl]-2-piperazinone Hydrochloride (37d). ES+ve *m*/*z* 452 (M + H)$^+$; ^{1}H NMR δ (CD_3OD) 7.60 (2H, dd, J = 9, 6 Hz), 7.28 (2H, d, J = 9 Hz), 7.23 (2H, t, J = 9 Hz), 7.06 (2H, d, J = 9 Hz), 4.83–4.76 (0.5H, m), 4.70–4.50 (0.5H, m), 4.45–4.30 (2H, m), 4.30–3.70 (5H, m), 3.70–3.30 (2H, m), 3.20–2.90 (2H, m), 2.40–2.18 (6H, m), 2.15–2.00 (2H, m), 1.95–1.78 (2H, m). When the solution was heated to 55 °C, the spectrum sharpened: 4.77–4.63 (1H, m), 4.37 (2H, s), 4.00–3.90 (2H, m), 3.83–3.67 (3H, m). HRMS ES+ve *m*/*z* found 452.2353, calcd for $C_{26}H_{31}FN_3O_3$ 452.2349.

Methyl 4-(Azetidin-1-ylcarbonyl)benzoate (40). Methyl 4-chlorocarbonylbenzoate (**39**) (4.98 g, 25.1 mmol) was stirred with triethylamine (8.7 mL, 63 mmol) in dry DCM (50 mL) at 13 °C. Azetidine hydrochloride (2.8 g, 30.2 mmol) was added portionwise over 5 min. Cooling in ice was required to keep the temperature below 20 °C. After being stirred at room temperature under nitrogen for 3.5 h, the solution was diluted with DCM and partitioned with aqueous sodium bicarbonate solution. The cloudy organic solution was then washed with dilute hydrochloric acid, water, and brine and dried. The solution was evaporated to give **40** (3.97 g, 72%): ES+ve *m*/*z* 220 (M + H)$^+$; ^{1}H NMR δ (DMSO-d_6) 8.00 (2H, d, J = 8 Hz), 7.73 (2H, d, J = 8 Hz), 4.28 (2H, t, J = 7.5 Hz), 4.05 (2H, t, J = 7.5 Hz), 3.87 (3H, s), 2.31–2.20 (2H, m).

4-(Azetidin-1-ylcarbonyl)benzoic Acid (41). A 2 M NaOH solution (10.8 mL, 21.6 mmol) was added to a solution of **40** (2.38 g, 10.8 mmol) in MeOH (24 mL) and stirred for 0.5 h. The mixture was acidified with 2 M hydrochloric acid and extracted with ethyl acetate. The aqueous phase was extracted with more ethyl acetate, and the combined organic solutions were washed with water and brine, dried, and evaporated under reduced pressure to give **41** (1.92 g, 86%): ES+ve *m*/*z* 206 (M + H)$^+$; ^{1}H NMR δ (DMSO-d_6) 13.21 (1H, br s), 7.99 (2H, d, J = 8 Hz), 7.71 (2H, d, J = 8 Hz), 4.28 (2H, t, J = 8 Hz), 4.05 (2H, t, J = 8 Hz), 2.31–2.21 (2H, m).

Determination of log *D*. A stock solution of the test compounds in DMSO (10 mM, 10 μL) was added to octanol saturated pH 7.4 phosphate buffer (740 μL) in a deep-well microtiter plate. Buffer saturated octanol (160 μL) was added (via a Beckman Biomek 2000 robot). The plate was sealed with a silicon rubber mat and then inverted, 3 sets of 50 inversions each set. The plate was centrifuged at 300*g* for 15 min to separate the two phases. The sample concentration in both phases was quantified by HPLC and log *D* calculated using the following equation:

$$\log D = \log[\text{HPLC sample peak area octanol phase/octanol phase sample injection volume } (1\mu\text{L})]/[\text{HPLC sample peak area aqueous phase/aqueous phase sample injection volume } (25\mu\text{L})]$$

Human Serum Albumin Binding Assay. The HSA binding of test compounds was determined using HPLC on chemically bonded protein stationary phases according to the method of Valko.[62]

Histamine H_3 Functional Antagonist Assay. Membranes derived from a CHO cell line stably transfected with human H_3 receptor (5 μg membranes/well) were adhered to wheat germ agglutinin polystyrene LeadSeeker (WGA PS LS) scintillation proximity assay (SPA) beads (250 μg/well) in assay buffer and GDP (16.6 μM). Assay buffer contained *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES) (20 mM), $MgCl_2$ (10 mM), 100 mM NaCl, and the pH was adjusted to 7.4 using 5 M KOH. After 1 h of precoupling at room temperature, the bead/membrane suspension (30 μL/well) was dispensed into a white Greiner polypropylene 384-well plate containing stock solution of the test compounds in DMSO (10 mM, 0.5 μL) or 0.5 μL of DMSO as a control. [^{35}S]-GTPγS (Amersham, radioactivity concentration 37 MBq mL^{-1}; specific activity = 1160 Ci mmol^{-1}) made up in assay buffer containing histamine (at a concentration that results in the final assay concentration of histamine being EC_{80}) was then added (15 μL/well, 0.4 nM final radioligand concentration), and the assay plates were sealed and centrifuged at 1000 rpm for 1 min. The plates were left to equilibrate and to adapt in the dark between 2 and 6 h before reading on a ViewLux luminescence imager using a 613/55 filter for 5 min plate^{-1}. Data were analyzed using a four-parameter logistical equation to determine the antagonist IC_{50}. Antagonist dissociation constants (K_i) were derived from the IC_{50} using a generalized form of the Cheng−Prusoff equation and expressed as the negative log of the K_i (pK_i).[63,64]

Histamine H_1 and Adrenergic α_{1A} and α_{1B} Receptor Selectivity Assays. CHO cells stably expressing the human recombinant histamine H_1 receptor or Rat1 fibroblast cells stably expressing the adrenergic α_{1A} or α_{1B} receptors were seeded into black-walled clear-bottom 384-well plates at a density of 10 000 cells/well. The following day the cells were washed and incubated with Tyrodes buffer (NaCl 145 mM, KCl 2.5 mM, HEPES 10 mM, glucose 10 mM, $MgCl_2$ 1.2 mM, $CaCl_2$ 1.5 mM, probenecid 2.5 mM, pH adjusted to 7.40 with 1 M NaOH) containing Fluo-4AM (10 μM) and brilliant black (500 μM) for 60 min at 37 °C. An amount of 10 μL of antagonist was added (concentration range 30 μM to 30 pM, 2% v/v DMSO), and the cells were incubated for 30 min at 37 °C to allow equilibration of the test compound with the receptor. An amount of 10 μL of histamine (H_1) or phenylephrine (α_{1a} and α_{1b}), at a concentration approximately equivalent to an EC_{80} on the agonist concentration–response curve, was added to each well using the fluorescence imaging plate reader (FLIPR; Molecular Devices, Sunnyvale, CA), and fluorescence emissions were monitored for 1 min. Agonist responses were measured as the peak stimulated fluorescence expressed as a percentage of the basal fluorescence, and antagonist affinity was determined as described in the previous section.

Guinea Pig and Rat Cortex Histamine Receptor Radioligand Binding Assays. Membranes prepared from rat and guinea pig cerebral cortex (7 mg of wet weight tissue in 0.4 mL) were incubated with 50 μL of radioligand (H_1, [^{3}H]mepyramine 0.5–1 nM; H_3, [^{3}H]-*R*-α-methylhistamine 1–2 nM final assay concentration) and increasing concentrations of antagonist (50 μM to 0.5 nM and 1% v/v DMSO final assay concentration) for 2 h at room temperature. Samples were then filtered through Whatman GF/C filter paper using a Brandell cell harvester and washed three times using distilled water at 4 °C. Bound radioligand was determined

using standard liquid scintillation spectrometry. Antagonist inhibition curves were analyzed using nonliner regression to obtain an IC_{50} as described above and converted to antagonist dissociation constants (K_i) using the method of Cheng and Prusoff.[63]

Isolated Guinea Pig Ileum Longitudinal Smooth Muscle H_1 and H_3 Functional Assays. Isolated guinea pig ileum longitudinal smooth muscle was prepared for the measurement of isometric contractions as described elsewhere.[65] For H_1 receptors, cumulative concentration–response curves for direct smooth muscle contraction in response to histamine were constructed in the absence and presence of test compound. For H_3 receptors, tissues were electrically stimulated (0.1 Hz, 0.5 ms pulse width, submaximal voltage) and cumulative concentration–response curves for inhibition of electrical field stimulation by (*R*)-α-methylhistamine were constructed in the absence and presence of test compound. Antagonist pA_2 values were calculated as described previously.[66]

^{3}H-Dofetilide Radioligand Binding Assay for hERG. hERG-expressing CHO-K1 membranes (2 μg membranes/well) were adhered to wheat germ agglutinin-coated SPAPEI imaging beads (120 μg/well) in assay buffer containing HEPES (25 mM) and $MgCl_2$ (1.2 mM), and pH was adjusted to 7.4 using 5 M KOH. The final potassium concentration in the assay was 13 mM. After 1 h of mixing at 4 °C, the bead/membrane suspension (20 μL/well) was dispensed into a white Nunc polypropylene 384-well plate containing 0.5 μL of test compound in DMSO. Tritiated dofetilide, made up in assay buffer containing 0.2% pluronic, was then added (20 μL/well, 5 nM final radioligand concentration), and the assay plates were sealed and centrifuged at 1000 rpm for 1 min The plates were left to equilibrate and to adapt in the dark for 4 h before reading on a ViewLux luminescence imager. Single-shot data were generated from a single concentration of 12.5 μM. pIC_{50} data were generated from an 11-point inhibition curve (top assay concentration of 60 μM and a 1:3 step dilution), a 4- parameter curvefit being applied.

In Vitro Inhibition of CYP450 System. Test compounds were incubated using a concentration range from 0.1 to 100 μM with human CYP expressed in *E. coli* or Gentest microsomes. The compounds were preincubated at 37 °C for 5 or 10 min with the CYP enzyme isoforms, in the presence of 50 mM phosphate buffer (pH 7.4) and fluorescent probes for each CYP tested. The incubations were started by addition of a cofactor solution containing NADP, glucose 6-phosphate and glucose 6-phosphate dehydrogenase. The plates were incubated at 37 °C for 10 min reading the fluorescence signal on a plate reader throughout. The fluorescence data were then used to calculate the IC_{50} values for each compound and each CYP.

CNS Penetration. Compounds were dosed intravenously at a nominal dose level of 1 mL kg^{-1} to male CD Sprague-Dawley rats. The test compounds were formulated as solutions in DMSO−PEG 200−water (5:45:50). Blood samples were taken under terminal anesthesia with isoflurane at 5 min postdose, and the brains were also removed for assessment of brain penetration. Blood samples were taken directly into heparinised tubes and prepared for analysis using protein precipitation, and brain samples were prepared using extraction of drug from brain by homogenization and subsequent protein precipitation. The concentration of parent drug in blood and brain extracts was determined by quantitative LCMS/MS analysis using compound-specific mass transitions.

Pharmacokinetics. Pharmacokinetics were determined in male CD Sprague-Dawley rats and male Beagle dogs following single oral and single intravenous administration. In the rat, doses of 1 and 3 mg kg^{-1} were used for iv and po, respectively. In the dog, doses of 1 and 2 mg kg^{-1} were used for iv and po, respectively. For all doses the compounds were formulated as solutions in DMSO−PEG 200−water (5:45:50). Profiles were obtained by taking serial and terminal blood samples over a range of time points up to 7, 12, or 24 h postdose. Blood samples were taken directly into heparinized tubes, prepared by protein precipitation, and subjected to quantitative analysis by LCMS/MS using compound-specific mass transitions. Drug concentration−time profiles were generated and noncompartmental PK analysis used to generate estimates of half-life, clearance, volume of distribution, and oral bioavailability.

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Supporting Information Available: NMR data for **10–13**, **15**, **18**, **19a−n**, **20d−i**, **21d−i**, **22d−h**, **23d−i**, **31**, **32**, **34**, **35**, **36c−i**, **37a−j**, **38c−i**, LCMS retention times and purity of target compounds, and LCMS traces for **19d**, **22i**, and **37d**. This material is available free of charge via the Internet at http://pubs.acs.org.

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