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Synthesis and evaluation of a new series of 1′-cyclobutyl-6-(4-piperidyloxy) spiro[benzopyran-2,4′-piperidine] derivatives as high affinity and selective histamine-3 receptor (H_3R) antagonists

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ABSTRACT

A novel class of 1′-cyclobutyl-6-(4-piperidyloxy)spiro[benzopyran-2,4′-piperidine] derivatives with low nanomolar affinity for the human and rat histamine-3 receptors (H_3Rs) are described. The spirobenzopyran piperidine ether analogs demonstrated excellent H_3R affinity and selectivity against histamine receptor subtypes (H_1R, H_2R, and H_4R), were stable in liver microsomes, and had selectivity against CYP P450 enzymes. Compounds **10**, **13**, **15**, and **16** demonstrated high H_3R affinity, in vitro liver microsomal stability, selectivity against CYP isoforms, moreover, these ether analogs exhibited acceptable iv pharmacokinetic (PK) properties but had poor oral exposure in rat.

Histamine-3 receptors (H_3Rs) are located primarily in the brain presynaptically, where they negatively regulate the synthesis and release of histamine. In addition, H_3Rs are also located on nonhistaminergic neurons, acting as heteroreceptors to regulate the releases of acetylcholine, dopamine, serotonin, and norepinephrine. Regulation of these multiple neurotransmitters is believed to be involved in attention, sleep, and cognition.[1] Since the cloning of the histamine-3 receptor cDNA in 1999,[1j] the histamine-3 receptor has gained the interest of several pharmaceutical/biotechnology companies as a potential drug target for the treatment of various disorders such as, attention-deficit hyperactivity disorder, Alzheimer's disease and schizophrenia.[1] The preliminary structure–activity relationships (SAR) and characterization of our clinical candidate **1** (Fig. 1, CEP-26401, irdabisant) were recently revealed[2] as well as several clinical compounds from other companies that are under active clinical investigation.[1g,h] Recently, a series of modifications to **1** were disclosed, exploring 4,5-dihydropyridazinone substitutions, 4,5-fused ring pyridazinones, central and linker structure–activity relationships, and constrained amine series.[3] In this Letter we present the synthesis and SAR for a series of constrained 6-(4-piperidyloxy)spiro[benzopyran-2,4′-piperidine] and the identification of a set of compounds that advanced into preliminary PK evaluation.

The H_3R target **10** (1′-cyclobutyl-6-[(1-methylsulfonyl-4-piperidyl)oxy]spiro[2*H*-1-benzopyran-2,4′-piperidine]) was synthesized in seven steps as outlined in Scheme 1. The synthesis of the key intermediate **7** commenced from commercially available 2,5-dihydroxyacetophenone **3** and *tert*-butyl 4-oxopiperidine-1-carboxylate **4**. Spiro-condensation of **3** and **4** in the presence of catalytic pyrrolidine led to the spirobenzopyran piperidine **5** in excellent yield and purity. Reduction of the spirobenzopyran ketone **5** with $NaBH_4$ followed by facile reductive deoxygenation and N-Boc deprotection with TFA and Et_3SiH[4b] gave spiro[2*H*-1benzopyran-2,4′-piperidine]-6-ol **6** in 72% yield. Reductive amination of **6** with cyclobutanone and $NaCNBH_3$ proceeded smoothly to produce the key intermediate **7** in moderate yield. Mitsunobu reaction of **7** with *tert*-butyl 4-hydroxypiperidine-1-carboxylate in the presence of triphenylphosphine and diethyl azodicarboxylate produced the desired ether derivative **8**. Subsequent N-Boc deprotection effected smoothly with TFA afforded **9** in excellent yield, which in turn was reacted with methanesulfonyl chloride and diisopropylamine to deliver 1′-cyclobutyl-6-[(4-methylsulfonyl-1-piperidyl)oxy]spiro[2*H*-1-benzopyran-2,4′-piperidine] **10** in 80% yield. Similarly, other alkyl sulfonamides (**11**–**13**) and alkyl amides (**14**–**16**) were synthesized successfully from piperidine ether **9** using the above reaction conditions in excellent yields.

The 1′-cyclobutyl-6-(4-piperidyloxy)spiro[2*H*-1-benzopyran-2,4′-piperidine] H_3R target compounds (**7**–**16**) were tested using in vitro binding assays by displacement of [^{3}H]NAMH in membranes isolated from CHO cells transfected with cloned human H_3 or rat H_3 receptors (Table 1).[2,3] While investigating the SAR of the spirobenzopyran-piperidine series, analogs with 6-(4-piperidyloxy)spiro[benzopyran-2,4′-piperidine] derivatives demonstrating excellent H_3R affinity

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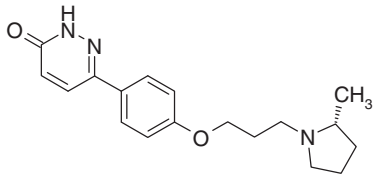
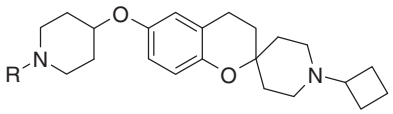


1 CEP-26401 (Irdabisant)



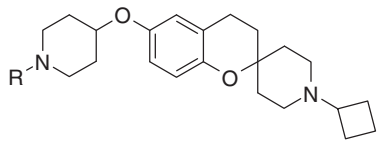
2 1'-Cyclobutyl-6-(4-piperidyloxy)spiro[benzopyran-2,4'-piperidine] series

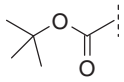
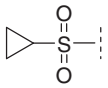
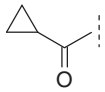
Figure 1. Structures of irdabisant (CEP-26401) and 1′-cyclobutyl-6-(4-piperidyloxy)spiro[benzopyran-2,4′-piperidine] series.

were identified. While exploring the SAR of **2**, the cyclobutyl group on the piperidine ring was fixed for comparison based on earlier SAR optimization studies.[5] The early SAR exploration was focused at the R position of the 1′-cyclobutyl-6-(4-piperidyloxy)spiro[benzopyran-2,4′-piperidine] to establish the discovery flow properties. In the discovery flow, compounds meeting binding criteria (hH_3R K_i <15 nM; rH_3R K_i <50 nM) were screened for selectivity against hH_1, hH_2, and hH_4 receptor subtypes. Subsequently, compounds were tested for aqueous solubility (pH_2 and $pH_{7.4}$), in vitro microsomal stability (rat, mouse, dog, and human), and inhibition of cytochrome P450 isoforms (CYP 1A2, 2C9, 2C19, 2D6, and 3A4). Selected compounds were further screened for in vivo rat PK properties including iv intrinsic pharmacokinetic parameters ($t_{½}$, V_d, and CL), oral bioavailability, and brain penetration. Quality molecules at this stage were progressed into in vivo efficacy models.

The simple spirobenzopyran piperidine phenol **7** had very weak affinity (50–60% inhibition of [^{3}H]NAMH binding at 10 μM) for both H_3R receptors (Table 1). Addition of a boc-piperidine ether **8** and NH piperidine **9** showed 50-fold and >500-fold improvement in affinity. Even though compound **9** exhibited excellent affinity for the human and rat H_3 receptors, it was known diamines had a number of liabilities such as, long half-lives, high tissues distribution and long brain residence times.[6] Therefore, the initial focus was to synthesize several simple *N*-sulfonamide (**10**–**13**) and *N*-amide (**14**–**16**) analogs of **9**. Simple alkyl sulfonamide analogs **10**–**13** exhibited excellent affinity for the human and rat H_3Rs. Simple methyl sulfonamide **10** exhibited high affinity for the human (K_i <7 nM) and rat (K_i <17 nM) receptors and similarly ethyl **11**, isopropyl **12**, and cyclopropyl **13** sulfonamides demonstrated equally high affinity. The simple amide analogs such as acetamide **14**, isobutylamide **15**, cyclopropylamide **16** also demonstrated excellent affinity for both H_3Rs (h K_i <6 nM; r K_i <16 nM). These results suggest that significant bulk was tolerated at the piperidine nitrogen. Based on the initial binding SAR data, sulfonamides (**10**–**13**) and amides (**14**–**16**) H_3R targets were selected for further evaluation of selectivity, metabolic stability, cytochrome P450 inhibition, and rat PK parameters. Compound **10** exhibited excellent in vitro metabolic stability across the species in liver microsomes ($t_{½}$ mouse, rat, dog, and human >40 min and had IC_{50} values of >30 μM for cytochrome P450 isoforms, 1A2, 2C9, 2C19, 2D6 (18 μM), and 3A4. Furthermore, compound **10** had excellent selectivity against hH_1, hH_2, and hH_4 receptor subtypes (<20% inhibition at 10 μM). In the rat, compound **10** exhibited acceptable iv pharmacokinetic parameters ($t_{½}$ = 0.6 h, V_d = 0.5 L/kg, CL = 9 mL/min/kg), but had poor oral exposure, (Table 2). Cyclopropyl sulfonamide **13** also exhibited very good metabolic stability ($t_{½}$ >40 min) and had IC_{50} values of >30 μM for cytochrome P450 enzymes. Moreover, compound **13** demonstrated favorable iv PK properties

Table 1
1′-Cyclobutyl-6-(4-piperidyloxy)spiro[benzopyran-2,4′-piperidne] derivatives H_3R binding data



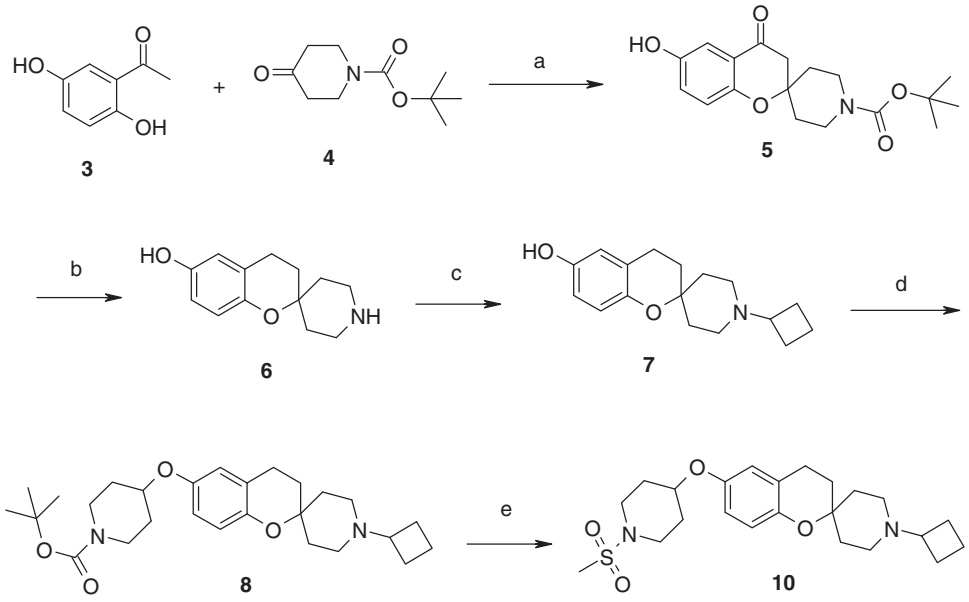
Compd	R	hH_3 (K_i nM)[a]	rH_3 (K_i nM)[a]
1	—	2	7
7	—	>3000	>3000
8		47	40
9	H	3	6
10		7	17
11		7	24
12		7	27
13		9	18
14		4	10
15		2	6
16		6	16

[a] K_i values are an average of at least two determinations. The assay-to-assay variation was typically within 2.5-fold.

Table 2
Rat pharmacokinetic profiles of **10**, **13**, **15**, and **16**[a]

		10[c]	**13**[c]	**15**[c]	**16**[c]
iv	$t_{½}$ (h)	0.6 ± 0	1.8 ± 0.2	1.1 ± 0.4	1.6 ± 0.4
	V_d (L/kg)	0.5 ± 0.1	1.6 ± 0.2	1.9 ± 0.9	2.7 ± 0.5
	CL (mL/min/kg)	9 ± 1	10 ± 1	18 ± 1	20 ± 2
p.o.	AUC(ng h/mL)$_{?\infty}$	392 ± 111	435 ± 69	410 ± 1	359 ± 27
	C_{max} (ng/mL)	83 ± 23	76 ± 14	80 ± 4	71 ± 12
	F (%)	4 ± 1	5 ± 0	9 ± 0	8 ± 1
	B/P[b]	1.5 ± 0.02	2.0 ± 0.04	2.3 ± 0.07	2.3 ± 0.1

[a] Administration at 1 mg/kg iv and 5 mg/kg p.o.
[b] B/P = brain to plasma ratio 1 h post IP dosing at 10 mg/kg, IP formulation (0.5% methylcellulose, 0.2% tween 80).
[c] iv formulation (3% DMSO, 30% solutol, 67% phosphate buffered saline); oral formulation (50% tween 80, 40% propylene carbonate and 10% propylene glycol).



Scheme 1. Reagents and conditions: (a) pyrrolidine, MeOH, reflux, 23 h, 82%; (b) (i) $NaBH_4$, EtOH, rt, 2 h, 90%, (ii) TFA, Et_3SiH, 5 °C to rt, 14 h, 72%; (c) cyclobutanone, $NaCNBH_3$, DMF–MeOH, rt, 5 h, 50%; (d) *tert*-butyl 4-hydroxypiperidine-1-carboxylate, Ph_3P, DEAD, THF, rt, 72%; (e) (i) TFA, CH_2Cl_2, rt, 2 h, 85% → **9**, (ii) H_3CSO_2Cl, DIEA, CH_2Cl_2, 0 °C to rt, 2 h, 80%.

($t_{½}$ = 1.8 h, V_d = 1.6 L/kg, CL = 10 mL/min/kg) but exhibited poor oral PK in rat. The amide H_3R targets (**14**–**16**) exhibited excellent liver microsomal stability ($t_{½}$ >40 min) and also had very good selectivity against cytochrome P450 enzymes (IC_{50} >30 μM) demonstrated acceptable iv PK properties but displayed poor oral exposure in rat, (Table 2). Furthermore, compounds **10**, **13**, **15**, and **16** exhibited good aqueous solubility ($PH_{7.4}$ >0.1 to 0.2 mg/mL).

In summary, a new series of 1′-cyclobutyl-6-(4-piperidyloxy)spiro[benzopyran-2,4′-piperidine] derivatives was identified as high affinity H_3R ligands. Simple alkyl sulfonamide and alkyl amide analogs of 1′-cyclobutyl-6-(4-piperidyloxy)spiro[benzopyran-2,4′-piperidine] exhibited high H_3R target affinity, receptor subtype (hH_1, hH_2, and hH_4) selectivity, metabolic stability, CYP isoforms selectivity, aqueous solubility, and favorable iv pharmacokinetic properties but demonstrated poor oral exposure in rat. Further optimization of spirobenzopyran piperidine ether series to improve the oral bioavailability and lead to a nomination candidate will be reported in due course.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2012.01.139.

References and notes

1. For reviews see (a) Berlin, M.; Boyce, C. W.; De Lera Ruiz, M. *J. Med. Chem.* **2011**, *54*, 26; (b) Brown, R. E.; Stevens, D. R.; Haas, H. L. *Prog. Neurobiol.* **2001**, *63*, 637; (c) Leurs, R.; Bakker, R. A.; Timmerman, H.; de Esch, I. *J. Nat. Rev. Drug Discov.* **2005**, *4*, 107; (d) Wijtmans, M.; Leurs, R.; de Esch, I. *Expert Opin. Investig Drugs* **2007**, *16*, 967; (e) Esbenshade, T. A.; Fox, G. B.; Cowart, M. D. *Mol. Interv.* **2006**, *6*, 77; (f) Esbenshade, T. A.; Browman, K. E.; Bitner, R. S.; Strakhova, M.; Cowart, M. D.; Brioni, J. D. *Br. J. Pharmacol.* **2008**, *154*, 1166; (g) Hudkins, R. L.; Raddatz, R. *Ann. Rep. Med. Chem.* **2007**, *42*, 49; (h) Raddatz, R.; Tao, M.; Hudkins, R. L. *Curr. Top. Med. Chem.* **2010**, *10*, 153; (i) Brioni, J. D.; Esbenshade, T. A.; Garrison, T. R.; Bitner, S. R.; Cowart, M. D. *J. Pharmacol. Exp. Ther.* **2011**, *336*, 38; (j) Lovenberg, T. W.; Roland, B. L.; Wilson, S. J.; Jiang, X.; Pyati, J.; Huvar, A.; Jackson, M. R.; Erlander, M. G. *Mol. Pharmacol.* **1999**, *55*, 1101.
2. (a) Bacon, E. R.; Bailey, T. R.; Becknell, N. C.; Chatterjee, S.; Dunn, D.; Hostetler, G. A.; Hudkins, R. L.; Josef, K. A.; Knutsen, L.; Tao, M.; Zulli, A. L. US2010273779, **2010**.; (b) Hudkins, R. L.; Raddatz, R.; Tao, M.; Mathiasen, J. R.; Aimone, L. D.; Becknell, N. C.; Prouty, C. P.; Knutsen, L.; Yazdanian, M.; Moachon, G.; Ator, M. A.; Mallamo, J. P.; Marino, M. J.; Bacon, E. R.; Williams, M. *J. Med. Chem.* **2011**, *54*, 4781; (h) Raddatz, R.; Hudkins, R. L.; Mathiasen, J. R.; Gruner, J. A.; Flood, D. G.; Aimone, L. D.; Le, S.; Schaffhauser, H.; Gasior, M.; Bozyczko-Coyne, D.; Marino, M. J.; Ator, M. A.; Bacon, E. R.; Mallamo, J. P.; Williams, M. *J. Pharmacol. Exp. Ther.* **2012**, *340*, 124.
3. (a) Hudkins, R. L.; Aimone, L. D.; Bailey, T. R.; Bendesky, R. J.; Dandu, R.; Dunn, D. D.; Gruner, J. A.; Josef, K. A.; Lin, Y.; Lyons, J.; Marcy, V. R.; Mathiasen, J. R.; Sundar, B. G.; Tao, M.; Zulli, A. L.; Raddatz, R.; Bacon, E. R. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 5493; (b) Sundar, B. G.; Bailey, T.; Bacon, E.; Aimone, L.; Huang, Z.; Lyons, J.; Raddatz, R.; Hudkins, R. L. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 5543; (c) Tao, M.; Raddatz, R.; Aimone, L. D.; Hudkins, R. L. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 6126; (d) Dandu, R.; Gruner, J. A.; Mathiasen, J. R.; Aimone, L. D.; Hostetler, G. A.; Benfield, C.; Bendesky, R. J.; Marcy, V. R.; Raddatz, R.; Hudkins, R. L. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 6362; Becknell, N. C.; Lyons, J. A.; Aimone, L. D.; Gruner, J. A.; Mathiasen, J. R.; Rita Raddatz, R.; Hudkins, R. L. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 7076; (f) Hudkins, R. L.; Aimone, L. D.; Dandu, R.; Dunn, D.; Gruner, J. A.; Huang, Z.; Josef, K. A.; Lyons, J.; Mathiasen, J. R.; Tao, M.; Zulli, A. L.; Raddatz, R. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 194; (g) Becknell, N. C.; Dandu, R.; Lyons, J. A.; Aimone, L. D.; Raddatz, R.; Hudkins, R. L. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 186.
4. (a) Yang, L.; Morriello, G.; Prendergast, K.; Cheng, K.; Jacks, T.; Chan, W. W.; Schleim, K. D.; Smith, R. G.; Patchett, A. A. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 107; (b) Jean-philippe, L.; Chun Sing, L.; Oscar Miguel, M. WO2010094120, **2010**.
5. These studies will be published elsewhere.
6. (a) Dvorak, C. A.; Apodaca, R.; Barbier, A. J.; Berridge, C. W.; Wilson, S. J.; Boggs, J. D.; Xiao, W.; Lovenberg, T. W.; Carruthers, N. J. *J. Med. Chem.* **2005**, *48*, 2229; (b) Bonaventure, P.; Letavic, M.; Dugovic, C.; Wilson, S.; Aluisio, L.; Pudiak, C.; Lord, B.; Mazur, C.; Kamme, F.; Nishino, S.; Carruthers, N.; Lovenberg, T. *Biochem. Pharmacol.* **2007**, *73*, 1084; (c) Dvorak, C. A.; Apodaca, R.; Xiao, W.; Jablonowski, J. A.; Bonaventure, P.; Dugovic, C.; Shelton, J.; Lord, B.; Miller, K.; Dvorak, L. K.; Lovenberg, T. W.; Carruthers, N. J. *Euro. J. Med. Chem.* **2009**, *44*, 4098.