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Synthesis of constrained benzocinnolinone analogues of CEP-26401 (irdabisant) as potent, selective histamine H_3 receptor inverse agonists

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ABSTRACT

A novel class of benzocinnolinones analogs of irdabisant were designed and synthesized as histamine H3R antagonists/inverse agonists. Modifications to the pyridazinone portion of the core and linker led to the identification of molecules with excellent target potency and selectivity with improved rat pharmacokinetic properties and reduced potential hERG liabilities.

Histamine has been established as a modulator of neuronal activity with at least four subtypes of the G-protein coupled receptors (H_1R–H_4R) reported.[1] Of these, the H_3 receptor is predominately expressed in the CNS, while H_1, H_2, and H_4 receptors are more widely distributed in both central and peripheral tissues. The H_1 and H_2 receptors have been successfully targeted with pharmacological agents to treat allergic response and control gastric acid secretion, respectively, while the H_4 receptor is predominately expressed in inflammatory cells, suggesting its critical role in the regulation of inflammatory and immune responses.[2] In the CNS, H_3 receptors are involved in presynaptic regulation of the release of neurotransmitters such as acetylcholine (ACh), dopamine (DA), norepinephrine (NE), and serotonin (5-HT) making this receptor an attractive target for indications such as attention-deficit hyperactivity disorder (ADHD), Alzheimer's disease (AD), mild cognitive impairment and schizophrenia.[1c–j] The H_3 receptor demonstrates a high degree of constitutive or spontaneous activity (receptor is active in the absence of agonist stimulation) in vitro and in vivo, therefore, ligands to the receptor can display agonist, neutral antagonist or inverse agonist effects. Initial work in the field focused on imidazole analogs of the natural ligand histamine, but has evolved into a search for amine-based compounds with more drug-like properties. These scaffolds usually contain a tertiary-amine linked to a lipophilic aryl group as a pharmacophore that can be broadly modified to give a diversity of structural classes yet still possess a binding affinity to the H_3 receptor. Several H_3R candidates have advanced into clinical evaluation (Fig. 1).[1h–j]

We have identified a novel class of pyridazin-3-one H_3R antagonists/inverse agonists,[3] and recently disclosed the structure–activity relationships (SAR) and characterization of clinical compound **1** (CEP-26401, irdabisant).[4] As part of our H_3 discovery project studying the SAR around **1**, we actively pursued a variety of structural core modifications.[5] One strategy, which is the focus of this paper, was to develop a synthesis and evaluate the effect of constraining the left-hand (western region) A–B rings by linking the pyridazinone C5 and A-ring phenyl of irdabisant to form constrained 2*H*-benzo[*h*]cinnolin-3-ones, or the pyridazinone C6 to form 3*H*-benzo[*f*]cinnolin-2-ones.

Scheme 1 outlines the general procedure for the synthesis of N2-substituted benzocinnolinones **3–6**. The aldol condensation product of 6-methoxy-1-tetralone with glyoxylic acid monohydrate in refluxing acetic acid was isolated in 30% yield. This was subsequently cyclized with the N-substituted hydrazines in isopropanol to give the 5,6-dihydro-2*H*-benzo[*h*]cinnolin-3-one intermediates **2** in 30–50% yield.[6] Demethylation with boron tribromide in cold dichloromethane, followed by alkylation with 1-bromo-3-chloro-propane, and then substitution with (*R*)-2-methylpyrrolidine (**3–5**) or piperidine (**6**) gave the targets **3–6** in 8% to 10% overall yield for the three steps. The N2-H analogs of both the 5,6-dihydrocinnolinone and the 4,4a,5,6-tetrahydrocinnolinone, as illustrated in Scheme 2, were synthesized via the chloropropyl ether intermediate **7** obtained from the alkylation of 6-hydroxy-1-tetralone with1-bromo-3-chloro-propane in a 73% yield. Cyclocondensation providing the dihydro target **8** was achieved in a similar manner as described above, except that the intermediate aldol product was not isolated. Condensation with glyoxylic acid in refluxing acetic acid proceeded for 2 h, was then cooled, and

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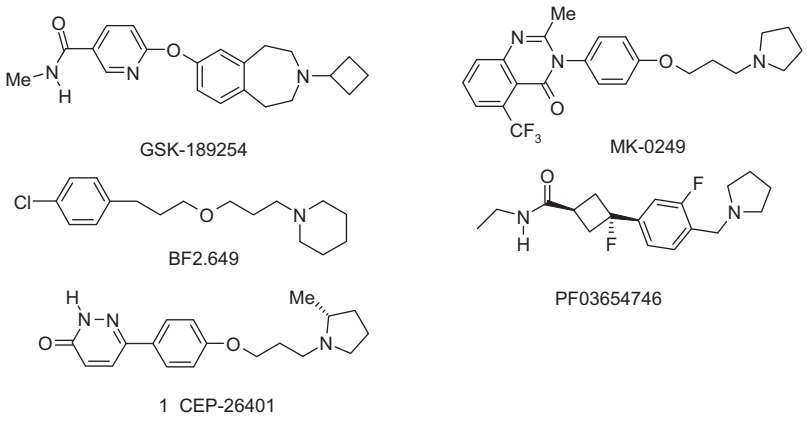
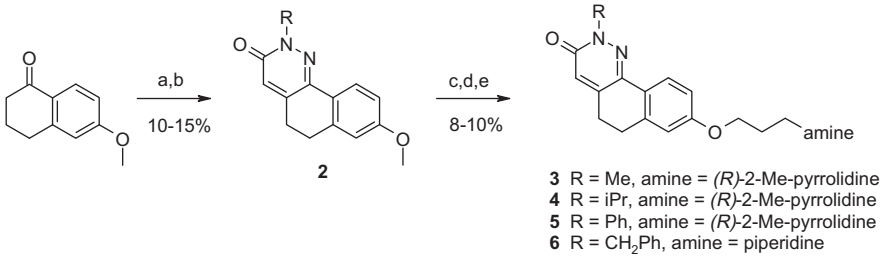
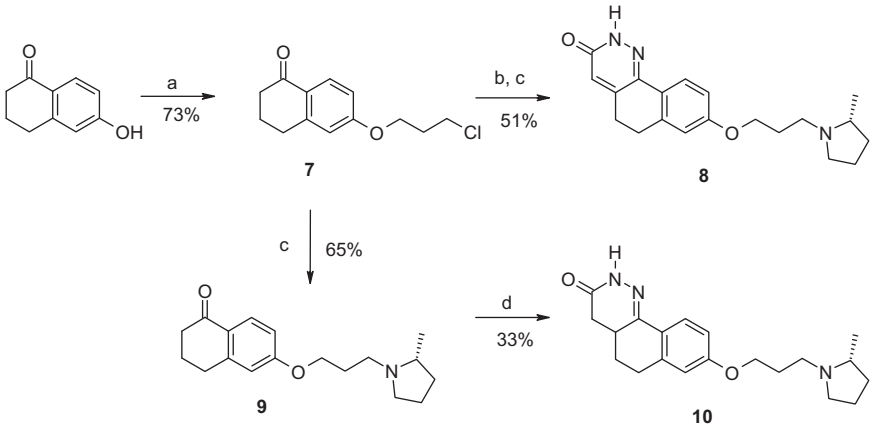


Figure 1. Structures of clinical H_3R clinical antagonists.

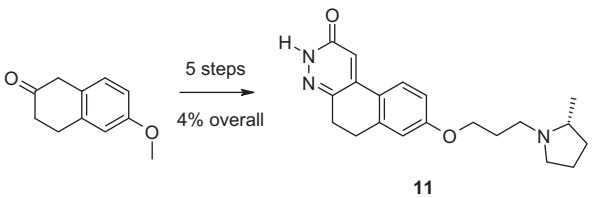


Scheme 1. Reagents and conditions: (a) glyoxylic acid monohydrate, AcOH, 120 °C; (b) H_2NNHR, *i*PrOH, 110 °C; (c) BBr_3, CH_2Cl_2, 0–25 °C; (d) $Br(CH_2)_3Cl$, K_2CO_3, ACN, 100 °C; (e) (*R*)-2-methylpyrrolidine or piperidine, K_2CO_3, NaI, ACN, 100 °C.

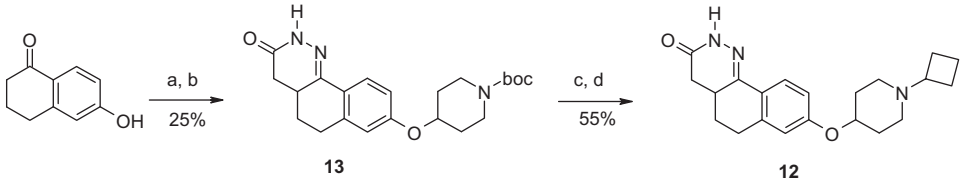


Scheme 2. Reagents and conditions: (a) $Br(CH_2)_3Cl$, K_2CO_3, ACN, 100 °C; (b) glyoxylic acid monohydrate, AcOH, 120 °C; hydrazine monohydrate, 90 °C; (c) (*R*)-2-methylpyrrolidine, K_2CO_3, NaI, ACN, 100 °C; (d) glyoxylic acid monohydrate, AcOH, 120 °C; zinc dust, 100 °C; hydrazine monohydrate, 90 °C.

hydrazine monohydrate was added directly to the reaction to give the benzocinnolinone in 51% yield. Subsequent substitution with (*R*)-2-methylpyrrolidine gave target **8**. In the case of the tetrahydro analog **10**, the chloropropylether **7** was not stable to a later reduction step and was therefore first converted to the (*R*)-2-methylpyrrolidyl-propoxy-tetralone **9** in 65% yield. This intermediate was transformed into target **10** using a single reaction vessel in which a stepwise addition of glyoxylic acid, followed by two equivalents of zinc dust,[7] and then hydrazine monohydrate, all in refluxing acetic acid, gave a 33% yield of **10**. The isomeric 5,6-dihydro-3*H*-benzo[*f*]cinnolin-2-one analog **11** was synthesized with an overall yield of 4% using similar procedures described in Schemes 1 and 2, but starting with the 6-methoxy-2-tetralone (Scheme 3). The cyclobutyl-piperidinyl target **12** was obtained by first coupling 6-hydroxy-1-tetralone and N-boc-4-hydroxypiperidine using DEAD and triphenylphosphine in cold tetrahydrofuran (70% yield, Scheme 4). Conversion to the tetrahydro-benzocinnolinone using

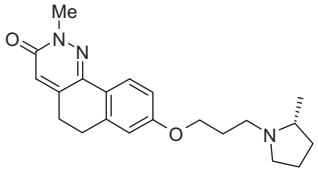
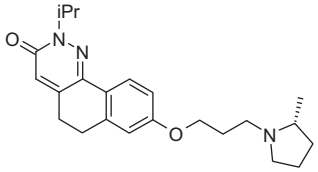
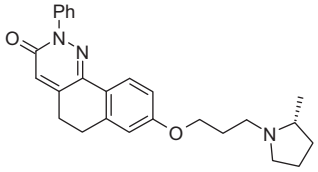
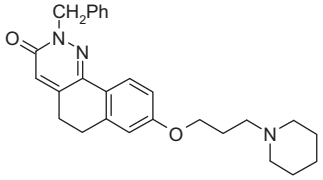
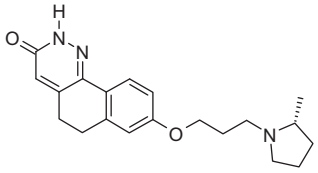
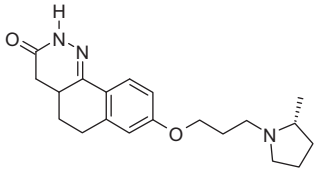
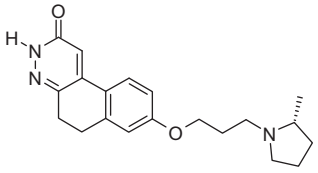
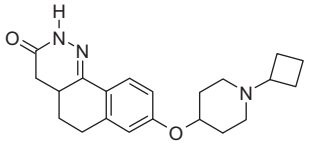


Scheme 3. Reagents and conditions: glyoxylic acid monohydrate, AcOH, 120 °C; hydrazine monohydrate, 90 °C; BBr_3, CH_2Cl_2, 0–25 °C; $Br(CH_2)_3Cl$, K_2CO_3, ACN, 100 °C; (*R*)-2-methylpyrrolidine, K_2CO_3, NaI, ACN, 100 °C.



Scheme 4. Reagents and conditions: (a) DEAD, PPh_3, N-Boc-4-OH-piperadine, THF, 0–25 °C; (b) glyoxylic acid monohydrate, AcOH, 120 °C; zinc dust, 100 °C; hydrazine monohydrate, 90 °C; (c) TFA, 25 °C; (d) cyclobutanone, $Na(CN)BH_3$, MeOH, DMF, AcOH, 60 °C.

Table 1
Pyridazin-3-one central ring and linker H_3R binding data

Entry	Structure	hH_3 (K_i nM)	rH_3 (K_i nM)	*c*log*P*
3		3.6 ± 0.3	8.3[a]	3.3
4		4.2 ± 0.7	17 ± 6	4.1
5		16 ± 4	23 ± 2	4.8
6		105 ± 44	140 ± 25	5.2
8		4.3 ± 1.1	14 ± 3	2.7
10		15 ± 2	53 ± 10	2.6
11		6.2 ± 0.6	19 ± 4	2.5
±12		17 ± 0.4	26 ± 10	1.9

[a] $n = 2$; for all others, $n = 3$–5.

the one pot reductive cyclocondensation procedure described in Scheme 2 gave intermediate **13** in 35% yield. Partial de-protection of the piperidine moiety occurred under the refluxing acetic acid conditions. Complete removal of the Boc group was achieved in trifluoroacetic acid at ambient temperature for 2 h. The subsequent reductive amination with cyclobutanone and sodium cyanoborohydride in dimethylformamide and methanol gave **12** in 55% yield.

All target compounds 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.[3,4] In the series of **1** and its analogs,[4,5] we investigated substitution around the central phenyl ring and the pyridazinone head group. It was hypothesized that some restricted rotation around these two aryl ring systems may enhance the activity and further improve the selectivity of the series. As previously described, H_3R binding affinity was not significantly affected by substitution of increasing steric bulk at the N2 position.[4] This SAR trend was also reflected in the benzocinnoline series, Table 1. With the *c*log*P*, a measure of the lipophilicity, of the minimally substituted **8** already at 2.7, increasing the size of the N2-R group, even to the methyl analog **3**, resulted in *c*log*P* values over 3.0. These higher *c*log*P* numbers and corresponding higher molecular weights can decrease the ligand efficiency (LE) and the ligand lipophilic efficiency (LLE)[8] as demonstrated in the N2-R set of **8** (N–H; LLE = 5.70), **3** (*N*-methyl; LLE = 5.14), and **4** (*N*-isopropyl; LLE = 4.28). Elevated *c*log*P* values have also been shown to enhance hERG activity, the propensity for high tissue distribution, and the induction of phospholipidosis.[9] Binding affinity was fairly consistent for N–H and lower alkyl substitution, with minimal differences for hH_3R and rH_3R between the N–H (**8**), *N*-methyl (**3**) and *N*-isopropyl (**4**) analogs. Selectivity of these compounds compared to the histamine receptor isoforms hH_1, hH_2, and hH_4 was high with less than 40% inhibition of radioligand binding at 10 μM concentration (>1000-fold selectivity). Binding affinity differences between the N2-phenyl **5** and N2-benzyl **6** analogs were likely due to the change in the amine moiety from (*R*)-2-methylpyrrolidine (**5**) to the piperidine (**6**) as seen previously.[4] The methyl analog **3** was screened for potential hERG liabilities using the astemizole binding assay (MDS Pharma Services) and showed 34% inhibition at 10 μM concentration. Functionally, **3** showed potent antagonist activity ($K_{b,app}$ = 0.4 nM) and displayed full inverse agonist activity in the [^{35}S]GTPγS hH_3R binding assay[3,4] by decreasing basal activity with an EC_{50} = 1.7 nM. Compound **3** had IC_{50} values >30 μM for inhibition of cytochrome P450 enzymes CYP1A2, 2C9, 2C19, 2D6 and 3A4, indicating minimal potential for drug–drug interactions. Unfortunately, when screened in the rat for pharmacokinetic (PK) properties compound **3** showed unacceptable iv intrinsic properties with a short half-life and high clearance ($t_{½}$ = 0.4 h, CL = 36 mL/min/kg, V_d = 1.3 L/kg) and also showed poor oral bioavailability (*F* = 3%; Table 2), consistent with the in vitro metabolic stability in rat liver microsomes ($t_{½}$ = 23 min). Similar to **3**, the N–H analog **8** also displayed poor in vitro metabolic stability and oral bioavailability in rat, and was not suitable for further advancement into in vivo testing. The tetrahydro-benzocinnolinone analog **10** showed slightly weaker binding affinity (hH_3R K_i = 15 nM and rH_3R K_i = 53 nM) compared with **3** or **8** and there was little difference in the iv PK properties of the dihydro and tetrahydro analogs **8** and **10** (Table 2). Encouragingly, there was a modest increase in bioavailability (*F* = 11%) for **10**. At this stage, based on its profile, no attempt was made to isolate the diastereomers of the tetrahydro benzocinnolinone **10**. The regiomeric 5,6-dihydro-3*H*-benzo[*f*]-cinnolin-2-one **11** showed comparable H_3R affinity (hH_3R K_i = 6.2 nM, rH_3R K_i = 19 nM) with the 5,6-dihydro-2*H*-benzo[*h*]- cinnolin-3-one **8**. Compound **11** also demonstrated good selectivity for hH_3R compared to the other hHistamine receptor subtypes (hH_1R, hH_2R, and hH_4R) with less than 45% inhibition of radioligand binding at 10 μM concentration. In an attempt to improve the PK profile and advanced compounds from the benzocinnolinone series into in vivo proof of concept studies, the (*R*)-2-methylpyrrolidyl-propyl moiety was switched to the constrained *N*-cyclobutylpiperidine **12**. Compound **12** had essentially equivalent binding affinity (hH_3R K_i = 17 nM and rH_3R K_i = 26 nM) compared to its tetrahydro counterpart **10**. Further, compound **12** showed an acceptable in vitro metabolic stability across species ($t_{½}$ >40 min) and a clean CYP inhibition selectivity profile (IC_{50} >30 μM). Based on the in vitro data, compound **12**, as a mixture of diastereomers, was screened in the rat for PK properties and showed improved oral exposure and C_{max} values (*F* = 44%; Table 2). However, the iv profile showed a high volume of distribution (V_d = 6.6) and short $t_{½}$ consistent with the high clearance. The high V_d for compound **12** also was reflected in high brain exposure with a 5 mg/kg oral dose producing brain concentrations of 780 nM 6 h post and a high brain to plasma ratio (B/P) of 5.2.[10] Compared to the phenoxypropyl core, the piperidine chemotype had a lower calculated *c*log*P* of 1.9 (*c*log$D_{7.4}$ = 0.7), which was with a key component in our earlier disclosed relationship between *c*log*P* and hERG activity.[4] Compound **12** showed weak hERG activity with an IC_{50} = 18 μM in the patch clamp assay (MDS Pharma Services, PatchExpress).

In summary, the tricyclic benzocinnolinone pyridazinone core was designed and synthesized showing high H_3R binding affinity with excellent selectivity against the H_1R, H_2R and H_4R subtype. Modification to the linker/amine region of the pharmacophore resulted in ±**12**, which showed improved metabolic stability and rat pharmacokinetics following oral administration. Presently, methods were developed to scale up **12** for separation and evaluation of the individual isomers.

Table 2
Pharmacokinetic properties in rat

	3	8	10	±12
iv[a] $t_{½}$ (h)	0.4 ± 0.0	1.4 ± 0.6	0.7 ± 0.1	0.9 ± 0.1
V_d (L/kg)	1.3 ± 0.1	1.2 ± 0.4	6.0 ± 1.0	6.6 ± 1.4
CL (mL/min/kg)	36 ± 2	11 ± 3	93 ± 6	84 ± 17
p.o.[b] AUC (ng h/mL)	80 ± 11	191 ± 30	96 ± 18	468 ± 111
C_{max} (ng/mL)	24 ± 3	34 ± 3	27 ± 5	75 ± 22
F (%)	3 ± 0	2 ± 0	11 ± 2	44 ± 10

[a] 1 mg/mg iv (3% DMSO, 30% solutol, 67% phosphate buffered saline.
[b] 5 mg/mg p.o. (50% Tween 80, 40% propylene carbonate and 10% propylene glycol).

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