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Synthesis and evaluation of a spiro-isobenzofuranone class of histamine H_3 receptor inverse agonists

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ABSTRACT

Spiro-isobenzofuranones **1a** and **1b** were discovered as potent, selective, and brain-penetrable non-imidazole H_3 receptor inverse agonists. Our corporate sample collection was screened to identify **2a** as a lead. Recognizing the right-hand portion of **2a** as an essential pharmacophore, an extensive screen of the left-hand piperidine portion was carried out to yield the potent spiro-derivatives **2t**–**x**. Spiro-isobenzofuranone **2x**, the most potent among the derivatives, was converted to the corresponding amide **1a**, which possessed dramatically improved H_3 activity (IC_{50} = 0.72 nM; more than 20-fold improvement over **2x**). Further elaboration led to the identification of **1b**, a 5-methoxy derivative with an IC_{50} of 0.54 nM. Our studies demonstrated that derivatives **1a** and **1b** to be potent, selective, and brain-penetrable H_3 inverse agonists.

Despite the pharmacological discovery of the H_3 receptor in 1983,[1] it was not until 1999, with its genetic identification,[2] that the H_3 receptor gained widespread attention and redirected the detailed pharmacological characterization of the receptor and drug discovery efforts from academia and pharmaceutical industries.[3] Studies have shown that signaling through the H_3 receptor activates G-proteins that inhibit adenylate cyclase activity and reduces intracellular cAMP levels.[2,4] The H_3 receptors, which are predominantly expressed in the CNS, are localized on the presynaptic membrane as autoreceptors, and negatively regulate the release and synthesis of histamine.[1] The H_3 receptor is also known to modulate the release of other neurotransmitters such as norepinephrine, dopamine, acetylcholine, serotonin, and GABA,[5] and accordingly, it has been suggested that H_3 antagonists/inverse agonists could serve as effective therapeutics for several CNS-related disorders.[6] In animal models, H_3 receptor antagonists/inverse agonists have been shown to enhance wakefulness, improve attentive and cognitive behaviors, and reduce feeding and body weight.[7,8] It has recently been reported that BF2.649 (Chart 1), a potent and selective H_3 receptor inverse agonist, can suppress excessive daytime sleep among narcoleptic patients.[9]

First-generation imidazole-based H_3 antagonists, which possess inhibitory actions on cytochrome P_{450} activity, may cause drug–drug interactions against co-administered drugs by inhibiting hepatic clearance.[10] Because of these liabilities, current efforts have focused on non-imidazole classes of H_3 receptor antagonists/inverse agonists. Since the identification of the H_3 receptor genes, various classes of non-imidazole H_3 receptor antagonists have been developed to target the CNS H_3 receptors.[3,7,11] Among them, BF2.649,[9,12] ABT-834,[13] and GSK189254,[14] as shown in Chart 1, target CNS disorders such as excessive daytime sleepiness, schizophrenia, and cognitive dysfunctions, and have successfully entered clinical trials.

Recently, our corporate chemical collection was screened against the human H_3 receptor, and resulted in the identification of **2a**. Subsequently, the substituted piperidine component (left-hand portion of **2a**) was extensively screened for H_3 affinity, and resulted in the identification of potent spiro-isobenzofuranones **1a** and **1b**. In this report, the discovery and characterization of these potent, selective, and brain-penetrable H_3 inverse agonists are described.

The synthetic route for the derivatives reported herein is illustrated in Schemes 1–4. Carbamoylchloride **4**, which was generated in situ from *N*-methyl-2-(1-piperidinyl)ethanamine (**3**) and triphosgene, was coupled with substituted piperidines and piperazines **5** to afford **2a**–**y** in 20–30% yield (Scheme 1). The substituted piperidines and piperazines **5** employed in the present study were either commercially available or reported in the literature.[15] The low yields for these coupling reactions can be improved by reversing the reaction sequence. For instance, compound **2x** was prepared in 81% yield by the generation of the carbamoylchloride from 3*H*-spiro[2-benzofuran-1,4′-piperidin]-3-one followed by coupling with amine **3**. For the preparation of alcohol **8** (Scheme

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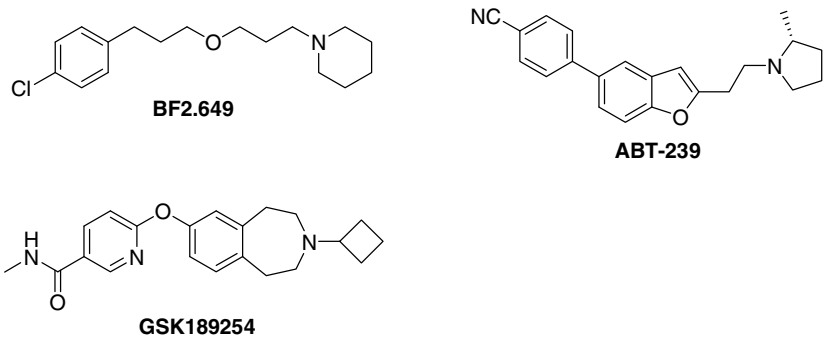
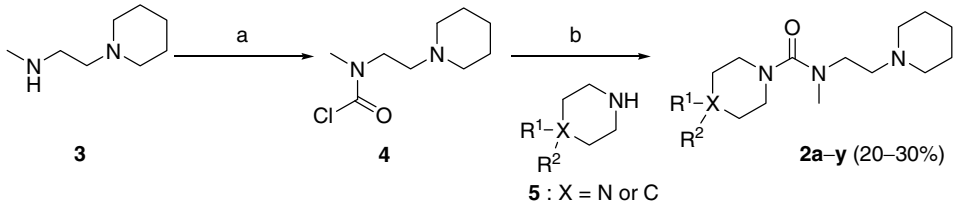
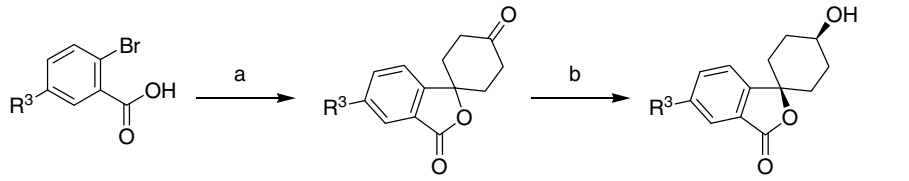


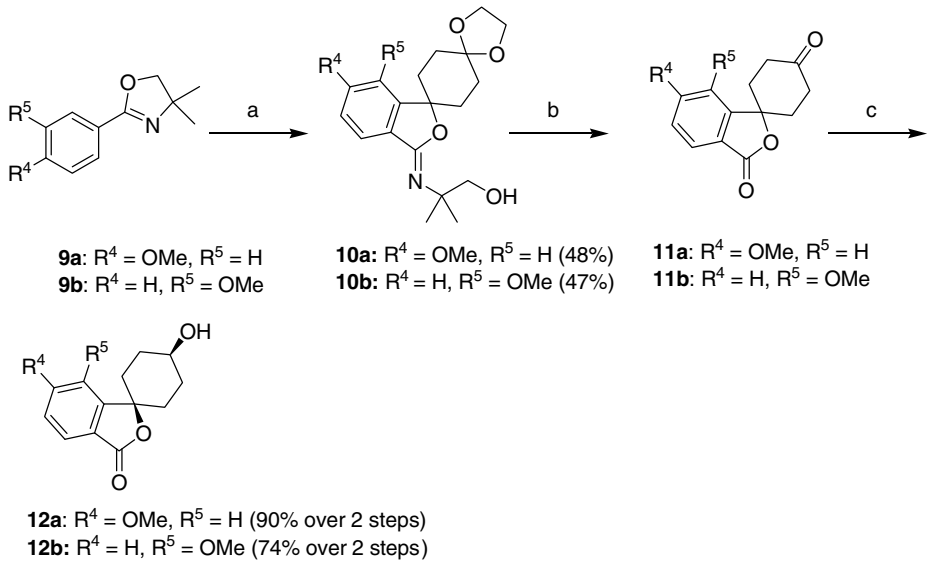
Chart 1. Structures of non-imidazole H_3 antagonists and inverse agonists.



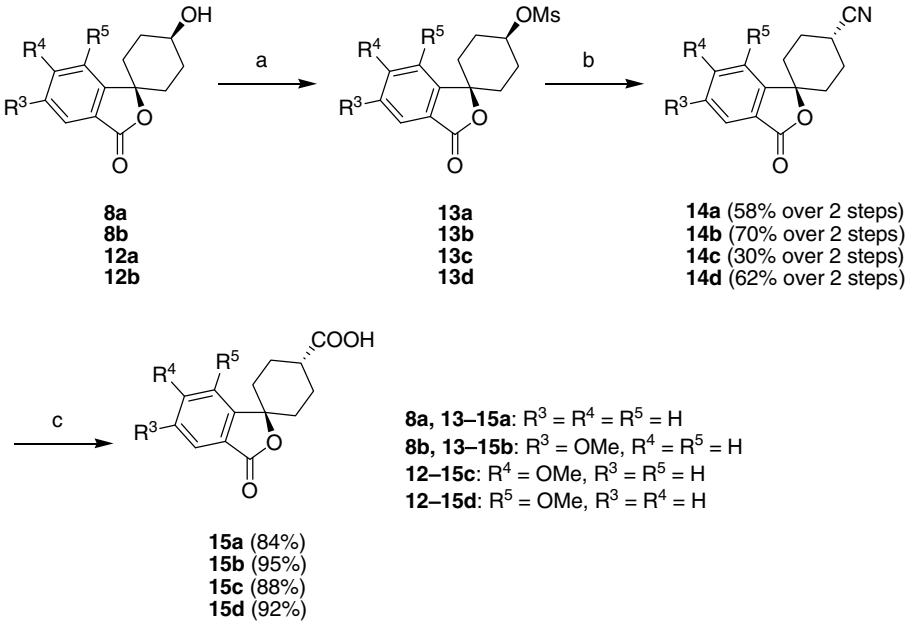
Scheme 1. Reagents and conditions: (a) triphosgene, *N*,*N*-diisopropylethylamine, $CHCl_3$, 0 °C, 3 h; (b) *N*,*N*-diisopropylethylamine, $CHCl_3$, 70 °C, 4 h.



Scheme 2. Reagents and conditions: (a) i—*n*-BuLi, THF, −78 °C, 1 h, then 4,4-ethylenedioxycyclohexanol, rt, 18 h; ii—concd HCl, acetone, H_2O, reflux, 18 h; (b) $NaBH_4$, H_2O/THF (v/v = 1/10), 0 °C, 1 h, diastereomeric ratio > 4:1.



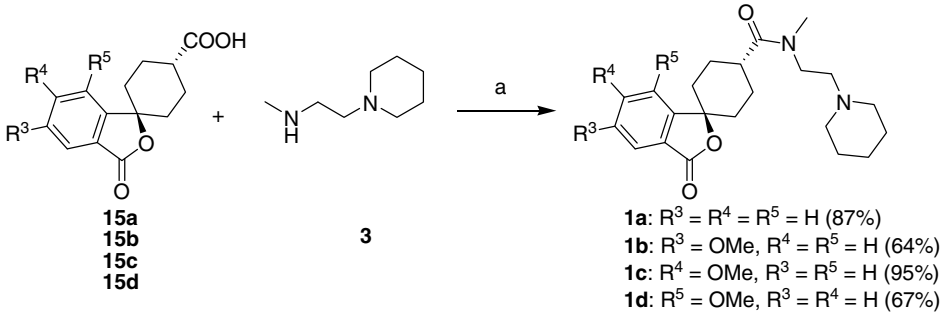
Scheme 3. Reagents and conditions: (a) *n*-BuLi, THF, rt, 2 h then 4,4-ethylenedioxycyclohexanol, rt, 18 h; (b) 2 N H_2SO_4, acetone, 80 °C, 18 h; (c) $NaBH_4$, H_2O/THF (v/v = 1/10), 0 °C, 4 h, diastereomeric ratio > 4:1.



Scheme 4. Reagents and conditions: (a) methanesulfonyl chloride, Et_3N, THF, 0 °C, 30 min; (b) Et_4NCN, DMF, 100 °C, 18 h; (c) 30% H_2SO_4, dioxane, 100 °C, 48 h.

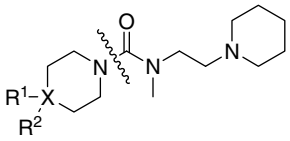
2), 2-bromobenzoic acid **6** was reacted with *n*-butyllithium, then treated with 4,4-ethylenedioxycyclohexanol, followed by hydrolysis of the ketal group under acidic conditions to afford **7** (**7a**, 44%; **7b**, 65%). Ketone **7** was reduced using sodium borohydride to give the corresponding alcohol **8** in good yields. For the preparation of alcohols **12** (Scheme 3), substituted 2-phenyloxazole **9** was reacted with *n*-butyllithium followed by treatment with 4,4-ethylenedioxycyclohexanol to afford **10** (**10a**, 48%; **10b**, 47%). The imidate and ketal groups of **10** were hydrolyzed under acidic conditions to give ketone **11**, which was reduced using sodium borohydride to give the corresponding alcohol **12** in good yields. It is important to note that the reduction of ketones **7** and **11** using sodium borohydride was diastereoselective—the desired *cis*-isomers **8** and **12** were obtained with a diastereomeric ratio of greater than 4:1. The hydroxy group of **8** and **12** was mesylated and displaced by a cyanide ion to give **14**, which was converted to the spiro-isobenzofuranone carboxylic acids **15a**–**d** by subsequent treatment with 30% sulfuric acid in dioxane (Scheme 4). The minor diastereomer in **15** was separated by silica gel chromatography without difficulty. Finally, **15a**–**d** were coupled with amine **3** in the presence of 2-chloro-1,3-dimethylimidazolinium chloride and triethylamine to afford **1a**–**d** (Scheme 5).

The compounds described herein were tested in the [^{35}S]GTPγS binding assay in membranes isolated from cells transfected with cloned human H_3 receptors.[16] All the quinazolinone derivatives reported herein reduced basal GTPγS binding, indicating that they are inverse agonists. Selected compounds were evaluated for hERG K^+ channel inhibitory activity using the [^{35}S]*N*-[(4*R*)-1′-[(2*R*)-6-cyano-1,2,3,4-tetrahydro-2-naphthalenyl]-3,4-dihydro-4-hydroxyspiro[2*H*-1-benzopyran-2,4′-piperidin]-6-yl]methanesulfonamide binding assay to assess QTc prolongation liability.[17] Screening of Merck sample collection led to the identification of lead **2a,** which possessed an IC_{50} value of 110 nM (Table 1). Based on previous SAR studies,[3,7,11] the structure of **2a** can be defined as a basic piperidine pharmacophore with an ethylene linker and a lipophilic left-hand pharmacophore tethered by a urea linkage. Consequently, our studies focused on modifying the lipophilic left-hand component, and by taking advantage of the urea linkage, a variety of 4-substituted piperidine and piperazine analogs were prepared and evaluated (Table 1). Compared to compound **2a**, the 1-naphthyl derivative **2b** was less potent, while replacement of the naphthyl with a phenyl group as in **2c** resulted in marked loss of potency. The tetrahydroquinoline derivative **2d** showed weak activity. Introduction of spacers between the piperidine and phenyl rings as in **2e**–**g** proved to be ineffective. Inclusion of another 4-substitutent, in addition to the phenyl group, showed mixed results—the 4,4-diphenyl and 4-cyano-4-phenyl derivatives **2i** and **2h** displayed similar activities as that of lead **2a**, whereas the 4-hydroxy-4-phenyl derivative **2j** exhibited a complete loss of potency. The 4-phenyl piperazine derivative **2k** showed weak activity



Scheme 5. Reagents and conditions: (a) 2-chloro-1,3-dimethylimidazolinium chloride, Et_3N, $CHCl_3$, rt, 1 h.

Table 1
Human H_3 activities of the urea derivatives **2a**–**y**[a]



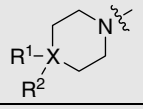
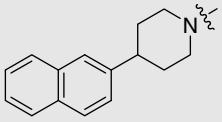
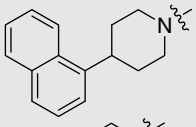
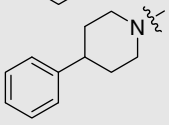
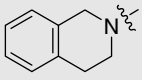
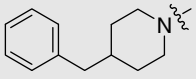
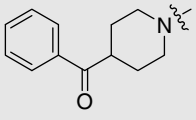
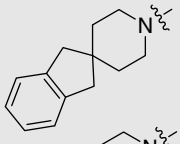
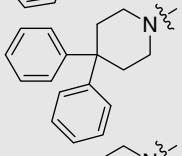
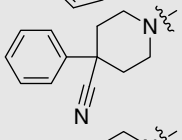
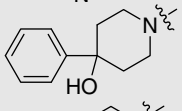
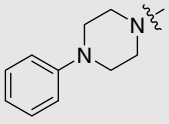
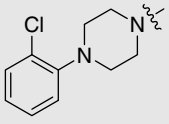
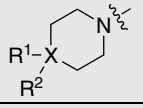
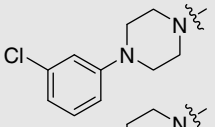
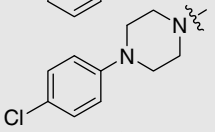
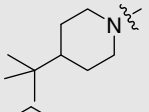
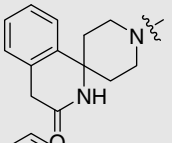
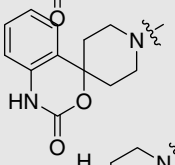
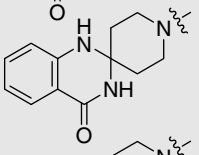
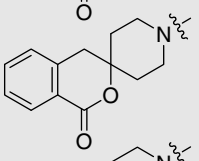
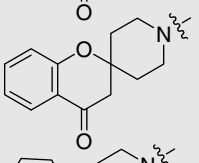
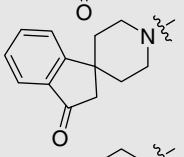
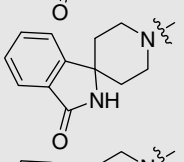
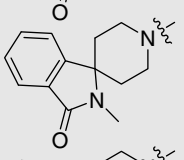
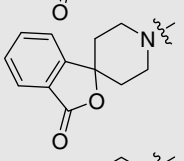
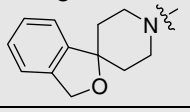
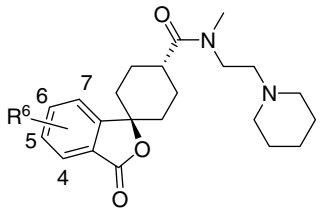
Compound		Human H_3[b] (IC_{50}, nM)
2a		110 ± 10
2b		343 ± 97
2c		893 ± 47
2d		680 ± 61
2e		393 ± 26
2f		237 ± 27
2g		530 ± 65
2h		98 ± 19
2i		267 ± 81
2j		>1000
2k		506 ± 147
2l		>1000

Table 1 (*continued*)

Compound		Human H_3[b] (IC_{50}, nM)
2m		297 ± 55
2n		160 ± 31
2o		>1000
2p		783 ± 213
2q		>1000
2r		560 ± 195
2s		380 ± 95
2t		68 ± 27
2u		43 ± 4
2v		53 ± 6
2w		45 ± 12
2x		21 ± 1
2y		387 ± 34

[a] The values represent the mean ± SE for $n \geqslant 3$.
[b] Inhibition of *R*-α-methylhistamine-induced binding of [35S]GTPγS at human H_3 receptor.

Table 2
In vitro profiles of **1a**–**d**[a]



Compound	R^6	Human H_3[b] IC_{50} (nM)	hERG[c] IC_{50} (nM)
1a	H	0.72 ± 0.03	>10,000
1b	5-MeO	0.54 ± 0.06	>10,000
1c	6-MeO	18 ± 2	d
1d	7-MeO	3.6 ± 0.8	d

[a] The values represent the mean ± SE for $n \geqslant 3$.
[b] Inhibition of *R*-α-methylhistamine-induced binding of [^{35}S]GTPγS at human H_3 receptor.
[c] Inhibition of [^{35}S]*N*-[(4*R*)-1′-[(2*R*)-6-cyano-1,2,3,4-tetrahydro-2-naphthalenyl]-3,4-dihydro-4-hydroxyspiro[2*H*-1-benzopyran-2,4′-piperidin]-6-yl]methanesulfonamide binding to hERG in HEK293 cells.
[d] Not tested.

(IC_{50} = 506 nM). Slight improvements in potency were observed for the *para*-chloro and *meta*-chloro derivatives **2n** and **2m**, whereas the *ortho*-chloro derivative **2l** was devoid of potency. Introduction of a bulky alkyl group such as a *tert*-butyl group as in **2o** was ineffective.

Next, we turned our attention to various spiro-derivatives. Among the 3-azaspiro[5,5]undecane-based derivatives **2p**–**t**, spiro-benzopyranone **2t** displayed the highest IC_{50} value (68 nM). Several more potent derivatives were identified among the 8-azaspiro[4,5]decane-based derivatives **2u**–**y**. The indanone and isoindolinone derivatives **2u** and **2v** possessed IC_{50} values of 43 and 53 nM, respectively. Methylation of **2v** resulted in the slightly more potent derivative **2w**. Within our urea screening study, spiro-isobenzofuranone **2x** was found to be the most potent derivative (IC_{50} = 21 nM). The carbonyl group of **2x** is pivotal for potency since the isobenzofuran derivative **2y** showed a 10-fold loss in potency. Consequently, in hopes of further enhancing the potency, the corresponding amide derivative of **2x** was synthesized and evaluated. The active *trans*-isomer **1a** showed a dramatic 30-fold increase in potency (IC_{50} = 0.72 nM), relative to the corresponding urea derivative **2x**. Furthermore, **1a** displayed excellent selectivity over other histamine receptor subtypes, such as H_1, H_2, and H_4 (IC_{50} > 10 μM) and 60 CNS receptors (IC_{50} > 10 μM), along with an absence of any significant hERG inhibitory activities (IC_{50} > 10 μM). Using the serum incubation method, the hepatic clearance of **1a** was found to be very poor (18 and 59 mL/min/kg for human and rat hepatocytes, respectively).[18] Based on a pharmacokinetic study involving iv administration of 1 mg/kg of **1a** in rats, the plasma clearance in rats was determined as 97 mL/min/kg. Upon iv administration of 1 mg/kg of **1a** in rats, the plasma, brain, and cerebrospinal fluid levels were 1.5 μM, 2.4 nmol/g, and 0.36 μM, respectively (Table 3), and therefore, it is reasonable to consider **1a** as brain-penetrable in rats. Moreover, **1a** is neither a human nor a mouse P-gp substrate, and therefore the effects of P-gp-mediated efflux in humans and mice should be negligible (Table 3).[19] Based on these data, the development of **1a** as an oral drug would be challenging; in contrast, however, development as an iv tool such as a positron emission tomography (PET) tracer may be feasible.

Table 4
Pharmacokinetic parameters of **1b** in SD rats[a]

iv (1 mg/kg)	$t_{1/2}$ = 1.4 h V_{dss} = 7.2 L/kg CLp = 74 mL/min/kg
po (3 mg/kg)	$t_{1/2}$ = 1.4 h T_{max} = 0.3 h C_{max} = 0.06 μM *F* = 7%

[a] The values represent the mean for n = 3.

As a potential candidate for carbon isotope labeling, a methoxy group was introduced into various positions in the phenyl ring of the spiro-isobenzofuranone moiety of **1a** (Table 2). Although the 6-methoxy and 7-methoxy derivatives **1c** and **1d** displayed significant losses in potency, the 5-methoxy derivative **1b** showed enhanced activity (IC_{50} = 0.54 nM). The profiles of **1b** and **1a** were comparable, where **1b** showed good selectivity over other histamine receptor subtypes, such as H_1, H_2, and H_4 (IC_{50} > 10 μM), and 115 diverse, unrelated binding sites (IC_{50} > 10 μM for all the binding sites tested). Again, using the serum incubation method, the hepatic clearance of **1b** was found to be very poor (18 and 54 mL/min/kg for human and rat hepatocytes, respectively).[18] The pharmacokinetic profile of **1b** in rats was indeed poor (Table 4). As in the case of **1a**, good brain permeability in rats was observed for **1b** (Table 3)—upon oral administration of 10 mg/kg of **1b** in rats, the plasma, brain, and cerebrospinal fluid levels at 2 h were 0.27 μM, 0.48 nmol/g, and 0.058 μM, respectively. Although **1b** was a significant mouse P-gp substrate, the P-gp-mediated efflux should not be an issue in humans based on the transcellular transport ratio for human *MDR1* (Table 3).[19]

In summary, spiro-isobenzofuranones **1a** and **1b** were determined as potent and selective non-imidazole H_3 receptor inverse agonists. Our studies showed that **1b** is brain-penetrable in rats, and is not a human P-gp substrate. Moreover, the structure of **1b**, which is amenable to carbon isotope labeling, is distinctly different from the current quinazolinone class of clinical development candidates.[20] On the basis of these features, we believe **1b** to be an ideal clinical PET tracer candidate. The synthesis and preclinical characterization of tracers based on **1b** for imaging H_3 receptors are currently in progress. Additionally, efforts to optimize the structure of **1** to improve its metabolic stability are underway to develop a candidate from this class of spiro-isobenzofuranone H_3 receptor inverse agonists for clinical development.

Table 3
Brain-penetration and P-gp susceptibility of **1a** and **1b**

Compound	Brain-penetration in SD rats[a]			P-gp transporter assay[b] Transcellular transport ratio (B-to-A)/(A-to-B)	
	Plasma (μM)	Brain (nmol/g)	CSF (μM)	*MDR1*	*mdr1a*
1a	1.5	2.4	0.37	1.2	2.0
1b	0.27	0.48	0.058	2.3	7.0

[a] The values represent the mean for n = 3. The concentrations were determined at 10 min after 1 mg/kg iv for **1a** and at 2 h after 10 mg/kg po for **1b**.
[b] Transcellular transport ratio ((B-to-A)/(A-to-B)) was obtained from human *MDR1*- and mouse *mdr1a*-transfected LLC-PK1 cell monolayers. The values represent the mean for $n \geqslant 3$. The value above 3.0 indicates that a compound is a P-gp substrate.

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