Bioorganic & Medicinal Chemistry Letters 20 (2010) 2916–2919

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

The discovery and structure–activity relationships of 2-(piperidin-3-yl)-1*H*-benzimidazoles as selective, CNS penetrating H_1-antihistamines for insomnia

Karine Lavrador-Erb, Satheesh Babu Ravula *, Jinghua Yu, Said Zamani-Kord, Wilna J. Moree, Robert E. Petroski, Jianyun Wen, Siobhan Malany, Samuel R. J. Hoare, Ajay Madan, Paul D. Crowe, Graham Beaton *

Neurocrine Biosciences, 12780 El Camino Real, San Diego, CA 92130, USA

ARTICLE INFO

Article history:
Received 21 January 2010
Revised 3 March 2010
Accepted 5 March 2010
Available online 10 March 2010

Keywords:
H_1-Antihistamines
H_1-Antagonist
Insomnia, hERG
CNS
Benzimidazole
SAR
Sedative
Selective

ABSTRACT

A series of 2-(3-aminopiperidine)-benzimidazoles were identified as selective H_1-antihistamines for evaluation as potential sedative hypnotics. Representative compounds showed improved hERG selectivity over a previously identified 2-aminobenzimidazole series. While hERG activity could be modulated via manipulation of the benzimidazole N1 substituent, this approach led to a reduction in CNS exposure for the more selective compounds. One example, **9q**, retained a suitable selectivity profile with CNS exposure equivalent to known centrally active H_1-antihistamines.

Insomnia is one of the most common CNS disorders particularly in industrialized nations.[1] Sleep disorders have significant economic impact on both managed care[2] and nations' workforces as a consequence of higher work absenteeism and decreased job performance.[3] We have been interested in the discovery of selective, centrally acting H_1-antihistamines for development of an effective insomnia therapeutic that is free of the issues exhibited by over-the-counter (OTC) sedating antihistamines. These include selectivity for muscarinic receptors, a property thought to contribute to undesirable side effects such as dry mouth, blurred vision, constipation, tachycardia, urinary retention.[4] Next-day impairment after bedtime use of these antihistamines is also common and has been attributed to long plasma half-lives and protracted CNS exposure.[5]

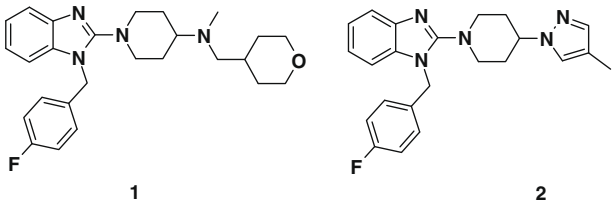


Figure 1. Early 2-aminobenzimidazole H_1-antihistamine leads.

As described in our previous publication,[6] we used the core of the known H_1-antihistamine mizolastine[7] to identify the sedating compound, **1** (Fig. 1). This compound and other basic analogs in this series were, however, determined to be potent hERG channel inhibitors, as has been observed for other H_1-antihistamine series.[8] Optimization of this class for the hERG liability resulted in the 2-aminobenzimidazole **2** (Fig. 1), a relatively selective antihistamine with acceptable blood–brain barrier (BBB) penetration (30 mpk oral dose at 4 h: brain concentration 262 ng/g; B/P ratio of 2.3).[6] However, **2** displayed a less than optimal profile with high metabolic clearance and poor solubility (<0.01 mg/mL at pH 7.4).[9] In an effort to improve properties of the 2-aminobenzimidazole series while maintaining selectivity, replacement of the 4-aminopiperidine moiety in **1** by alternative diamines was examined, using chemistry previously described.[6] Compounds were assessed for selectivity versus monoamine receptors as well as inhibition of CYP2D6, CYP3A4 enzymes

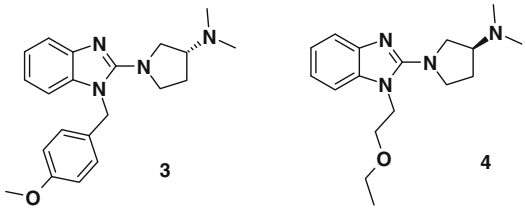


Figure 2. 3-Aminopyrrolidin-1-yl-2-aminobenzimidazoles.

* Corresponding authors. Tel.: +1 619 200 5689 (S.B.R.); tel.: +1 858 337 1801 (G.B.).
E-mail addresses: satheesh.ravula@gmail.com (S.B. Ravula), beaton.graham@gmail.com (G. Beaton).

0960-894X/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2010.03.027

Table 1
H_1-Antihistamine activity, M_1 selectivity, CYP2D6 inhibition, predicted intrinsic clearance and hERG electrophysiology assay results for compounds **1**–**4**

Compd	H_1 K_i[a] (nM)	M_1 K_i[a] μM)	CYP2D6 IC_{50}[b] (μM)	Pred. Cl Int. (mL/min/kg)	hERG IC_{50} (nM)
1	3.9 ± 0.3	4.8 ± 0.8	11.0	106	29
2	6.9 ± 0.9	>10	5.4	242	809
3	8.4 ± 2.0	2.5 ± 0.5	>10	61	492
4	4.8 ± 0.6	1.5 ± 0.1	>10	21	233

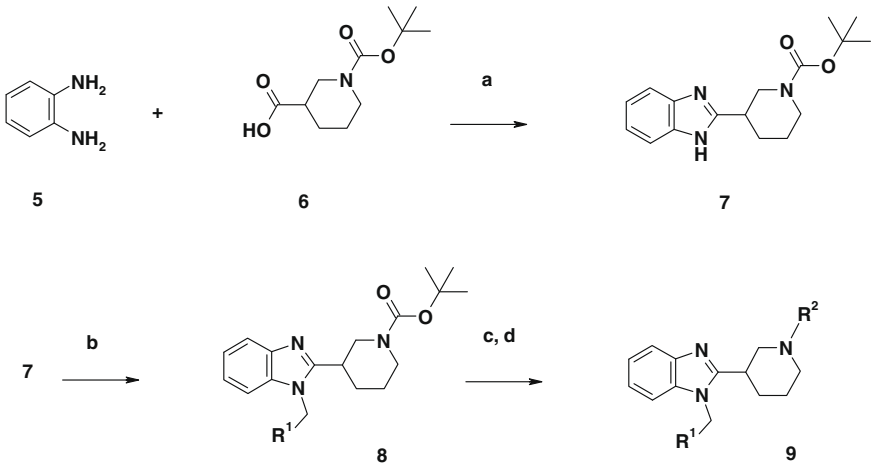
[a] SEM for K_i values derived from dose–response curves generated from triplicate or more data points.
[b] IC_{50} for CYP3A4 inhibition >5 μM for all compounds.

and the hERG channel. From this effort we identified 3-aminopyrrolidines **3** and **4** (Fig. 2) with profiles of interest. Data for these examples is shown in comparison to compounds **1** and **2** (Table 1).

Both compounds **3** and **4** retained selectivity for M_1 and CYP2D6. Interestingly hERG selectivity for these analogs was significantly improved compared to compound **1** from the 4-aminopiperdine series. From our previous studies,[5] hERG selectivity was only slightly influenced by changes to the N1 substituents employed and suggested that the introduction of a basic moiety with alternative orientation relative to the benzimidazole core could facilitate improved hERG selectivity. Both **3** and **4** were also significantly more stable in a human liver microsome (HLM) assay. This data was encouraging and offered the potential to access compounds with improved solubility and enhanced bioavailability. Based on this information we focused our attention for further studies on the more novel benzimidazole scaffold **9** shown in Scheme 1. Our intent was to first validate the hypothesis that selected analogs based on **9** were potent H_1-antihistamines with suitable selectivity against a range of key targets. To achieve similar selectivity to our work in a parallel series that was described earlier,[10] candidate compounds were required to demonstrate high H_1 binding affinity (K_i <10 nM) with at least 100-fold binding selectivity versus the representative muscarinic M_1 receptor. Selectivity of the order of 1000-fold was deemed acceptable for CYP enzyme inhibition. While our leads from this work demonstrated weak hERG channel inhibition (hERG IC_{50}/H_1 K_i selectivity of 336[10]), in vivo characterization indicated an absence of cardiovascular risks and safety margins significantly higher than previous guidelines for the assessment of hERG inhibitors.[11] Based on these data we reasoned that hERG IC_{50}/H_1 K_i selectivity of 400 or greater would be acceptable for candidate compounds. With this accomplished we planned to assess stability and in vivo central exposure for key compounds to determine their candidacy for evaluation as sleep agents.

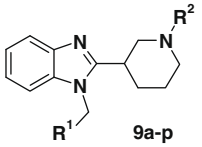
Synthesis of these benzimidazole analogs is outlined in Scheme 1. We started with *O*-phenylenediamine **5**, which was treated with *N*-BOC-piperidinecarboxylic acid **6** to give benzimidazole **7**. N-alkylation of **7** was accomplished using the corresponding alkyl bromide to give the analog **8**. Removal of the BOC group was achieved with TFA to afford the intermediate amine that was further modified by reductive amination or alkylation to yield the final benzimidazoles **9a**–**p**.

From preliminary binding data for the racemic compounds **9a**–**p**, we confirmed that this alternative orientation of the basic center within the 3-piperidinyl moiety was accommodated in the H_1 pharmacophore, all compounds with the exception of **9g** showing potent H_1 affinity (Table 2). Within this set both benzylic (**9a**,**b**, **9h**–**l**) and extended alkyl (**9c**–**d**, **9m**–**n**) substitution was tolerated at the benzimidazole N1 position. Replacement of the arene with a heterocycle within the N1 benzyl moiety (**9e**–**f**, **9o**–**p**) was also possible with some reduction in affinity. In addition, substitution at the piperidine nitrogen was well tolerated for the analogs assessed. Selectivity of this series was evaluated for M_1 binding affinity, P450 enzyme inhibition (CYP2D6, CYP3A4) and hERG binding.[6] From this assessment, the compounds were determined to be generally very selective for M_1, with only the phenoxyethyl analog, **9c**, showing significant binding affinity. In addition, we were encouraged with the observation that the series showed a general selectivity on the basis of hERG binding, similar to that determined for the original lead, **2** (K_i 3849 nM). [6] Selectivity for CYP2D6 inhibition was, however, reduced. While the compounds **9a** and **9e**–**f** were reasonably selective for CYP2D6, other similar analogs exhibited significant inhibition of the P450 enzyme. Two trends were noted in the structure–activity relationships for CYP2D6. First, enzyme inhibition appeared to increase with increasing hydrophobicity at R^2, as exemplified for comparisons of **9a** with **9h**–**i**, **9d** with **9m** and **9f** with **9o**. In the case of the *p*-methoxybenzyl series CYP2D6 inhibition was significant for all comparable samples. The

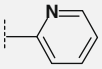
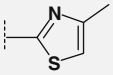
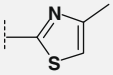
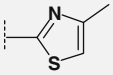


Scheme 1. Reagents and conditions: (a) neat, 120 °C (65%); (b) R^1-CH_2-Br, K_2CO_3, DMF, 80 °C (70–85%); (c) TFA, CH_2Cl_2, (95%); (d) R^2(=O)H, $Na(OAc)_3BH$ or R^2-X, Et_3N, THF (45–90%).

Table 2
H_1-Antihistamine activity, M_1 selectivity, CYP2D6 inhibition and hERG dofetilide assay results for compounds **9a–p**



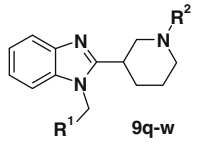
9a-p

Compd	R^1	R^2	H_1 K_i[a] (nM)	M_1 K_i[b] (μM)	CYP2D6 IC_{50}[c] (nM)	hERG K_i[d] (nM)
9a	*p*-F-Ph	Me	0.5 ± 0.4	2.2 ± 0.7[a]	3065	>10,000
9b	*p*-MeOPh	Me	1.2 ± 0.3	9.7 ± 0.2	465	>5000
9c	CH_2OPh	Me	0.8 ± 0.3	0.04 ± 0.05	NT[e]	5291
9d	CH_2OEt	Me	4.6 ± 0.3	2.60 ± 0.03	1556	1262
9e		Me	14 ± 2[b]	4.9 ± 0.1	>10,000	>10,000
9f		Me	3.3 ± 0.9	2.4 ± 0.1	15,989	>10,000
9g		Me	605 ± 63[b]	NT[e]	>10,000	638
9h	*p*-F-Ph	$CH(CH_3)_2$	0.8 ± 0.2	5.4 ± 0.9	618	1373
9i	*p*-F-Ph	Cyclohexyl	1.9 ± 0.2[b]	3.7	195	926
9j	*p*-MeOPh	$CH(CH_3)_2$	1.8 ± 0.2	12 ± 1	717	2649
9k	*p*-MeOPh	Cyclohexyl	4.7 ± 0.5[b]	13	122	1840
9l	*p*-MeOPh	Tetrahydropyran-4-yl	5.0 ± 0.9[b]	11.9 ± 0.01	1901	>10,000
9m	CH_2OEt	Cyclohexyl	5.2 ± 0.9[b]	>10	526	>10,000
9n	CH_2OEt	Tetrahydropyran-4-yl	14 ± 2	>10	4393	>3000
9o		Cyclohexyl	3.8 ± 0.5	1.9 ± 0.2	480	1183
9p		Tetrahydropyran-4-yl	10 ± 1	3.2 ± 0.5	6453	>3000

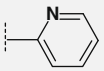
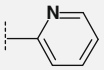
[a] SEM for K_i values derived from dose response curves generated from triplicate or more data points.
[b] K_i values average of two data points.
[c] IC_{50} for CYP3A4 inhibition >5 μM for all compounds except **9k** [IC_{50} = 3.3 μM].
[d] K_i values were derived from single or duplicate data points.
[e] NT = not tested.

addition of hydrophobic moieties at the piperidino nitrogen also increased hERG binding affinity. In all cases, the reduction of hydrophobicity by substitution of the tetrahydropyranyl moiety for cyclohexyl showed a general improvement in CYP2D6 IC_{50} and a decrease in hERG binding affinity. Comparison of analogs **9a** with **9c–f** suggested a second trend in which CYP2D6 inhibition could also be influenced by the substitution at N1 of the benzimidazole. In particular, replacement of the R^1 arene with a heterocycle was beneficial in providing significant reductions in enzyme inhibition.

Table 3
H_1-Antihistamine activity, M_1 selectivity, CYP2D6 inhibition and hERG dofetilide assay results for the enantiomeric compounds **9q–w**



9q-w

Compd	R^1	R^2	Stereo-chemistry	H_1 K_i[a] (nM)	M_1 K_i[b] (μM)	CYP2D6 IC_{50}[c] (μM)	hERG K_i[d] (nM)
9q	*p*-F-Ph	Me	*R*	0.9 ± 0.2	2.8 ± 0.2	2.3	3860
9r	*p*-F-Ph	Me	*S*	1.10 ± 0.03	3.5 ± 0.5	2.8	6515
9s	CH_2OEt	Me	*R*	2.6 ± 0.4	1.7 ± 0.1	15.5	>10,000
9t	CH_2OEt	Me	*S*	32.9 ± 0.1[b]	>10,000	6.6	>10,000
9u		Me	*R*	9 ± 2	7 ± 2a	53.3	10,336
9v		Me	*S*	12 ± 1	3.7 ± 0.1	47.5	>10,000
9w	CH_2OEt	Tetrahydropyran-4-yl	*R*	6.4 ± 0.3	>10	32.2	>3000

[a] SEM for K_i values derived from dose response curves generated from triplicate or more data points.
[b] K_i values average of two data points.
[c] None of the compounds showed appreciable inhibition of CYP3A4.
[d] K_i values were derived from single or duplicate data points.

While initial data indicated suitable selectivity profiles were achievable, CYP2D6 inhibition was identified as a significant potential liability. Preferred substitutions for selectivity at M_1 were *p*-F benzyl, ethoxyethyl, pyridine-2-ylmethyl and 2-methylthiazol-4-ylmethyl with R^2 as methyl. Tetrahydropyran-substituted piperidine analogs were also of potential interest given the ability of this feature to modulate off-target interactions. Prior to testing exposure and stability of these compounds a more detailed selectivity study was conducted on the enantiomers of selected key compounds which were prepared according to Scheme 1 using the appropriate chiral BOC 3-piperidine carboxylic acid. Data for compounds **9q**–**w** is summarized in Table 3. From this data, chirality had little impact on H_1 affinity for analogs with R^1 as the benzyl or pyridin-2-ylmethyl motifs. Chiral preference for the *R* enantiomer was observed for the ethoxyethyl substitution, **9s** being approximately 10-fold more potent than **9t**. No significant binding was observed for any of the compounds for either H_3 or M_3 receptors. In general the more potent compounds were approximately 1000-fold selective for H_1 over the other targets assessed. Patch clamp analysis of **9q** indicated a hERG IC_{50} of 721 nM. This result was in line with previous observations of trends comparing hERG binding and electrophysiology data[6] and indicated that **9q** demonstrated improved selectivity compared to the lead **2** previously identified [hERG IC_{50}/H_1 K_i = 800 for **9q** compared to 117 for **2**]. Compound **9w** had weaker inhibition in the patch clamp assay with an IC_{50} value of 2.1 μM. **9u** was a still weaker inhibitor [39% at 10 μM].

In the absence of a high throughput in vivo assessment, suitable CNS exposure was required of the leading compounds prior to their evaluation for sedative hypnotic potential in a rat electroencephalography (EEG) model. To assess suitable CNS penetration in potential lead compounds, representative analogs were evaluated for their ability to penetrate the BBB in rodents using cassette PK studies.[6] Groups of five compounds including the short-acting brain penetrating antihistamine triprolidine[6] (**3**) were administered (iv) to rats and brain levels and B/P ratios were determined. Analogs with CNS penetration similar or better than triprolidine were required for the candidate compound to be further evaluated in the EEG model. Cassette data for representatives of the compounds synthesized is summarized in Table 4.

Of the compounds studied, only **9q** displayed significant brain levels that were similar to the sedating antihistamine triprolidine. Neither the heteroaryl substituted compounds **9u**, **9f** or **9p** nor ethoxyethyl analogs **9d** (racemate of **9s**) or **9n** (racemate of **9w**) achieved significant brain exposure greater than 10 ng/g at the iv dose. These exposures were less than 25% of the relative CNS exposure achieved by the sedating antihistamine control. This data indicated that, despite excellent selectivity profiles, compounds **9s** and **9u**–**w** were unsuitable as candidates because of poor CNS penetrability.[12] The enantiomer of **9n** (**9w**), was assessed in a discrete PK study to determine whether increasing oral doses could achieve suitable exposure. However, no measurable brain exposure could be detected at doses up to 30 mg/kg after 4 h. The reduction in exposure for this and other analogs appears to be dominated by effects of the benzimidazole R^1 substituent, presumably by local reductions in hydrophobicity and increases in PSA and H-bond acceptors.[13] Calculations of log *P* and polar surface area[14] indicated that estimates of log *P* for the compounds in this class were similar [2.73–4.25] to those of the known sedating antihistamines diphenhydramine [3.35], doxepin [3.77] and triprolidine [3.38]. In contrast, only polar surface area for **9q** [21.1] was similar to that for the sedating antihistamines [12.5–16.1] with the other analogs exhibiting significantly higher values [30.2–71.4]. While these R^1 substitutions improved in vitro selectivity for H_1 over hERG and reduced CYP2D6 inhibition compared to **9q**, central exposure was significantly decreased. Nevertheless, **9q** retained a suitable selectivity profile and central exposure as a representative of a new class of selective brain penetrating H_1-antihistamines.

Table 4
CNS exposure results for selected analogs compared to triprolidine

Compd	Pred. hCl int.[a] (ml/min/kg)	[B][b] ng/g	[P][b] ng/ml	B/P[b]	[B][b] ng/g Triprolidine
9q	21.0	39.5	14.7	2.7	46.5
9u	23.4	4.9	3.5	1.4	46.5
9d	34.1	10	13.2	0.8	89.4
9n	80.0	7.5	3.4	2.6	37.0
9f	32.9	5.4	5.6	1.0	37.0
9p	98.3	3.5	4.1	0.9	37.0

[a] Predicted based on HLM stability studies.
[b] Cassette dose 1 mg/kg, iv.

In summary, starting from a series of 2-aminobenzimidazoles we identified a novel class of 2-(piperidin-3-yl)-1H-benzimidazoles as potent and selective H_1-antihistamines as potential agents for the treatment of insomnia. SAR studies within this class indicated that manipulation of the N1 substituent on the benzimidazole led to selective compounds with reduced hERG activity although these analogs lacked CNS penetration. One compound, **9q**, retained CNS exposure equivalent to known sedating antihistamines with a suitable selectivity profile to warrant further optimization.

Acknowledgments

The authors wish to thank John Harman and Chris DeVore for analytical support, Dr. Jaimie K. Rueter for solubility data and Dr. John Saunders, Dr. Paul Conlon, Dr. Wendell Wierenga and Dr. Haig Bozigian for program support.

References and notes

1. Ohayon, M. M.; Lemoine, P. *L'Encephale* **2004**, *30*, 135.
2. Fullerton, D. S. P. *Am. J. Manag. Care* **2006**, *12*, S246.
3. Zammit, G. K.; Weiner, J.; Damato, N.; Sillup, G. P.; McMillan, C. A. *Sleep* **1999**, *22*, S379.
4. (a) Kubo, N.; Shirakawa, O.; Kuno, T.; Tanaka, C. *Jpn. J. Pharmacol.* **1987**, *43*, 277; (b) Meolie, A. L.; Rosen, C.; Kristo, D.; Kohrman, M.; Gooneratne, N.; Aguillard, R. N.; Fayle, R.; Troell, R.; Townsend, D.; Claman, D.; Hoban, T.; Mahowald, M. *Clin. Sleep Med.* **2005**, *1*, 173.
5. Kay, G. G.; Plotkin, K. E.; Quig, M. B.; Starbuck, V. N.; Yasuda, S. *Am. J. Manag. Care* **1997**, *3*, 1843.
6. Coon, T.; Moree, W. J.; Li, B.; Yu, J.; Zamani-Kord, S.; Malany, S.; Santos, M. A.; Hernandez, L. M.; Petroski, R. E.; Sun, A.; Wen, J.; Sullivan, S.; Haelewyn, J.; Hedrick, M.; Hoare, S. J.; Bradbury, M. J.; Crowe, P. D.; Beaton, G. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 4380. and references cited therein.
7. Benavides, J.; Schoemaker, C.; Dana, C.; Laustre, Y.; Delahaye, M.; Prouteau, M.; Manoury, P.; Allen, J.; Scatton, B.; Langer, S. Z.; Arbilla, S. *Arzneim.-Forsch/Drug Res.* **1995**, *45*, 551.
8. Aslanian, R.; Piwinski, J. J.; Zhu, X.; Priestley, T.; Sorota, S.; Du, X.-Y.; Zhang, X.-S.; McLeod, R. L.; West, R. E.; Williams, S. M.; Hey, J. A. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 5043.
9. Measured using GLPKa instrumentation (pION Inc.): potentiometric method using 0.15 M KCl buffer.
10. Moree, W. J.; Li, B.; Jovic, F.; Coon, T.; Yu, J.; Gross, R. S.; Tucci, F. C.; Marinkovic, D.; Malany, S.; Bradbury, M. J.; Hernandez, L. M.; O'Brien, L.; Wen, J.; Wang, H.; Hoare, S. R. J.; Petroski, R. E.; Sacaan, A.; Madan, A.; Crowe, P. D.; Beaton, G. *J. Med. Chem.* **2009**, *52*, 5307.
11. Redfern, W. S.; Carlsson, L.; Davis, A. S.; Lynch, W. G.; MacKenzie, I.; Palethorpe, S.; Siegl, P. K. S.; Strang, I.; Sullivan, A. T.; Wallis, R.; Camm, A. J.; Hammond, T. G. *Cardiovasc. Res.* **2003**, *58*, 32.
12. In some cases, where insufficient amounts of enantiomer were available, the racemic compound was assessed in the cassette PK experiment. In control experiments representative enantiomers and their racemates were shown to exhibit similar CNS penetration properties in cassette studies. No differences were observed in clearance profile between racemate and enantiomers.
13. Feher, M.; Sourial, E.; Schmidt, J. M. *Int. J. Pharm.* **2000**, *201*, 239.
14. Calculated using ACD Labs Software Suite.