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A novel class of H₃ antagonists derived from the natural product guided synthesis of unnatural analogs of the marine bromopyrrole alkaloid dispyrin

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The neurotransmitter histamine exerts its action through four distinct Class A GPCRs (H_1-H_4) .¹⁻⁷ The histamine H_3 receptor, a Gi/o-coupled receptor in the CNS, is a pre-synaptic auto- and heteroreceptor that not only controls the release of histamine, but also other neurotransmitters (acetylcholine, noradrenaline, dopamine, GABA and serotonin).¹⁻⁷ Preclinically, H₃ antagonists/inverse agonists have demonstrated efficacy in a number of CNS pathologies including schizophrenia, epilepsy, depression, pain, decreasing food intake, drug abuse and addiction, sleep disorders/narcolepsy and cognitive enhancement.¹⁻⁷ Early reference H₃ antagonists contained imidazole moieties, such as thioperimide 1 and Perceptin (GT-2331) 2 (Fig. 1). Effort from multiple companies then focused on non-imidazole H₃ antagonists and include compounds such as UCL 1972 3, ABT-239 4, JNJ's 5, Novo Nordisk's 6, Eli Lilly's 7 and GSK189254 8 to exemplify a few (Fig. 1). This intense effort from the pharmaceutical industry led to the evolution of a refined H₃ antagonist pharmacophore model 9.1-8

We recently completed the first total synthesis of dispyrin **10**,⁹ a bromopyrrole alkaloid with a novel bromotyramine core, isolated by Crews in 2007¹⁰ from the marine sponge *Agelas dispar* (Fig. 2). Upon recognition that dispyrin **10** possessed the basic features of the refined H₃ pharmacophore model **9**, we evaluated our synthetic dispyrin against the human H₃ receptor. Gratifyingly, dispyrin was found to have modest activity as an H₃

ABSTRACT

This Letter describes the natural product guided synthesis of unnatural analogs of the marine bromopyrrole alkaloid dispyrin, and the resulting SAR of H_3 antagonism. Multiple rounds of iterative parallel synthesis improved human H_3 IC₅₀ ~33-fold, and afforded a new class of H_3 antagonists based on the novel bromotyramine core of dispyrin.

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antagonist ($IC_{50} = 2.35 \ \mu$ M, $K_i = 1.04 \ \mu$ M).⁹ Based on these data, we initiated a natural product guided synthesis effort, employing iterative parallel synthesis¹¹ for molecular editing, aimed at improving H₃ inhibition and binding; moreover, we wanted to validate the marine natural product dispyrin **10** as a viable lead molecule due to the novel scaffold providing intellectual property in extremely crowded chemical space.

The first generation 25-member library was based on a 5×5 two-dimensional design wherein the core was held constant and the amide R¹ and aminoalkyl moieties R² varied (Scheme 1). The library synthesis began with a simple DIC amide coupling employing commercially available 3-bromo-4-methoxyphenylethylamine **11** with one of five heterocyclic carboxylic acids R¹. These five scaffolds were then treated with BBr₃ to remove the methyl ether liberating the free phenols **13**. Each of the five phenols **13** was then alkylated with one of five aminoalkyl chlorides to install R² under microwave-assisted conditions to afford unnatural dispyrin analogs **14** (Table 1).

This first generation library was highly informative. In general, all R¹s and R²s afforded modestly potent (K_i s and IC₅₀s in the low micromolar range) H₃ antagonists. Potent H₃ antagonists (K_i s < 200 nM, IC₅₀s < 430 nM) resulted for all of the heterocyclic amides R¹ in combination with the ethyl pyrrolidinyl R² (**14c**, **14h**, **14m**, **14r** and **14w**). In contrast, the ethyl morpholino congeners (**14d**, **14i**, **14n**, **14s** and **14x**) were uniformly weak (K_i s > 12 µM, IC₅₀s > 29 µM). The most potent H₃ antagonist from the first generation library was **14r** (R¹ = 4-bromo-thiophene,

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Figure 1. Imidiazole and non-imidazole H₃ antagonists 1–8 leading to a refined H₃ pharmacophore model 9.



Figure 2. Dispyrin **10**, a novel bromopyrrole alkaloid from *Agelas dispar* with a bromotyramine core unprecedented in marine natural products.



Scheme 1. First generation library synthesis. Reagents and conditions: (a) R¹COOH, DIC, HOBt, DIEA, DCM, rt, 12 h (69–99%); (b) BBr₃, DCM, -78 °C-rt, 1.5 h (50–95%); (c) 1.3 equiv CIR², CsCO₃, KI, DMF, mw, 160 °C, 20 min (72–93%). All analogs purified to >98% by mass-directed preparative HPLC.¹²

 R^2 = ethyl pyrrolidine) with a K_i of 80 nM and an IC₅₀ of 180 nM—a 13-fold improvement over the parent natural product dispyrin **10** (IC₅₀ = 2.35 μ M, K_i = 1.04 μ M). Based on these data,

the next library maintained R^1 = 4-bromo-thiophene and surveyed functionalized pyrrolidines at R^2 (Scheme 2).

Following Scheme 1, a large quantity of **15** was prepared. Then, the phenol was alkylated with 2-bromo-1,1-dimethoxy ethane to provide **16**, which was then converted to the corresponding aldehyde **17** by treatment with tosylic acid. Finally,

Table 1

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$R^{1} \downarrow N \downarrow O Br O R^{2}$							
14							
Compd	R ¹	R ²	$H_3 K_i^a (\mu m)$	$H_{3} IC_{50}{}^{a} (\mu m)$			
14a	Br N P	کر <u>N</u>	1.04	2.35			
14b		5 ~ - N	1.61	3.72			
14c		ζ, , , , , , , , , , , , , , , , , , ,	0.19	0.43			
14d		N N	12.9	29.1			
14e		⁵ 2, N	1.33	2.94			
			(cont	inued on next page)			

Table 1 (continued)

Compd	R ¹	R ²	$H_3 K_i^a (\mu m)$	$H_{3} IC_{50}{}^{a} (\mu m)$
14f	N 355	کم ا	1.36	2.92
14g	Η '	ب _ک N	1.55	3.43
14h		×××××N√	0.15	0.38
14i		ζ ζ νζ νζ νζ	26.4	59.2
14j		کر N	1.52	3.63
14k	N	۶ <u></u> N	1.76	3.99
141	/ ~	برN	2.13	4.54
14m		×××××N√	0.13	0.31
14n		°2∼∕N√O	17.6	39.6
140		₹ N	1.65	3.62
14p	Br	کرN	1.61	3.61
14q	S ⁻ ³	۲ ۲ ۲	0.67	1.52
14r		× ××∽∼N√	0.08	0.18
14s		×××××××××××××××××××××××××××××××××××××	13.5	30.4
14t		کر N	1.25	3.73
14u	Br - S	۶ <u></u> N	2.29	4.81
14v	- ,	۰ ۲	2.72	5.93
14w		ν ν _ζ ∕νγγγγγγγγγγγγγγγγγγγγγγγγγγγγγγγγγγγγ	0.17	0.39
14x		ېرN	32.5	73.2
14y		ζN_	2.11	4.56

^a Average of three independent determinations with human H₃.

Table 2

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Compd	R ¹	$H_3 K_i^a (\mu m)$	H ₃ IC ₅₀ ^a (μm)
18a	(S)-2-Me	0.31	0.71
18b	(S)-2-Me	0.33	0.75
18c	(S)-3-F	1.10	2.11
18d	(S)-3-F	1.15	2.52

^a Average of three independent determinations.

reductive amination employing a functionalized pyrrolidine and MP-B(OAc)₃H provided analogs **18**. As shown in Table 2, analogs **18** were weaker H₃ antagonists than **14r**, and there was no evidence of enantioselective inhibition (**18a** vs **18b**). In agreement with the H₃ pharmacophore model, incorporation of β -fluorine atoms such as in **18c** and **18d**, which lowers the pK_a on the pyrrolidine nitrogen from 11 to 9, afforded diminished H₃ inhibition.¹³

We then prepared two singleton compounds following the synthetic route depicted in Scheme 1 with the appropriate substitutions, wherein the bromine in **14r** was replaced with a chlorine **19** and a truncated version **20** (Fig. 3). A twofold diminution in potency was noted for **19**, relative to **14r**, and the truncated benzyl version lost over 13-fold compared to **14r**; however, this highlighted that the heavy bromine atom was not required for H_3 inhibition.

The final library iteration was directed at surveying a wider range of alternative amides (heterocycles and functionalized aromatic moieties) while holding the preferred ethyl pyrrolidine ether and bromotyramine core constant. The synthesis began with **11** and conversion to the phthalimide congener **21**. Standard BBr₃ deprotection provided **22** which was alkylated with chloroethyl pyrrolidine to deliver **23**. Hydrolysis of the phthalimide with hydrazine, followed by amide coupling with a diverse collection of aryl and heteroaryl carboxylic acids generated library **24** (Scheme 3).

This third generation library was uniformly active, providing H_3 antagonists in the sub-micromolar range. Six-member heterocycles, such as pyridine **24a**, were active, as were aryl amides



Scheme 2. Second generation library synthesis. Reagents and conditions: (a) BrCH₂CH(OMe)₂, Cs₂CO₃²⁻, KI, DMF, reflux (70%); (b) TosOH, mw, 160 °C, 10 min (60%); (c) functionalized pyrrolidine, MP-(OAc)₃H, DCM, rt (40–85%). All analogs purified to >98% by mass-directed preparative HPLC.¹²



Figure 3. Chlorinated version and chlorinated truncated version of 14r.



Scheme 3. Third generation library synthesis. Reagents and conditions: (a) 1,2-dicarboxybenzene, DIC, HOBt, DIEA, DCM, rt, 12 h (99%); (b) BBr₃, DCM, -78 °C-rt, 1.5 h (95%); (c) 1.3 equiv Chloroethyl pyrrolidine, CsCO₃, Kl, DMF, mw, 160 °C, 20 min (93%); (d) (i) N₂H₄, mw, 160 °C; (ii) SCX; (e) R¹COOH, DIC, HOBt, DIEA, DCM, rt, 12 h (64–98%). All analogs purified to >98% by mass-directed preparative HPLC.¹²

with halogens (Cl and Br) or trifluoromethyl groups in the 3-position (**24f-h**). Five-member heterocycles (**24b-e**) proved optimal, with a 5-oxazole **24b** (K_i = 32 nM, IC₅₀ = 83 nM) and 2thiazole **24d** (K_i = 32 nM, IC₅₀ = 72 nM) affording the most potent H₃ antagonists of the unnatural dispyrin analogs. For example, **24d** improved the H₃ K_i and IC₅₀ ~33-fold over the natural product dispyrin, and required only three iterations of molecular editing and 40 analogs. Moreover, as dispyrin represented a novel chemotype, we were able to obtain composition of matter patents for the dispyrin analogs as H₃ antagonists within an incredibly crowded intellectual property landscape.¹⁴ This effort highlights the value of employing natural products as leads for therapeutically relevant targets (Table 3).

In summary, a natural products guided synthesis effort in molecular editing, employing iterative parallel synthesis, quickly optimized the weak H_3 antagonism of the marine natural product dispyrin **10** over 30-fold to afford unnatural analog **24d** with low nanomolar potency and binding. By employing a novel natural product scaffold for lead optimization, we were able to establish an intellectual property position in an incredibly crowded intellectual property landscape. Although the role of natural products drug discovery efforts within the pharmaceutical industry is being significantly reduced, despite overwhelming success, the biological activity of dispyrin and its analogs argue further that natural products are viable drug leads and offer patenting advantages.

Table 3 Structures and activities of dispyrin analogs 24



Compd	R ¹	$H_3 K_i^a (\mu m)$	$H_{3} IC_{50}{}^{a} (\mu m)$
24a	F ₃ C N J ^{s⁵}	0.27	0.56
24b	N J ss	0.03	0.08
24c	N∽N ℓ	0.43	0.97
24d	∑ ^N S ^S S ^S	0.03	0.07
24e	N S S	0.12	0.26
24f	Cl	0.25	0.55
24g	Br	0.21	0.45
24h	F ₃ C	0.44	0.98

^a Average of three independent determinations.

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