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Synthesis and optimization of 2-pyridin-3-yl-benzo[*d*][1,3]oxazin-4-one based inhibitors of human neutrophil elastase

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ABSTRACT

The hit-to-lead optimization of the HNE inhibitor 5-methyl-2-(2-phenoxy-pyridin-3-yl)-benzo[*d*][1,3]oxazin-4-one is described. A structure–activity relationship study that focused on the 5 and 7 benzoxazinone positions yielded the optimized 5-ethyl-7-methoxy-benzo[*d*][1,3]oxazin-4-one core structure. 2-[2-(4-Methyl-piperazin-1-yl)-pyridin-3-yl] derivatives of this core were shown to yield HNE inhibitors of similar potency with significantly different stabilities in rat plasma.

Human neutrophil elastase (HNE) is a serine protease, stored in and secreted from neutrophils, that is capable of degrading a variety of structural proteins of the extracellular matrix.[1] Infiltration of activated neutrophils has been implicated in several lung diseases, including ARDS and emphysema.[2] Thus, HNE inhibitors that target the excess production of this protease in these indications have the potential as therapeutic agents. A variety of structural classes of HNE inhibitors have been developed, including those based on benzoxazinones[3] and related thienoxazinones[4] and several HNE inhibitors have advanced to the clinic.[5]

As part of an effort to find novel HNE inhibitors, we conducted a high throughput screen of an internal compound library which led to the identification of compound **1** as a potent HNE inhibitor (Table 1).[6] We then set out to determine what structural elements of this compound contributed to its potency. With that information in hand, the core benzoxazinone scaffold was optimized for potency and stability. A position of the core structure was identified where polar and non-polar groups could be incorporated without significantly impacting HNE inhibitor potency. Taking advantage of this position, HNE inhibitors were synthesized that had similar HNE IC_{50} values but with a wide range of rat plasma stabilities.

The generalized synthesis of 2-pyridin-3-yl-benzoxazinones used in the exploratory SAR study is shown in Scheme 1. Such benzoxazinones could be obtained in a one-pot fashion by the activation of nicotinic acid derivatives with carbonyldiimidazole (CDI) followed by the addition of an anthranilic acid (1.2 equiv). The resulting amide was then ring-closed to the benzoxazinone using *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDCI). Furthermore, aryl acid starting materials or intermediates could be conveniently removed at the end of the reaction using a solid phase extraction column containing an anion exchange resin (Varian, PL-HCO_3 MP SPE Resin) prior to a final C_{18} reverse phase HPLC purification (CH_3CN/H_2O, 0.1% AcOH).

Compounds for the initial SAR study were designed to probe the absence, position, or modification of substituents present in the hit compound **1**. The impact of deleting substituents was investigated first. The importance of the pyridyl nitrogen was demonstrated by comparing the HNE IC_{50} value of compound **2** to the original hit; substitution of the pyridine ring of compound **1** with a phenyl ring resulted in a threefold loss in potency. When compounds that represented the deletion from the hit **1** of the B5 methyl group (compound **3**) or the phenoxy group (compound **4**) were synthesized and tested, a 150-fold and 15-fold loss of potency, respectively, were seen relative to compound **1**. When the phenoxy group of compound **1** was substituted with a methoxy group (compound **5**), the resulting IC_{50} value was similar to compound **1**. To further explore the impact of substitution in this position, compounds **6** and **7** were synthesized and compared. The P2 methyl derivative **6** yielded an inhibitor with potency comparable to the hit **1**. By contrast, the P6 methyl derivative **7** resulted in a 12-fold loss in potency relative to the hit **1**. Additionally, substitution of the B5

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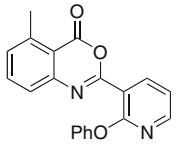
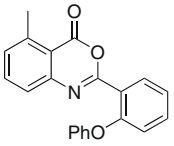
Table 1
HNE IC_{50} values for compounds **1–9** and **13–16**



1



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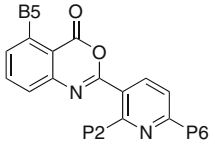
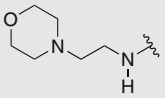
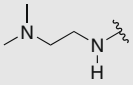
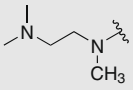
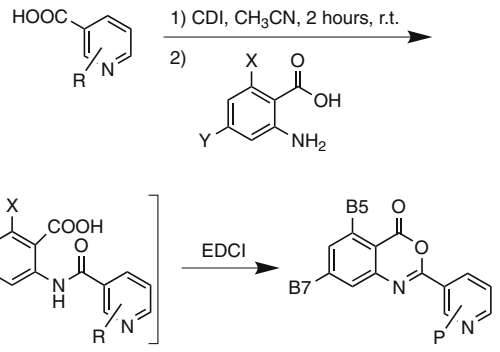


3-9, 13-17

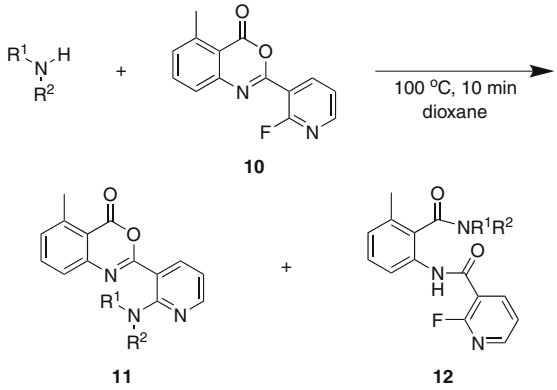
Compound	B5	P2	P6	HNE IC_{50} (nM)
1				61
2				170
3	–H	PhO–	–H	9300
4	–Me	H–	–H	900
5	–Me	MeO–	–H	85
6	–Me	Me–	–H	87
7	–Me	H–	–Me	710
8	–Cl	PhO–	–H	160
9	$–OCH_3$	PhO–	–H	390
13	–Me		–H	6000
14	–Me		–H	8100
15	–Me		–H	110
16	–Me		–H	64



Scheme 1. Generalized synthesis of 2-pyridin-3-yl-benzo[*d*][1,3]oxazin-4-ones. The positions of the substituents on the benzoxazinone ring are identified trivially with a B. Positions on the pyridin-3-yl group are identified trivially with a P.

methyl group of hit **1** was explored. The replacement of this methyl group with the isosteric chloro (compound **8**) or methoxy (compound **9**) resulted in HNE inhibitors of decreased potency relative to the hit **1** (2.6-fold and 6.4-fold, respectively). This SAR foray led us to conclude (a) the B2 pyridin-3-yl group was preferred over a B2 phenyl group (b) P2 substitution (vs P6 substitution or a H in the P2 position) yielded more potent inhibitors (c) the P2 position was tolerant of groups that varied considerably in size and (d) varying the B5 substituent could dramatically modulate HNE inhibitory activity.

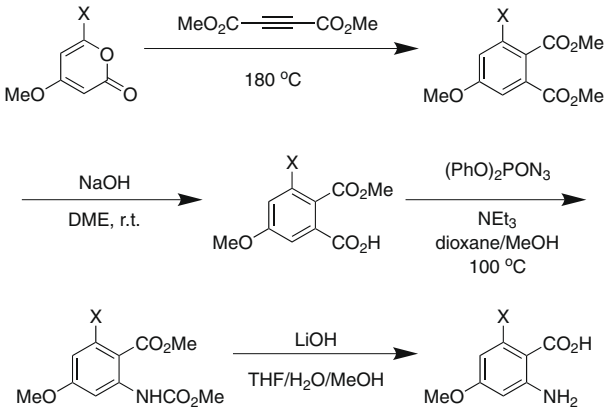
Replacement of the phenoxy group of the hit **1** with amines was also investigated using the route shown in Scheme 2. The condensation of 2-fluoro-nicotinic acid and 6-methyl-anthranilic acid according to Scheme 1 produced compound **10**. Subsequent displacement of the fluoride in a S_NAr fashion with aliphatic amines readily occurred at 100 °C in dioxane to yield compounds of the form **11** and **12**. Secondary amines gave the desired product **11** and only a trace to none of the tertiary amide **12**. By contrast, primary amines yielded the unwanted secondary amide **12** as the major product.



Scheme 2. Generalized synthesis of 5-methyl-2-(2-amino-pyridin-3-yl)-benzo[*d*]-[1,3]oxazin-4-one derivatives.

The use of the primary amines 4-(2-aminoethyl)morpholine or *N*,*N*-dimethylaminoethylenediamine as shown in Scheme 2 produced compounds **13** and **14**, respectively. Both compounds exhibited micromolar HNE IC_{50} values. However, when the secondary amine *N*,*N*,*N*′-trimethylethylenediamine was used, the resulting product **15** exhibited a 110 nM HNE IC_{50} value. A direct comparison between compounds **14** and **15**, which differ by a single N–Me group, led us to the preferred use of secondary amines versus primary amines in our efforts to design potent inhibitors. Interestingly, when 6-methyl-anthranilic acid was added to two equivalents of carbonyldiimidazole (CDI) activated 2-fluoro-nicotinic acid and the reaction was heated to 70 °C, compound **16** was obtained via fluoride displacement by imidazole, a byproduct of CDI activation. Compound **16** had an HNE IC_{50} value of 64 nM, a value similar to the hit **1**, and indicated that heteroaryl groups also were tolerated in the P2 position.

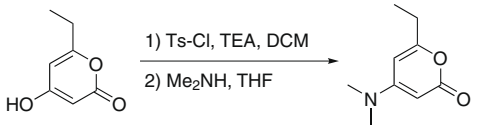
With the importance of a methyl group at the B5 position established, an effort was undertaken to evaluate the effect of other small alkyl and cycloalkyl groups in this same position. In parallel with this effort, electron donating groups were incorporated into the 7-position, a known strategy to improve the chemical stability of benzoxazinones.[3d] For these studies, the requisite anthranilic acids were synthesized using three different routes. 6-Ethyl-anthranilic acid was produced from the oxidative cleavage of 4-ethyl isatin.[7] 4-Methoxy anthranilic acids were synthesized (see Scheme 3) from the Diels–Alder reaction of 6-substituted 4-methoxy-2-pyrones[8] with dimethyl acetylenedicarboxylate followed by the regiospecific saponification of the resulting dimethyl phthalate with NaOH (1 M). The reaction of the resulting acid with diphenylphosphoryl azide in MeOH yielded the methylcarbamate protected amine via a Curtius rearrangement. We chose not to produce the free amine directly (by treating the intermediate isocyanate with water instead of MeOH) for the following reasons: Comparing the isolated yields of these two strategies, we found the yields of the carbamate to be higher. In addition, when choosing to store an intermediate, the methyl carbamate was found to be stable versus the 4-methoxy-anthranilic acids which decarboxylated upon storage. Ultimately, the carbamate could be deprotected using LiOH to yield the required 4-methoxy-6-substituted anthranilic



Scheme 3. Synthesis of 6-substituted 4-methoxy-anthranilic acids from 6-substituted 4-methoxy-2-pyrones.

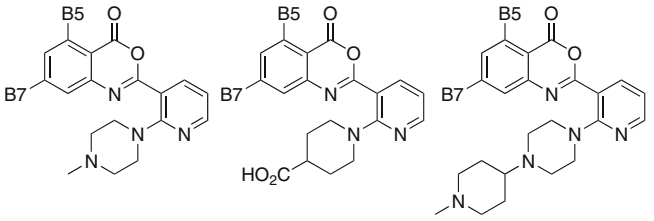
acids. Finally, the one-pot tosylation of 4-hydroxy-6-ethyl 2-pyrone followed by displacement of the intermediate tosyl group with dimethylamine yield 4-dimethylamino-6-ethyl-2-pyrone (Scheme 4). This 2-pyrone was then converted to 4-dimethylamino-6-ethyl-2-anthranilic acid as shown in Scheme 3.

The B5 and B7 positions of the benzoxazinone were shown to be important modulators of HNE inhibitor potency. Three benzoxazinone series, derivatized in the P2 position with *N*-methyl piperazine (Series A), isonipecotic acid (Series B), and 1-(*N*-methyl-piperidin-4-yl)piperazine (Series C) were examined. Starting with the same core present in hit **1** (B5 = methyl and B7 = H, compounds **17**–**19**) we found that despite the charge and steric differences present in these series, the HNE IC_{50} values remained close, ranging from 74 nM to 93 nM. Substituting the B5 methyl group with an ethyl group resulted in inhibitors with single digit nM IC_{50} values (**20–22**). Fixing this B5 ethyl group, we then examined the impact of placing electron donating groups in the B7 position on hydrolytic stability.[9] Previously, the addition of electron donating groups to the 7-position of benzoxazinones have been shown to enhance their hydrolytic stability by tempering the reactivity of the carbonyl to nucleophilic attack.[3d] Two electron donating groups were investigated, methoxy and dimethylamino, and both were shown to increase hydrolytic stability (Table 2, compare the $t_{1/2}$ values of compound sets **20–22** to **23**–**25** to **26**–**27**). While the dimethylamino containing inhibitors **26** and **27** exhibited the longest half-lives, they unfortunately also exhibited micromolar HNE IC_{50} values. In contrast, the 7-methoxy derivatives **23**–**25** yielded HNE IC_{50} values from 20 to 28 nM. In order to choose a benzoxazinone scaffold with a balance between hydrolytic stability and potency, we locked in on 7-methoxy derivatives while continuing to examine the B5 position. Ultimately, the 5-ethyl-7-methoxy scaffold was found to be optimal. Extending the B5 ethyl group of compounds **23**–**25** with *n*-propyl (**28–30**) or replacing the ethyl group with isopropyl (**31–33**), cyclopropyl (**34–36**), or cyclobutyl (**37–39**) resulted in HNE inhibitors with diminished potency by a factor that ranged from 3 to 46 relative to their ethyl congeners. In the one case when the B5 ethyl group was replaced with *tert*-butyl group, a 750-fold reduction in potency was observed (compare compounds **23** and **40**).



Scheme 4. Synthesis of 4-dimethylamino-6-ethyl-2-pyrone.

Table 2
HNE IC_{50} values for compounds **17–40** and half-life data in 200 mM sodium borate buffer, pH 9.2 for compounds **20**–**27**



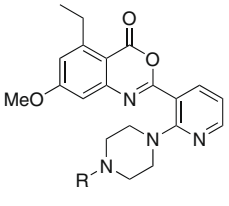
Compound	Series	B5	B7	HNE IC_{50} (nM)	Half-life pH 9.2 (min)
17	A	$-CH_3$	–H	93	n.d.
18	B	$-CH_3$	–H	79	n.d.
19	C	$-CH_3$	–H	74	n.d.
20	A	$-CH_2CH_3$	–H	8.1	65
21	B	$-CH_2CH_3$	–H	8.6	89
22	C	$-CH_2CH_3$	–H	8.5	56
23	A	$-CH_2CH_3$	–OMe	20	240
24	B	$-CH_2CH_3$	–OMe	28	1000
25	C	$-CH_2CH_3$	–OMe	28	1400
26	A	$-CH_2CH_3$	$-N(CH_3)_2$	5300	7300
27	C	$-CH_2CH_3$	$-N(CH_3)_2$	2600	6900
28	A	$-CH_2CH_2CH_3$	–OMe	250	n.d.
29	B	$-CH_2CH_2CH_3$	–OMe	180	n.d.
30	C	$-CH_2CH_2CH_3$	–OMe	71	n.d.
31	A	$-CH(CH_3)_2$	–OMe	210	n.d.
32	B	$-CH(CH_3)_2$	–OMe	330	n.d.
33	C	$-CH(CH_3)_2$	–OMe	140	n.d.
34	A		–OMe	120	n.d.
35	B		–OMe	140	n.d.
36	C		–OMe	110	n.d.
37	A		–OMe	150	n.d.
38	B		–OMe	1300	n.d.
39	C		–OMe	850	n.d.
40	A	$-C(CH_3)_3$	–OMe	15,000	n.d.

n.d. = not determined.

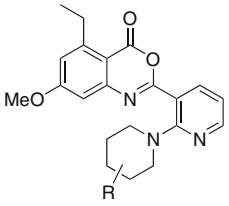
In anticipation of using these compounds in rodent efficacy models, the rat plasma stabilities of selected derivatives were determined[10] and shown to vary considerably based on structure (Table 3). All compounds examined contained the same, optimized 2-(pyridin-3-yl)-5-ethyl-7-methoxy-benzoxazinone core and differed by the amine used to derivatize the P2 position. Series D was derived from N-substituted piperazines and Series E was derived from carboxy-substituted piperidines. HNE IC_{50} values were also measured for these compounds and found not to vary more than a factor of two among the 14 compounds in Table 3. By contrast, plasma stability $t_{1/2}$ values varied considerably, from a high of 270 min for compound **41** to a low of 7.8 min for compound **52**. Structure–activity relationship information could be gleaned from this data set. Carboxy-substituted derivatives (compounds **24** and **41–43**) whether from series D or E were found to be among the most plasma-stable inhibitors. For series D, non-polar N-substituents of the piperazine ring were found to yield more stable inhibitors versus polar N-substituents (compounds **49–52**).

In conclusion, the synthesis and optimization of novel 2-pyridin-3-yl-benzo[*d*][1,3]oxazin-4-one derived HNE inhibitors have been described. From among the 5,7-substituted benzoxazinones

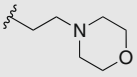
Table 3
Half-life in rat plasma and HNE IC_{50} values for compounds **23**, **24**, **41–52**



D



E

Compound	Series—R group		Rat plasma $t_{1/2}$ (min)	HNE IC_{50} (nM)
	D	**E**		
41	$-CH_2CO_2H$	–	270	23
42	—	(*R*)-3-CO_2H	150	19
43	—	(*S*)-3-CO_2H	140	25
44		—	90	34
23	$-CH_3$	—	84	20
24	—	4-CO_2H	81	28
45		—	69	28
46	$-(CH_2)_2OCH_3$	—	52	16
47	$-(CH_2)_3CH_3$	—	51	27
48	$-CH_2CH(CH_3)_2$	—	48	21
49		—	19	22
50	$-(CH_2)_2OH$	—	17	16
51	$-(CH_2)_2CN$	—	11	24
52	$-(CH_2)_2N(CH_3)_2$	—	7.8	21

examined, the 5-ethyl-7-methoxy scaffold was found to have the best balance of chemical stability and potency. Furthermore, using this optimized core derivatization of the P2 position with various piperazines and piperidines resulted in inhibitors with very different rat plasma stabilities. This data indicates that the P2 position can be modified to modulate biochemical properties. Studies on the cell permeability of these novel HNE inhibitors as well as their efficacy in lung injury models will be reported in due course.

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6. Human sputum neutrophil elastase (Elastin Products Co.) was diluted into Assay Buffer A (200 mM Tris pH 7.4, 1 mg/ml BSA) to a working concentration of 0.55 U/ml. Inhibitors dissolved and diluted in DMSO at 50× were added to the elastase in Assay Buffer A at final concentrations ranging from 1×10^{-4} M to 7×10^{-12} M and preincubated for 20 min at room temperature. DMSO alone was used as the negative control. MeOSuc-AAPV-AMC (Bachem) substrate was dissolved in DMSO to 20 mM and further diluted to 1 mM in Assay Buffer A immediately before use. Substrate was added to the elastase assay at a final concentration of 1×10^{-4} M. The reaction was allowed to proceed for 20 min at room temperature and then quenched with acetic acid at a final concentration of 3% (v/v). The AMC fluorescence was measured using a Wallac (Perkin Elmer) Victor2 plate reader equipped with excitation/emission filters of 355/460 nm. Fluorescence intensity versus inhibitor concentration was plotted and fit to the Hill equation to quantify IC_{50} values.
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8. 4-Hydroxy-2-pyrones were readily prepared using a modification of the Katritzky method in which 2,2,6-trimethyl-1,3-dioxin-4-one was acylated using short chain alkyl or cycloalkyl acid chlorides. Subsequent O-methylation with dimethylsulfate yielded the 4-methoxy-pyrones. See: (a) Katritzky, A. R.; Wang, Z.; Wang, M.; Hall, C. D.; Suzuki, K. *J. Org. Chem.* **2005**, *70*, 4854; (b) Deshpande, V. H.; Khan, R. A.; Ayyanagar, N. R. *Indian J. Chem.* **1996**, *35B*, 790.
9. Alkaline hydrolysis rates were measured by the disappearance of the characteristic absorbance maximum for the intact benzoxazinone ring (340 nm ± 10 nm) in 200 mM, pH 9.2 sodium borate and 1% DMSO. Hydrolysis rates were determined through linear regression analysis of the progress curves and then converted to half-life data. See: Stein, R. L.; Strimpler, A. M.; Viscarello, B. R.; Wildonger, R. A.; Mauger, R. C.; Trainor, D. A. *Biochemistry* **1987**, *26*, 4126.
10. Test compounds (2 μM) were incubated in heparin treated rat plasma at 37 °C. Following precipitation of the plasma proteins with acetonitrile, the concentration of test compound was quantified against an internal standard using ESI-MS. Half-life data was calculated from a fit of compound concentration versus time to a first order rate constant.