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Selective Heterocyclic Amidine Inhibitors of Human Inducible Nitric Oxide Synthase

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Abstract—The potency and selectivity of a series of 5-hetero-2-iminohexahydroazepines were examined as inhibitors of the three human NOS isoforms. The effect of ring substitution of the 5-carbon for a heteroatom is presented. Potencies (IC_{50}'s) for these inhibitors are in the low micromolar range for hi-NOS with some examples exhibiting a 500× selectivity versus hec-NOS.

The production of nitric oxide (NO) from the induced expression of i-NOS has been shown to play a role in many pathological processes, while the constitutive production of NO from endothelial NOS (e-NOS) plays a role in the regulation of vascular smooth muscle tone. Therefore, the selective inhibition of i-NOS versus e-NOS is desirable and such a selective inhibitor could find utility for controlling many pathological states.[1–3]

The cyclic amidine, **1** (Fig. 1) and its carbon homologues exhibit binding selectivity in favor of i-NOS.[4–7] Molecular modeling of **1** in the active site of murine i-NOS, suggested that the 5-carbon of the amidine ring is in close proximity to the heme iron of the enzyme. Heteroatoms were incorporated into the ring at the 5-position in an attempt to increase potency and selectivity. The 5-carbon was replaced by heteroatoms, compounds **2**–**4**, in an attempt to interact with the enzyme's heme iron. The heteroatom can alter the sterics, bond lengths, angles, and solvation.

The carbon analogues chosen for this study were easily synthesized cyclic amidines with good potency and selectivity. These carbon analogues were compared to the corresponding analogue with the heteroatom incorporated into the 5-position of the ring.

The compounds were screened for binding against the human isoforms of NOS.[8]

The compounds where X = CH_2, O, and NMe were synthesized, as shown in Scheme 1. The ketone **5** was converted to the oxime **6**. Beckmann rearrangement of oxime **6** produced a 3:7 mixture of lactams **7** and **8**. When the desired lactam **8** did not crystallize, the lactams were isolated using silica chromatography, eluting with MeCN/EtOAc, lactam **7** eluting first. The lactam **8** was converted first to the iminoether **9** using Meerwein's reagent, then to the amidines **1**–**3**, **10**–**15** with NH_4Cl in refluxing EtOH. Compounds **16** and **17** were synthesized by reduction of the olefins **12** and **13**, respectively. Compounds **18** and **19** were synthesized by reduction of the nitro group of compounds **14** and **15**, respectively.

Because Meerwein's reagent was found to alkylate the sulfur, the sulfur heterocycles were synthesized as shown

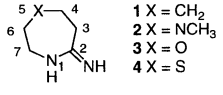

Figure 1.

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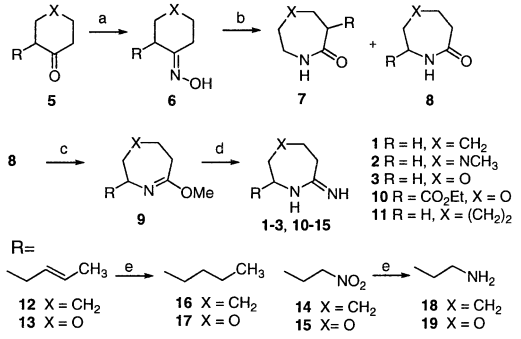
in Scheme 2. The seven-member ring was synthesized as follows: a Michael addition of *l*-cysteine ethyl ester **20** to acrylonitrile in DMF produced the nitrile **21**. This nitrile was stirred with EtOH saturated with HCl at 0 °C to form the iminoether **22**, which cyclized to the cyclic amidine **23** upon treatment with Amberlyst A-21 ion exchange resin. Compound **24** was synthesized in like manner from *d*-cysteine ethyl ester. The seven-member ring **4** was synthesized in like manner starting with 2-aminoethanethiol. The eight-member ring **28** was synthesized as follows: bromide **26** was alkylated with the sodium salt of 3-thiopropionitrile[9] then refluxed with hydrazine hydrate in EtOH to produce the aminonitrile **27**. This nitrile was converted with HCl/EtOH to the iminoether followed by treatment with Amberlyst A-21 ion exchange resin to produce the cyclic amidine **28**.

As shown in Table 1, nitrogen substitution, **2**, produced a compound with greatly diminished activity. The energy to desolvate the amine and the steric bulk of the methyl probably prevents the molecule from entering the active site.



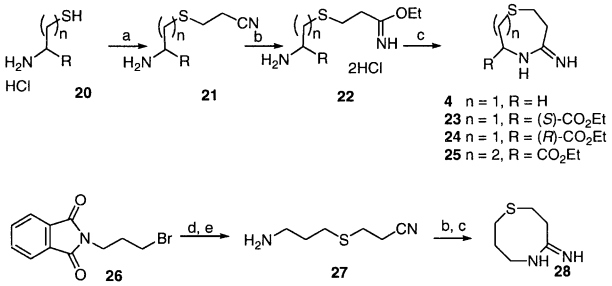
Scheme 1. (a) NH_2OH*HCl/NaHCO in EtOH; (b) benzene sulfonylchloride (1.1 equiv)/2.5 N NaOH (1.1 equiv) in acetone at 0 °C warming to ambient temperature for 18 h. Column eluting with MeCN/EtOAc; (c) Me_3OBF_4/CH_2Cl_2 18 h at ambient temperature; quenched with satd $NaHCO_3$; (d) 1.0 equiv NH_4Cl refluxed in EtOH for 18 h; (e) 10% Pd/C–H_2 in EtOH.

Incorporation of oxygen (**3**, **13**, **15**, **17**, and **19**) did not increase potency, but did increase the selectivity. Compound **17** is noteworthy in that the increase in selectivity of i-NOS versus e-NOS is 560 times, and versus n-NOS is 10.6 times.

Incorporation of sulfur (**4**, **23**, **24**, **25**, and **28**) produced mixed results. Compound **4**, when compared to the carbon analogue **1**, exhibited no difference in i-NOS potency, but a modest increase in selectivity versus e-NOS was observed. The 7-ethyl carboxylate analogues, **23** and **24**, produced compounds comparable in potency to the carbon analogue, **10**. The enantiomers were compared: the *S*-isomer (**23**) exhibited potency comparable to the *R*-isomer (**24**) and exhibited slightly better selectivity for i-NOS versus e-NOS.

The eight-member ring analogue **11**, was modestly active. The sulfur analogue, **28**, lost some potency, but did exhibit an increased selectivity for i-NOS versus e-NOS. The 8-ethyl carboxylate analogue, **25**, lost potency.

Incorporation of the heteroatom did not increase the potency toward i-NOS when compared to the



Scheme 2. (a) Ethyl *l*-cysteine (1 equiv)/acrylonitrile (2 equiv)/K_2CO_3 (1.1 equiv) in DMF; (b) EtOH saturated with HCl at 0 °C; **21** added and warmed to ambient temperature; concentrated to dryness; (c) Amberlyst A-21 (3 equiv) in EtOH; (d) sodium-3-thiopropionitrile[9] in EtOH; (e) hydrazine hydrate (1.1 equiv) refluxed in EtOH.

Table 1. SAR for the heterocyclic amidines[a]

Compd	i-NOS IC_{50}, μM	e-NOS IC_{50}, μM (Selectivity)	n-NOS IC_{50}, μM (Selectivity)
1	2.06	15.25 (7.4)	3.5 (1.7)
2	32.1% @100 μM	6.42% @100 μM	25.89% @100 μM
3	14.03	72.68 (5.2)	17.74 (1.2)
4	2.7	43.6 (16.1)	6.8 (2.5)
10	74.9% @100 μM	11.45% @100 μM	71.4% @100 μM
11	9.98	58.9 (5.9)	10.15 (1.01)
12	0.89	205.9 (230)	3.39 (3.8)
13	9.26	709 (76)	108 (11.6)
14	0.152	2.77 (18.2)	0.339 (2.2)
15	0.979	49.3 (50.3)	6.24 (6.4)
16	0.517	102.5 (198)	0.85 (1.6)
17	2.43	1363 (561)	25.9 (10.6)
18	0.701	39.07 (55.7)	0.932 (1.3)
19	6.66	247.8 (37.2)	8.39 (1.2)
23	70.9% @100 μM	13.9% @100 μM	82.6% @100 μM
24	70.9% @100 μM	8.66% @100 μM	84.78% @100 μM
25	55.7% @100 μM	2.4% @100 μM	13.9% @100 μM
28	13.1	186.3 (14.2)	21.0 (1.6)

[a]The enzyme binding was performed as described in ref 8.

corresponding carbon analogue. The lack of enhanced potency implies that the heme iron does not interact with the heteroatom.

The incorporation of the oxygen or sulfur atom, in some cases, increased the selectivity of i-NOS versus e-NOS when compared to the corresponding carbon analogue. In the one occurrence for a direct comparison, sulfur **3** was more selective than oxygen **4** and carbon **1**. One interpretation of this increase in selectivity is an overall conformational or steric change caused by the sulfur atom containing analogue, which favors the i-NOS enzyme versus the e-NOS enzyme.

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9. Synthesis of the sodium salt of 3-thiopropionitrile: 3-bromopropionitrile (13.3 g, 0.1 mol) and potassium thioacetate (14.25 g, 0.125 mol) was dissolved in DMF (125 mL) and stirred for 16 h at ambient temperature. The reaction mixture was quenched with brine (700 mL) and the product extracted 3× Et_2O (200 mL) then hexane (200 mL). The combined organic extracts were washed with brine, dried over $MgSO_4$, and concentrated. The residue was chromatographed over silica, eluting with EtOAc/hexane to yield 10.8 g of the *S*-3-acetylthiopropionitrile. C_5H_7NOS (MW 129.17); MS: $M + NH_4$ @ 147. *S*-3-Acetylthiopropionitrile (5.8 g; 0.045 mol) was dissolved in MeOH (100 mL) and H_2SO_4 (10 drops) was stirred for 2 days. The reaction mixture was quenched at 0 °C with NaOMe prepared from Na metal (1.03 g; 0.45 mol) and MeOH (30 mL). The resulting mixture was concentrated and used as is.