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Bioorganic & Medicinal Chemistry Letters 14 (2004) 5907–5911
Bioorganic & Medicinal Chemistry Letters

Synthesis of analogs of (1,4)-3- and 5-imino oxazepane, thiazepane, and diazepane as inhibitors of nitric oxide synthases☆

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Received 14 July 2004; revised 8 September 2004; accepted 8 September 2004
Available online 30 September 2004

Abstract—A series of 3- and 5-imino analogs from oxazepane, thiazepane, and diazepane was prepared and evaluated as inhibitors of human nitric oxide synthesis (NOS). The most potent iNOS inhibitor was the thiazepane analog **25** (IC_{50} = 0.19 μM).

1. Introduction

The isozymes of nitric oxide synthase (NOS) produce nitric oxide (NO), a reactive inorganic radical gas, which plays central role in physiological functions in various mammalian tissues.[2–5] All three NOS enzymes bring about a five electron oxidation of L-arginine to NO and L-citrulline, as shown in Figure 1. To date three isoforms of NOS enzymes have been identified. Of these, two isoforms are constitutive and are implicated in the cardiovascular and neuronal activities (eNOS and nNOS). The third isoform, which is linked with host defense, is induced by inflammatory cytokines and hence the name iNOS. Unwarranted production of nitric oxide by iNOS has been associated with tissue damage leading to inflammatory diseases such as arthritis and inflammatory bowl disease. Thus selective inhibition of iNOS may offer possible treatments for such diseases.

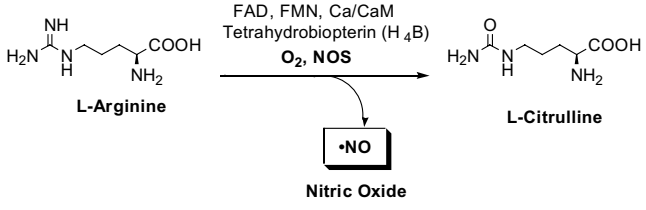

Figure 1. Nitric oxide biosynthesis pathway.

Efforts toward NOS inhibitors have led to amino acids as well as nonaminoacid based inhibitors, including aminoguanidines, isothioureas, amidines benzoxazolones, and 2-amino-pyridines, respectively.[6] Previously, pyrrolidin-2-imines[7a,8] and piperidin-2-imines[9] as inhibitors of NOS displaying selectivity to varying degrees has been reported (Fig. 2). In an obvious extension from the pyrrolidinyl and piperidinyl-2-imines, the analogous of 3- and 5-imino derivatives of oxazepane, thiazepane, and diazepane were prepared (Fig. 2).[7b] The synthesis and SAR of these classes of inhibitors are the subject of this report.

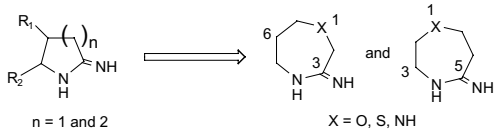

Figure 2.

Keyword: NOS synthase.
☆See Ref. 1.
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doi:10.1016/j.bmcl.2004.09.019

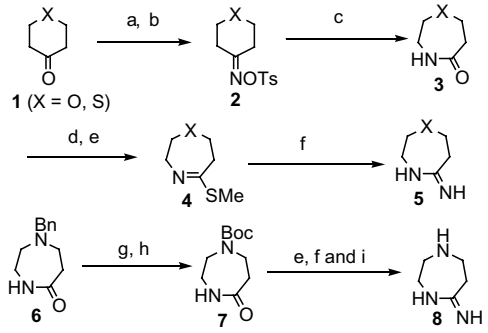
2. Chemistry

The preparation of unsubstituted 5-imino analogs of oxazepane, thiazepane, and diazepane described herein are shown in Scheme 1. In an identical sequence of reactions both tetrahydropyran-4-one (X = O) and tetrahydrothiopyran-4-one (X = S) (**1**) were subjected to oximation followed by tosylation to give the desired tosylates (**2**). These tosylates (**2**) undergo smooth Beckmann rearrangement[10] to afford the amides **3**. The amides **3** were transformed to the thioethers **4** in two steps consisting of initial formation of the thioamide[11] from **3** that was followed by S-alkylation in the subsequent step. The thioethers **4** on reflux with ammonium chloride gave the amidines **5**. The preparation of diazepane **8** took a different route starting from the known amide **6**. Removal of the benzyl protecting group at nitrogen on **6** followed by reprotection gave **7**. The transformation of **7** to the desired amidine **8** was accomplished in three steps that involve formation of iminoether, amidine, and then eventually *N*-Boc removal to afford **8** as shown below.

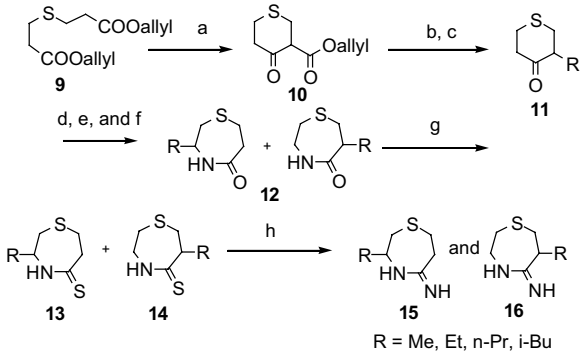
The synthesis of C-3 and C-6 substituted analogs of (1,4)-5-imino thiazepanes are shown in Scheme 2. The bis-allylester **9** was transformed to **11** in three steps involving the Dieckmann cyclization[12] of **9** to afford **10** that was followed by its alkylation and the eventual removal of allyloxycarbonyl moiety to furnish the tetrahydrothiopyran-4-one (**11**). Transformation of **11** to the oxime followed by the Beckmann rearrangement of the resultant oxime tosylate gave a mixture of two amides (**12**), that could not be separated. The mixture of **12** was converted to the thioamides **13** and **14**, at which point they could be separated by flash chromatography. The separated thioamides **13** and **14** were then transformed individually to their respective amidines **15** and **16** in a sequences of reaction similar to the conversion **3** to **5** described in Scheme 1.

The preparation of C-2 and C-7-methyl analogs of (1,4)-5-imino thiazepanes **19** and **20** were carried out as in Scheme 3. Oxidation of the β-ketoester **10** to the enone **17** was accomplished under active MnO_2 conditions as reported in the literature.[13] The enone **17** underwent smooth Cu-catalyzed 1,4-addition of MeLi followed by Pd-mediated removal of the allyloxycarbonyl in subsequent step to give **18**. The conversion of **18** to the desired amidines **19** and **20** was analogous to Scheme 1.

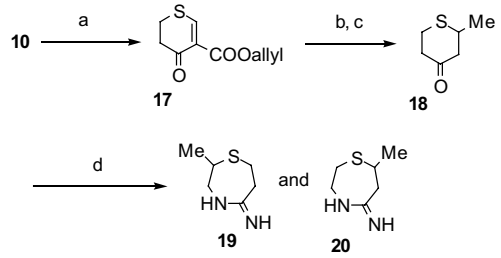
The preparation of homochiral 3-(*S*)-propyl-(1,4)-5-imino thiazepane **25** is shown in Scheme 4. The readily available *N*-Boc alcohol **21** undergoes facile aziridation[14] under Mitsunobu protocol giving **22**. The nucleophilic ring opening of **22** by β-mercapto propionic acid gave the desired acid, that was subsequently deprotected



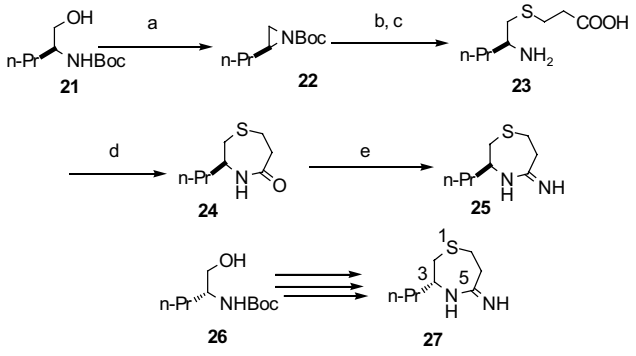
Scheme 1. Reagents and conditions: (a) NH_2OH, Py; (b) *n*-BuLi, TsCl, THF, −78 °C to rt; (c) dioxane, NEt_3, rt; (d) Lawesson's regent, Tol., 90 °C; (e) Me_3OBF_4, CH_2Cl_2; (f) NH_4Cl, EtOH, reflux; (g) $Pd(OH)_2$, H_2, EtOH, rt; (h) Boc_2O, CH_2Cl_2, rt; (i) HCl, EtOAc, rt.



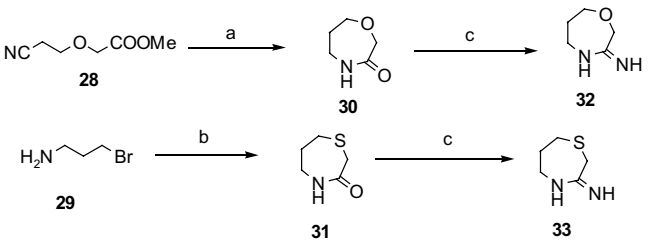
Scheme 2. Reagents and conditions: (a) NaH, ether, allyl alcohol; (b) NaH, DMF, RI; (c) $Pd(PPh_3)_4$, THF, morpholine; (d) NH_2OH, Py; (e) *n*-BuLi, TsCl, THF, −78 °C to rt; (f) dioxane, NEt_3, rt; (g) Lawesson's regent, Tol., 90 °C; (h) Scheme 1 and steps e and f.



Scheme 3. Reagents and conditions: (a) active MnO_2, $CHCl_3$, rt; (b) MeLi, CuI–Me_2S, ether, −78 °C to rt; (c) $Pd(PPh_3)_4$, THF, morpholine; (d) Scheme 1 and steps a–f.



Scheme 4. Reagents and conditions: (a) PPh_3, THF, DIAD, rt; (b) β-mercaptopropionic acid, Cs_2CO_3, DMF, 60 °C; (c) EtOAc, HCl (g), rt; (d) EDC·HCl, DMF, NMM, rt; (e) Scheme 1 and steps d–f.



Scheme 5. Reagents and conditions: (a) RaNi, H_2, 1000 psi, 100 °C; (b) NaOEt, $HSCH_2COOEt$, EtOH; (c) Scheme 1.

to the amino acid **23**.[15] Transformation of **23** to the lactam **24** was accomplished using EDC, *N*-methylmorpholine in DMF. Conversion of **24** to the amidine **25** was analogous to Scheme 1. A similar sequence of reactions was in employment to transform **26** to the enantiomeric 3-(*R*)-propyl-(1,4)-5-imino thiazepane amidine **27**.

The preparation of isomeric analogs of (1,4)-3-imino oxazepane (**32**) from **28** and the thiazepane (**33**) from **29** are shown in Scheme 5. This involved routine chemistry leading to the preparation of intermediate lactams **30** and **31** that was followed by steps given under Scheme 1 to give **32** and **33**.

3. Results and discussion

The hydrochloride salts of synthesized (1,4)-3- and 5-imino analogs were evaluated for NOS binding activity in an assay protocol that used recombinant versions of the human enzymes.[16] Results tabulated below describing the SAR for this class of inhibitors were compared with *N*-iminoethyl lysine (L-NIL) and primarily centered on the placement of substituents at all available position of the heterocycles. The IC_{50} from the parent heterocycles are shown in Table 1. In the (1,4)-5-imine class, clearly the thiazepane analog **35** was relatively more potent and also had a better selectivity than the oxazepane analog **34**, while the diazepane analog **8** was inactive ($IC_{50} > 50\,\mu M$). The binding results for the (1,4)-3-imine analogs **32** and **33** are also displayed in Table 1. The relative comparison of the oxazepane analogs **34** and **32** suggested that the later was significantly more potent for the all three isoforms but neither displayed selectivity. Interestingly, the thiazepane analogs **35** and **33** were both equipotent and, unlike their oxazepane counterparts displayed slightly better selectivity. Since the selectivity data for both thiazepanes (**33** and **35**) appeared better, the effects of substituents on (1,4)-5-imino thiazepane (**35**) in order to enhance its potency and also to increase the selectivity for the iNOS over the other isoforms was explored. The assay results of these studies were compared with **35** and are shown in Table 2.

The thiazepane analog **36** with methyl at C-2 resulted in 2-folds loss of potency for iNOS compared to **35** and gain in the potency for other isoforms. The placement of methyl at C-3 (**37**) led to a gain in some potency for the all three isoforms. Analog **38** with a methyl at C-6 resulted in a substantial loss versus all isoforms, especially for the iNOS and eNOS, respectively. The presence of a methyl substituent at C-7 (**39**) gave an analog that was as potent as **35** or **36**. Since the presence of methyl at C-3 generated more potent analog (**37**), further alterations were carried out at this position that led to the preparation of additional analogs bearing linear and branched alkyls, as in **40**–**45**. The C-3 hydroxymethyl and bicyclic analogs **40** and **41** were unimpressive in that the former displayed no improvement compared to **37** while the later lost all activity. A 2–3-fold gain in the iNOS potency (compared to **37**) was observed in the analogs **42**–**45**. In general the gain in the selectivity over eNOS was substantial for these

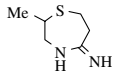
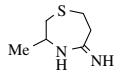
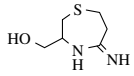
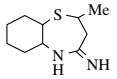
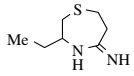
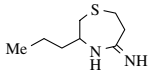
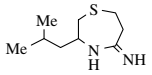
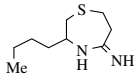
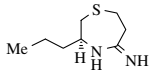
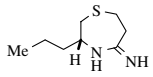
Table 1. NOS inhibition by (1,4)-3- and 5-imino oxazepane, thiazepane, and diazepane[a]

#	IC_{50} (μM)					
	Structure[b]	iNOS	eNOS	Selectivity eNOS/iNOS	nNOS	Selectivity eNOS/iNOS
—	L-NIL	2.1	7.9	3.7	17.5	8.3
8		>50	>50	ND	>50	ND
32		0.16	0.45	2.8	0.16	1
33		1.2	10.5	8.8	2.8	2.3
34		11.8	25	2.1	8.7	0.7
35		1.4	13.4	9.5	4.3	3

[a] IC_{50}s values are obtained from a 10 point titration using Sigma Plot, where each individual point is an average of duplicate determinations at that concentration.

[b] The analogs in Table 1 were synthesized according to Schemes 1 and 5, ND = not determined, L-NIL = L-*N*-iminoethyl lysine.

Table 2. NOS inhibition by substituted (1,4)-5-imino thiazepanes[a]

#	IC_{50} (μM)					
	Structure[b]	iNOS	eNOS	Selectivity eNOS/iNOS	nNOS	Selectivity eNOS/iNOS
35		1.4	13.4	9.5	4.3	3.0
36		3.5	4.1	1.1	0.83	0.24
37		0.81	5.0	6.1	1.8	2.2
38		>50	>50	ND	>50	ND
39		3.0	5.0	1.6	1	0.3
40		1.1	6	5.3	2.7	2.4
41		>50	>50	ND	>50	ND
42		0.31	1.1	3.7	0.59	2
43		0.29	50	172	2.8	10
44		0.32	60	186	1.9	6
45		0.5	77	164	1.2	2.6
25		0.19	1.0	5.2	0.44	2.3
27		1.2	50	42	7.1	6

[a] IC_{50}s values are obtained from a 10 point titration using Sigma Plot, where each individual point is an average of duplicate determinations at that concentration.

[b] In Table 2 the analogs were synthesized according to Schemes 2 and 3 (compounds **35**–**39**) and Scheme 4 (compounds **40**–**45** and including **25** and **27**), ND = not determined.

analogs while the selectivity over the nNOS was modest. As the *n*-propyl analog **43** was among the best, it was probed further to broaden the scope of these observations.

Since **43** was racemic, the chiral synthesis of enantiomers **25** and **27** were undertaken to see if an additional potency/selectivity are to be gained. Table 2 reveals that *S*-isomer (**25**) displayed 6-fold more potency for the iNOS compared to the (*R*)-isomer **27**.

Paradoxically, the selectivity (over eNOS) data for the **25** was modest in comparison to **27**. Interestingly, the selectivity for neither compound matched that of the racemic analog **43** or other analogs displayed in Table 2.

In conclusion, limited SAR studies described herein established the (1,4)-5-imino thiazepanes as potent and selective iNOS inhibitors. We have also shown that iNOS potency can be improved by the placement of *n*-propyl substituent at C-3 of thiazepane and that the analog **25** was more potent and **27** was more selective. Our observations described herein is similar to an analogous studies conducted previously on these systems by Mooremann et al.[7b] Nevertheless studies described herein when compared with the earlier observations suggest

that the pyrrolidin-2-imine[6h] or piperidin-2-imine[6h,9] were significantly more potent and had a more balanced inhibitory profile than the analogs described herein.

References and notes

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14. (a) Pfister, J. R. *Synthesis* **1984**, 969; (b) Mitsunobu, O. *Synthesis* **1981**, *1*; and also see Hughes, D. L. *Org. React.* **1992**, *42*, 335.
15. The crude acid **23** was taken as is to the next step to afford lactam **24** whose purification was facile.
16. The NOS inhibitory activity of the compound was determined by comparing the conversion of ^{3}H-L-arginine to ^{3}H-citrulline in the presence of inhibitor with control. The assay mixture containing 1 μM of ^{3}H-L-arginine, cofactors and the inhibitor or aqueous DMSO (control) was incubated for 30 min at room temperature. The reaction was quenched by adding a slurry of Dowex 50W-K8 resin to complex and remove the unreacted substrate. The concentration of the ^{3}H-L-citrulline product in the supernatant fluid was determined on a scintillation counter. Under the assay conditions the production of L-citrulline was linear with time for the duration of the experiment.