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Evaluation of pyrrolidin-2-imines and 1,3-thiazolidin-2-imines as inhibitors of nitric oxide synthase ☆

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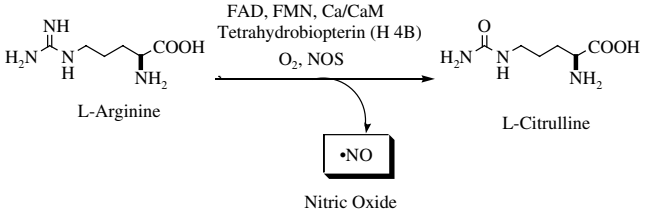
Abstract—Syntheses and evaluation of pyrrolidin-2-imines and 1,3-thiazolidin-2-imines as inhibitors of nitric oxide synthase (NOS) are discussed. An extensive SAR was established for pyrrolidin-2-imines class of compounds. The amidines came out as the most potent inhibitors in addition to displaying selectivity.

1. Introduction

Nitric oxide synthases (NOS) are a group of oxidative enzymes, which produce nitric oxide (NO) from L-arginine.[2] These enzymes bring about a five electron oxidation of L-arginine to nitric oxide and L-citrulline as shown below.



To date three isoform of NOS enzymes have been discovered. Of these, the two constitutive versions namely, neuronal (nNOS or NOS1) and endothelial (eNOS or NOS3) isoform are involved in neurotransmission, and vasodilatation, respectively.[3] The inducible form of nitric oxide synthase (iNOS or NOS2) is concerned with host defense, septic shock, and inflammatory diseases.[4] Thus, selective inhibition of iNOS over the other isoforms may offer a new therapy for these diseases. Pursuit of NOS inhibitors has lead to amino acid analogs[5] as well as nonamino acid based inhibitors including aminoguanidines,[6] isothioureas,[7] and amidines,[8] benzoxazolones,[9] and pyridines.[10] A recent communication[11a] disclosed that the analogs of 1,3-oxazolidin-2-imine derivatives (**2**) were shown to be inhibitors of the NOS enzymes prompted us to communicate our findings in these area.

We had previously reported that piperidin-2-imines (**1**) are potent inhibitors[12] of NOS enzymes displaying varying degree of selectivity. In parallel efforts we[1] and others studied pyrrolidin-2-imines as NOS inhibitors and toward this end Hagen and co-workers has already disclosed their results.[11b] In addition to pyrrolidin-2-imines we also probed the related thiazolidin-2-imines and it was hoped that these classes (**3**) might provide selective iNOS inhibitors. The results of such a study are disclosed herein. While work related to the 1,3-thiazolidin-2-imines lead class was minimal in scope, a detailed structure–activity relationship was established for the pyrrolidin-2-imines.

Keywords: NOS; Pyrrolidin-2-imine; 1,3-Thiazolidin-2-imine.
☆ See Ref. 1.
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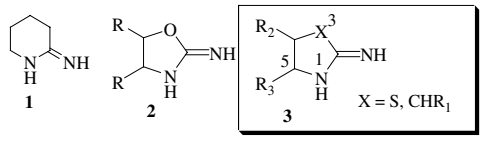
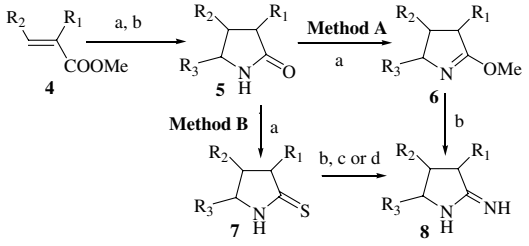


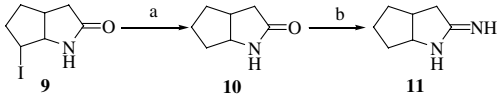
2. Chemistry

Scheme 1 shows the preparation of mono- and disubstituted pyrrolidin-2-imines essentially in three steps. The first two steps consists of Michael addition of various nitroalkanes to the acrylates **4** followed by the catalytic hydrogenation of the intermediate nitroesters to give the lactams **5**.[13] The lactams **5** were transformed to the desired amidines following one of the several conditions shown below. Method A, which involved the conversion of **5** to the iminoether **6** followed by reflux with ammonium chloride in ethanol to furnish **8**, as a mixture of stereoisomers was the first choice. In some incidences, the iminoether **6** was too volatile to be isolated and for these systems method B was preferred. This required the initial conversion of the amides **5** to the thioamides **7** by Lawesson's reagent.[14] The thioamides **7** were then quaternized with excess iodomethane followed by reaction with methanolic ammonia to afford the amidines **8** as a hydroiodide salt. Alternatively, the thioamides **7** could be directly converted to the amidines **8** in a procedure utilizing mercuric chloride and gaseous ammonia as reported in the literature.[15]

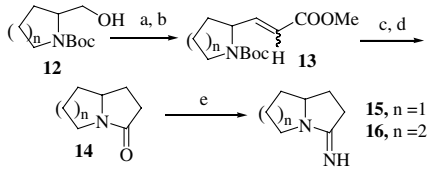
The preparation of fused amidine **11** from the known[16] iodolactam **9** is shown in Scheme 2. The reduction of **9** to **10** was efficiently accomplished under tinhydride conditions. The transformation of **10** to the amidine **11** was analogous to the transformation **5** to **8** (method B) shown in Scheme 1.



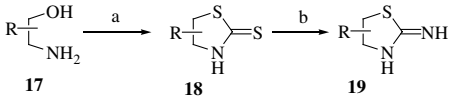
Scheme 1. Reagents and conditions: (a) $R_3CH_2NO_2$, DBU, MeOH; (b) PtO_2, H_2, EtOH; (c) *Method A* (a) Me_3OBF_4, CH_2Cl_2, rt; (b) NH_4Cl, EtOH, reflux; *Method B* (a) Lawesson's reagent, toluene, 90 °C; (b) MeI, rt; (c) NH_3, MeOH; (d) NH_3, $HgCl_2$, THF, 60 °C.



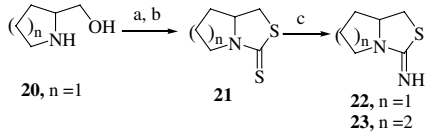
Scheme 2. Reagents and conditions: (a) *n*-Bu_3SnH, AIBN, PhH reflux; (b) *Method B*, steps (a) and (d) (Scheme 1).



Scheme 3. Reagents and conditions: (a) Oxalyl chloride, DMSO, DCM, NEt_3; (b) $Ph_3P{=}CHCOOMe$, DCM, rt; (c) Pd/C, H_2, MeOH; (d) HCl and then K_2CO_3; (e) *Method B*, steps (a), (b), and (c) (Scheme 1).



Scheme 4. Reagents and conditions: (a) KOH, CS_2, EtOH reflux; (b) *Method A*, steps (a) and (b) (Scheme 1).



Scheme 5. Reagents and conditions: (a) $SOCl_2$, $CHCl_3$, reflux; (b) NEt_3, CS_2, CH_2Cl_2; (c) *Method B*, steps (b) and (c) (Scheme 1).

The fused amidine **15** was prepared from *N*-Boc protected aminol **12** as shown in Scheme 3. In a sequence of reaction that involved Swern oxidation of **12** followed by the Wittig–Horner reaction gave a mixtures of esters **13**. Catalytic reduction of **13** followed by *N*-Boc removal led to a rapid cyclization affording **14**. The amide **14** was transformed to **15** by the method B as in Scheme 1. Likewise, starting from the corresponding piperidinol and following the same procedure for **15** gave **16** ($n = 2$).

A general synthesis of 1,3-thiazolidin-2-imines is shown in Scheme 4. This involved the reaction of aminol **17** and carbon disulfide under basic conditions[17] to afford **18**. The thiazolidine-2-thiones **18** was transformed to 1,3-thiazolidin-2-imines **19** in two steps that involved initial reaction with Meerwein salt (to afford thioiminoether) that was followed by refluxing with ammonium chloride (Scheme 1, method A).

The bicyclic 1,3-thiozol-3-imine analogs **22** and **23** were accessed by a similar approach shown above for **19**, but with a slight variation as shown in Scheme 5. A two step reaction that involved the transformation of aminol **20** to the intermediate chloro compound followed by reaction with CS_2/triethylamine gave **21**. The thione **21** was transformed to **22** by method B. Similarly, starting from 2-piperidinemethanol and following the aforesaid steps gave **23**.

3. Results and discussion

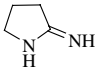
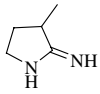
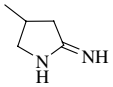
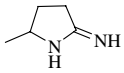
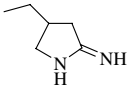
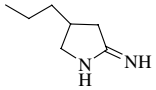
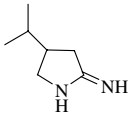
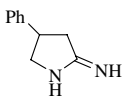
The hydrochloride/hydroiodide salts of pyrrolidine-2-imines and 1,3-thiazolidin-2-imines were evaluated for

NOS activity by an assay protocol[18] that used recombinant human version of all three isoform of NOS. The binding results for both the pyrrolidin-2-imines and 1,3-thiazolidin-2-imines classes are compared with the standard (L)-*N*-iminoethyl-lysine (L-NIL). The SAR studies on the pyrrolidin-2-imines analogs **8** centered on the placement of alkyl substituents at all the available positions of pyrrolidine core. The results from such a study, compared to the parent amidine (**8a**) are displayed in Table 1. The analog (**8c**) with the methyl group at C-4 of the pyrrolidine ring lead to significant improvement in the binding potency for the iNOS (20-folds) and nNOS (12-folds) as compared to **8a**. Compounds **8b** and **8d** bearing the methyl at C-3 and C-5 showed a modest 2- to 3-folds improvement in potency for iNOS, however these compounds (**8b–d**) displayed no selectivity. In probing other alkyl residues at C-4, it became clear that the amidine analog **8e** (with ethyl at C-4) led to somewhat less potent compound compared to **8c**. More importantly, **8e** displayed better selectivity for iNOS (over eNOS) while the selectivity over nNOS was not as good. The gain in potency for **8e** led to the preparation of other analogs **8f–h**. The *n*-propyl analog **8f** had lost significant activity compared to either **8c** or **8e**. This was also true for **8g** but it still maintained good selectivity for iNOS over eNOS. Finally, the compound with phenyl at C-4 (**8h**) was inactive.

An observation (from Table 1) that the amidine **8e** was not only potent (iNOS) but also displayed modest selectivity encouraged us to prepare analogs of **8e** with additional substituents on pyrrolidine core to improve its profile further. The results of binding data from such a study compared with **8e** are shown in Table 2.

The placement of alkyl substituents at C-3 and C-4 leading to **8i** resulted in an analog that was less potent for iNOS but surprisingly was more potent for nNOS. This was also reflected to some extent in **8j**, whose selectivity for iNOS over eNOS was very good. Despite slight loss of potency, the selectivity displayed by **8j** (eNOS/iNOS) was the best we have seen for these analogs. The C-3/C-5 disubstituted compound **8k** had lost significant binding for the iNOS as a result this substitution pattern was not further pursued. The C-4/C-5 substituted compounds were explored in greater depth (**8l–p**). The data for **8l**,**m**, and **8o** clearly indicates that small alkyl substituents at C-4 or C-5 were the most preferred as it lead to potent analogs (compared to **8e**) in addition to maintaining good selectivity. The amidines

Table 1. Inhibition of NOS by monosubstituted pyrrolidin-2-imines ($IC_{50} = \mu M$)[a]

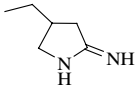
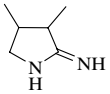
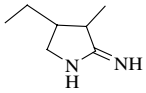
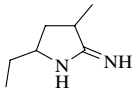
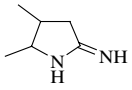
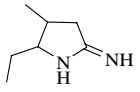
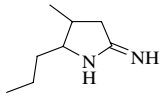
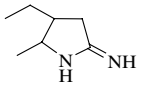
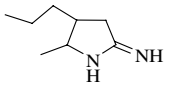
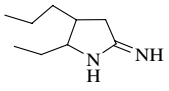
Entry	Structure[b]	iNOS	eNOS	Selectivity eNOS/iNOS	nNOS	Selectivity nNOS/iNOS
—	L-NIL	2.1	7.9	3.7	17.5	8.3
8a		4.1	4.6	1.1	9.6	2.3
8b		1.5	1.8	1.2	0.9	0.6
8c		0.2	2.6	13	0.8	0.3
8d		1.3	1.9	1.4	3.1	2.3
8e		0.5	14.5	27	2.6	4.8
8f		38.7	53	1.4	53	1.4
8g		3.5	87.8	25	10.6	3
8h		>50	>50	ND	>50	ND

ND = not determined.

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

[b] The pyrrolidin-2-imines were synthesized according to Scheme 1 and the IC_{50}s are reported for racemic mixture.

Table 2. Inhibition of NOS by disubstituted pyrrolidin-2-imines ($IC_{50} = \mu M$)[a]

Entry	Structure[b]	iNOS	eNOS	Selectivity eNOS/iNOS	nNOS	Selectivity nNOS/iNOS
8e		0.5	14.5	27	2.6	4.8
8i		1.2	8.7	7.2	0.61	0.5
8j		0.93	60.6	65	0.61	0.65
8k		23	5.5	0.24	2.4	0.1
8l		0.3	0.6	17	0.13	3.8
8m		0.02	0.52	26	0.08	4.0
8n		2	49	24	4.8	2.4
8o		0.16	6.3	38	0.14	0.8
8p		13.1	104	8	27	2
8q		10.2	227	22	17.3	1.7

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

[b] The pyrrolidin-2-imines were synthesized according to Scheme 1 and the IC_{50}s are reported for the diastereomeric and racemic mixture.

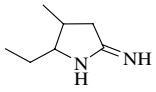
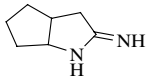
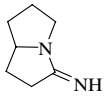
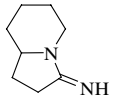
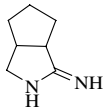
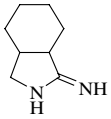
8n,**p**, and **8q** with *n*-propyl group at both C-5 and C-4 led to significant loss in iNOS activity. This result was akin to the one seen for **8f** (Table 1) as discussed previously. It is also worth noticing that both **8n** and **8q** still retained good selectivity for iNOS over eNOS despite loss of potency.

While we had achieved significant potency going from **8e** (Table 1) to **8m** (Table 2) the selectivity remained unchanged. We felt that perhaps the preparation of rigid analogs might overcome the mediocre selectivity (over nNOS) seen for **8m**. The results of binding for the select fused amidines are displayed in Table 3. In general the fused amidines displayed binding activity that was drastically less for the all three isoforms and more so for the iNOS compared to **8m**. In a dramatic reversal, all fused analogs consistently maintained better binding potency for the nNOS over iNOS. Thus, the amidine analog **11** (fused version of **8m**) was practically inactive and fared much worse than we had hoped. Similar behavior was noticed for **15** and **16**. The bicyclic analogs **17** and **18** were considerably more potent for nNOS while the potency for the iNOS was unimpressive.

While we had modest success in the pyrrolidin-2-imines lead class, the results of the binding assay for the 1,3-thiazolidin-2-imines analogs as shown below (Table 4) were far from satisfactory and stand in sharp contrast with the results of pyrrolidin-2-imines discussed above.

To start with, the lead compound **19a** was equipotent in all three isoform, and showed lack of selectivity. Partial attempts to improve the potency and selectivity specifically for the iNOS enzyme suggests that this lead class was quite sensitive to the substituents on the thiazole ring as exemplified by the analogs **19b**,**c**, and **19d**. All three analogs had lost significant activity in binding assay compared to **19a**. Notice also that the fused analog **22** and **23** were less impressive and share the trait similar to **15** and **16** (Table 3). But for the fused analogs,

Table 3. Inhibition of NOS by fused pyrrolidin-2-imines ($IC_{50} = \mu M$)[a]

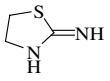
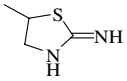
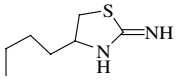
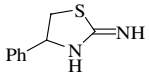
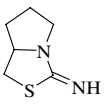
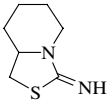
Entry	Structure[b]	iNOS	eNOS	Selectivity eNOS/iNOS	nNOS	Selectivity nNOS/iNOS
8m		0.02	0.52	26	0.08	4.0
11		>50	>50	ND	9.9	ND
15		>50	33	ND	9.6	ND
16		>50	>50	ND	>50	ND
17		3.1	4.7	1.5	0.31	0.1
18		1.2	24	20	0.66	0.55

ND = not determined.

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

[b] The amidine **11** was synthesized according to Scheme 2, **15** and **16** were prepared according to Scheme 3 and **17** and **18** by Scheme 1. The IC_{50} s are reported for the racemic mixture.

Table 4. Inhibition of NOS by disubstituted 1,3-thiazolidin-2-imines ($IC_{50} = \mu M$)[a]

Entry	Structure[b]	iNOS	eNOS	Selectivity eNOS/iNOS	nNOS	Selectivity nNOS/iNOS
—	L-NIL	2.1	7.9	3.7	17.5	8.3
19a		0.9	1.1	1.2	1.8	2
19b		>50	>50	ND	>50	ND
19c		5.2	>50	ND	13.2	2.5
19d		>50	>50	ND	>50	ND
22		>50	>50	ND	14	ND
23		>50	>50	ND	>50	ND

ND = not determined.

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

[b] The 1,3-thiazolidin-2-imines were synthesized according to Schemes 4 and 5 and the IC_{50}s are reported for the racemic mixture.

the pyrrolidin-2-imines, which were investigated in parallel with 1,3-thiazolidin-2-imines had a more encouraging outcome. For these reasons 1,3-thiazolidin-2-imine as a lead class was not pursued further.

In conclusion, the synthesis and evaluation of pyrrolidin-2-imines and 1,3-thiazolidin-2-imines as iNOS inhibitor have been demonstrated. Among the pyrrolidin-2-imines, **8l** and **8m** displayed superb potency for iNOS and was the best with in this class. Our limited efforts in the 1,3-thiazolidin-2-imines suggest that they were far less significant both in potency and selectivity compared to the pyrrolidin-2-imines or for that matter 1,3-oxazolidin-2-imines class of compounds that was reported recently.[11a]

In the final analysis, it has been our experience that, in general, the pyrrolidin-2-imines lead class was less potent and considerably less selective compared to the piperidin-2-imines as iNOS inhibitors.[12]

References and notes

1. The part of results of the work described herein was presented at the 214th ACS National Meeting, Las Vegas, NV, 1997 (*Abstract* #037).
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18. 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.