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Design and Synthesis of Orally Bioavailable Inhibitors of Inducible Nitric Oxide Synthase. Synthesis and Biological Evaluation of Dihydropyridin-2(1*H*)-imines and 1,5,6,7-Tetrahydro-2*H*-azepin-2-imines

Yasufumi Kawanaka,[a,*] Kaoru Kobayashi,[b] Shinya Kusuda,[b] Tadashi Tatsumi,[b] Masayuki Murota,[b] Toshihiko Nishiyama,[b] Katsuya Hisaichi,[b] Atsuko Fujii,[b] Keisuke Hirai,[b] Minoru Nishizaki,[b] Masao Naka,[b] Masaharu Komeno,[b] Hisao Nakai[b] and Masaaki Toda[b]

[a]*Fukui Research Institute, Ono Pharmaceutical Co., Ltd., Technoport, Yamagishi, Mikuni, Sakai, Fukui 913-8538, Japan*
[b]*Minase Research Institute, Ono Pharmaceutical Co., Ltd., Shimamoto, Mishima, Osaka 618-8585, Japan*

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Abstract—The process of discovery and biological evaluation of α,β-unsaturated cyclic amidines, as selective inhibitors of inducible nitric oxide synthase (iNOS), is reported. Dihydropyridin-2(1*H*)-imines and 1,5,6,7-tetrahydro-2*H*-azepin-2-imines were synthesized and biologically evaluated both in vitro and in vivo using a nitric oxide synthase inhibition assay. Compounds **1**, **5**, **6**, **8**–**12** and **16** exhibited potent inhibition of iNOS. Among these, compounds **6**, **7**, **10**, **11** and **16** showed 5- to 19-fold isoform selectivity. Compounds **1**, **6**, **10**, **11** and **16** also showed potent inhibitory activity in the NOx accumulation assay in mice. Compounds **1** and **6** showed excellent bioavailability (BA) in rats when administered orally. Full details are presented here, including the structure–activity relationship (SAR) studies, the chemistry of these compounds, and the pharmacokinetic data and the computer-aided docking study of **10** with hiNOS.

Introduction

In recent years, nitric oxide (NO) has emerged as one of the important biological mediators that plays a significant role in various physiological processes.[1,2] NO is produced by nitric oxide synthase (NOS), which catalyzes the oxidation of L-arginine to L-citrulline, and is synthesized by two major classes of NOS, termed constitutive NOS (cNOS) and inducible NOS (iNOS).[3] The cNOS is essential to maintain homeostasis and comprises both neuronal NOS (nNOS) and endothelial NOS (eNOS). The nNOS is believed that it appears to function as a neurotransmitter, which regulates neuronal transmission. The eNOS is found predominantly in the vascular endothelium and it continuously produces low concentrations of NO, which have an important role in blood pressure regulation and control of platelet aggregation. In contrast, the iNOS is found in activated macrophages and other tissues, and it seems to play a role in the pathogenesis of various diseases. Overexpression of NO, which damages tissues during acute and chronic inflammation, is caused by iNOS. Identification of potent and selective iNOS inhibitors has been a subject of intense interest, because of their therapeutic potential for the treatment of diseases mediated by overexpression of NO.[4,5] The natural substrate for NOS, arginine, has been the obvious basis for the molecular design of NOS inhibitors.

Structural analogues of L-arginine, such as N^G-methyl-L-arginine (L-NMA),[6,7] N^G-nitro-L-arginine,[8] *N*-iminoethyl-L-ornithine (L-NIO),[9] and L-thiocitrulline,[10] have been shown to inhibit the various forms of NOS, but lack selectivity for iNOS. Abundant pharmacological and biological data have been obtained utilizing some of the early nonspecific arginine analogue inhibitors. A number

*Corresponding author. Tel.: +81-776-82-6161; fax: +81-776-82-8420; e-mail: kawanaka@ono.co.jp

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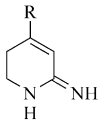
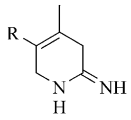
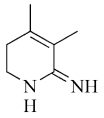
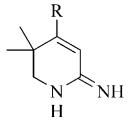
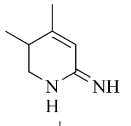
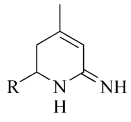
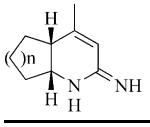
of non-amino acid inhibitors of iNOS, such as aminoguanidine,[11] isothioureas,[12] 2-amino-5,6-dihydro-6-methyl-*4H*-1,3-thiazine,[13] 2-iminopiperidine[14] and 2-aminopyridine,[15] have also been described, and we recently reported on 5,6-dihydropyridin-2(1*H*)-imine.[16] As shown in Chart 1, our molecular design process started with structural hybridization of 2-amino-4-methylpyridine **20**[15] and (4*R*)-4-methylpiperidin-2(1*H*)-imine **21**.[14] This modification led us to the discovery of 4-methyl-5,6-dihydropyridin-2(1*H*)-imine **1**,[16] which is structurally new and possesses no asymmetric center. Further chemical modification was continued. Ring expansion of **1**, followed by introduction of alkyl group at position-7, gave **16**, which showed increased iNOS inhibition and better isoform selectivity (Chart 1). Two series of cyclic amidines were synthesized and biologically evaluated in an attempt to identify a new selective inhibitor of human iNOS (hiNOS). Here we report on a series of 5,6-dihydropyridin-2(1*H*)-imines and 1,5,6,7-tetrahydro-*2H*-azepin-2-imines, which are structurally new iNOS inhibitors.

Table 1. Inhibitory activities of **1**–**13** against hiNOS, heNOS and their isoform selectivity[c]

Structure	Compd	hiNOS IC_{50} (μM)	heNOS IC_{50} (μM)	Selectivity heNOS/hiNOS[a]
	**1** R = Me	0.20	0.23	1.2
	2 R = Et	1.2	1.5	1.3
	**3** R = H	0.70	0.44	0.6
	4 R = Me	2.7	4.8	1.8
	**5**	0.31	0.32	1.0
	**6** R = Me	0.42	2.1	5.0
	7 R = H	2.8	31.0	11.1
	**8**[b]	0.51	0.52	1.0
	**9** R = Me[b]	0.18	0.24	1.3
	10 R = *n*-Pr[b]	0.06	0.58	9.7
	**11** R = Allyl[b]	0.05	0.36	7.2
	12 n = 1[b]	0.17	0.51	3.0
	13 n = 2[b]	1.4	7.4	5.3

[a]Selectivity was evaluated as the rate of the IC_{50} values for heNOS and hiNOS.
[b]Synthesized as a mixture of racemates.
[c]All compounds were prepared as their hydrochlorides.

Chemistry

Synthesis of the compounds listed in Tables 1 and 2 is outlined in Schemes 1–5b. As shown in Scheme 1, compounds **1** and **3** were synthesized from **20** by Birch reduction, followed by the appropriate work up procedure, at a 66% yield (Method A) and a 64% yield (Method B), respectively.[17] Application of this partial reduction procedure to **22**[15] and **23**[18] resulted in **2** and **4** at a yield of 79 and 75%, respectively.

As described in Scheme 2a, the common intermediates [substituted 5,6-dihydropyridin-2(1*H*)-ones **27a**–**i**] were prepared from the corresponding cyclopentenones **24a**–**i** by conventional Beckmann rearrangement. Conversion of **24a** and **24e**–**i** to their corresponding oximes **25a** and **25e**–**i**, followed by the *O*-tosylation, provided **26a** and **26e**–**i**. Then Beckmann rearrangement of these compounds was successfully carried out to give **27a** and **27e**–**i** (Method C: 22–48% yield), respectively. Direct Beckmann rearrangement of **24b**–**d** also resulted in **27b**–**d** (Method D: 44–60% yield), respectively.

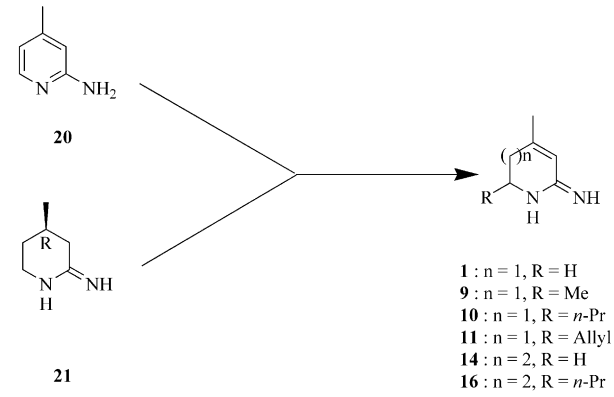
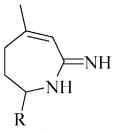
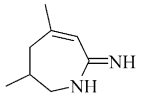
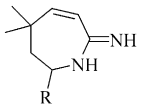
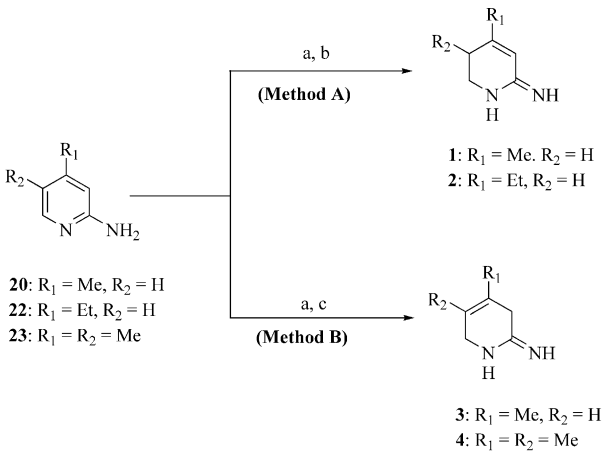


Chart 1. Molecular design of 5,6-dihydropyridin-2(1*H*)-imine (**1** and **9**–**11**) and 1,5,6,7-tetrahydro-2*H*-azepin-2-imine (**14** and **16**).

Table 2. Inhibitory activities of **14**–**19** against hiNOS, heNOS and their isoform selectivity[c]

Structure	Compd	hiNOS IC_{50} (μM)	heNOS IC_{50} (μM)	Selectivity heNOS/hiNOS[a]
	**14** R = H	1.1	4.6	4.2
	15 R = Me[b]	0.68	0.42	0.6
	16 R = *n*-Pr[b]	0.052	1.0	19.2
	**17**[b]	0.82	2.1	2.6
	**18** R = H	1.4	24.6	17.6
	19 R = *n*-Pr[b]	1.6	17.4	10.9

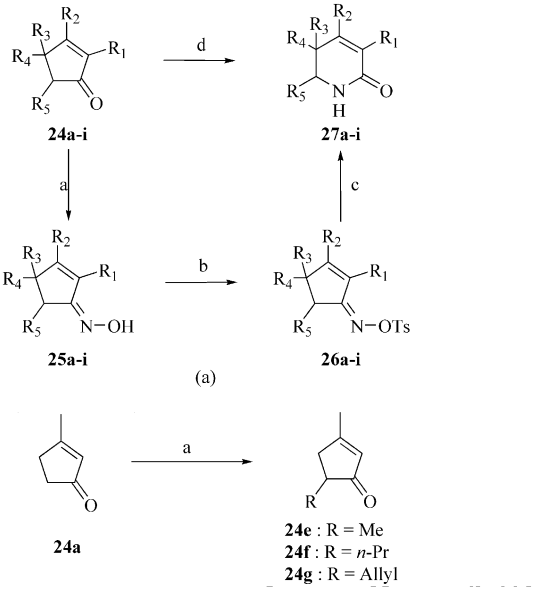
[a]Selectivity was evaluated as the rate of the IC_{50} values for heNOS and hiNOS.
[b]Synthesized as a mixture of racemates.
[c]All compounds were prepared as their hydrochlorides.



Scheme 1. Synthesis of 5,6-dihydropyridin-2(1*H*)-imines (**1** and **2**) and 3,6-dihydropyridin-2(1*H*)-imines (**3** and **4**). Reagents: (a) Li, liquid NH_3, THF/EtOH, −78°C; (b) NH_4Cl; (c) H_2O (64–79%).

Among the cyclopentenones used in the above-mentioned synthesis, **24e**,[19a] **24f** and **24g**[19b] were prepared by alkylation of **24a** (28–55% yield), as described in Scheme 2b. Compounds **24a** and **24b** are commercially available. The methods for synthesis of **24c–d**[19c,19d] **24h**[19e] and **24i**[19f] are reported. As described in Scheme 3, another dihydropyridin-2(1*H*)-one **30** was prepared by oxidative dehydrogenation of **29**[20a] (70% yield), which was prepared from **28**[20b] by direct Beckmann rearrangement (52% yield).

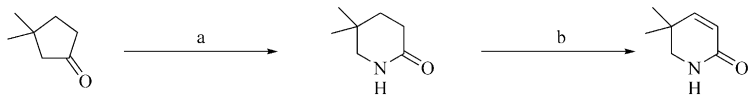
Synthesis of 5,6-dihydropyridin-2(1*H*)-imines **1** and **5–13** is described in Scheme 4. Dihydropyridin-2(1*H*)-ones **27a–i** and **30** were converted to the corresponding compounds **31a–i** and **32**, after which aminolysis and treatment with hydrogen chloride provided **1** and **5–13** (8–70% yield), respectively.



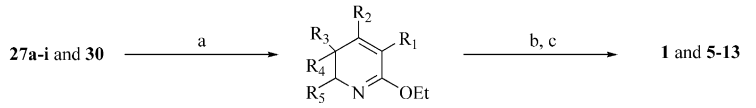
Compound	R_1	R_2	R_3	R_4	R_5
a	H	Me	H	H	H
b	Me	Me	H	H	H
c	H	Me	Me	Me	H
d	H	Me	(H, Me)		H
e	H	Me	H	H	Me
f	H	Me	H	H	*n*-Pr
g	H	Me	H	H	Allyl
h	H	Me	H	-$(CH_2)_3$-	
i	H	Me	H	-$(CH_2)_4$-	

(b)

Scheme 2. a. Synthesis of 5,6-dihydropyridin-2(1*H*)-ones (**27a–i**). Method C: (a) NH_2OH·HCl, NaOAc, MeOH, reflux; (b) p-TsCl, Pyridine, 0°C; (c) concd HCl, MeOH, reflux (**27a** and **27e–i**: 22–48% in three steps). Method D: (d) NaN_3, TFA, reflux (**27b–d**: 44–60%). b. Synthesis of substituted 2-cyclopenten-1-ones (**24e–g**). Reagents: (a) LDA. RX (RX = MeI or *n*-PrI or CH_2=$CHCH_2Br$), THF-DMPU, −78°C (28–55%).



Scheme 3. Synthesis of 5,5-dimethyl-5,6-dihydropyridin-2-one (**30**). Reagents: (a) NH_2OSO_3H, HCO_2H, reflux (52%); (b) DDQ, BSTFA, dioxane, reflux (70%).



Compound	R_1	R_2	R_3	R_4	R_5
a	H	Me	H	H	H
b	Me	Me	H	H	H
c	H	Me	Me	Me	H
d	H	Me	(H, Me)		H
e	H	Me	H	H	Me
f	H	Me	H	H	*n*-Pr
g	H	Me	H	H	Allyl
h	H	Me	H	-$(CH_2)_3$-	
i	H	Me	H	-$(CH_2)_4$-	

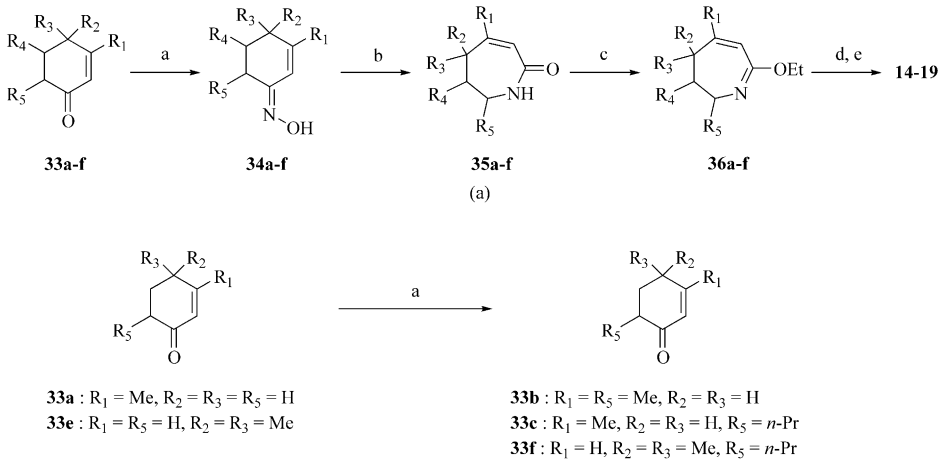
Scheme 4. Synthesis of 5,6-dihydropyridin-2(1*H*)-imines (**1** and **5–13**). Reagents: (a) $Et_3O^+BF_4^-$, CH_2Cl_2; (b) NH_3, EtOH; (c) 4 M HCl dioxane (8–70% in three steps).

Synthesis of 1,5,6,7-tetrahydro-2*H*-azepin-2-imines **14–19** is outlined in Scheme 5a. Cyclohexenone **33a–f** were converted to their corresponding oximes **34a–f**, and Beckmann rearrangement of **34a–f** afforded **35a–f** at a 39–48% yield, respectively. Formation of imidates using **35a–f** resulted in **36a–f**, aminolysis of which gave **14–19** (10–26% yield). Among the cyclohexenones used in the above-described synthesis, **33b–c**[21] and **33f** were prepared by alkylation of **33a** or **33e** (23–82% yield), as described in Scheme 5b. Compounds **33a**, **33d** and **33e** are commercially available.

Results and Discussion

As shown in Tables 1 and 2, compounds **1–19** were evaluated for their ability to inhibit two isoforms of human NOS, that is human iNOS (hiNOS) and human eNOS (heNOS). As expected, 4-methyl-5,6-dihydropyridin-2(1*H*)-imine **1** demonstrated potent inhibition of both hiNOS and heNOS without any selectivity.

Replacement of the 4-methyl group with a 4-ethyl group gave compound **2**, with a nearly 6-fold reduction of inhibitory activity and still no isoform selectivity. Shifting the double bond of compound **1** from position 3,4 to 4,5 resulted in compound **3**, with reduced inhibitory activity for both isoforms as well as reduced selectivity. Introduction of another methyl group at position 5 of compound **3** afforded compound **4**, which showed a nearly 4-fold reduction of inhibitory activity against hiNOS and a 10-fold reduction of activity against heNOS as compared with **3**. However, the isoform selectivity (heNOS/hiNOS) of compound **4** was nearly 3 times greater than that of compound **3**. Based on this information, our chemical modification was carried out by fixing the double bond at position 3,4, as illustrated in compounds **5–13**. Introduction of another methyl group at position 3 of compound **1** gave compound **5**, with little change of inhibitory activity or isoform selectivity. Introduction of a *gem*-dimethyl group at position 5 of compound **1** provided compound **6**, which showed a 2-fold reduction of inhibitory activity for hiNOS and a 10-fold reduction of heNOS inhibition. As a result, its isoform selectivity was 5 times greater than that of **1**. Removal of the 4-methyl group from **6** afforded compound **7**, showing a 14-fold reduction of hiNOS inhibition and a 135-fold reduction of heNOS inhibition. As a



Compound	R_1	R_2	R_3	R_4	R_5
a	Me	H	H	H	H
b	Me	H	H	H	Me
c	Me	H	H	H	*n*-Pr
d	Me	H	H	Me	H
e	H	Me	Me	H	H
f	H	Me	Me	H	*n*-Pr

(b)

Scheme 5. a. Synthesis of 1,5,6,7-tetrahydro-2*H*-azepin-2-imines (**14**–**19**). Reagents: (a) $NH_2OH{\cdot}HCl$, NaOAc, MeOH, reflux; (b) PPA 120°C (39–48% in two steps); (c) $Et_3O^+BF_4^-$, CH_2Cl_2; (d) NH_3, EtOH; (e) 4 M HCl dioxane (10–26% in three steps). b. Synthesis of substituted 2-cyclohexen-1-ones (**33b**, **33c** and **33f**). Reagents: (a) LDA, RX (RX = MeI or *n*-PrI), THF-DMPU, −78°C (23–82%).

result, its isoform selectivity (heNOS/hiNOS) was nearly 10 times greater than that of **1**. Introduction of another methyl group at position-5 of **1** afforded compound **8**, which showed a nearly 2-fold reduction of both hiNOS and heNOS inhibition, but retained the same isoform selectivity. Introduction of another methyl group at position 6 of **1** gave compound **9**, which retained inhibitory activity for both isoforms as well as selectivity. Replacement of the 6-methyl group of **9** with a 6-propyl group produced compound **10**, with a 3-fold increase of inhibitory activity against hiNOS and a 2.4-fold reduction of activity against heNOS, resulting in 7.5-fold higher isoform selectivity than that of **9**. Nearly the same result was obtained with the corresponding allyl derivative, compound **11**.

Two *cis*-fused bicyclic analogues were prepared and evaluated as iNOS inhibitors. The *cis*-fused 5,6-bicyclic analogue **12** demonstrated nearly the same hiNOS inhibition and 2.2-fold less heNOS inhibition when compared with **1**, while the isoform selectivity of **12** was better than that of **1**. This was also true for the *cis*-fused 6,6-bicyclic analogue **13**, although its IC_{50} values for both isoforms indicated that it was much less potent than **12**.

The biological activity profile of 1,5,6,7-tetrahydro-*2H*-azepin-2-imines **14**–**19** is described in Table 2. Ring expansion of **1** provided a seven-membered amidine derivative **14**, which showed reduced inhibitory activity. Introduction of an alkyl group at position 7 of the seven-membered compound **14** was effective for increasing the inhibition of both isoforms. Introduction of a methyl group at position 7 of **14** afforded compound **15**, which showed a slight increase, 10-fold increase, and 7-fold reduction of hiNOS inhibition, heNOS inhibition, and isoform selectivity, respectively. Introduction of a *n*-propyl group at position 7 of **14** resulted in compound **16**, which showed a 20-fold increase and a 4.6-fold increase of hiNOS inhibition and heNOS inhibition, respectively. It was also interesting that a 5-fold increase of isoform selectivity was obtained by this chemical modification. Analogous results were observed in the biological evaluation of compounds **9**–**10**. Introduction of a methyl group at position-6 of **14** provided compound **17**, with slight increases of hiNOS

and heNOS inhibition, but less isoform selectivity. Removal of the 4-methyl group of **14**, followed by introduction of a 5,5-dimethyl moiety, gave **18**, which retained hiNOS inhibition and showed nearly 5-fold less heNOS inhibition, resulting in nearly 4-fold more isoform selectivity. Introduction of an *n*-propyl group at position 7 of **18** did not increase either its iNOS inhibitory activity or isoform selectivity.

As a result, introduction of a *n*-propyl group at the carbon adjacent to the nitrogen was found to be effective for improving the iNOS inhibitory activity and isoform selectivity of these two series of inhibitors, as illustrated in compounds **10** and **16**, although this was not always true for 5,5-dimethyl-1,5,6,7-tetrahydro-2*H*-azepin-2-imine derivatives, as illustrated in compound **19**.

Among the compounds described in Tables 1 and 2, compound **1** is structurally representative of this series and shows potent iNOS inhibitory activity. It was selected together with compounds **3**, **5**–**6**, **8**–**12** and **14**–**17** for further biological and pharmacodynamic evaluation in animal models, on the basis of their in vitro potency and isoform selectivity, as well as the acute toxicity in mice (maximum tolerated dose) and the structural features. Compounds **10**, **11** and **16** exhibited greater potency and more isoform selectivity than the above-mentioned compounds **1** and **6**. As shown in Table 3, compounds **10** and **11** demonstrated a higher acute toxicity than **1** and **6**, as evaluated by the ratio between the maximum tolerated dose (MTD) and the ID_{50} value for NOx[22] accumulation (MTD/NOx).

In order to assess compounds **1**, **3**, **5**–**6**, **8**–**12** and **14**–**17** for their ability to inhibit iNOS in vivo, mice were given a single compound subcutaneously (sc) at 3 h after lipopolysaccharide (LPS) injection. Then the plasma NOx accumulation from 3 to 6 h after LPS injection was determined. As shown in Table 3, all of the tested compounds exhibited more potency in NOx accumulation assay in mice. Of these, compounds **1**, **6**, **9**, **10**, **11** and **16** inhibited NOx accumulation in plasma and their ID_{50} values were 0.16, 0.28, 0.09, 0.36, 0.16 and 0.80 mg/kg, sc, respectively. To assess acute toxicity, the MTD was determined for each compound. As shown in Table 3, the MTD was 20 mg/kg for both compound **1** and compound **6**, when a single intravenous (iv) dose was given to normal mice. The MTD values of five compounds **1**, **6**, **9**, **10** and **11** were lower than that of L-NMMA (3000 mg/kg), although the MTD/ID_{50} ratio for NOx accumulation in mice was 130 for **1**, 71 for **6**, 330 for **9**, 14 for **10** and 31 for **11**, respectively. The MTD of **16** was not evaluated, because it was unexpectedly less potent in vivo. Compound **14** also showed unexpectedly less potent ID_{50} value in NOx accumulation assay for its potent IC_{50} value in mouse. 1,5,6,7-Tetrahydro-2*H*-azepin-2-imines exhibited relatively less potency than dihydropyridin-2(1*H*)-imines in both the in vitro and in vivo assays.

Table 3. Pharmacological evaluation of **1**, **3**, **5**–**6**, **8**–**12** and **14**–**17**

Compd	mouse iNOS	Nox	MTD	MTD/NOx
	IC_{50} (μM)	ID_{50} (mg/kg, sc)	mg/kg, iv	
1	0.022	0.16	20	130
3	0.066	0.38	50	130
5	0.12	0.30	30	100
6	0.10	0.28	20	71
8	0.067	0.30	30	100
9	0.14	0.09	30	330
10	0.10	0.36	5	14
11	0.025	0.16	5	31
12	0.038	0.18	10	56
14	0.10	3 mg/kg (47.9%)	30	<10
15	0.17	0.83	10	12
16	0.20	0.80	NT[a]	NT[a]
17	0.22	0.84	30	36
L-NMMA	3.5	26	3000	120

[a]Not tested.

As shown in Table 4, compounds **1** and **6** had a promising pharmacokinetic profile in rats and these compounds (2 mg/kg, iv; 10 mg/kg, po) achieved good plasma levels. Intravenous administration of compounds **1** and **6** yielded the following values for mean pharmacokinetic half-life ($t_{1/2}$) (**1**: 1.08±0.40 h and **6**: 1.93±0.21 h, respectively), plasma clearance (CL) (**1**: 3.084±0.573 L/h/kg and **6**: 2.127±0.447 L/h/kg, respectively) and steady-state volume of distribution (*Vss*) (**1**: 3.305±1.258 L/kg and **6**: 4.589±0.116 L/kg, respectively). After oral administration, the mean maximum plasma concentration of unchanged drug in plasma (C_{max}), time of maximum concentration (t_{max}), pharmacokinetic half-life ($t_{1/2}$) and oral bioavailability (BA) were 1.082±0.156 μg/mL, 0.67±0.29 h, 2.18±1.18 h and 88% for compound **1** versus 1.312±0.225 μg/mL, 1.000±0.000 h, 2.41±0.26 h and 84% for compound **6**, respectively.

Figure 1 demonstrates a stereo view of the docking study of **10** with hiNOS (PDB code 2NSI). Clear-cut interaction between the basic amidine moiety with the acidic Glu377 residue and insertion of the 4-methyl group of **10** into one of the small pockets of the enzyme were observed. Additionally, 6-*n*-propyl moiety, which played a role in an increased hiNOS inhibition and isoform selectivity as illustrated in **10** and **16**, was found to

Table 4. Rat pharmacokinetic data for compounds **1** and **6**[a] (N [b] = 3)

Route (Dose)	Parameters	**1**	**6**
		Mean±SD	Mean±SD
iv (2 mg/kg)	$t_{1/2}$[c] (1 or 2 → 4) (h)	1.08±0.40	1.93±0.21
	AUC[d] (0 → ∞) (μg h/mL)	0.665±0.137	0.969±0.207
	CL[e] (L/h/kg)	3.084±0.573	2.127±0.447
	Vss[f] (L/kg)	3.305±1.258	4.589±0.116
po (10 mg/kg)	C_{max}[g] (μg/mL)	1.082±0.156	1.312±0.225
	t_{max}[h] (h)	0.67±0.29	1.000±0.000
	$t_{1/2}$[c] (1 or 2 → 8) (h)	2.18±1.18	2.41±0.26
	AUC[d] (0 → ∞) (μg h/mL)	2.927±0.760	4.067±0.218
	BA[i]	88.0±22.9	83.9±4.5

[a]The mean values are reported. Standard deviations are reported.
[b]*N* is the number of animals used in the study.
[c]Pharmacokinetic half-life.
[d]Integrated area under plasma concentration versus time curve from time 0 to time infinity.
[e]Plasma clearance.
[f]Steady-state volume of distribution.
[g]Maximum plasma concentration of unchanged drug in plasma.
[h]Time of maximum concentration.
[i]Oral bioavailability.

occupy the space close to the entrance of the hole in which the inhibitor is inserted.

Summary

In summary, we explored the SAR for a series of substituted 5,6-dihydropyridin-2(1*H*)-imines and substituted 1,5,6,7-tetrahydro-2*H*-azepin-2-imines and obtained a significant increase of both isoform selectivity and iNOS inhibition. 4-Methyl and 4-methyl-5,5-dimethyl substitution gave compound, **1** and **6**, which were potent and/or selective inhibitors of iNOS. The oral bioavailability of **1** and **6** in rats was excellent (**1**:88% and **6**:84%).

Compounds **10** and **11** exhibited greater inhibitory activity and isoform selectivity than **1** and **6**, but their acute toxicity in mice was also relatively higher. Compound **16** exhibited potent hiNOS inhibitory activity and excellent isoform selectivity relative to other inhibitors, but it showed unexpectedly week miNOS inhibition.

The computer-aided docking study of **10** with hiNOS demonstrates that the 4-methyl moiety is inserted into one of the small pockets and the 6-*n*-propyl moiety occupies the space at the entrance of the hole in which the inhibitor is inserted (Fig. 1).

Based on the currently available data, pharmacological evaluation of some of these compounds suggested that they might become clinically useful drugs with a good safety margin.

Experimental

General directions

Analytical samples were homogeneous as confirmed by TLC, and afforded spectroscopic results consistent with the assigned structures. All ^{1}H NMR spectra were obtained on a Varian Gemini-200, VXR-200s spectrometer. Fast atom bombardment mass spectra (FAB-MS) and electron ionization (EI) were obtained on a JEOL JMS-DX303HF or PerSeptive Voyager Elite spectrometer. Atmospheric pressure chemical ionization (APCI) was determined on a Hitachi M1200H spectrometer. Matrix assisted laser desorption ionization-time of flight high-resolution mass spectra (MALDI-TOF HRMS) were obtained on a PerSeptive Voyager Elite spectrometer. IR spectra were measured on a Perkin–Elmer FT-IR 1760X or Jasco FT/IR-430 spectrometer. Melting points (mp) were determined by Yanaco micro melting point apparatus MP-500D and are uncorrected. Column chromatography was carried out on silica gel [Merck silica gel 60 (0.063–0.200 mm) or Wako Gel C200 or Fuji Silysia FL60D]. Thin layer chromatography was performed on silica gel (Merck TLC plate, silica gel 60 F_{254}).

Method A (Birch reduction)

4-Methyl-5,6-dihydropyridin-2(1*H*)-imine hydrochloride salt (1). To a stirred solution of 2-amino-4-methylpyridine (**20**) (2 g, 18.5 mmol) in THF (6.2 mL) and EtOH (2.2 mL) was added liquid NH_3 (60 mL) at −78 °C. Small pieces of lithium metal (320 mg, 46.2 mmol) were added to the reaction mixture and the solution stirred for an additional 1.5 h at −78 °C before quenched with solid ammonium chloride (75 g). The reaction mixture was slowly warmed up to room temperature removing the ammonia with a stream of nitrogen and then treated with aqueous ammonium chloride. The reaction mixture was extracted with $CHCl_3$. The combined organic layers were dried over anhydrous sodium sulfate and evaporated under reduced pressure. The residue was converted to its hydrochloride with 4 M HCl/EtOAc–EtOH at 0 °C and further purified by column chromatography on silica gel ($CHCl_3$/MeOH, 5/1) to afford **1** as a pale yellow powder (1.8 g, 66%). TLC R_f 0.38

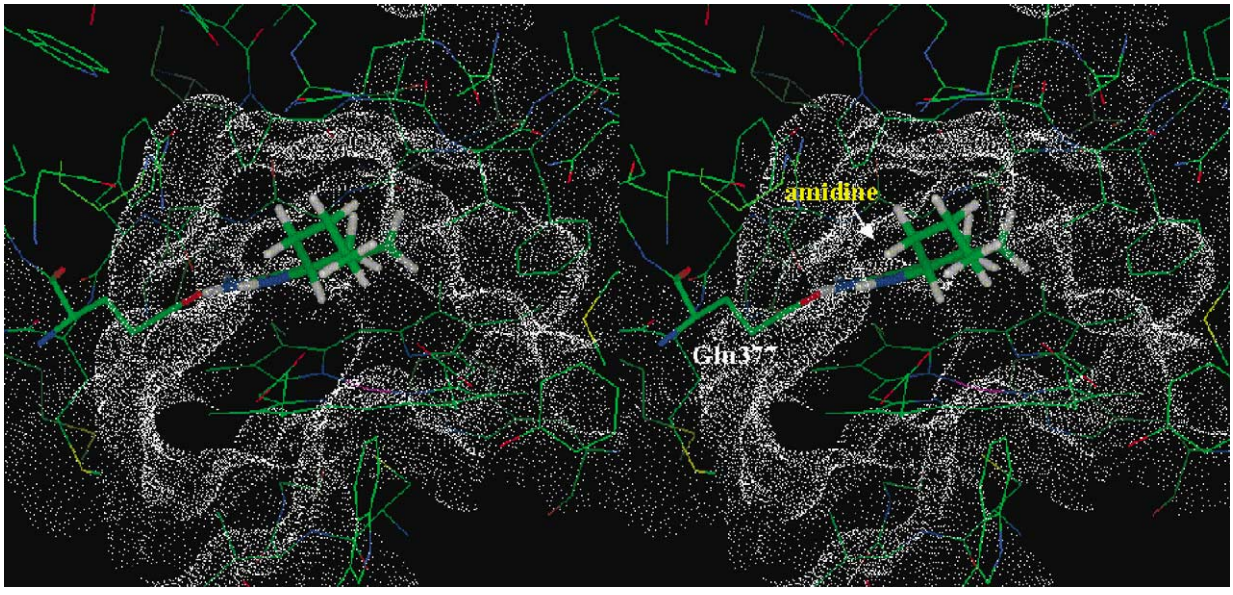


Figure 1. Stereo view of a docking model of compound **10** with human iNOS (PDB code 2NSI).

($CHCl_3$/MeOH/AcOH, 10/1/1); IR (KBr) 3327, 3138, 1692, 1639, 1614, 1528, 1443, 1383, 1370, 1344, 1178, 1052, 987, 849, 785, 677 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 9.30–9.15 (brs, 1H), 9.15–8.95 (brs, 1H), 8.65–8.50 (brs, 1H), 6.24 (s, 1H), 3.50 (t, $J=7.5$ Hz, 2H), 2.43 (t, $J=7.6$ Hz, 2H), 2.05 (s, 3H); mp: 157–158 °C; MS and HR-MS analysis showed only the ion from $C_6H_{10}N_2$ corresponding to the loss of HCl, MS (APCI, Pos.) m/z 111 $(M+H)^+$; HR-MS (MALDI-TOF, Pos.) calcd for $C_6H_{10}N_2+H^+$: 111.0922; found: 111.0949.

4-Ethyl-5,6-dihydropyridin-2(1*H*)-imine hydrochloride salt (2). Compound **2** was prepared from 4-ethyl-2-aminopyridine (**22**)[15] in 79% yield according to the same procedure as described for the preparation of **1** from **20**. White powder; TLC R_f 0.45 ($CHCl_3$/MeOH/AcOH, 10/1/1); IR (KBr) 3100, 1688, 1649, 1607, 1425, 1380, 1338, 1249, 1212, 1164, 1054, 992, 976, 867, 765, 707, 680 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 9.48–9.18 (brs, 1H), 8.98–8.75 (brs, 1H), 8.74–8.52 (brs, 1H), 6.30 (s, 1H), 3.49 (t, $J=7.2$ Hz, 2H), 2.42 (t, $J=7.2$ Hz, 2H), 2.32 (q, $J=7.4$ Hz, 2H), 1.14 (t, $J=7.4$ Hz, 3H); mp: 113–114 °C; MS and HR-MS analysis showed only the ion from $C_7H_{12}N_2$ corresponding to the loss of HCl, MS (APCI, Pos.) m/z 125 $(M+H)^+$; HR-MS (MALDI-TOF, Pos.) calcd for $C_7H_{12}N_2+H^+$: 125.1079; found: 125.1080.

Method B (Birch reduction)

4-Methyl-3,6-dihydropyridin-2(1*H*)-imine hydrochloride salt (3). To a stirred solution of 2-amino-4-methylpyridine (**20**) (1.0 g, 9.2 mmol) in THF (3.1 mL) and EtOH (1.1 mL) was added liquid NH_3 (30 mL) at −78 °C. After the addition of small pieces of lithium metal (210 mg, 30.3 mmol), the reaction mixture was stirred for an additional 1.5 h at −78 °C before quenched with a small amount of water (4 mL). The reaction mixture was slowly warmed up to room temperature removing the ammonia with a stream of nitrogen and then treated with additional water (20 mL). The reaction mixture was extracted with $CHCl_3$. The combined organic layers were dried over anhydrous sodium sulfate and evaporated under reduced pressure. The residue was treated with 4 M HCl/EtOAc–EtOH at 0 °C and further purified by column chromatography on silica gel ($CHCl_3$/MeOH, 5/1) to afford **3** as a pale yellow powder (860 mg, 64%). TLC R_f 0.38 ($CHCl_3$/MeOH/AcOH, 10/1/1); IR (KBr) 3292, 3137, 2979, 2944, 2878, 1718, 1682, 1544, 1417, 1356, 1305, 1154, 1016, 946, 866, 812, 600 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 10.15–9.95 (brs, 1H), 9.05–8.95 (brs, 1H), 8.90–8.65 (brs, 1H), 5.55 (brs, 1H), 4.05–3.90 (m, 2H), 3.22 (t, $J=5.1$ Hz, 2H), 1.80 (s, 3H); mp: 158–159 °C; MS and HR-MS analysis showed only the ion from $C_6H_{10}N_2$ corresponding to the loss of HCl, MS (APCI, Pos.) m/z 111 $(M+H)^+$; HR-MS (MALDI-TOF, Pos.) calcd for $C_6H_{10}N_2+H^+$: 111.0922; found: 111.0896.

4,5-Dimethyl-3,6-dihydropyridin-2(1*H*)-imine hydrochloride salt (4). Compound **4** was prepared from 4,5-dimethyl-2-aminopyridine (**23**)[18] in 75% yield according to the same procedure as described for the preparation of **3** from **20**. White powder; TLC R_f 0.42 ($CHCl_3$/MeOH/AcOH, 10/1/1); IR (KBr) 3137, 3004, 2941, 2880, 1721, 1687, 1621, 1546, 1440, 1417, 1389, 1363, 1326, 1141, 839, 585 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 10.20–10.00 (brs 1H), 8.90–8.70 (brs, 1H), 8.60–8.50 (brs, 1H), 3.90–3.75 (m, 2H), 3.30–3.05 (m, 2H), 1.72 (s, 3H), 1.70(s, 3H); mp: 68–69 °C; MS and HR-MS analysis showed only the ion from $C_7H_{12}N_2$ corresponding to the loss of HCl, MS (APCI, Pos.) m/z 125 $(M+H)^+$; HR-MS (MALDI-TOF, Pos.) calcd for $C_7H_{12}N_2+H^+$: 125.1079; found: 125.1061.

Method C (Beckmann rearrangement)

4-Methyl-5,6-dihydropyridin-2(1*H*)-one (27a). To a stirred solution of 3-methyl-2-cyclopenten-1-one (**24a**) (10.3 g, 107 mmol) in MeOH (200 mL) were added NaOAc (21.2 g, 258 mmol), $NH_2OH{\cdot}HCl$ (8.7 g, 125 mmol) and water (40 mL). The reaction mixture was heated at reflux temperature for 2 h. After completing the reaction, the reaction mixture was cooled to room temperature and evaporated under reduced pressure. The residue was treated with water and extracted with $CHCl_3$. The combined organic layers were washed with saturated aqueous sodium bicarbonate, brine, dried over anhydrous magnesium sulfate and evaporated to afford **25a** (8.43 g), which was used for the subsequent reaction without further purification.

To a stirred solution of the compound obtained above in pyridine (57 mL) was added *p*-TsCl (17.3 g, 90.7 mmol) at 0 °C under an argon atmosphere. After stirring for 1 h at room temperature, the reaction mixture was treated with saturated aqueous sodium bicarbonate and evaporated under reduced pressure. The residue was treated with water and extracted with EtOAc. The combined organic layers were washed with 2 M HCl, water and brine, dried over anhydrous magnesium sulfate, and evaporated to afford **26a** (14.5 g), which was used for the subsequent reaction without further purification.

The residue was dissolved in MeOH (412 mL) and treated with conc HCl (28 mL). The reaction mixture was stirred at room temperature for 12 h, then at reflux temperature for 12 h, and was concentrated under reduced pressure. The residue was purified by column chromatography on silica gel ($CHCl_3$/MeOH, 20/1) to afford **27a** as a white powder (1.3 g, 11% in three steps). TLC R_f 0.38 ($CHCl_3$/MeOH, 10/1); IR (KBr) 3418, 1671, 1615, 1484, 1455, 1380, 1339, 1157, 1109, 996, 857, 672, 506, 470 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 5.90 (brs, 1H), 5.73–5.69 (m, 1H), 3.42 (dt, $J=7.0$, 2.6 Hz, 2H), 2.30 (t, $J=7.1$ Hz, 2H), 1.94 (d, $J=1.4$ Hz, 3H); mp: 58–59 °C; MS (APCI, Pos.) m/z 112 $(M+H)^+$, 92, 83; HR-MS (MALDI-TOF, Pos.) calcd for $C_6H_9N_1O_1+H^+$: 112.0762; found: 112.0775.

Method D (Beckmann rearrangement)

3,4-Dimethyl-5,6-dihydropyridin-2(1*H*)-one (27b). To a stirred solution of 2,3-dimethyl-2-cyclopenten-1-one (**24b**) (1.0 g, 9.1 mmol) in TFA (27 mL) was added NaN_3

(0.89 g, 13.7 mmol) and the reaction mixture was heated at reflux temperature for 15 h. After completing the reaction, the reaction mixture was cooled to room temperature and evaporated under reduced pressure. The resulting mixture was treated with water and extracted with $CHCl_3$. The combined organic layers were washed with saturated aqueous sodium bicarbonate, water, brine, dried over anhydrous magnesium sulfate and evaporated under reduced pressure. The residue was purified by column chromatography on silica gel (EtOAc) to afford **27b** as a white powder (682 mg, 60%). TLC R_f 0.40 ($CHCl_3$/MeOH, 10/1); IR (KBr) 3202, 3066, 2929, 1667, 1632, 1479, 1415, 1375, 1344, 1248, 1228, 1158, 1091, 1003, 903, 838, 796, 669, 539, 505, 456 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 5.76 (brs, 1H), 3.34 (dt, $J=7.0$, 2.8 Hz, 2H), 2.31 (t, $J=7.0$ Hz, 2H), 1.89 (q, $J=1.0$ Hz, 3H), 1.87–1.83 (m, 3H); mp: 75–76 °C; MS (APCI, Pos.) m/z 126 $(M+H)^+$; HR-MS (MALDI-TOF, Pos.) calcd for $C_7H_{11}N_1O_1+H^+$: 126.0919; found: 126.0931.

4,5,5-Trimethyl-5,6-dihydropyridin-2(1*H*)-one (27c). Compound **27c** was prepared from 3,4,4-trimethyl-2-cyclopenten-1-one (**24c**)[19c] in 47% yield according to the same procedure as described for the preparation of **27b** from **24b**. White powder; TLC R_f 0.36 ($CHCl_3$/MeOH, 10/1); ^1H NMR (200 MHz, $CDCl_3$) δ 5.88 (brs, 1H), 5.63–5.60 (m, 1H), 3.14 (d, $J=2.8$ Hz, 2H), 1.86 (d, $J=1.4$ Hz, 3H), 1.10 (s, 6H); MS (APCI, Pos.) m/z 140 $(M+H)^+$, 124, 111, 98, 83; HR-MS (MALDI-TOF, Pos.) calcd for $C_8H_{13}N_1O_1+H^+$: 140.1075; found: 140.1049.

4,5-Dimethyl-5,6-dihydropyridin-2(1*H*)-one (27d). Compound **27d** was prepared from 3,4-dimethyl-2-cyclopenten-1-one (**24d**)[19d] in 44% yield according to the same procedure as described for the preparation of **27b** from **24b**. White powder; TLC R_f 0.36 ($CHCl_3$/MeOH, 10/1); ^1H NMR (200 MHz, $CDCl_3$) δ 6.17 (brs, 1H), 5.66 (s, 1H), 3.54 (ddd, $J=12.2$, 5.3, 1.8 Hz, 1H), 3.1 (ddd, $J=12.2$, 4.9, 3.7 Hz, 1H), 2.41–2.23 (m, 1H), 1.92 (d, $J=1.4$ Hz, 3H), 1.15 (d, $J=7.0$ Hz, 3H); MS (APCI, Pos.) m/z 126 $(M+H)^+$; HR-MS (MALDI-TOF, Pos.) calcd for $C_7H_{11}N_1O_1+H^+$: 126.0919; found: 126.0929.

4,6-Dimethyl-5,6-dihydropyridin-2(1*H*)-one (27e). Compound **27e** was prepared from 3,5-dimethyl-2-cyclopenten-1-one (**24e**)[19a] in 34% yield according to the same procedure as described for the preparation of **27a** from **24a**. White powder; TLC R_f 0.65 ($CHCl_3$/MeOH, 10/1); IR (KBr) 3239, 2974, 2928, 1672, 1623, 1440, 1340, 1188, 1156, 1074, 879, 847, 769, 627, 532, 491, 436 cm^{-1}; ^1H NMR (200 MHz, CDCl3) δ 5.70 (s, 1H), 5.68–5.35 (br, 1H), 3.83–3.60 (m, 1H), 2.33–2.00 (m, 2H), 1.91 (s, 3H), 1.08 (s, 3H), 1.24 (d, $J=6.6$ Hz, 3H); mp: 41–43 °C; MS (APCI, Pos.) m/z 126 $(M+H)^+$; HR-MS (MALDI-TOF, Pos.) calcd for $C_7H_{11}N_1O_1+H^+$: 126.0919; found: 126.0913.

3-Methyl-5-propyl-2-cyclopenten-1-one (24f). To a stirred solution of 3-methyl-2-cyclopenten-1-one (**24a**) (962 mg, 10 mmol) in THF (15 mL), DMPU (2 mL) and *n*-propyl iodide (2.0 mL, 20 mmol) was slowly added LDA (2 M in heptane/THF/ethylbenzene, 5.5 mL, 11 mmol) at −78 °C under an argon atmosphere. The reaction mixture was warmed up to room temperature over 2 h and stirred for an additional 30 min at this temperature. After completing the reaction, the reaction mixture was treated with saturated aqueous ammonium chloride and extracted with EtOAc. The combined organic layers were washed with brine, dried over anhydrous magnesium sulfate and evaporated under reduced pressure. The residue was purified by column chromatography on silica gel (*n*-hexane/EtOAc, 10/1) to afford **24f** as a pale yellow oil (483 mg, 35%). TLC R_f 0.42 (*n*-hexane/EtOAc, 5/1); IR (neat) 3481, 2958, 2930, 2871, 1736, 1697, 1625, 1432, 1379, 1323, 1281, 1178, 925, 838, 532, 438 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 5.91 (s, 1H), 2.74 (dd, $J=18.4$, 6.6 Hz, 1H), 2.64–2.32 (m, 1H), 2.25 (d, $J=18.4$ Hz, 1H), 2.12 (s, 3H), 1.90–1.66 (m, 1H), 1.50–1.10 (m, 3H), 0.93 (t, $J=6.6$ Hz, 3H); MS (APCI, Pos.) m/z 139 $(M+H)^+$; HR-MS (MALDI-TOF, Pos.) calcd for $C_9H_{14}O_1+H^+$: 139.1123; found: 139.1106.

4-Methyl-6-propyl-5,6-dihydropyridin-2(1*H*)-one (27f). Compound **27f** was prepared from **24f** in 24% yield according to the same procedure as described for the preparation of **27a** from **24a**. Brown oil; TLC R_f 0.38 ($CHCl_3$/MeOH, 20/1); IR (neat) 3235, 2960, 2874, 2354, 1661, 1602, 1464, 1382, 1344, 1160, 1004, 882, 492 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 5.69 (s, 1H), 5.50 (br, 1H), 3.70–3.45 (m, 1H), 2.35–2.00 (m, 2H), 1.92 (s, 3H), 1.63–1.20 (m, 4H), 0.95 (t, $J=7.0$ Hz, 3H); MS (APCI, Pos.) m/z 154 $(M+H)^+$; HR-MS (MALDI-TOF, Pos.) calcd for $C_9H_{15}N_1O_1+H^+$: 154.1232; found: 1541266.

6-Allyl-4-methyl-5,6-dihydropyridin-2(1*H*)-one (27g). Compound **27g** was prepared from 5-allyl-3-methyl-2-cyclopenten-1-one (**24g**)[19b] in 48% yield according to the same procedure as described for the preparation of **27a** from **24a**. Brown oil; TLC R_f 0.50 ($CHCl_3$/MeOH, 10/1); IR (neat) 3353, 2970, 2930, 2357, 1697, 1621, 1444, 1381, 1328, 1098, 1054, 932, 887, 825, 775, 720, 455 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 5.90–5.55 (m, 3H), 5.21 (br, 1H), 5.15–5.10 (m, 1H), 3.70–3.55 (m, 1H), 3.00–1.70 (m, 4H), 1.92 (s, 3H); MS (APCI, Pos.) m/z 152 $(M+H)^+$; HR-MS (MALDI-TOF, Pos.) calcd for $C_9H_{13}N_1O_1+H^+$: 152.1075; found: 152.1062.

(*dl*)-(4a*R*,7a*R*)-4-Methyl-1,4a,5,6,7,7a-hexahydro-2*H*-cyclopenta[*b*]pyridin-2-one (27h). Compound **27h** was prepared from (*dl*)-(3a*S*,6a*R*)-3-methyl-4,5,6,6a-tetrahydropentalen-1(3a*H*)-one (**24h**)[19e] in 33% yield according to the same procedure as described for the preparation of **27a** from **24a**. White powder; TLC R_f 0.48 ($CHCl_3$/MeOH, 10/1); ^1H NMR (200 MHz, $CDCl_3$) δ 5.79 (brs, 1H), 5.62–5.57 (m, 1H), 4.08–3.98 (m, 1H), 2.43 (q, $J=7.8$ Hz, 1H), 2.20–1.52 (m, 6H), 1.92 (s, 3H); MS (APCI, Pos.) m/z 152 $(M+H)^+$; HR-MS (MALDI-TOF, Pos.) calcd for $C_9H_{13}N_1O_1+H^+$: 152.1075; found: 152.1073.

(*dl*)-(4a*R*,8a*R*)-4-Methyl-4a,5,6,7,8,8a-hexahydroquinolin-2(1*H*)-one (27i). Compound **27i** was prepared from (*dl*)-(3a*S*,7a*R*)-3-methyl-3a,4,5,6,7,7a-hexahydro-1*H*-

inden-1-one (**24i**)[19f] in 22% yield according to the same procedure as described for the preparation of **27a** from **24a**. White powder; TLC R_f 0.48 ($CHCl_3$/MeOH, 10/1); ^{1}H NMR (200 MHz, $CDCl_3$) δ 5.69–5.63 (m, 1H), 5.23 (brs, 1H), 3.77–3.72 (m, 1H), 2.07–1.95 (m, 1H), 1.92 (d, J = 1.6 Hz, 3H), 1.80–1.11 (m, 8H); MS (APCI, Pos.) m/z 166 $(M+H)^+$; HR-MS (MALDI-TOF, Pos.) calcd for $C_{10}H_{15}N_1O_1 + H^+$: 166.1232; found: 166.1223.

5,5-Dimethylpiperidin-2-one (29).[20a] To a stirred solution of 3,3-dimethylcyclopentanone (**28**)[20b] (8.5 g, 75.8 mmol) in HCO_2H (76 mL) was added NH_2OSO_3H (12.8 g, 113.2 mmol) and the reaction mixture was heated at reflux temperature for 12 h. After cooling at room temperature, the reaction mixture was diluted with water, then treated with 5 M aqueous sodium hydroxide and extracted with $CHCl_3$. The organic layer was washed with brine, dried over anhydrous magnesium sulfate and evaporated under reduced pressure. The residue was purified by recrystallization (EtOAc) to afford **29** as a white powder (5.0 g, 52%). TLC R_f 0.54 ($CHCl_3$/MeOH, 10/1); ^{1}H NMR (200 MHz, $CDCl_3$) δ 5.88 (brs, 1H), 3.02 (d, J = 2.5 Hz, 2H), 2.38 (t, J = 7.0 Hz, 2H), 1.61 (t, J = 7.0 Hz, 2H), 1.05 (s, 6H); MS (APCI, Pos.) m/z 128 $(M+H)^+$.

5,5-Dimethyl-5,6-dihydropyridin-2(1*H*)-one (30). To a stirred solution of **29** (2.5 g, 19.7 mmol) in 1,4-dioxane (65 mL) were added DDQ (4.7 g, 20.6 mmol) and BSTFA (Bis(trimethylsilyl)trifluoroacetamide) (21 mL, 79.1 mmol) and the reaction mixture was heated at reflux temperature for 16 h. After cooling up to room temperature, the reaction mixture was diluted with CH_2Cl_2 (100 mL) and treated with in aqueous sodium bicarbonate carefully. After stirring for 15 min, insoluble substances were removed by filtration. The organic layer was separated and washed with 2 M HCl, brine, dried over anhydrous magnesium sulfate and evaporated under reduced pressure. The residue was purified by column chromatography on silica gel (EtOAc to $CHCl_3$/MeOH, 10/1) to afford **30** as a reddish solid (1.72 g, 70%). TLC R_f 0.36 ($CHCl_3$/MeOH, 10/1); ^{1}H NMR (200 MHz, $CDCl_3$) δ 6.37 (d, J = 10.0 Hz, 1H), 5.76 (dd, J = 10.0, 1.8 Hz, 1H), 5.58 (br, 1H), 3.22–3.17 (m, 2H), 1.14 (s, 6H); MS (APCI, Pos.) m/z 126 $(M+H)^+$; HR-MS (MALDI-TOF, Pos.) calcd for $C_7H_{11}N_1O_1 + H^+$: 126.0919; found: 126.0892.

4-Methyl-1,5,6,7-tetrahydro-2*H*-azepin-2-one (35a). To a stirred solution of 3-methyl-2-cyclohexen-1-one (**33a**) (331 mg, 3 mmol) in MeOH (6 mL) were added NaOAc (310 mg, 3.8 mmol), $NH_2OH{\cdot}HCl$ (260 mg, 3.7 mmol), water (1 mL) and the reaction mixture was heated at reflux temperature for 2 h. After completing the reaction, the reaction mixture was cooled to room temperature and evaporated under reduced pressure. The reaction mixture was treated with water and extracted with $CHCl_3$. The combined organic layers were washed with saturated aqueous sodium bicarbonate, brine, dried over anhydrous magnesium sulfate and evaporated to afford **34a** (307 mg), which was used for the subsequent reaction without further purification.

A solution of **34a** obtained above in PPA (5 mL) was heated at 120 °C for 4 h under stirring. The reaction mixture was cooled up to room temperature and diluted with water and extracted with $CHCl_3$. The combined organic layers were washed with brine, dried over anhydrous magnesium sulfate and evaporated under reduced pressure. The residue was purified by column chromatography on silica gel ($CHCl_3$/MeOH, 20/1) to afford **35a** as a brown powder (158 mg, 42% in two steps). TLC R_f 0.16 ($CHCl_3$/MeOH, 10/1); IR (KBr) 3236, 2930, 1661, 1614, 1479, 1339, 1278, 1142, 1056, 1016, 889, 841, 770, 651, 502, 434, 417 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 6.60–6.40 (brs, 1H), 5.75 (s, 1H), 3.28–3.10 (m, 2H), 2.65–2.42 (m, 1H), 2.18–1.90 (m, 2H), 1.78–1.60 (m, 1H), 1.14 (d, J = 7.0 Hz, 3H); MS (APCI, Pos.) m/z 126 $(M+H)^+$; mp: 74–75 °C; HR-MS (MALDI-TOF, Pos.) calcd for $C_7H_{11}N_1O_1 + H^+$: 126.0919; found: 126.0923.

4,7-Dimethyl-1,5,6,7-tetrahydro-2*H*-azepin-2-one (35b). Compound **35b** was prepared from 3,5-dimethy-2-cyclohexen-1-one (**33b**)[21] in 39% yield according to the same procedure as described for the preparation of **35a** from **33a**. Pale yellow powder; TLC R_f 0.49 ($CHCl_3$/MeOH, 15/1); IR (KBr) 3419, 2971, 2939, 1656, 1609, 1458, 1320, 1006, 866, 806, 646, 578, 528, 442, 416 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 5.98 (brs, 1H), 5.78 (s, 1H), 3.50 (m, 1H), 2.41 (m, 1H), 2.30 (m, 1H), 1.90 (s, 3H), 1.85 (m, 2H), 1.23 (d, J = 6.6 Hz, 3H); mp: 53–54 °C; MS (APCI, Pos.) m/z 140 $(M+H)^+$; HR-MS (MALDI-TOF, Pos.) calcd for $C_8H_{13}N_1O_1 + H^+$: 140.1075; found: 140.1103.

3-Methyl-6-propyl-2-cyclohexen-1-one (33c). Compound **33c** was prepared from 3-methyl-2-cyclohexen-1-one (**33a**) in 38% yield according to the same procedure as described for the preparation of **24f** from **24a**. Pale yellow oil; TLC R_f 0.72 (*n*-hexane/EtOAc, 2/1); IR (neat) 3852, 3749, 3648, 3393, 2957, 2930, 2871, 2365, 1710, 1667, 1541, 1456, 1379, 1200, 889 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 5.83 (brs, 1H), 2.29 (t, J = 6.2 Hz, 2H), 2.26–2.00 (m, 1H), 1.93 (s, 3H), 1.88–1.64 (m, 2H), 1.50–1.25 (m, 4H), 0.92 (t, J = 6.8 Hz, 3H); MS (APCI, Pos.) m/z 153 $(M+H)^+$; HR-MS (MALDI-TOF, Pos.) calcd for $C_{10}H_{16}O_1 + H^+$: 153.1279; found: 153.1289.

4-Methyl-7-propyl-1,5,6,7-tetrahydro-2*H*-azepin-2-one (35c). Compound **35c** was prepared from **33c** in 45% yield according to the same procedure as described for the preparation of **35a** from **33a**. Brown oil; TLC R_f 0.29 ($CHCl_3$/MeOH, 20/1); IR (neat) 3226, 2958, 2930, 2872, 1660, 1439, 1381, 1192, 1110, 744, 536 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 5.78 (s, 1H), 5.65 (brs, 1H), 3.32 (m, 1H), 2.45 (m, 1H), 2.23 (m, 1H), 1.90 (s, 3H), 2.00–1.70 (m, 2H), 1.55–1.25 (m, 4H), 0.93 (t, J = 7.2 Hz, 3H); MS (APCI, Pos.) m/z 168 $(M+H)^+$; HR-MS (MALDI-TOF, Pos.) calcd for $C_{10}H_{17}N_1O_1 + H^+$: 168.1388; found: 168.1343.

4,6-Dimethyl-1,5,6,7-tetrahydro-2*H*-azepin-2-one (35d). Compound **35d** was prepared from 3,5-dimethyl-2-cyclohexen-1-one (**33d**) in 48% yield according to the same procedure as described for the preparation of **35a**

from **33a**. Pale yellow powder; TLC R_f 0.41 ($CHCl_3$/MeOH, 10/1); IR (KBr) 3419, 2958, 1660, 1617, 1457, 1379, 1355, 1310, 1284, 1118, 942, 905, 842, 688, 538, 421 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 6.95–6.65 (brs, 1H), 5.76 (s, 1H), 3.17 (ddd, J = 14.2, 6.6, 4.0 Hz, 1H), 2.94 (ddd, J = 14.2, 7.4, 5.0 Hz, 1H), 2.42 (dd, J = 15.8, 5.5 Hz, 1H), 2.35–2.11 (m, 1H), 2.01 (dd, J = 15.8, 7.8 Hz, 1H), 1.92 (s, 3H), 0.97 (d, J = 6.7 Hz, 3H); mp: 52–54 °C; MS (APCI, Pos.) m/z 140 $(M+H)^+$; HR-MS (MALDI-TOF, Pos.) calcd for $C_8H_{13}N_1O_1+$ H^+: 140.1075; found: 140.1059.

5,5-Dimethyl-1,5,6,7-tetrahydro-2*H*-azepin-2-one (35e). Compound **35e** was prepared from 4,4-dimethyl-2-cyclohexen-1-one (**33e**) in 43% yield according to the same procedure as described for the preparation of **35a** from **33a**. Pale yellow powder; TLC R_f 0.71 ($CHCl_3$/MeOH, 10/1); IR (KBr) 3195, 3036, 2961, 1657, 1618, 1476, 1427, 1394, 1360, 1337, 1308, 1269, 1249, 1205, 1156, 1111, 985, 904, 825, 695, 666, 602, 516 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 6.50–6.18 (brs, 1H), 5.94 (d, J = 12.8 Hz, 1H), 5.70 (dd, J = 12.8, 2.2 Hz, 1H), 3.24 (q, J = 5.2 Hz, 2H), 1.83 (t, J = 4.7 Hz, 2H), 1.11 (s, 6H); mp: 102–103 °C; MS (APCI, Pos.) m/z 140 $(M+H)^+$; HR-MS (MALDI-TOF, Pos.) calcd for $C_8H_{13}N_1O_1+$ H^+: 140.1075; found: 140.1081.

4,4-Dimethyl-6-propyl-2-cyclohexen-1-one (33f). Compound **33f** was prepared from 4,4-dimethyl-2-cyclohexen-1-one (**33e**) in 23% yield according to the same procedure as described for the preparation of **24f** from **24a**. Pale yellow oil; TLC R_f 0.45 (*n*-hexane/EtOAc, 4/1); IR (neat) 3901, 3852, 3838, 3734, 3689, 3675, 3649, 3566, 2958, 2871, 2369, 1681, 1558, 1541, 1507, 1457, 1376, 1237, 1199, 1121, 910, 813, 419 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 6.57 (dd, J = 10.0, 2.2 Hz, 1H), 5.81 (d, J = 10.0 Hz, 1H), 2.50–2.30 (m, 1H), 2.00–1.78 (m, 2H), 1.70–1.10 (m, 4H), 1.18 (s, 3H), 1.14 (s, 3H), 0.93 (t, J = 6.6 Hz, 3H); MS (APCI, Pos.) m/z 167 $(M+H)^+$; HR-MS (MALDI-TOF, Pos.) calcd for $C_{11}H_{18}O_1+$ H^+: 167.1436; found: 167.1454.

5,5-Dimethyl-7-propyl-1,5,6,7-tetrahydro-2*H*-azepin-2-one (35f). Compound **35f** was prepared from **33f** in 46% yield according to the same procedure as described for the preparation of **35a** from **33a**. Brown powder; TLC R_f 0.50 ($CHCl_3$/MeOH, 10/1); IR (KBr) 3175, 2964, 2929, 1663, 1616, 1435, 1389, 1258, 1202, 824, 742, 679, 538 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 5.95 (d, J = 10.4 Hz, 1H), 5.90–5.70 (brs, 1H), 5.73 (dd, J = 10.4, 0.8 Hz, 1H), 3.50–3.32 (m, 1H), 1.90–1.50 (m, 6H), 1.10 (s, 3H), 1.08 (s, 3H), 0.95 (t, J = 6.6 Hz, 3H); mp: 63–64 °C; MS (APCI, Pos.) m/z 182 $(M+H)^+$; HR-MS (MALDI-TOF, Pos.) calcd for $C_{11}H_{19}N_1O_1+$ H^+: 182.1545; found: 182.1553.

General procedure for preparation of compounds 1 and 5–19

4-Methyl-5,6-dihydropyridin-2(1*H*)-imine hydrochloride salt (1). To a stirred solution of **27a** (1.2 g, 10.8 mmol) in CH_2Cl_2 (10 mL) was added triethyloxonium tetrafluoroborate (2 M in CH_2Cl_2, 6.0 mL, 12 mmol) under an argon atmosphere, and the reaction mixture was stirred at room temperature for 5 h. Concentration of the reaction mixture under reduced pressure gave the **31a**, which was used for the subsequent reaction without further purification.

To a stirred solution of **31a** in EtOH (5 mL) was added saturated ethanolic ammonia (20 mL) under an argon atmosphere at room temperature and stirring was continued for an additional 24 h. The reaction mixture was diluted with $CHCl_3$ and the resulting precipitates were removed by filtration. The filtrate was concentrated under reduced pressure. The reaction mixture was treated with 2 M aqueous sodium hydroxide and extracted with $CHCl_3$. The combined organic layers were dried over anhydrous sodium sulfate and evaporated under reduced pressure. The residue was converted to its hydrochloride with 4 M HCl/EtOAc–EtOH at 0 °C and further purified by column chromatography on silica gel ($CHCl_3$/MeOH, 5/1) to afford **1** as a pale yellow powder (126 mg, 8%).

The spectral data of **1** (prepared by Method C) were identical with that of **1** prepared by Method A.

3,4-Dimethyl-5,6-dihydropyridin-2(1*H*)-imine hydrochloride salt (5). Compound **5** was prepared from **27b** in 72% yield according to the same procedure as described for the preparation of **1** from **27a**. White powder; TLC R_f 0.51 ($CHCl_3$/MeOH/AcOH, 10/2/1); IR (KBr) 3312, 1679, 1633, 1599, 1525, 1427, 1362, 1241, 1165, 1141, 1107, 904, 603 cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ 9.41 (brs, 1H), 8.90 (brs, 1H), 8.44 (brs, 1H), 3.29 (dt, J = 7.6, 2.8 Hz, 2H), 2.38 (t, J = 7.6 Hz, 2H), 1.97 (s, 3H), 1.86 (s, 3H); mp: 72–74 °C; MS and HR-MS analysis showed only the ion from $C_7H_{12}N_2$ corresponding to the loss of HCl, MS (EI, Pos.) m/z 124 $(M)^+$, 109 $(M-CH_3)^+$, 94 $(M-2CH_3)^+$; HR-MS (MALDI-TOF, Pos.) calcd for $C_7H_{12}N_2+$ H^+: 125.1079; found: 125.1067.

4,5,5-Trimethyl-5,6-dihydropyridin-2(1*H*)-imine hydrochloride salt (6). Compound **6** was prepared from **27c** in 68% yield according to the same procedure as described for the preparation of **1** from **27a**. Pale pink powder; TLC R_f 0.43 ($CHCl_3$/MeOH/AcOH, 10/2/1); IR (KBr) 3421, 3208, 2968, 2053, 1690, 1521, 1473, 1442, 1379, 1366, 1348, 1277, 1159, 1072, 1026, 1015, 854, 812, 716, 642, 515, 446 cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ 9.30 (brs, 1H), 8.98 (brs, 1H), 8.60 (brs, 1H), 5.95–5.90 (m, 1H), 3.15 (d, J = 3.0 Hz, 2H), 1.96 (d, J = 1.2 Hz, 3H), 1.05 (s, 6H); mp: 127–128 °C; MS and HR-MS analysis showed only the ion from $C_8H_{14}N_2$ corresponding to the loss of HCl, MS (APCI, Pos.) m/z 139 $(M+H)^+$, 124 $(M-CH_3+H)^+$; HR-MS (MALDI-TOF, Pos.) calcd for $C_8H_{14}N_2+$ H^+: 139.1235; found: 139.1216.

5,5-Dimethyl-5,6-dihydropyridin-2(1*H*)-imine hydrochloride salt (7). Compound **7** was prepared from **30** in 21% yield according to the same procedure as described for the preparation of **1** from **27a**. White powder; TLC R_f 0.44 ($CHCl_3$/MeOH/AcOH, 10/1/1); IR (KBr) 3408,

3258, 3086, 2970, 2875, 1688, 1600, 1523, 1463, 1395, 1366, 1336, 1168, 796, 667 cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ 9.28 (brs, 1H), 9.12 (brs, 1H), 8.73 (brs, 1H), 6.80 (d, $J=9.8$ Hz, 1H), 6.11 (dd, $J=9.8$, 2.0 Hz, 1H), 3.19 (d, $J=2.6$ Hz, 2H), 1.07 (s, 6H); mp: 131–133 °C; MS and HR-MS analysis showed only the ion from $C_7H_{12}N_2$ corresponding to the loss of HCl, MS (EI, Pos.) m/z 124 $(M)^+$, 123 $(M-H)^+$, 109 $(M-CH_3)^+$, 94 $(M-2CH_3)^+$; HR-MS (MALDI-TOF, Pos.) calcd for $C_7H_{12}N_2+H^+$: 125.1079; found: 125.1055.

4,5-Dimethyl-5,6-dihydropyridin-2(1*H*)-imine hydrochloride salt (8). Compound **8** was prepared from **27d** in 21% yield according to the same procedure as described for the preparation of **1** from **27a**. White powder; TLC R_f 0.50 ($CHCl_3$/MeOH/AcOH, 10/2/1); IR (KBr) 3387, 1686, 1607, 1533, 1457, 1436, 1386, 1360, 1332, 1294, 1181, 1019, 966, 849, 636 cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ 9.13 (brs, 1H), 8.92 (brs, 1H), 8.53 (brs, 1H), 5.97–5.92 (m, 1H), 3.43 (ddd, $J=13.2$, 6.0, 1.8 Hz, 1H), 3.17 (ddd, $J=13.2$, 5.0, 4.4 Hz, 1H), 2.51–2.40 (m, 1H), 2.01 (s, 3H), 1.05 (d, $J=7.2$ Hz, 3H); mp: 109–110 °C; MS and HR-MS analysis showed only the ion from $C_7H_{12}N_2$ corresponding to the loss of HCl, MS (EI, Pos.) m/z 124 $(M)^+$, 109 $(M-CH_3)^+$, 94 $(M-2CH_3)^+$; HR-MS (MALDI-TOF, Pos.) calcd for $C_7H_{12}N_2+H^+$: 125.1079; found: 125.1053.

4,6-Dimethyl-5,6-dihydropyridin-2(1*H*)-imine hydrochloride salt (9). Compound **9** was prepared from **27e** in 34% yield according to the same procedure as described for the preparation of **1** from **27a**. Beige amorphous; TLC R_f 0.15 ($CHCl_3$/MeOH/AcOH, 10/1/1); IR (KBr) 3373, 1680, 1631, 1543, 1417, 1371, 1336, 1060, 881, 840, 762, 689, 620 cm^{-1}; ^1H NMR (200 Mz, DMSO-d_6) δ 8.98–8.82 (brs, 1H), 8.80–8.64 (brs, 1H), 8.08–7.92 (brs, 1H), 5.91 (s, 1H), 3.80–3.58 (m, 1H), 2.58–2.40 (m, 1H), 2.18 (dd, $J=18.0$, 10.0 Hz, 1H), 1.98 (s, 3H), 1.18 (d, $J=6.6$ Hz, 3H); MS and HR-MS analysis showed only the ion from $C_7H_{12}N_2$ corresponding to the loss of HCl, MS (APCI, Pos.) m/z 125 $(M+H)^+$; HR-MS (MALDI-TOF, Pos.) calcd for $C_7H_{12}N_2+H^+$: 125.1079; found: 125.1085.

4-Methyl-6-propyl-5,6-dihydropyridin-2(1*H*)-imine hydrochloride salt (10). Compound **10** was prepared from **27f** in 67% yield according to the same procedure as described for the preparation of **1** from **27a**. Brown oil; TLC R_f 0.30 ($CHCl_3$/MeOH/AcOH, 20/1/1); IR (neat) 3393, 2961, 1685, 1638, 1605, 1536, 1439, 1382, 1351, 1177, 835, 654 cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ 9.38 (brs, 1H), 8.98 (brs, 1H), 8.42 (brs, 1H), 5.97 (s, 1H), 3.68–3.45 (m, 1H), 2.60–2.40 (m, 1H), 2.21 (dd, $J=18.0$, 10.0 Hz, 1H), 1.98 (s, 3H), 1.70–1.10 (m, 4H), 0.88 (t, $J=7.0$ Hz, 3H); MS and HR-MS analysis showed only the ion from $C_9H_{16}N_2$ corresponding to the loss of HCl, MS (EI, Pos.) m/z 152 $(M)^+$, 109, 92; HR-MS (MALDI-TOF, Pos.) calcd for $C_9H_{16}N_2+H^+$: 153.1392; found: 153.1364.

6-Allyl-4-methyl-5,6-dihydropyridin-2(1*H*)-imine hydrochloride salt (11). Compound **11** was prepared from **27g** in 51% yield according to the same procedure as described for the preparation of **1** from **27a**. Brown oil; TLC R_f 0.50 ($CHCl_3$/MeOH/AcOH, 15/2/1); IR (neat) 3079, 1683, 1643, 1605, 1532, 1440, 1353, 1174, 1001, 926 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 9.40–9.05 (brs, 2H), 8.70–8.50 (brs, 1H), 6.37 (s, 1H), 5.89–5.68 (m, 1H), 5.24–5.15 (m, 2H), 3.76–3.63 (m, 1H), 2.54–2.30 (m, 4H), 2.03 (s, 3H); MS and HR-MS analysis showed only the ion from $C_9H_{14}N_2$ corresponding to the loss of HCl, MS (APCI, Pos.) m/z 151 $(M+H)^+$, 109; HR-MS (MALDI-TOF, Pos.) calcd for $C_9H_{14}N_2+H^+$: 151.1235; found: 151.1240.

(*dl*)-(4a*R*,7a*R*)-4-Methyl-1,4a,5,6,7,7a-hexahydro-2*H*-cyclopenta[*b*]pyridin-2-imine hydrochloride salt (12). Compound **12** was prepared from **27h** in 70% yield according to the same procedure as described for the preparation of **1** from **27a**. White powder; TLC R_f 0.44 ($CHCl_3$/MeOH/AcOH, 10/1/1); IR (KBr) 3398, 3122, 2965, 1687, 1645, 1620, 1536, 1443, 1391, 1365, 1312, 1196, 1051, 851, 780, 672 cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ 9.41 (brs, 1H), 9.0 (brs, 1H), 8.37 (brs, 1H), 5.92 (s, 1H), 4.05–3.92 (m, 1H), 2.70 (q, $J=8.6$ Hz, 1H), 2.25–1.90 (m, 2H), 2.01 (s, 3H), 1.87–1.34 (m, 4H); mp: 121–122 °C; MS and HR-MS analysis showed only the ion from $C_9H_{14}N_2$ corresponding to the loss of HCl, MS (FAB, Pos.) m/z 337 $(2M+HCl+H)^+$, 151 $(M+H)^+$, 122, 109; HR-MS (MALDI-TOF, Pos.) calcd for $C_9H_{14}N_2+H^+$: 151.1235; found: 151.1249.

(*dl*)-(4a*R*,8a*R*)-4-Methyl-4a,5,6,7,8,8a-hexahydroquinolin-2(1*H*)-imine hydrochloride salt (13). Compound **13** was prepared from **27i** in 44% yield according to the same procedure as described for the preparation of **1** from **27a**. White powder; TLC R_f 0.36 ($CHCl_3$/MeOH/AcOH, 10/1/1); IR (KBr) 3447, 3248, 2940, 2861, 1689, 1620, 1594, 1532, 1450, 1437, 1367, 1332, 1315, 1286, 1208, 1167, 1104, 1065, 1031, 992, 960, 917, 893, 856, 728, 640, 565, 509, 480 cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ 9.26 (brs, 1H), 8.90 (brs, 1H), 8.13 (brs, 1H), 5.96–5.92 (m, 1H), 3.70–3.61 (m, 1H), 2.35–2.21 (m, 1H), 2.02 (s, 3H), 1.95–0.95 (m, 8H); mp: 76–77 °C; MS and HR-MS analysis showed only the ion from $C_{10}H_{16}N_2$ corresponding to the loss of HCl, MS (EI, Pos.) m/z 164 $(M)^+$, 149 $(M-CH_3)^+$, 135, 121, 109; HR-MS (MALDI-TOF, Pos.) calcd for $C_{10}H_{16}N_2+H^+$: 165.1392; found: 165.1379.

4-Methyl-1,5,6,7-tetrahydro-2*H*-azepin-2-imine hydrochloride salt (14). Compound **14** was prepared from **35a** in 22% yield according to the same procedure as described for the preparation of **1** from **27a**. Beige viscous amorphous; TLC R_f 0.25 ($CHCl_3$/MeOH/AcOH, 10/1/1); IR (KBr) 3115, 1672, 1616, 1438, 1386, 1084, 694 cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ 9.48–9.32 (brs, 1H), 8.67–8.46 (brs, 1H), 8.26–8.13 (brs, 1H), 5.88 (s, 1H), 3.32–3.24 (m, 2H), 2.52–2.43 (m, 2H), 1.98 (s, 3H), 1.95–1.78 (m, 2H); MS and HR-MS analysis showed only the ion from $C_7H_{12}N_2$ corresponding to the loss of HCl, MS (FAB, Pos.) m/z 125 $(M+H)^+$; HR-MS (MALDI-TOF, Pos.) calcd for $C_7H_{12}N_2+H^+$: 125.1079; found: 125.1077.

4,7-Dimethyl-1,5,6,7-tetrahydro-2*H*-azepin-2-imine hydrochloride salt (15). Compound **15** was prepared from **35b** in 44% yield according to the same procedure

as described for the preparation of **1** from **27a**. Yellow amorphous; TLC R_f 0.37 ($CHCl_3$/MeOH/AcOH, 15/2/1); IR (KBr) 3292, 1676, 1616, 1540, 1457, 1387, 1321 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 9.71 (brs, 1H), 8.53 (brs, 1H), 8.35 (brs, 1H), 6.17 (s, 1H), 3.63 (m, 1H), 2.49 (m, 2H), 2.04 (s, 3H), 1.99–1.91 (m, 2H), 1.38 (d, J = 6.8 Hz, 3H); MS and HR-MS analysis showed only the ion from $C_8H_{14}N_2$ corresponding to the loss of HCl, MS (APCI, Pos.) m/z 139 $(M+H)^+$; HR-MS (MALDI-TOF, Pos.) calcd for $C_8H_{14}N_2 + H^+$: 139.1235; found: 139.1213.

4-Methyl-7-propyl-1,5,6,7-tetrahydro-2*H*-azepin-2-imine hydrochloride salt (16). Compound **16** was prepared from **35c** in 19% yield according to the same procedure as described for the preparation of **1** from **27a**. White powder; TLC R_f 0.58 ($CHCl_3$/MeOH/AcOH, 15/2/1); IR (KBr) 3234, 3117, 1678, 1640, 1609, 1534, 1438, 646 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 9.82 (brs, 1H), 8.91 (brs, 1H), 7.82 (brs, 1H), 6.08 (s, 1H), 3.44 (m, 1H), 2.48 (m, 2H), 2.04 (s, 3H), 2.10–1.40 (m, 6H), 0.95 (t, J = 7.0 Hz, 3H); mp: 103–104 °C; MS and HR-MS analysis showed only the ion from $C_{10}H_{18}N_2$ corresponding to the loss of HCl, MS (APCI, Pos.) m/z 167 $(M+H)^+$; HR-MS (MALDI-TOF, Pos.) calcd for $C_{10}H_{18}N_2 + H^+$: 167.1548; found: 167.1552.

4,6-Dimethyl-1,5,6,7-tetrahydro-2*H*-azepin-2-imine hydrochloride salt (17). Compound **17** was prepared from **35d** in 34% yield according to the same procedure as described for the preparation of **1** from **27a**. Orange viscous oil; TLC R_f 0.38 ($CHCl_3$/MeOH/AcOH, 10/1/1); IR (neat) 3292, 3152, 2964, 1678, 1641, 1615, 1537, 1454 cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ 9.64–9.54 (brs, 1H), 8.78–8.63 (brs, 1H), 8.49–8.37 (brs, 1H), 5.91 (s, 1H), 3.40–2.94 (m, 2H), 2.62–2.42 (m, 1H), 2.25–2.06 (m, 2H), 1.98 (s. 3H), 0.91 (d, J = 6.6 Hz, 3H); MS and HR-MS analysis showed only the ion from $C_8H_{14}N_2$ corresponding to the loss of HCl, MS (APCI, Pos.) m/z 139 $(M+H)^+$; HR-MS (MALDI-TOF, Pos.) calcd for $C_8H_{14}N_2 + H^+$: 139.1235; found: 139.1227.

5,5-Dimethyl-1,5,6,7-tetrahydro-2*H*-azepin-2-imine hydrochloride salt (18). Compound **18** was prepared from **35e** in 16% yield according to the same procedure as described for the preparation of **1** from **27a**. Yellow powder; TLC R_f 0.40 ($CHCl_3$/MeOH/AcOH, 10/1/1); IR (KBr) 3401, 1681, 1624, 1534, 1474, 1385, 1350, 1309, 1253, 1222, 1182, 1125, 1085 cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ 10.07–9.89 (brs, 1H), 9.13–8.94 (brs, 1H), 8.70–8.53 (brs, 1H), 6.45 (d, J = 12.8 Hz, 1H), 5.90 (d, J = 12.8 Hz, 1H), 3.38–3.20 (m, 2H), 1.77–1.72 (m, 2H), 1.07 (s, 6H); mp: 98–100 °C; MS and HR-MS analysis showed only the ion from $C_8H_{14}N_2$ corresponding to the loss of HCl, MS (APCI, Pos.) m/z 139 $(M+H)^+$; HR-MS (MALDI-TOF, Pos.) calcd for $C_8H_{14}N_2 + H^+$: 139.1235; found: 139.1220.

5,5-Dimethyl-7-propyl-1,5,6,7-tetrahydro-2*H*-azepin-2-imine hydrochloride salt (19). Compound **19** was prepared from **35f** in 10% yield according to the same procedure as described for the preparation of **1** from **27a**. Brown oil; TLC R_f 0.25 ($CHCl_3$/MeOH, 10/1); IR (neat) 3243, 3099, 2961, 2873, 1681, 1608, 1539, 1471, 1455, 1049, 817, 667 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 9.65–9.20 (brs, 1H), 8.75–8.43 (brs, 1H), 8.40–8.00 (brs, 1H), 6.33 (d, J = 12.8 Hz, 1H), 6.08 (d, J = 12.8 Hz, 1H), 3.52–3.33 (m, 1H), 2.00–1.38 (m, 6H), 1.14 (s, 3H), 1.13 (s, 3H), 0.97 (d, J = 6.4 Hz, 3H); MS and HR-MS analysis showed only the ion from $C_{11}H_{20}N_2$ corresponding to the loss of HCl, MS (APCI, Pos.) m/z 181 $(M+H)^+$; HR-MS (MALDI-TOF, Pos.) calcd for $C_{11}H_{20}N_2 + H^+$: 181.1705; found: 181.1685.

Preparation of partially purified enzyme and determination of K_i values

Human eNOS was overexpressed in Sf-21 cells, by infecting the cells with baculovirus carrying heNOS cDNA. The hiNOS was overexpressed in A549 by stimulation with LPS (10 μg/mL) plus cytokines (10 ng/mL TNF-α, 5 ng/mL IL-1 β and 100 ng/mL interferon-γ). Human eNOS and iNOS were partially purified by chromatography on 2′, 5′-ADP-Sepharose gels. NOS activity was determined by the method for the conversion of [^{14}C]-L-arginine to L-citrulline with a minor modification. The conversion rates for various concentrations of the test compounds and L-arginine were measured. Dixon and Lineweaver–Burk plots were constructed to determine the K_i values and the mode of inhibition.

Enzyme assay with recombinant mouse iNOS

Recombinant mouse iNOS was purchased from Cayman Chemical (Cat. No. 60862) and the inhibitory activity of each test compound was evaluated by measuring the conversion rate from [^{14}C]-L-arginine to [^{14}C]-L-citrulline. Then the IC_{50} values of the compounds were determined.

The ID_{50} values were determined from log-logit transformation of the dose–response curves (**1**, **3**, **5–6**, **8–12** and **14–17**; 0.1, 0.3, 1 mg/kg, sc, L-NMMA; 10, 30 and 100 mg/kg, sc). The ID_{50} value was defined as the dose of a test compound that produced 50% inhibition in the NOx accumulation induced by LPS treatment alone. The MTD was defined as the maximum dose at which no death was observed within 24 h after an intravenous injection administration. The doses used were 5, 10, 20, 30, 40, and 50 mg/kg for **1**, **3**, **5–6**, **8–12** and **14–17**, while 1000, 2000, 3000, 4000, and 5000 mg/kg were used for L-NMMA.

Inhibition of NOx accumulation and the maximum tolerated dose (MTD) in mice

The test compounds or saline were administered subcutaneously at 3 h after LPS (10 mg/kg, iv) injection into 7 weeks old Balb/c mice (Charles River Japan, Inc.). Blood was collected by venipuncture from the abdominal aorta under light anesthesia at 6 h after LPS treatment. Plasma was obtained by centrifugation and the concentration of accumulated NOx over 3 h was determined by the method described below. To evaluate the acute toxicity, the MTD (iv maximum dose where no

death was observed within 24 h after the administration) of the test compound was determined.

Measurement of nitrite/nitrate

Nitrite and nitrate, the oxidized form of nitric oxide that accumulated in the culture medium and plasma were determined by the use of nitrite/nitrate colorimetric assay kit (Cayman Chemical, Cat. No. 780001). Basically, the nitrate in the sample was reduced to nitrite with a nitrate reductase contained in the assay kit; nitrite levels were then determined spectrophotometrically as the total NOx concentration.

Pharmacokinetic (PK) studies

Pharmacokinetic parameters were determined in rats after intravenous (2 mg/kg, iv) or oral (10 mg/kg, po) administration. Rats ($N=3$) were given the test compounds intravenously or orally in physiological saline (1 mL/kg, iv or 2.5 mL/kg, po). Blood samples (250 μL) were collected from the jugular veins at 0.25, 0.5, 1, 2, 3, 4, 6 and 8 h after dosing.

Plasma samples were analyzed after extraction of the test compounds by simple liquid–liquid extraction. LC/MS/MS analysis was performed with a Quattro II (Micromass Co., Ltd.) mass spectrometer consisting of a Perkin–Elmer series 200 solvent delivery system, a Perkin–Elmer ISS 200 autoinjector, and a Waters Symmetry octyl mini-bore column (2.1×50 mm).

Docking study

Docking study was performed using InsightII/Discover molecular modeling package (Accelrys, San Diego, CA) with CVFF force field on a SGI Octane2 workstation with R12000 processors. Simulated-annealing procedure in gas phase was used to search the complex model of the lowest-energy. Initial protein structure of hiNOS (PDB code 2NSI) was obtained from the Protein Data Bank. All the hydrogens were added and their positions were energetically optimized. During simulation, all the heavy atoms were fixed (dielectric constant: $\varepsilon=4r$).

First of all, a complex model of low-energy, consisting of **10** with hiNOS was constructed, in which **10** was put near the Glu377 residue of the enzyme to form a salt bridge with its amidine group. A complex model was first equilibrated by running dynamics (dielectric constant: $\varepsilon=4r$) for 6 ps while increasing the temperature from 50 to 1200 K by time steps of 1 fs, after which the resulting conformations were sampled every 5000 steps over a span of 300 ps at 1200 K to yield 60 snapshots. Each snapshot was then equilibrated for 4 ps while decreasing the temperature from 1200 to 200 K, followed by 200 K simulation for 4 ps. Each annealed model was energetically optimized using the steepest descent method followed by the conjugate-gradient method to an energy difference of 0.001 kcal/mol between successive iterations.

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