Synthesis and Ocular Effects of Imidazole Nitrolic Acids

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Novel 1-R-imidazole-2-nitrolic acids and 1-R-imidazole-5-nitrolic acids (R: H, Me, Bn) were synthesized from oximes by treatment with a mixture of fuming nitric acid and acetic acid. The effects of these potential nitric oxide-donating compounds were tested on ocular variables such as intraocular pressure and formation of cyclic guanosine-3,5′-monophosphate in the incubation of porcine iris-ciliary body.

Introduction

In the past, nitrolic acids (Figure 1) have been considered relatively unstable compounds. However, recent studies indicate that some of them are stable enough to be heated several hours, purified in column chromatography, and crystallized for X-ray crystallographic measurements.[1−3] It has been suggested that the stability of nitrolic acids depends on the substituents; electron-donating character of the R group (Figure 1) increases the stability of the compound. For some of the previously studied nitrolic acids, the stereochemistry of the nitrogen−carbon double bond is suggested to be crucial for the stability; (*E*)-isomers are more stable.[1]

Nitrolic acids have been prepared using various compounds as starting materials, among them nitro- and halogen-substituted compounds, oximes, and olefins.[1,3,4] Since nitrolic acids decompose to carboxylic acids, they have been used as intermediates in the preparation of carboxylic acids from nitro- and halogen-substituted compounds.[3] Since they decompose via nitrile oxides, they have also been used as a source of nitrile oxides for 1,3-dipolar cycloadditions.[5,6] Some nitrolic acids have been tested for antithrombotic and blood pressure-lowering activities. The compounds studied were poor or inactive antiplatelet agents in vitro, but, depending on the substituents, they exhibited strong or moderate antithrombotic effects in vivo. These effects were suggested to be a consequence of the nitric oxide (NO) donation of nitrolic acids. Effects on blood pressure were not significant.[7]

In the eye, NO is involved in a wide range of physiological events, such as regulation of aqueous humor dynamics, neuronal visual processing, and ocular hemodynamics. Both underproduction and overproduction of NO may contribute to the pathogenesis of degenerative eye diseases (glaucoma, retinal degeneration, cataract) or inflammatory eye diseases (uveitis, retinitis).[8,9] It has been shown that NO-releasing compounds and cyclic guanosine 3′,5′-monophosphate (cGMP), the second messenger of NO, lower intraocular pressure (IOP).[10−17] They may be involved in the modulation of aqueous humor dynamics through enhancing the outflow facility of aqueous humor, which results in lowered IOP.[13,14,18]

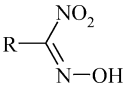
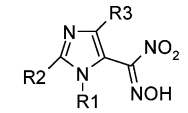
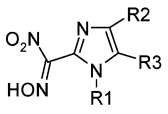


Figure 1. General formula for nitrolic acids.



3a-e

	R1	R2	R3
a	Bn	H	H
b	Me	H	H
c	H	H	H
d	H	Ph	H
e	H	H	Me
f	Bn	H	H
g	Me	H	H
h	H	H	H



3f-h

Figure 2. Prepared nitrolic acids.

Aware of the various biological activities of imidazole compounds, we became interested in attaching potential nitric oxide (NO)-donating functionalities to the imidazole scaffold. In the present study we report the preparation of novel, sufficiently stable imidazole nitrolic acids (Figure 2) and their ocular effects measured by various methods.

Chemistry

All nitrolic acids were prepared by nitration of corresponding oximes (Scheme 1). Oximes were prepared by treating aldehydes with hydroxylamine hydrochloride in neutralized water solutions, and the nitration of the oximes was carried out in a mixture of acetic acid and fuming nitric acid. The nitrolic acids precipitated from the reaction mixture when pH was adjusted to about 3. If the precipitate was filtered out before pH

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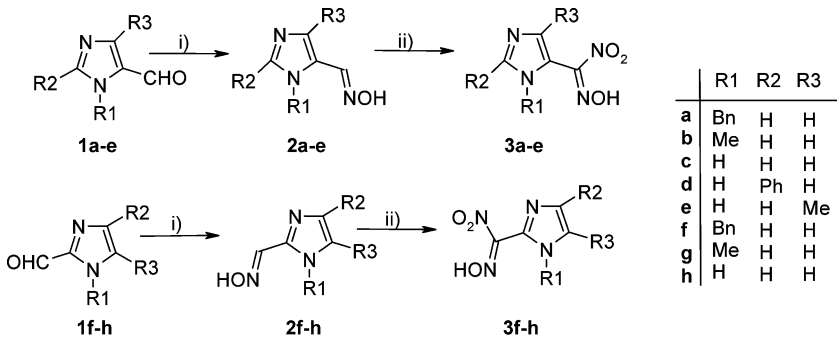
Scheme 1[a]



	R1	R2	R3
a	Bn	H	H
b	Me	H	H
c	H	H	H
d	H	Ph	H
e	H	H	Me
f	Bn	H	H
g	Me	H	H
h	H	H	H

[a] Reagents: (i) $NH_2OH{\cdot}HCl$, H_2O, Na_2CO_3; (ii) fuming HNO_3, AcOH.

Table 1. Effects of Imidazole Nitrolic Acids on IOP in Rabbits and Increase of Concentrations of NO_x, Nitrite, and cGMP in Porcine Iris-Ciliary Body Incubation (mean ± SEM). Statistics are Calculated Based on the Respective Controls of the Experiment

compound	maximal decrease in IOP (time) ($n = 4$) mmHg	iris-ciliary body incubation ($n = 6$)		
		NO_x (μM)	nitrite (μM)	cGMP (pmol/mg prot.)
control		0.36 ± 0.08	0.15 ± 0.04	0.46 ± 0.03
1a	NE	NE[d,e]	NE[d,e]	0.30 ± 0.04[d]
				0.38 ± 0.05[e]
1b	NE	0.63 ± 0.25[d]	0.34 ± 0.04[d]	0.38 ± 0.03[d]
		0.09 ± 0.04[e]	0.62 ± 0.18[e]	0.50 ± 0.05[e]
2a	NE	NE[d]	NE[d]	0.30 ± 0.04[d]
2a·HCl	NE	1.97 ± 0.10[d]	NE[d,e]	0.58 ± 0.06[d]
		2.21 ± 0.07 [c,e]		0.58 ± 0.03[e]
2b	NE	NE[d,e]	NE[d,e]	0.51 ± 0.04[d]
				0.58 ± 0.03[e]
3a	NE	2.01 ± 0.10[c,d]	2.15 ± 0.04[c,d]	0.46 ± 0.03[d]
		24.60 ± 0.17[c,e]	24.44 ± 0.28[c,e]	3.29 ± 0.40[c,e]
3b	NE	3.38 ± 0.10[c,d]	3.46 ± 0.09[c,d]	0.87 ± 0.30[d]
		29.96 ± 1.80[c,e]	32.07 ± 0.52 [c,e]	1.19 ± 0.15[a,e]
3b·HNO_3	ivtr: −4.0 [g]	4.87 ± 0.27[c,d]	2.68 ± 0.05[c,d]	1.01 ± 0.10[c,d]
	(28 h)	51.95 ± 0.98[c,e]	28.05 ± 0.38[c,e]	2.76 ± 0.40[c,e]
3c	ivtr: −7.3[a, g]	NE[d,e]	0.18 ± 0.04[d]	0.51 ± 0.04[d]
	(24 h)		0.14 ± 0.04[e]	0.55 ± 0.03[e]
3d	NE	NA	NA	NA
3e	NE	3.06 ± 0.04[c,d]	3.62 ± 0.14[c,d]	0.60 ± 0.08[a,d]
		30.62 ± 0.57[c,e]	31.22 ± 0.61[c,e]	1.22 ± 0.17[a,e]
3f	ivtr: −5.6 [f]	0.06 ± 0.001[d]	0.44 ± 0.03[d]	0.44 ± 0.03[d]
	(24 h)	22.18 ± 1.01[c,e]	20.79 ± 0.39[c,e]	1.17 ± 0.14[b,e]
3g	ivtr: −6.5 [f]	3.51 ± 0.06[c,d]	4.30 ± 0.11[c,d]	0.61 ± 0.07[a,d]
	(24 h)	37.39 ± 0.51[c,e]	43.35 ± 0.61[c,e]	1.44 ± 0.18[c,e]
3h	NE	NE[d,e]	0.12 ± 0.04[d]	0.72 ± 0.12[a,d]
			0.10 ± 0.03[e]	0.54 ± 0.07[e]

Abbreviations: ivtr = intravitreal injection of test compound, NE = no effect on IOP or increase in concentration as compared to control, NA = not assayed. [a] $p < 0.05$. [b] $p < 0.01$. [c] $p < 0.001$. [d] Concentration 10 μM. [e] Concentration 100 μM. [f] Concentration 10 mM. [g] Concentration 20 mM.

adjustment, 1-methylimidazole-5-nitrolic acid **3b** was also obtained as an HNO_3 salt. All nitrolic acids were obtained as a single isomer. It may be that both isomers are formed in the reaction, but the other isomer decomposes during isolation or is much more soluble. This could be the reason for the relatively low yields of nitrolic acids (30−68%).

The studied nitrolic acids were sufficiently stable and could be stored in a fridge several weeks or even months without significant decomposition. In DMSO solution, however, the decomposition was much more rapid. 1-Benzyl-substituted nitrolic acids **3a** and **3f** completely decompose within 24 and 48 h, respectively. 1-Methyl-substituted nitrolic acids **3b** and **3g** decomposed within 24 h, while compounds **3c** and **3h** were stable in DMSO solution several days. It has been suggested that the configuration may affect the stability of nitrolic acids.[1] Previously attempts have been made to assign the configuration by preparing *O*-esters and assigning the configuration according to the *O*-acetyl signals, as have been done for oximes.[1] However, the X-ray crystallographic data showed that assignments made by this method were not unambiguous for nitrolic acids.[1] 1(*H*)-Imidazole-2-nitrolic acid **3h** and the HNO_3 salt of 1-methylimidazole-5-nitrolic acid **3b** could be crystallized, enabling determination of X-ray structures. Both compounds were (*E*)-isomers.

Pharmacology

IOP Measurements. Table 1 shows the in vivo IOP data obtained for the imidazole compounds in normotensive rabbits after topical and intravitreal administration of the test compounds. Intravitreal injections of **3b**·HNO_3, **3c**, **3f**, and **3g** lowered IOP.

Incubation of Porcine Iris-Ciliary Bodies. NO_x, nitrite, and cGMP data in incubation of porcine iris-ciliary bodies measured after administration of test compounds (concentrations 10 μM and 100 μM) are shown in Table 1.

Table 2. NO_x, Nitrite, and cGMP Concentrations in Aqueous Humor and in Plasma after Topical (**3a**, **3f**) and Intravitreal (**3b**·HNO_3, **3c**) Administration in Rabbits (mean ± SEM). Statistics are Calculated Based on the Respective Controls of the Experiment

		aqueous humor ($n = 4$)			plasma ($n = 4$)	
compound		NO_x (μM)	nitrite (μM)	cGMP (pmol/mg prot.)	NO_x (μM)	nitrite (μM)
					control	control
					408.59 ± 47.90	0.98 ± 0.15
3a	drug	302.19 ± 52.29	1.61 ± 0.16	16.79 ± 1.97	379.43 ± 63.69	0.87 ± 0.13
	control	309.09 ± 45.89	1.31 ± 0.17	16.10 ± 2.88		
3b·HNO_3	drug	524.29 ± 48.06	10.32 ± 0.41[b]	9.41 ± 1.34	425.49 ± 50.35	0.75 ± 0.07
	control	500.86 ± 17.90	1.32 ± 0.12	9.17 ± 1.38		
3c	drug	451.94 ± 27.95	2.49 ± 0.87	21.77 ± 1.79[a]	469.42 ± 16.06	1.38 ± 0.48
	control	450.31 ± 26.92	2.40 ± 0.91	14.31 ± 1.49		
3f	drug	607.73 ± 51.05	2.91 ± 0.70[a]	13.23 ± 0.73	522.07 ± 58.76	1.69 ± 0.46
	control	574.79 ± 30.56	2.55 ± 0.65	11.26 ± 0.84		

[a] $p < 0.05$. [b] $p < 0.001$.

Nitrite, Nitrate, and Cyclic GMP in Aqueous Humor and Plasma. Table 2 presents the change in concentration of NO metabolites NO_x (nitrate + nitrite) and nitrite and cGMP in aqueous humor 1 h after topical (**3a** and **3f**, concentration 20 mM) and 5 h/24 h after intravitreal (**3b**·HNO_3/**3c**, respectively, concentration 20 mM) administration of test compounds.

Aqueous Humor Outflow Facility. Aqueous humor outflow facility was measured after the intracameral injection of compound **3c** ($n = 3$, concentration 10 mM), but compared with a contralateral controls eye, the compound had no significant effect.

Discussion

Nitric oxide has numerous sites of action in the eye, including the ciliary muscle, the trabecular meshwork, and endothelial and vascular smooth muscle cells in the aqueous drainage system.[8] Recent studies have demonstrated that NO-donating compounds lower IOP in animals.[13,14,16,17] The elevation of cGMP concentration by guanylate cyclase activators or cGMP analogues is also associated with a reduction of IOP.[12,15,17,19−21] Furthermore, NO-donating compounds and cGMP analogues increase aqueous humor outflow,[18,20] indicating that this may be one of the main mechanisms of action of these compounds. There is, therefore, accumulating evidence to suggest a role for NO in the control of IOP, but no current antiglaucomatous drugs are based on the nitric oxide-cGMP pathway.

IOP was measured with a pneumatonometer calibrated to the eye of the rabbit. Pneumatonometry is the most reliable approach in IOP determination of anesthetized eyes in vivo.[22] The limitation of the method is that it slightly disturbs normal aqueous humor dynamics. To minimize this disturbance, IOP measurements were made simultaneously for the experimental and control eyes of the rabbit. In addition, measurements were made by the same person at the same time of day. The physicochemical properties of imidazole nitrolic acids may restrict the penetration of these compounds into the eye after topical administration. Positive effects on IOP were observed after intravitreal injections of imidazole nitrolic acids. These compounds had a fairly long-lasting effect on IOP with the duration of up to 6 days.

Most of the effects of NO donors are mediated via the activation of guanylate cyclase. These effects were determined in the porcine eye by a simple incubation method by measuring the production of cGMP. The iris-ciliary body is the target tissue of NO in the modulation of aqueous humor flow. This novel method of porcine iris-ciliary body incubation was introduced by Kotikoski et al.[24] In our previous studies NO donors and cGMP activators, which lower IOP in rabbits in vivo,[17] clearly increased the production of cGMP in the porcine iris-ciliary body in vitro.[24] In our present study, all studied imidazole nitrolic acids with IOP-lowering ability except **3c** increased cGMP production in the porcine iris-ciliary body.

Aqueous humor outflow facility was measured by the two-level constant pressure infusion method according to Bárány.[23] This method is accurate and even small changes in the outflow facility can be detected.

Under various in vitro conditions, the primary decomposition products of nitrolic acids are nitrile oxides.[1,3,5] Nitrile oxides are formed after a formal loss of HNO_2. In an investigation of volatile decomposition products of nitrolic acids by mass spectrometry and gas chromatography, Rehse et al. found the main product to be nitrous oxide (N_2O).[7] In the same study, several alkyl nitrolic acids were tested for antithrombotic effects and the lowering of blood pressure. The lowering of blood pressure was not significant. The nitrolic were poor or inactive antiplatelet agents in vitro; however, in vivo exhibited strong or moderate antithrombotic effects. These effects were suggested to be a consequence of the NO donation of the nitrolic acids.[7] Our results, especially the elevated cGMP concentrations in the iris ciliary incubation experiments, support the suggestion that NO is involved. Although there is no direct evidence that nitrolic acids donate NO in vitro, different decomposition mechanisms could well exist in vivo. This could also explain the differences between the in vivo and in vitro experiments found by of Rehse's group.

Nitrolic acid **3c** lowered the IOP but had no effect on the concentration of cGMP in iris-ciliary incubation. It is possible that **3c** lowers IOP by a different mechanism because not all the effects of NO are mediated by cGMP. Compounds **3a**,**b**, **3e**, **3h** had no effect on IOP while they showed elevated NO_x, nitrite and/or cGMP concentrations in iris-ciliary incubation. This finding might be due to their inability to reach the site of action at effective concentrations, rather than inability to donate NO.

Conclusions

Sufficiently stable differently substituted imidazole nitrolic acids were prepared by nitrating corresponding oximes. Nitrolic acids were isolated as single isomers. Of the studied nitrolic acids, compounds **3b**·HNO_3, **3c**, **3f**, and **3g** lowered IOP when administered intravitre-

ally. Topical administration of the nitrolic acids had no effect on IOP. Elevated concentrations of cGMP in iris-ciliary body incubation were measured for all nitrolic acids except **3c** (**3d** not assayed). This finding may be interpreted as evidence of nitric oxide donation of these compounds.

Experimental Section

Chemistry. Unless otherwise indicated, all common reagents and solvents together with the compounds **1c, 1g**, and **1h** were obtained from commercial suppliers and were used without further purification. 1-Benzylimidazole-5-carbaldehyde (**1a**),[25] 1-methylimidazole-5-carbaldehyde (**1b**),[25] 1(*H*)-imidazole-4(5)-carbaldehyde oxime (**2c**),[26] 1-benzylimidazole-2-carbaldehyde (**1f**),[27] and 1-benzylimidazole-2-carbaldehyde oxime (**2f**)[27] were prepared according to literature procedures and oximes **2b**[28] and **2g**[29] according to slightly modified ones. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance 250 NMR spectrometer. The mass spectrometry measurements were performed on a Bruker BioApex 47e Fourier transform ion cyclotron resonance (FTICR) mass spectrometer (Bruker Daltonics, Billerica, MA), equipped with an Infinity cell, 4.7 T 160-mm-bore superconducting magnet (Magnex Scientific Ltd., Abingdon, UK), and an external electron ionization (EI) or electrospray ion source (ESI) (Analytica of Branford Inc., Branford, CT). Elemental analyses were performed on a CE Instruments EA 1110 elemental analyzer.

General Method for Preparation of Oximes. Hydroxylamine hydrochloride was dissolved in water and neutralized with Na_2CO_3. Aldehyde was added, and the solution was stirred at 70 °C for 1−4 h. Oxime was filtered out and washed with water.

(*E*)- and (*Z*)-1-Benzylimidazole-5-carbaldehyde Oxime (2a). Prepared according to the general method. Aldehyde **1a** 1 g (5.4 mmol), $NH_2OH\cdot HCl$, 1.5 g (21.6 mmol), water (3 mL), Na_2CO_3 1.14 g (10.8 mmol), reaction time 1 h at 70 °C. The product was a mixture of (*E*)- and (*Z*)-isomers and was used for the next step without further purification. Yield 70%. ^{1}H NMR (DMSO-d_6): δ = 5.42 (s, 2 H), 7.14−7.36 (m, 5 H), 7.46 (s, 1 H), 7.80 (s, 1 H), 7.96 (s, 1H), 11.67 (s, 1 H); 5.47 (s, 2 H), 7.14−7.36 (m, 5 H), 7.90 (s, 1H), 8.08 (s, 1H), 11.10 (s, 1H); ^{13}C NMR (DMSO-d_6): δ = 47.69, 122.67, 126.78, 127.77, 128.81, 133.43, 135.99, 137.29, 139.38; 48.97, 125.29, 127.21, 127.51, 128.63, 132.05, 137.74, 139.17, 141.16; MS (EI): Calcd for $C_{11}H_{11}N_3O_1$ *m/z* = 201.08966. Found *m/z* = 201.09093. Anal. ($C_{11}H_{11}N_3O$): C, H, N.

***(E)*- and *(Z)*-1-Methylimidazole-5-carbaldehyde Oxime (2b).** Prepared according to the general method. Aldehyde **1b** 1 g (9.1 mmol), $NH_2OH\cdot HCl$ 1.26 g (18.1 mmol), Na_2CO_3 0.96 g (9.1 mmol), water (7 mL), reaction time 1.5 h at 70 °C. Yield 68%. The product was a mixture of (*E*)- and (*Z*)-isomers. The compound was used for the next step without further purification. Recrystallization for elemental analyses from methanol. ^{1}H NMR (DMSO-d_6): δ = 3.71 (s, 3H), 3.74 (s, 3H), 7.17 (s, 1H), 7.53 (s, 1H), 7.69 (2H), 7.74 (s, 1), 8.12 (s, 1H), 11.12 (s, br, 1H), 11.65 (s, Br, 1H); ^{13}C NMR (DMSO-d_6): δ = 31.76 (q), 33.80 (q), 123.5 (s), 125.86 (s), 131.87 (d), 133.61 (d), 135.54 (d) 139.39 (d) 139.63 (d) 141.31 (d); MS (EI): Calcd for $C_{11}H_{11}N_3O_1$ *m/z* = 125.05836. Found *m/z* = 125.05808; Anal. ($C_5H_7N_3O$): C, H, N.

1(*H*)-2-Phenylimidazole-4(5)-carbaldehyde Oxime (2d). Prepared according to the general method. Aldehyde **1d** 1 g (5.81 mmol), $NH_2OH\cdot HCl$ 0.81 g (11.6 mmol), water (10 mL), Na_2CO_3 0.62 g (5.81 mmol), reaction time 1.5 h at ambient temperature. Yield 82%. ^{1}H NMR (DMSO-d_6): δ = 7.32−7.52 (m, 4H), 7.99−8.05 (m, 3H). MS (EI): Calcd for $C_{10}H_9N_3O$ *m/z* = 187.07401. Found 187.07434.

***1*(*H*)-4(5)-Methylimidazole-5(4)-carbaldehyde Oxime (2e).** Prepared according to the general method. Aldehyde **1e** 1 g (9.08 mmol), $NH_2OH\cdot HCl$ 1.26 g (18.2 mmol), water (8 mL), Na_2CO_3 0.96 g (9.08 mmol), reaction time 1.5 h at ambient temperature. Yield 69% ^{1}H NMR (DMSO-d_6): δ = 2.25 (s, 3H), 7.55 (s, 1H), 8.02 (s, 1H), 10.80 (s, 1H); ^{13}C NMR (DMSO-d_6): δ = 11.37 (q); 125.65 (s); 131.42 (s); 135.47 (d); 141.91 (d); MS (EI): Calcd for $C_5H_7N_3O$ *m/z* =125.05836. Found 125.05861. Anal. ($C_5H_7N_3O\cdot H_2O$): C, H, N.

1-Methylimidazole-2-carbaldehyde Oxime (2g). Prepared according to the general method. Aldehyde **1g** 1 g (9.1 mmol), $NH_2OH\cdot HCl$ 0.96 g (13.8 mmol) in water (8 mL), $NaHCO_3$ 1.16 g (13.8 mmol), reaction time 1 h at 70 °C. Yield 80%. ^{1}H NMR (DMSO-d_6): δ = 3.80 (s, 3H), 6.98 (s, 1H), 7.26 (s, 1H), 8.05 (s, 1H), 11.53 (s, 1H) ^{1}H NMR shifts are in agreement with the literature.[29] MS (ESI): Calcd for $C_5H_8N_3O_1$ $[M + H]^+$ = 126.06619. Found $[M + H]^+$ = 126.06637.

(*E*)- and (*Z*)-1-(*H*)-Imidazole-2-carbaldehyde Oxime (2h). Prepared according to the general method. Aldehyde **1h** 1 g (10.4 mmol), $NH_2OH\cdot HCl$ 1.44 g (20.7 mmol), water (8 mL), Na_2CO_3 1.1 g (10.4 mmol), reaction time 1 h at ambient temperature. Yield 44%. ^{1}H NMR (DMSO-d_6): δ = 7.09 (s), 7.14 (s), 7.45 (s), 7.95 (s); MS (EI): Calcd for $C_4H_5N_3O$ *m/z* = 111.04271. Found 111.04260. Anal. ($C_4H_5N_3O$): C, H.

General Method for Preparation of Nitrolic Acids. The solution of the oxime in glacial acetic acid was cooled in an ice bath. Fuming nitric acid was added dropwise. The ice bath was removed, and the mixture was stirred at ambient temperature. The reaction mixture was poured into ice and NaOH (2 M) was added dropwise until the pH was about 3−4. The precipitate was filtered and washed with cold water.

1-Benzylimidazole-5-nitrolic Acid (3a). Prepared according to the general method. Oxime **2a** (0.25 g, 1.24 mmol), glacial acetic acid (1 mL), fuming nitric acid (0.15 mL), reaction time 1 h. Yield 48%. ^{1}H NMR (DMSO-d_6): δ = 5.14 (s, 2H), 7.02−7.06 (m, 2H), 7.26−7.34 (m, 3H), 7.36 (s, 1H), 8.12 (s, 1H); ^{13}C NMR (DMSO-d_6): δ = 49.59 (t), 115.84 (s), 127.28 (d), 128.07 (d), 128.65 (d), 133.81 (d), 135.95 (s), 140.96 (d), 151.58 (s). MS(ESI): Calcd for $C_{11}H_{11}N_4O_3$ $[M + H]^+$ = 247.08257. Found $[M + H]^+$ = 247.08180. Anal. ($C_{11}H_{10}N_4O_3$): C, H, N.

1-Methylimidazole-5-nitrolic Acid (3b). Prepared according to the general method. Oxime **2b** (0.25 g, 2 mmol), AcOH (1 mL), fuming HNO_3 (0.25 mL), reaction time 0.5 h. Yield 47%. ^{1}H NMR (DMSO-d_6): δ = 3.54 (s, 3H), 7.36 (s, 1H), 7.91 (s, 1H). ^{13}C NMR (DMSO-d_6): δ = 32.54 (k), 116.69 (s), 133,73 (d), 141.15 (d), 151.79 (s). MS(ESI): Calcd for $C_5H_7N_4O_3$ $[M + H]^+$ = 171.05148. Found $[M + H]^+$ = 171.05127. Anal. ($C_5H_6N_4O_3$): C, H.

1-Methylimidazole-5-nitrolic Acid (3b) HNO_3 Salt. The salt form could be obtained by filtering the resulting precipitate after pouring the reaction mixture in water. ^{1}H NMR (DMSO-d_6): δ = 3.74 (q, 3H, CH_3), 8.04 (s, 1H), 9.20 (s, 1H) ^{13}C NMR (DMSO-d_6): δ = 34.74 (q), 118.85 (s), 124.93 (d), 138.62 (d), 148.78 (s). Anal. ($C_5H_7N_5O_6$): C, H, N.

1-(*H*)-Imidazole-5-nitrolic Acid (3c). Prepared according to the general method. Oxime **2c** 0.25 g (2.25 mmol), AcOH (1.5 mL), fuming HNO_3 (0.1 mL), reaction time 0.5 h. After the pH was adjusted to 3, NaCl was added until **3c** precipitated. Yield 46%. ^{1}H NMR (DMSO-d_6): δ = 7.68 (s, 1H), 8.10 (s, 1H), 12.76 (s, br); ^{13}C NMR (DMSO-d_6): δ = 123.7 (s), 124.7 (d), 136.5 (d), 156.5 (s); MS (ESI): Calcd for $C_4H_5N_4O_3$ $[M + H]^+$ = 157.03562. Found $[M + H]^+$ = 157.03564. Anal. ($C_4H_4N_4O_3$): C, H.

1(*H*)-2-Phenylimidazole-4(5)-nitrolic Acid (3d). Prepared according to the general method. Oxime **2d** 0.25 g (1.34 mmol), AcOH (0.70 mL), fuming HNO_3 (0.18 mL). The reaction time was 0.5 h. Yield 68%. ^{1}H NMR (DMSO-d_6): δ = 7.38−7.51 (m, 3H); 7.92−7.95 (m, 2H), 8.23 (s, 1H); ^{13}C NMR (DMSO-d_6): δ = 124.50 (s), 125.56 (d), 126.25 (d), 128.96 (d), 129.11 (s), 129.32 (d), 146.36 (s), 156.41 (s). MS(ESI): Calcd for $C_{10}H_9N_4O_3$ $[M + H]^+$ = 233.0669. Found 233.0663.

1(*H*)-4(5)-Methylimidazole-4(5)-nitrolic Acid (3e). Prepared according to the general method. Oxime **2e** 0.25 g (2 mmol), AcOH (1 mL); fuming nitric acid (0.25 mL), reaction time 0.5 h. Yield 30%. ^{1}H NMR (DMSO-d_6): δ = 2.25 (s, 3H); 7.69 (s, 1H); 12.68 (br); ^{13}C NMR (DMSO-d_6): δ = 11.54 (k), 121.09 (s, br), 132.88 (s, br), 135.82 (d), 157.02 (s). MS (ESI): Calcd for $C_5H_7N_4O_3$ $[M + H]^+$ = 171.05127. Found $[M + H]^+$ = 171.05284.

1-Benzylimidazole-2-nitrolic Acid (3f). Prepared according to the general method. Oxime **2f** 0.25 g, (1.24 mmol), AcOH (1 mL), fuming HNO_3 (0.15 mL), reaction time 1 h. Yield 43%. ^{1}H NMR (DMSO-d_6): δ = 5.16 (s, 2H), 7.14−7.20 (m, 3H), 7.29−7.37 (m, 3H), 7.50 (s, 1H); ^{13}C NMR (DMSO-d_6): δ = 50.32 (t), 123.60 (d), 127.79 (d), 128.79 (d), 128.26 (d), 128.84 (d), 129.93 (d), 132.34 (s), 136.29 (s), 152.06 (s); MS (ESI): Calcd for $C_{11}H_{11}N_4O_3$ $[M + H]^+ = 247.08256$. Found $[M + H]^+ = 247.08295$. Anal.($C_{11}H_{10}N_4O_3 \cdot 0.25\ H_2O$): C, H, N.

1-Methylimidazole-2-nitrolic Acid (3g). Prepared according to the general method. Oxime **2g** 0.5 g (4.0 mmol), AcOH (2 mL), fuming HNO_3 (0.4 mL). Yield 58%. ^{1}H NMR (DMSO-d_6): δ = 3.61 (s, 3H), 7.16 (s, 1H), 7.47 (s, 1H); ^{13}C NMR (DMSO-d_6): δ = 33.54 (q),124.17 (d), 129.28 (d), 132.72 (s),151.97 (s); Anal. ($C_5H_6N_4O_3$): C, H, N.

1-(*H*)-imidazole-2-nitrolic Acid (3h). Prepared according to the general method. Oxime **2h** 0.25 g (2.25 mmol), AcOH (1.5 mL), fuming HNO_3 (0.27 mL). Yield 57%. ^{1}H NMR (DMSO-d_6): δ = 7.36 (s), 13.23 (br); ^{13}C NMR (DMSO-d_6): δ = 125.65 (d), 130.67 (s), 152.58 (s); MS (ESI): Calcd for $C_4H_5N_4O_3$ $[M + H]^+ = 157.03562$. Found $[M + H]^+ = 157.03525$. Anal. ($C_4H_4N_4O_3$): C, H, N.

X-ray Structure Determinations. The X-ray diffraction data (compounds **2a**,**b**, **2h 3b**, **3b**·HNO_3, and **3g**) were collected with a Nonius KappaCCD diffractometer using Mo Kα radiation (λ = 0.71073 Å). The Denzo-Scalepack[30] program package was used for cell refinements and data reduction. All structures were solved by direct methods using the SHELXS-97 program and the WinGX graphical user interface.[31,32] Structural refinements were carried out with SHELXL-97.[31] In all structures OH hydrogens were located from the difference Fourier map. In **2b**, **2h**, and **3b**·HNO_3 they were refined isotropically. In **2a** and **3g** OH hydrogens were refined with constant U_{iso} = 0.05. All hydrogens were placed in idealized positions and constrained to ride on their parent atom.

Pharmacology. Intraocular Pressure Measurements. New Zealand White (NZW) rabbits of both sexes with normal IOP were used in the experiments (four animals were used for each imidazole nitrolic acid). The Guiding Principles in the Care and Use of Animals (DHEW Publication, NIH 80-23) was adhered to the protocol of the study, and the protocol was approved by the local Animal Experimentation Committee. IOP was measured with a pneumatonometer (Modular One Tonometer, Mentor, Cambridge, MA) after topical anesthesia with 0.4% oxybuprocain (Oftan Obucain, Santen Oy, Tampere, Finland). Measurements were taken at the same time of day by the same investigator. One hour before application of the test compound, a control measurement was taken for both eyes. Thirty microliters of the test compounds (experimental eye) or vehicle (control eye) was administered by micropipet in the inferior conjunctival sac, or 50 μL of the test compounds or vehicle was injected by Hamilton precision syringe in the vitreous humor. Different concentrations of imidazole nitrolic acids were tested. IOP was measured three times at each time interval, and the means were reported.

Incubation of Porcine Iris-Ciliary Bodies. Porcine eyes were obtained from an abattoir and were prepared for the experiments within 3 h of enucleation. The data measurements have been described in our recent article.[24] Acetylated cGMP in the samples was assayed with the [^{125}I]-cyclic GMP RIA kit (Amersham International, Little Chalfont, Buckinghamshire, UK). Nitric oxide release from the test compounds was determined spectrophotometrically by measuring nitrate + nitrite (NO_x) in the incubation medium with the Nitrate/Nitrite Colorimetric Assay Kit (Cayman Chemical, Ann Arbor, MI).

The Measurement of Nitrite, Nitrate and Cyclic GMP in Aqueous Humor and Plasma. In the experiments for the biochemical assays, the rabbits were euthanized by 300 mg of pentobarbital (Mebunat, Orion Oy, Espoo, Finland) intravenously 1−24 h after the last measurements depending on test compounds, and the aqueous humor was taken by injection syringe for measurements. The procedure is presented in detail previously.[33]

Measurement of Aqueous Humor Outflow Facility. New Zealand White (NZW) rabbits of both sexes with normal IOP were used in the experiments. The Guiding Principles in the Care and Use of Animals (DHEW Publication, NIH 80-23) was adhered to the protocol of the study, and the protocol was approved by the local Animal Experimentation Committee. Anesthesia was initiated with an intramuscular injection of a combination of ketamine (Ketalar 50 mg/mL, Parke-Davis Warner Lambert Nordic AB, Solna, Sweden) and xylazine (RompunVet 20 mg/mL, Bayer AG, Leverkusen, Germany) and maintained by intravenous infusion of the same combination. Measurements have been described previously.[18]

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Supporting Information Available: Crystallographic data and elemental analyses. This material is available free of charge via the Internet at http://pubs.acs.org.

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