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Synthesis and ocular effects of imidazole nitrolic acid and amidoxime esters

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Abstract—Esters of 1-(*H*)-imidazole-5-nitrolic acid and 1-methyl-imidazole-5-carboxamide oxime were prepared to study the effect of esterification on the ocular effects of these compounds. Esterifications were performed with acid chloride. Acid chloride also reacts with the ring nitrogen of 1-(*H*)-imidazole-5-nitrolic acid, but the desired esters could be selectively prepared by adjustment of the reaction conditions. Esterification led to loss of the ocular effects exhibited by the parent compounds.

Nitric oxide (NO) is a gaseous messenger molecule, which plays an important role in diverse physiological processes in the eye, for example, regulation of aqueous humor dynamics, local modulation of ocular blood flow, neuronal visual processing, and ocular immunological responses.[1–3] NO is also involved in several diseases of the eye such as uveitis, retinitis, glaucoma, and retinal degeneration.[2] Cyclic guanosine-3′,5′-monophosphate (cGMP) mediates many but not all the intracellular processes induced by NO. Compounds affecting the NO–cGMP pathway have been shown to lower intraocular pressure (IOP) in animal and human experiments by reducing the production of aqueous humor and/or by increasing the outflow of aqueous humor.[4–19]

Previously, we have studied the ocular effects of potential nitric oxide donating imidazole nitrolic acids and amidoximes. Several of these compounds had IOP-lowering properties and they increased the cGMP concentrations in iris–ciliary incubation, suggesting NO donation. However, in vivo the compounds lowered IOP only as intravitreal injections and were mostly ineffective when administered topically.[20,21] Prodrugs are

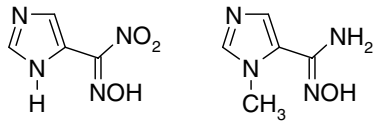

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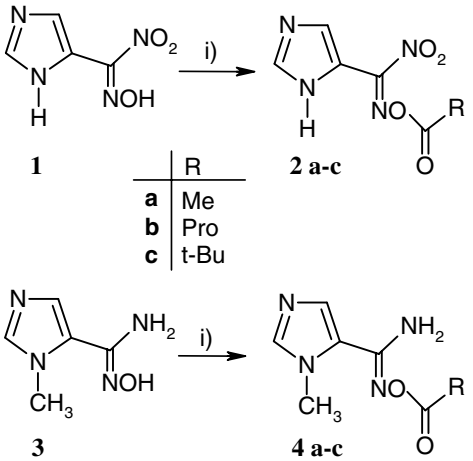
Figure 1. Imidazole nitrolic acid **1** and amidoxime **3**.



Scheme 1. Preparation of compounds **2a–c** and **4a–c**. Reagents: (i) EtO_2, RCOCl, Et_3N.

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inactive derivatives of drug molecules, which are metabolized to their active form in the body. A common way to prepare them from molecules containing hydroxyl groups is esterification. Esters are hydrolyzed by esterases, which are expressed in almost all tissues in the body including ocular tissue.[22] We chose the two most potent

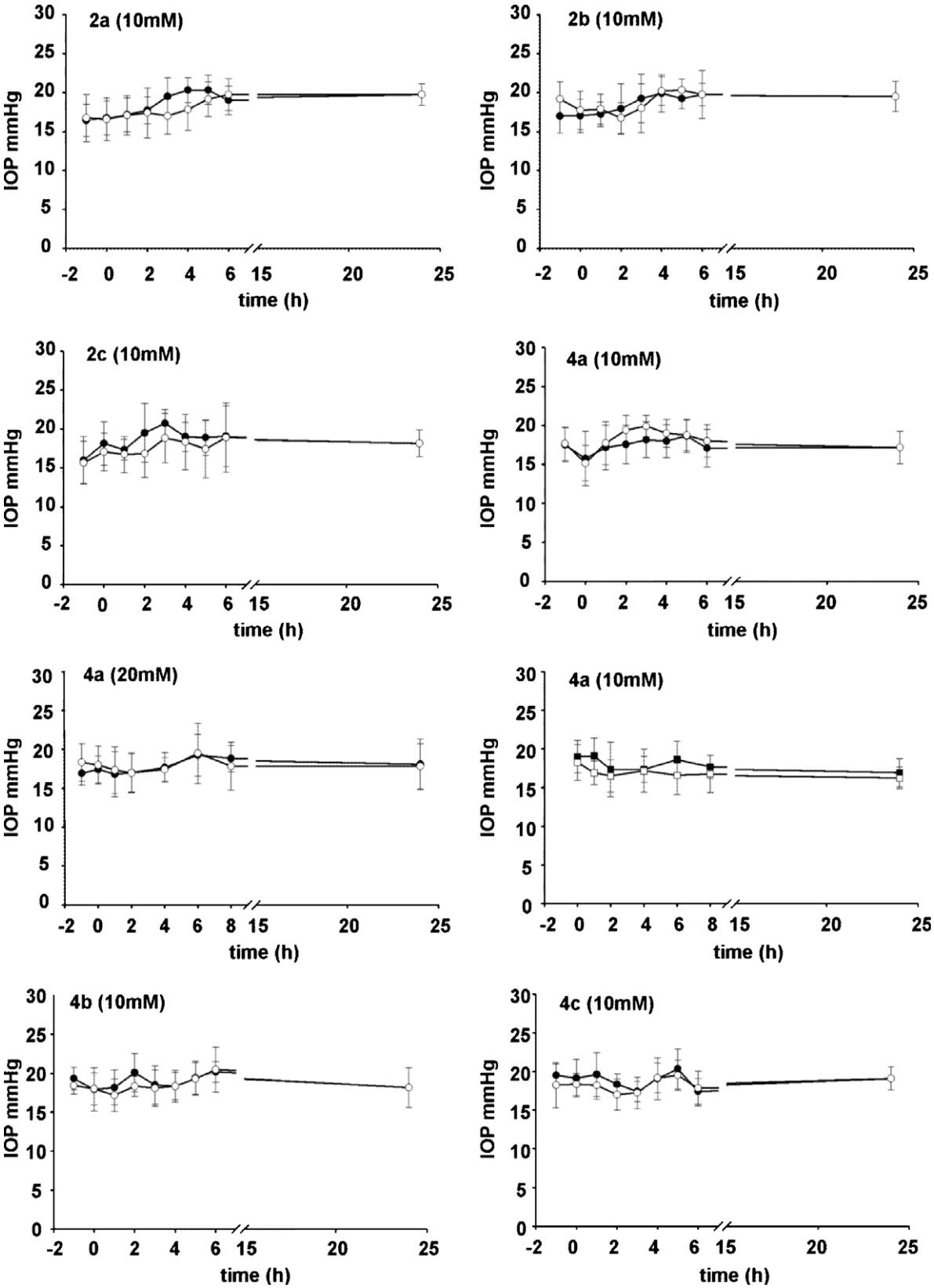

Figure 2. Effects of imidazole nitrolic acids and amidoxime esters in IOP in rabbits (means ± SD, $n = 4$). ● and ○, test compound and control administered topically; ■ and □, test compound and control injected intravitreally.

compounds from our previous studies, 1-(*H*)-imidazole-5-nitrolic acid **1** and 1-methyl-imidazole-5-carboxamide oxime **3** (Fig. 1), for further modification. Acetyl, propyl, and pivaloyl esters of these compounds were prepared and tested for their ocular effects.

All esterifications were performed with acid chloride and triethylamine in ethereal solution (Scheme 1). In the case of nitrolic acid **1**, addition of the acid chloride could also occur to the imidazole ring nitrogen. The reacting position could be controlled by adjusting the reaction conditions. The desired esterification products **2a–c** were afforded with use of 1 equiv acid chloride and 1.5 equiv triethylamine. Reactions had to be performed in an ice bath with short reaction times (5–15 min). The appropriate conditions were found with the acylation reaction, and the same conditions were suitable for the other esterifications.[23] When longer reaction times or different proportions of reagents were used, the product was either a compound that was concluded to be a diacylation product[24] or a mixture of unreacted nitrolic acid, the desired ester, and the diacylation product. Amidoxime esters **4a–c** were prepared as nitrolic acid esters only using longer reaction times.[23]

One shift was missing in the carbon spectra of pivaloyl ester **2c** in DMSO. Since the tertiary carbon of the *t*-butyl group has a chemical shift in the same region as the DMSO signal, a further spectrum was measured in deuterated methanol. Compound **2c** was only sparingly soluble in methanol and only a low-quality carbon spectrum could be obtained. However, the missing peak could be identified.[23]

The previously tested parent compounds **1** and **3** lowered IOP when administered as intravitreal injections in rabbits but had no effect when administered topically.[20,21] Eyes are well protected against chemicals, so poor bioavailability of topically administered ophthalmic drugs is a quite common problem. Esterifications were performed to prepare prodrugs of compounds **1** and **3** with the aim of improving ocular penetration. The effect of esterification was not the desired one since the studied activities were lost. When compounds **2a–c** and **4a–c** were administered topically (concentration 10 mM on the rabbit eye),[25] they were not able to lower IOP (Fig. 2). Compound **4a** was not able to lower IOP tested as intravitreal injection (concentration 10 mM).

The formation of cGMP by activating guanylate cyclase accounts for many of the physiological effects of NO.[26–30] NO has been shown to be a transmitter of smooth muscle relaxation in the chamber angle of the eye and it might thus be involved in the regulation of aqueous humor dynamics. This relaxation of the ciliary muscle and trabecular meshwork is mediated via an increase in intracellular cGMP.[31,32] In our previous studies, NO donors and cGMP activators, that lowered IOP in rabbits in vivo, were shown to increase the production of cGMP in the porcine iris–ciliary body in vitro.[18,33] In the present study, the concentration of cGMP was measured in iris–ciliary bodies after administration of **2a–c**, **4b**, and **4c** (concentrations 10 and 100 μM) (Table 1).[34] Cyclic GMP production was slightly increased after administration of **2a** (100 μM), **2b** (10 μM), **2c** (100 μM), and **4c** (10 μM), but the changes were not statistically significant.

Table 1. Effects of nitrolic acid and amidoxime esters (10 and 100 μM) on cGMP levels in porcine iris–ciliary body incubation (means ± SEM, $n = 6$)

Compound	*c* (μm)	cGMP (pmol/mg prot.)	*p*-value
Control		0.37 ± 0.05	
2a	10	0.45 ± 0.05	0.88
	100	0.78 ± 0.11	0.11
2b	10	0.50 ± 0.05	0.57
	100	0.38 ± 0.04	0.68
2c	10	0.36 ± 0.03	0.54
	100	0.73 ± 0.14	0.21
4a		NA	
4b	10	0.23 ± 0.03	0.21
	100	0.24 ± 0.01	0.53
4c	10	0.63 ± 0.18	0.13
	100	0.24 ± 0.03	0.55

Statistics were calculated on the basis of the respective controls of the experiment.
NA, not assayed.

In conclusion, esters of 1-(*H*)-imidazole-5-nitrolic acid and 1-methyl-imidazole-5-carboxamide oxime were prepared to study the effect of esterification on ocular effects of these compounds. 1-(*H*)-Imidazole-5-nitrolic acid esters were prepared selectively, without substitution on the ring nitrogen, by adjustment of the reaction conditions. Esterification led to loss of the possibly beneficial ocular effects shown in our previous study.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2006.01.068.

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