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ScienceDirect
Bioorganic & Medicinal Chemistry Letters 17 (2007) 5881–5885
Bioorganic & Medicinal Chemistry Letters

NO donors. Part 16: Investigations on structure–activity relationships of organic mononitrates reveal 2-nitrooxyethylammoniumnitrate as a high potent vasodilator☆

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Received 24 June 2007; revised 30 July 2007; accepted 1 August 2007
Available online 28 August 2007

Abstract—The vasoactive properties of 14 organic mononitrates were investigated in vitro using $PGF_{2\alpha}$-precontracted porcine pulmonary arteries. A surprisingly wide range of vasorelaxant potencies was observed (pD_2: 3.36–7.50). Activities showed to be highly sensitive to the molecular structure and the substituents at the molecular carrier of the nitrate group. A correlation between lipophilicity and vasorelaxant potency could not be recognized. 2-Nitrooxyethylammoniumnitrate (**1**) was found to be slightly superior to the high potency trinitrate GTN.

Organic nitrates are useful drugs for the treatment of ischemic heart diseases. The vasoactive response is mediated by a catenary process which includes bioactivation to nitric oxide (NO) and/or related species, and intracellular accumulation of cyclic GMP causing relaxation of vascular smooth muscle cells. Four organic nitrates are used therapeutically: pentaerithrityl tetranitrate (PETN), glyceryl trinitrate (GTN), isosorbide dinitrate (ISDN), and isosorbide mononitrate (ISMN).[2,3] Clinically, GTN represents the most potent compound (daily dosage about 1 mg) but it induces nitrate tolerance. PETN, although having more nitrate groups than the other nitrates, requires the highest daily dosage (50–240 mg), but on the other hand it does not develop significant tolerance.[4] However, experimental in vitro studies (tension measurements) yielded different results. Using $PGF_{2\alpha}$-precontracted porcine pulmonary arteries we have shown that there is a clear correlation between the number of nitrate groups in the molecule and the vasodilator potency and PETN showed to be the most potent nitrate (pD_2: 8.18).[1] Low systemic bioavailability for PETN and its active metabolite PEtriN[5,6] as well as a limited absorption of its less active metabolites PEdiN and PEmonoN is obviously responsible for the clinical profile of PETN.[1]

Besides the number of nitrate groups, the lipophilicity ($\log P$) and stereochemistry of the nitrate carrier molecule may influence the vasorelaxant potency.[7,8] Furthermore it was reported that the vasoactivities of cyclic organic dinitrate isomers like isoidide dinitrate, isosorbide dinitrate, and isomannide dinitrate differ significantly, although their $\log P$ were similar.[9,10] The authors assumed that compounds with a nitrate group in the endo position were less potent than exonitrates due to the steric hindrance to endogenous bioactivating ligands. It is supposed that the bioactivating enzyme requires two attachment sites for interaction with the nitrate compound, one involves the oxygen in the nitrate group, while the other seeks another electron-rich center.[11] Moreover, there are two different bioactivation pathways for organic nitrates. Recent results revealed that only the 'high potency nitrates' GTN and PETN but not the 'low potency nitrates' ISDN and ISMN were bioactivated predominantly by the mitochondrial aldehyde dehydrogenase (ALDH-2).[12–14] At higher concentration (>1 μM), GTN and PETN are also subjected

Keywords: Organic nitrates; Vasorelaxation; Structure–activity relationships.
☆ See Ref. 1.
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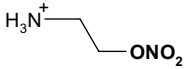
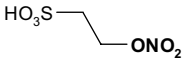
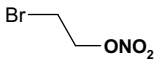
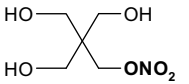
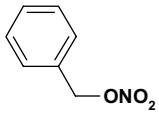
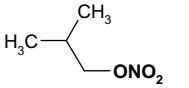
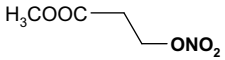
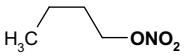
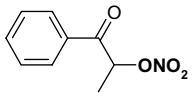
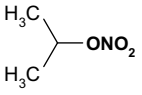
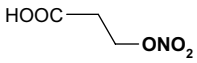
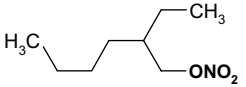
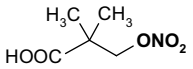
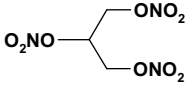
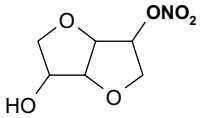
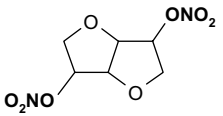
doi:10.1016/j.bmcl.2007.08.046

Table 1. Vasodilatory effects (pD_2-value and EC_{50}) of organic mononitrates, ISDN and GTN, on $PGF_{2\alpha}$-precontracted porcine pulmonary arteries with intact endothelium

Compound	Structure	pD_2	EC_{50} (M)	n	Log P	Compound	Structure	pD_2	EC_{50} (M)	n	Log P
1[a]		7.50 (±0.02)	3.15×10^{-8}	6	0.06[a]	**9**		4.48 (±0.03)	3.35×10^{-5}	4	−0.74
2		6.42 (±0.04)	3.77×10^{-7}	3	1.59	**10**		4.14 (±0.02)	7.25×10^{-5}	7	−0.37
3		6.34 (±0.04)	4.12×10^{-7}	5	2.37	**11**		4.01 (±0.03)	8.77×10^{-5}	4	1.97
4		5.99 (±0.05)	1.03×10^{-6}	6	0.90	**12**		3.85 (±0.02)	1.41×10^{-4}	5	2.15
5		5.81 (±0.03)	1.54×10^{-6}	5	2.48	**13**		3.36 (±0.04)	4.35×10^{-4}	4	1.43
6		5.02 (±0.06)	9.56×10^{-6}	7	0.44	**14**		<DMSO	>DMSO	8	4.09
7		4.83 (±0.03)	1.47×10^{-5}	9	1.14	GTN		7.44 (±0.02)	3.56×10^{-8}	6	2.21
8		4.48 (±0.03)	3.32×10^{-5}	9	−0.30	ISDN		6.37 (±0.03)	4.24×10^{-7}	7	0.90

The pD_2-value presents the negative logarithm of the molar concentration producing 50% of the maximum relaxation (EC_{50}). Values are means ± SEM from n separate experiments.

[a] Applied as 2-nitrooxyethylammoniumnitrate.

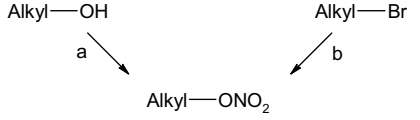
additionally to the 'low potency nitrates' pathway. Most probably, the low potency mononitrates are bioactivated exclusively by cytochrome P450 in the endoplasmic reticulum for all concentrations.[13]

The correlation between the number of nitrate functions in the molecule and the vasorelaxant potency[1,14,15] prompted us to perform further investigations of structure–activity relationships (SAR) in this class of bioactive compounds, but this time by structural alterations with a fixed number of nitrate groups. Especially organic mononitrates are appropriate for this study. They are less explosive, easy to handle, and the break down to active metabolites can be excluded. In addition, the chemical variation of the organic nitrate carrier molecule is easier than by using di- or trinitrates.

Several methods have been applied for the synthesis of organic nitrates.[16] In this study, the preparation of the mononitrates (Table 1) was accomplished by esterification of the corresponding alkyl alcohols with fuming nitric acid or from the corresponding alkyl bromides by reaction with silver nitrate in acetonitrile, respectively (Scheme 1). The following nitrates were prepared according to procedures reported in the literature: **1**[17,18] from 2-aminoethanol, **2**[19] from 2-bromoethanol, **3**[7] from benzylbromide, **4**[20] from methyl 3-hydroxypropanoate, **5**[21] from 2-hydroxy-1-phenylpropanone, **6**[18] from 3-hydroxypropionic acid, **7**[22] from methyl 3-hydroxy-2,2-dimethylpropanoate and subsequent ester hydrolysis in methanolic sodium hydroxide, **9**[23] from 2-hydroxyethanesulfonic acid, **10** from 2-(bromomethyl)-2-(hydroxymethyl)propane-1,3-diol (this synthesis will be reported in detail later), and **12**[24] from 1-bromobutane.

The following nitrates were purchased: **11**, **13**, and **14** (Sigma–Aldrich, Steinheim, Germany), GTN (Merck, Darmstadt, Germany). Compound **8** and ISDN were obtained as gift from Schwarz-Pharma AG (Monheim, Germany).

To study SAR without the influence of any pharmacokinetic parameters, tension experiments were performed in organ baths using $PGF_{2\alpha}$-precontracted porcine pulmonary arteries and the vasorelaxant responses for the nitrates were measured and are given in Table 1. According to a previously described protocol,[1] lungs from adult pigs were obtained from the local slaughterhouse. Small branches from pulmonary arteries were prepared and cut into rings (length 2–3 mm, diameter 1.5–2 mm). In experiments with endothelium-denuded rings, the endothelium was removed by gently rubbing the inner surface with a rough plastic rod. The rings were suspended between two L-shaped platinum hooks and mounted in 10 mL organ baths filled with modified Krebs–Henseleit solution. The solution was kept at 37 °C and aerated with 95% O_2/5% CO_2. The vasorelaxant effects were measured using the $PGF_{2\alpha}$ (3 μM)-precontracted rings with a resting tension of 20 mN. The test compounds were added to the organ bath at the contraction plateau in a cumulative manner. Endothelial integrity was assessed by bradykinin (10 nM)-induced relaxation of $PGF_{2\alpha}$-precontracted vessels. In mechanically endothelium-denuded arterial rings, pretreated with 0.2 mM L-NAME, the relaxation induced by bradykinin was less than 10%. Relaxation responses were expressed as a percentage of the $PGF_{2\alpha}$ (3 μM)-induced contraction in each tissue.



Scheme 1. Synthesis of mononitrates from alkyl alcohols and alkyl bromides, respectively: Reagents and conditions: (a) fuming nitric acid, 2 h, <10 °C for **1**, **3**, **7**, and **9**; (b) silver nitrate/acetonitrile, 2–28 d, rt 50 °C (depending on the compound) for **2**, **4**, **5**, **6**, **10**, and **12**.

All of the compounds were able to elicit a significant vasorelaxation in a concentration-dependent manner (Fig. 1) with exception of compound **14**, which induced a very weak relaxation in the range of the pure solvent DMSO. The maximum of vasorelaxation has been achieved throughout for the compounds **1**–**12** ($E_{max} \sim 100\%$), compound **13** only performed ~70% relaxation after adding the selected maximum concentration of 1 mM. The sigmoidal concentration–response curves (Fig. 1) were consistent with the Hill slope for these mononitrates. The concentration–response curves resulting from experiments with endothelium-denuded vessels did not shift significantly, compared to the vessels with intact endothelium (data not shown).

In order to recognize any relationship between vasodilator potency and lipophilicity the pD_2-values were plotted against the calculated log *P*-values (ACD, Toronto, Canada) (Table 1 and Fig. 2).

According to previous investigations, it had to be expected that the vasorelaxant potency of the organic nitrates investigated should increase with their lipophilicity.[8,9] We have found the calculated log *P*-values for the bioactive nitrates in a wide range of three orders of magnitude (−0.74 to 2.48), but no clear correlation with the vasodilator activity could be recognized (Fig. 2).

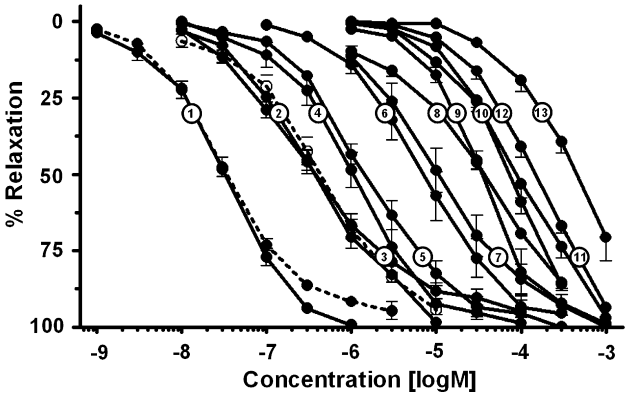


Figure 1. Concentration–response curves for the relaxation of $PGF_{2\alpha}$-precontracted porcine pulmonary arteries with intact endothelium induced by the mononitrates given in Table 1. GTN (-•-) and ISDN (-o-). Mean ± SEM.

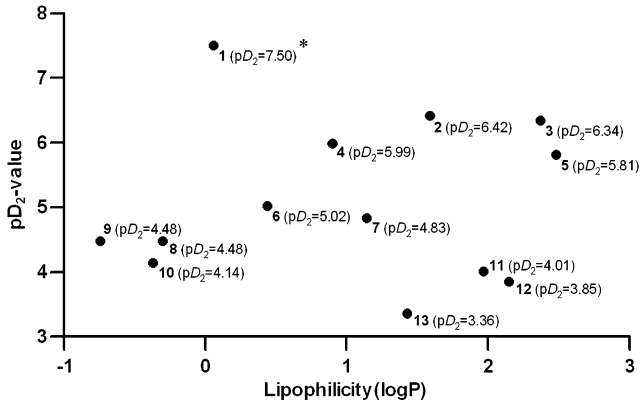
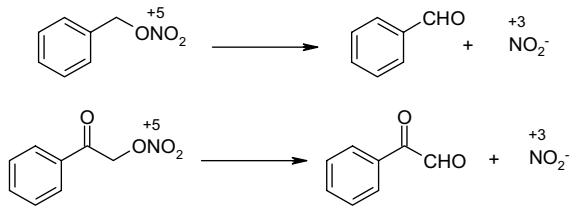


Figure 2. Relationship between lipophilicity (log *P*) and the concentrations for half maximal relaxation (expressed as pD_2-values) of the organic mononitrates. *Calculated for the uncharged molecule.

Nevertheless there is a significant influence of the nitrate carrier structures on the vasodilator potency. Compounds bearing the nitrate function attached to bulky and lipophilic carrier molecules without any further functional groups (**11**–**14**) displayed the lowest activities, particularly the most lipophilic compound **14** which even failed to outperform the solvent DMSO.

Polarization of the nitrate carriers by hydroxylation (**8** and **10**), introduction of hydrophilic cyclic ether (**8**), carboxy (**6** and **7**) or sulfonic acid moieties (**9**) enhances the vasodilator activities significantly. Acidity in general seems to be less crucial. The sulfonic acid **9** is more acidic but less active than the carboxylic acids **6** and **7**. Remarkably, the lipophilic ester **4** is more active than the corresponding less lipophilic acid **6**. It can be speculated that chemically reactive functions such as a carboxylic ester (**4**) or an alkyl bromide (**2**) are able to support the bioactivation of the attached nitrate. Compounds **3** and **5** extremely drop out of any potency/lipophilicity correlation (Fig. 2). We suggest that these unexpected high potencies may be based on a lability of the nitrate functions due to the specific chemical properties of these compounds which are not present in all of the other structures. Both, benzylnitrates and α-nitratophenones are capable to decompose in terms of a disproportionation.[21] Scheme 2 demonstrates that both structures may be considered as 'self-reducing nitrates'. Independently whether disproportionations as outlined in Scheme 2 will be performed under physiological conditions, we suggest that these properties support the bioactivation of the organic nitrate to break-down species.



Scheme 2. Disproportionation of benzylnitrates and α-nitratophenones.[21]

The 2-amino-substituted ethylmononitrate **1** was found to be the most potent mononitrate with pD_2 = 7.50. This surprising result indicates that in addition to previous observations[1] the number of nitrate moieties in the vasodilator compound is not necessarily the leading parameter for vasodilator potency. Recently, we speculated that the reactivity of oligonitrates, which is directly correlated with the number of nitrate groups, determines the vasodilator potency of organic nitrates and whether they are bioactivated by ALDH-2 or not.[12] Probably, the reactivity can also be increased by insertion of other functional groups (Table 1). Compound **1** showed to be more potent than all of the dinitrates investigated up to now and furthermore it reaches the efficacy of the most potent trinitrate GTN (pD_2 = 7.44).

Contradictory to the idea that the structure of the organic carrier is more or less negligible with regard to the biological activity, the present results reveal that the carrier molecule and its substituents are highly important. The vasoactivity of nitrovasodilators is definitively more sensitive to the functional groups rather than to the lipophilicity of the compounds. Therefore, we conclude that this is due to the significant differences in the binding affinities for the nitrate bioactivating enzymes ALDH-2 and cytochrome P450. It is suggested that these affinities for the binding areas of the enzymes are determined not only by the stereochemistry and the physicochemical properties of the compounds but in first line by polar substituents which are able to perform additional binding options. In particular, the primary protonated amino group in compound **1** is capable of improving the binding to the target enzyme.

Acknowledgment

A Ph.D. scholarship for A.K. by Graduiertenförderung (Thuringia, Germany) is gratefully acknowledged.

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