Oximes of quinuclidin-3-ones as nitric oxide donors

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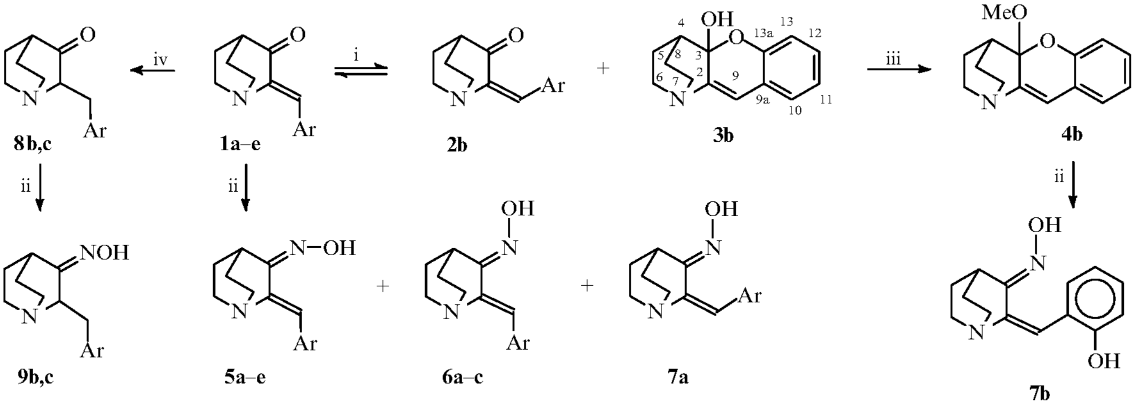
Oximes of quinuclidin-3-ones give NO under mild biomimetic oxidative conditions and activate soluble guanylate cyclase.

The identification of 'endothelium-derived relaxing factor' as endogenous NO, produced in vascular endothelium from L-arginine[1], initiated the quest for a novel generation of cardiovascular agents.[2–5] It is already evident that biochemical formation of NO can proceed *via* different pathways. Organic nitrates (nitroglycerine, isosorbide nitrates and other classical vasodilators) are subjected to reduction,[6] and the same is true for new NO precursors — *N*-nitropyrazoles[7] and furoxans.[8] On the contrary, the main source of NO in organism L-arginine is oxidized by an enzyme nitric oxide synthase (NOS) giving NO and L-citrulline.[2] NOS also releases NO from β-mercaptoethylguanidine.[9] In-depth study of the action of the known vasodilator 'Molsidomine' showed that it generates NO on reacting with atmospheric oxygen.[3] It has been reported recently that 1,2-diazetidine-1,2-*N*,*N*-dioxides form NO by thermolysis and (or) hydrolysis.[10] A year ago use of (±)-3-(*E*)-4-ethyl-2[(*E*)-hydroxyimino]-5-nitro-3-hexenamide (FK 409) as a spontaneous NO donor in solutions at pH ~7.5 was documented but the authors could not determine whether oxime, nitro or both groups was the source of NO.[11]

In order to clarify the problem and to design new NO precursors we have chosen a set of known (**5**/**6b,e**)[12] and new (**5**/**6c,d,7b**) oximes of 2-arylmethylenequinuclidin-3-one and of their hydrogenated analogues **9b,c**.[†] A compact rigid-cage structure of quinuclidine (1-azabicyclo[2.2.2]octane) provides spatial fixation of the substituents and good solubility, while the double bond in position 2 is essential for obtaining pairs of *Z*/*E*-isomers for an elucidation of the proximity effects of the aromatic group and of their interaction with the enzyme active site.



Scheme 1 *Reagents and conditions*: i, HCl, $CHCl_3$, 20 °C; ii, $NH_2OH{\cdot}HCl$, EtOH; iii, MeOH, HCl, bp; iv, H_2, Pd/C, EtOH, 20 °C. Ar = **a**: Ph, **b**: $2\text{-}HOC_6H_4$, **c**: $2\text{-}MeOC_6H_4$, **d**: $4\text{-}HOC_6H_4$, **e**: $4\text{-}MeOC_6H_4$.

The starting (*Z*)-2-arylmethylenequinuclidin-3-ones **1a**–**e** were synthesized according to a known method — the condensation of quinuclidin-3-one hydrochloride with aromatic aldehydes in the presence of NaOH.[13,14] New compound **1c**, yield 90%, mp 114–116 °C (Pr^{i}OH) had ^{1}H NMR spectrum δ ($CDCl_3$): 2.00 (m, 5, 8-CH_2), 2.60 (qn, 4-H), 2.97–3.12 (m, 6, 7-CH_2), 3.83 (s, MeO), 6.85 (m, 3′-H), 6.94 (m, 5′-H), 7.28 (m, 4′-H), 8.48 (q, 6′-H) similar to that published for **1a–b,d–e**.[15] An attempt to convert 2-HO-substituted *Z*-ketone **1b** into the corresponding *E*-isomer **2b** in acidic media as described for **1a**[12] led to its cyclic isomer — benzo[*b*]pyrano[3,2-*b*]quinuclidine **3b**, yield 30%, mp (decomp.) 210 °C (heptane). ^{1}H NMR δ ([$^2H_6$]DMSO): 1.50–1.95 (m, 5,8-CH_2), 2.29 (qn, 4-H), 2.70–2.90 (m, 6,7-CH_2), 6.23 (s, 9-H), 6.90–7.20 (m, 4H, Ar).

3b readily converts into **4b** in MeOH with traces of HCl, yield 52%, mp 65–66 °C (heptane). ^{1}H NMR δ ($CDCl_3$): 1.50–1.90 (m, 5,8-CH_2), 2.58 (qn, 4-H), 2.90–3.05 (m, 6,7-CH_2), 3.25 (s, MeO), 6.35 (s, 9-H), 6.93 (m, 11-H), 6.99 (m, 13-H), 7.13 (m, 12-H), 7.14 (q, 10-H); ^{13}C NMR δ ($CDCl_3$): 22.2, 22.7 (t, 5,8-C), 30.1 (d, 4-C), 46.9, 51.3 (t, 6,7-C), 49.7 (q, MeO), 96.7 (s, 3-C), 114.5 (d, 9-C), 116.0 (d, 12-C), 121.6 (d, 11-C), 122.6 (s, 9a-C), 127.1, 128.1 (d, 11,13-C), 143.04 (s, 2-C), 150.6 (s, 13a-C). Strong shielding of 3-C for **4b** (^{13}C δ = 96.7 in comparison to δ ~200 for ketones **1**,**2**[16]) proves its ketal structure.

Reaction of ketones **2b–e** with $NH_2OH{\cdot}HCl$ does not change the *Z*-configuration of the aryl groups and gives hydrochlorides of the corresponding oximes as mixtures of *anti*-**5b–e** and *syn*-**6b–e** isomers identified on the basis of published NMR spectra.[16] The ratio of **5**/**6** (%, **b** 50/50, **c** 60/40, **d** 26/74, **e** 10/90) in the crystalline samples obtained is close to that found in solutions and reflects their relative thermodynamic stability. Yield of **5c**/**6c**·HCl 18%, mp 194–195 °C (Pr^{i}OH). ^{1}H NMR δ (D_2O): **5c** 3.88 (qn, 4-H), 8.37 (s, 9-H); **6c** 3.03 (qn, 4-H), 7.27 (s, 9-H); signals at 2.05–2.20 (m, 5,8-CH_2), 3.45–3.65 (m, 6,7-CH_2), 7.10–7.50 (m, 3′,4′,5′,6′-H) are overlapping. Yield of **5d**/**6d**·HCl 44%, mp 170–171 °C (Pr^{i}OH). ^{1}H NMR δ (D_2O): **5d** 3.02 (qn, 4-H), 6.97 (m, 3′,5′-H), 7.22 (m, 2′,6′-H), 8.45 (s, 9-H); **6d** 3.88 (qn, 4-H), 6.84

[†] The purity of all compounds was proved by microanalysis.

Table 1 Hydrochlorides of oximes as NO donors: oxidation ($c = 2\times10^{-4}$ M, 4% EtOH–H_2O) by $K_3[Fe(CN)_6]$ or atmospheric oxygen and activation of soluble guanylate cyclase.

Compound	Yield of NO = $h_{test}/h_{ref} \times 100\%$		Soluble guanylate cyclase activation		
	2×10^{-3} M $K_3[Fe(CN)_6]$ + 0.2 M KOH	O_2, borate buffer pH 9.2	Concentrations tested (M)		
			10^{-5}	10^{-4}	10^{-3}
5/6b	4	0	1.40 ± 0.19	2.30 ± 0.51 (30%)	2.50 ± 0.84
5/6c	6	0	–	–	–
5/6d	4	0	–	–	–
5/6e	2	0	1.30 ± 0.06	1.50 ± 0.06 (19%)	1.30 ± 0.06
7a	2	0	–	–	–
7b	17	4	3.60 ± 1.02	5.70 ± 1.05 (74%)	3.00 ± 0.96
9b	10	0.5	–	–	–
9c	4	0	–	–	–
10	2	0	–	–	–
SNP	100	–	–	7.70 ± 0.69 (100%)	–

(m, 3′,5′-H), 7.14 (m, 2′,6′-H), 7.37 (s, 9-H); signals at 2.05–2.20 (m, 5,8-CH_2), 3.45–3.65 (m, 6,7-CH_2) are overlapping.

Quite unexpectedly, in the above reaction **2a** gives a ~1 : 1 : 1 mixture of **5a, 6a** and **7a** from which **7a**·HCl is obtained by crystallization from MeOH, yield 37%, mp (decomp.) 207 °C. **7b** is the only product of reaction of **4b** and NH_2OH·HCl, yield 87%, mp 191–193 °C (MeOH). ^1H NMR δ ([$^2H_6$]DMSO): 1.90–2.05 (m, 5,8-CH_2), 3.83 (qn, 4-H), 3.40–3.65 (m, 6,7-CH_2), 7.39 (s, 9-H), 7.39 (m, 3′,5′-H), 7.79 (m, 6′-H + 2′-OH).

Catalytic hydrogenation[13,17] of **1b,c** leads to 2-arylmethyl-3-oxoquinuclidines **8b,c** which were transformed into oximes **9b,c**. Yield of **9b**·HCl (86% of *anti*-isomer) 85%, mp 185–186 °C (EtOH–acetone 1:2), ^1H NMR δ (CD_3OD): 2.00–2.20 (m, 5,8-CH_2), 2.89 (qn, 4-H), 3.05–4.05 (m, 6,7,9-CH_2), 4.95 (m, 2-H), 6.89 (m, 5′-H), 6.90 (m, 3′-H), 7.16 (m, 4′-H), 7.30 (m, 6′-H); yield of **9c**·HCl (95% of syn-isomer) 57%, mp (decomp.) 185 °C (EtOH), ^1H NMR δ (CD_3OD): 1.95–2.15 (m, 5,8-CH_2), 3.15–3.95 (m, 6,7,9-CH_2), 3.79 (qn, 4-H), 4.56 (m, 2-H), 6.97 (m, 5′-H), 7.03 (m, 3′-H), 7.32 (m, 6′-H), 7.33 (m, 4′-H).

For a quick test of the ability of the above oximes and oxime of quinuclidin-3-one **10** to serve as NO donors under biomimetic conditions a special non-direct electrochemical method has been developed. Alkaline $K_3[Fe(CN)_6]$ at 80 °C was used as an oxidizer and after 5 min the reaction was quenched by adjusting the pH to 5.0 with a saturated solution of citric acid at room temperature. Though $[Fe(CN)_6]^{4-}$ anion is formed in the reaction, to ensure conversion of NO^+ into stable $[Fe(CN)_5NO]^{2-}$ (nitroprusside) anion $K_4[Fe(CN)_6]$ (2 mM) was added to the mixture and kept at 80 °C for 5 min:

$$RR' = NOH + [Fe(CN)_6]^{3-} \longrightarrow RR'C = O + NO + [Fe(CN)_6]^{4-}$$

$$[Fe(CN)_6]^{3-} + NO \longrightarrow NO^+ + [Fe(CN)_6]^{4-}$$

$$NO^+ + [Fe(CN)_6]^{4-} \longrightarrow [Fe(CN)_5NO]^{2-} + CN^-$$

The nitroprusside obtained is reduced on a dropping mercury electrode giving two characteristic polarographic waves with $E_{1/2} = -0.30$ and -0.70 V. For higher selectivity pulsed differential polarography was used. The yield of NO (%) calculated as a ratio of peaks corresponding to the second wave of the tested oxime (h_{test}) and reference solution of sodium nitroprusside (SNP) of the same concentration (h_{ref}) is given in Table 1.

Non-substituted oxime **10** and conjugated oximes **7a** (Ph) and **5/6e** (4-MeOC_6H_4) proved to be equally poor NO donors. Hydroxy substitution of the benzene ring increases the efficiency of oximes as NO donors with maximum values for 2-OH compounds **7b, 9b**. The striking difference between 2-HO-substituted *Z*- and *E*-isomers **5/6b**, **7b** and the moderate variance between **7b** and its saturated analogue **9b** clearly demonstrate that it is not conjugation but proximity of the HO group to the =NOH fragment which governs the efficiency of NO generation.

The best NO precursors **7b** and **9b** are subjected to oxidation even by atmospheric oxygen in alkaline solution (Table 1). In this case NO fixation and polarographic detection is based on the following reactions:[18]

$$2NO + O_2 \longrightarrow NO_2$$

$$NO + NO_2 + 2OH^- \longrightarrow 2NO_2^- + H_2O$$

$$[Fe(CN)_6]^{4-} + NO_2^- + H_2O \longrightarrow [Fe(CN)_5NO]^{2-} + CN^- + 2OH^-$$

It is well known that biological action of NO depends on activation of soluble guanylate cyclase — the enzyme catalysing synthesis of cyclic 3′,5′-guanosine monophosphate which results in vascular relaxation and arterial pressure drop. Thus, we studied the effect of oximes on the activity of soluble guanylate cyclase from human platelets. This was determined by radioimmunoassay as described earlier relative to SNP.[10] The dependence of the response on concentration is a typical ‘bell-shaped’ curve with a maximum at about 10^{-4} mol dm^{-3} and the values of enzyme activation by oximes correlate with results of polarographic testing (Table 1).

Thus, all the oximes tested generate NO under mild oxidation and the most active of them, **7b**, activates soluble guanylate cyclase *in vitro* with an activity close to that of SNP. The presence of an aromatic substituent, especially one bearing a 2-HO-group, increases the activity. A moderate drop in activity for **9b** compared to **7b** and its considerable decrease for the *Z*-isomer **5/6b**, 4-HO isomer **5/6d** and MeO-derivatives **5/6c,e** leads to the conclusion that phenolic group proximal to the oxime fragment participates in some oxidation steps, possibly by stabilizing ‘hot’ intermediates. The nature of this effect is being studied.

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Received: Moscow, 8th December 1995
Cambridge, 15th February 1996; Com. 5/08186F