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Synthesis, antinociceptive activity and pharmacokinetic profiles of nicorandil and its isomers

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ABSTRACT

Nicorandil (*N*-(2-hydroxyethyl)nicotinamide nitrate) is an antianginal drug, which activates guanylyl cyclase and opens the ATP-dependent K^+ channels, actions that have been suggested to mediate its vasodilator activity. We synthesized nicorandil and its two isomers, which vary in the positions of the side chain containing the nitric oxide (NO) donor, and also their corresponding denitrated metabolites. The activities of these compounds were evaluated in an experimental model of pain in mice. Pharmacokinetic parameters of nicorandil and its isomers, as well as the plasma concentrations of the corresponding denitrated metabolites and also nicotinamide and nitrite were determined. Nicorandil exhibited the highest antinociceptive activity, while the *ortho*-isomer was the least active. Nicorandil and *para*-nicorandil, which induced higher plasma concentrations of nitrite, exhibited higher antinociceptive activity, which suggests that the release of NO may mediate this activity.

1. Introduction

Nicorandil [*N*-(2-hydroxyethyl)nicotinamide nitrate (**1**; Fig. 1) was synthetized in 1976[1] and has been marketed since 1984 in Japan and other countries for the prevention and treatment of chronic angina pectoris.[2–4] It has been demonstrated that nicorandil releases nitric oxide (NO) and opens ATP-dependent K^+ channels, actions that have been suggested to mediate its vasodilator activity.[5] It is still unclear whether nicorandil induces its NO-independent effects directly or indirectly.[5] Regarding the opening of ATP-dependent K^+ channels induced by nicorandil, Simpson and Wellington (2004)[4] have demonstrated that *N*-(2-hydroxyethyl)-nicotinamide (**1A**; Fig. 1), the major metabolite, may mediate this effect. In addition, **1A** enhances the vascular effects of endogenous vasodilators, such as adenosine and adrenomedullin.[6,7] Another metabolite, nicotinamide, may be formed when the side chain of **1A** is cleaved.[3] Nicotinamide, an amide derivative of vitamin B3, similarly to nicorandil, induces vasodilation, but its potency is much lower than that of the parent compound.[8]

As NO and ATP-dependent K^+ channels are important molecules mediating many other biological actions, including inflammation[9,10] and nociception,[11] it would not be surprising to identify new activities of nicorandil beyond those in the cardiovascular system. Indeed, the inhibitory effects induced by nicorandil on the overactive bladder in animal models,[12] proteinuria and glomerular injury in a model of diabetic nephropathy[13] and intraocular pressure in the anterior chamber of the eye of humans[14] have been demonstrated. Although the investigation of the effects induced by nicorandil in experimental models of pain is still too preliminary, it is warranted. Many compounds that release NO[15–17] and activate ATP-dependent K^+ channels,[18–20] and also nicotinamide,[21] a nicorandil metabolite, exhibit activities in different experimental models of pain. In addition, we have recently demonstrated that systemic administration of nicorandil inhibits the nociceptive response induced by formaldehyde in mice.[22]

In the present study, we synthesized the three isomers of nicorandil, which vary in the positions of the side chain that contains the $-ONO_2$ group (Fig. 1), and investigated their activities in an experimental model of nociceptive and inflammatory pain in mice.

Abbreviations: NO, nitric oxide; p.o., *per os*; T_{max}, maximum time; AUC_{0-inf}, area under curve (time 0 > infinite); CMC, carboxymethyl cellulose; NMR, nuclear magnetic resonance; C_{max}, maximum concentration; AUC_{0-t}, area under curve (time 0 > last time).

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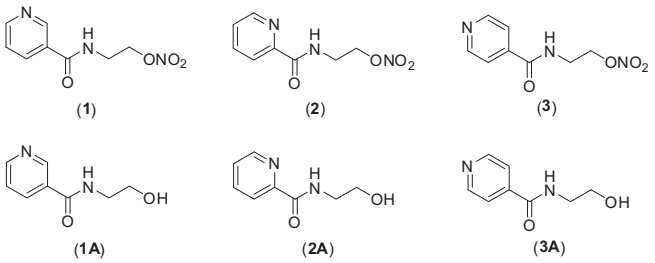


Figure 1. Chemical structures of nicorandil (**1**), *ortho*-nicorandil (**2**) and *para*-nicorandil (**3**) and their respective main metabolites *N*-(2-hydroxyethyl)nicotinamide (NHN) (**1A**), *N*-(2-hydroxyethyl)picolinamide (NHP) (**2A**) and *N*-(2-hydroxyethyl)isonicotinamide (NHI) (**3A**).

To further explore the structure-activity relationships, we determined some pharmacokinetic parameters of the three isomers, as well as the plasma concentrations of the corresponding main denitrated metabolites (Fig. 1) and also the plasma concentrations of nicotinamide and nitrite after the administration of each isomer in mice.

2. Material and methods

2.1. Chemical and reagents

All chemicals were obtained from commercial suppliers and used without further purification. Melting points were determined in a Gehaka PF 1500 apparatus and are uncorrected. ^{1}H nuclear magnetic resonance (NMR) spectra and ^{13}C NMR spectra were recorded on a Bruker Avance DPX/200. Chemical shift values (δ) were given in parts per million (ppm). Infrared (IR) spectra were recorded on a Spectro One Perkin Elmer. The elemental analyses were performed with a Perkin-Elmer apparatus.

2.2. Syntheses of nicorandil and its isomers

Nicorandil (**1**) and its positional isomers (**2** and **3**) were synthesized, as shown in Scheme 1. In summary, the corresponding esters were treated with ethanolamine under reflux, which furnished the hydroxylated precursors **1A** to **3A**. Compounds **1A**–**3A** were treated with fuming nitric acid at −5 °C, which lead to the nitrates **1** to **3**. All synthesized compounds were characterized by ^{1}H and ^{13}C NMR, infrared, melting point and elemental analysis; the data are in accordance with previous reports.[23,24]

2.3. Chemistry and biological assays

For the synthesis of the hydroxylated precursors, ethanolamine (1.5 mmol) was added slowly to the esters (1 mmol) at 55 °C and stirred for 3 h. The reaction mixture was stirred at room temperature for 15 h. The residue was purified by silica gel column chromatography (eluent/ethyl acetate/hexane 8:2) or recrystallized from ethyl acetate. The progress of the reaction was monitored by TLC.

2.3.1. *N*-(2-Hydroxyethyl)nicotinamide (1A)

(Yield 64%): mp 88.0 °C. IR (ATR): 3321, 3173, 2876, 1659, 1550, 1422, 1296, 1057, 1028, 702. ^{1}H NMR (200 MHz, DMSO-d_6): 3.34–3.39 (m, 2H), 3.57 (t, 2H, J = 5.8 Hz), 4.83 (br s, 1H), 7.42–7.50 (m, 1H), 8.19 (d, 1H, J = 8.0 Hz), 8.66–8.68 (m, 2H), 9.02 (s, 1H). ^{13}C NMR (50 MHz, DMSO-d_6): 42.3, 59.8, 123.4, 130.1, 135.0, 148.5, 151.7, 165.1. Anal. Calcd for $C_8H_{10}N_2O_2$. Calcd (%): C, 57.82; H, 6.07; N, 16.86. Found (%): C, 56.94; H, 6.03; N, 16.88.

2.3.2. *N*-(2-Hydroxyethyl)picolinamide (2A)

(Yield 92%): mp 36.0 °C. IR (ATR): 3364, 3297, 2932, 2876, 1645, 1569, 1436, 1365, 1298, 1060, 1044, 821, 752. ^{1}H NMR (200 MHz, $CDCl_3$): 3.60–3.68 (m, 2H), 3.84 (t, 2H, J = 5.0 Hz), 4.85 (br s, 1H), 7.32–7.41 (m, 1H), 7.78 (td, 1H, J = 7.6, 1.6 Hz), 8.10 (d, 1H, J = 7.8 Hz), 8.47 (d, 1H, J = 4.3 Hz), 8.64 (br s,1H). ^{13}C NMR (50 MHz, $CDCl_3$): 41.6, 60.8, 121.6, 125.7, 136.9, 147.6, 149.0, 164.6. Anal. Calcd for $C_8H_{10}N_2O_2$. Calcd (%): C, 57.82; H, 6.07; N, 16.86. Found (%): C, 57.78; H, 6.12; N, 16.85.

2.3.3. *N*-(2-Hydroxyethyl)isonicotinamide (3A)

(Yield 88%): mp 135.5 °C. IR (ATR): 3317, 3173, 3058, 2931, 1660, 1548, 1410, 1314, 1237, 1065, 1041, 1003, 816, 751. ^{1}H NMR (200 MHz, DMSO-d_6): 3.32–3.40 (m, 2H), 3.47–3.59 (m, 2H), 4.81 (t, 1H, J = 5.4 Hz), 7.76 (d, 2H, J = 4.8 Hz), 8.69-8.75 (m, 3H). ^{13}C NMR (50 MHz, DMSO-d_6): 42.3, 59.5, 121.3, 141.5, 150.1, 164.8. Anal. Calcd for $C_8H_{10}N_2O_2$. Calcd (%): C, 57.82; H, 6.07; N, 16.86. Found (%): C, 58.72; H, 6.04; N, 16.97.

The nitrated compounds were obtained by mixing fuming nitric acid (10 mmol) and the hydroxylated precursors (1 mmol) at −5.0 °C and stirring for 2 h. The reaction mixture was poured into a mixture of water and ice. The pH was adjusted to 6.0 by adding $CaCO_3$. The obtained solid was vacuum filtered and recrystallized in ethanol, which furnished a white solid in all cases.[23,24]

2.3.4. *N*-(2-Nytroxyethyl)nicotinamide (nicorandil; 1)

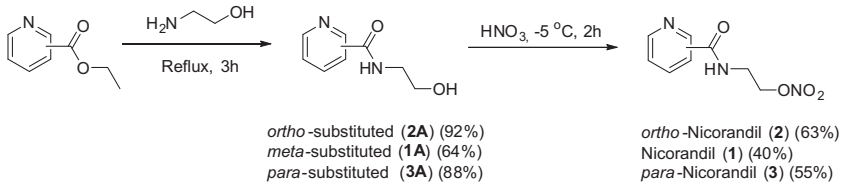
(Yield 40%): mp 91.0 °C. IR (ATR): 3241, 3073, 1717, 1627, 1590, 1554, 1372, 1361, 1319, 1286, 1012, 1000, 860, 824, 705. ^{1}H NMR (200 MHz, DMSO-d_6): 3.65 (q, 2H, J = 5.1 Hz), 4.67 (t, 2H, J = 5.1 Hz), 7.46–7.55 (m, 1H), 8.18 (d, 1H, J = 7.8 Hz), 8.71 (d, 1H, J = 4.4 Hz), 8.94–9.02 (m, 2H). ^{13}C NMR (50 MHz, DMSO-d_6): 36.8, 72.1, 123.5, 129.5, 135.0, 148.3, 152.0, 165.2. Anal. Calcd for $C_8H_9N_3O_4$. Calcd (%): C, 45.50; H, 4.30; N, 19.90. Found (%): C, 45.62; H, 4.31; N, 20.03.

2.3.5. *N*-(2-Nytroxyethyl)picolinamide (*ortho*-nicorandil; 2)

(Yield 63%): mp 62.5 °C. IR (ATR): 3391, 2956, 1664, 1630, 1520, 1275, 1012, 858, 752. ^{1}H NMR (200 MHz, DMSO-d_6): 3.64–3.74 (m, 2H), 4.69 (t, 2H, J = 5.1 Hz), 7.55–7.61 (m, 1H), 7.94–8.07 (m, 2H), 8.62 (d, 1H, J = 4.4 Hz), 9.08–9.10 (m, 1H). ^{13}C NMR (50 MHz, DMSO-d_6): 36.4, 72.2, 121.9, 126.6, 137.7, 148.3, 149.5, 164.3. Anal. Calcd for $C_8H_9N_3O_4$. Calcd (%): C, 45.50; H, 4.30; N, 19.90. Found (%): C, 45.45; H, 4.21; N, 19.86.

2.3.6. *N*-(2-Nytroxyethyl)isonicotinamide (*para*-nicorandil; 3)

(Yield 55%): mp 112 °C. IR (ATR): 3265, 1647, 1618, 1545, 1322, 1280, 1018, 877, 754. ^{1}H NMR (200 MHz, DMSO-d_6): 3.65 (q, 2H,



Scheme 1. Synthesis of nicorandil (**1**) and its isomers **2** and **3**.