

Supporting Information

HPLC-MS aided PC12 cell systems: To quantitatively monitor the conversion of nitronyl nitroxide in biological systems with and without NO·

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Agents¹⁻³

3-Nitro-NTIO was prepared according to the literature. Yield: 93%, Mp: 161-162 °C. $R_f = 0.69$ (CHCl₃/CH₃OH, 20:1). ESI-MS (m/z): 279 [M + H]⁺. IR (KBr): 1530, 1350, 1600, 1450, 880, 790, 680 cm⁻¹. ESR: a five-line pattern with intensity ratios of 1:2:3:2:1, $a_N = 8.10$ G, $g = 2.00998$. HPLC purity was more than 99.0%.

3-Nitro-PTIH was prepared according to the literature. Yield 66%, Mp: 97-99 °C. $R_f = 0.13$ (CHCl₃/CH₃OH, 20:1). ESI-MS (m/z): 264 [M + H]⁺. IR (KBr): 3345, 1602, 1513, 1455, 1370, 811, 790, 680 cm⁻¹. ¹H NMR (500 MHz, CDCl₃-d): δ /ppm=8.549 (s, 1H), 8.219 (d, J=7.20Hz, 1H), 7.989 (d, J=7.21Hz, 1H), 7.545 (t, J=7.21Hz, 1H), 2.646 (s, 1H), 1.153 (s, 6H), 1.131 (s, 6H).

3-Nitro-PTI was prepared according to the literature. Yield 73%, Mp: 71-72 °C.

$R_f = 0.78$ ($\text{CHCl}_3/\text{CH}_3\text{OH}$, 20:1). ESI-MS (m/z): 263 $[\text{M} + \text{H}]^+$. IR (KBr): 3348, 1604, 1533, 1452, 1359, 810, 791, 684 cm^{-1} .

MS and HPLC spectra of 3-nitro-PTIO, 3-nitro-PTIH and 3-nitro-PTI

The MS and UV based HPLC spectra of 3-nitro-PTIO, 3-nitro-PTIH and 3-nitro-PTI are given in Figure S1- S3, respectively.

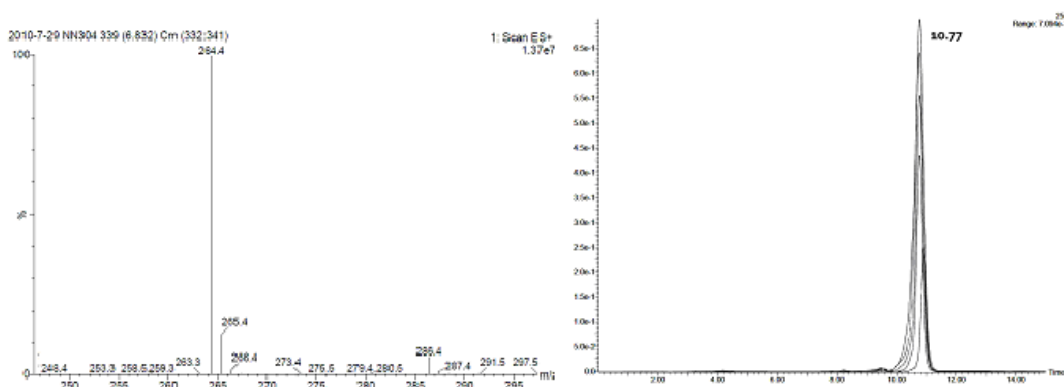


Figure S1 MS and HPLC spectra of 3-nitro-PTIO.

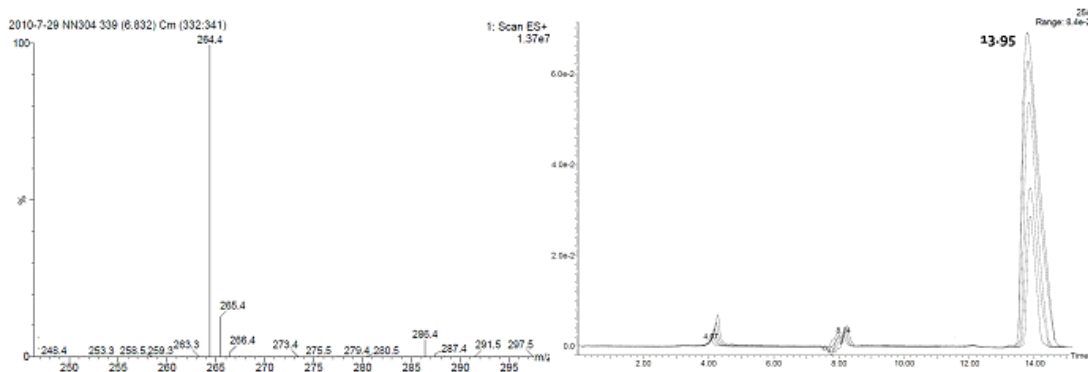


Figure S2 MS and HPLC spectra of 3-nitro-PTIH.

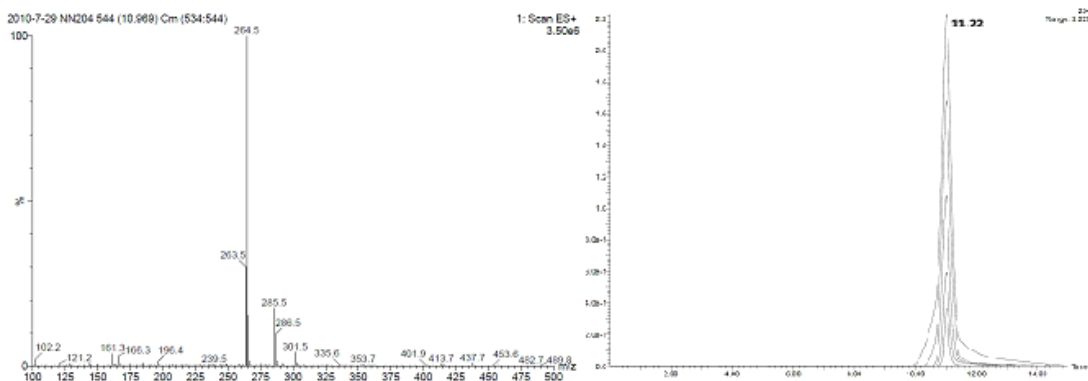


Figure S3 MS and HPLC spectra of 3-nitro-PTI.

The UV based HPLC spectrum of a 1:1:1 mixture of 3-nitro-PTIO, 3-nitro-PTIH and 3-nitro-PTI is given in Figure S4.

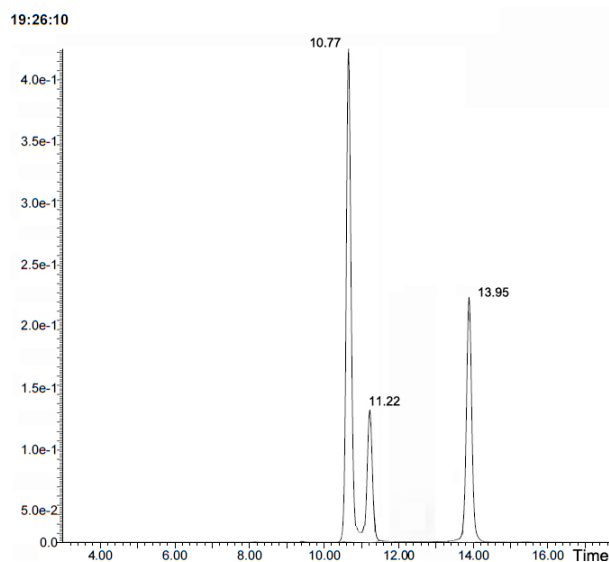


Figure S4 HPLC spectrum of 1:1:1 mixture of 3-nitro-PTIO, 3-nitro-PTIH and 3-nitro-PTI.

The MS and ion chromatography based HPLC spectra of 3-nitro-PTIO, 3-nitro-PTIH and 3-nitro-PTI are given in Figure S5 - S7, respectively.

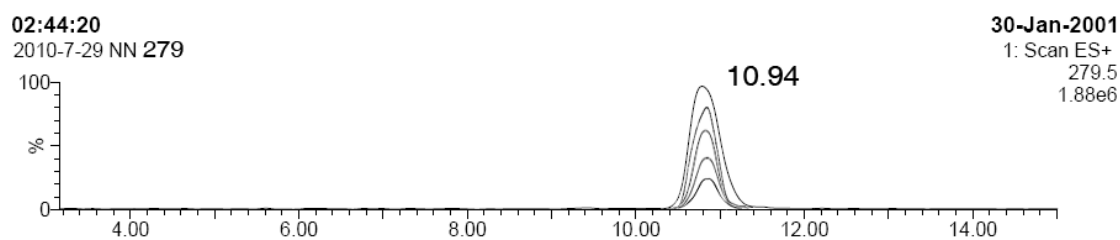


Figure S5 Ion chromatography of 3-nitro-PTIO

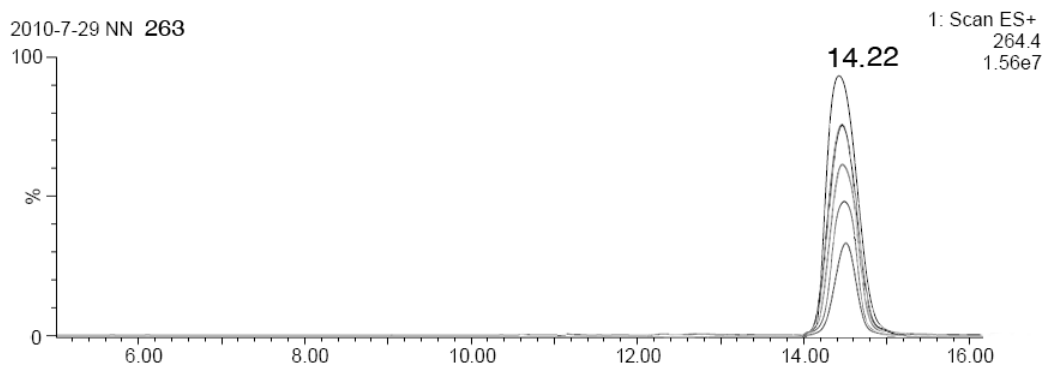


Figure S6 Ion chromatography of 3-nitro-PTIH



Figure S7 Ion chromatography of 3-nitro-PTI.

The ion chromatography based HPLC spectrum of a 1:1:1 mixture of 3-nitro-PTIO, 3-nitro-PTIH and 3-nitro-PTI is given in Figure 8.

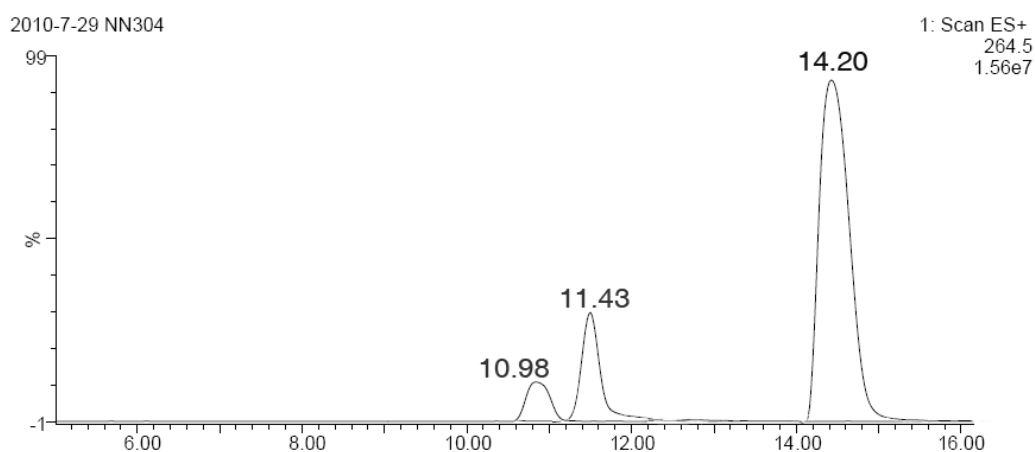


Figure S8 Ion chromatography of 1:1:1 mixture of 3-nitro-PTIO, 3-nitro-PTIH and 3-nitro-PTI.

Conversion of 3-nitro-PTIO and its conversion forms PBS or DMEM

To examine the effect of cultural medium on the conversion of 3-nitro-PTIO in the cultural system of PC12 cells, the conversion of 3-nitro-PTIO in blank PBS and DMEM was monitored with HPLC-MS method, the conversion is shown as Figure S9.

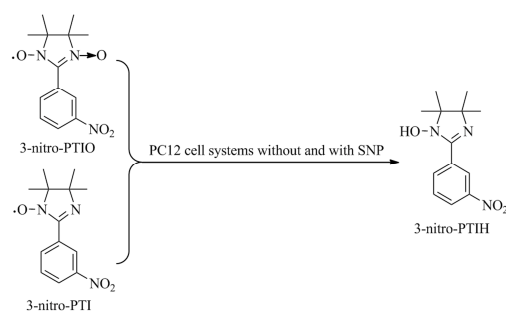


Figure S9 Conversion of 3-nitro-PTIO in PBS and DMEM.

Laser scanning confocal microscope image

Endothelium cells were seeded in 6-well plates at a density of 5×10^5 cells/mL and grown in DMEM (consisted of 10% of heat inactivated horse serum, 5% of fetal bovine serum, 100 U/mL penicillin, and 100 μ g/mL streptomycin) at 37 °C and 5% CO₂ atmosphere for 24 h. After removing the medium 50 μ L of DMEM (blank control) or 50 μ L of the solution of SNP in DMEM (final concentration 1 mM, oxidation control) or 50 μ L of the solution of 1 mM H₂O₂/30 M Fe (II) in DMEM were added into the residual cells, in other respective group 50 μ L of 3-nitro-PTIO in DMEM (treated control, final concentration 50 μ M) or 50 μ L of the solution of 3-nitro-PTIO plus SNP in DMEM (prevention group, the final concentration of 3-nitro-PTIO and SNP were 50 μ M and 1 mM, respectively) or 50 μ L of the solution of 3-nitro-PTIO plus 1 mM H₂O₂/30 M Fe (II) in DMEM (prevention group, the final concentration of

3-nitro-PTIO was 50 μM) were added to the residual cells, which were incubated at 37 $^{\circ}\text{C}$ in 5% CO_2 atmosphere for 2 h. After removing the medium the residual cells were washed with DMEM ($100\text{ }\mu\text{L} \times 2$) and to the residual cells 50 μL of the solution of DCFH-DA in DMEM (final concentration 5 μM) were added, which were at 37 $^{\circ}\text{C}$ in dark and 5% CO_2 atmosphere incubated for 20 min. After removing the medium the residual cells were washed with DMEM ($100\text{ }\mu\text{L} \times 2$) and tested on laser scanning confocal microscope (LSCM, Leica, Model TCS NT, Germany). The used parameters were wavelength 488 nm, channels BP530/30, power 100 W, iris 6.2, gain 1139 and offset 0. For each test 200 PC12 cells or 200 endothelium cells were selected from various visual fields and their fluorescence intensities were averaged. Some images are shown in Figure S10.

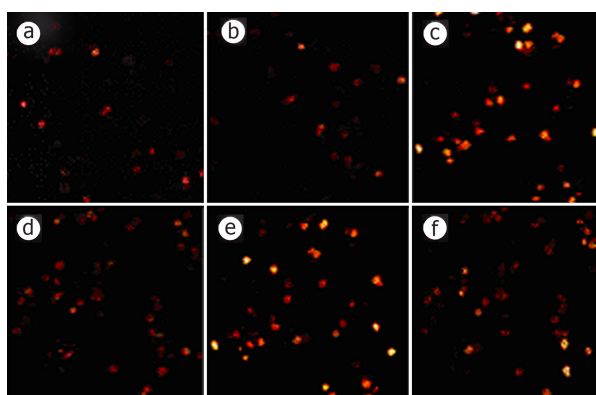


Figure S10 LSCM photo of DCFH-DA labeled endothelium cells, a) Image of endothelium cells labeled with DCFH-DA in DMEM alone (blank control); b) Image of endothelium cells labeled with DCFH-DA in DMEM plus 50 μM of 3-nitro-PTIO (treated control); c) Image of endothelium cells labeled with DCFH-DA in DMEM plus 1 mM of SNP (oxidation control of NO); d) Image of endothelium cells labeled with DCFH-DA in DMEM plus 50 μM of 3-nitro-PTIO and 1 mM of SNP; e) Image of endothelium cells labeled with DCFH-DA in DMEM plus 1 mM H_2O_2 /30 M Fe (II) (oxidation control of $\cdot\text{OH}$); f) Image of endothelium cells labeled with DCFH-DA in DMEM plus 50 μM of 3-nitro-PTIO and 1 mM H_2O_2 /30 M Fe (II).

Effect of 3-Nitro-PTIO on gel electrophoresis of SC pBR32 DNA

PTIO has been known as the scavenger and is able to prevent DNA from damage. To know whether 3-nitro-PTIO protects DNA from damage a solution of SC pBR322 DNA in 10 μ L of Tris-acetate buffer (final concentration 50 mg/L) and the solutions of SC pBR322 DNA (final concentration 50 mg/L) plus 100, 200 and 400 ng of 3-nitro-PTIO in 10 μ L of Tris-acetate buffer as well as 1 μ L of SNP in Tris-acetate buffer (1 mM) were incubated at 37 $^{\circ}$ C for 24 h. The gel electrophoresis is shown in Figure S11. SC pBR322 DNA gives two bands (form I and II). The quantitative data of the bands in Figure S11 were read with Quantity One- 4.6.2 (BIO-RAD, software) and are listed in Table 1. The comparison of the band intensity of SC pBR32 DNA to those of SC pBR322 DNA plus SNP (1 mM) and 100, 200 and 300 ng of 3-nitro-PTIO a decrease of the intensity of the second band was observed. This is a direct evidence that 3-nitro-PTIO could prevent DNA from cleavage induced by NO donor, since the increase of the band number is the result of DNA cleavage.

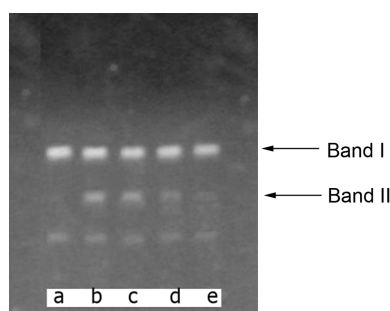


Figure S11 Effect of 3-Nitro-PTIO on gel electrophoresis of SC pBR32 DNA, a) SC pBR322 DNA alone; b) SC pBR322 DNA plus 1 μ L of SNP (1 mM); c) SC pBR322 DNA plus 1 μ L of SNP (1 mM) and 100 ng of 3-nitro-PTIO; d) SC pBR322 DNA plus 1 μ L of SNP (1 mM) and 200 ng of 3-nitro-PTIO; e) SC pBR322 DNA plus 1 μ L of SNP (1 mM) and 400 ng of 3-nitro-PTIO.

Table S1 Relative quantities of band I and band II in lanes

	Relative quantity (%)*				
	Lane a	Lane b	Lane c	Lane d	Lane e
Band I	100.0	71.6	73.9	86.9	96.4
Band II	0	28.4	26.1	13.1	3.6

*The quantity of a particular band was measured and represented as the percentage of the total intensity of all the bands in the lane. The calculation method (%Bands in Lane) is set in the Preferences dialog. The parameters were set as Band Sens. 2.8, Band Width 10, Band Min. Dens. 0, Band Filter 4, Band Shoulder 1 and Band Size 5.

References

1. Y. Wu, L. Bi, W. Bi, Z. Li, M. Zhao, C. Wang, J. Ju, S. Peng, Novel 2-substituted nitronyl nitroxides as free radical scavengers: Synthesis, biological evaluation and structure–activity relationship. *Bioorg & Med Chem.*, 2006, **14**, 5711-5720.
2. M. Zhao, Z. Li, L. Peng, Y. Tang, C. Wang, Z. Zhang, S. Peng, A new class of analgesic agents toward prostacyclin receptor inhibition: synthesis, biological studies and QSAR analysis of 1-hydroxyl-2-substitutedphenyl-4,4,5,5-tetramethylimidazolines. *Eur J Med Chem.*, 2008, **43**, 1048-1058.
3. M. Zhao, Z. Li, L. Peng, Y. Tang, C. Wang, Z. Zhang, S. Peng, Novel 1-oxyl-2-substitutedphenyl-4,4,5,5-tetramethylimidazolines: Synthesis, selectively analgesic action and QSAR analysis. *Bioorg & Med Chem.*, 2007, **15**, 2815-2826.