Herein $^1$H and $^{13}$C NMR spectra of ERJ-500, a new hybrid aspirin derivative, covalently conjugated to nitrogen monoxide donor linsidomine are presented as well as NMR spectra of its synthetic intermediate compounds. HPLC-MS measurements data are also included, demonstrating the stability of the linsidomine-aspirin hybrid in oxidation reactions. This data article also concerns miscellaneous myocardial parameters of isolated rat hearts as a complementation of the tables shown in the paper entitled “A new, vasoactive hybrid aspirin containing nitrogen monoxide-releasing molsidomine moiety” Szoke et al., 2019. Column tables represent data of aorta flow, aortic pressure, derivated aortic pressure and cardiac output.

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1. Data

Spectra from \(^1\)H and \(^{13}\)C NMR measurements are reported to prove the structure of the synthesized compounds [1] (see Figs. 1–9). HPLC-MS measurements were used to study the oxidative stability of the compound ERJ-500, representative total ion chromatogram of the oxidation by synthetic porphyrin and the Chemical Fenton System can be seen (Figs. 10, 11). Cardiac parameters (aorta flow, aortic pressure, derivated aortic pressure, and cardiac output) were registered (Fig. 12) in "isolated working hearts" treated with ERJ-500.

1.1. Characterization of new compounds

The \(^1\)H NMR (400 MHz) and \(^{13}\)C NMR spectra were recorded with a Bruker DRX-400 spectrometer at 25 °C. HPLC-MS measurements were used to study the oxidative stability of the compound ERJ-500, representative total ion chromatogram of the oxidation by synthetic porphyrin and the Chemical Fenton System can be seen (Figs. 10, 11). Cardiac parameters (aorta flow, aortic pressure, derivated aortic pressure, and cardiac output) were registered (Fig. 12) in "isolated working hearts" treated with ERJ-500.

1.1. Characterization of new compounds

The \(^1\)H NMR (400 MHz) and \(^{13}\)C NMR (101 MHz) spectra were recorded with a Bruker DRX-400 spectrometer at 25 °C. Chemical shifts are referenced to Me₄Si (0.00 ppm for \(^1\)H) and to the residual solvent signals (CDCl₃: 77.1 ppm for \(^{13}\)C).

1.2. Compound 5

\(^1\)H NMR (400 MHz, CDCl₃): \(\delta 8.03 (dd, J = 7.8 \text{ Hz}, J = 1.8 \text{ Hz}, 1H, arom), 7.53 (td, J = 7.8 \text{ Hz}, J = 1.8 \text{ Hz}, 1H, arom), 7.47−7.45 (m, 6H, arom), 7.30–7.19 (m, 10H, arom), 7.08 (dd, \(J = 8.1 \text{ Hz}, J = 0.8 \text{ Hz}, 1H), 4.41–4.39 (m, 2H, TEG-CH₂), 3.78–3.76 (m, 2H, TEG-CH₂), 3.70–3.65 (m, 10H, 5 \times \text{TEG-CH₂}), 3.23 (t, \(J = 5.2 \text{ Hz}, 2H, \text{TEG-CH₂})

2.34 (t, 3H, CH₃ Ac), \(^{13}\)C NMR (101 MHz, CDCl₃): \(\delta 169.9 (1\text{C, C₉ Ac}), 164.5 (1\text{C, COO}), 150.8 (1\text{C, C₉ arom}), 144.2 (3\text{C, C₉ arom}), 134.0, 132.0, 128.8, 127.9, 127.0, 126.1, 123.9 (19\text{C, arom}), 123.3 (1\text{C, C₉ arom}), 86.6 (1\text{C, C₉ Tr}), 70.9, 70.8, 70.7, 69.2, 64.4, 63.4 (8\text{C, 8 \times \text{TEG-CH₂}}, 21.1 (1\text{C, CH₃ Ac})

1.3. Compound 6

\(^1\)H NMR (400 MHz, CDCl₃): \(\delta 8.05 (dd, J = 7.8 \text{ Hz}, J = 1.6 \text{ Hz}, 1H, arom), 7.56 (td, J = 7.9 \text{ Hz}, J = 1.6 \text{ Hz}, 1H, arom), 7.32 (td, J = 7.6 \text{ Hz}, J = 1.2 \text{ Hz}, 1H, arom), 7.11 (dd, J = 8.1 \text{ Hz}, J = 1.2 \text{ Hz}, 1H, arom), 4.45–4.43
(m, 2H, TEG-CH₂), 3.81–3.78 (m, 2H, TEG-CH₂), 3.74–3.65 (m, 10H, 5 × TEG-CH₂), 3.60–3.58 (m, 2H, TEG-CH₂), 2.62 (s, 1H, TEG-OH), 2.36 (s, 3H, CH₃ Ac); ¹³C NMR (101 MHz, CDCl₃): δ 169.9 (1C, Cq COO), 164.5 (1C, Cq Ac), 150.8 (1C, Cq arom), 134.1, 132.0, 126.1, 123.9 (4C, arom), 123.2 (1C, Cq arom), 72.5, 70.8, 70.7, 70.6, 70.4, 69.2, 64.3, 61.8 (8C, 8 × TEG-CH₂), 21.1 (1C, CH₃ Ac).

1.4. Compound 7

¹H NMR (400 MHz, CDCl₃): δ 8.41 (s, 1H, CH sydnone), 8.27 (d, J = 9.1 Hz, 2H, arom), 7.42 (d, J = 9.1 Hz, 1H, arom), 3.87–3.85 (m, 2H, CH₂ morpholine), 3.64–3.61 (m, 2H, CH₂ morpholine); ¹³C NMR (101 MHz, CD₃OD): δ 183.5 (1C, Cq carbamate), 167.0 (1C, Cq sydnone), 134.5, 132.1, (4C, arom), 125.3 (1C, CH), 74.3, 62.9 (4C, 4 × morpholine-CH₂).

1.5. Compound ERJ-500

¹H NMR (400 MHz, CDCl₃): δ 8.03 (dd, J = 7.9 Hz, J = 1.7 Hz, 1H, arom), 7.70 (s, 1H, CH sydnone), 7.56 (ddd, J = 8.1, 7.4, 1.8 Hz, 1H, arom), 7.31 (td, J = 7.7 Hz, 1.1 Hz, 1H, arom), 7.10 (dd, J = 8.1 Hz, J = 1.1 Hz, 1H,
Fig. 3. $^{13}$C NMR spectra of compound 5.

Fig. 4. $^1$H NMR spectra of compound 6.
Fig. 5. $^{13}$C NMR spectra of compound 6.

Fig. 6. $^1$H NMR spectra of compound 7.
Fig. 7. $^{13}$C NMR spectra of compound 7.

Fig. 8. $^1$H NMR spectra of compound ERJ-500.
Fig. 9. $^{13}$C NMR spectra of compound ERJ-500.

Fig. 10. Representative total ion chromatogram of the oxidation by synthetic porphyrin. On the control chromatogram (red) the peak at 6.59 represents ERJ-500. After oxidation (green chromatogram) the peak of ERJ-500 at 6.58 remained unchanged.
arom), 4.43–4.41 (m, 2H, CH₂ morpholine), 4.26–4.24 (m, 2H, CH₂ morpholine), 3.94–3.92 (m, 4H, 2 × TEG-CH₂), 3.80–3.78 (m, 2H, CH₂ morpholine), 3.74–3.72 (m, 2H, CH₂ morpholine), 3.68–3.63 (m, 8H, 4 × TEG-CH₂), 3.51–3.49 (m, 4H, 2 × TEG-CH₂), 2.35 (s, 3H, CH₃ Ac); ¹³C NMR (101 MHz, CDCl₃): δ 174.2 (1C, Cq carbamate), 169.7 (1C, Cq COO), 164.4 (1C, Cq Ac), 161.2 (1C, Cq sydnone), 150.6 (1C, Cq arom), 133.8, 131.8, 125.9, 123.7 (4C, arom), 123.2 (1C, Cq arom), 70.6, 70.5, 69.3, 69.0, 65.4, 64.6, 64.3, 54.6 (13C, 1 × sydnone-C, 4 × morpholine-CH₂, 8 × TEG-CH₂), 20.9 (1C, CH₃ Ac).

1.6. Representative chromatograms of oxidative stability assays

Non-significant myocardial parameters in working heart preparation.

| Aorta flow | Aortic pressure | Derivated aortic pressure | Cardiac output |
|------------|-----------------|---------------------------|---------------|
| Control    | ERJ-500         | Control                   | ERJ-500       | Control | ERJ-500 |
| 22         | 46              | 87                        | 100           | 740     | 1361    |
| 31         | 50              | 92.7                      | 110           | 924     | 2159    |
| 25         | 32              | 110,9                     | 104           | 1670    | 1666    |
| 48         | 38              | 99                        | 106           | 1357    | 1938    |
| 42         | 42              | 102                       | 102,6         | 1568    | 1783    |
| 34         | 42              | 105                       | 109           | 1756    | 1786    |
| 40         |                 | 106                       | 1879          | 54      | 66      |
| 34         |                 | 104                       | 1873          | 52      |         |
| 46         |                 | 99                        | 1424          | 65      |         |
| 46         |                 | 99                        | 1558          | 65      |         |
| 46         |                 | 113,5                     | 1956          | 65      |         |

Fig. 11. Representative total ion chromatogram of the oxidation by the Chemical Fenton System. On the control chromatogram (red) the peak at 6.54 represents ERJ-500. After oxidation (green chromatogram) the peak of ERJ-500 at 6.53 remained unchanged.
2. Experimental design, materials and methods

2.1. LC-MS measurements

The reaction mixture was analyzed with an LTQ-XL linear ion trap mass spectrometer coupled with the Accela LC system (Thermo Fisher Scientific, Waltham, MA, USA). The HPLC separation was performed using a Kinetex XB-C18 2.6 μm column, 0.1% formic acid in water, and ACN with 0.1% formic acid with gradient elution, and the flow rate was set to 300 μL/min. The method parameters for mass spectrometry were the followings: 35 a.u. sheath gas flow rate, 5000 V spray voltage, 275 °C capillary temperature, 31 V capillary voltage, 150 V tube lens voltage, and 34 V skimmer voltage.

2.2. Oxidation by synthetic porphyrin and the chemical Fenton system

Two reactions were carried out to test the stability of ERJ-500 molecule under oxidative conditions, based on the method as reported by Csepanyi et al. [2] recently, with minor modifications as follows: 50 μL of ERJ-500 dissolved in acetonitrile was used for synthetic porphyrin oxidation in 10 mM concentration. 400 μL of ERJ-500 in 2.5 mM concentration for the Fenton reaction. Samples were drawn at 1 h in the Fenton reactions prior to injecting them instantly to the HPLC and further investigation. Reaction mixtures for blank contained acetonitrile only without ERJ-500. The control mixtures contained no peroxide.

Fig. 12. Myocardial function. The results show aorta flow, aortic pressure, derivated aortic pressure, and cardiac output in control and ERJ-500 treated hearts.
2.3. **Isolated working heart preparation to assess cardiac parameters**

To measure cardiac function (Aortic flow, Aortic pressure, Derivated aortic pressure, and Cardiac output), isolated working heart preparations were carried out based on a previously described method by Czompa et al. [3] on Sprague Dawley female rat hearts (n = 11 in the control group, n = 6 in the treated group). After completing the isolated working heart preparation procedure followed by 10 min washout period, and aorta flow, aortic pressure, derivated aortic pressure, and cardiac output were registered (Fig. 12). Cardiac output was calculated by the sum of aortic and coronary flow represented in the associated research article [1]. In the treated group, **ERJ-500** was added to the KHB buffer by a dilution of a previously prepared stock solution leading to a 100 μM concentration of **ERJ-500** in the inflow line. The molecule-containing KHB buffer was presented after the washout and baseline registration period for 5 min, followed by a 30 min ischemia and 90 min of reperfusion.

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**Conflict of interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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