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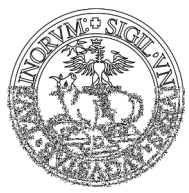

Physicochemical Profile and PAMPA Study of New NO-Donor Edaravone Co-Drugs

Barbara Rolando[a], Andrea Filieri[a], Konstantin Chegaev[a], Loretta Lazzarato[a], Marta Giorgis[a], Roberta Fruttero[a*], Sophie Martel[b], Pierre-Alain Carrupt[b], Alberto Gasco[a]

[a)] Dipartimento di Scienza e Tecnologia del Farmaco, Università degli Studi di Torino, Via Pietro Giuria 9, 10125 Torino, Italy

[b)] School of Pharmaceutical Sciences, University of Geneva, University of Lausanne, 30 Quai Ernest-Ansermet, CH 1211 Geneva 4, Switzerland

*E-MAIL ADDRESS: roberta.fruttero@unito.it

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Abstract

A new class of co-drugs obtained by joining antioxidant edaravone with a substructure containing NO-donor nitrooxy functions were synthesised and characterized for their stability, lipophilicity and permeability profile. These products display widely modulated lipophilicity and PAMPA studies predict for a number of these compounds a very good gastrointestinal absorption. When incubated in human serum, these co-drugs quickly afford edaravone and the related NO-donor hydrolysis products, the latter displaying good vasodilator properties.

Keywords

Nitrooxy-acyl derivatives of edaravone; Co-drugs; Lipophilicity profile; In vitro permeability profile.

1. Introduction

Edaravone (MC-186) (**1**) is an acid pyrazoline drug that can exist in three tautomeric forms **a**, **b**, **c** (Figure 1). It was developed in Japan for the treatment of patients in the acute stage of cerebral thrombosis or embolism and has been reported to be effective also in the acute stage of stroke. Edaravone displays potent antioxidant properties, and consequently it is potentially useful for the management of other pathological processes involving oxidative stress (Watanabe et al., 2008). Generally these processes are complex diseases which have to be treated with a cocktail of drugs. An alternative could be the use of polyvalent drugs, namely single products active at more than one target, and consequently, able to display more than one action simultaneously (Morphy et al., 2009). The design of a polyvalent drug is carried out by fusing or by joining through appropriate linkers two drugs, or crucial parts of them. The linker can be susceptible to metabolic cleavage (co-drug), or can be a hard linker (dual drug). Another possibility is to overlap substructures which are common to the two leads. Recently we proposed a new class of polyvalent drugs: the nitric oxide (NO)-donor antioxidants (Cena et al., 2004; Chegaev et al., 2009; Tosco et al., 2008). These compounds were obtained by combining known antioxidants with moieties able to release NO. All products display both antioxidant and NO-dependent activities. They are potentially useful for the treatment of reactive oxygen species (ROS)-related pathologies accompanied by a decreased NO availability. Typical examples of such pathologies are atherosclerosis and related diseases. Among the hybrid products we designed, there is also a series of derivatives obtained by combining edaravone with nitrooxy (ONO_2) or furoxan (1,2,5-oxadiazole 2-oxide) NO-donor moieties through a hard linker (Chegaev et al., 2009).

As an extension of this work, here we describe a new class of co-drugs obtained by joining edaravone through a vulnerable carbonate (**4a**-**c**) or ester (**7a**-**i**) linker with alcohols (**2a-c**) or carboxylic acids (**5a**-**i**) containing ONO_2 functions (Schemes 1 and 2). In contrast with the compounds described above, the antioxidant activity is linked to the edaravone part recovered after the hydrolysis of the vulnerable linker. This difference may lead to different in vivo pharmacokinetic and pharmacodynamic profiles. The synthesis of these products, their stability in water solution and in human serum, their lipophilicity as well as the prediction of their human gastrointestinal permeation using parallel artificial membrane permeability assay (PAMPA), are described. Vasodilator properties of the NO-donor related alcohols and acids are also reported.

2. Materials and methods

2.1 Chemistry

Melting points were measured with a capillary apparatus (Büchi 540). ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance 300 instrument, with $Si(CH_3)_4$ as an internal standard. The following abbreviations were used to indicate the peak multiplicity: s = singlet; d = doublet; t = triplet; q = quartet; qi = quintet; m = multiplet. Low resolution mass spectra were recorded with a Finnigan-Mat TSQ-700 instrument. Flash column chromatography was performed on silica gel (Merck Kieselgel 60, 230-400 mesh ASTM) using the indicated eluents. Petroleum ether 40-60 °C (PE) was used as coeluent. The progress of the reactions was followed by thin layer chromatography (TLC) on 5×20 cm plates with a layer thickness of 0.2 mm. Anhydrous magnesium sulphate was used as a drying agent for the organic phases. Organic solvents were removed under vacuum at 35-40 °C. Analysis (C, H, N) of the new compounds dried at 20 °C, pressure < 10 mmHg for 24 h were performed at the University of Geneva and the results are within ± 0.4% of the theoretical values. Preparative HPLC was performed on a Lichrospher® C_{18} column (250 × 25 mm, 10 μm) (Merck Darmstadt, Germany) with a Varian ProStar mod-210 with Varian UV detector mod-325.

Structures **2a**, **2b** (Kawashima et al., 1993), **2c** (Cena et al., 2008), **5a** (Garvey et al., 2006), **5b** (Lazzarato et al., 2005), **5c** (Kartasasmita et al., 2002), **5f**, **5g** (Lazzarato et al., 2009), **5h** and **5i** (Chegaev et al., 2007) were synthesized according to published methods. Methyl 2,3-dimethylhex-5-enoate (**9**) was obtained using described procedure (Dasse et al., 2000) starting from methyl isobutyrate.

General procedure for preparation of carbonate derivatives 4a-c. A solution of the appropriate alcohol (**2a-c**) (2.5 mmol) and anhydrous pyridine (0.20 mL, 2.5 mmol) in dry CH_2Cl_2 (10 mL) was added dropwise to a stirred solution of triphosgene (0.29 g, 1.0 mmol) in dry CH_2Cl_2 (5 mL) at 0 °C. The reaction mixture was stirred at 0 °C until reaction completion. Organic phase was washed with 1M HCl (10 mL), brine and dried. The CH_2Cl_2 solution of the crude cloroformate (**3a-c**) thus obtained was added dropwise to a solution of **1** (0.45 g, 2.6 mmol) and Et_3N (0.36 mL, 2.6 mmol) in dry CH_2Cl_2 (20 mL) at 0 °C. The reaction mixture was stirred at r.t. for 1 h, then it was washed with H_2O (20 mL), 0.1M HCl (50 mL), brine, dried and the solvent was evaporated. The product was purified by flash chromatography using the indicated eluents. Solid substances were further purified by crystallization from the reported solvents.

3-Methyl-1-phenyl-1H-pyrazol-5-yl 3-(nitrooxy)propyl carbonate (4a): eluent PE/EtOAc 8/2 v/v; yellow oil which solidified in freezer. The obtained solid was crystallized from i-Pr_2O to give the title compound as a white solid. Yield 51%. Mp 42-43 °C. ^{1}H NMR ($CDCl_3$) δ: 2.11 (qi, 2H, CH_2), 2.33 (s, 3H, CH_3), 4.33 (t, 2H, CH_2), 4.51 (t, 2H, CH_2), 6.10 (s, 1H, CH), 7.30-7.35 (m, 1H),

7.42-7.47 (m, 2H), 7.54-7.57 (m, 2H) (C_6H_5); ^{13}C NMR ($CDCl_3$) δ: 14.5, 26.2, 65.5, 69.0, 95.4, 122.9, 127.3, 129.2, 137.8, 144.4, 149.0, 150.7. CI-MS: 322 $(M+1)^+$. Anal. calcd for $C_{14}H_{15}N_3O_6$: C 52.34, H 4.70, N 13.08. Found: C 52.36, H 4.73, N 13.12.

3-Methyl-1-phenyl-1H-pyrazol-5-yl 6-(nitrooxy)hexyl carbonate (4b): eluent PE/EtOAc 8/2 v/v; yellow oil which solidified in freezer. The obtained solid was crystallized from hexane to give the title compound as a white solid. Yield 60%. Mp 39-40 °C. ^{1}H NMR ($CDCl_3$) δ: 1.36-1.42 (m, 4H, $2CH_2$), 1.66-1.74 (m, 4H, $2CH_2$), 2.32 (s, 3H, CH_3), 4.22 (t, 2H, CH_2), 4.43 (t, 2H, CH_2), 6.09 (s, 1H, CH), 7.29-7.34 (m, 1H), 7.41-7.46 (m, 2H), 7.55-7.58 (m, 2H) (C_6H_5); ^{13}C NMR ($CDCl_3$) δ: 14.5, 25.1, 25.3, 26.6, 28.2, 69.5, 73.0, 95.4, 122.9, 127.2, 129.1, 137.9, 144.6, 148.9, 151.0. CI-MS: 363 $(M+1)^+$. Anal. calcd for $C_{17}H_{21}N_3O_6$: C 56.19, H 5.82, N 11.56. Found: C 56.23, H 5.86, N 11.58.

5,6-Bis(nitrooxy)hexyl 3-methyl-1-phenyl-1H-pyrazol-5-yl carbonate (4c): eluent PE/EtOAc 8/2 v/v; yellow oil. Yield 64%. ^{1}H NMR ($CDCl_3$) δ: 1.43-1.56 (m, 2H, CH_2), 1.68-1.79 (m, 4H, $2CH_2$), 2.32 (s, 3H, CH_3), 4.23 (t, 2H, CH_2), 4.44 (dd, 1H, CHH), 4.71 (dd, 1H, CHH), 5.23-5.27 (m, 1H, CH), 6.09 (s, 1H, CH), 7.29-7.35 (m, 1H), 7.41-7.47 (m, 2H), 7.54-7.58 (m, 2H) (C_6H_5); ^{13}C NMR ($CDCl_3$) δ: 14.5, 21.3, 28.0, 28.9, 68.9, 71.0, 78.7, 95.4, 122.9, 127.2, 129.1, 137.9, 144.5, 148.9, 150.9. EI-MS: 424 $(M)^+$. Anal. calcd for $C_{17}H_{20}N_4O_9$: C 48.12, H 4.75, N 13.20. Found: C 47.9, H 4.80, N 13.09.

General procedure for preparation of ester derivatives 7a-i. To a solution/suspension of the appropriate acid (**5a-i**) (3.5 mmol) in dry toluene (5 mL) anhydrous DMF (2 drops) was added, followed by $SOCl_2$ (0.3 mL, 4.2 mmol). The reaction mixture was stirred at r.t. until reaction completion (monitored by TLC). Solvent was evaporated and the obtained acylchloride (**6a-i**) was dissolved in dry CH_2Cl_2 (15 mL), then added dropwise to a solution of edaravone (0.55 g, 3.2 mmol) and Et_3N (0.50 mL, 3.2 mmol) in dry CH_2Cl_2 (20 mL) at 0 °C. The reaction mixture was stirred at r.t. for 1 h, then it was washed with H_2O (20 mL), 0.1M HCl (50 mL), brine, dried and the solvent was evaporated. The product was purified by flash chromatography with the indicated eluent. Solid substances were further purified by crystallization.

3-Methyl-1-phenyl-1H-pyrazol-5-yl 6-(nitrooxy)hexanoate (7a): eluent PE/EtOAc 9/1 v/v; yellow oil which solidified in freezer. The obtained solid was crystallized from hexane to give the title compound as a white solid. Yield 45%. Mp 39-40 °C. ^{1}H NMR ($CDCl_3$) δ: 1.34-1.44 (m, 2H, CH_2), 1.64-1.75 (m, 4H, $2CH_2$), 2.32 (s, 3H, CH_3), 2.52 (t, 2H, CH_2), 4.39 (t, 2H, CH_2) 6.08 (s, 1H, CH), 7.29-7.34 (m, 1H), 7.40-7.46 (m, 2H), 7.50-7.53 (m, 2H) (C_6H_5); ^{13}C NMR ($CDCl_3$) δ: 14.5, 24.0, 25.0, 26.4, 33.7, 72.8, 95.8, 123.3, 127.2, 129.1, 138.0, 144.3, 149.0, 168.6. EI-MS: 333 $(M)^+$. Anal. calcd for $C_{16}H_{19}N_3O_5$: C 57.65, H 5.74, N 12.60. Found: C 57.56, H 5.66, N 12.56.

3-Methyl-1-phenyl-1H-pyrazol-5-yl 5,6-bis(nitrooxy)hexanoate (7b): eluent PE/EtOAc 8/2 v/v; yellow oil. Yield 75%. ^{1}H NMR ($CDCl_3$) δ: 1.68-1.82 (m, 4H, $2CH_2$), 2.32 (s, 3H, CH_3), 2.56-2.60 (m, 2H, CH_2), 4.40 (dd, 1H, CHH), 4.67 (dd, 1H, CHH), 5.21-5.22 (m, 1H, CH), 6.08 (s, 1H, CH), 7.35-7.36 (m, 1H), 7.41-7.52 (m, 4H) (C_6H_5); ^{13}C NMR ($CDCl_3$) δ: 14.5, 19.9, 28.4, 33.1, 70.9, 78.5, 95.8, 123.3, 127.4, 129.1, 138.0, 144.0, 149.0, 168.1. EI-MS: 394 $(M)^+$. Anal. calcd for $C_{16}H_{18}N_4O_8$: C 48.73, H 4.60, N 14.21. Found: C 48.74, H 4.70, N 14.16.

3-Methyl-1-phenyl-1H-pyrazol-5-yl 2,2-dimethyl-3-(nitrooxy)propanoate (7c): eluent PE/EtOAc 9/1 v/v; yellow oil which solidified on standing. The obtained solid was crystallized from hexane to give the title compound as a white solid. Yield 92%. Mp 35.5-36.0 °C. ^{1}H NMR ($CDCl_3$) δ: 1.33 (s, 6H, $2CH_3$), 2.33 (s, 3H, CH_3), 4.49 (s, CH_2), 6.10 (s, 1H, CH), 7.34-7.48 (m, 5H) (C_6H_5); ^{13}C NMR ($CDCl_3$) δ: 14.5, 22.2, 42.5, 76.8, 95.7, 123.7, 127.7, 129.0, 137.7, 143.9, 149.0, 170.0. EI-MS: 319 $(M)^+$. Anal. calcd for $C_{15}H_{17}N_3O_5$: C 56.42, H 5.37, N 13.16. found: C 56.40, H 5.44, N 12.99.

3-Methyl-1-phenyl-1H-pyrazol-5-yl 2,2-dimethyl-6-(nitrooxy)hexanoate (7d): eluent PE/EtOAc 9/1 v/v; yellow oil which solidified on standing. The obtained solid was crystallized from hexane to give the title compound as a white solid. Yield 90%. Mp 51.0-51.5 °C. ^{1}H NMR ($CDCl_3$) δ: 1.17-1.28 (m, 8H, $2CH_3$, CH_2), 1.54-1.63 (m, 4H, $2CH_2$), 2.33 (s, 3H, CH_3), 4.32 (t, 2H, CH_2), 6.06 (s, 1H, CH), 7.30-7.52 (m, 5H) (C_6H_5); ^{13}C NMR ($CDCl_3$) δ: 14.5, 21.2, 24.9, 27.0, 39.8, 42.7, 72.8, 95.6, 123.8, 127.4, 128.9, 137.9, 144.5, 149.0, 173.0. EI-MS: 361 $(M)^+$. Anal. calcd for $C_{18}H_{23}N_3O_5$: C 59.82, H 6.41, N 11.63. Found: C 59.95, H 6.41, N 11.65.

3-Methyl-1-phenyl-1H-pyrazol-5-yl 2,2-dimethyl-5,6-bis(nitrooxy)hexanoate (7e): eluent PE/EtOAc 85/15 v/v. Obtained oil was further purified by reverse phase flash chromatography (RP-18, eluent MeCN/H_2O 6/4 v/v); yellow oil. Yield 45%. ^{1}H NMR ($CDCl_3$) δ: 1.25 (s, 3H, CH_3), 1.27 (s, 3H, CH_3), 1.45-1.70 (m, 4H, $2CH_2$), 2.33 (s, 3H, CH_3), 4.31 (dd, 1H, CHH), 4.58 (dd, 1H, CHH), 5.06-5.10 (m, 1H, CH), 6.07 (s, 1H, CH), 7.32-7.51 (m, 5H) (C_6H_5); ^{13}C NMR ($CDCl_3$) δ: 14.5, 24.5, 24.9, 25.3, 35.2, 42.4, 70.9, 78.8, 95.6, 124.0, 127.7, 129.0, 137.8, 144.3, 149.1, 172.5. EI-MS: 422 $(M)^+$. Anal. calcd for $C_{18}H_{22}N_4O_8$: C 51.18, H 5.25, N 13.26. Found: C 51.01, H 5.24, N 13.12.

3-Methyl-1-phenyl-1H-pyrazol-5-yl 4-[3-(nitrooxy)propyl]benzoate (7f): eluent PE/EtOAc 8/2 v/v. The obtained oil was further purified by preparative HPLC (eluent MeCN/H_2O 7/3 v/v); colorless oil which solidified in freezer. Yield 79%. The obtained solid was crystallized from hexane to give the title compound as a white solid. Mp 38.5-39.5 °C. ^{1}H NMR ($CDCl_3$) δ: 2.06-2.11(m, 2H, CH_2), 2.36 (s, 3H, CH_3), 2.82 (t, 2H, CH_2), 4.45 (t, 2H, CH_2), 6.27 (s, 1H, CH), 7.28-7.33 (m, 3H), 7.41-7.46 (m, 2H), 7.58-7.62 (m, 2H), 8.01-8.03 (m, 2H) (C_6H_5 + C_6H_4); ^{13}C NMR

($CDCl_3$) δ: 14.6, 27.9, 31.9, 71.9, 95.8, 123.2, 126.2, 127.2, 128.9, 129.1, 130.8, 138.2, 144.5, 147.3, 149.1, 161.7. EI-MS: 381 $(M)^+$. Anal. calcd for $C_{20}H_{19}N_3O_5$: C 62.98, H 5.02, N 11.02. Found: C 62.99, H 5.02, N 11.04.

3-Methyl-1-phenyl-1H-pyrazol-5-yl 4-[3-(nitrooxy)propoxy]benzoate (7g): eluent PE/EtOAc 8/2 v/v. The obtained oil was further purified by preparative HPLC (eluent MeCN/H_2O 6/4 v/v); colourless oil which solidified in freezer. Yield 79%. The obtained solid was crystallized from hexane to give the title compound as a white solid. Mp 61.5-62.5 °C. ^{1}H NMR ($CDCl_3$) δ: 2.25 (qi, 2H, CH_2), 2.36 (s, 3H, CH_3), 4.14 (t, 2H, CH_2), 4.67 (t, 2H, CH_2), 6.24 (s, 1H, CH), 6.94 (d, 2H), 7.30-7.32 (m, 1H), 7.40-7.45 (m, 2H), 7.58-7.61 (m, 2H), 8.03 (d, 2H) (C_6H_5 + C_6H_4); ^{13}C NMR ($CDCl_3$) δ: 14.6, 26.9, 63.9, 69.6, 95.8, 114.5, 120.6, 123.2, 127.1, 129.1, 132.6, 138.2, 144.6, 149.1, 161.5, 163.2. EI-MS: 397 $(M)^+$. Anal. calcd for $C_{20}H_{19}N_3O_6$: C 60.45, H 4.82, N 10.57. Found: C 60.42, H 4.84, N 10.57.

3-Methyl-1-phenyl-1H-pyrazol-5-yl 4-[2,3-bis(nitrooxy)propyl]benzoate (7h): eluent PE/EtOAc 8/2 v/v. The obtained oil was further purified by preparative HPLC (eluent MeCN/H_2O 7/3 v/v); colourless oil. Yield 64%. ^{1}H NMR ($CDCl_3$) δ: 2.37 (s, 3H, CH_3), 3.11-3.15 (m, 2H, CH_2), 4.45 (dd, 1H, CHH), 4.73 (dd, 1H, CHH), 5.46-5.49 (m, 1H, CH), 6.28 (s, 1H, CH), 7.37-7.47 (m, 5H), 7.58-7.61 (m, 2H), 8.05-8.07 (m, 2H) (C_6H_5 + C_6H_4); ^{13}C NMR ($CDCl_3$) δ: 14.6, 35.7, 70.0, 78.7, 95.8, 123.3, 127.3, 127.6, 129.1, 129.8, 131.1, 138.1, 140.9, 144.3, 149.1, 161.3. EI-MS: 442 $(M)^+$. Anal. calcd for $C_{20}H_{18}N_4O_8$: C 54.30, H 4.10, N 12.66. Found: C 54.32, H 4.06, N 12.58.

3-Methyl-1-phenyl-1H-pyrazol-5-yl 4-[2,3-bis(nitrooxy)propoxy]benzoate (7i): eluent PE/EtOAc 8/2 v/v. Obtained oil was further purified by preparative HPLC (eluent MeCN/H_2O 7/3 v/v); colourless oil. Yield 61%. ^{1}H NMR ($CDCl_3$) δ: 2.36 (s, 3H, CH_3), 4.31 (d, 2H, CH_2), 4.78 (dd, 1H, CHH), 4.92 (dd, 1H, CHH), 5.59-5.65 (m, 1H, CH), 6.25 (s, 1H, CH), 6.97 (d, 2H), 7.26-7.33 (m, H), 7.40-7.45 (m, 2H), 7.57-7.60 (m, 2H) 8.06 (d, 2H) (C_6H_5 + C_6H_4); ^{13}C NMR ($CDCl_3$) δ: 14.6, 64.8, 68.5, 76.3, 95.8, 114.6, 121.7, 123.2, 127.2, 129.1, 132.8, 138.2, 144.5, 149.1, 161.3, 162.0. EI-MS: 458 $(M)^+$. Anal. calcd for $C_{20}H_{18}N_4O_9$: C 52.40, H 3.96, N 12.22. Found: C 52.37, H 4.02, N 12.09.

Methyl 2,2-dimethyl-5,6-bis(nitrooxy)hexanoate (10): to a vigorously stirred solution of **9** (1.0 g, 6.4 mmol) and $AgNO_3$ (3.3 g, 19.0 mmol) in MeCN (20 mL) I_2 (1.6 g, 6.4 mmol) was added in one portion. The reaction mixture was stirred at r.t. until all iodine dissolved and then refluxed for 6 h. The precipitate was filtered, filtrate was poured into H_2O (50 mL) and extracted with EtOAc (100 mL). Organic phase was washed with H_2O (50 mL), brine, dried and solvent was evaporated. The obtained oil was purified by flash chromatography (eluent PE/EtOAc 9/1 v/v) to give the title compound as a colourless liquid. Yield 74%. ^{1}H NMR ($CDCl_3$) δ: 1.21 (m, 6H, $2CH_3$), 1.61-1.74

(m, 4H, $2CH_2$), 3.69 (s, 3H, CH_3), 4.48 (dd, 1H, CHH), 4.75 (dd, 1H, CHH), 5.23-5.26 (m, 1H, CH); ^{13}C NMR ($CDCl_3$) δ: 24.8, 25.2, 25.5, 35.3, 41.9, 52.0, 71.1, 79.3, 177.5. CI-MS: 281 $(M+1)^+$.

2,2-Dimethyl-5,6-bis(nitrooxy)hexanoic acid (5e): a mixture of **10** (1.3 g, 3.7 mmol) in 1,4-dioxane (5 mL), 6M HCl (5 mL) and DMSO (1 mL) was heated at reflux for 6 h. Then the reaction mixture was cooled, poured into H_2O (20 mL) and extracted with EtOAc (25 mL). Organic phase was washed with brine, dried and solvent was evaporated. The obtained oil was purified by flash chromatography (eluent CH_2Cl_2 + 0.1% HCOOH) to give the title compound as a colourless oil which solidified on standing. Yield 36%. Obtained solid was crystallized from hexane/toluene mixture. Mp 87.5–88.5 °C. ^{1}H NMR ($CDCl_3$) δ: 1.24-1.25 (m, 6H, $2CH_3$), 1.65-1.80 (m, 4H, $2CH_2$), 4.49 (dd, 1H, CHH), 4.75 (dd, 1H, CHH), 5.24-5.26 (m, 1H, CH); ^{13}C-NMR ($CDCl_3$) δ: 24.6, 25.1, 25.3, 35.1, 41.7, 71.0, 79.2, 174.7. CI-MS: 267 $(M+1)^+$.

Methyl 6-hydroxy-2,2-dimethylhexanoate (11): to a vigorously stirred mixture of $NaBH_4$ (2.9 g, 76 mmol), 2-methylbut-1-ene (30 mL, 0.28 mol) and dry THF (30 mL) kept under positive N_2 pressure $BF_3{\cdot}Et_2O$ (7.1 mL, 56 mmol) was added dropwise at -10 °C. The reaction mixture was stirred at r.t. for 5h, cooled in an ice-bath and a solution of **9** (0.84 g, 5.6 mmol) was added dropwise. The cooling bath was removed and the reaction mixture was stirred at r.t. overnight. The following day the reaction was cooled at -10 °C and after the sequential addition of H_2O (36 mL), 3M NaOH (36 mL) and H_2O_2 30% (54 mL) it was heated at 40 °C for 2 h. The organic phase was separated and the water phase was extracted with Et_2O (2×75 mL). Combined organic extracts were washed with brine, dried and solvent was evaporated. The obtained oil was purified by flash chromatography (eluent PE/EtOAc 8/2 v/v) to give the title compound as a colourless liquid. Yield 71%. ^{1}H NMR ($CDCl_3$) δ: 1.17 (s, 6H, $2CH_3$), 1.26-1.34 (m, 2H, CH_2), 1.50-1.57 (m, 4H, $2CH_2$), 1.79 (br. s, 1H, OH), 3.62-3.66 (m, 5H, CH_2 + CH_3); ^{13}C NMR ($CDCl_3$) δ: 21.2, 25.2, 33.0, 40.4, 42.3, 51.7, 62.6, 178.6. MS-CI: 175 $(M+1)^+$.

2,2-Dimethyl-6-(nitrooxy)hexanoic acid (5d): KOH (0.59 g, 11 mmol) was added to a solution of **11** (0.66 g, 3.8 mmol) in MeOH (15 mL) and H_2O (15 mL). The reaction mixture was heated at reflux for 6 h, then it was cooled to r.t., poured into H_2O (30 mL) and extracted with Et_2O (2×15 mL). The water phase was then acidified with 6M HCl until pH ≈ 1 and extracted again with Et_2O (2×25 mL). Organic phases were washed with brine, dried and solvent was evaporated. The obtained 6-hydroxy-2,2-dimethylhexanoic acid was used further without any purification.

To vigorously stirred ice-cooled fuming HNO_3 (2 mL), conc. H_2SO_4 (2 mL) was added dropwise at t < 15 °C. To the obtained sulfonitric mixture CH_2Cl_2 (10 mL) was added followed by the dropwise addition of the solution of hydroxyl derivative in CH_2Cl_2 (5 mL) at t < 10 °C. Ice bath was removed and the reaction mixture was stirred at r.t. for 1 h. The organic phase was decanted, washed with

H_2O (2×20 mL), 10% urea solution (15 mL), brine, dried and solvent was evaporated. The obtained oil was purified by flash chromatography (eluent CH_2Cl_2) to give the title compound as a colourless oil. Yield 85%. ^{1}H NMR ($CDCl_3$) δ: 1.20 (s, 6H, $2CH_3$), 1.35-1.46 (m, 2H, CH_2), 1.56-1.61 (m, 2H, CH_2), 1.68-1.77 (m, 2H, CH_2), 4.46 (t, 2H, CH_2); ^{13}C NMR ($CDCl_3$) δ: 21.3, 24.9, 27.2, 49.8, 42.1, 73.1, 184.7. MS-CI: 206 $(M+1)^+$.

2.2 Stability studies

Stability in water solution. A solution of each compound (10 mM) in DMSO was added to water (0.1 M KCl) in order to obtain 5% DMSO (v/v) in the mixture. The suspension was filtered using 4 mm, 0.2 μm nylon filters (Titan), obtaining the solution used in the experiment. The resulting solution was maintained at r.t., and at appropriate time intervals, a 100 μL aliquot of reaction mixture was analyzed by RP-HPLC. The reverse-phase HPLC procedure allowed for the separation and quantitation of the compounds and hydrolysis products. HPLC analyses were performed with a Hitachi Elite LaChrom equipped with a L-2130 pump, a L-2200 autosampler, a L-2400 UV detector, a L-7614 degasser and a Gynkotek STH585 oven. Data were analyzed using EZChromElite v. 3.1.7. The analytical column was a Zorbax Extend C18 (150 × 4.6 mm, 5 μm particle size). The mobile phase consisted of methanol/water at 1.0 mL min^{-1} under gradient conditions: 70% methanol until 2.5 min, from 70 to 80% methanol between 2.5 and 5.0 min, 80% methanol for next 3 min and from 80 to 70% methanol between 8 and 12 min. The injection volume was 100 μL. The column effluent was monitored at 240 nm referenced against a 600 nm wavelength. Quantitation was performed by a comparison of peak areas with standards chromatographed under the same conditions.

Stability in human serum. A solution of each compound (10 mM) in acetonitrile was added to human serum (from human male AB plasma, Sigma) preheated at 37 °C. The final concentration of the compound was 250 μM. The resulting solution was incubated at 37 ± 0.5 °C and, at appropriate time intervals, a 300 μL aliquot of the reaction mixture was withdrawn and added to 450 μL of acetonitrile containing 0.1% trifluoroacetic acid in order to deproteinize the serum. The sample was sonicated, vortexed, and then centrifuged for 10 min at 2150g. The clear supernatant was filtered by 0.45 μm PTFE filters (Alltech) and analyzed by RP-HPLC. HPLC analyses were performed with a HP1100 chromatograph system (Agilent Technologies, Palo Alto, CA, USA) equipped with a quaternary pump (model G1311A), a membrane degasser (model G1379A), and a diode-array detector (DAD) (model G1315B). Data analysis was accomplished using a HP ChemStation system (Agilent Technologies). The analytical column was a Zorbax Eclipse C18 (150 × 4.6 mm, 5 μm particle size). The mobile phase consisted of acetonitrile/water at 1.2 mL min^{-1} under gradient

conditions: 40% acetonitrile until 2.0 min, from 40 to 70% acetonitrile between 2.0 and 5.0 min, 70% acetonitrile for the next 5 min and from 70 to 40% acetonitrile between 10 and 12 min. The injection volume was 20 μL (Rheodyne, Cotati, CA). The column effluent was monitored at 240 nm referenced against a 600 nm wavelength. Quantitation was performed by a comparison of peak areas with standards chromatographed under the same conditions.

2.3 Lipophilicity studies

The log P_{oct} of all the compounds was obtained by a RP-HPLC method using the HP1100 chromatograph system described in the "human serum stability" section. Retention time measurements were performed on a Discovery RP-amide-C16 column (150 × 4.6 mm i.d., 5 mm; Supelco, Bellefonte, PA, USA) thermostated at 30 °C. The flow rate was 1.0 mL min^{-1}. The column effluent was monitored at 226 and 240 nm referenced against a 600 nm wavelength. Each analysis was performed isocratically using pH 7.0 phosphate buffer and methanol mixtures in proportions varying from 30 to 70% (v/v), with final ionic strength of 20 mM. Before use, the mobile phase was filtered under vacuum through a 0.45 μm HA Millipore filter (Millipore, Milford, MA, USA). Stock solutions (10 mM) of compounds were prepared in methanol and diluted (1–0.1 mM) in the mobile phase for injection (20 μL). All samples were injected at least three times for each mobile phase. Uracil was used as the non-retained compound. The logarithms of the capacity factor (log k) for a minimum of four different methanol/buffer ratios were measured for each compound. Log k_w, namely the logarithm of the capacity factor corresponding to 0% methanol modifier, was obtained by linear extrapolation ($r^2 > 0.99$, for all the compounds). Log P_{oct} values of edaravone and derivatives were determined using the reference line obtained from the correlation between log P_{oct} and log k_w of 63 known structurally diverse compounds (Tosco et al., 2008).

2.4 Permeability studies

Permeation experiments were carried out in 96-well microtiter polycarbonate filter plates obtained from Millipore AG (MPC4NTR10, Volketswil, Switzerland). Polycarbonate filter specifications were as follows: 0.45 μm pore size, 10 μm thickness, and 5-20% porosity. An average porosity value of 12.5% was used for permeability calculations. Each well of the filter plate was impregnated with 15 μL of 5% hexadecane dissolved in hexane and shaken for at least 10 min to achieve complete evaporation of the hexane. Subsequently, the donor compartments were hydrated with 280 μL of solution (prepared as described in the "water solution stability" section) of test compound in water, containing 5% DMSO and 100 mM KCl, and placed upon a Teflon acceptor plate (MSSACCEPTOR, Millipore, Volketswil, Switzerland), which had been prefilled with water

containing 5% DMSO and 100 mM KCl. The resulting sandwich was incubated at room temperature under constant light shaking (150 rpm). After appropriate time intervals (varying between 1 and 6h), the sandwich was disassembled and a 100 μL aliquot of solutions in the acceptor and donor compartment were analyzed by RP-HPLC. Analyses were performed using the RP-HPLC method and Hitachi EliteLaChrom system described in the "water solution stability" section.

2.5 Vasodilation Studies

Thoracic aortas were isolated from male Wistar rats weighing 180-200 g. As few animals as possible were used. The purposes and the protocols of our studies have been approved by Ministero della Salute, Rome, Italy. The endothelium was removed, the vessels were helically cut, and three strips were obtained from each aorta. The tissues were mounted under 1.0 g tension in organ baths containing 30 mL of Krebs-bicarbonate buffer with the following composition (mM): NaCl 111.2, KCl 5.0, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.0, $NaHCO_3$ 12.0, glucose 11.1, maintained at 37 °C and gassed with 95% O_2 -5% CO_2 (pH = 7.4). The aortic strips were allowed to equilibrate for 1.5 h and then contracted with 1 μM L-phenylephrine. When the response to the agonist reached a plateau, cumulative concentrations of the vasodilator agent were added. Results are expressed as EC_{50} (± SEM) μM. The effects of 1 μM ODQ on relaxation were evaluated in a separate series of experiments in which it was added 5 min before the contraction. With this protocol, the inhibitor is preincubated for at least 30 min before the addition of the vasodilator compound. Responses were recorded by an isometric transducer connected to the MacLab System PowerLab. Addition of the drug vehicle, DMSO, had no appreciable effect on contraction level. At least five experiments for each compound were performed.

3. Results and discussion

3.1 Chemistry

The carbonates **4a**-**c** were obtained according to the procedure reported in Scheme 1. The already known NO donor alcohols **2a**-**c** (see Experimental Section) were transformed into the corresponding chloroformates **3a**-**c** by action of triphosgene in dichloromethane, in the presence of pyridine. Crude compounds thus obtained were immediately treated with edaravone in dichloromethane solution in the presence of triethylamine, to give the expected final carbonates as principal reaction products. The assigned pyrazole structures were confirmed by NMR spectroscopy. Indeed, ^{13}C NMR and ^{1}H NMR spectra of the compounds show a signal occurring at

δ 95.6-95.8, attributable to the 4-C aromatic carbon, and a signal occurring at δ 6.09 or 6.10, attributable to 4-CH methyne proton. Additional evidence of the assigned structures derives from the capacity of the products to undergo hydrolysis affording edaravone. Synthesis of the final esters **7a**-**i** required the preliminary preparation of the NO-donor acids **5a**-**i** (Scheme 2). Compounds **5a**-**c** and **5f,g** are products known in literature (see Experimental section), while acids **5d,e** were obtained according to the pathway reported in Scheme 3. Methylisobutyrate (**8**) was alkylated by action of 4-bromobut-2-ene in the presence of lithium diisopropylamide, prepared in situ. The obtained methyl 2,3-dimethylhex-5-enoate (**9**) was treated with $AgNO_3$ and I_2 in CH_3CN to give dinitrooxy substituted ester **10**. Finally, acid hydrolysis of this ester afforded the corresponding acid **5e**. Hydroboration of the unsaturated ester **9** by action of diisoamylborane, prepared in situ, followed by oxidation with hydrogen peroxide, gave **11**. Basic hydrolysis of this intermediate followed by the action of sulphonitric mixture in dichloromethane afforded the corresponding final nitrooxy substituted acid **5d**.

NO-donor acids **5a**-**i** were transformed into the corresponding acyl chlorides **6a**-**i** by action of thionyl chloride in toluene, in the presence of a catalytic amount of DMF (Scheme 2). These intermediates were not purified and characterised but immediately transformed into the desired products **7a**-**i** by reaction with edaravone, in the presence of triethylamine. The pyrazole structures assigned to these final compounds were confirmed by NMR spectroscopy. Indeed, ^{13}C NMR and ^{1}H NMR spectra of the products show a signal occurring in the range δ 95.6-95.8, attributable to the 4-C aromatic carbon, and a signal occurring in the range δ 6.06-6.28 attributable to 4-CH methyne proton. Also for this class of products additional evidence of the assigned structures derives from their capacity to undergo hydrolysis yielding edaravone.

3.2 Stability studies

The stabilities of the co-drugs object of the present work were assessed by high-performance liquid chromatography (HPLC), in human serum and in water. In both media the products underwent hydrolysis following pseudo-first-order kinetics, affording edaravone and the related nitrooxy substituted alcohol and acid moieties. The observed pseudo-first-order rate constants (k_{obs}) and the half-lives ($t_{1/2}$, Table 1) were determined by fitting the data with one phase exponential decay equation (Graph Pad, Prism software vers. 5).

Analysis of the table shows that half-lives are quite different in the two media used. The greatest stabilities occur in water/DMSO 5% (v/v). In this medium $t_{1/2}$ values of the three tested carbonates lie in the range 25 to 6 hours and rank the order **4b > 4a > 4c**, indicating that the stability of the products increases moving from propyl to hexyl chain bearing at the ω-position the nitrooxy

function and decreases when on the hexyl chain are present two of these groups at ω- and ω-1 positions. The two esters **7a**,**b** are slowly hydrolyzed and again the dinitrooxy compound **7b** is less stable than its monosubstituted analogue **7a**. The introduction of two methyl groups at *α*-position of the ester function gives rise to products which display high $t_{1/2}$ values according to the order **7c** > **7d** > **7e.** The low water solubility prevented us from studying the aromatic esters **7f**-**i**. By contrast, all the products are quickly metabolized in serum, in which the hydrolysis of a variety of esters is catalysed by carboxylesterases. These enzymes are ubiquitous and display broad substrate specificity. Frequently the same ester can be hydrolyzed by more than one of these enzymes for which a precise classification so far remains an unsolved problem (Buchwald, 2001). Humans express carboxylesterases in several compartments including liver, plasma, small intestine, brain, stomach, colon macrophages, and monocytes (Satoh et al., 1998). Analysis of the table shows that carbonates and unbranched aliphatic esters display $t_{1/2}$ values < 1 min, while these values for branched aliphatic esters and aromatic esters lie in the range 1.9 min to 16.2 min.

3.3 Lipophilicity studies

The lipophilicity (log P_{oct}) of the new products (Table 2) was determined by a RP-HPLC method (see Experimental section for details). A calibration of the method (Eq. 1) was obtained by plotting log P_{oct} values of 63 reference compounds against the corresponding extrapolated retention log k_w factors (Tosco et al., 2008). This equation was used for the evaluation of log *P* of edaravone and edaravone derivatives from their log k_w factors, measured under the same conditions as the reference compounds.

$$\log P_{oct} = 1.098\ (\pm 0.022) \log k_w + 0.335\ (\pm 0.047) \qquad (1)$$

$$N = 63,\ r^2 = 0.98,\ s = 0.15,\ F = 2531$$

The experimental log P_s, of edaravone derivatives are widely modulated across the series (from 3.35 to 5.14) and are higher compared to the parent compound (edaravone, log P_{oct} = 1.44). Most of them are in accordance with the calculated values (CLOGP, Bio-Loom for Windows v. 1.5, BioByte Corp., Claremont, CA, U.S.A) within ± 0.4, other exceed this range slightly.

3.4 Permeability studies.

PAMPA assay is a recent procedure developed for rapid determination of passive transport permeability that is widely accepted in pharmaceutical research. In this method, the donor compartment, containing a buffer solution of the tested compound, and the acceptor compartment, containing an initial fresh buffer solution, are separated by a 96-well filter plate coated with a liquid artificial membrane. To predict gastrointestinal absorption, phospholipids (Kansy et al., 1998) or

hexadecane (Wohnsland and Faller, 2001) were proposed as artificial membrane. Although the PAMPA-HDM (hexadecane membrane) does not contain phospholipids, the formation of a bilayer membrane has never been characterized in the phospholipid-based PAMPA assay. As a consequence PAMPA-HDM was used as a simpler and more robust alternative to predict GI permeation.

Equations used to calculate effective permeability P_e can be deduced in several ways according to experimental conditions and to the design of the in vitro assay. When retention cannot be neglected, no sink conditions in acceptor wells are fixed and compounds degrade following first-order kinetics under experimental conditions, thus the effective permeability coefficients P_e (cm/s) can be calculated using the following equation (see Supporting information for details):

$$P_e = -\frac{2.303 V_d}{(1+r_v)(t-\tau_{Lag})A} \log_{10}\left(1-\frac{(1+r_v^{-1})}{(1-R)e^{-kt}}\frac{C_{a(t)}}{C_{d(0)}}\right) \qquad (2)$$

where $r_v = V_d/V_a$, A (cm^2) is the filter area, t is the incubation time (s), τ_{lag} is the steady-state time (s), namely the time needed for the permeant's concentration gradient to became stabilised, V_a and V_d (cm^3) are the volumes in the acceptor and the donor wells respectively, $C_{a(t)}$ is the concentration of the compound (mol cm^{-3}) in the acceptor well at time t, $C_{d(0)}$ is the concentration of the compound (mol cm^{-3}) in the donor well at time 0 and k the pseudo-first-order rate constant of the hydrolysis. R is the retention factor defined as the mole fraction of compound that is lost in the membrane and in the microplates (filters and plate material) and calculated according to equation 3:

$$R = 1 - \frac{C_{d(t)}}{C_{d(0)}e^{-kt}} - \frac{V_a C_{a(t)}}{V_d C_{d(0)} e^{-kt}} \qquad (3)$$

where $C_{a(t)}/C_{d(0)}$ represents the fraction of compound that reached the acceptor compartment after the incubation time *t* (for $V_a = V_d$).

PAMPA experiments were carried out on edaravone and its new derivatives using filters impregnated with hexadecane to evaluate the gastrointestinal passive permeability of these new compounds (PAMPA-HDM experiments). The values of log P_e and R obtained using equations 2 and 3 are collected in Table 2 together with the human gastrointestinal absorption (A%) predicted from the reference sigmoid described in the work of Wohnsland et al (Wohnsland and Faller, 2001). Indeed, the authors described a quite good sigmoidal correlation for a set of 32 chemically diverse drugs between their log P_e measured by PAMPA-HDM and human absorption. The predicted absorption through gastrointestinal tract for these new edaravone NO-donor co-drugs (Table 2) varies in the series of compounds studied as illustrated by A% values of compounds (near 100% for compounds **4a**, **7b**, **7c**, in the range 60-85% for **4c**, **7d**, **7e**, 34% for **4b** and 14% for **7a**).

The retention factors R are quite high for most derivatives. However, since P_e is an "effective" value of permeation it does not depend on loss of compound due to membrane retention or adsorption processes. Figure 2A highlights that the mechanisms governing gastrointestinal permeation (A%) and the membrane retention (R) processes are different for this series of derivatives. In particular, the permeation appears to be diminished for compounds **4b**, **7a** and in a lesser extent for compound **4c**, while their retention factor remains high. Several molecular determinants may be responsible for these differences. The predicted intestinal absorption (A%) appears to be limited by an increasing number of free CH_2 groups (Figure 2B). By contrast the retention is linearly correlated with the number of free CH_2 groups for most compounds, but not for **7c**, **7d** and **7e** (Figure 2C), probably due to their steric hindrance caused by branching. The high retention of most compounds with respect to edaravone can be partly due to adsorption processes on the plastic material of filter-microplates. In fact, it is known that molecules bearing nitrooxy groups can be adsorbed on plastic materials (Cossum et al., 1978). However, the variation of retention factors was not correlated with the number of ONO_2 substituents as shown on Figure 2A and 2B where black symbols correspond to compounds having a unique ONO_2 group, and grey symbols to compounds having two ONO_2 groups.

In addition, no clear relationship exists between these permeation parameters and the related log P_{oct} measured by HPLC technique (Figure 3A and 3B). Globally intestinal absorption decreases and membrane retention increases with lipophilicity. However a number of compounds deviate from this behaviour, suggesting that the intestinal absorption is lowered by the presence of long linear hydrophobic CH_2 chains (compounds **4b** and **7a**). By contrast, the retention is lower for compounds with small substituents (compounds **4a** and **7b)**, and for compound **4c** having a borderline complex permeation profile. Additional studies would be necessary to confirm this preliminary picture.

3.5 Vasodilation studies.

The vasodilator activity of the NO-donor nitrooxy substituted alcohols and acids which are quickly generated when the related edaravone co-drugs are incubated in human serum, was evaluated on denuded rat aorta strips precontracted with phenylephrine. All the products were able to relax the contracted tissue in a concentration-dependent manner. Their potencies, expressed as EC_{50}, are collected in Table 3. The dinitrooxy substituted compounds **5b**, **5h**, **5i**, **2c** appear to be more potent than the monosubstituted analogues **5a**, **5f**, **5g** and **2b**, respectively. Alcohols **2b** and **2c** are better vasodilators than the structurally related acids **5a** and **5b**, and this might be partly due to their greater ease to penetrate into smooth muscle cells, as a consequence of their higher lipophilicity. When the experiments were repeated in the presence of 1 μM ODQ (1H-[1,2,4]oxadiazolo[4,3-

a]quinoxalin-1-one), a decrease in the potencies was observed, in keeping with a NO-induced activation of the sGC as mechanism which underlies the vasodilator effect.

4. Conclusion

In conclusion we were able to prepare a new series of NO-donor edaravone co-drugs by joining edaravone through a vulnerable carbonate or ester linker with alcohols or carboxylic acids containing NO-donor nitrooxy functions. These products display widely modulated lipophilicity and quickly afford edaravone and the related NO-donor hydrolysis products when incubated in human serum. PAMPA studies predict for a number of these compounds a very good gastrointestinal absorption. The related NO-donor alcohols and acids used for designing these products are able to relax rat aorta strips precontracted with phenylephrine in a concentration-dependent manner. The described co-drugs represent a new class of polyvalent agents which combine edaravone dependent activities with the NO-dependent effects of the nitrates. They are interesting tools potentially useful for the treatment of ROS-related pathologies accompanied by decreased NO availability.

Acknowledgement

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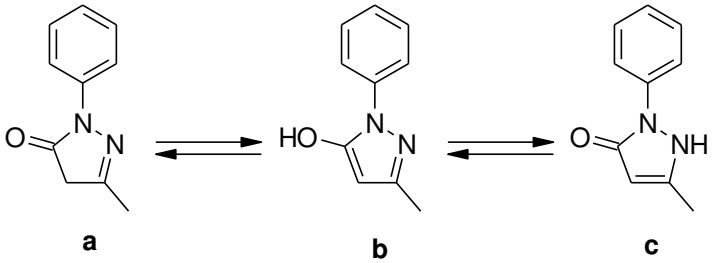
Captions to figures

Figure 1. Edaravone and its tautomeric forms.

Figure 2. (A) Relationship between predicted absorption (A%) from PAMPA experiments and membrane retention (R). (B) Relationship between predicted absorption (A%) and number of CH_2 groups. (C) Relationship between membrane retention factor (R) and number of CH_2 groups. ● Compounds with up to three free unsubstituted CH_2 groups; ▲ compounds with four or more free unsubstituted CH_2 groups; ○ compounds with substituted CH_2 groups. Black symbols correspond to compounds having a unique ONO_2 group, and grey symbols to compounds having two ONO_2 groups.

Figure 3. Relationships between octanol-water partition coefficients and predicted intestinal absorption (A%) from PAMPA experiments (A) and membrane retention (B). ● Compounds with up to three free unsubstituted CH_2 groups; ▲ compounds with four or more free unsubstituted CH_2 groups; ○ compounds with substituted CH_2 groups.

Figure 1. Edaravone and its tautomeric forms.



a **b** **c**

1

Figure 2. (A) Relationship between predicted absorption (A%) from PAMPA experiments and membrane retention (R). (B) Relationship between predicted absorption (A%) and number of CH_2 groups. (C) Relationship between membrane retention factor (R) and number of CH_2 groups. ● Compounds with up to three free unsubstituted CH_2 groups; ▲ compounds with four or more free unsubstituted CH_2 groups; ○ compounds with substituted CH_2 groups. Black symbols correspond to compounds having a unique ONO_2 group, and grey symbols to compounds having two ONO_2 groups.

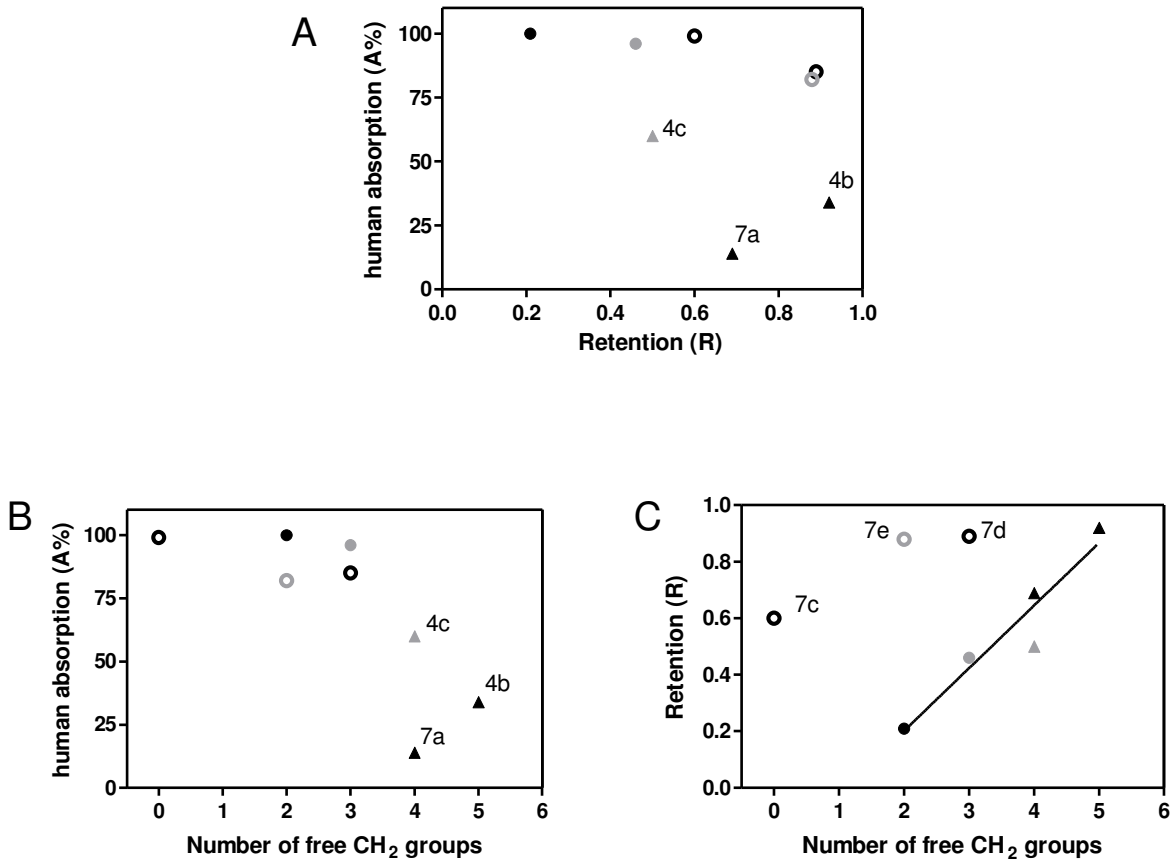


Figure 3. Relationships between octanol-water partition coefficients and predicted intestinal absorption (A%) from PAMPA experiments (A) and membrane retention (B). ● Compounds with up to three free unsubstituted CH_2 groups; ▲ compounds with four or more free unsubstituted CH_2 groups; ○ compounds with substituted CH_2 groups.

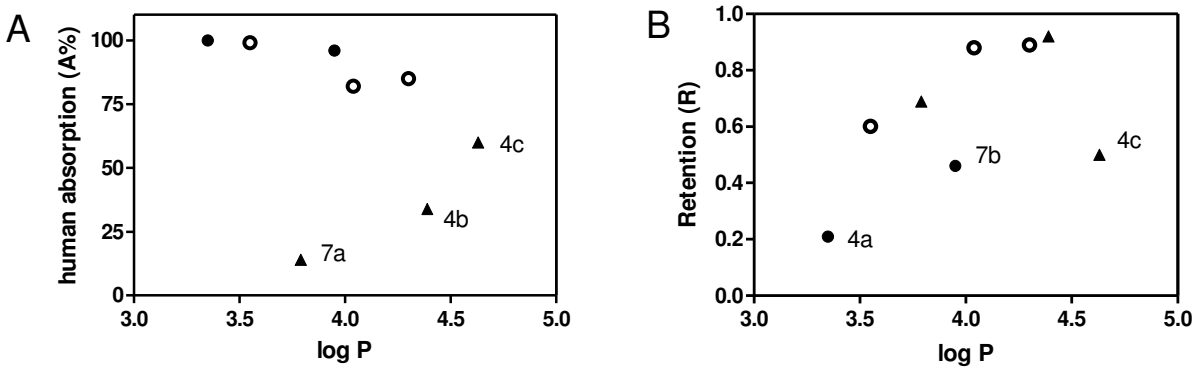


Table 1. Stability in aqueous media (5% DMSO v/v, 0.1 M KCl) and in human serum.

Compound	$t_{1/2}$	
	Water (5% DMSO, 0.1 M KCl)	**Human serum**
edaravone	stable	Stable
4a	13 h	<1 min
4b	25 h	<1 min
4c	6 h	<1 min
7a	31 h	<1 min
7b	10 h	<1 min
7c	36 h	1.9 min
7d	22 h	13.3 min
7e	16 h	16.2 min
7f	a)	4.0 min
7g	a)	5.0 min
7h	a)	7.1 min
7i	a)	7.8 min

a) not determined due to low solubility under the experimental conditions

Table 2. Calculated and measured log P_{oct}, retention factor (R), effective permeability coefficient (log P_e) and human absorption values (A%) predicted by Faller sigmoid curve interpolation.

Compound	CLOGP $_{oct}$ [a)]	log P_{oct} [b)]	PAMPA G.I.		
			R (±SD)	log P_e (±SD)	A%
edaravone	1.33	1.44	0.12 ± 0.00	-3.52 ± 0.01	100
4a	2.85	3.35	0.21 ± 0.03	-3.51 ± 0.05	100
4b	4.15	4.39	0.92 ± 0.04	-4.68 ± 0.24	34
4c	3.96	4.63	0.50 ± 0.01	-4.40 ± 0.08	60
7a	3.84	3.79	0.69 ± 0.08	-4.93 ± 0.10	14
7b	3.65	3.95	0.46 ± 0.01	-3.75 ± 0.08	96
7c	3.36	3.55	0.60 ± 0.05	-3.56 ± 0.05	99
7d	4.55	4.30	0.89 ± 0.02	-4.06 ± 0.12	85
7e	4.36	4.04	0.88 ± 0.04	-4.12 ± 0.15	82
7f	5.41	4.76	c)	c)	c)
7g	5.17	4.74	c)	c)	c)
7h	5.37	4.88	c)	c)	c)
7i	5.26	5.14	c)	c)	c)

a) Bio-Loom for Windows v.1.5, Bio Byte Corp., Claremont, CA, USA;

b) determined by RP-HPLC (S.E. ≤ 0.05);

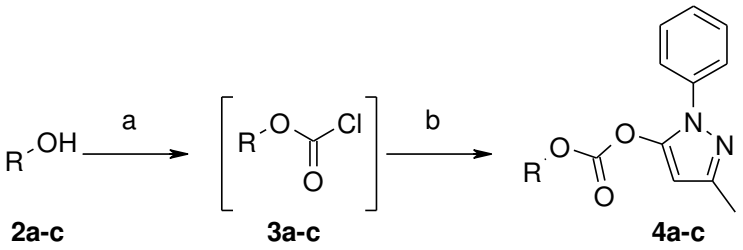
c) not determined due to low solubility under the experimental conditions.

Table 3. Vasodilator activity of nitrooxy substituted alcohols **2a-c** and acids **5a-i** used for the preparation of edaravone co-drugs.

Compounds	EC_{50} (μM) ± SEM	EC_{50} (μM) ± SEM + 1 μM ODQ
2a	4.0 ± 0.6	> 100
2b	1.5 ± 0.4	> 100
2c	0.83 ± 0.17	> 100
5a	8.3 ± 1.5[a)]	> 100
5b	5.8 ± 0.8[a)]	> 100
5c	13 ± 2	> 100
5d	8.9 ± 2.2	> 100
5e	4.2 ± 1.2	> 100
5f	0.51 ± 0.08[a)]	> 100
5g	0.62 ± 0.07[a)]	66 ± 12
5h	0.33 ± 0.06[a)]	> 100
5i	0.28 ± 0.04[a)]	67 ± 6

a) taken from Cena et al., 2008.

Scheme 1. Synthesis of carbonates **4a**-**c**

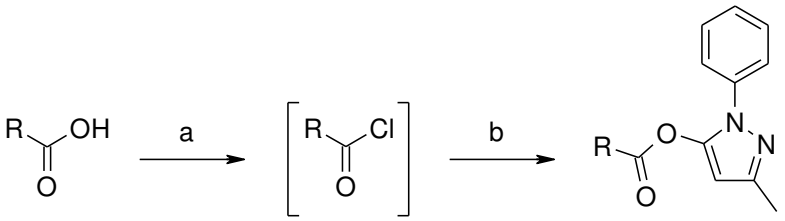


2a-c **3a-c** **4a-c**

a R = $(CH_2)_3ONO_2$
b R = $(CH_2)_6ONO_2$
c R = $(CH_2)_4CH(ONO_2)CH_2ONO_2$

Reaction conditions: a) $(Cl_3CO)_2CO$, Py, CH_2Cl_2, 0 °C to rt; b) edaravone, Et_3N, CH_2Cl_2, 0 °C to rt.

Scheme 2. Synthesis of esters **7a**-**i**

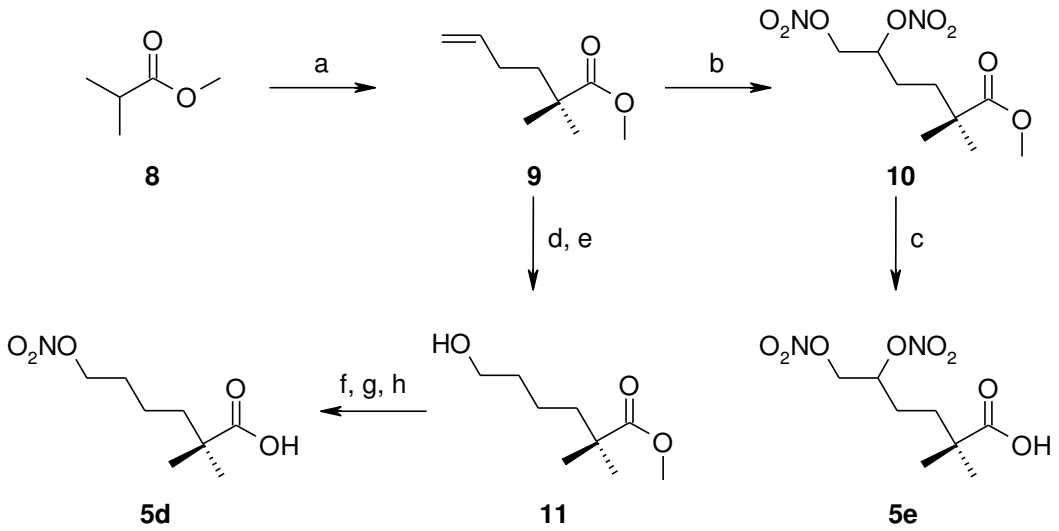


5a-i **6a-i** **7a-i**

a R = $(CH_2)_5ONO_2$
b R = $(CH_2)_3CH(ONO_2)CH_2ONO_2$
c R = $C(CH_3)_2CH_2ONO_2$
d R = $C(CH_3)_2(CH_2)_4ONO_2$
e R = $C(CH_3)_2(CH_2)_2CH(ONO_2)CH_2ONO_2$
f R = *p*-$C_6H_4(CH_2)_3ONO_2$
g R = *p*-$C_6H_4O(CH_2)_3ONO_2$
h R = *p*-$C_6H_4CH_2CH(ONO_2)CH_2ONO_2$
i R = *p*-$C_6H_4OCH_2CH(ONO_2)CH_2ONO_2$

Reaction conditions: a) $SOCl_2$, toluene, DMF, rt; b) edaravone, Et_3N, CH_2Cl_2, 0 °C to rt.

Scheme 3. Synthesis of NO-donor acids **5d,e**



Reaction conditions: a) BuLi, DPA, 4-bromobut-1-en, HMPA, THF dry, -78 °C; b) $AgNO_3$, I_2, CH_3CN, rt, then Δ; c) HCl 6M, dioxane, Δ; d) $NaBH_4$, $BF_3 \cdot Et_2O$, 2-methylbut-1-en, dry THF, 0 °C rt; e) NaOH, H_2O_2, 40 °C f) NaOH, MeOH, Δ; g) HCl; h) HNO_3, H_2SO_4, CH_2Cl_2, 0 °C.

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