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Edaravone derivatives containing NO-donor functions

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SUPPORTING INFORMATION

Edaravone derivatives containing NO-donor functions

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Elemental analyses

| Compound | Formula | Calculated | | | Found | | |
|-----------|---|------------|------|-------|-------|------|-------|
| | | %C | %H | %N | %C | %H | %N |
| 3a | C ₄ H ₉ NO ₄ | 35.56 | 6.71 | 10.37 | 35.47 | 6.68 | 10.35 |
| 3b | C ₇ H ₁₇ NO ₄ | 47.45 | 8.53 | 7.90 | 47.32 | 8.52 | 7.82 |
| 3c | C ₇ H ₁₄ N ₂ O ₇ | 35.30 | 5.92 | 11.76 | 35.19 | 5.81 | 11.80 |
| 4c | C ₅ H ₇ N ₃ O ₄ | 34.69 | 4.07 | 24.27 | 34.83 | 4.08 | 24.25 |
| 4d | C ₅ H ₅ N ₃ O ₃ | 38.72 | 3.25 | 27.09 | 38.52 | 3.41 | 26.94 |
| 9a | C ₁₃ H ₁₅ N ₃ O ₅ | 53.24 | 5.16 | 14.33 | 53.04 | 5.22 | 14.06 |
| 9b | C ₁₆ H ₂₁ N ₃ O ₅ | 57.30 | 6.31 | 12.53 | 57.66 | 6.32 | 12.52 |
| 9c | C ₁₆ H ₂₀ N ₄ O ₈ | 48.49 | 5.09 | 14.14 | 48.38 | 5.19 | 13.69 |
| 9d | C ₂₁ H ₂₀ N ₄ O ₅ | 61.76 | 4.94 | 13.72 | 61.94 | 4.94 | 13.59 |
| 9e | C ₂₁ H ₂₀ N ₄ O ₇ S | 53.38 | 4.27 | 11.86 | 53.16 | 4.27 | 11.65 |
| 9f | C ₁₄ H ₁₃ N ₅ O ₅ | 49.86 | 4.08 | 20.77 | 49.82 | 3.97 | 20.63 |
| 9g | C ₁₄ H ₁₁ N ₅ O ₄ | 53.67 | 3.54 | 22.36 | 53.59 | 3.66 | 22.22 |

Supplementary Experimental Section

Chemistry. Melting points were measured with a capillary apparatus (Büchi 540). All the compounds were routinely checked by ^1H and ^{13}C -NMR (Bruker Avance 300) and mass spectrometry (Finnigan-Mat TSQ-700). The following abbreviations have been used to indicate the peak multiplicity: s = singlet, d = doublet, t = triplet, m = multiplet. Flash column chromatography was performed on silica gel (Merck Kieselgel 60, 230-400 mesh ASTM) using the reported eluents. Thin layer chromatography (TLC) was carried out on 5×20 cm plates with a 0.2 mm layer thickness. Anhydrous magnesium sulfate was used as the drying agent for the organic phases. Analysis (C, H, N) of the target compounds was performed by REDOX (Monza) and the results were within $\pm 0.4\%$ of theoretical values unless otherwise stated. Tetrahydrofuran (THF) was distilled immediately before use from Na and benzophenone. Petroleum ether (PE) 40-70 °C was used. Compounds **4a**,¹ **4b**,² **5**³ were synthesised according to literature. Synthesis of the reference compounds **3a-c**, **4c**, **4d**, **8g**, of intermediates **6** and **7**, spectral characterisation data for intermediates **6**, **7**, **8a-f** and final compounds **9a-g** are reported.

3-Methoxypropyl nitrate (3a). HNO_3 (5.0 mL, 0.12 mol) was added dropwise to H_2SO_4 (5.0 mL, 0.09 mol) keeping reaction temperature below 10 °C (ice-water bath). Subsequently, dry CH_2Cl_2 was added, followed by 3-methoxypropan-1-ol (5.0 mL, 0.05 mol). After removing the cooling bath, the reaction mixture was vigorously stirred at r.t. for 2 h. The organic phase was decanted and washed with H_2O , urea 10% solution, NaHCO_3 sat. sol., brine, dried and evaporated. The crude product was distilled under reduced pressure (66-68 °C, 31 mbar) to give the title product as a colourless volatile liquid; yield 4.0 g (60%); ^1H -NMR (δ , CDCl_3): 1.95-2.03 (m, 2H, CH_2), 3.35 (s, 3H, CH_3), 3.47 (t, 2H, CH_2), 4.56 (t, 2H, CH_2); ^{13}C -NMR (δ , CDCl_3): 27.3, 58.8, 68.2, 70.5. Anal. ($\text{C}_4\text{H}_9\text{NO}_4$) C, H, N.

6-Methoxyhexyl nitrate (3b). A solution of 6-hydroxyhexyl nitrate⁴ (0.40 g, 2.5 mmol) in dry THF (10 mL) was placed under positive N_2 pressure. NaH (0.13 g, 3.3 mmol) was added at 0 °C, then the reaction mixture was vigorously stirred for 15 min; subsequently, methyl iodide (0.6 mL, 9.6 mmol) was added. After stirring at r.t overnight, the reaction mixture was poured into H_2O and extracted with

Et₂O. The organic phase was washed with brine, dried and evaporated. The crude yellow oil was purified by flash chromatography (eluent: 7/3 PE/CH₂Cl₂) to give the title product as a colourless liquid; yield 0.23 g (52%); ¹H-NMR (δ, CDCl₃): 1.37-1.44 (m, 4H, 2CH₂), 1.54-1.63 (m, 2H, CH₂), 1.68-1.78 (m, 2H, CH₂), 3.33 (s, 3H, CH₃), 3.38 (t, 2H, CH₂), 4.45 (t, 2H, CH₂); ¹³C-NMR (δ, CDCl₃): 25.5, 25.8, 26.7, 29.4, 58.6, 72.6, 73.3. Anal. (C₇H₁₅NO₄) C, H, N.

6-Methoxyhexane-1,2-diyl dinitrate (3c). To a solution of 6-methoxyhex-1-ene (0.45 g, 3.9 mmol) in MeCN (30 mL) AgNO₃ (2.0 g, 12 mmol) and I₂ (1.0 g, 3.9 mmol) were added. After vigorous stirring at r.t. for 1 h, the reaction mixture was refluxed for 8 h, then filtered. The filtrate was poured into H₂O and extracted with AcOEt; the organic phase was washed with H₂O, brine, dried and evaporated. The crude oil was purified by flash chromatography (eluent: 1/1 PE/CH₂Cl₂) to give the title product as a yellow liquid; yield 0.46 g (50%); ¹H-NMR (δ, CDCl₃): 1.48-2.81 (m, 6H, 3CH₂), 3.33 (s, 3H, CH₃), 3.39 (t, 2H, CH₂), 4.47 (dd, 1H, CHH), 4.74 (dd, 1H, CHH), 5.26-5.34 (m, 1H, CH); ¹³C-NMR (δ, CDCl₃): 21.9, 29.2, 58.7, 71.3, 72.1, 79.2. Anal. (C₇H₁₄N₂O₇) C, H, N.

4-(Methoxymethyl)furoxan-3-carboxamide (4c). Dry MeOH (20 mL) was placed under positive N₂ pressure and sodium (0.060 g, 2.7 mmol) was added under stirring. After complete dissolution 4-(bromomethyl)furoxan-3-carboxamide⁵ (0.40 g, 1.8 mmol) was added, and stirring was continued for 1 h. The reaction mixture was then concentrated under reduced pressure, poured into H₂O and extracted with AcOEt; the organic phase was washed with H₂O, brine, dried and evaporated. The residue was recrystallised from a *i*PrOH/*n*-hexane mixture to give the title product as a white solid; yield 0.27 g (87%); m.p. 115-117 °C (*i*PrOH/*n*-hexane); ¹H-NMR (δ, DMSO-*d*₆): 3.39 (s, 3H, CH₃), 4.71 (s, 2H, CH₂), 7.80, 8.42 (2s, 2H, NH₂); ¹³C-NMR (δ, DMSO-*d*₆): 58.4, 64.7, 110.4, 155.6, 155.9; MS (CI) *m/z* 174 ([M+H]⁺). Anal. (C₅H₇N₃O₄) C, H, N.

4-(Methoxymethyl)furoxan-3-carbonitrile (4d). To a stirred solution of **4c** (0.15 g, 0.90 mmol) in dry THF (20 mL), dry pyridine (0.22 mL, 2.6 mmol) was added at 0 °C, followed by TFAA (0.37 mL, 2.6 mmol). The cooling bath was removed, and stirring was continued at r.t. for 2 h. The reaction mixture was then concentrated under reduced pressure, poured into H₂O and extracted with AcOEt; the

organic phase was washed with 1 N HCl, NaHCO₃ 10% sol., brine, dried and evaporated. The crude oil was purified by flash chromatography (eluent: 9/1 PE/AcOEt) to give the title product as a colourless oil; yield 0.10 g (75%); ¹H-NMR (δ, DMSO-*d*₆): 3.40 (s, 3H, CH₃), 4.72 (s, 2H, CH₂); ¹³C-NMR (δ, DMSO-*d*₆): 58.6, 63.9, 106.3, 155.9; MS (CI) *m/z* 156 ([M+H]⁺). Anal. (C₅H₅N₃O₃) C, H, N.

***tert*-Butyl 1-(4-benzyloxyphenyl)-3-methyl-1*H*-pyrazol-5-yl carbonate (6).** Boc₂O (1.5 g, 6.9 mmol) was added to a stirred suspension of **5** (1.4 g, 5.0 mmol) in CH₂Cl₂ (30 mL). The reaction mixture was cooled in an ice bath and DMAP (60 mg, 0.5 mmol) was added. After 20 min the ice bath was removed and the reaction stirred at r.t. for 1 h. The reaction mixture was diluted with CH₂Cl₂, then the organic phase was washed with NaHCO₃ sat. sol., brine, dried and evaporated. The obtained solid was recrystallised from MeOH to give the title product as a light-yellow crystalline solid; yield 1.4 g (75%); m.p. 105-106 °C (MeOH); ¹H-NMR (δ, CDCl₃): 1.44 (s, 9H, C(CH₃)₃), 2.30 (s, 3H, CH₃), 5.08 (s, 2H, CH₂), 6.01 (s, 1H, CH), 6.99-7.04 (m, 2H), 7.32-7.47 (m, 7H) (C₆H₅, C₆H₄); ¹³C-NMR (δ, CDCl₃): 14.5, 27.4, 70.2, 85.1, 95.0, 115.2, 127.7, 127.4, 128.0, 128.6, 131.4, 136.7, 144.8, 148.4, 149.0, 157.8; MS (EI) *m/z* 380 (M⁺).

***tert*-Butyl 1-(4-hydroxyphenyl)-3-methyl-1*H*-pyrazol-5-yl carbonate (7).** To a suspension of **6** (6.7 g, 18 mmol) in MeOH (100 mL), Pd/C (0.60 g) was added, then the vigorously stirred reaction mixture was placed under positive H₂ pressure. After 3 h the reaction mixture was filtered through a Celite[®] pad and the solvent was removed under reduced pressure. The obtained solid was dissolved in CH₂Cl₂, then the organic phase was washed with H₂O, NaHCO₃ sat. sol., brine, dried and evaporated. The obtained white solid was desiccated and used without further purification; yield 4.7 g (92%); ¹H-NMR (δ, CDCl₃): 1.46 (s, 9H, C(CH₃)₃), 2.33 (s, 3H, CH₃), 6.05 (s, 1H, CH), 6.68 (d, 2H), 7.21 (d, 2H) (C₆H₄); ¹³C-NMR (δ, CDCl₃): 14.1, 27.4, 85.4, 95.0, 116.0, 125.7, 129.1, 145.2, 148.5, 149.0, 156.9; MS (EI) *m/z* 290 (M⁺).

***tert*-Butyl 3-methyl-1-{4-[3-(nitrooxy)propoxy]phenyl}-1*H*-pyrazol-5-yl carbonate (8a).** Eluent 85/15 PE/AcOEt. Yellow oil; yield 72%. ¹H-NMR (δ, CDCl₃): 1.46 (s, 9H, C(CH₃)₃), 2.21-2.27 (m, 2H,

CH₂CH₂CH₂ONO₂), 2.30 (s, 3H, CH₃), 4.09 (t, 2H, CH₂CH₂CH₂ONO₂), 4.68 (t, 2H, CH₂CH₂CH₂ONO₂), 6.02 (s, 1H, CH), 6.91-6.96 (m, 2H), 7.43-7.48 (d, 2H) (C₆H₄); ¹³C-NMR (δ, CDCl₃): 14.5, 27.0, 27.4, 63.9, 69.9, 85.2, 95.1, 114.8, 124.7, 131.7, 144.8, 148.5, 149.0, 157.5; MS (EI) *m/z* 393 (M⁺).

***tert*-Butyl 3-methyl-1-(4-{{6-(nitrooxy)hexyl}oxy}phenyl)-1*H*-pyrazol-5-yl carbonate (8b).** Eluent 9/1 PE/AcOEt. Yellow oil; yield 81%. ¹H-NMR (δ, CDCl₃): 1.46-1.56 (m, 13H, C(CH₃)₃, 2CH₂), 1.75-1.83 (m, 4H, 2CH₂), 2.31 (s, 3H, CH₃), 3.98 (t, 2H, OCH₂), 4.47 (t, 2H, CH₂ONO₂), 6.02 (s, 1H, CH), 6.91-6.94 (m, 2H), 7.41-7.44 (m, 2H) (C₆H₄); ¹³C-NMR (δ, CDCl₃): 14.5, 25.5, 25.7, 26.7, 27.4, 29.0, 67.9, 73.2, 85.1, 95.0, 114.7, 124.8, 131.0, 144.8, 148.3, 149.0, 158.1; MS (EI) *m/z* 435 (M⁺).

***tert*-Butyl 1-(4-{{5,6-bis(nitrooxy)hexyl}oxy}phenyl)-3-methyl-1*H*-pyrazol-5-yl carbonate (8c).** Eluent 50/45/5 PE/CH₂Cl₂/AcOEt. Yellow oil; yield 76%. ¹H-NMR (δ, CDCl₃): 1.46 (s, 9H, C(CH₃)₃), 1.62-1.72 (m, 2H, CH₂), 1.79-1.87 (m, 2H, CH₂), 2.30 (s, 3H, CH₃), 4.00 (t, 2H, OCH₂), 4.49 (dd, 1H, CHHONO₂), 4.76 (dd, 1H, CHHONO₂), 5.30-5.34 (m, 1H, CHONO₂), 6.02 (s, 1H, CH), 6.91-6.94 (m, 2H), 7.42-7.45 (m, 2H) (C₆H₄); ¹³C-NMR (δ, CDCl₃): 14.5, 21.8, 27.5, 28.8, 29.1, 67.5, 71.1, 79.0, 85.1, 95.0, 114.7, 124.7, 131.4, 144.8, 148.4, 149.1, 159.9; MS (EI) *m/z* 496 (M⁺).

***tert*-Butyl 3-methyl-1-(4-{{3-[(3-phenylfuroxan-4-yl)oxy]propoxy}phenyl)-1*H*-pyrazol-5-yl carbonate (8d).** Eluent 50/45/5 PE/CH₂Cl₂/AcOEt. Yellow oil; yield 83%. ¹H-NMR (δ, CDCl₃): 1.45 (s, 9H, C(CH₃)₃), 2.30 (s, 3H, CH₃), 2.41-2.45 (m, 2H, CH₂), 4.20 (t, 2H, CH₂), 4.73 (t, 2H, CH₂), 6.03 (s, 1H, CH), 6.94-6.97 (m, 2H), 7.42-7.51 (m, 5H), 8.10-8.13 (m, 2H) (C₆H₄, C₆H₅); ¹³C-NMR (δ, CDCl₃): 14.5, 27.4, 28.7, 64.3, 67.7, 85.2, 95.1, 114.7, 122.4, 124.7, 126.1, 129.0, 130.5, 131.5, 144.9, 148.5, 149.0, 157.6, 162.2; MS (EI) *m/z* 508 (M⁺).

***tert*-Butyl 3-methyl-1-(4-{{3-[(3-phenylsulfonylfuroxan-4-yl)oxy]propoxy}phenyl)-1*H*-pyrazol-5-yl carbonate (8e).** Partially purified with eluent 7/3 PE/AcOEt, finally purified with eluent 95/5 CH₂Cl₂/AcOEt. Colourless foam; yield 40%. ¹H-NMR (δ, CDCl₃): 1.45 (s, 9H, C(CH₃)₃), 2.31 (s, 3H, CH₃), 2.33-2.42 (m, 2H, CH₂), 4.20 (t, 2H, CH₂), 4.65 (t, 2H, CH₂), 6.04 (s, 1H, CH), 6.95-7.00 (m,

2H), 7.45-7.55 (m, 4H), 7.67-7.73 (m, 1H), 7.97-8.00 (m, 2H) (C_6H_4 , C_6H_5); ^{13}C -NMR (δ , $CDCl_3$): 14.5, 27.4, 28.4, 63.6, 67.9, 85.2, 95.1, 114.8, 124.7, 128.4, 129.7, 131.5, 135.7, 138.0, 144.8, 148.5, 149.0, 157.6, 158.9; MS (EI) m/z 572 (M^+).

tert-Butyl 1-{4-[(3-carbamoylfuroxan-4-yl)methoxy]phenyl}-3-methyl-1H-pyrazol-5-yl carbonate (8f). Partially purified with eluent 75/35 PE/AcOEt, finally purified with eluent 9/1 CH_2Cl_2 /AcOEt. Glass-like colourless oil; yield 45%. 1H -NMR (δ , $CDCl_3$): 1.46 (s, 9H, $C(CH_3)_3$), 2.30 (s, 3H, CH_3), 5.45 (s, 2H, CH_2), 6.03 (s, 1H, CH), 6.40 (br. s, 1H), 7.06-7.11 (m, 2H), 7.47-7.53 (m, 3H) (C_6H_4 , NH_2); ^{13}C -NMR (δ , $CDCl_3$): 14.5, 27.4, 61.5, 85.3, 95.2, 110.3, 115.4, 124.7, 132.5, 144.8, 148.6, 149.0, 154.6, 155.9, 156.6; MS (EI) m/z 431 (M^+).

tert-Butyl 1-{4-[(3-cyanofuroxan-4-yl)methoxy]phenyl}-3-methyl-1H-pyrazol-5-yl carbonate (8g). To the solution of **8f** (1.0 g, 2.4 mmol) in dry THF (10 mL) under positive N_2 pressure, Py (0.40 mL, 5.0 mmol) was added at 0 °C, followed by TFAA (0.50 mL, 3.6 mmol). After 15 min, the reaction mixture was diluted with Et_2O , then the organic phase was washed with NH_4Cl sat. sol., $NaHCO_3$ sat. sol., brine, dried and evaporated. The obtained solid was purified by column chromatography (8/2 PE/AcOEt) to give the title compound as a white solid; yield 92%; m.p. 110-111.5° (*n*-hexane). 1H -NMR (δ , $CDCl_3$): 1.47 (s, 9H, $C(CH_3)_3$), 2.31 (s, 3H, CH_3), 5.27 (s, 2H, CH_2), 6.06 (s, 1H, CH), 7.05-7.10 (m, 2H), 7.52-7.57 (m, 2H) (C_6H_4); ^{13}C -NMR (δ , $CDCl_3$): 14.5, 27.4, 60.9, 85.4, 95.3, 104.9, 115.3, 124.7, 133.2, 144.9, 148.9, 153.4, 155.6; MS (EI) m/z 413 (M^+).

3-[4-(3-Methyl-5-oxo-4,5-dihydro-1H-pyrazol-1-yl)phenoxy]propyl nitrate (9a). The product was recrystallised from EtOH, pale-yellow solid; yield 96%; m.p. 117.5-118 °C (EtOH). 1H -NMR (δ , $CDCl_3$): 2.16-2.24 (m, 5H, CH_3 , $CH_2CH_2CH_2$), 3.41 (s, 2H, $COCH_2$), 4.06 (t, 2H, OCH_2), 4.67 (t, 2H, CH_2ONO_2), 6.88-6.93 (m, 2H), 7.71-7.77 (m, 2H) (C_6H_4); ^{13}C -NMR (δ , $CDCl_3$): 17.0, 27.0, 42.9, 63.8, 67.0, 114.6, 120.7, 131.8, 155.8, 156.2, 170.3; MS (EI) m/z 293 (M^+). Anal. ($C_{13}H_{15}N_3O_5$) C, H, N.

6-[4-(3-Methyl-5-oxo-4,5-dihydro-1H-pyrazol-1-yl)phenoxy]hexyl nitrate (9b). The product was recrystallised from a MeOH/*i*Pr $_2$ O mixture, white solid; yield 67%; m.p. 123.5-125.5 °C (MeOH/*i*Pr $_2$ O).

$^1\text{H-NMR}$ (δ , CDCl_3): 1.42-1.57 (m, 4H), 1.71-1.83 (m, 4H) ($\text{CH}_2(\text{CH}_2)_4\text{CH}_2$), 2.18 (s, 3H, CH_3), 3.41 (s, 2H, COCH_2), 3.96 (t, 2H, OCH_2), 4.46 (t, 2H, CH_2ONO_2), 6.88-6.93 (m, 2H), 7.69-7.74 (m, 2H) (C_6H_4); $^{13}\text{C-NMR}$ (δ , CDCl_3): 17.0, 25.5, 25.7, 27.0, 29.0, 42.9, 67.8, 73.3, 114.6, 120.8, 131.3, 156.2, 156.4, 170.3; MS (EI) m/z 335 (M^+). Anal. ($\text{C}_{16}\text{H}_{21}\text{N}_3\text{O}_5$) C, H, N.

6-[4-(3-Methyl-5-oxo-4,5-dihydro-1H-pyrazol-1-yl)phenoxy]hexane-1,2-diyl dinitrate (9c). The product was purified by flash chromatography (eluent 95/5 $\text{CH}_2\text{Cl}_2/\text{AcOEt}$), yellow oil; yield 84%. $^1\text{H-NMR}$ (δ , CDCl_3): 1.61-1.86 (m, 6H, $\text{CH}_2(\text{CH}_2)_3\text{CHONO}_2$), 2.19 (s, 3H, CH_3), 3.42 (s, 2H, COCH_2), 3.98 (t, 2H, OCH_2), 4.48 (dd, 1H), 4.76 (dd, 1H) (CH_2ONO_2), 5.30-5.34 (m, 1H, CHONO_2), 6.88-6.91 (m, 2H), 7.71-7.74 (m, 2H) (C_6H_4); $^{13}\text{C-NMR}$ (δ , CDCl_3): 17.0, 21.8, 28.8, 29.1, 43.0, 67.4, 71.2, 80.6, 114.5, 120.8, 131.5, 156.2, 156.2, 170.3; MS (EI) m/z 396 (M^+). Anal. ($\text{C}_{16}\text{H}_{20}\text{N}_4\text{O}_8$) (396.36) C, H, N: calcd, 14.14; found, 13.69.

5-Methyl-2-(4-{3-[(3-phenylfuroxan-4-yl)oxy]propoxy}phenyl)-2,4-dihydro-3H-pyrazol-3-one (9d). The product was purified by flash chromatography (eluent 95/5 $\text{CH}_2\text{Cl}_2/\text{AcOEt}$). The obtained oil was triturated with $i\text{Pr}_2\text{O}$ to give the title compound as a white powder; yield 80%; m.p. 139.5-140.5 °C (at 133 °C the solid changes shape). $^1\text{H-NMR}$ (δ , CDCl_3): 2.19 (s, 3H, CH_3), 2.37-2.45 (m, 2H, $\text{CH}_2\text{CH}_2\text{CH}_2$), 3.41 (s, 2H, COCH_2), 4.18 (t, 2H, CH_2), 4.72 (t, 2H, CH_2), 6.91-6.94 (m, 2H), 7.46-7.51 (m, 3H), 7.72-7.75 (m, 2H), 8.10-8.13 (m, 2H) (C_6H_4 , C_6H_5); $^{13}\text{C-NMR}$ (δ , CDCl_3): 17.0, 28.7, 43.0, 64.2, 67.8, 107.6, 114.6, 120.8, 122.4, 126.1, 128.9, 130.5, 131.7, 155.9, 156.2, 162.2, 170.3; MS (EI) m/z 408 (M^+). Anal. ($\text{C}_{21}\text{H}_{20}\text{N}_4\text{O}_5$) C, H, N.

5-Methyl-2-(4-{3-[(3-phenylsulfonylfuroxan-4-yl)oxy]propoxy}phenyl)-2,4-dihydro-3H-pyrazol-3-one (9e). The product was purified by flash chromatography (eluent 9/1 $\text{CH}_2\text{Cl}_2/\text{AcOEt}$). The obtained oil was triturated with Et_2O to give the title compound as a white powder; yield 77%; m.p. 139-143 °C. $^1\text{H-NMR}$ (δ , CDCl_3): 2.19 (s, 3H, CH_3), 2.31-2.39 (m, 2H, $\text{CH}_2\text{CH}_2\text{CH}_2$), 3.42 (s, 2H, COCH_2), 4.17 (t, 2H, CH_2), 4.63 (t, 2H, CH_2), 6.91-6.97 (m, 2H), 7.50-7.55 (m, 2H), 7.68-7.79 (m, 2H), 7.97-8.00 (m, 2H) (C_6H_4 , C_6H_5); $^{13}\text{C-NMR}$ (δ , CDCl_3): 15.6, 28.8, 43.3, 63.9, 68.4, 110.8, 114.9, 121.2,

128.7, 130.0, 132.1, 136.0, 138.3, 156.3, 156.6, 159.2, 170.7; MS (EI) m/z 472 (M^+). Anal. ($C_{21}H_{20}N_4O_7S$) C, H, N.

4-{[4-(3-Methyl-5-oxo-4,5-dihydro-1H-pyrazol-1-yl)phenoxy]methyl}-furoxan-3-carboxamide

(9f). The product was purified by flash chromatography (eluent 98/2 $CH_2Cl_2/MeOH$). The obtained oil was triturated with Et_2O to give the title compound as a white powder; yield 79%; m.p. 181-183 °C (dec.). 1H -NMR (δ , $CDCl_3$): 2.20 (s, 3H, CH_3), 3.43 (s, 2H, OCH_2), 5.44 (s, 2H, $COCH_2$), 7.03-7.08 (m, 2H), 7.77-7.81 (m, 2H) (C_6H_4), 7.55 (br. s., 2H, NH_2); ^{13}C -NMR (δ , $CDCl_3$): 17.0, 43.0, 61.6, 110.4, 115.4, 120.7, 132.7, 154.8, 154.9, 155.6, 156.3, 170.3; MS (EI) m/z 331 (M^+). Anal. ($C_{14}H_{13}N_5O_5$) C, H, N.

4-{[4-(3-Methyl-5-oxo-4,5-dihydro-1H-pyrazol-1-yl)phenoxy]methyl}-furoxan-3-carbonitrile

(9g). The product was recrystallised from a 1,2-dichloroethane/*n*-hexane mixture, white solid; yield 74%; m.p. 137-138 °C (1,2-dichloroethane/*n*-hexane, dec.). 1H -NMR (δ , $CDCl_3$): 2.20 (2s, 3H, CH_3), 3.43 (s, 2H, $COCH_2$), 5.24 (s, 2H, OCH_2), 7.00-7.05 (m, 2H), 7.81-7.88 (m, 2H) (C_6H_4); ^{13}C -NMR (δ , $CDCl_3$): 17.0, 43.0, 61.0, 96.3, 105.0, 115.1, 120.7, 133.3, 153.6, 153.9, 156.5, 170.4; MS (CI) m/z 314 ($[M+H]^+$). Anal. ($C_{14}H_{11}N_5O_4$) C, H, N.

Theoretical ΔH_{HAT} and ΔH_{SET} calculations. All molecular models were constructed using standard bond lengths and angles with the MOE software package.⁶ Following truncated Newton-Raphson geometry optimization with the MMFF94s force field (MMFF94 charges, dielectric constant $\epsilon = 1r$) until the gradient was lower than $0.001 \text{ kcal mol}^{-1}$, a stochastic conformational search through the StochasticCSearch module as implemented in MOE was performed in order to find low-energy starting conformers for subsequent quantum-mechanical (QM) calculations. Conformations were rejected if the RMS deviation from the already existing ones was lower than 0.5 \AA , or if their energy was more than 7 kcal above the global minimum. A maximum of 10^4 iterations were performed on each molecule, and the search was abandoned after 100 consecutive failures to find a new conformer. After eliminating duplicate conformers, the lowest energy conformer was chosen for further optimisation by an *ab initio* RHF/6-31G(d) method; vibrational frequencies were determined at the same level of theory to characterise the stationary points as true minima, as well as to obtain the zero-point vibrational energy (ZPVE) and the thermal contribution to enthalpy at 298.15 K , scaled by a factor of 0.9135 as suggested by Scott and Radom.⁷ All QM calculations were accomplished with the GAMESS-US software package.⁸ Subsequently, on the RHF geometry a single-point DFT calculation was run at the RB3LYP/6-311+G(2d,2p) level; the electronic energy thus obtained was then corrected by the scaled ZPVE and the thermal contribution to enthalpy, yielding ΔH_f for the edaravone derivatives. The radicals R^\bullet were built by H-atom abstraction from position 4, as suggested by previous investigators.^{9,10} Geometry optimizations, vibrational analysis and single-point energy calculations were run at the same level of theory used for the parent structures, adopting the Restricted Open-shell method, yielding ΔH_f for the radical species. ΔH_{HAT} for each compound was then calculated according to Equation 1:

$$\Delta H_{\text{HAT}} = \Delta H_f(\text{edaravone-H}) + \Delta H_f(\text{R}^\bullet) - \Delta H_f(\text{edaravone}^\bullet) - \Delta H_f(\text{R-H}) \quad (1)$$

The values thus obtained (Table 1) represent 4-C-H bond homolytic cleavage enthalpies relative to edaravone. The ionised forms of edaravone derivatives were built out of the neutral radical geometries and optimised by a RHF/6-31+G(d) method; diffuse sp shells were added to heavy atoms to properly

account for the anionic status. Vibrational analysis was accomplished at the same level of theory, using 0.9153 as a scaling factor,⁷ while single-point energy calculations were run as previously described for the neutral molecules. Neutral radical geometries and hessian matrices were computed at the ROHF/6-31+G(d) level, while ROB3LYP/6-311+G(2d,2p) was used for electronic energies; ΔH_{SET} (Table 2) were calculated out of the ΔH_f values for anionic and neutral radical species as outlined in Equation 2:

$$\Delta H_{\text{SET}} = \Delta H_f(\text{edaravone}^-) + \Delta H_f(\text{R}^\bullet) - \Delta H_f(\text{edaravone}^\bullet) - \Delta H_f(\text{R}^-) \quad (2)$$

Table 1. ΔH_f of neutral and radical species (hartree) and ΔH_{HAT} values (kcal mol⁻¹) relative to edaravone **1** for compounds **1**, **2**, **9a-g**.

| Compd | Electronic energy (hartree, neutral species) ^a | ZPVE and thermal contribution at 25 °C (hartree, neutral species) ^b | ΔH_f (hartree, neutral species) ^c | Electronic energy (hartree, radical species) ^d | ZPVE and thermal contribution at 25 °C (hartree, radical species) ^e | ΔH_f (hartree, radical species) ^c | ΔH_{HAT} relative to edaravone (1) (kcal mol ⁻¹) ^f |
|-----------|---|--|--|---|--|--|---|
| 1 | -571.639959 | 0.189678 | -571.450281 | -570.998387 | 0.176641 | -570.821746 | 0.00 |
| 2 | -686.138971 | 0.224217 | -685.914754 | -685.498523 | 0.211946 | -685.286577 | -0.22 |
| 9a | -1044.395894 | 0.294185 | -1044.101709 | -1043.754504 | 0.281977 | -1043.472528 | 0.41 |
| 9b | -1162.288566 | 0.382184 | -1161.906382 | -1161.648107 | 0.369008 | -1161.279099 | -0.79 |
| 9c | -1441.941802 | 0.393431 | -1441.548372 | -1441.301137 | 0.380306 | -1440.920831 | -0.62 |
| 9d | -1406.881429 | 0.407921 | -1406.473508 | -1406.240600 | 0.394780 | -1405.845820 | -0.53 |
| 9e | -1955.405778 | 0.420397 | -1954.985381 | -1954.764197 | 0.406707 | -1954.357489 | -0.40 |
| 9f | -1190.772513 | 0.289337 | -1190.483175 | -1190.130242 | 0.276363 | -1189.853879 | 0.48 |
| 9g | -1114.302208 | 0.260442 | -1114.041766 | -1113.659947 | 0.246595 | -1113.413353 | -0.08 |

^a Single point calculation at the RB3LYP/6-311+G(2d,2p) level on the RHF/6-31G(d) geometry. ^b Computed at the RHF/6-31G(d) level, then scaled by a 0.9135 factor. ^c Calculated by summing ZPVE and thermal contribution at 25 °C to the electronic energy. ^d Single point calculation at the ROB3LYP/6-311+G(2d,2p) level on the ROHF/6-31G(d) geometry. ^e Computed at the ROHF/6-31G(d) level, then scaled by a 0.9135 factor. ^f Calculated according to equation 1.

Table 2. ΔH_f of anionic and radical species (hartree) and ΔH_{SET} values (kcal mol⁻¹) relative to edaravone **1** for compounds **1**, **2**, **9a-9g**.

| Compd | Electronic energy (hartree, anionic species) ^a | ZPVE and thermal contribution at 25 °C (hartree, anionic species) ^b | ΔH_f (hartree, anionic species) ^c | Electronic energy (hartree, radical species) ^d | ZPVE and thermal contribution at 25 °C (hartree, radical species) ^e | ΔH_f (hartree, radical species) ^c | ΔH_{SET} relative to edaravone (1) (kcal mol ⁻¹) ^f |
|-----------|---|--|--|---|--|--|--|
| 1 | -571.095331 | 0.178253 | -570.917079 | -570.991652 | 0.179256 | -570.812396 | 0.00 |
| 2 | -685.590908 | 0.212465 | -685.378444 | -685.491388 | 0.213446 | -685.277942 | -2.62 |
| 9a | -1043.854833 | 0.279777 | -1043.575056 | -1043.749277 | 0.280829 | -1043.468448 | 1.21 |
| 9b | -1161.745743 | 0.366822 | -1161.378921 | -1161.643265 | 0.367627 | -1161.275638 | -0.88 |
| 9c | -1441.405047 | 0.374908 | -1441.030138 | -1441.300049 | 0.375877 | -1440.924172 | 0.80 |
| 9d | -1406.332092 | 0.394908 | -1405.937185 | -1406.227813 | 0.394069 | -1405.833744 | -0.78 |
| 9e | -1954.861784 | 0.406432 | -1954.455352 | -1954.760251 | 0.405772 | -1954.354479 | -2.39 |
| 9f | -1190.231139 | 0.275254 | -1189.955886 | -1190.126396 | 0.274956 | -1189.851439 | -0.15 |
| 9g | -1113.763776 | 0.245432 | -1113.518344 | -1113.658613 | 0.244677 | -1113.413936 | -0.17 |

^a Single point calculation at the RB3LYP/6-311+G(2d,2p) level on the RHF/6-31+G(d) geometry. ^b Computed at the RHF/6-31+G(d) level, then scaled by a 0.9153 factor. ^c Calculated by summing ZPVE and thermal contribution at 25 °C to the electronic energy. ^d Single point calculation at the ROB3LYP/6-311+G(2d,2p) level on the ROHF/6-31+G(d) geometry. ^e Computed at the ROHF/6-31+G(d) level, then scaled by a 0.9153 factor. ^f Calculated according to equation 2.

Ionisation constants and lipophilicity descriptors. The ionisation constants and lipophilicity descriptors of compounds were determined by potentiometric titration with the GlpKa apparatus (Sirius Analytical Instruments Ltd, Forrest Row, East Sussex, UK). All titrations were performed under argon at 25.0 ± 0.1 °C. Ionisation constants of edaravone **1** and his methoxy derivative **2** were obtained by aqueous titrations; the measurement of ionisation constants for all the other compounds was performed in methanol-water mixtures according to the following procedure. At least five different hydro-organic solutions (ionic strength adjusted to 0.15 M with KCl) of the compounds (20 mL, about 1 mM in 25-57 Wt% methanol) were initially acidified to pH 1.8 with 0.5 N HCl. The solutions were then titrated with standardized 0.5 N KOH to pH 10.5. The initial estimates of the apparent ionisation constants (p_sK_a s) were obtained by Bjerrum plots; these values were finally refined by a weighted non-linear least-squares procedure. Aqueous pK_a values were obtained by extrapolation at 0% methanol using the Yasuda-Shedlovsky procedure.¹¹

To obtain lipophilicity data of the compounds at least four separate titrations of the compounds (about 1 mM) were carried out in the pH range 1.8 to 10.5 using various volumes of *n*-octanol (volume ratios *r* of organic solvent/water ranging from 0.05 to 3, ionic strength adjusted to 0.15 M with KCl). In the presence of *n*-octanol new ionisation constants (p_oK_a s) were determined; the shifts in the pK_a were used to determine $\log P^N$ by the multiset approach.¹² Distribution coefficient *D* was calculated according to Equation 3:

$$D = P^N \cdot \frac{1}{1 + 10^{pK_a - pH}} + P^I \cdot \frac{10^{pK_a - pH}}{1 + 10^{pK_a - pH}} \quad (3)$$

$\log P$ and $\log D^{7.4}$ between *n*-octanol and water were also obtained by shake-flask technique at room temperature. In the shake-flask experiments 0.1 M HCl (pH = 1 for $\log P^N$ determination) and 0.05 M phosphate buffer (pH = 7.4 for $\log D^{7.4}$ determination) were used as aqueous phases; ionic strength was adjusted to 0.15 M with KCl. The organic (*n*-octanol) and aqueous phases were mutually saturated by shaking for 4 h. The compounds were solubilised in the buffered aqueous phase at a concentration of about 0.1 mM and an appropriate amount of *n*-octanol was added. The two phases were shaken for about 20 min, by which time the partitioning equilibrium of solutes is reached, and then centrifuged (10000

rpm, 10 min). The concentration of the solutes was measured in the aqueous phase by UV spectrophotometer (UV-2501PC, Shimadzu) at λ_{max} . Each log P or log D value is an average of at least six measurements.

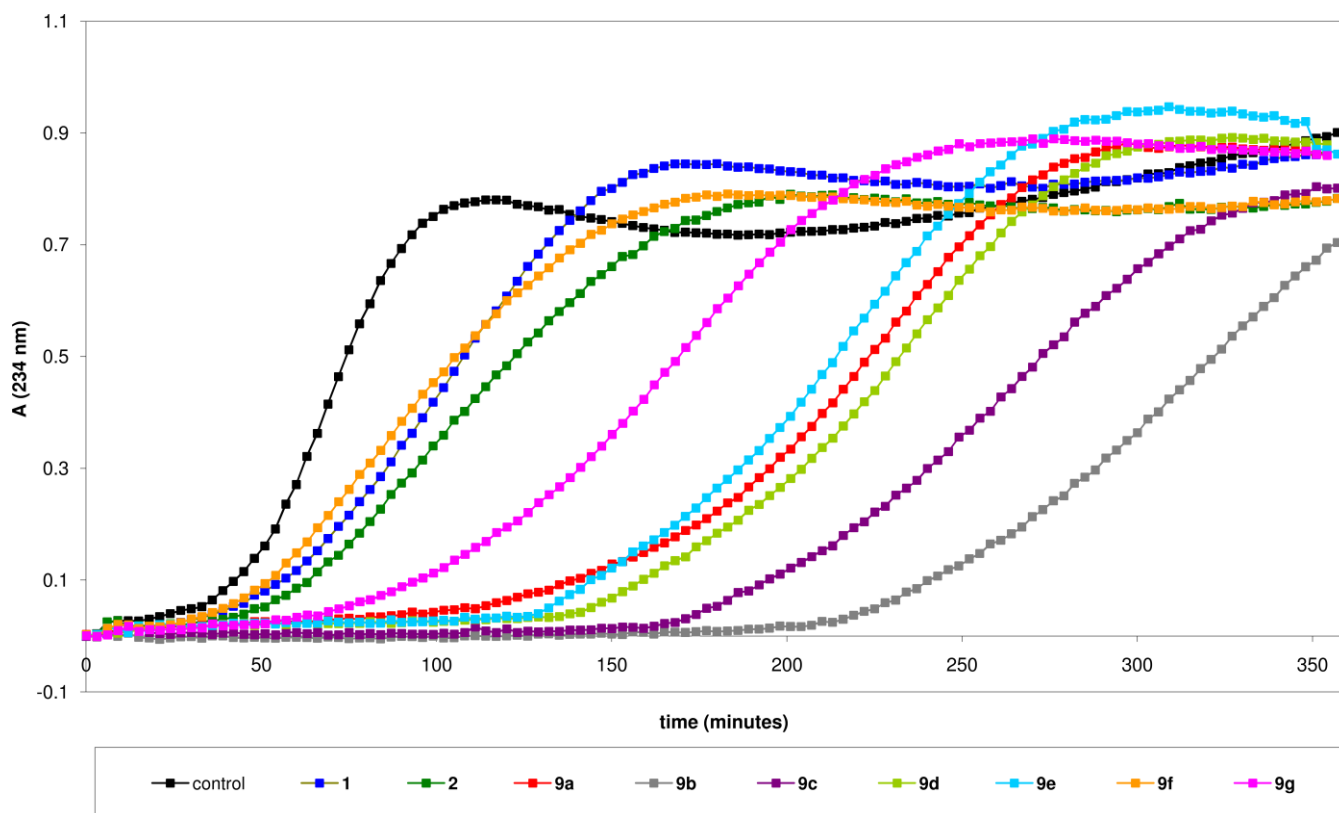


Figure 1. Effect of edaravone **1** and compounds **2**, **9a-9g** on kinetics of conjugated diene formation during copper-induced LDL oxidation. The figure shows typical experimental kinetic profiles obtained incubating the compounds (10 μM) at 37 $^{\circ}\text{C}$ with 50 $\mu\text{g mL}^{-1}$ of LDL in PBS (2 mL in total) in the presence of 2.5 μM CuSO_4 . Conjugated diene formation was assessed monitoring over 6 h the changes in absorbance at 234 nm.

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