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Evaluation of Liposome-Encapsulated Hemoglobin/LR16
Formulations as a Potential Blood Substitute

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Performance Report/3rd of 1991
November 8, 1991

Attached are two summary sheets showing the structures of several, new LR16 analogues recently synthesized with the support of Department of the Navy Grant No. N000014-90-J-1648. Preliminary testing has shown compounds 19, 22, and 25 to be active, achieving P_{50} values in LEH suspensions of 29.5, 28.5, and 21.0 mm Hg, respectively, at drug concentrations of 3 mM. Testing of the compounds listed on the second sheet (01 series) is presently underway. The two lists of structures is followed by the experimental section outlining synthetic methodologies.

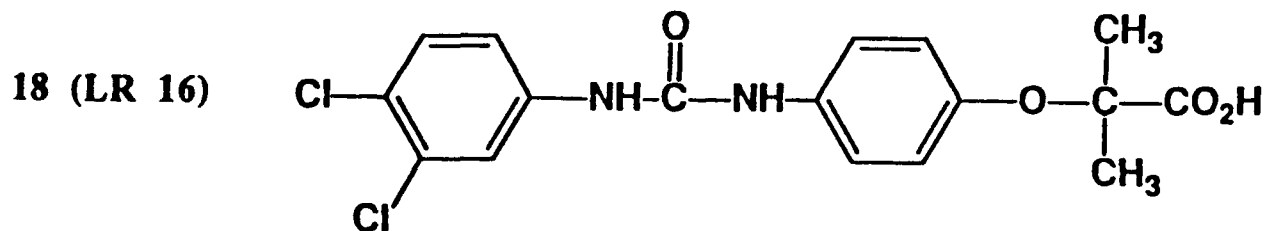
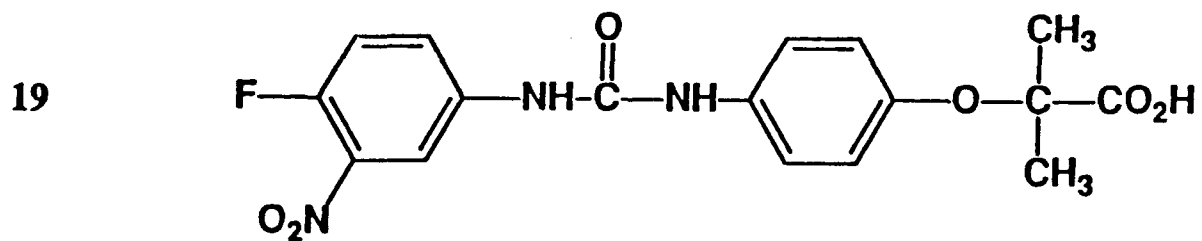
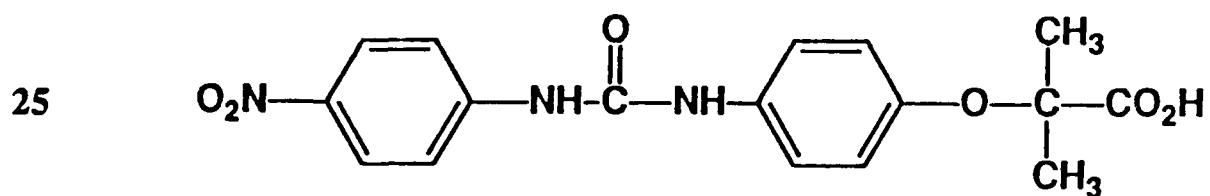
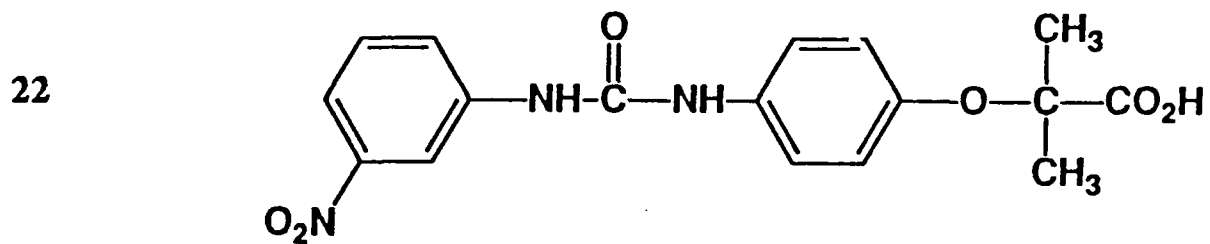
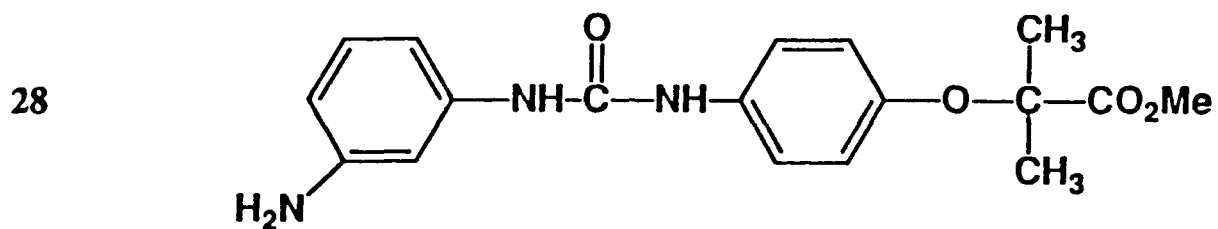
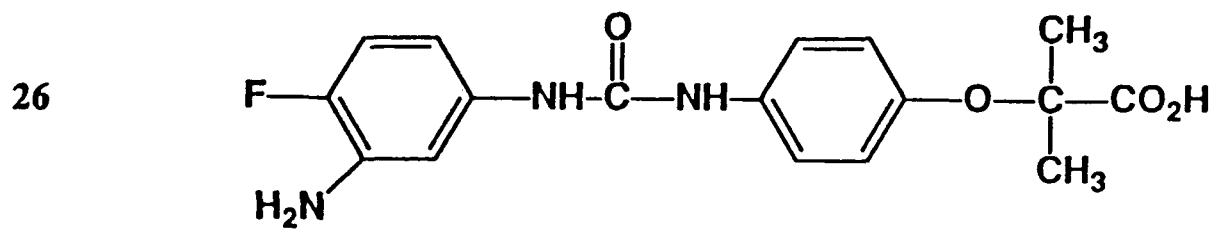
The final three pages of this report summarize a synthetic scheme and NMR data concerning our first analogue which contains a quaternary, permanently-charged nitrogen moiety (which should significantly reduce drug leakage from liposome). We are presently testing the ability of this agent to modulate the P_{50} value of hemoglobin.

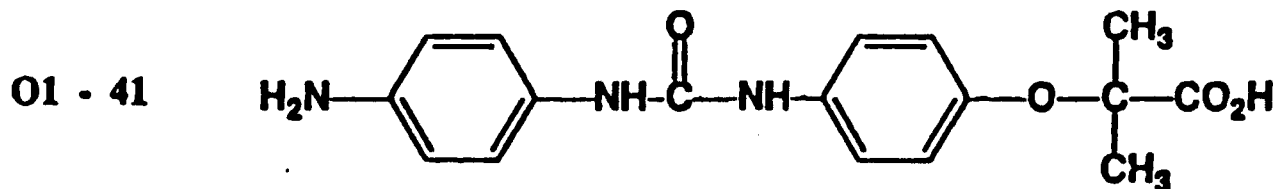
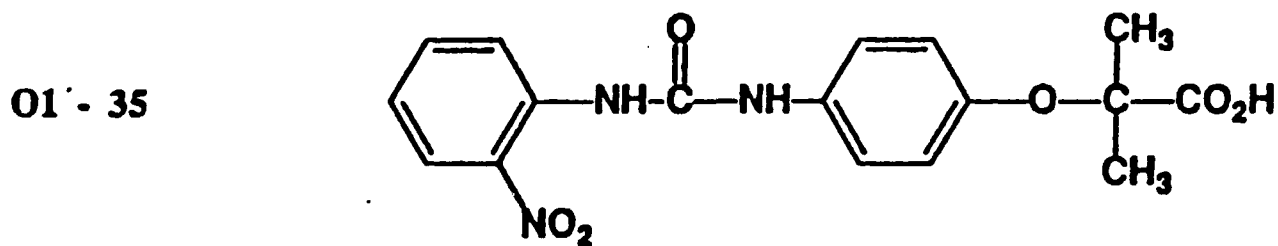
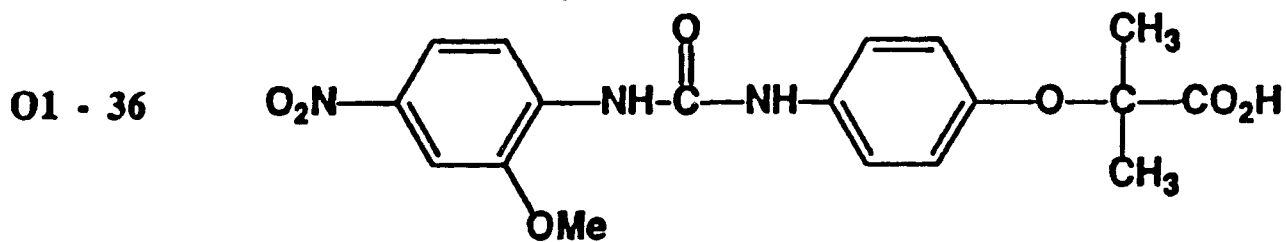
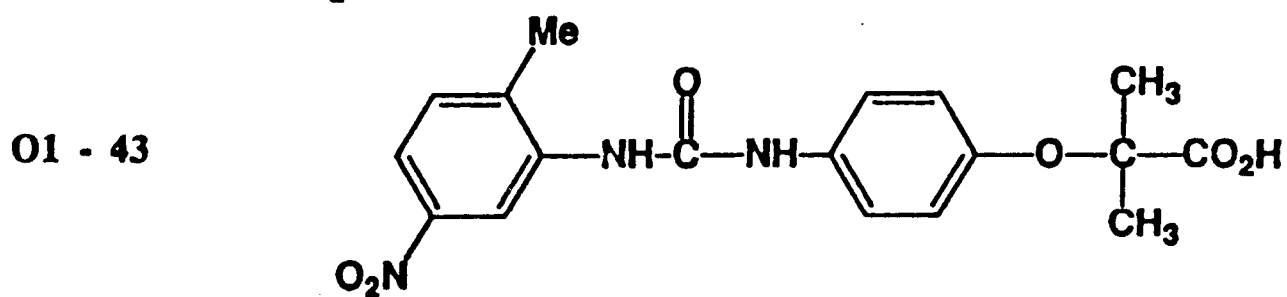
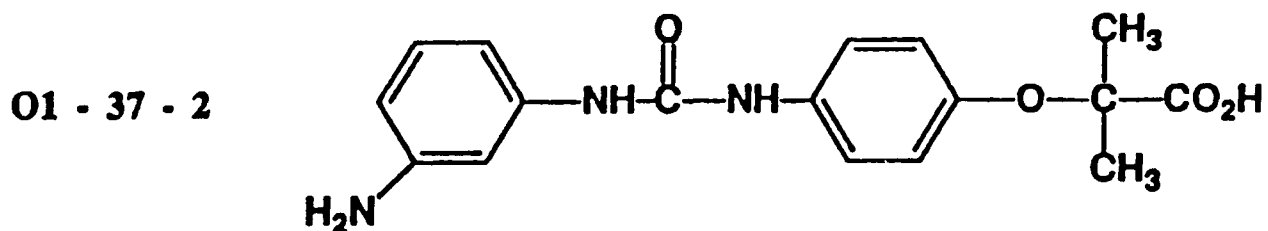
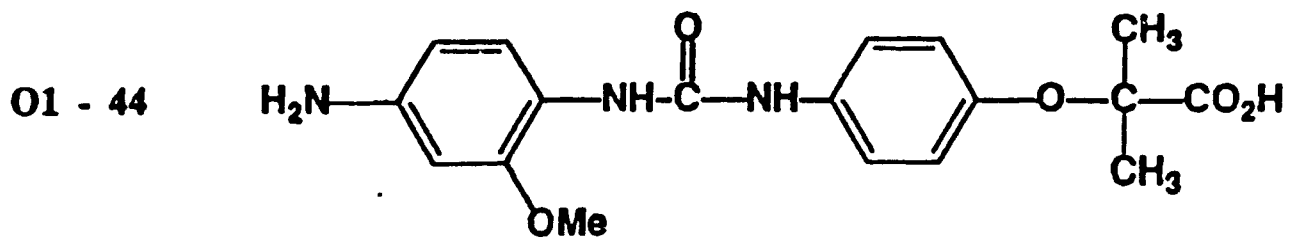
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EXPERIMENTAL

TLC was performed on precoated plastic sheets (0.2 mm) of silica gel 60 F-254 (E. Merck AG, Darmstadt, West Germany); compounds were detected by UV lamps (254 nm). Hydrogenations were carried out in Parr Apparatus. Melting points were determined with a Buchi 530 apparatus and are uncorrected. NMR spectra were recorded for solution in DMSO-d₆ or CDCl₃/DMSO-d₆ (internal standard Me₄Si) with a QE -300-MHz spectrometer.

Synthesis of 1-aryl-3-(4-hydroxyphenyl)urea derivatives. General Procedure.

Aryl isocyanate 2-9 (30 mmol) was added (as a pure substance or as a solution in 1-2 mL of pyridine) to an intensively stirred solution of 4-aminophenol (3.3 g, 30 mmol) in pyridine (8-10 mL) at 0°C. The reaction was kept at this temperature for 15 min. Then, the ice bath was removed and the reaction continued for another 15 min at room temperature. Water (250 mL) was then added, and the pyridine was neutralized with a small excess of 12% HCl (180 mL). The suspension was left with stirring for 0.5 h, the precipitate was separated by filtration, and dried to give products 10-17. If the product was not pure enough (TLC: CHCl₃/MeOH - 10:1) it was dissolved in methanol and the insoluble white solid (products of polymerization of isocyanates) were filtered off. The filtrate was concentrated and dried on the oil pump. Analytical samples were recrystallized from acetone, methanol or methanol/chloroform mixture.

10: Yield - 94%; m.p. 206-208°C (MeOH); ¹H NMR (300 MHz, CDCl₃/DMSO-d₆ - 10:1) 8.18 (s, 1 H, NH), 7.72 (s, 1 H, NH), 7.52 (d, J = 2.4 Hz, 1 H), 7.02 (d, J = 8.7 Hz, 1 H), 6.98-6.82 (m, 3 H), 6.50 (d, J = 8.7 Hz, 2 H); ¹³C NMR (75 MHz, CHCl₃/DMSO-d₆ - 5:1) 152.8 (CO), 139.2, 131.7, 130.1, 129.7, 123.9, 121.1 (2xC), 119.6, 119.4, 117.4, 115.2 (2xC) (C-Ar).

11: Yield - 91%; m.p. 202-202.5°C (acetone); ¹H NMR (300 MHz, CDCl₃/DMSO-d₆ - 10:1) 8.69 (s, 1 H, OH), 8.65 (s, 1H, NH), 8.26 (dd, J_{HF} = 6.6 Hz, J = 2.7 Hz, 1 H), 7.92 (s, 1 H, NH), 7.72 (dt, J = 9.0, 3.3 Hz, J_{HF} = 3.3 Hz, 1 H), 7.22 (d, J = 8.7 Hz, 2 H), 7.20 (t, J_{HF} = 10.2 Hz, 1 H), 6.79 (d, J = 8.7 Hz, 2 H); ¹³C NMR (75 MHz, CDCl₃/DMSO-d₆ - 10:1) 152.9 (CO), 147.9, 136.4, 124.6 (2xC), 121.1 (2xC), 117.7 (d, J = 18.7 Hz), 115.2 (2xC), 114.3



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(C-Ar).

12: Yield - 56%; m.p. 221-225°C (acetone); ¹H NMR (300 MHz, DMSO-d₆) 9.14 (s, 1 H, NH), 8.97 (s, 1 H, NH), 8.97 (d, *J* = 2.4 Hz, 1 H), 8.16 (s, 1 H, OH), 7.76 (dd, *J* = 8.4, 2.4 Hz, 1 H), 7.42 (d, *J* = 8.4 Hz, 1 H), 7.28 (d, *J* = 8.7 Hz, 2 H), 6.74 (d, *J* = 8.7 Hz, 2 H), 2.36 (s, 3 H, CH₃); ¹³C NMR (75 MHz, DMSO-d₆) 152.8, 152.6, 146.1, 138.9, 134.0, 130.9 (d), 130.7, 120.4 (d, 2xC), 116.2 (d), 115.3 (d, 2xC), 113.3 (d) (C-Ar).

13: Yield - 99%; m.p. 215-217°C (CHCl₃/MeOH); ¹H NMR (300 MHz, CDCl₃/DMSO-d₆ - 10:1) 9.16 (dd, *J*_{H,F} = 7.0, *J* = 2.8 Hz, 1 H), 8.45 (s, 1 H, NH), 8.37 (s, 1 H, NH), 7.66 (ddd, *J* = 8.8, 3.2 Hz, *J*_{HF} = 4.1 Hz, 1 H), 7.10 (d, *J* = 8.75 Hz, 2 H), 7.02 (t, *J*_{HF} = 9.8 Hz, 1 H), 6.65 (d, *J* = 8.75 Hz, 2 H); ¹³C NMR (75 MHz, DMSO-d₆) 154.8 (d, *J* = 252 Hz), 153.1 (CO), 152.1, 144.0, 130.4, 129.2 (d, *J* = 11.7 Hz), 120.6 (2xC), 117.2, 115.8 (d, *J* = 24.5 Hz), 115.4 (2xC), 114.3 (d, *J* = 21.3 Hz) (C-Ar).

14: Yield - 91%; m.p. 221°C (acetone); ¹H NMR (300 MHz, CDCl₃/DMSO-d₆ - 10:1) 8.49 (s, 1 H, NH), 8.22 (t, *J* = 2.0 Hz, 1 H), 7.78 (s, 1 H, NH), 7.73-7.64 (m, 2 H), 7.29 (t, *J* = 8.0 Hz, 1 H), 7.12 (d, *J* = 8.7 Hz, 2 H), 6.69 (d, *J* = 8.7 Hz, 2 H); ¹³C NMR (75 MHz, DMSO-d₆) 153.1 (CO), 147.9, 141.2, 130.3 (2xC), 124.6, 123.6, 120.5 (2xC), 116.3, 115.3 (2xC), 112.2 (C-Ar).

15: Yield - 97%; m.p. 209-211°C (acetone); ¹H NMR (300 MHz, CDCl₃/DMSO-d₆ - 10:1) 9.76 (s, 1 H, OH), 8.96 (s, broad, 1 H, NH), 8.58 (d, *J* = 8.6 Hz, 1 H), 8.48 (s, broad, 1 H, NH), 8.08 (dd, *J* = 8.2, 1.0 Hz, 1 H), 7.52 (pseud. t, *J* = 8.0, 1.0 Hz, 1 H), 7.24 (d, *J* = 8.7 Hz, 2 H), 6.99 (pseudo t, *J* = 8.0, 0.8 Hz, 1 H), 6.77 (d, *J* = 8.7 Hz, 2 H); ¹³C NMR (75 MHz, DMSO-d₆) 153.1 (CO), 151.9, 137.2, 135.4, 135.0, 130.5, 125.4, 122.3, 121.8, 120.9 (2xC), 115.3 (2xC) (C-Ar).

16: Yield - 97%; m.p. 201°C (acetone); ¹H NMR (300 MHz, CDCl₃/DMSO-d₆ - 10:1) 8.62 (s, broad, 1 H, NH), 8.47 (d, *J* = 9.1 Hz, 1 H), 8.32 (s, broad, 1 H, NH), 7.85 (dd, *J* = 9.1, 2.4 Hz, 1 H), 7.68 (d, *J* = 2.4 Hz, 1 H), 7.22 (d, *J* = 8.8 Hz, 2 H), 6.77 (d, *J* = 8.8 Hz, 2 H), 3.96 (s, 3 H, OCH₃); ¹³C NMR (75 MHz, DMSO-d₆) 153.1 (CO), 146.9, 140.7, 136.0, 130.3, 120.2, 120.2 (2xC), 117.8 (2xC), 116.4, 115.3, 105.5 (C-Ar), 56.3 (OCH₃).

17: Yield - 63%; m.p. 216-217°C (CHCl₃/MeOH); ¹H NMR (300 MHz, CDCl₃/DMSO-d₆ - 10:1) 8.93 (s, 1 H, NH), 8.84 (s, 1 H, NH), 8.15 (s, 1 H, OH), 8.13, 7.62, 7.12, and 6.89 (4 x AA'XX' system, 8 H); ¹³C NMR (75 MHz, CDCl₃/DMSO-d₆ - 10:1) 153.0 (CO), 152.3, 146.0, 140.9, 129.8, 124.5 (2xC), 121.0 (2xC), 116.7 (2xC), 115.2 (2xC) (C-Ar).

Synthesis of 2-[4-[[[(aryl)amino]carbonyl]amino]phenoxy-2-methyl propionic acid derivatives. General Procedure.

Sodium hydroxide (2.8 g, 71.6 mmol) was suspended in acetone (35 mL) and 1-aryl-3-(4-hydroxyphenyl)urea derivative (13.7 mmol) was added to this mixture, while heated to reflux. Then chloroform (5.5 mL, 68 mmol) was added dropwise for 10 min. The reaction was continued for an additional 4 h in reflux. Then, the solvents were evaporated and to the residue water (90 mL) was added. The mixture was suspended and filtered. The filtrate was shaken with ethyl acetate (2x50 mL), heated with charcoal at 50°C, filtered through Celite and then acidified with 12% HCl to pH ~1. The precipitate was isolated by filtration, washed with water and dried to give crude products 18-25.

The products were purified according the following procedure: The crude compound was dissolved in a small amount of acetone and then ether was added dropwise until a dark solid started to precipitate. Then the solution was left for 0.5 h, and a dark precipitate was filtered off. The remaining solution was concentrated to dryness. This procedure was repeated 2-3 times. Finally, the product was precipitated from a concentrated acetone solution by addition of ethyl ether or crystallized from acetone or acetone/ether mixture.

18: Yield - 62%; m.p. 178-179°C (acetone/Et₂O); ¹H NMR (300 MHz, DMSO-d₆) 8.96 (s, 1 H, NH), 8.68 (s, 1 H, NH), 7.87 (d, *J* = 2.4 Hz, 1 H), 7.51 (d, *J* = 8.8 Hz, 1 H), 7.33 (d, *J* = 8.9 Hz, 2 H), 7.31 (dd, *J* = 8.8, 2.4 Hz, 1 H), 6.81 (d, *J* = 8.9 Hz, 2 H), 1.47 (s, 6 H, 2xCH₃); ¹³C NMR (75 MHz, DMSO-d₆) 175.1 (CO₂H), 152.4 (CO), 150.4, 140.1, 133.6, 131.0, 130.5, 123.0, 119.9 (2xC), 119.8 (2xC), 119.2, 118.2 (C-Ar), 78.7 (CCO₂H), 25.1 (2xCH₃).

19: Yield - 28%; m.p. 170.5-171°C (acetone/Et₂O); ¹H NMR (300 MHz, DMSO-

d₆ 9.14 (s, 1 H, NH), 8.73 (s, 1 H, NH), 8.42 (dd, $J_{H,F} = 6.8$ Hz, $J = 2.5$ Hz, 1 H), 7.69 (dt, $J = 9.3, 3.3$ Hz, $J_{H,F} \sim 3.3$ Hz, 1 H), 7.51 (t, $J_{H,F} = 10.1$ Hz, $J = 9.3$ Hz, 1 H), 7.34 (d, $J = 8.8$ Hz, 2 H), 6.82 (d, $J = 8.8$ Hz, 2 H), 1.46 (s, 6 H, 2xCH₃); ¹³C NMR (75 MHz, DMSO-d₆) 175.0 (CO₂H), 152.6 (CO), 150.5, 149.4 (d, $J = 255$ Hz), 136.8, 136.4 (d, $J = 8$ Hz), , 133.5, 125.5 (d, $J = 7$ Hz), 120.0 (2xC), 119.8 (2xC), 118.6 (d, $J = 22$ Hz), 114.3 (C-Ar), 78.8 (CCO₂H), 25.0 (2xCH₃).

21: Yield - 59%; m.p. 158-162°C (acetone/Et₂O).

22: Yield - 38%; m.p. 175-178°C (acetone); ¹H NMR (300 MHz, DMSO-d₆) 13.00 (s, broad, 1 H, CO₂H), 9.30 (s, 1 H, NH), 8.81 (s, 1 H, NH), 8.56 (t, $J = 1.8$ Hz, 1 H), 7.81 (dd, $J = 8.0, 1.5$ Hz, 1 H), 7.70 (d, $J = 8.3$ Hz, 1 H), 7.56 (t, $J = 8.1$ Hz, 1 H), 7.36 (d, $J = 8.9$ Hz, 2 H), 6.82 (d, $J = 8.9$ Hz, 2 H), 1.47 (s, 6 H, 2xCH₃); ¹³C NMR (75 MHz, DMSO-d₆) 175.1 (CO₂H), 152.5 (CO), 150.4, 148.1, 141.2, 133.5, 129.9, 124.1, 119.9 (2xC), 119.7 (2xC), 116.0, 112.0 (C-Ar), 78.7 (CCO₂H), 25.0 (2xCH₃).

23: Yield - 38%; m.p. 170-172°C (acetone); ¹H NMR (300 MHz, DMSO-d₆) 12.84 (s, broad, 1 H, CO₂H), 9.70 (s, 1 H, NH), 9.55 (s, 1 H, NH), 8.30 (d, $J = 8.4$ Hz, 1 H), 8.09 (dd, $J = 8.7, 1.2$ Hz, 1 H), 7.69 (m, 1 H), 7.37 (d, $J = 9.0$ Hz, 2 H), 7.19 (m, 1 H), 6.83 (d, $J = 9.0$ Hz, 2 H), 1.48 (s, 6 H, 2xCH₃).

24: Yield - 49%; m.p. 182-183°C (acetone); ¹H NMR (300 MHz, DMSO-d₆) 12.95 (s, broad, 1 H, CO₂H), 9.46 (s, 1 H, NH), 8.76 (s, 1 H, NH), 8.43 (d, $J = 9.0$ Hz, 1 H), 7.90 (dd, $J = 9.0, 2.4$ Hz, 1 H), 7.79 (d, $J = 2.4$ Hz, 1 H), 7.36 (d, $J = 9.0$ Hz, 2 H), 6.83 (d, $J = 9.0$ Hz, 2 H), 4.03 (s, 3 H, OCH₃) 1.48 (s, 6 H, 2xCH₃); ¹³C NMR (75 MHz, DMSO-d₆) 175.0 (CO₂H), 151.8 (CO), 150.5, 146.9, 140.7, 136.0, 133.4, 119.8 (2xC), 119.5 (2xC), 117.6, 116.1, 105.5 (C-Ar), 78.7 (CCO₂H), 56.5 (OCH₃), 25.0 (2xCH₃).

25: Yield - 35%; m.p. 209-210.5°C (acetone/ether); ¹H NMR (300 MHz, DMSO-d₆) 12.95 (s, broad, 1 H, CO₂H), 9.38 (s, 1 H, NH), 8.77 (s, 1 H, NH), 8.18, 7.68, 7.35, and 6.83 (4 x AA'XX' system, 8 H), 1.48 (s, 6 H, 2xCH₃); ¹³C NMR (75 MHz, DMSO-d₆) 174.9 (CO₂H), 152.0 (CO), 150.6, 146.5, 140.9, 133.3, 125.1 (2xC), 119.9 (2xC), 119.7 (2xC), 117.3 (2xC) (C-Ar), 78.7 (CCO₂H), 25.0 (2xCH₃).

2-[4-[[3-Amino-4-fluorophenyl]amino]carbonyl]amino]phenoxy-2-methyl propionic acid (26).

2-[4-[[3-Nitro-4-fluorophenyl]amino]carbonyl]amino]phenoxy-2-methyl propionic acid (190 mg, 0.5 mmole) was hydrogenated in MeOH (10 mL) using 10% Pd/C as a catalyst (10 psi, 2.5 h). After the reduction, the catalyst was filtered off through Celite, washed with MeOH, and concentrated. Chloroform (15 mL) was added and the solution was left overnight. Dark oily tar was filtered off, the filtrate was concentrated and dried on the oil pump to give crude 2-[4-[[3-amino-4-fluorophenyl]amino]carbonyl]amino]phenoxy-2-methyl propionic acid (119 mg, 69%). 100 mg of the product was chromatographed (30 g SiO₂, CHCl₃/MeOH - 10:1 to 1:1) to give 40 mg of pure product: m.p. 220-225°C (decomp., MeOH/ether); ¹H NMR (300 MHz, DMSO-d₆) 8.40 (s, 1 H, NH), 8.36 (s, 1 H, NH), 7.31 (d, *J* = 8.9 Hz, 2 H),

6.94 (dd, *J*_{H,F} = 8.3 Hz, *J* = 2.5 Hz, 1 H), 6.86 (dd, *J*_{H,F} = 11.3 Hz, *J* = 8.8 Hz, 1 H), 6.79 (d, *J* = 8.9 Hz, 2 H), 6.52 (ddd, *J* = 8.8, 2.9 Hz, *J*_{H,F} = 3.6 Hz, 1 H), 5.11 (s, broad, NH₂), 1.46 (s, 6 H, 2xCH₃); ¹³C NMR (75 MHz, DMSO-d₆) 175.0 (CO₂H), 152.5 (CO), 149.8, 146.2 (d, *J* = 231 Hz), 136.2 (d, *J* = 15 Hz), 136.1, 134.3, 119.9 (2xC), 119.2 (2xC), 114.5 (d, *J* = 19 Hz), 106.0 (d, *J* = 5 Hz), 105.7 (C-Ar), 78.7 (CCO₂H), 25.0 (2xCH₃).

2-[4-[[[3-(N,N-Dimethylamino)-4-fluorophenyl]amino]carbonyl]amino]phenoxy-2-methyl propionic acid methyl ester (27).

2-[4-[[3-Amino-4-fluorophenyl]amino]carbonyl]amino]phenoxy-2-methyl propionic acid (37 mg, 0.11 mmole), methyl iodide (296 mg, 0.13 mL, 2.1 mmole) and NaHCO₃ (180 mg, 2.1 mmole) were stirred in DMF (5 mL) during 18 h and the reaction was controlled by TLC (CHCl₃/MeOH - 4:1). To this solution ether (30 mL) was added and the heavy oily precipitated fraction was separated and washed twice with ether (2 x 20 mL). This oil rapidly solidified. The solid was dissolved in MeOH (20 mL), the inorganic salt was filtered off, and the filtrate was evaporated to dryness. After an addition of ether (30 mL), CH₂Cl₂ was added dropwise to give a white precipitate, it was filtered off and dried on an oil pump to give 2-[4-[[[3-(N,N-dimethylamino)-4-fluorophenyl]amino]carbonyl]ami-

no]phenoxy-2-methyl propionic acid methyl ester: m.p. ~225°C (decomp., ether/CH₂Cl₂); ¹H NMR (300 MHz, DMSO-d₆) 9.20 (s, 1 H, NH), 8.84 (s, 1 H, NH), 8.18-8.15 (m, 1 H), 7.60-7.50 (m, 2 H), 7.39-7.30 (m, 2 H), 6.81-6.72 (m, 2 H), 3.70 (s, 3 H, CO₂CH₃), 3.15 (s, 6 H, N(CH₃)₂), 1.46 (s, 6 H, 2xCH₃).

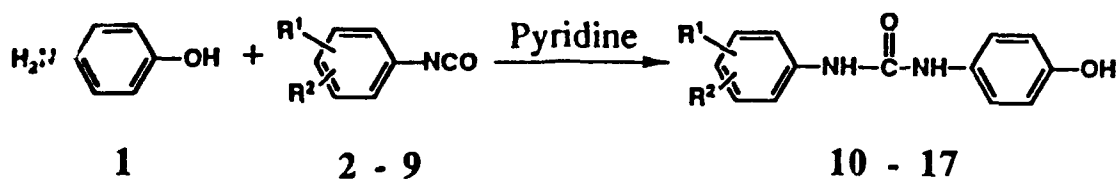
2-[4-[[[(3-Aminophenyl)amino]carbonyl]amino]phenoxy-2-methyl propionic acid methyl ester (28).

2-[4-[[[(3-Nitrophenyl)amino]carbonyl]amino]phenoxy-2-methyl propionic acid (750 mg, 2.09 mmole) was hydrogenated in MeOH (20 mL) using 10% Pd/C as a catalyst (20 mg, 10 psi, 3 h). After reduction the catalyst was filtered off through silica gel. In the filtrate a mixture of a few compounds was found. Then, the silica was washed with the diluted HCl in MeOH, the filtrate was concentrated and chromatographed (CHCl₃/MeOH - 20:1) to give 2-[4-[[[(3-aminophenyl)amino]carbonyl]amino]phenoxy-2-methyl propionic acid methyl ester (275 mg, 38%): semisolid; ¹H NMR (300 MHz, CDCl₃/DMSO-d₆ - 5:1) 10.23 (s, broad, 2 H, NH₂), 9.15 (s, 1 H, NH), 8.71 (s, 1 H, NH), 7.65 (s, 1 H), 7.51 (d, *J* = 7.9 Hz, 1 H), 7.35 (d, *J* = 8.4 Hz, 2 H), 7.19 (t, *J* = 7.5 Hz, 1 H), 6.94 (d, *J* = 6.9 Hz, 1 H), 6.77 (d, *J* = 8.4 Hz, 2 H), 3.77 (s, 3 H, CH₃), 1.54 (s, 6 H, 2xCH₃); ¹³C NMR (75 MHz, DMSO-d₆) 173.8 (CO₂H), 152.5 (CO), 149.4, 149.1, 140.3, 134.8, 129.0, 120.3 (2xC), 119.2 (2xC), 108.0, 106.1, 103.7 (C-Ar), 79.0 (CCO₂H), 52.3 (CO₂CH₃), 24.9 (2xCH₃).

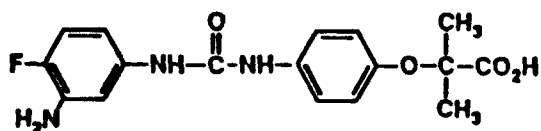
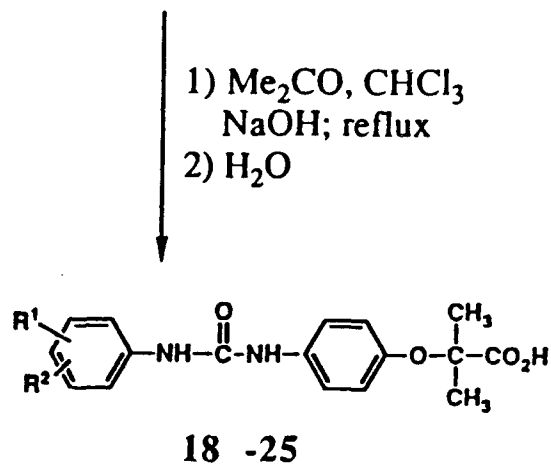
2-[4-[[[(4-Aminophenyl)amino]carbonyl]amino]phenoxy-2-methyl propionic acid (29).

2-[4-[[[(4-Nitrophenyl)amino]carbonyl]amino]phenoxy-2-methyl propionic acid (100 mg, 0.28 mmole) was hydrogenated in MeOH (20 mL) using 10% Pd/C as a catalyst (20 mg, 11 psi, 32 h). The catalyst was filtered off and washed with MeOH. The filtrate was evaporated to dryness to give 29 mg of product. The remaining product adsorbed on the catalyst was dissolved in diluted HCl in MeOH and separated from the catalyst by filtration. Then, it was neutralized with ammonia and left for crystallization to give an additional 10 mg of 2-[4-[[[(4-aminophenyl)amino]carbonyl]amino]phenoxy-2-methyl propionic acid. Total yield - 39 mg, 42%: m.p. 236-243°C (decomp., H₂O/MeOH); ¹H NMR (300

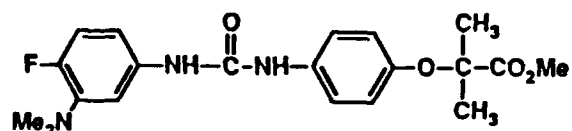
MHz, DMSO-d₆) 8.44 (s, 1 H, NH), 8.17 (s, 1 H, NH), 7.30 (d, $J = 8.8$ Hz, 2 H), 7.06 (d, $J = 8.5$ Hz, 2 H), 6.78 (d, $J = 8.8$ Hz, 2 H), 6.51 (d, $J = 8.5$ Hz, 2 H), 1.45 (s, 6 H, 2xCH₃), NH₂ - undetected; ¹³C NMR (75 MHz, DMSO-d₆) 175.1 (CO₂H), 153.1 (CO), 149.6, 143.7, 134.7, 128.8, 120.6 (2xC), 119.9 (2xC), 119.1 (2xC), 114.2 (2xC) (C-Ar), 78.7 (CCO₂H), 25.0 (2xCH₃).



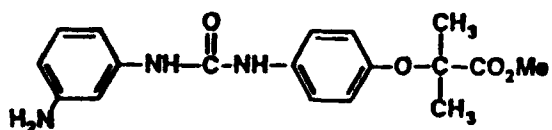
Compound	R ¹	R ²
2,10,18	3-Cl	4-Cl
3,11,19	3-NO ₂	4-F
4,12	2-Me	5-NO ₂
5,13,21	2-F	5-NO ₂
6,14,22	3-NO ₂	H
7,15,23	2-NO ₂	H
8,16,24	2-OMe	4-NO ₂
9,17,25	4-NO ₂	H



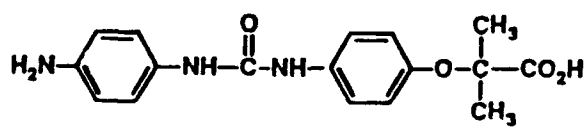
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Preparation of 2-[4-[[2-methoxy-4-trimethylaminophenyl)amino]carbonyl]amino]phenoxy-2-methyl propionic acid tetrafluoroborate.

2-[4-[[2-Methoxy-4-aminophenyl)amino]carbonyl]amino]phenoxy-2-methyl propionic acid (2). 2-[4-[[2-Methoxy-4-nitrophenyl)amino]carbonyl]amino]phenoxy-2-methyl propionic acid (1; 410 mg, 1.05 mmole) was hydrogenated in Parr Apparatus in MeOH (20 mL) using 10% Pd/C as a catalyst (40 mg, 10 psi, 3 h). The catalyst was filtered off and washed four times with MeOH. The filtrate was evaporated to dryness to give 275 mg of 2-[4-[[2-methoxy-4-aminophenyl)amino]carbonyl]amino]phenoxy-2-methyl propionic acid. Yield - 73%; m.p. 177°C (decomposition, crystallized from MeOH); ¹H NMR (300 MHz, DMSO-d₆) 8.86 (s, 1 H, NH), 7.64 (s, 1 H, NH), 7.53 (d, J = 8.5 Hz, 1H), 7.27 (d, J = 8.9 Hz, 2 H), 6.77 (d, J = 8.6 Hz, 2 H), 6.28 (s, 1H), 6.10 (d, J = 8.5 Hz, 1H), 3.74 (s, 3 H, OCH₃), 1.43 (s, 6 H, 2xCH₃); ¹³C NMR (75 MHz, DMSO-d₆) 175.1 (CO₂H), 152.9 (CO), 149.8, 149.7, 144.5, 134.7, 121.4, 120.0 (2xC), 118.8 (2xC), 117.6, 105.5, 98.0 (C-Ar), 78.8 (CCO₂H), 55.3 (OCH₃), 25.1 (2xCH₃).

2-[4-[[2-Methoxy-4-trimethylaminophenyl)amino]carbonyl]amino]phenoxy-2-methyl propionic acid tetrafluoroborate (3). 2-[4-[[2-Methoxy-4-aminophenyl)amino]carbonyl]amino]phenoxy-2-methyl propionic acid (75 mg, 0.21 mmol) and diethylaniline (109 mg, 0.73 mmol) were dissolved in DMF (1 mL) and then methyl iodide (170 mg, 1.20 mmol) was added. The reaction was left with stirring for 3 days. It was filtered, the filtrate was concentrated to dryness and dried on oil pump. Then, it was dissolved in water (20 mL), and 5% NaOH was added to pH~13. Such mixture was extracted with ethyl acetate (15 x 10 mL). The water phase was concentrated on evaporator until precipitate appeared. Then, the solution was acidified with HBF₄ to pH~1. The precipitate was filtered, washed with H₂O and dried on oil pump. Yield - 36 mg (36%); m.p. 188°C (H₂O); ¹H NMR (300 MHz, DMSO-d₆) 9.33 (s, 1 H, NH), 8.48 (s, 1 H, NH), 8.28 (d, J = 9.1 Hz, 1H), 7.54 (d, J = 2.6 Hz, 1 H), 7.42 (dd, J = 9.1, 2.6 Hz, 1 H), 7.31 (d, J = 8.9 Hz, 2 H), 6.79 (d, J = 8.9 Hz, 2 H), 3.98 (s, 3 H, OCH₃), 3.57 (s, 9 H, N(CH₃)₃), 1.44 (s, 6 H, 2xCH₃); ¹³C NMR (75 MHz, DMSO-d₆)

175.0 (CO₂H), 152.3 (CO), 150.3, 148.1, 147.7, 133.7, 130.3, 119.9 (2xC),
119.3 (2xC), 117.1, 112.2, 103.8 (C-Ar), 78.8 (CCO₂H), 60.9 (OCH₃), 56.5
(N(CH₃)₃), 25.0 (2xCH₃).