POTENTIAL NOOTROPIC AGENTS: SYNTHESIS OF SOME 1,4-DISUBSTITUTED 2-OXOPYRROLIDINES AND SOME RELATED COMPOUNDS

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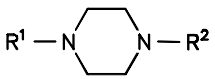
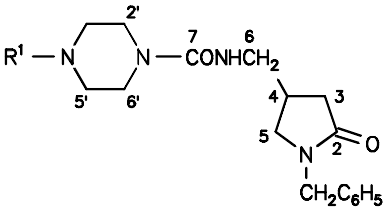
4-(Aminomethyl)-1-benzyl-2-oxopyrrolidine (*VI*) was transformed by treatment with (4-benzhydrylpiperazin-1-yl)carbonyl chlorides *IIIb – IIId* and with (4-methylpiperazin-1-yl)carbonyl chloride (*IIIa*) to the carboxamides *IVa – IVd*. Heating of 1-(ethoxycarbonylmethyl)-2,4-dioxopyrrolidine (*XIX*) in acetonitrile in the presence of water afforded *XVIIIa*. Treatment with ammonia led to the diamide *XVIIIc*, while alkaline hydrolysis of *XVIIIa* gave the dicarboxylic acid *XVIIIb*. 4-(Aminomethyl)-1-(4-methylthiobenzyl)-2-oxopyrrolidine (*XII*) was prepared by the reaction of 4-(methylthio)benzylamine with itaconic acid and the following sequence of reactions starting from the obtained carboxylic acid *VII* including esterification, reduction and treatment the obtained alcohol *IX* with thionyl chloride, synthesis of phthalimido derivative *XI* and hydrazinolysis. Amine *XII* added to 4-chlorophenyl isocyanate formed *XIII*. The compounds prepared were tested for nootropic activity.

In recent communications we dealt with series of 2-oxopyrrolidin-1-ylacetic acid piperazides[1] and some amides and choline derivatives[2] as potential nootropic agents and their pharmacological effects. Our present communication concerns some of the derivatives of 1,4-disubstituted 2-oxopyrrolidines synthesized within the scope of our investigation.

In this series (4-hydroxy-2-oxopyrrolidin-1-yl)acetamide (oxiracetam, Neuromet®) (*XVII*, ref.[3]) and 4-(aminomethyl)-1-benzyl-2-oxopyrrolidine (nebracetam fumarate, WEB-1881, Memolog®) (*VI*, ref.[4]) were described as nootropic drugs. Our preceeding experience with piperazine derivatives of 2-oxopyrrolidines led us to synthesize some piperazine derivatives in a series of 1,4-disubstituted 2-oxopyrrolidines. 4,4′-Difluorobenzhydryl chloride and 2,3′-dichlorobenzhydryl chloride, respectively, reacted with 1-ethoxycarbonylpiperazine in the presence of a catalytic amount of potassium iodide and an excess of potassium carbonate in boiling *N*,*N*-dimethylformamide to afford compounds *Ic* and *Id*, respectively. Reaction of 2,3′-dichlorobenzhydryl chloride with





I, $R^2 = COOC_2H_5$

II, $R^2 = H$

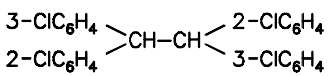
III, $R^2 = COCl$

IV

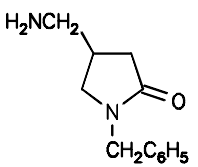
In formulae *I–IV*:

a, $R^1 = CH_3$; *b*, $R^1 = CH(C_6H_5)_2$; *c*, $R^1 = CH(4\text{-}FC_6H_4)_2$

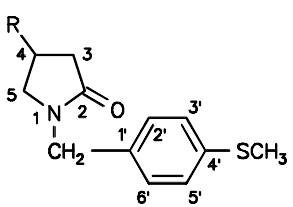
d, $R^4 = CH(2\text{-}ClC_6H_4)(3\text{-}ClC_6H_4)$



V



VI, Nebracetam^R

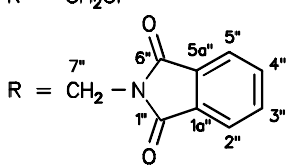


VII, R = COOH

VIII, $R = COOC_2H_5$

IX, $R = CH_2OH$

X, $R = CH_2Cl$

XI, 

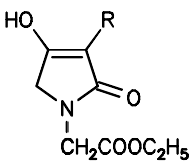
XII, $R = CH_2NH_2$

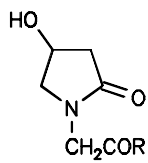
XIII, $R = CH_2NHCONH(4\text{-}ClC_6H_5)$

1-ethoxycarbonylpiperazine in *N*,*N*-dimethylformamide led to the isolation of a crystalline by-product, identified as 1,2-bis(2-chlorophenyl)-1,2-bis(3-chlorophenyl)ethane (*V*). The same compound but with a different melting point was previously prepared by the reaction of 2,3′-dichlorobenzhydryl chloride with 2-(4-methyl-piperazin-1-yl)ethanol and sodium hydride in dioxane[5]. Discrepancy in the melting points may be explained by a different ratio of the racemate and the *meso*-form in the product.

The piperazines *IIb* – *IId* were prepared from the 1-ethoxycarbonyl derivatives *Ib* (ref.[6]), *Ic* and *Id* by refluxing with alcoholic potassium hydroxide. The substituted (4-benzhydrylpiperazin-1-yl)carbonyl chlorides *IIIb* – *IIId* were obtained by treatment of the piperazines *IIb* – *IId* with excess of phosgene. Carbonyl chlorides *IIIa* (ref.[7]) and *IIIb* – *IIId* reacting with the amine *VI* in equimolar amounts in the presence of triethylamine in boiling dioxane afforded compounds *IVa* – *IVd*.

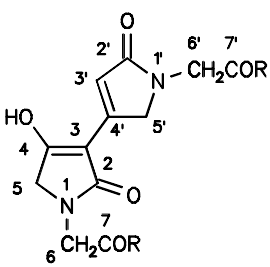
Refluxing 4-(methylthio)benzylamine[8] with an equimolecular amount of itaconic acid in xylene afforded the acid *VII*. The sequence of esterification, reduction and treat-

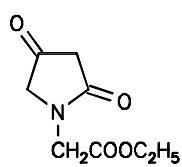




XIV, R = $COOC_2H_5$
XV, R = H

XVI, R = OC_2H_5
XVII, R = NH_2 (Oxiracetam^R)





XVIIIa, R = OC_2H_5
XVIIIb, R = OH
XVIIIc, R = NH_2

XIX

ment the obtained alcohol *IX* with thionyl chloride led to chloride *X*. Its reaction with potassium phthalimide in *N*,*N*-dimethylformamide at 150 – 152 °C yielded *XI*, which treated with hydrazine hydrate, gave the amine *XII*. By addition of 4-chlorophenyl isocyanate[9] to the amine *XII* in toluene the urea *XIII* was obtained.

3-(Ethoxycarbonyl)-1-(ethoxycarbonylmethyl)-4-hydroxy-2-oxopyrrolidine (*XIV*), an intermediate in the synthesis of oxiracetam[3] (*XVII*), refluxed in acetonitrile in the presence of water for more then 20 min yielded compound *XVIIIa*. The same compound was obtained by keeping a mixture of ethyl (2,4-dioxopyrrolidin-1-yl)acetate (*XIX*) and acetonitrile at room temperature for four months. Refluxing mixture of *XIV* in acetonitrile for a shorter time led to *XIX*, which was reduced with sodium borohydride to ethyl (4-hydroxy-2-oxopyrrolidin-1-yl)acetate (*XVI*). Following reaction of the last mentioned compounds with ammonia afforded oxiracetam[3] (*XVII*). A reaction of *XVIIIa* with ammonia in methanol led to a diamide *XVIIIc*. Alkaline hydrolysis of *XVIIIa* gave the acid *XVIIIb*. Elemental analysis and spectra provide a good evidence for the structure *XVIIIa* and in this way also for the related structures *XVIIIb* and *XVIIIc*.

Compounds *IVa – IVd*, *XII*, *XIII*, *XVIIIa – XVIIIc* were tested by the methods of behavioral pharmacology. The detailed results will be reported in a separate communication. Serotonin, muscarine, glutamate and NMDA receptor activity was investigated by methods biochemical pharmacology. Some compounds significantly inhibit the binding of [^{3}H]MK-801 to the brain cortex membranes (compounds were used in concentrations of 1 000 nmol l^{-1}, inhibition of binding in % or IC_{50} are given): *IVb* 85, IC_{50} 38.3 μM; *IVc* 68; *XIII* 42; *XVIIIa* 98; *XVIIIb* 96; *XVIIIc* 34.

The prepared compounds were also tested for antimicrobial activity in vitro. Microorganism and the minimum inhibitory concentrations in mg ml^{-1} are given unless they exceed 150 μg/ml: *Proteus vulgaris*, *IVd* 32; *Streptococcus* D, *IVa* 32; *Streptococcus pyogenes*, *IVa* 64, *IVd* 64, *XVIIIa* 128, *XVIIIb* 128; *Escherichia coli*, *XVIIIb* 128.

EXPERIMENTAL

The melting points of analytical samples were determined on a Kofler block and are uncorrected; the samples were dried in vacuo of about 40 Pa at a room temperature or at a suitably elevated temperature. UV spectra (in methanol), λ_{max} in nm (log ε) were recorded with the Unicam SP 8000 spectrophotometer; IR spectra (wavenumbers in cm^{-1}) with Unicam SP 2000 or Perkin–Elmer 298 spectrophotometers, NMR spectra on a Tesla BS 567A (^{1}H at 100 MHz, ^{13}C at 25.14 MHz) or on a Varian XL-200 (^{1}H at 200,057 MHz, ^{13}C at 50,309 MHz), chemical shifts are given in ppm (δ-scale), coupling constants (*J*) in Hz. The mass spectra were measured on a Varian-MAT 44S (GC-MS) spectrometer. The homogeneity of the products and composition of the mixtures were checked by thin-layer chromatography (TLC) on silica gel (Silufol UV_{254}). Preparative chromatographic separations were carried out on columns of silica gel (Fluka 60). The extracts were dried with Na_2SO_4 or K_2CO_3 and evaporated under reduced pressure on a rotary evaporator.

4,4′-Difluorobenzhydryl Chloride

A solution of 4,4′-difluorobenzhydrol[10] (15.0 g, 68.1 mmol) and thionyl chloride (10.7 g, 90 mmol) in toluene (60 ml) was refluxed for 2 h. The excess of thionyl chloride and toluene was evaporated in vacuo. The residue distilled in vacuo gave 15.2 g (94%) product, b.p. 88 – 90 °C/27 Pa (ref.[11], b.p. 130 – 133 °C/200 Pa).

1-Ethoxycarbonyl-4-(4,4′-difluorobenzhydryl)piperazine (*Ic*)

A mixture of 1-(ethoxycarbonyl)piperazine (10.0 g, 63 mmol), 4,4′-difluorobenzhydryl chloride (15.2 g, 64 mmol) and triethylamine (13.0 g, 128 mmol) in chloroform (100 ml) was refluxed for 6 h, cooled and washed with 0.1 M aqueous tartaric acid (100 ml) and water (100 ml), dried and evaporated. The residue was crystallized from cyclohexane (30 ml) to give 11.5 g (49%) of *Ic*, m.p. 120 – 123 °C. IR spectrum (Nujol): 830 (2 adjacent Ar–H), 1 120, 1 220, 1 243, (COOR); 1 502, 1 600, 1 685 (NCOOR); 2 760, 2 805 (N–CH), 3 030, 3 050, 3 070, 3 105 (Ar). ^{1}H NMR spectrum ($CDCl_3$, 100 MHz): 3.24 t, 3 H, (C–CH_3, J = 7); 2.32 bt, 4 H (2 × H-3 and H-5 from piperazine); 3.48 bt, 4 H (2 × H-2 and H-6 from piperazine); 4.11 q, 2 H (OCH_2CH_3, J = 7); 4.22 s, 1 H (Ar_2CH–N); 6.98 t, 4 H (2 × H-3 and H-5 from 4-FC_6H_4, J(H,H) = 9; J(H,F) = 9); 7.35 dd, 4 H (2 × H-2 and H-6 from 4-FC_6H_4, J(H,H) = 9; J(H,F) = 6). For $C_{20}H_{22}F_2N_2O$ (360.4) calculated: 66.65% C, 6.15% H, 10.54% F, 7.77% N; found: 66.62% C, 6.32% H, 10.25% F, 7.57% N.

1-Ethoxycarbonyl-4-(2,3′-dichlorobenzhydryl)piperazine (*Id*)

A mixture of 2,3′-dichlorobenzhydryl chloride[5] (19.0 g, 70 mmol), 1-(ethoxycarbonyl)piperazine (13.5 g, 85 mmol), anhydrous potassium carbonate (13.8 g, 100 mmol) and potassium iodide (0.3 g) in *N*,*N*-dimethylformamide (60 ml) was stirred at 145 °C for 5 h and left overnight at a room temperature. The solid was filtered off and the solution evaporated in vacuo. Oily residue (29.9 g) was chromatographed on silica gel (Fluka). Elution with chloroform gave first 1.27 g less polar fraction, m.p. 185 – 189 °C. Repeated crystallization from benzene gave 0.9 g (6%) of 1,2-bis(2-chlorophenyl)-1,2-bis(3-chlorophenyl)ethane (*V*), m.p. 195 – 200 °C. Mass spectrum, *m*/*z*(%): 470 (M^+, $C_{26}H_{18}Cl_4$, 0.1), 237 (62), 235 (100), 200 (23), 199 (23), 165 (69). UV spectrum: 261 (3.03), 266.5 (3.13), 274.5 (3.02). IR spectrum (Nujol) : 690, 776, 750, 883, (3 and 4 adjacent and solitary Ar–H); 1 569, 1 590, 3 005, 3 050 (Ar). For $C_{26}H_{18}Cl_4$ (472.2) calculated: 66.12% C, 3.84% H, 30.03% Cl; found: 65.84% C, 3.89% H, 29.99% Cl. The next fraction (chloroform) provided 29.9 g (80%) oily crude *Id* which was used for the next step.

1-(4,4′-Difluorobenzhydryl)piperazine (*IIc*)

A solution of *Ic* (15.0 g, 41 mmol) and potassium hydroxide (16.0 g, 280 mmol) in ethanol (25 ml) was refluxed for 3 h. Ethanol was evaporated and the residue distributed between water (30 ml) and toluene (160 ml). The organic layer was washed with brine (50 ml) and evaporated. The residue was triturated with petroleum ether (25 ml) to yield crystalline *IIc* (8.4 g, 71%), m.p. 85 – 91 °C. Analytical sample m.p. 91 – 94 °C (hexane). ^{1}H NMR spectrum ($CDCl_3$, 100 MHz): 1.58 bs, 1 H, (NH); 2.34 bt, 4 H (2 × H-2 and H-6 of piperazine); 2.90 bt, 4 H (2 × H-3 and H-5 of piperazine); 4.20 s, 1 H (Ar_2CH); 6.98 t, 4 H (2 × H-3 and H-5 from 4-FC_6H_4, J(H,H) = J(H,F) = 8); 7.32 dd, 4 H (2 × H-2 and H-5 from 4-FC_6H_4, J(H,H) = 8, J(H,F) = 6). ^{13}C NMR spectrum ($CDCl_3$, 25.14 MHz): 46.31 t (C-3 and C-5 of piperazine); 53.26 t (C-2 and C-6 of piperazine); 74.99 d (Ar_2CH); 115.37 d (C-2 from 4-FC_6H_4, J(F,C) = 20.6); 129.37 d (C-3 from 4-FC_6H_4, J(F,C) = 7.5); 138.19 s (C-1 from 4-FC_6H_4); 161.83 s (C-4 from 4-FC_6H_4, J(F,C) = 246). For $C_{17}H_{18}F_2N_2$ (288.3) calculated: 70.81% C, 6.29% H, 13.18% F, 9.72% N; found: 71.07% C, 6.31% H, 12.85% F, 9.76% N.

1-(2,3′-Dichlorobenzhydryl)piperazine (*IId*)

A mixture of crude *Id* (5.4 g, 14 mmol) and potassium hydroxide (14.0 g, 210 mmol) in ethanol (15 ml) was stirred and refluxed for 1 h. Ethanol was distilled of and the residue was distributed between benzene (60 ml) and water (20 ml). The benzene layer was evaporated in vacuo to give 4.2 g (94%) oily base *IId*, which was characterized as the maleate hemihydrate, m.p. 157.5 – 160 °C (ethanol). Mass spectrum *m/z*: 320 (M^+, $C_{17}H_{18}Cl_2N_2$, 4), 285 (11), 277 (13), 275 (20), 264 (11), 240 (12), 237 (29), 235 (50), 165 (32). For $C_{21}H_{22}Cl_2N_2O_4$. 0.5 H_2O (446.3) calculated: 56.50% C, 5.19% H, 15.88% Cl, 6.28% N; found: 56.30% C, 4.91% H, 15.77% Cl, 6.06% N.

(4-Benzhydrylpiperazin-1-yl)carbonyl Chloride (*IIIb*)

A solution of *IIb* (ref.[6]; 14.4 g, 57 mmol) in chloroform (70 ml) was added dropwise to a stirred solution of phosgene (8.9 g, 900 mmol) in chloroform (16 ml) at –1 °C for 1.5 h. The mixture was stirred at 0 °C for another 1.5 h. The precipitate was filtered and washed twice with hexane (25 ml); 17.6 g crude product, m.p. 120 – 145 °C. It was dissolved in dichloromethane (200 ml), washed with 50% aqueous K_2CO_3 (10 ml) and water (10 ml) and evaporated in vacuo. The residue was dissolved in benzene (20 ml) and precipitated with) hexane (5 ml). A by product was collected (4.1 g, m.p. 180 – 221 °C). The filtrate was evaporated, the residue was dissolved in hexane (20 ml) and precipitated with petroleum ether (20 ml) giving 6.4 g (43%) of *IIIb*, m.p. 133 – 135 °C (ref.[5], m.p. 140 – 141 °C).

[4-(4,4′-Difluorobenzhydryl)piperazin-1-yl]carbonyl Chloride (*IIIc*)

A solution of *IIc* (8.0 g, 27.8 mmol) in chloroform (40 ml) was added dropwise into a solution of phosgene (6.6 g, 670 mmol) in chloroform (40 ml) at 0 °C over 40 min. The mixture was stirred at 0 °C for 2 h. A precipitated *IIIc* hydrochloride was filtered and washed with ether (15 ml) yield 7.2 g (66%), m.p. 127 – 130 °C. Analytical sample m.p. 127 – 129 °C (chloroform–ether). For $C_{18}H_{18}Cl_2F_2N_2O$ (387.3) calculated: 55.83% C, 4.68% H, 18.31% Cl, 9.80% F, 7.23% N; found: 56.12% C, 4.74% H, 18.57% Cl, 10.10% F, 7.23% N.

[4-(2,3′-Dichlorobenzhydryl)piperazin-1-yl]carbonyl Chloride (*IIId*)

Similar reaction of *IId* (11.4 g, 35.5 mmol) in chloroform (60 ml) with phosgene (10.5 g, 106 mmol) in chloroform (65 ml) afforded 7.5 g (50%) of *IIId* hydrochloride, m.p. 150 – 153 °C. Analytical sample m.p. 153 – 155 °C (chloroform–ether). For $C_{18}H_{48}Cl_4N_2O$ (420.2) calculated: 51.45% C, 4.32% H, 33.75% Cl, 6.67% N; found: 51.68% C, 4.30% H, 33.76% Cl, 6.90% N.

1-Benzyl-4-[(4-methylpiperazin-1-yl)carbonylaminomethyl]-2-oxopyrrolidine (*IVa*)

A solution of *IIIa* (ref.[7]; 3.4 g, 20 mmol) in anhydrous dioxane (25 ml) was dropped into an vigorous stirred suspension of *VI* (ref.[4]; 4.1 g, 20 mmol) and triethylamine (2.0 g, 20 mmol) in anhydrous dioxane (50 ml) over 30 min at 30 °C. The mixture was then stirred and refluxed for 2.5 h and left overnight at room temperature. The solvent was evaporated in vacuo, the oily residue was disolved in ethyl acetate (30 ml), the solution was washed with 50% aqueous K_2CO_3 (10 ml), and twice with 12% brine and precipitated with petroleum ether (20 ml). After standing overnight at a room temperature, the solid product was filtered; 4.7 g (71%), m.p. 104 – 114 °C. Crystallization from ethyl acetate yielded 3.2 g (48%) of pure *IVa*, m.p. 119 – 122 °C. The compound crystallized with a half molecule of ethyl acetate. Crystallization from toluene gave *IVa* melting at 121 – 123 °C. IR spectrum (Nujol): 700, 730 (5 adjacent Ar–H); 1 482, 3 030, 3 050, 3 075 (Ar); 1 530, 1 666 (NCONHR); 1 632 (NC=O in the ring); 2 740, 2 780 (NCH_3). ^1H NMR spectrum ($CDCl_3$, 200 MHz): 2.26 s, 3 H

(CH_3N); 2.29 – 2.56 m, 7 H; 3.01 dd, 1 H (J = 10.7, J' = 4.2, H-5); 3.14 t, 1 H (J = 6,6); 3.24 – 3.38 m, 6 H; 4.30 and 4.48, 2 H (AB system, J = 15.2, $ArCH_2$); 5.73 t, 1 H (NH, J = 5.8); 7.15 – 7.36 m, 5 H (Ar–H). ^{13}C NMR spectrum ($CDCl_3$, 50.3 MHz): 31.4 d (C-4); 34.9 t (C-3); 43.4 t (C-2′ and C-6′); 43.9 t (C-5); 45.8 q (CH_3N); 46.2 t (C-6); 49.9 t (NCH_2Ar); 54.4 t (C-3′and 5′); 127.5 d (C-3 and C-5 of benzyl); 127.3 d (C-4 of benzyl); 128.4 d (C-2 and C-6 of benzyl); 135.9 s (C-1 of benzyl); 157.6 s (C-7); 173.6 s (C-2). For $C_{18}H_{26}N_4O_2$ (330.4) calculated: 65.42% C, 7.93% H, 16.96% N; found: 65.63% C, 7.99% H, 16.79% N.

4-(4-Benzhydrylpiperazin-1-ylcarbonylaminomethyl)-1-benzyl-2-oxopyrrolidine (*IVb*)

A mixture of *IIIb* (4.4 g, 14 mmol), *VI* (2.9 g, 14 mmol), and triethylamine (2.1 g, 21 mmol) in anhydrous dioxane (40 ml) was refluxed for 3.5 h. After cooling, the solid was collected and the filtrate evaporated. The oily residue was chromatographed on a column of silica gel (60 g). Elution with dichloromethane gave 4.5 g (67%) base *IVb*, m.p. 164 – 165 °C (benzene). IR spectrum (Nujol): 708, 740 (5 adjacent Ar–H); 1 250, 1 490, 1 599, 1 604, 3 025, 3 060, 3 080 (Ar–H); 1 519, 1 643 (NCONH), 1 679 (N–CO in the ring); 3 408 (NH). Mass spectrum, *m*/*z*: 482 (M^+, $C_{30}H_{34}N_4O_2$, 0.01), 315 (0.1), 230 (29), 207 (37), 168 (62), 91 (99), 56 (100). ^{1}H NMR spectrum ($CDCl_3$, 100 MHz): 7.10 – 7.50 m, 15 H (Ar–H); 4.86 bt, 1 H (NH); 4.50 and 4.30, 2 H (AB system, J = 14, $PhCH_2N$); 4.22 s, 1 H (Ar_2CH–N). ^{13}C NMR spectrum ($CDCl_3$, 25.14 MHz): 30.11 t (C-3); 31.60 d (C-4); 43.85 t (C-2′ and C-6′); 44.14 t (C-5); 46.46 t (C-6); 50.12 t (CH_2 of benzyl); 51.46 t (C-3′and C-5′); 75.89 d (Ar_2CH); 127.13 d (C-4 of benzhydryl); 127.58 d (C-4 of benzyl); 127.88 d, 128.55 d, 128.70 d (C-2, C-3, C-5 and C-6 of benzhydryl); 136,20 s (C-1 of benzyl); 142.22 s (C-1 of benzhydryl); 157.83 s (C-7); 173.82 s (C-2). For $C_{30}H_{34}N_4O_2$ (482.6) calculated: 74.65% C, 7.10% H, 11.61% N; found: 74.78% C, 7.31% H, 11,43% N.

1-Benzyl-4-[4-(4,4′-difluorobenzhydryl)piperazin-1-ylcarbonylaminomethyl]-2-oxopyrrolidine (*IVc*)

A similar procedure starting from *VI* (ref.[4]; 1.9 g, 9.5 mmol) and *IIIc* (3.4 g, 9.5 mmol) in dioxane (35 ml) gave 5.4 g of crude oily product which was chromatographed on silica gel (50 g). Elution with chloroform saturated with ammonia gave 2.0 g (40%) of pure base *IVc*, m.p. 180 – 183 °C. Analytical sample m.p. 179 – 181 °C (benzene–hexane). IR spectrum (Nujol): 707, 749, 831 (5 and 2 adjacent Ar–H); 1 220 (Ar–F); 1 519, 1 575 (NCONH); 1 646 (NRCO in five membered cycle); 1 502, 1 600, 3 030, 3 065 (Ar); 3 395 (NH). ^{1}H NMR spectrum ($CDCl_3$, 200 MHz): 2.18 – 2.55 m, 7 H; 2.97 dd, 1 H (J = 10.2, J' = 4.3; H-5); 3.11 t, 1 H (J = 6.2); 3.22 t, 1 H (J = 5.6); 3.32 m, 5 H; 4.19 s, 1 H (Ar_2CH); 4.29 and 4.45, 2H (AB system, J = 14.8, $PhCH_2$); 5.17 t, 1 H (J = 5.7, NH); 6.95 dd, 4 H, J(H,H) = 8.9, J(H,F) = 8.9, 2 × H-3 and H-5 from 4-FC_6H_4); 7.14 – 7.34 m, 5 H (ArH of benzyl); 7.29 dd, 4 H (J(H,H) = 8.9, J(H,F) = 2.8, 2 × H-2 and H-6 from 4-FC_6H_4). ^{13}C NMR spectrum ($CDCl_3$, 50.3 MHz): 31.6 d (C-4); 35.1 t (C-3); 43.8 t (C-2′ and C-6′); 44.1 t (C-5); 46.4 t (C-6); 50.1 t (C-6); 51.3 t (C-3′, C-5′); 74.2 d (Ar_2CH); 115.4 d (2 × C-3 and C-5 from 4-FC_6H_4, J(F,C) = 21.2); 127.5 d (C-4 from $PhCH_2$); 127.9 d (C-3 and C-5 from $PhCH_2$); 128.6 d (C-2 and C-6 from $PhCH_2$); 129.1 d (2 × C-2 and C-6 from 4-FC_6H_4, J(C,F) = 7.7); 136.2 s (C-1 of $PhCH_2$); 137.7 s (2 × C-1 of 4-FC_6H_4, J(C,F) = 3.2); 157.7 s (C-7); 161.8 s (2 × C-4 of 4-FC_6H_4, J(C,F) = 245.9); 173.8 s (C-2). For $C_{30}H_{32}F_2N_4O_2$ (518.6) calculated: 69.48% C, 6.22% H, 7.33 F, 10.80% N; found: 69.43 % C, 6.25% H, 7.05% F, 10.58% N.

1-Benzyl-4-(4-(2,3′-dichlorobenzhydryl)piperazin-1-ylcarbonylaminomethyl)-2-oxopyrrolidine (*IVd*)

A similar procedure starting from *IIId* (6.5 g, 16.9 mmol) and *VI* (ref.[4]; 3.5 g, 16.9 mmol) in dioxane (60 ml) gave 10.1 g of crude product. Similar processing like in the preparation of *IVc* yielded 3.5 g

(38%) of *IVd*, m.p. 147 – 152 °C. Analytical sample m.p. 152 – 155 °C (benzene–hexane). IR spectrum (Nujol): 700, 736, 756, 780, 886 (5, 4 and 2 adjacent and solitary Ar–H); 1 487, 1 569, 1 590, 3 010, 3 050 (Ar); 1 530, 1 670 (NCONH); 1 623 (NCO in the ring); 3 330 (NH). ^{1}H NMR spectrum ($CDCl_3$, 100 MHz): 4.28 and 4.48 2 H (AB system, J = 13.0, $PhCH_2$); 4.82 s, 1 H (Ar_2CH); 4.86 bt, 1 H (NH); 7.00 – 7.80 m, 13 H (Ar–H). ^{13}C NMR spectrum ($CDCl_3$, 25.14 MHz): 31.67 d (C-4); 35.18 t (C-3); 43.85 t (C-2′, C-6′); 44.22 t (C-5); 46.54 t ($PhCH_2$); 50.20 t (C-6); 51.39 t (C-3′, C-5′); 69.62 d (Ar_2CH); 126.61 d; 127.36 d (C-4 of $PhCH_2$); 127.65 d (C-2 and C-6 of $PhCH_2$); 128.03 d, 128.33 d, 128.78 d (C-3 and C-5 of $PhCH_2$); 129.90 d, (C-5 of 3-ClC_6H_4); 133.93 s (C-2 of 2-ClC_6H_4); 134.45 s (C-3 of 3-ClC_6H_4); 136.39 s (C-1 of $PhCH_2$); 138.86 s (C-1 of 2-ClC_6H_4); 142.99 s (C-1 of 3-ClC_6H_4); 157.76 s (C-7); 173.89 s (C-2). For $C_{30}H_{32}Cl_2N_4O_2$ (551.5) calculated: 65.33% C, 5.85% H, 12.86% Cl, 10,16% N; found: 64.96% C, 5.71% H, 12.80% Cl, 9.99% N.

1-(4-Methylthio)benzyl-2-oxopyrrolidine-4-carboxylic Acid (*VII*)

A solution of 4-methylthiobenzylamine[8] (5.1 g, 33 mmol) and itaconic acid (4.3 g, 33 mmol) in xylene (30 ml) was refluxed for 3 h. Water (0.6 ml) was separated, the mixture was cooled and extracted with 10% NaOH (45 ml). The alkaline extract was washed with ether and treated with concentrated hydrochloric acid; the precipitate was collected and washed with water; yield 8.4 g (95%) of crude *VII*, m.p. 141 – 144 °C. Analytical sample m.p. 143.5 – 144.5 °C (ethanol). IR spectrum (Nujol): 784 (2 solitary Ar-H); 967, 1 269, 1 276, 1 709, 2 500 (RCOOH); 1 491, 1 500 (Ar); 1 619 (RNC=O in the five-membered ring). IR spectrum (chloroform): 1 675 (RNC=O); 1 719 (RCOOH). ^{1}H NMR spectrum ($CDCl_3$, 100 MHz): 2.50 s, 3 H (S–CH_3); 4.42 and 4.23 d, 2 H (AB system, J = 13, $ArCH_2$); 7.18 d, 2 H (J = 8.5, H-3′, H-5′); 7.30 d, 2 H (H-2′, H-6′, J = 8.5). ^{13}C NMR spectrum (CD_3SOCD_3, 25.14 MHz): 14.79 q (S–CH_3); 33.54 t (C-3); 35.33 d (C-4); 44.97 t (C-5); 48.33 t (C-6); 126.27 d (C-3′, C-5′); 128.48 d (C-2′, C-6′); 133.41 s (C-1′); 137.22 s (C-4′); 172.10 s (C-2); 174.56 s (COOH). For $C_{13}H_{15}NO_3S$ (265.3) calculated: 58.84% C, 5.70% H, 5.28% N, 12.09% S; found: 58.71% C, 5.80% H, 5.20% N, 11.88% S.

Ethyl 1-(4-Methylthio)benzyl-2-oxopyrrolidin-4-carboxylate (*VIII*)

Anhydrous HCl was introduced into a stirred boiling solution of *VII* (8.0 g, 30 mmol) in ethanol (90 ml) for 2 h. The reaction mixture was evaporated in vacuo, the residue was dissolved in dichloromethane (40 ml) and the solution was washed with 10% $NaHCO_3$ (15 ml) and water (2 × 30 ml). The organic layer was evaporated in vacuo and gave 8.8 g of crude liquid, which was distilled. A fraction, collected at 192 – 196 °C/47 Pa, crystallized; 7.9 g (90%), m.p. 38 – 41 °C. ^{1}H NMR spectrum ($CDCl_3$, 100 MHz): 1.26 t, 3 H (J = 7, CH_3 in Et); 2.72 m, 2 H (H-3); 3.18 m, 1 H (H-4); 3.45 d, 2 H (J = 6, H-5); 2.50 s, 3 H (CH_3S); 4.16 q, 2 H (J = 7, CH_2 in Et); 4.52 s, 2 H (H-6); 7.12 and 7.21, 4 H (AB system, J = 8.5, H-2′, H-3′, H-5′, H-6′). ^{13}C NMR spectrum ($CDCl_3$, 25.14 MHz): 14.04 q (CH_3 in Et); 15.76 q (CH_3S); 33.99 t (C-3); 36.06 d (C-4); 46.01 t (C-5); 48.46 t (C-6); 61.32 t (CH_2 in Et); 126.91 d (C-3′, C-5′); 128.70 d (C-2′, C-6′); 132.81 s (C-4′); 138.11 s (C-1′); 172.40 s (COOR); 172.70 s (C-2). For $C_{15}H_{19}NO_3$ (293.4) calculated: 61.40% C, 6.53% H, 4.77% N, 10.93% S; found: 61.15% C, 6.69%% H, 4.83% N, 11.03% S.

1-(4-Methylthio)benzyl-4-hydroxymethyl-2-oxopyrrolidine (*IX*)

A cool solution of sodium borohydride (3.1 g, 82 mmol) in water (30 ml) was dropped into a solution of ester *VIII* (10.0 g, 34 mmol) in methanol (100 ml) at 0 °C. The reaction mixture was stirred at 4 °C for 4 h, for 1 h at 10 °C and then left to reach laboratory temperature. After acidification at

10 °C with acetic acid to pH 5.5, the mixture was evaporated in vacuo to dryness and the residue distributed between dichloromethane (50 ml) and water (50 ml). The organic layer was evaporated; yield 8.1 g (95%), m.p. 77 – 82 °C. Analytical sample m.p. 83 – 85 °C (benzene). IR spectrum (Nujol): 791, 822 (2 adjacent Ar–H); 1 027, 1 057 (CH_2OH); 1 492, 1 562, 3 013, 3 075 (Ar); 1 643 (N–CO in the ring); 3 265 (OH). UV spectrum (methanol): 256 (4.14). ^{1}H NMR spectrum ($CDCl_3$, 100 MHz): 2.20 – 2.60 m, 3 H (H-3, H-4); 2.50 s, 3 H (CH_3S); 3.20 m, 2 H (H-5); 3.58 m, 2 H (CH_2–O); 4.41 s, 2 H (CH_2Ar); 7.12 and 7.26, 4 H (AB system, J = 8.5, H-2′, H-3′, H-5′, H-6′). ^{13}C NMR spectrum ($CDCl_3$, 25.14 MHz): 15.69 q (CH_3S); 33.09 d (C-4); 33.91 t (C-3); 46.09 t (C-6); 49.22 t (C-5); 64.31 t (CH_2–O); 126.76 d (C-3′, C-5′); 128.63 d (C-2′, C-6′); 133.03 s (C-4′); 137.89 s (C-1′); 174.27 s (C-2). For $C_{13}H_{17}NO_2S$ (251.4) calculated: 62.12% C, 6.82% H, 5.57% N, 12,76% S; found: 61.95% C, 7.10% H, 5.56 % N, 12.95% S.

1-(4-Methylthio)benzyl-4-phthalimidomethyl-2-oxopyrrolidine (*XI*)

A solution of *IX* (6.7 g, 27 mmol) and $SOCl_2$ (3.6 g, 30 mmol) in dichloromethane (50 ml) was refluxed for 7 h. The solvent was evaporated, the oily residue dissolved in benzene and evaporated; yield 7.2 g (100%) crude chloride *X* which was used without characterization for the next step. Crude chloride *X* (7.2 g) and potassium phthalimide (5.0 g, 27 mmol) in *N*,*N*-dimethylformamide (80 ml) were stirred and refluxed for 2.5 h. The reaction mixture was evaporated in vacuo, the residue (13.4 g) was distributed between dichloromethane and water, the organic layer was filtered with charcoal and evaporated. The solid residue (7.8 g, 77%, m.p. 126 – 130 °C) was crystallized from toluene–petroleum ether and yielded 5.4 g (53%) of *XI*, m.p. 131 – 133 °C (crystalline modification A). UV spectrum (methanol): 217 (4.66), 239 (4.14), 256 (4.17), 288 inflex (3.50). IR spectrum (Nujol): 732, 795, 810, 830 (4 and 2 adjacent Ar–H); 1 484, 1 493, 1 563, 1 600, 1 610, 3 010, 3 028, 3 050 (Ar); 1 672 (N–CO in five membered cycle); 1 702, 1 769 (CO–N–CO in the ring). ^{1}H NMR spectrum ($CDCl_3$, 100 MHz): 2.20 – 2.50 m, 2 H (H-3); 2.48 s, 3 H (CH_3S); 2.80 m, 1 H (H-4); 3.22 m, 2 H (H-5); 3.72 d, 2 H (J = 7, H-7″); 4.12 and 4.55, 2 H (AB system, J = 14, Ar–CH_2); 7.12 and 7.24, 4 H (AB system, J = 8.5, H-2′, H-3′, H-5′, H-6′); 7.80 m, 4 H (Ar–H from phthalimide). ^{13}C NMR spectrum ($CDCl_3$, 25.14 MHz): 15.69 q (CH_3S); 31.22 d (C-4); 35.18 t (C-3); 41.01 t (C-9″); 46.01 t (C-5); 49.82 t (C-6); 123.40 d (C-2″, C-5″); 126.76 d (C-3′, C-5′); 128.63 d (C-2′, C-6′); 131.69 s (C-4′); 133.03 s (C-1a″, C-5a″); 134.15 d (C-3″, C-4″); 137.81 s (C-1′); 168.29 s (C-1″, C-6″); 173.07 s (C-2). For $C_{21}H_{20}N_2O_3$ (380.5) calculated: 66.29% C, 5.30% H, 7.36% N, 8.43% S; found: 66.58% C, 5.25 % H, 7.61% N, 8.56% S.

Crystallization from toluene provided a crystalline modification B (37%) m.p. 144 – 146 °C. For $C_{21}H_{20}N_2O_3$ (380.5) calculated: 66.29% C, 5.30% H, 7.36% N, 8.43% S; found: 66.46% C, 5.47% H, 7.32% N, 8.73% S.

4-(Aminomethyl)-1-(4-methylthio)benzyl-2-oxopyrrolidine (*XII*)

A suspension of *XI* (5.0 g, 13 mmol) in ethanol (100 ml) was intensively stirred at room temperature and 100% hydrazine hydrate (2.6 g, 52 mmol) was added. A clear solution was formed within 15 min. The reaction mixture was stirred for 4 h at 20 °C. During this time a white solid precipitated was filtered off and the filtrate was evaporated in vacuo. The oily residue (3.3 g) was dissolved in methanol (10 ml), the boiling solution was treated with fumaric acid (1.6 g) and the salt was precipitated with ether (20 ml). The crude hemifumarate of *XII* (2.7 g, 68%, m.p. 195 – 198 °C) was recrystallized from methanol giving 2.6 g (65%) of the pure product, m.p. 201 – 203 °C. Mass spectrum, *m*/*z* (%): 250 (M^+, $C_{13}H_{18}N_2OS$, 13), 233 (8), 205 (7), 152 (10), 137 (100), 122 (18), 91 (10), 74 (18), 72 (21). For $C_{15}H_{20}N_2O_3S$ (308.4) calculated: 58.41% C, 6.54% H, 9.08% N, 10.40% S; found: 58.28% C, 6.84% H, 8.89% N, 10.47% S.

4-((4-Chlorophenylaminocarbonyl)aminomethyl)-1-(4-(methylthio)benzyl)-2-oxopyrrolidine (*XIII*)

A solution of 4-chlorophenylisocyanate[10] (2.1 g, 14 mmol) in toluene (15 ml) was dropped over 10 min into a stirred solution of *XII* (3.4 g, 14 mmol) in toluene (20 ml) at 60 °C. After 40 min stirring at 80 °C, the mixture was cooled to 20 °C, the product was precipitated by addition of petroleum ether (20 ml). The solid was filtered at 10 °C and washed with petroleum ether; yield 4.8 g (87%), m.p. 144 – 147 °C. Analytical sample m.p. 146 – 147.5 °C (benzene). IR spectrum (Nujol): 819, 831 (2 H adjacent Ar–H); 1 490, 1 600, 3 020, 3 030, 3 050, 3 095 (Ar); 1 550, 1 658 (ArNH-CONH); 1 658 (N–CO in the five membered ring); 3 300 (NH). ^{1}H NMR spectrum ($CDCl_3$, 100 MHz): 2.00 – 3.20 m; 2.40 s, 3 H (CH_3S); 4.36 bs, 2 H (Ar–CH_2–N); 6.08 bt, 1 H (C–NHCO); 7.12, 4 H and 7.20, 4 H (Ar–H); 7.86 s, 1 H (Ar–NH–CO). For $C_{20}H_{22}ClN_3O_2S$ (403.9) calculated: 59.46% C, 5.49% H, 8.78% Cl, 10.40% N, 7,94% S; found: 59.16% C, 5.39% H, 8.82% Cl, 10.30% N, 8.15% S.

3-(1-(Ethoxycarbonylmethyl)-2-oxo-3-pyrrolin-4-yl)-1-(ethoxycarbonylmethyl)-4-hydroxy-2-oxo-3-pyrroline (*XVIIIa*)

A) A suspension of *XIV* (ref.[3]; 41.0 g, 161 mmol) in acetonitrile (400 ml) and water (3.6 ml) was stirred, gradually warmed during 0.5 h and refluxed for further 0.5 h. The solvent was evaporated under reduced pressure. The solid residue was crystallized from ethanol (100 ml); yield 16.1 g (57 %) of *XVIIa*, m.p. 190 – 192 °C. Analytical sample m.p. 194 – 195 °C (methanol). Mass spectrum, *m*/*z* (%): 352 (M^+, $C_{16}H_{20}N_2O_7$). IR spectrum (Nujol): 1 251, 1 738 (R–COOR); 1 677 (R–N–CO–C=C– in the ring); 1 620 (C=C); 2 480 (C–OH in a conjugated system). ^{1}H NMR spectrum (CD_3SOCD_3, 200 MHz): 1.20 t, 6 H (J = 6.9, 2 × CH_3 in Et); 4.06 – 4.20 m, 10 H (2 × CH_2 in Et, H-6, H-6′, H-5); 4.39 s, 2 H (H-5′); 6.36 s, 1 H (H-3′). ^{13}C NMR spectrum (CD_3SOCD_3, 50.3 MHz): 14.3 q (2 × CH_3 in Et); 42.7 t, 43.1 t (C-6, C-6′); 50.4 t (C-5); 53.7 t (C-5′); 61.0 t (2 × CH_2 in Et); 99.2 s (C-3); 116.0 d (C-3′); 147.9 s (C-4′); 169.8 s (C-7, C-7′); 170.2 s, 171.9 s, 172.7 s (C-2, C-2′, C-4). For $C_{16}H_{20}N_2O_7$ (352.4) calculated: 54.53% C, 5.72% H, 7.95% N; found: 54.28% C, 5.79% H, 7.78 % N.

B) A suspension of *XIX* (22.0 g, 86 mmol) in acetonitrile (200 ml) was allowed to stand for 4 month at room temperature. The crude product (5.2 g, 17%, m.p. 190 – 191 °C) was crystallized from methanol (40 ml), m.p. 195 – 197 °C. IR and ^{1}H NMR spectra were identical with those of the product prepared by method *A*.

3-(1-(Carboxymethyl)-2-oxo-3-pyrrolin-4-yl)-4-hydroxy-2-oxo-3-pyrroline-1-acetic Acid (*XVIIIb*)

A mixture of *XVIIIa* (9.3 g, 26 mmol) and potassium hydroxide (5.6 g, 10 mmol) in 90% ethanol (150 ml) was refluxed for 2 h. After cooling, the solid was filtered, extracted with chloroform (100 ml) and filtered again. The crude solid potassium salt of *XVIIIb* (9.8 g) was suspended in water (50 ml) and treated with concentrated hydrochloric acid (14 ml) over 0.5 h at 35 °C under vigorous stirring. The precipitate was filtered and washed four times with water (20 ml) until pH 4.5. Crystallization of the crude product (7.7 g) from 50% ethanol (80 ml) gave 7.1 g (91%) acid *XVIIIb*, m.p. 260 °C (decomposition). ^{1}H NMR spectrum (CD_3SOCD_3, 200 MHz): 4.08 s, 4 H (H-6, H-6′); 4.13 s, 2 H (H-5); 4.40 s, 2 H (H-5′); 6.36 s, 1 H (H-3′); 11.59 bs, 2 H (2 × COOH). ^{13}C NMR spectrum (CD_3SOCD_3, 50.3 MHz): 43.0 t (C-6′); 43.3 t (C-6); 50.7 t (C-5); 54.1 t (C-5′); 99.4 s (C-3); 116.1 d (C-3′); 148.2 s (C-4′); 170.5 s, 171.5 s, 172.4 s, 172.9 s (C-2, C-2′, C-4, C-7, C-7′). For $C_{12}H_{12}N_2O_7$ (296.2) calculated: 48.65% C, 4.09% H, 9.46% N; found: 48.44% C, 4.16% H, 9.46 % N.

3-(1-(Carbamoylmethyl)-2-oxo-3-pyrrolin-4-yl)-2-oxo-4-hydroxy-3-pyrroline-1-acetamide (*XVIIIc*)

Ethyl ester *XVIIIa* (4.7 g, 13 mmol) was stepwise added into methanol (300 ml) saturated with ammonia at 0 °C. The clear solution was stirred at 0 °C for 4 h and left overnight at room temperature. The reaction mixture was evaporated in vacuo to give 4.7 g solid residue. The product was extracted with ethanol (2 × 50 ml) which provided 3.1 g (80%) of *XVIIIc*, m.p. 280 °C (decomposition). IR spectrum (Nujol): 1 668 (RNC=O in the ring); 3 190, 3 370 ($CONH_2$). ^{1}H NMR spectrum (CD_3SOCD_3, 200 MHz): 3.68 s, 2 H; 3.81 s, 2 H; 3.88 s, 2 H (H-6, H-6′ H-5); 4.32 s, 2 H (H-5′); 4.66 bs, 3 H (OH and H_2O); 6.05 s, 1 H (H-3′); 7.00 s, 2 H and 7.31 s, 2 H (2 × NH_2). ^{13}C NMR spectrum (CD_3SOCD_3, 50.3 MHz): 39.3 t, 44.4 t (C-6, C-6′); 53.2 t (C-5); 54.3 t (C-5′); 95.3 s (C-3); 108.6 d (C-3′); 151.0 s (C-4′); 171.3 s, 171.7 s (C-7, C-7′); 172.8 s, 173.4 s (C-2, C-2′); 181.4 s (C-4). For $C_{12}H_{14}N_4O_5 \cdot H_2O$ (312.3) calculated: 46.15% C, 5.16% H, 17.94% N; found: 46.27% C, 5.16% H, 17.86% N.

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