HETEROCYCLES, Vol. 60, No. 4, 2003, pp. 833 - 842
Received, 9th December, 2002, Accepted, 17th February, 2003, Published online, 3rd March, 2003

SYNTHESIS AND BIOLOGICAL EVALUATION OF TRIAZOLO[4,5-*g*]QUINOLINES, IMIDAZO[4,5-*g*]QUINOLINES AND PYRIDO[2,3-*g*]QUINOXALINE. Part II[#]

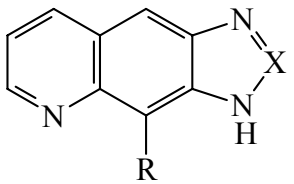
Antonio Carta,*[a] Gianpiero Boatto,[a] Giuseppe Paglietti,[a] Graziella Poni,[b] Maria Giovanna Setzu,[b] and Paolo Caredda[b]

[a]Department of Medicinal and Toxicological Chemistry, University of Sassari, Via Muroni 23, 07100 Sassari, Italy

[b]Department of Experimental Biology-Division of Microbiology, University of Cagliari, Cittadella Universitaria, 09174 Monserrato, Italy

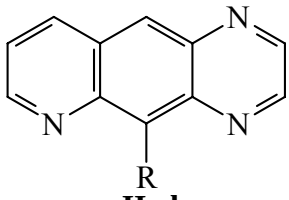
Abstract – Synthesis of triazolo[4,5-*g*]quinolines, imidazo[4,5-*g*]quinolines and pyrido[2,3-*g*]quinoxaline has been described. Antimycobacterial, antibacterial and antimycotic activity were also reported. Compounds (**14**) and (**IIb**) exhibited an interesting antimycobacterial activity (against *M. tuberculosis* and *M. smegmatis*).

In continuation of our research program devoted to the discovery of pharmacological activities on new three ring heterocycles,[1-7] we recently described the synthesis of imidazo[4,5-*g*]- (**Ia,b**) and triazolo[4,5-*g*]quinolines (**Ic,d)** and pyrido[2,3-*g*]quinoxalines (**IIa,b**) of Figure 1.[8]



Ia-d

a) R = H, X = CH;
b) R = Cl, X = CH;
c) R = H, X = N;
d) R = Cl, X = N



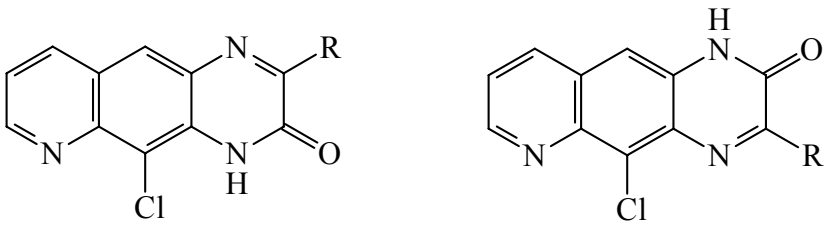
IIa,b

a) R = H;
b) R = Cl;

Figure 1

[#]*Dedicated to the memory of Professor Paolo Sanna[a], died on the 28th March 2002.*

As first application of molecular modification of compounds (**II**) for pharmacological purposes, we have described a series of pyrido[2,3-*g*]quinoxalin-2(3)-ones (**IIIa-d,f,g** and **IIIb-g**) of Figure 2 that showed an encouraging *in vitro* antibacterial and anticandida activities.[9]



IIIa-d,f,g **IIIb-g**

a) R = CH_3; **b**) R = CF_3; **c**) R = CH_2CH_3; **d**) R = $CH(CH_3)_2$;
e) R = CH_2Br; **f)** R = $CO_2CH_2CH_3$; **g**) R = $CH(CH_3)CO_2CH_2CH_3$.

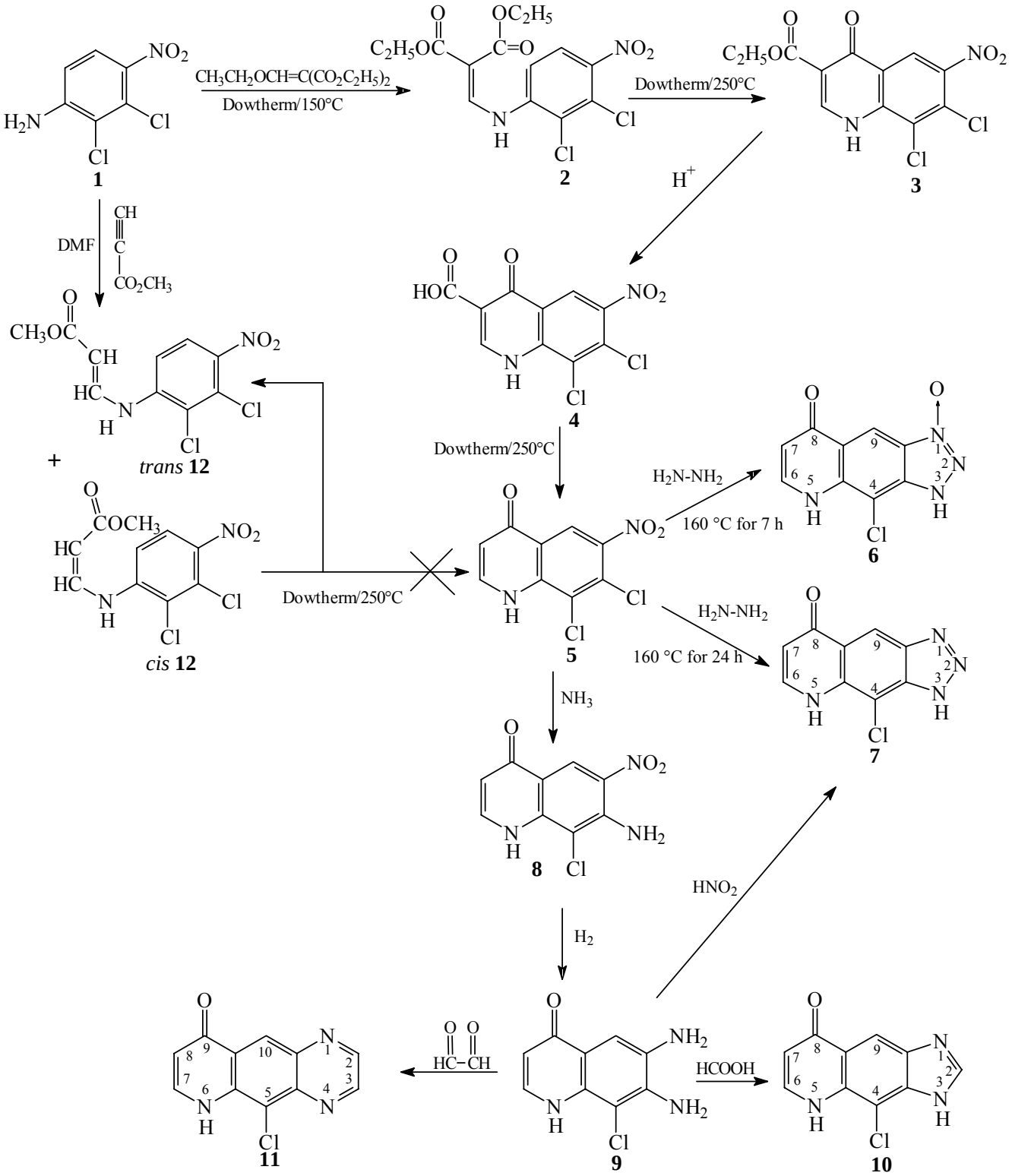
Figure 2

Now, in connection with this research program we have synthesised new linear three ring heterocycles applying the previously reported procedure.[8] According to the route depicted in Scheme 1 we were able to obtain the triazolo[4,5-*g*]quinolines (**6,7**), imidazo[4,5-*g*]quinoline (**10**) and pyrido[2,3-*g*]quinoxaline (**11**), while *via* Scheme 2 we obtained the pyrido[2,3-*g*]quinoxaline (**14**) and 2,3-dihydro-1*H*-imidazo[4,5-*g*]quinoline (**15**). The multistep reactions reported deserve some comments.

The key intermediate (**1**), obtained according to a described procedure,[8] was reacted with diethyl ethoxy-methylenemalonate (EMME) in Dowtherm at 150°C to give the anilinoacrylate (**2**) that in turn underwent ring closure to **3** at 250°C in the same solvent. After its acidic hydrolysis into **4,** the latter was decarboxylated in Dowtherm at 250°C to give the quinolone (**5**). A parallel route to afford **5** was also explored. From the addition of **1** upon methyl propiolate we obtained a mixture of *trans*/*cis* isomer in 1:1 ratio (61%) accompanied by a moderate yield (17%) of pure *trans* **12**. The attempted thermal cyclization of the above Michael adduct mixture failed, but we could observe that an interconversion of *cis* component into *trans* isomer had taken place thus preventing ring closure.

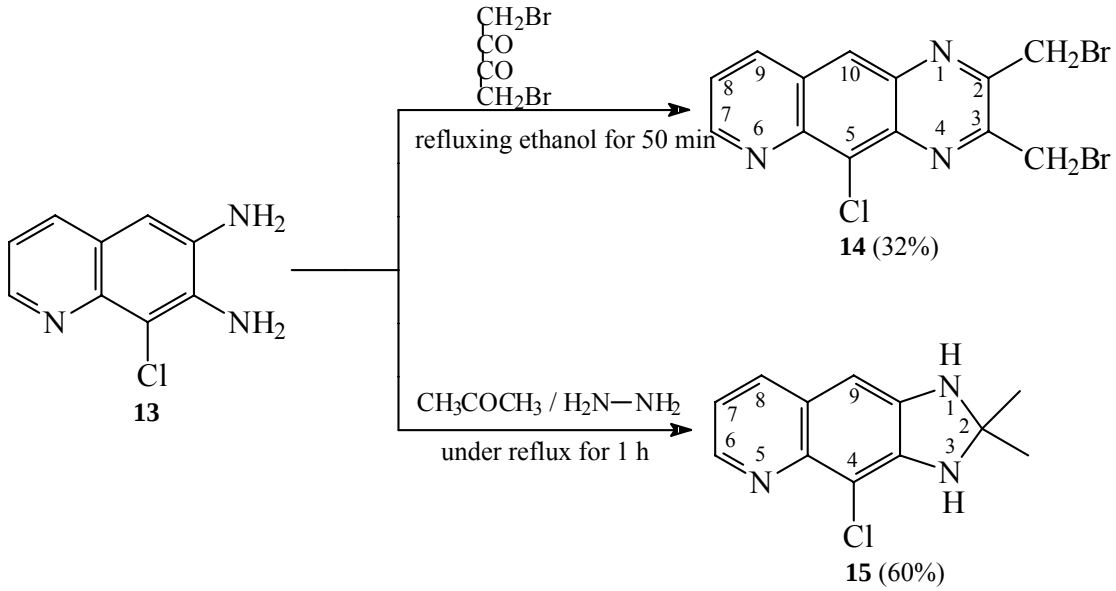
Treatment of compound (**5**) with hydrazine hydrate under varying conditions gave us the chance to obtain either the triazolo[4,5-*g*]quinolin-8-one–N_1-oxide (**6**) or the triazolo[4,5-*g*]quinolin-8-one (**7**). In fact heating a mixture of **5** with a strong excess of hydrazine for 7 h at 160°C in a sealed steel vessel we obtained compound (**6**) in 57% yield, while the same reaction after a prolonged time (24 h) gave compound (**7**) in 35% yield. From these results it seems evident that formation of **7** was due to the reducing conditions coming from the decomposition of hydrazine. The intermediate (**5**) underwent nucleophilic displacement of 6-chlorine atom with ethanolic ammonia to give compound (**8**) then converted into the diamine (**9**) by treatment with palladium-charcoal and hydrogen under moderate

pressure. Ring closure of **9** either with formic acid or glyoxal gave the novel imidazo[4,5-*g*]quinolin-8-one (**10**) or the pyrazino[2,3-*g*]quinolin-9-one (**11**) in fair yields whereas with nitrous acid we reobtained compound (**7**) identical to the above specimen.



Scheme 1

In addition, we have investigated the reaction of the previously described diamine (**13**)[8] with 1,4-dibromo-butane-2,3-dione that afforded compound (**14**) in moderate yield (Figure 2). It is interesting to note that during the preparation of **13** by reduction of the nitro group with hydrazine hydrate a small amount of a stable unknown compound was also formed. In fact when the residue of evaporation of the reaction mixture was taken up with acetone a different solid from the diamine (**13**) was separated after column chromatography. The compound was identified as the 2,3-dihydro-1*H*-imidazo[4,5-*g*]quinoline (**15**) by its ^{1}H and ^{13}C-NMR spectra and MS spectral analysis. In the light of this result, we submitted the diamine (**13**) to reaction with a strong excess of acetone and hydrazine under reflux for 1 h and compound (**15**) was obtained in 60% yield. The latter result is surprising since usually oxidation to imidazole takes place during cyclization and dihydroimidazolines are formed and isolated in particular circumstances.[10]



Scheme 2

MICROBIOLOGICAL ASSAYS

All the new (**6**, **7**, **10**, **11**, **14**, and **15**) and the previously reported compounds (**Ia-d** and **IIb**) were evaluated *in vitro* for antimycobacterial activity (against *M. tuberculosis* ATCC 27294 and *M. smegmatis* ATCC 19420), against representative strains of Gram-positive and Gram-negative bacteria (*S. aureus* and *Salmonella spp* clinical isolates), and for antimycotic activity (against *C. albicans* ATCC 10231 and *A. fumigatus* clinical isolate). Ciprofloxacin, Isoniazid, Streptomycin and Miconazole were used as reference drugs. In antimycobacterial assays the minimum inhibitory concentrations (MICs) were assessed in microtiter plates by adding 20 μL aliquots of a culture suspension to 80 μL of Middlebrook 7H9 medium containing serial dilutions of test compounds. At the end of incubation, the number of

viable mycobacteria was determined by the MTT method.[11] For antibacterial and antimycotic activity, the assays were carried out in Triptosio agar for *S. aureus*, *Salmonella spp* and Sabouraud dextrose broth for *C. albicans* and *A. fumigatus*, with an inoculum of 10^3 bacteria/mL and $5x10^3$ yeast/mL. *A. fumigatus* inocula were obtained from cultures grown at 37 °C for 1 days and then diluting to 0.05 OD_{50}/mL. MICs were determined after incubations at 37 °C for 18 h in the presence of serial dilutions of test compounds. Furthermore, all compounds were evaluated for cytotoxicity against MT-4 cells by the MTT method.[12]
In antibacterial and antimycotic tests only compound (**14**) showed moderate activity against *C. albicans* (MIC = 33.3 μM), while in antimycobacterial assays the compounds (**14**) and (**IIb**) exhibited an interesting activity against *M. tuberculosis* (MIC_{50} = 22.9 and 6.0 μM respectively) and *M. smegmatis* (MIC_{50} = 9.8 and 9.5 μM respectively). However, compound (**IIb**) exhibited low citotoxicity against MT-4 cells (CC_{50} = 82 μM), whereas **14** resulted moderately citotoxic (CC_{50} = 16 μM). None of compounds showed interesting antiproliferative activity (in citotoxicity assay against MT-4 cells) CC_{50} ranging beetwen 16 and >100 μM. In conclusion, from these results seem to emerge that the pyrido[2,3-*g*]quinoxaline nucleus is a good lead for the development of new antitubercular agents.

EXPERIMENTAL

Melting points are uncorrected and were taken in open capillaries in a Digital Electrothermal IA9100 melting point apparatus. IR spectra were recorded as nujol mulls or film on a Perkin Elmer 781 spectrophotometer and are expressed in cm^{-1}. UV spectra are qualitative and were recorded in nm for ethanol solution with a Perkin-Elmer Lambda 5 spectrophotometer. ^{1}H-NMR spectra were recorded at 200 MHz using a Varian XL-200 spectrometer. Chemical shifts are reported in ppm (δ) relative to tetramethylsilane as internal standard. MS spectra of **2-5**, **8**, **9**, **12** and **15** were performed on a combined HP 5790 (GC)-HP 5970 (MS) apparatus, while the products (**6**, **7**, **10**, **11** and **14**) were performed with a combined Liquid Chromatograph-Agilent 1100 series Mass Selective Detector (MSD). Column chromatography was performed using 70-230 mesh (Merck silica gel 60). The progress of the reactions and the purity of the final compounds were monitored by TLC using Merck F-254 commercial plates. Light petroleum refers to the fraction with bp 40-60°C. Elemental analyses were performed at the Laboratorio di Microanalisi, Dipartimento di Scienze Farmaceutiche, University of Padua-Italy.

Preparation of diethyl [(2,3-dichloro-4-nitrophenyl)amino]methylenemalonate (2). To the amine (**1**) (3.0 g, 14.5 mmol) prepared as described,[8] suspended in Dowtherm (45 g) at 50°C, an excess of EMME (4.0 g, 18.5 mmol) was added. Then, the mixture was heated at 150 °C under stirring for 17 h. On cooling, it was taken up with 300 mL of hexane and stirred for an additional 30 min. The precipitate

formed, was filtered off and washed with diethyl ether to give **2** (4.36 g, 80 %) as a solid, mp 134-135 °C (ether); IR (cm^{-1}): 1690, 1660, 1610, 1590; UV (nm): λ_{max} 347, 259, 209; ^{1}H-NMR (DMSO-d_6): δ 11.61 (d, 1H, *J* = 12.6 Hz, NH), 8.44 (d, 1H, *J* = 12.6 Hz, C*H*=), 7.95 (d, 1H, *J* = 9.2 Hz, H-5), 7.32 (d, 1H, *J* = 9.2 Hz, H-6), 4.35 (m, 4H, 2 CH_2), 1.39 (m, 6H, 2 CH_3); MS: *m/z* 380, 378, 376 (M^+); Anal. Calcd for $C_{14}H_{14}N_2O_6Cl_2$: C, 44.58; H, 3.74; N, 7.43; Cl, 18.90. Found C, 44.71; H, 3.63; N, 7.65; Cl, 18.74.

Preparation of ethyl 7,8-dichloro-6-nitro-4-oxo-1,4-dihydroquinoline-3-carboxylate (3). Compound (**2**) (2.0 g, 5.3 mmol) was suspended in Dowtherm (50 g) and heated at 250 °C under stirring for 2.5 h. On cooling, it was taken up with 200 mL of hexane and stirred for an additional 30 min. The precipitate formed, was filtered off and washed with hexane to give **3** (1.75 g, 100 %) as a solid, mp 335 °C (DMSO); IR (cm^{-1}): 1710, 1610, 1600; UV (nm): λ_{max} 336, 239, 218; ^{1}H-NMR (DMSO-d_6): δ 12.31 (s, 1H, NH), 8.67 (s, 1H, H-5), 8.45 (s, 1H, H-2), 4.27 (q, 2H, *J* = 7.2 Hz, CH_2), 1.30 (t, 3H, *J* = 7.2 Hz, CH_3); MS: *m/z* 334, 332, 330 (M^+); Anal. Calcd for $C_{12}H_8N_2O_5Cl_2$: C, 43.53; H, 2.43; N, 8.46; Cl, 21.41. Found C, 44.21; H, 2.64; N, 8.21; Cl, 21.09.

Preparation of 7,8-dichloro-6-nitro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (4). The ester (**3**) (2.5 g, 7.6 mmol) was dissolved in 250 mL of H_2SO_4 (95-97 %) and heated at 100-110 °C under stirring for 2 h. On cooling, the solution was poured onto crushed ice (500 g) and stirred for an additional 15 min. A precipitate was collected, thoroughly washed with water and eventually dried to give **4** (2.20 g, 96 %) as a solid, mp 299-300 °C (DMSO); IR (cm^{-1}): 1730, 1610, 1550; UV (nm): λ_{max} 332, 240, 196; ^{1}H-NMR (DMSO-d_6): δ 8.78 (s, 1H, H-5), 8.64 (s, 2H, H-2 + NH), 4.00 (s, 1H, COOH); MS: *m/z* 306, 304, 302 (M^+); Anal. Calcd for $C_{10}H_4N_2O_5Cl_2$: C, 39.63; H, 1.33; N, 9.25; Cl, 23.40. Found C, 39.38; H, 1.52; N, 9.04; Cl, 23.09.

Preparation of 7,8-dichloro-6-nitro-4-oxo-1,4-dihydroquinoline (5). The acid (**4**) (1.0 g, 3.0 mmol) was suspended in Dowtherm (60 g) and heated at 250 °C under stirring for 15 h. On cooling, A precipitate was collected and washed with hexane to give **5** (0.77 g, 98 %) as a solid, mp 332-333 °C (DMSO); IR (cm^{-1}): 1665, 1630, 1600, 1590; UV (nm): λ_{max} 380, 326, 264, 222, 195; ^{1}H-NMR ($CDCl_3$+DMSO-d_6): δ 11.85 (s, 1H, NH), 8.65 (s, 1H, H-5), 7.94 (d, 1H, *J* = 6.2 Hz, H-2), 6.27 (d, 1H, *J* = 6.2 Hz, H-3); MS: *m/z* 262, 260, 258 (M^+); Anal. Calcd for $C_9H_4N_2O_3Cl_2$: C, 41.73; H, 1.56; N, 10.82; Cl, 18.53. Found C, 42.06; H, 1.38; N, 10.59; Cl, 18.22.

Preparation of 4-chloro-1,8-dioxo-5,8-dihydro-3*H*-triazolo[4,5-*g*]quinoline (6). A mixture of **5** (1.0 g, 3.9 mmol) and hydrazine hydrate (99%, 3.0 g, 60 mmol) in ethanol (100 mL) was heated in a sealed steel

vessel at 160 °C under stirring for 7 h. The reaction mixture was allowed to reach rt and the solvent was removed *in vacuo*. The solid residue was dissolved in water and the resulting solution was made acidic by HCl (37 %) to give a precipitate of **6** (0.52 g, 56 %) as a solid, mp 310-320 °C (ethanol, decomp); IR (cm^{-1}) : 3340, 1710, 1640, 1580; UV (nm): λ_{max} 388, 370, 329, 296, 231, 206; ^{1}H-NMR (DMSO-d_6): δ 8.43 (s, 1H, H-9), 8.13 (d, 1H, *J* = 7.4 Hz, H-6), 6.26 (d, 1H, *J* = 7.4 Hz, H-7); LC/MS: 237 (M+H), 259 (M+Na); Anal. Calcd for $C_9H_5N_4O_2Cl$: C, 45.68; H, 2.13; N, 23.68; Cl, 14.98. Found C, 46.06; H, 1.99; N, 23.50; Cl, 15.01.

Preparation of 4-chloro-8-oxo-5,8-dihydro-3*H*-triazolo[4,5-*g*]quinoline (7).

Method A: from **5** using hydrazine hydrate.

An identical run as reported for preparation of **6**, was reacted for 24 h. The work-up of the reaction mixture gave an oily residue that was taken up with 15 mL of acetone and stirred for 30 min. A solid was collected and dried to give **7** (0.30 g, 35 %) mp > 320 °C (ethanol); IR (cm^{-1}): 1630, 1600, 1570; UV (nm): λ_{max} 394, 379, 325, 294, 246, 226; ^{1}H-NMR (DMSO-d_6): δ δ 11.60 (s, 1H, NH),8.62 (s, 1H, H-9), 7.98 (d, 1H, *J* = 6.2 Hz, H-6), 6.11 (d, 1H, *J* = 6.2 Hz, H-7); LC/MS: 221 (M+H), 243 (M+Na); Anal. Calcd for $C_9H_5N_4OCl$: C, 48.99; H, 2.28; N, 25.40; Cl, 16.07. Found C, 49.27; H, 2.09; N, 25.31; Cl, 15.84.

Method B: from **9** using sodium nitrite.

A solution of sodium nitrite (1.0 g, 144 mmol) in water (8 mL) was added dropwise to an ice-cooled stirred solution of **9** (0.3 g, 14.3 mmol) in 2 M hydrochloric acid aqueous solution (10 mL). After the addition was complete, the mixture was allowed to warm at rt and stirring continued for an additional 4 h. The resulting precipitate was filtered off to give **7** (0.14 g, 44 %), identical with an authentic specimen as above described.

Preparation of 7-amino-8-chloro-6-nitro-4-oxo-1,4-dihydroquinoline (8). A solution of **5** (1.0 g, 3.9 mmol) in ethanol saturated with dry gaseous ammonia (100 mL) was heated in a sealed steel vessel at 160 °C under stirring for 24 h. Then the reaction mixture was cooled to rt, the solvent was removed *in vacuo* and the solid residue purified by column chromatography over silica gel, using a 95:5 mixture of ether-ethanol as eluent, to yield **8** (0.81 g, 87 %) as solid, mp > 330 °C (DMSO); IR (cm^{-1}): 3390, 3190, 1640, 1610, 1570; UV (nm): λ_{max} 373, 285, 247, 222; ^{1}H-NMR (DMSO-d_6): δ 11.20 (s, 1H, NH), 8.75 (s, 1H, H-5), 7.75 (d, 1H, *J* = 7.6 Hz, H-2), 7.46 (s, 2H, NH_2), 6.00 (d, 1H, *J* = 7.6 Hz, H-3); MS: *m/z* 241, 239 (M^+); Anal. Calcd for $C_9H_6N_3O_3Cl$: C, 45.11; H, 2.52; N, 17.54; Cl, 14.80. Found C, 45.27; H, 2.48; N, 18.00; Cl, 14.67.

Preparation of 6,7-diamino-8-chloro-4-oxo-1,4-dihydroquinoline (9). A suspension of **8** (1.0 g, 4.2 mmol) and 10% palladium-charcoal (0.20 g) in ethanol (150 mL) was hydrogenated in Parr at 20-25 °C and 3 atm for 3 h. After filtration of the catalyst, the solvent was evaporated *in vacuo* and the oily residue obtained was taken up with 15 mL of acetone. The precipitate obtained, filtered off and dried, afforded **9** (0.76 g, 88 %) as solid, mp 140-141 °C (ethanol); IR (cm^{-1}): 3360-3100, 1650, 1640, 1610, 1570; UV (nm): λ_{max} 332, 244, 231, 198; ^{1}H-NMR (DMSO-d_6): δ 10.77 (s, 1H, NH), 7.55 (d, 1H, *J* = 7.4 Hz, H-2), 7.18 (s, 1H, H-5), 5.83 (d, 1H, *J* = 7.4 Hz, H-3), 5.70 (s, 2H, NH_2), 5.10 (s, 2H, NH_2); MS: *m/z* 211, 209 (M^+); Anal. Calcd for $C_9H_8N_3OCl$: C, 51.56; H, 3.85; N, 20.04; Cl, 16.91. Found C, 51.22; H, 4.09; N, 20.20; Cl, 17.17.

Preparation of 4-chloro-8-oxo-5,8-dihydro-3*H*-imidazo[4,5-*g*]quinoline (10). A mixture of **9** (0.5 g, 2.4 mmol) and an excess of formic acid (9.8 g, 212 mmol) was stirred at 100 °C for 2 h. After cooling to rt, the solution was cautiously neutralised with 50% sodium hydroxide aqueous solution untill pH ≅ 5. A precipitate was filtered off, washed with ethanol and dried to give **10** (0.35 g, 66 %) as solid, mp > 300 °C (DMSO); IR (cm^{-1}): 1620, 1590, 1570; UV (nm): λ_{max} 360, 350, 307, 262, 246, 224, 200; ^{1}H-NMR (DMSO-d_6): δ 8.60 (s, 1H, H-9), 8.33 (s, 1H, H-2), 7.94 (d, 1H, *J* = 5.8 Hz, H-6), 6.09 (d, 1H, *J* = 5.8 Hz, H-7), 4.58 (br s, 1H, NH); LC/MS: 220 (M+H), 242 (M+Na), 258 (M+K); Anal. Calcd for $C_{10}H_6N_3OCl$: C, 54.68; H, 2.75; N, 19.13; Cl, 16.14. Found C, 54.30; H, 3.01; N, 18.84; Cl, 15.91.

Preparation of 5-chloro-9-oxo-6,9-dihydropyrido[2,3-*g*]quinoxaline (11). Glyoxal (6.0 g, 41.3 mmol, 40% in water) was slowly dropwise added, under stirring, to a refluxing solution of **9** (0.47 g, 2.24 mmol) in ethanol (12 mL) and stirring continued for an additional 1 h. After cooling the resulting solution was evaporated to dryness *in vacuo* to give an oily residue. Purification of this was accomplished by flash chromatography on silica gel using a 9:1 mixture of ether-ethanol as eluent to give **11** (0.34 g, 65 %) as solid, mp > 300 °C (DMSO); IR (cm^{-1}): 1730, 1660, 1610, 1590; UV (nm): λ_{max} 413, 270, 237, 206; ^{1}H-NMR (DMSO-d_6): δ 11.61 (s, 1H, NH), 9.14 (s, 1H, H-3), 9.07 (s, 1H, H-2), 8.76 (s, 1H, H-10), 8.06 (d, 1H, *J* = 7.6 Hz, H-7), 6.20 (d, 1H, *J* = 7.6 Hz, H-8);); LC/MS: 232 (M+H), 254 (M+Na); Anal. Calcd for $C_{11}H_6N_3OCl$: C, 57.03; H, 2.61; N, 18.14; Cl, 15.31. Found C, 57.29; H, 2.60; N, 18.00; Cl, 18.41.

Preparation of 5-chloro-2,3-dibromomethylpyrido[2,3-*g*]quinoxaline (14). To a solution of 6,7-diamino-8-chloroquinoline (**13**)[8] (0.35 g, 1.8 mmol) in ethanol (12 mL), 1,4-dibromobutane-2,3-dione (0.53 g, 2.2 mmol) was added under stirring, and the mixture was refluxed for 50 min. After evaporation of the solvent the crude solid was crystallized by acetone obtaining **14** (0.23 g, 32 %) as solid, mp > 300

°C; IR (cm^{-1}): 1610, 1580; UV (nm): λ_{max} 374, 356, 272, 243, 207; ^{1}H-NMR ($CDCl_3$): δ 9.27 (dd, 1H, *J* = 4.2 and 1.6 Hz, H-7), 8.60 (s, 1H, H-10), 8.48 (d, 1H, *J* = 8.4 Hz, H-9), 7.62 (dd, 1H, *J* = 8.4 and 4.2 Hz, H-8), 5.07 (s, 2H, C*H*$_2$Br), 5.01 (s, 2H, C*H*$_2$Br);); LC/MS: 402 (M+H), 424 (M+Na); Anal. Calcd for $C_{13}H_8N_3Br_2Cl$: C, 38.89; H, 2.01; N, 10.47; Cl, 8.83. Found C, 40.12; H, 2.17; N, 1021; Cl, 8.51.

Preparation of 4-chloro-2,2-dimethyl-2,3-dihydro-1*H*-imidazo[4,5-*g*]quinoline (15). A solution of 6,7-diamino-8-chloroquinoline (**13**)[8] (0.50 g, 2.6 mmol) and hydrazine hydrate (99%, 1.0 g, 20 mmol) in acetone (20 mL) was stirred under reflux for 1 h. After evaporation of the solvent the crude solid was purified by column chromatography over silica gel, using a 4:3 mixture of ether-light petroleum as eluent, to yield **15** (0.35 g, 60 %) as solid, mp 175-176°C (acetone); IR (cm^{-1}): 3440, 3410, 1640, 1610; UV (nm): λ_{max} 359, 241, 209; ^{1}H-NMR ($CDCl_3$): δ 8.61 (d, 1H, *J* = 4.2 Hz, H-6), 7.74 (d, 1H, *J* = 7.8 Hz, H-8), 7.10 (dd, 1H, *J* = 7.8 and 4.2 Hz, H-7), 6.47 (s, 1H, H-9), 4.67 (s, 1H, NH), 4.43 (s, 1H, NH), 1.59 (s, 6H, 2 CH_3); MS: *m/z* 235, 233 (M^+); Anal. Calcd for $C_{12}H_{12}N_3Cl$: C, 61.67; H, 5.18; N, 17.98; Cl, 15.17. Found C, 61.52; H, 5.04; N, 18.29; Cl, 15.36.

Reaction of 2,3-dichloro-4-nitroaniline (1) with methyl propiolate. To a solution of **1**[8] (0.5 g, 2.42 mmol) in N,N-dimethylformamide (5 mL) were dropwise added, under stirring at rt, methyl propiolate (1.1 mL, 12.1 mmol) and triethylamine (0.2 mL, 1.4 mmol). Then the mixture was heated at 70 °C for 15 h. On cooling, the solution was poured into water (50 mL) and extracted with dichloromethane (30 mL for 3 times). The solvent of the resulting solution was removed *in vacuo* and the solid residue was purified by flash chromatography on silica gel using a 7:3 mixture of ether-light petroleum as eluent, to give in the order, a mixture of about 1:1 ratio (^{1}H-NMR evidence) of *cis*- and *trans*-methyl [(2,3-dichloro-4-ntrophenyl)amino]propenoate (**12**) (0.43 g, 61 %) and an additional amount of pure *trans* **12** (0.12g, 17 %) as solid, mp 188-189 °C (ether); IR (cm^{-1}): 3380, 1710, 1660, 1500; UV (nm): λ_{max} 292, 216, 208; ^{1}H-NMR ($CDCl3_3$): δ 7.87 (d, 1H, *J* = 8.6 Hz, H-5′), 7.73 (d, 1H, *J* = 13.8 Hz, H-2), 7.37 (d, 1H, *J* = 8.6 Hz, H-6′), 4.66 (d, 1H, *J* = 13.8 Hz, H-3), 3.71 (s, 3H, OCH_3); MS: *m/z* 294, 292, 290 (M^+); Anal. Calcd for $C_{10}H_8N_2O_4Cl_2$: C, 41.26; H, 2.77; N, 9.62; Cl, 24.36. Found C, 41.44; H, 2.58; N, 10.01; Cl, 24.11.

Attemps of thermal cyclization of the *cis*-*trans* mixture of 12. A mixture of about 1:1 ratio of *cis* and *trans* **12** (0.20g, 0.69 mmol) was suspended in Dowtherm (5 g) and heated at 250 °C under stirring for 1 h. On cooling, it was taken up with 30 mL of hexane, the precipitate formed, was filtered off and washed with hexane to give *trans* **12** (0.18 g, 90 %), identical with an authentic specimen as above described.

REFERENCES

1. A. Nuvole, P. Sanna, G. Paglietti, C. Juliano, S. Zanetti, and P. Cappuccinelli, *Il Farmaco*, 1989, **44**, 619.
2. P. Sanna and G. Paglietti, *Il Farmaco*, 1989, **44**, 609.
3. P. Sanna, A. Carta, G. Paglietti, S. Zanetti, and G. Fadda, *Il Farmaco*, 1992, **47**, 1001.
4. P. Sanna, P.A. Sequi, and G. Paglietti, *Il Farmaco*, 1995, **50**, 47.
5. P. Sanna, P.A. Sequi, S. Piras, and G. Paglietti, *Heterocycles* , 1995, **41**, 2459.
6. P. Sanna, A. Carta, and G. Paglietti, *Heterocycles* , 1999, **50**, 693.
7. P. Sanna, A. Carta, and G. Paglietti, *Heterocycles* , 1999, **51**, 2171.
8. P. Sanna, A. Carta, and G. Paglietti, *Heterocycles*, 2000, **53**, 423.
9. A. Carta, P. Sanna, L. Gherardini, D. Usai and S. Zanetti, *Il Farmaco*, 2001, **56**, 993.
10. D. M. Smith, Dihydrobenzimidazoles, Benzimidazoles, Benzimidazolethiones and Related Compounds, in The Chemistry of Heterocyclic Compounds, Benzimidazoles and Congeneric Tricyclic Compounds, ed. by P. N. Preston, Vol. 40, part 1, p. 331, J. Willey and Sons-Interscience, New York, 1981.

11 J. W. Baker and H.B. Hopkins, *J. Chem. Soc*., 1949, 1089.

12 R. Pauwels, J. Balzarini, M. Baba, R. Snoeck, D. Schols, P. Herdewijn, J. Desmyster, and E. De Clercq, *J. Virol. Methods*, 1988, **20** 309.