ELSEVIER

www.elsevier.com/locate/farmac

Il Farmaco 56 (2001) 933–938

IL FARMACO

# Novel functionalized pyrido[2,3-*g*]quinoxalinones as antibacterial, antifungal and anticancer agents☆

Antonio Carta [a], Paolo Sanna [a,*], Laura Gherardini [a], Donatella Usai [b], Stefania Zanetti [b]

[a] *Dipartimento Farmaco Chimico Tossicologico, via Muroni 23/a, 07100 Sassari, Italy*
[b] *Dipartimento di Scienze Biomediche, viale S. Pietro, 07100 Sassari, Italy*

Received 15 February 2001; accepted 30 May 2001

**Abstract**

A series of twelve novel pyrido[2,3-*g*]quinoxalinones (**3**–**14**), variously substituted at the C-3 position, was synthesized, structurally determined and submitted to a preliminary in vitro evaluation for antibacterial, anticandida and anticancer activities. Results of the antimicrobial screening showed that all compounds, with the exception of **6**, **11** and **12**, exhibited interesting activity against all strains tested; while compound **10** was found to have encouraging in vitro anticancer activity at a concentration of $10^{-4}$ M. 

*Keywords:* Pyrido[2,3-*g*]quinoxalinones; Antibacterial; Anticandida; Anticancer activities

## 1. Introduction

Intercalation represents one of the most applied strategies in anticancer therapy [2]. Polycyclic molecules having a large planar structure like acridines, amsacrines, aminoanthraquinones and related tricyclic systems are generally used for this purpose. Moreover, a number of heterocyclic quinones, derived from the simplification of streptonigrin, have been reported to possess a wide spectrum of pharmacological activities, including antimicrobial and anticancer activities [3–6]. A bioreductive alkylation mechanism together with an intercalation was suggested for these molecules [6,7]. As part of our interest for the chemistry and the biological properties of the quinoxaline nucleus and its derivatives, we have reported the synthesis and the antimicrobial and anticancer activities of more than 100 derivatives, the structure of which is summarized in Fig. 1. Among them, various terms exhibited interesting anticandida and antiproliferative activities, particularly when $R = CF_3$ or $CH_2Br$, and a withdrawing group is present in the 6 or 7 position [8–10]. In connection with this research project we have thought that an extension of quinoxaline with an extra pyridine ring might be profitable for a potential intercalating activity of our quinoxalinones. Thus, we have preliminarily explored the chemistry of the new heterocycle pyrido[2,3-*g*]quinoxaline of Fig. 2 [11] that purposely functionalized would be suitable to our aim.

In this context we have examined the preparation of a series of 5-chloropyrido[2,3-*g*]quinoxalinones depicted in Scheme 1, in order to verify if this extension of the linear cyclic system could positively influence the promising antifungal and anticancer activity previously recorded. Substituents in the pyrazine ring of the molecular skeleton ($CH_3$, $CH_2CH_3$, $CH(CH_3)_2$, $CF_3$, $CH_2Br$, $CO_2Et$, $CH(CH_3)CO_2Et$) were selected among those which exhibited good activity in previous research.

## 2. Chemistry

The preparation of pyridoquinoxalinones (**3**–**14**), depicted in Scheme 1, was carried out by condensation

☆ Part of this work was presented as a poster communication at the XVI International Symposium on Medicinal Chemistry, Bologna, Italy, 18–22 September 2000 [1].

* Corresponding author.
*E-mail address:* psanna@ssmain.uniss.it (P. Sanna).

0014-827X/01/$ - see front matter 
PII: S0014-827X(01)01161-2

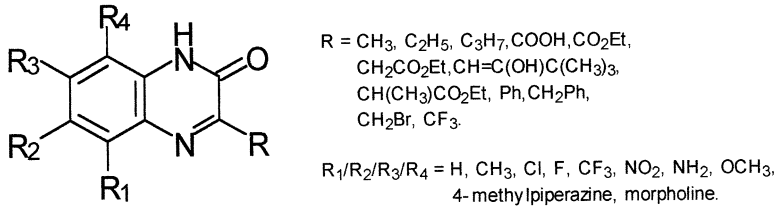



Fig. 1. Chemical structure of quinoxalinones.

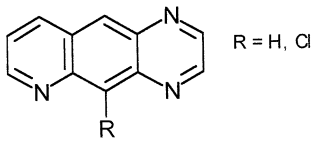



Fig. 2. Chemical structure of pyrido[2,3-*g*]quinoxalines.

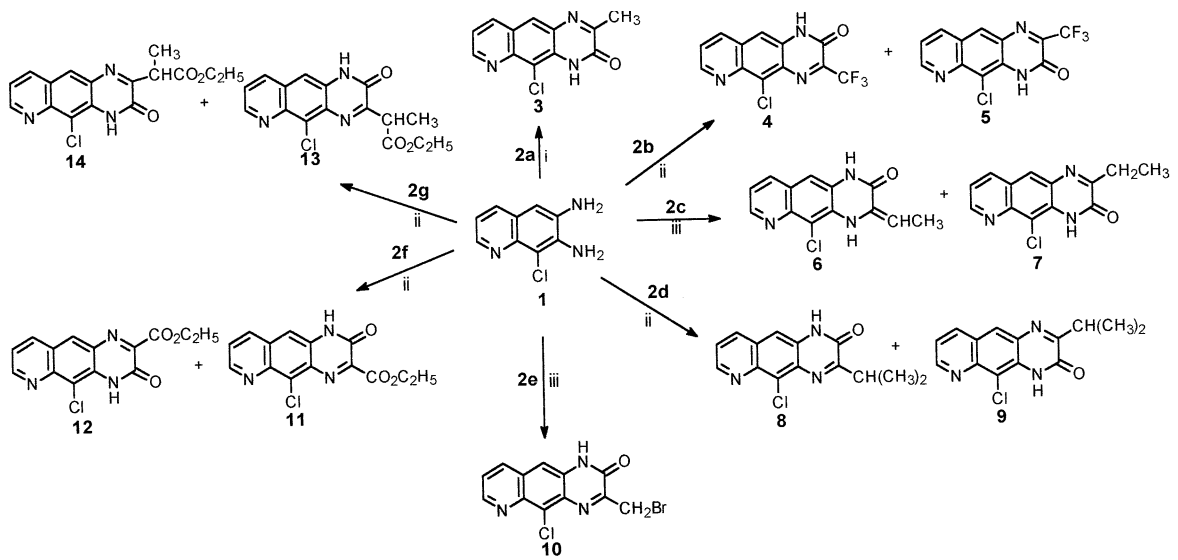
between 6,7-diamino-8-chloroquinoline (**1**) and the appropriate α-ketoester **2a**,**b**,**d**,**f** and **g** in ethanol at reflux to give compounds **3**–**5**, **8**, **9** and **11**–**14**, while the condensation of **1** with **2c**,**e** in these conditions failed. Therefore the preparation of compounds **6**, **7** and **10** was performed starting from the same intermediates, by reaction at a moderate temperature (45–50 °C) in 10% aqueous solution of sulfuric acid.

According to results previously reported by us, in all cases we observed the formation of a mixture of two isomers which were isolated by chromatography. In the case of compounds **3** and **10** their isomers were detected by $^1$H NMR, but not isolated in the reaction mixture. An interesting behavior was observed for the pair of isomers **6** and **7** which, differently to that observed by us for analogous compounds [9], were, respectively, obtained only as ethyliden and ethyl derivatives, but not as tautomeric mixture, as evidenced by their $^1$H NMR spectra. The key intermediate **1** was previously obtained by us, starting from 7-amino-8-chloro-6-nitroquinoline by catalytic reduction under very critical conditions [11]. We have now simplified this procedure by carrying out the reduction in a sealed steel vessel with 98% hydrazine monohydrate in absolute ethanol.

Structures of all the new pyridoquinoxalinones (**3**–**14**) are also supported by analytical and spectroscopical data (IR, UV, $^1$H NMR). The assignment of the structure to 5-chloropyridoquinoxalinones (**3**–**14**) has been obtained by application of $^1$H NMR spectroscopy to all compounds according to that previously reported by us for the quinoxalinone derivatives [8]. In particular the H-10 proton of the pyridoquinoxalin-2-ones shows a diamagnetic shift owing to the *peri* effect determined from NH-1, while this is not exhibited from the corresponding proton in the 3-one isomers.

## 3. Experimental

Melting points were determined by a Kofler hot stage or Digital Electrothermal apparatus, and are uncorrected. Infrared spectra are for nujol mulls and were




Scheme 1. Preparation of substituted pyrido[2,3-*g*]quinoxalinones (**3**–**14**). Reagents: (**2a**) $CH_3COCO_2Et$; (**2b**) $CF_3COCO_2Et$; (**2c**) $CH_3CH_2COCOONa$; (**2d**) $CH(CH_3)_2COCO_2Et$; (**2e**) $CH_2BrCOCO_2Et$; (**2f**) $CO(CO_2Et)_2$; (**2g**) $EtO_2CCOCH(CH_3)CO_2Et$. Conditions: (i) EtOH, under reflux for 15 h; (ii) EtOH, under reflux for 3 h; (iii) $H_2SO_4$ 10% aqueous solution at 45–50 °C for 1 h.

recorded using a Perkin–Elmer 781 spectrophotometer. UV spectra are qualitative and were recorded in nm for solutions in ethanol with a Perkin–Elmer Lambda 5 spectrophotometer. $^1$H NMR spectra were recorded on a Varian XL-200 (200 MHz) instrument, using TMS as an internal standard. The chemical shift values are reported in ppm ($\delta$) and coupling constants ($J$) in Hertz (Hz). Signal multiplicities are represented by: s (singlet), d (doublet), dd (double doublet), t (triplet), q (quadruplet), m (multiplet), and br s (broad singlet). Column chromatographies were performed using 230–400 mesh silica gel (Merck silica gel 60). Light petroleum refers to the fraction with b.p. 40–60 °C. Elemental analyses were performed by the Laboratorio di Microanalisi, Dipartimento di Scienze Farmaceutiche, Università di Padova (Padua). The analytical results for C, H, N, and halogen were within $\pm$ 0.4% of the theoretical values.

### 3.1. Materials

#### 3.1.1. 6,7-Diamino-8-chloroquinoline (**1**)

A solution of 7-amino-8-chloro-6-nitroquinoline [10] (1.0 g, 4.5 mmol) and hydrazine monohydrate 98% (3.05 g, 3.0 ml, 95 mmol) in ethanol (110 ml) was heated at 100 °C in a sealed steel vessel for 48 h. After this time the mixture reaction was cooled to room temperature and the solvent was removed under reduced pressure. The solid residue triturated with ether gave the known **1** (0.67 g, 80%); m.p. 162–163 °C [11].

### 3.2. General procedure for preparation of **3**–**5**, **8**, **9** and **11**–**14**

A solution of the intermediate **1** (0.5 g; 2.6 mmol) and the suitable α-ketoester **2a**,**b**,**d**,**f**,**g** (0.45–0.79 g; 3.9 mmol) in ethanol (15 ml) was refluxed for 3 h, while in the case of **2a** the reflux was continued for 15 h. After evaporation of the solvent, the crude solid residue was purified by chromatography on a silica gel column, eluting with a diethyl ether–acetone mixture with increasing percentage of acetone in the case of compounds **3**–**5** and **11**–**14** or with a 1:1 mixture of diethyl ether–light petroleum for compounds **8** and **9**. In general we observed that the pyridoquinoxalin-3-ones were collected in the eluate before the 2-one isomers.

#### 3.2.1. 5-Chloro-2-methyl-3,4-dihydropyrido[2,3-g]-quinoxalin-3-one (**3**)

0.07 g (10% yield); m.p. 302–304 °C (d); IR: $\nu$ 3180, 1680, 1620 cm$^{-1}$; UV: $\lambda$ 332, 264 infl., 249, 215 nm; $^1$H NMR (DMSO-$d_6$): $\delta$ 11.92 (1H, br s, NH), 9.05 (1H, d, $J = 4.4$ Hz, H-7), 8.56 (1H, d, $J = 8.4$ Hz, H-9), 8.38 (1H, s, H-10), 7.60 (1H, dd, $J = 8.4$ and 4.4 Hz, H-8), 2.51 (3H, s, $CH_3$).

#### 3.2.2. 5-Chloro-3-(trifluoromethyl)-1,2-dihydropyrido-[2,3-g]quinoxalin-2-one (**4**)

0.27 g (35% yield); m.p. > 360 °C; IR: $\nu$ 1700, 1690, 1650, 1625 cm$^{-1}$; UV: $\lambda$ 356, 338, 272 infl., 257, 225 nm; $^1$H NMR (DMSO-$d_6$): $\delta$ 13.20 (1H, br s, NH), 9.05 (1H, d, $J = 4.0$ Hz, H-7), 8.56 (1H, d, $J = 8.4$ Hz, H-9), 7.71 (1H, dd, $J = 8.4$ and 4.0 Hz, H-8), 7.63 (1H, s, H-10).

#### 3.2.3. 5-Chloro-2-(trifluoromethyl)-3,4-dihydropyrido-[2,3-g]quinoxalin-3-one (**5**)

0.23 g (30% yield); m.p. 355 °C (d); IR: $\nu$ 3150, 1710, 1690, 1680, 1620, 1590 cm$^{-1}$; UV: $\lambda$ 350, 267, 249, 222 nm; $^1$H NMR (DMSO-$d_6$): $\delta$ 12.54 (1H, br s, NH), 9.15 (1H, d, $J = 4.2$ Hz, H-7), 8.72 (1H, s, H-10), 8.65 (1H, d, $J = 8.4$ Hz, H-9), 7.67 (1H, dd, $J = 8.4$ and 4.2 Hz, H-8).

#### 3.2.4. 5-Chloro-3-isopropyl-1,2-dihydropyrido[2,3-g]-quinoxalin-2-one (**8**)

0.42 g (59% yield); m.p. 287–288 °C; IR: $\nu$ 1680, 1640, 1590 cm$^{-1}$; UV: $\lambda$ 340, 326, 267, 240, 216 nm; $^1$H NMR ($CDCl_3$ + DMSO-$d_6$): $\delta$ 12.51 (1H, br s, NH), 8.96 (1H, d, $J = 4.2$ Hz, H-7), 8.34 (1H, d, $J = 8.2$ Hz, H-9), 7.64 (1H, s, H-10). 7.56 (1H, dd, $J = 8.2$ and 4.2 Hz, H-8), 3.57 (1H, m, $J = 6.4$ Hz, CH), 1.35 (6H, d, $J = 6.4$ Hz, 2 $CH_3$).

#### 3.2.5. 5-Chloro-2-isopropyl-3,4-dihydropyrido[2,3-g]-quinoxalin-3-one (**9**)

0.20 g (28% yield); m.p. 274–275 °C; IR: $\nu$ 3270, 1720, 1640 cm$^{-1}$; UV: $\lambda$ 332, 264 infl., 249, 215 nm; $^1$H NMR ($CDCl_3$ + DMSO-$d_6$): $\delta$ 11.49 (1H, br s, NH), 9.04 (1H, d, $J = 4.2$ Hz, H-7), 8.44 (1H, d, $J = 8.4$ Hz, H-9), 8.35 (1H, s, H-10), 7.52 (1H, dd, $J = 8.4$ and 4.2 Hz, H-8), 3.60 (1H, m, $J = 6.8$ Hz, CH), 1.33 (6H, d, $J = 6.8$ Hz, 2 $CH_3$).

#### 3.2.6. 5-Chloro-3-(ethoxycarbonyl)-1,2-dihydropyrido-[2,3-g]quinoxalin-2-one (**11**)

0.35 g (45% yield); m.p. 246–247 °C; IR: $\nu$ 3200, 1740, 1730, 1670, 1620, 1610 cm$^{-1}$; UV: $\lambda$ 332, 275, 245, 220 nm; $^1$H NMR ($CDCl_3$ + DMSO-$d_6$): $\delta$ 13.08 (1H, br s, NH), 9.03 (1H, d, $J = 4.0$ Hz, H-7), 8.32 (1H, d, $J = 8.6$ Hz, H-9), 7.70 (1H, s, H-10), 7.60 (1H, dd, $J = 8.6$ and 4.0 Hz, H-8), 4.51 (2H, q, $J = 7.2$ Hz, $CH_2$), 1.46 (3H, d, $J = 7.2$ Hz, $CH_3$).

#### 3.2.7. 5-Chloro-2-(ethoxycarbonyl)-3,4-dihydropyrido-[2,3-g]quinoxalin-3-one (**12**)

0.04 g (5% yield); m.p. 263–265 °C; IR: $\nu$ 1725, 1670, 1610 cm$^{-1}$; UV: $\lambda$ 331, 271, 250, 216 nm; $^1$H NMR ($CDCl_3$): $\delta$ 9.53 (1H, br s, NH), 9.17 (1H, d, $J = 4.2$ Hz, H-7), 8.45 (1H, s, H-10), 8.37 (1H, d,

$J = 8.4$ Hz, H-9), 7.55 (1H, dd, $J = 8.4$ and 4.2 Hz, H-8), 4.56 (2H, q, $J = 7.2$ Hz, $CH_2$), 1.48 (3H, d, $J = 7.2$ Hz, $CH_3$).

*3.2.8. 5-Chloro-3-(1-ethoxycarbonylethyl)-1,2-dihydropyrido[2,3-g]quinoxalin-2-one (**13**)*

0.30 g (35% yield); m.p. 298–300 °C; IR: $\nu$ 1725, 1670, 1620 $cm^{-1}$; UV: $\lambda$ 343, 330, 271, 243, 220 nm; $^1H$ NMR ($CDCl_3$ + DMSO-$d_6$): $\delta$ 12.71 (1H, br s, NH), 8.99 (1H, d, $J = 4.0$ Hz, H-7), 8.38 (1H, d, $J = 8.4$ Hz, H-9), 7.69 (1H, s, H-10), 7.58 (1H, dd, $J = 8.4$ and 4.0 Hz, H-8), 4.24–4.10 (3H, m, C*H*–$CH_3$ + C$H_2$–$CH_3$), 1.60 (3H, d, $J = 7.0$ Hz, C$H_3$–CH), 1.22 (3H, t, $J = 7.2$ Hz, C$H_3$–$CH_2$).

*3.2.9. 5-Chloro-2-(1-ethoxycarbonylethyl)-3,4-dihydropyrido[2,3-g]quinoxalin-3-one (**14**)*

0.15 g (17% yield); m.p. 232–233 °C; IR: $\nu$ 3180, 1725, 1665, 1610, 1590 $cm^{-1}$; UV: $\lambda$ 332, 266 sh., 249, 215 nm; $^1H$ NMR ($CDCl_3$ + DMSO-$d_6$): $\delta$ 10.34 (1H, br s, NH), 9.11 (1H, d, $J = 4.2$ Hz, H-7), 8.35 (1H, d, $J = 8.2$ Hz, H-9), 8.33 (1H, s, H-10), 7.52 (1H, dd, $J = 8.2$ and 4.2 Hz, H-8), 4.30 (1H, q, $J = 7.2$ Hz, C*H*–$CH_3$), 4.21 (2H, q, $J = 7.2$ Hz, C$H_2$–$CH_3$), 1.65 (3H, d, $J = 7.2$ Hz, C$H_3$–CH), 1.26 (3H, t, $J = 7.2$ Hz, C$H_3$–$CH_2$).

*3.3. General procedure for preparation of **6**, **7** and **10***

The suitable α-ketoderivative **2c**,**e** (0.64–1.02 g; 5.2 mmol) was added to a stirred solution of **1** (0.5 g; 2.6 mmol) in 10% aqueous solution of sulfuric acid (15 ml). The mixture was then heated at 45–50 °C for 2 h. On cooling to room temperature, the solution was made alkaline (pH 11–12) by the addition of 2N NaOH aqueous solution and the resulting precipitate was collected by filtration. This was then purified by chromatography on a silica gel column, eluting with a 7:3 mixture of diethyl ether–acetone.

*3.3.1. 5-Chloro-3-ethylidene-1,2,3,4-tetrahydropyrido-[2,3-g]quinoxalin-2-one (**6**)*

0.16 g (24% yield); m.p. 279–280 °C; IR: $\nu$ 1690, 1640 $cm^{-1}$; UV: $\lambda$ 370, 326, 315, 255, 222 nm; $^1H$ NMR ($CDCl_3$ + DMSO-$d_6$): $\delta$ 10.91 (1H, br s, NH-1), 8.78 (1H, d, $J = 4.2$ Hz, H-7), 7.97 (1H, d, $J = 8.2$ Hz, H-9), 7.44 (1H, s, H-10), 7.24 (1H, dd, $J = 8.2$ and 4.2 Hz, H-8), 6.94 (1H, br s, NH-4), 5.91 (1H, q, $J = 7.4$ Hz, C*H*–$CH_3$), 1.85 (3H, d, $J = 7.4$ Hz, CH–C$H_3$).

*3.3.2. 5-Chloro-2-ethyl-3,4-dihydropyrido[2,3-g]-quinoxalin-3-one (**7**)*

0.08 g (12% yield); m.p. 269–270 °C; IR: $\nu$ 1680, 1620, $cm^{-1}$; UV: $\lambda$ 332, 256 sh., 248, 214 nm; $^1H$ NMR ($CDCl_3$): $\delta$ 9.21 (1H, br s, NH), 9.04 (1H, d, $J = 4.2$ Hz, H-7), 8.27 (1H, d, $J = 8.4$ Hz, H-9), 8.22 (1H, s, H-10), 7.43 (1H, dd, $J = 8.4$ and 4.2 Hz, H-8), 2.97 (2H, q, $J = 7.2$ Hz, $CH_2$), 1.32 (3H, t, $J = 7.2$ Hz, $CH_3$).

*3.3.3. 5-Chloro-3-(bromomethyl)-1,2-dihydropyrido-[2,3-g]quinoxalin-2-one (**10**)*

0.06 g (7% yield); m.p. 256–257 °C; IR: $\nu$ 1670, 1610 $cm^{-1}$; UV: $\lambda$ 351, 258, 246 nm; $^1H$ NMR (DMSO-$d_6$): $\delta$ 12.90 (1H, br s, NH), 9.04 (1H, d, $J = 4.2$ Hz, H-7), 8.55 (1H, d, $J = 8.2$ Hz, H-9), 7.74 (1H, s, H-10), 7.69 (1H, dd, $J = 8.2$ and 4.2 Hz, H-8), 4.75 (2H, s, $CH_2$).

*3.4. Microbiology*

*3.4.1. Antibacterial assay*

Antibacterial activity was investigated in vitro on Gram positive and Gram negative bacteria. The strains used were *Escherichia coli* ATCC 3853, *Pseudomonas aeruginosa* ATCC 27853, and *Staphylococcus aureus* ATCC 25923. The minimum inhibitory concentration (MIC) was determined according to the dilution method in broth with test tubes. All bacteria strains were cultured in Lb broth (Luria broth, Difco) and after overnight incubation at 37 °C, were diluted to the optical density of 0.5 McFarland turbidity standard (measured by spectrophotometer at 450 nm). The final inoculum concentration was $10^6$ CFU/ml. Each compound was dissolved (1 mg/ml) in dimethyl sulfoxide (DMSO) and then diluted with the test medium in order to obtain the required range of concentration (500–0.5 μg/ml). MICs were determined by the standard microbroth dilution method [12] as the lowest concentration of the compound which completely inhibited bacteria growth.

*3.4.2. Anticandida assay*

The anticandida tests were done by an in vitro method using various strains of *Candida* spp. (hospital isolated). All strains of *Candida* were cultured at 37 °C in Brain Heart infusion broth (Difco) for 24 h before use. Inocula were standardized by spectrophotometer to 0.5 McFarland turbidity standard, as reported above. Compounds were dissolved (1 mg/ml) in DMSO and then diluted with the test medium in order to obtain the required range of concentration (500–0.5 μg/ml). Susceptibility testing was determined using the National Committee for Clinical Laboratory Standards microbroth dilution method [13]. After the addition of the solution of tested compound to standardized inoculum the test tube was incubated overnight at 35 °C. The MICs were determined as the lowest concentration with at least an 80% reduction in turbidity when compared with the compound free control tube.

*3.4.3. In vitro antitumoral activity*

All new compounds were sent to the National Cancer

Table 1
Antimicrobial activity (MIC in μg/ml) of pyridoquinoxalinones **3**–**14**

| Comp. | *P. aeruginosa* ATCC 27853 | *S. aureus* ATCC 25923 | *E. coli* ATCC 3853 | *Candida* spp [a] |
|---|---|---|---|---|
| **3** | 62.5 | 15.6 | 62.5 | 125 |
| **4** | 62.5 | 62.5 | 62.5 | 62.5 |
| **5** | 62.5 | 62.5 | 62.5 | 62.5 |
| **6** | >500 | >500 | >500 | >500 |
| **7** | 62.5 | 62.5 | 62.5 | 62.5 |
| **8** | 62.5 | 31.25 | 62.5 | 125 |
| **9** | 62.5 | 62.5 | 125 | 125 |
| **10** | 31.25 | 31.25 | 31.25 | 31.25 |
| **11** | >500 | >500 | >500 | >500 |
| **12** | >500 | >500 | >500 | >500 |
| **13** | 62.5 | 31.25 | 62.5 | 62.5 |
| **14** | 62.5 | 31.25 | 62.5 | 62.5 |

[a] Strains hospital isolated.

Table 2
Percent tumor GI recorded on subpanel cell-lines at $10^{-4}$ and $10^{-5}$M of compound **10**

| Panel/cell-lines | $10^{-4}$ | $10^{-5}$ [a] | Panel/cell-lines | $10^{-4}$ | $10^{-5}$ |
|---|---|---|---|---|---|
| *Leukemia* | | | MALME-3M | 120 | |
| CCRF-CEM | 139 | 62 | M14 | 189 | |
| HL-60(TB) | 130 | | SK-MEL-5 | 173 | |
| K-562 | 99 | | UACC-257 | 128 | |
| MOLT-4 | 88 | 54 | UACC-62 | 198 | |
| RPMI-8226 | 153 | 62 | *Ovarian cancer* | | |
| SR | 105 | 67 | IGROV1 | 186 | 92 |
| *Non small cell lung cancer* | | | OVCAR-3 | 159 | |
| A549/ATCC | 168 | | OVCAR-4 | 193 | |
| EKVX | 128 | | OVCAR-5 | 190 | |
| HOP-62 | 196 | | OVCAR-8 | 145 | |
| HOP-92 | 157 | | SK-OV-3 | 123 | |
| NCI-H226 | 127 | | *Renal cancer* | | |
| NCI-H23 | 174 | | 786-0 | 156 | 62 |
| NCI-H322M | 127 | | ACHN | 194 | 58 |
| NCI-H460 | 163 | | CaKI-1 | 176 | |
| NCI-H522 | 166 | | RXF 393 | 179 | |
| *Colon cancer* | | | SN12c | 199 | |
| COLO 205 | 199 | | TK-10 | 123 | |
| HCT-116 | 186 | | UO-31 | 190 | |
| HCT-15 | 187 | nt | *Prostate cancer* | | |
| HT29 | 184 | | PC-3 | 156 | |
| KM12 | 172 | | DU-145 | 189 | |
| SW-620 | 154 | | *Breast cancer* | | |
| *CNS cancer* | | | MCF7 | 158 | 50 |
| SF-268 | 120 | | NCI/ADR-RES | 132 | |
| SF-295 | 133 | | MDA-MB-231/ATCC | 169 | |
| SF-539 | 178 | | HS 578T | 182 | |
| SNB-19 | 195 | 60 | MDA-MB-435 | 168 | |
| U251 | 176 | | MDA-N | 165 | |
| *Melanoma* | | | BT-549 | 104 | |
| LOX IMVI | 173 | | T-47D | 154 | 56 |

[a] Values not reported for GI below 50%; (nt): not tested at this molar concentration.

Institute (NCI) of Bethesda, Maryland (USA). The selected compounds (**4**, **5**, **7**, **9**, **10**, **11**, **13**, and **14**) were submitted to a primary screening for anticancer activity following the known in vitro disease-oriented antitumor screening program, against a panel of about 60 human tumor cell lines [14]. The activity of each compound tested was deduced from a dose–response curve according to the data provided by NCI.

## 4. Results and discussion

### 4.1. Antibacterial and anticandida activity

Results of antibacterial and anticandida assays of all new compounds are reported in Table 1. These data show that most of the tested compounds, with the exception of **6**, **11** and **12**, possess an encouraging activity against all selected strains with MIC values in the range 15.6–62.5 μg/ml against *S. aureus*, 31.25–62.5 μg/ml against *P. aeruginosa*, and 31.25–125 μg/ml against both *E. Coli* and *Candida* spp. In particular, of all tested compounds the 3-bromomethyl-5-chloro-derivative (**10**) exhibits a MIC of 31.25 μg/ml against all tested strains. These data, compared with those of the related bicyclic quinoxalinones, show a shift of antimicrobial activity from fungi to bacteria.

### 4.2. Antitumor activity

The results of the in vitro anticancer activity, concerning the selected pyridoquinoxalinones **4**, **5**, **7**, **9**, **10**, **11**, **13** and **14**, indicate that these compounds exhibited a general moderate percent growth inhibition (GI) activity at concentration of $10^{-4}$ M, however compounds **4** and **11** proved to have a good selective antiproliferative activity against various leukemia, non-small cell lung cancer, colon cancer, CNS cancer and melanoma cell lines. Finally, once more the bromomethyl derivative **10** proved the most interesting, showing an antiproliferative activity on all subpanel cell-lines at $10^{-4}$ M concentration, even if this activity decreases at $10^{-5}$ M; this behavior is showed in Table 2.

## 5. Conclusions

The present work describes the synthesis and the in vitro antimicrobial and antitumor evaluation of a series of novel tricyclic pyrido[2,3-*g*]quinoxalinone derivatives (**3**–**14**) prepared with the aim to evaluate the effect on the biological activities due to enlargement of the heterocyclic system in comparison with bicyclic quinoxalinones previously described. Most of the tested compounds exhibited a slightly more appreciable antibacterial activity than the reference compounds, but a lower antifungal activity. Regarding the antitumor activity of the new compounds, the increasing of the planar area of the system does not seem to influence this activity significantly, with the exception of the 3-bromomethyl derivative **10** which, as expected, resulted the most active. At this point of the research, it is hard to state if our compounds behave as alkylant or intercalator agents. Other derivatives will be synthesized and studied in order to evaluate more fully the structure–activity relationships and to define their mechanism.

## References

[1] A. Carta, P. Sanna, L. Gherardini, A. Deriu, L. Sechi, S. Zanetti, Novel functionalized pyrido[2,3-*g*]quinoxalin-2-ones as antibacterial, antifungal and anticancer agents, Bologna, Italy, 18–22 September 2000, Italian Chemical Society, Division of Medicinal Chemistry, Bologna, pc-86.
[2] W.A. Denny, in: W.O. Foye (Ed.), Cancer Chemotherapeutic Agents, ACS, Washington, DC, 1995, pp. 218–260.
[3] C.W. Schellhammer, S. Petersen, Uber derivate des chinolinchinons-(5.8), Ann, 624 (1959) 108–119.
[4] S. Giorgi-Renault, J. Renault, M. Baron, Heterocyclic quinones. VI: Synthesis and antitumoral effects of 5,10-benzo[*g*]quinoxalinediones and aza-analogues, Eur. J. Med. Chem. 20 (1985) 144–148.
[5] H.W. Yoo, M.E. Suh, S.W. Park, Synthesis and cytotoxicity of 2-methyl-4,9-dihydro-1-substituted-1*H*-imidazo[4,5-*g*]quinoxaline-4,9-diones and 2,3-disubstituted-5,10-pyrazino[2,3-*g*]quinoxalinediones, Eur. J. Med. Chem. 41 (1998) 4716–4722.
[6] M.E. Suh, M.J. Kang, H.W. Yoo, S.Y. Park, C.O. Lee, Synthesis and cytotoxicity of 2-methyl-1-substituted-imidazo[4,5-*g*]-quinoline-4,9-dione and 7,8-dihydro-10*H*-[1,4]oxazino-[3′,4′:2,3]imidazo[4,5-*g*]quinoline-5,12-dione derivatives, Bioorg. Med. Chem. 8 (2000) 2079–2083.
[7] H.W. Moore, Bioactivation as a model for drug design bioreductive alkylation, Science 197 (1977) 527–532.
[8] P. Sanna, A. Carta, M. Loriga, S. Zanetti, L. Sechi, Synthesis of substituted 2-ethoxycarbonyl- and 2-carboxyquinoxalin-3-ones for evaluation of antimicrobial and anticancer activity, Farmaco 53 (1998) 455–461.
[9] P. Sanna, A. Carta, M. Loriga, S. Zanetti, L. Sechi, Synthesis of 3,6,7-substituted-quinoxalin-2-ones for evaluation of antimicrobial and anticancer activity. Part 2, Farmaco 54 (1999) 161–168.
[10] P. Sanna, A. Carta, M. Loriga, S. Zanetti, L. Sechi, Preparation and biological evaluation of 6/7-trifluoromethyl(nitro)-, 6,7-difluoro-3-alkyl(aryl)-substituted-quinoxalin-2-ones. Part 3, Farmaco 54 (1999) 169–177.
[11] P. Sanna, A. Carta, G. Paglietti, Synthesis of two novel tricyclic rings: triazolo[4,5-*g*]quinolines and pyrido[2,3-*g*]quinoxalines derived from 6,7-diaminoquinolines, Heterocycles 53 (2000) 423–432.
[12] National Committee for Clinical Laboratory Standards, Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, Approved standard M7-A2, Villanova Pa, USA, 1990.
[13] National Committee for Clinical Laboratory Standards, Methods for determining bactericidal activity of antimicrobial agents. Tentative guidelines, National Committee for Clinical Laboratory Standards, document M26-T, Villanova Pa, USA, 1992.
[14] M.R. Boyd, Status of the NCI preclinical antitumor drug discovery screen, Principles Practice Oncol. 3 (1989) 1–12.