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Preparation and biological evaluation of 6/7-trifluoromethyl(nitro)-, 6,7-difluoro-3-alkyl (aryl)-substituted-quinoxalin-2-ones. Part 3☆

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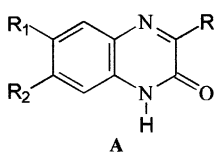
Abstract

A new series of quinoxalinones 6/7-trifluoromethyl or nitro- and 6,7-difluoro substituted bearing various side-chains (alkyl, halogenoalkyl, benzyl and phenyl groups) at C-3 of the ring system was synthesized and submitted to preliminary in vitro evaluation for antibacterial, antifungal, antimycobacterial, anticancer and anti-HIV activities. Results of these screenings showed that compounds **23–28** exhibited a good inhibition activity against various strains of *Candida*. Compound **24** showed also an interesting in vitro anticancer activity. © 1999 Elsevier Science S.A. All rights reserved.

Keywords: 2-Quinoxalinones; Antibacterial; Antifungal; Antimycobacterial activities; Anticancer activities

1. Introduction

In previous works we reported the synthesis and the antimicrobial and anticancer activities of various quinoxalinone derivatives [2,3]. Among these a moderate antimicrobial activity was observed for those compounds bearing an electronegative substituent (F, Cl, CF_3) at the 6 or 7 position of the ring and a carboxyethyl or ethyl group at C-3. The above-mentioned results prompted us to continue our investigation in order to achieve additional data for a structure–activity relationship. In this context a series of 27 new quinoxalinones of formula **A** has been prepared.



A

R=$CH_2CH_2CH_3$, $CH(CH_3)_2$,$CH{=}C(OH)C(CH_3)_3$, CH_2Ph, Ph, CH_2Br, CF_3
R_1/R_2=F, CF_3, NO_2

These compounds, structurally related to the above-mentioned leads, maintain an electron-withdrawing group (CF_3, NO_2, diF) in the benzene moiety, while in the C-3 position either an alkyl of different length and steric hindrance, or a benzyl or phenyl substituent was introduced with the aim to evaluate the effect of the increased lipophilicity on the biological activities. Furthermore, we deemed it interesting to study the influence of a further concomitant electronegative substitution (CH_2Br/CF_3) on C-3 position.

2. Chemistry

The quinoxalinones (**3–31**) listed in Table 1, were prepared following the reactions depicted in Scheme 1. According to our previously reported procedures [3], the diamines **1a–c** were condensed with the suitable α-keto acids (**2a**,**d**,**e**) or α-keto esters (**2b**,**c**,**f**,**g**) in refluxing ethanol to give compounds **8**, **9**, **12**, **13** and **22** in good yields. The reaction of **1a** with **2f** and **1c** with **2a** failed. However, when the same reactions were repeated using 10% aqueous solution of sulfuric acid, compounds **7**, **23** and **24** were obtained in good yields (51, 25 and 43%, respectively). This procedure allowed us to

☆ Part of this work was presented as a poster communication at the XIII Convegno Nazionale della Divisione di Chimica Farmaceutica, Paestum, Italy, 23–27 Sept. 1996 [1].

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Table 1
Compounds of Scheme 1

Compd.	R_1	R_2	Compd.	R_1	R_2	Compd.	R_1	R_2	Compd.	R_1	R_2
3	CF_3	H	**11**	F	F	**19**	H	CF_3	**27**	CF_3	H
4	H	CF_3	**12**	CF_3	H	**20**	NO_2	H	**28**	H	CF_3
5	NO_2	H	**13**	H	CF_3	**21**	H	NO_2	**29**	NO_2	H
6	H	NO_2	**14**	F	F	**22**	F	F	**30**	H	NO_2
7	F	F	**15**	CF_3	H	**23**	CF_3	H	**31**	F	F
8	CF_3	H	**16**	H	CF_3	**24**	H	CF_3			
9	H	CF_3	**17**	F	F	**25**	H	NO_2			
10	H	NO_2	**18**	CF_3	H	**26**	F	F			

ameliorate up to 77% yield the previous results reported for the reaction of **1a** with **2e**, when carried out in refluxing ethanol, which gave compounds **18** and **19** in 44% overall yield [4].

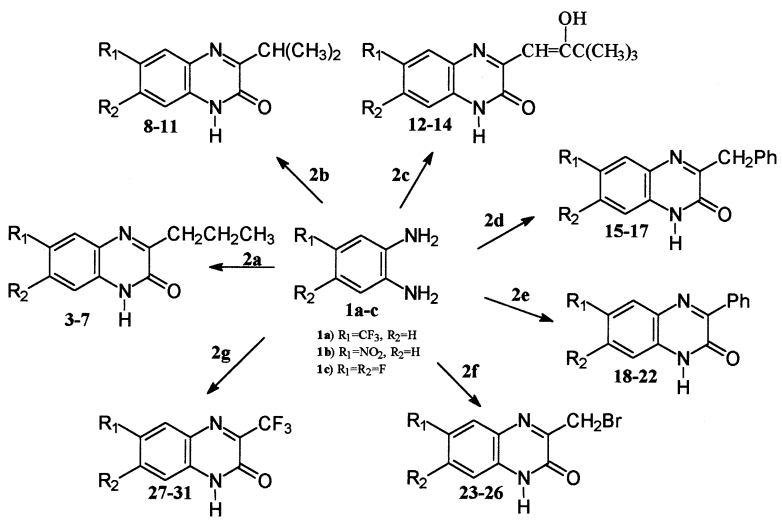
It is important to note that when the 4-monosubstituted diamines were condensed with the α-keto derivatives a mixture of 6/7 substituted isomers was obtained. Under neutral conditions, the formation of the 6-isomer was prevailing but these results were reversed in favor of 7-isomer under acidic conditions in line with the observations reported by others [4,5]. In particular, in the case of reactions of the diamine **1b** with keto acids (**2a**,**e**) and keto esters (**2b**,**c**,**f**,**g**), the 7-nitro isomers were greatly prevailing or, as in the case of **10** and **25**, extremely selective. Quinoxalinones **29** and **30** were known [6] and have been prepared again in order to evaluate their biological activities, thus allowing us to obtain a better characterization for these compounds. Despite several attempts the reaction of **1b** with **2d** was not successful.

Interestingly, during the isolation of compound **25** by recrystallization from hot acetone, we obtained additionally the compound **32**, the structure of which has been deduced by both ^1H and ^{13}C NMR spectra and mass data. The conversion of **25** into **32** by addition of acetone at C-3 position of the ring was attributed to acidic catalysis due to traces of sulfuric acid in the crude product (Scheme 2). This behavior, previously reported by Baxter et al. [7] for a similar compound, was not observed in the absence of acid. However, when the pure compound **25** was heated in acetone in the presence of one mole equivalent of sulfuric acid or hydrochloric acid, a complete conversion into compound **32** was obtained.

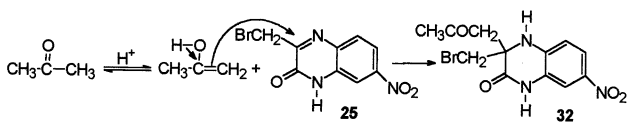
Structures of the new quinoxalinones **3–32** are supported by analytical and spectroscopical data (IR, UV, ^1H and ^{13}C NMR) as previously reported by us for similar compounds [2,3].

3. Experimental

Melting points were determined by a Kofler hot stage or Digital Electrothermal apparatus, and are uncorrected. IR spectra are for Nujol mulls and were recorded using a Perkin–Elmer 781 spectrophotometer.



Scheme 1. Preparation of substituted quinoxalin-2-ones: (**2a**) $CH_3(CH_2)_2COCOONa$; (**2b**) $(CH_3)_2CHCOCO_2Et$; (**2c**) $(CH_3)_3C–C(OH)=CHCOCO_2Et$; (**2d**) $Ph–CH_2COCOOH$; (**2e**) $Ph–COCOOH$; (**2f**) $CH_2BrCOCO_2Et$; (**2g**) CF_3COCO_2Et.



Scheme 2.

UV spectra are qualitative and were recorded in nm for solutions in ethanol with a Perkin–Elmer Lambda 5 spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded on a Varian XL-200 (200 MHz for ^{1}H and 50 MHz for ^{13}C) instrument, using TMS as internal standard. MS spectra were performed on a Finningan MatTSQ 700 spectrometer. Column chromatography was performed using 70–230 mesh silica gel (Merck Silica Gel 60). 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 when present, were within $\pm 0.4\%$ of the theoretical values.

3.1. Intermediates

The diaminobenzene derivatives **1a–c** were prepared following the procedures previously described [2].

3.2. General procedure for the preparation of 6- and 7-substituted 3-alkyl- (**3–14**), 3-benzyl- (**15–17**), 3-phenyl- (**18–22**) and 3-halogenomethyl-2(1H)-quinoxalinones (**23–31**)

3.2.1. Method A

A solution of the appropriate diamine **1a–c** (1 g, 5.7–6.9 mmol) and the suitable α-keto acid (**2a**,**d**,**e**) or α-keto ester (**2b**,**c**,**f**) (0.9–1.6 g, 6.5–7.4 mmol) in ethanol (20 ml) was refluxed for 1–4 h. After evaporation of the solvent, the crude solid residue was chromatographed on a silica gel column, eluting as reported below in order to separate the mixture of 6- and 7-isomers where present. In general we observed that the 6-isomers were collected in the eluate before the 7-ones. In the case of 6,7-difluoro derivatives the crude product was purified by recrystallization from a suitable solvent, when necessary.

*3.2.1.1. 6-Trifluoromethyl-3-isopropyl-2(1H)-quinoxalinone (**8**).* This compound (0.96 g, 66%) was obtained by eluting with a 1:1 mixture of diethyl ether/light petroleum; m.p. 208–210°C; IR: *v* 1660, 1620, 1570 cm^{-1}; UV: λ 346 sh, 330, 320, 274, 235, 205 nm; ^{1}H NMR ($CDCl_3$ + DMSO-d_6): δ 12.40 (1H, s, NH), 8.04 (1H, s, H-5), 7.60 (1H, dd, $J = 8.4$ and 1.8 Hz, H-7), 7.41 (1H, d, $J = 8.4$ Hz, H-8), 3.58 (1H, m, $CH(CH_3)_2$), 1.30 (6H, d, $J = 6.8$ Hz, $(CH_3)_2CH$).

*3.2.1.2. 7-Trifluoromethyl-3-isopropyl-2(1H)-quinoxalinone (**9**).* This compound (0.42 g, 28%) was obtained by eluting with a 1:1 mixture of diethyl ether/light petroleum; m.p. 252–253°C; IR: *v* 1660, 1570 cm^{-1}; UV: λ 348 sh, 326, 274, 254 sh, 228, 205 nm; ^{1}H NMR ($CDCl_3$ + DMSO-d_6): δ 12.42 (1H, s, NH), 7.88 (1H, d, $J = 8.2$ Hz, H-5), 7.58 (1H, s, H-8), 7.46 (1H, dd, $J = 8.2$ and 1.8 Hz, H-6), 3.58 (1H, m, $CH(CH_3)_2$), 1.30 (6H, d, $J = 6.8$ Hz, $(CH_3)_2CH$).

*3.2.1.3. 6-Trifluoromethyl-3-(2′-hydroxy-3′,3′-dimethyl-butenyl)-2(1H)-quinoxalinone (**12**).* This compound (1.11 g, 39%) was obtained by eluting with a 2:1 mixture of diethyl ether/light petroleum; m.p. 276–277°C; IR: *v* 3300–3150, 1680, 1610, 1570 cm^{-1}; UV: λ 407, 386, 370 sh, 309, 256 infl, 226, 203 nm; ^{1}H NMR ($CDCl_3$): δ 13.38 (1H, s, NH), 11.03 (1H, s, OH), 7.38 (1H, s, H-5), 7.34 (1H, d, $J = 8.4$ Hz, H-7), 7.20 (1H, d, $J = 8.4$ Hz, H-8), 6.54 (1H, s, *CH*=C), 1.27 (9H, s, $(CH_3)_3C$).

*3.2.1.4. 7-Trifluoromethyl-3-(2′-hydroxy-3′,3′-dimethyl-butenyl)-2(1H)-quinoxalinone (**13**).* This compound (0.6 g, 21%) was obtained by eluting with a 2:1 mixture of diethyl ether/light petroleum; m.p. 221–222°C; IR: *v* 3200–3100, 1680, 1610, 1570 cm^{-1}; UV: λ 405, 384, 368 sh, 300, 261, 231, 225, 204 nm; ^{1}H NMR ($CDCl_3$): δ 13.28 (1H, s, NH), 10.75 (1H, s, OH), 7.39 (1H, d, $J = 8.4$ Hz, H-5), 7.34 (1H, s, H-8), 7.16 (1H, d, $J = 8.4$ Hz, H-6), 6.58 (1H, s, *CH*=C), 1.28 (9H, s, $(CH_3)_3C$).

*3.2.1.5. 6,7-Difluoro-3-phenyl-2(1H)-quinoxalinone (**22**).* This compound was obtained in 92% yield (1.64 g); m.p. 276–277°C (from EtOH); IR: *v* 1650, 1630, 1600 cm^{-1}; UV: λ 364, 298, 224 sh, 205 nm; ^{1}H NMR ($CDCl_3$ + DMSO-d_6): δ 12.66 (1H, s, NH), 8.32 (2H, dd, $J = 6.0$ and 2.0 Hz, H-2′ + H-6′), 7.73 (1H, dd, $J = 9.2$ and 8.4 Hz, H-5), 7.48 (3H, m, H-3′ + H-4′ + H-5′), 7.23 (1H, dd, $J = 10.4$ and 7.4 Hz, H-8).

3.2.2. Method B

To a stirred solution of the appropriate diamine **1a–c** (1.0 g, 5.7–6.9 mmol) in 10% aqueous solution of sulfuric acid (20 ml), the suitable α-keto derivative **2a–g** (0.9–1.6 g, 6.5–7.4 mmol) was added. The mixture was then heated at 70–90°C for 1–2 h. On cooling to room temperature, the precipitate formed was collected by filtration. The crude solid product was then purified in the same manner indicated by Method A.

*3.2.2.1. 6-Trifluoromethyl-3-propyl-2(1H)-quinoxalinone (**3**).* This compound (0.96 g, 33%) was obtained by eluting with an 8:2 mixture of diethyl ether/light petroleum; m.p. 174–175°C; IR: *v* 1680, 1630, 1600, 1570 cm^{-1}; UV: λ 330, 276 infl, 236, 204 nm; ^{1}H NMR ($CDCl_3$): δ 12.61 (1H, s, NH), 8.15 (1H, s, H-5), 7.73

(1H, dd, $J = 8.4$ and 1.8 Hz, H-7), 7.47 (1H, d, $J = 8.4$ Hz, H-8), 2.99 (2H, t, $J = 7.2$ Hz, $CH_2CH_2CH_3$), 1.90 (2H, m, $CH_2CH_2CH_3$), 1.10 (3H, t, $J = 7.2$ Hz, $CH_3CH_2CH_2$).

3.2.2.2. 7-Trifluoromethyl-3-propyl-2(1H)-quinoxalinone ***(4)***. This compound (0.72 g, 49%) was obtained by eluting with an 8:2 mixture of diethyl ether/light petroleum; m.p. 224–225°C; IR: ν 1660, 1620, 1570 cm^{-1}; UV: λ 331, 272 sh, 229, 204 nm; ^1H NMR ($CDCl_3$): δ 12.51 (1H, s, NH), 7.96 (1H, d, $J = 8.2$ Hz, H-5), 7.63 (1H, s, H-8), 7.58 (1H, d, $J = 8.2$ Hz, H-6), 3.02 (2H, t, $J = 7.4$ Hz, $CH_2CH_2CH_3$), 1.90 (2H, m, $CH_2CH_2CH_3$), 1.10 (3H, t, $J = 7.4$ Hz, $CH_3CH_2CH_2$).

3.2.2.3. 6-Nitro-3-propyl-2(1H)-quinoxalinone ***(5)***. This compound (0.05 g, 3%) was obtained by eluting with a 7:3 mixture of diethyl ether/light petroleum; m.p. 188–189°C; IR: ν 1680, 1620, 1580 cm^{-1}; UV: λ 337, 267 infl, 203 nm; ^1H NMR ($CDCl_3$ + DMSO-d_6): δ 12.60 (1H, br s, NH), 8.56 (1H, d, $J = 2.2$ Hz, H-5), 8.23 (1H, dd, $J = 9.0$ and 2.2 Hz, H-7), 7.43 (1H, d, $J = 9.0$ Hz, H-8), 2.87 (2H, t, $J = 7.4$ Hz, $CH_2CH_2CH_3$), 1.82 (2H, m, $CH_2CH_2CH_3$), 1.05 (3H, t, $J = 7.4$ Hz, $CH_3CH_2CH_2$).

3.2.2.4. 7-Nitro-3-propyl-2(1H)-quinoxalinone ***(6)***. This compound (1.17 g, 77%) was obtained by eluting with a 7:3 mixture diethyl ether/light petroleum; m.p. 195–197°C; IR: ν 1660, 1570 cm^{-1}; UV: λ 360, 280, 232 sh, 222, 202 nm; ^1H NMR ($CDCl_3$ + DMSO-d_6): δ 12.53 (1H, br s, NH), 8.10 (1H, s, H-8), 7.97 (1H, dd, $J = 8.8$ and 1.8 Hz, H-6), 7.79 (1H, d, $J = 8.8$ Hz, H-5), 2.83 (2H, t, $J = 7.4$ Hz, $CH_2CH_2CH_3$), 1.76 (2H, m, $CH_2CH_2CH_3$), 0.98 (3H, t, $J = 7.4$ Hz, $CH_3CH_2CH_2$).

3.2.2.5. 6,7-Difluoro-3-propyl-2(1H)-quinoxalinone ***(7)***. This compound was obtained in 82% yield (1.36 g); m.p. 186–187°C; IR: ν 1680, 1570 cm^{-1}; UV: λ 333, 271, 225, 204 nm; ^1H NMR ($CDCl_3$): δ 12.77 (1H, br s, NH), 7.64 (1H, dd, $J = 10.2$ and 8.0 Hz, H-5), 7.20 (1H, dd, $J = 9.9$ and 7.8 Hz, H-8), 2.94 (2H, t, $J = 7.4$ Hz, $CH_2CH_2CH_3$), 1.85 (2H, m, $CH_2CH_2CH_3$), 1.08 (3H, t, $J = 7.4$ Hz, $CH_3CH_2CH_2$).

3.2.2.6. 7-Nitro-3-isopropyl-2(1H)-quinoxalinone ***(10)***. This compound (0.24 g, 16%) was obtained by eluting with a 2:1 mixture diethyl ether/light petroleum; m.p. 268–270°C; IR: ν 1670, 1620, 1570 cm^{-1}; UV: λ 359, 280, 232 infl, 221, 201 nm; ^1H NMR ($CDCl_3$ + DMSO-d_6): δ 12.62 (1H, s, NH), 8.16 (1H, d, $J = 2.4$ Hz, H-8), 8.03 (1H, dd, $J = 8.8$ and 2.4 Hz, H-6), 7.89 (1H, d, $J = 8.8$ Hz, H-5), 3.56 (1H, m, $CH(CH_3)_2$), 1.29 (6H, d, $J = 6.8$ Hz, $(CH_3)_2CH$).

No traces of the 6-isomer were ever detected.

3.2.2.7. 6,7-Difluoro-3-isopropyl-2(1H)-quinoxalinone ***(11)***. This compound was obtained in crystalline form in 69% yield (1.14 g); m.p. 246–247°C (from diethyl ether); IR: ν 1670, 1610, 1560 cm^{-1}; UV: λ 352 infl, 338, 326, 272, 226, 205 nm; ^1H NMR ($CDCl_3$ + DMSO-d_6): δ 12.28 (1H, s, NH), 7.57 (1H, dd, $J =$ 10.8 and 8.2 Hz, H-5), 7.16 (1H, dd, $J = 10.8$ and 8.2 Hz, H-8), 3.52 (1H, m, $CH(CH_3)_2$), 1.27 (6H, d, $J =$ 6.8 Hz, $(CH_3)_2CH$).

3.2.2.8. 6,7-Difluoro-3-(2′-hydroxy-3′,3′-dimethylbutenyl)-2(1H)-quinoxalinone ***(14)***. This compound was obtained in crystalline form in 88% yield (1.64 g); m.p. 242–244°C (from diethyl ether); IR: ν 3300–3100, 1680, 1610, 1570 cm^{-1}; UV: λ 412, 391, 374 sh, 258, 215 nm; ^1H NMR ($CDCl_3$ + DMSO-d_6): δ 13.27 (1H, s, NH), 11.87 (1H, s, OH), 7.18 (1H, dd, $J = 10.8$ and 7.4 Hz, H-5), 7.01 (1H, dd, $J = 10.8$ and 7.4 Hz, H-8), 6.37 (1H, s, CH=C), 1.23 (9H, s, $(CH_3)_3C$).

3.2.2.9. 6-Trifluoromethyl-3-benzyl-2(1H)-quinoxalinone ***(15)***. This compound (0.52 g, 30.2%) was obtained by eluting with a 7:3 mixture diethyl ether/light petroleum; m.p. 156–158°C; IR: ν 1660, 1620, 1570 cm^{-1}; UV: λ 335, 326, 276, 236, 206 nm; ^1H NMR ($CDCl_3$): δ 12.34 (1H, s, NH), 8.15 (1H, s, H-5), 7.71 (1H, dd, $J = 8.6$ and 1.8 Hz, H-7), 7.46 (2H, d, $J =$ 6.6 Hz, H-2′ + H-6′), 7.40–7.20 (4H, m, H-8 + 3 aromatic H), 4.32 (2H, s, CH_2).

3.2.2.10. 7-Trifluoromethyl-3-benzyl-2(1H)-quinoxalinone ***(16)***. This compound (0.88 g, 51%) was obtained by eluting with a 7:3 mixture diethyl ether/light petroleum; m.p. 210–211°C; IR: ν 1660, 1560 cm^{-1}; UV: λ 332, 278, 229, 206 nm; ^1H NMR ($CDCl_3$): δ 12.57 (1H, br s, NH), 7.97 (1H, d, $J = 8.4$ Hz, H-5), 7.59–7.45 (4H, m, H-6 + H-8 + 2 aromatic H), 7.33–7.20 (3H, m, 3 aromatic H), 4.32 (2H, s, CH_2).

3.2.2.11. 6,7-Difluoro-3-benzyl-2(1H)-quinoxalinone ***(17)***. This compound was obtained in 97% yield (1.80 g); m.p. 158–160°C; IR: ν 1650, 1600, 1560 cm^{-1}; UV: λ 360 infl, 331, 300 infl, 285, 276, 226, 204 nm; ^1H NMR ($CDCl_3$): δ 12.50 (1H, br s, NH), 7.66 (1H, dd, $J = 9.8$ and 8.0 Hz, H-5), 7.44 (2H, d, $J = 6.6$ Hz, H-2′ + H-6′), 7.35–7.22 (3H, m, H-3′ + H-4′ + H-5′), 7.05 (1H, dd, $J = 9.8$ and 7.0 Hz, H-8), 4.26 (2H, s, CH_2).

3.2.2.12. 6-Trifluoromethyl- ***(18)*** *and 7-trifluoromethyl-3-phenyl-2(1H)-quinoxalinone* ***(19)***. Using the route described previously [4], the following yields were obtained for title compounds: 0.75 g (30%) for **18** and 1.16 g (47%) for **19**, respectively.

3.2.2.13. 6-Nitro-3-phenyl-2(1H)-quinoxalinone ***(20)***. This compound (0.04 g, 2.3%) was obtained by eluting with a 7:3 mixture diethyl ether/light petroleum; m.p. 326–328°C; IR: ν 3300, 1650, 1600 cm^{-1}; UV: λ 360, 291, 228 infl, 204 nm; ^1H NMR ($CDCl_3$ + DMSO-d_6): δ 12.90 (1H, br s, NH), 8.65 (1H, d, $J = 2.4$ Hz, H-5), 8.36 (1H, d, $J = 8.0$ Hz, H-8), 8.30 (1H, dd, $J = 8.0$ and 2.4 Hz, H-7), 7.52 (5H, m, phenyl H).

3.2.2.14. 7-Nitro-3-phenyl-2(1H)-quinoxalinone ***(21)***. This compound (0.73 g, 41%) was obtained by eluting with a 7:3 mixture diethyl ether/light petroleum; m.p. 276–278°C; IR: ν 1670, 1600 cm^{-1}; UV: λ 383, 323, 240 infl, 222, 203 nm; ^1H NMR ($CDCl_3$ + DMSO-d_6): δ 12.85 (1H, br s, NH), 8.38 (2H, d, $J = 7.0$ Hz, H-2′ + H-6′), 8.16 (1H, s, H-8), 8.06 (1H, d, $J = 8.8$ Hz, H-6), 7.98 (1H, d, $J = 8.8$ Hz, H-5), 7.50 (3H, m, H-3′ + H-4′ + H-5′).

3.2.2.15. 6-Trifluoromethyl-3-bromomethyl-2(1H)-quinoxalinone ***(23)***. This compound (0.44 g, 25%) was obtained by eluting with a 1:1 mixture diethyl ether/light petroleum; m.p. 189–191°C; IR: ν 1670, 1620, 1570 cm^{-1}; UV: λ 331, 280, 240, 204 nm; ^1H NMR ($CDCl_3$): δ 12.45 (1H, br s, NH), 8.18 (1H, s, H-5), 7.80 (1H, d, $J = 8.4$ Hz, H-7), 7.53 (1H, d, $J = 8.4$ Hz, H-8), 4.69 (2H, s, CH_2Br).

3.2.2.16. 7-Trifluoromethyl-3-bromomethyl-2(1H)-quinoxalinone ***(24)***. This compound (0.75 g, 43%) was obtained by eluting with a 1:1 mixture diethyl ether/light petroleum; m.p. 215–217°C; IR: ν 1670, 1620, 1560 cm^{-1}; UV: λ 353, 281, 232, 204 nm; ^1H NMR ($CDCl_3$): δ 12.78 (1H, s, NH), 7.91 (1H, d, $J = 8.8$ Hz, H-5), 7.64 (1H, s, H-6), 7.51 (1H, d, $J = 8.8$ Hz, H-8), 4.62 (2H, s, CH_2Br).

3.2.2.17. 7-Nitro-3-bromomethyl-2(1H)-quinoxalinone ***(25)***. This compound (1.26 g, 68%) was obtained by eluting with a 7:3 mixture diethyl ether/light petroleum; m.p. > 400°C, recrystallized from acetone; IR: ν 1670, 1610, 1530 cm^{-1}; UV: λ 371, 289, 226, 202 nm; ^1H NMR ($CDCl_3$ + DMSO-d_6): δ 12.99 (1H, s, NH), 8.16 (1H, d, $J = 2.2$ Hz, H-8), 8.08 (1H, dd, $J = 8.8$ and 2.2 Hz, H-6), 7.97 (1H, d, $J = 8.8$ Hz, H-5), 4.63 (2H, s, CH_2Br). No traces of the 6-isomer were ever detected.

Recrystallization from acetone of crude **25** (0.5 g), containing traces of sulfuric acid, afforded 0.2 g (33% yield) of *7-nitro-3-acetonyl-3-bromomethyl-1,2,3,4-tetrahydroquinoxalin-2-one* (**32**); m.p. 150–151°C; IR: ν 1720, 1680, 1620, 1530 cm^{-1}; UV: λ 397, 285, 218 nm; ^1H NMR (DMSO-d_6): δ 10.97 (1H, s, NH-1), 7.80 (1H, dd, $J = 8.8$ and 2.2 Hz, H-6), 7.70 (1H, s, NH-4), 7.68 (1H, d, $J = 2.2$ Hz, H-8), 6.67 (1H, d, $J = 8.8$ Hz, H-5), 4.00 (1H, d, $J = 10.2$ Hz, CH_aH_bBr), 3.63 (1H, d, $J = 10.2$ Hz, CH_bH_aBr), 3.43 (1H, d, $J = 17.6$ Hz, $CH_aH_bCOCH_3$), 3.04 (1H, d, $J = 17.6$ Hz, $CH_bH_aCOCH_3$), 2.19 (3H, s, CH_3); ^{13}C NMR (DMSO-d_6): δ 204.36 (CO), 165.06 (CO), 140.33 (s), 136.40 (s), 123.48 (s), 120.52 (d), 110.18 (d), 109.62 (d), 60.45 (s), 50.15 (t), 42.39 (t), 30.24 (q); MS m/z 341 (M +).

3.2.2.18. 6,7-Difluoro-3-bromomethyl-2(1H)-quinoxalinone ***(26)***. This compound was obtained in 82% yield (1.56 g); m.p. 228–229°C; IR: ν 1660, 1620, 1600 cm^{-1}; UV: λ 345, 282, 229, 204 nm; ^1H NMR ($CDCl_3$ + DMSO-d_6): δ 12.74 (1H, s, NH), 7.65 (1H, dd, $J = 10.8$ and 8.2 Hz, H-5), 7.23 (1H, dd, $J = 10.8$ and 7.6 Hz, H-8), 4.57 (2H, s, CH_2Br).

3.2.2.19. 3,6-Bis(trifluoromethyl)-2(1H)-quinoxalinone ***(27)***. This compound (0.50 g, 31%) was obtained by eluting with a 7:3 mixture diethyl ether/light petroleum; m.p. 204–205°C; IR: ν 1700, 1630, 1580 cm^{-1}; UV: λ 355, 280, 237, 203 nm; ^1H NMR ($CDCl_3$ + DMSO-d_6): δ 12.80 (1H, br s, NH), 8.18 (1H, s, H-5), 7.82 (1H, d, $J = 8.6$ Hz, H-7), 7.57 (1H, d, $J = 8.6$ Hz, H-8).

3.2.2.20. 3,7-Bis(trifluoromethyl)-2(1H)-quinoxalinone ***(28)***. This compound (0.92 g, 58%) was obtained by eluting with a 7:3 mixture diethyl ether/light petroleum; m.p. 211–213°C; IR: ν 1690, 1630, 1570 cm^{-1}; UV: λ 363, 282, 276, 232, 202 nm; ^1H NMR ($CDCl_3$ + DMSO-d_6): δ 13.10 (1H, br s, NH), 8.06 (1H, d, $J = 8.4$ Hz, H-5), 7.70 (1H, d, $J = 1.8$ Hz, H-8), 7.58 (1H, dd, $J = 8.4$ and 1.8 Hz, H-7).

3.2.2.21. 6-Nitro-3-trifluoromethyl-2(1H)-quinoxalinone ***(29)***. This compound (0.34 g, 20%) was obtained by eluting with a 9:1 mixture diethyl ether/light petroleum; m.p. 229–230°C (Ref. [6], 226–228°C); IR: ν 1690, 1620, 1600, 1580 cm^{-1}; UV: λ 354, 344 infl, 310, 268, 205 nm; ^1H NMR ($CDCl_3$ + DMSO-d_6): δ 12.20 (1H, br s, NH), 8.74 (1H, d, $J = 2.4$ Hz, H-5), 8.44 (1H, dd, $J = 9.0$ and 2.4 Hz, H-7), 7.58 (1H, d, $J = 9.0$ Hz, H-8).

3.2.2.22. 7-Nitro-3-trifluoromethyl-2(1H)-quinoxalinone ***(30)***. This compound (1.19 g, 70%) was obtained by eluting with a 9:1 mixture diethyl ether/light petroleum; m.p. 218–219°C (Ref. [6], 219–220°C); IR: ν 1700, 1670, 1620, 1550 cm^{-1}; UV: λ 379, 364 infl, 285, 225, 201 nm; ^1H NMR ($CDCl_3$ + DMSO-d_6): δ 12.10 (1H, br s, NH), 8.28 (1H, d, $J = 2.0$ Hz, H-8), 8.17–8.07 (2H, m, H-5 + H-6).

3.2.2.23. 6,7-Difluoro-3-trifluoromethyl-2(1H)-quinoxalinone ***(31)***. This compound was obtained in 80% yield (1.38 g); m.p. 171–172°C; IR: ν 1680, 1630, 1600 cm^{-1}; UV: λ 362, 281, 229, 204 nm; ^1H NMR ($CDCl_3$ + DMSO-d_6): δ 13.20 (1H, br s, NH), 7.75 (1H, dd, $J = 10.8$ and 8.2 Hz, H-5), 7.28 (1H, dd, $J = 10.2$ and 7.4 Hz, H-8).

Table 2
Antifungal activity (MIC in μg/ml) of quinoxalinones **23**–**28**

Compd.	*C. albicans* ATCC 90029	*Candida* spp.[a]			*C. krusei* ATCC 6258	*C. parapsilosis* ATCC 20198	*C. tropicalis* ATCC 750	*C. glabrata* ATCC 90030
		1	2	3				
23	31.2	15.6	62.5	62.5	62.5	250	>500	62.5
24	>125	62.5	–	<3.5	125	>125	125	>125
25	8	15.6	31.2	15.6	62.5	15.6	31.2	15.6
26	31.2	62.5	125	500	125	62.5	500	125
27	8	125	125	125	250	125	250	250
28	8	15.6	125	125	250	15.6	125	125

[a] Hospital isolated strains.

*3.2.2.24. Conversion of 7-nitro-3-bromomethyl-2(1H)-quinoxalinone (**25**) into 7-nitro-3-acetonyl-3-bromomethyl-1,2,3,4-tetrahydroquinoxalin-2-one (**32**).* To a stirred solution of 0.45 g (1.59 mmol) of purified **25** in acetone (15 ml), 1.59 mmol of conc. sulfuric (or hydrochloric) acid were added and the solution was refluxed for 1 h. Evaporation of the solvent gave **32** (0.6 g, 100%) identical to the compound described above.

3.3. Microbiology

3.3.1. Antibacterial assay

Antibacterial activity was investigated in vitro on Gram positive and Gram negative bacteria at the Department of Biomedical Science of Sassari University. The strains used were *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *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, they 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 (500–0.5 μg/ml) of concentration. MICs were determined by the standard microbroth dilution method [8] as the lowest concentration of the compound which completely inhibited bacteria growth.

3.3.2. Antifungal assay

The antifungal tests were done by an in vitro method using the following model cultures: *Candida albicans* ATCC 90029, *C. krusei* ATCC 6258, *C. parapsilosis* ATCC 20198, *C. tropicalis* ATCC 750, *C. glabrata* ATCC 90030 and 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 above reported. Compounds were dissolved (1 mg/ml) in DMSO and then diluted with the test medium in order to obtain the required range (500–0.5 μg/ml) of concentration. Susceptibility testing was determined using the National Committee for Clinical Laboratory Standards microbroth dilution method [9]. After 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 80% reduction in turbidity when compared with the compound free control tube. Results of antifungal activity are reported in Table 2.

3.3.3. Antimycobacterial activity

Evaluation of antimycobacterial activity was performed at the Tuberculosis Antimicrobial Acquisition and Coordinating Facility (TAACF) of the National Institute of Allergy and Infection Diseases of the Colorado State University, Birmingham, CO. In the primary screening, compounds were tested in vitro at 12.5 μg/ml against *Mycobacterium tuberculosis* H37Rv in BACTEC 12B medium, using the BACTEC 460-radiometric system to determine bacteriostatic activity. Compounds demonstrating at least 90% inhibition in the primary screening are re-tested at lower concentrations to determine the actual MIC, the cytotoxicity (IC_{50}) and the activity on *M. avium* and on other naturally drug-resistant strains.

3.3.4. In vitro antitumoral and anti-HIV activity

All new quinoxalinones were sent to the National Cancer Institute (NCI) of Bethesda, MD. The selected compounds (**8**, **9**, **12**–**17**, **24**, **27** and **28**) were submitted to a primary evaluation for the anticancer and anti-HIV activities following the known in vitro disease-oriented antitumor screening program, against a panel of about 60 human tumor cell lines [10] and anti-HIV drug testing system [11]. The activity of each compound tested was deduced from dose–response curve according to the data provided by NCI. In Table 3 we report

Table 3
Percent tumor growth inhibition recorded on subpanel cell-lines at 10^{-4} M of tested quinoxalinones (**8**, **9**, **12–17**, **24**, **27** and **28**)[a]

Panel/cell-lines	**8**	**9**	**12**	**13**	**14**	**15**	**16**	**17**	**24**	**27**	**28**
Leukemia											
CCRF-CEM	55	–	–	106	–	91	–	–	87	132	88
HL-60(TB)	67	–	nt	131	40	85	–	–	125	nt	nt
K-562	42	–	–	65	–	98	60	80	85	96	75
MOLT-4	57	–	–	nt	71	137	45	77	83	122	88
RPMI-8226	40	–	44	–	46	108	–	65	116	97	87
SR	62	–	47	nt	43	44	66	–	90	85	72
Non-small cell lung cancer											
A549/ATCC	70	63	–	40	nt	nt	nt	nt	188	97	91
EKVX	92	40	–	–	–	95	48	–	199	94	85
HOP-62	55	200	–	–	–	92	102	–	197	127	109
HOP-92	72	–	–	45	–	135	99	48	186	153	140
NCI-H226	63	–	–	–	–	132	–	–	191	104	87
NCI-H23	–	–	–	–	–	144	117	42	158	140	102
NCI-H322M	–	50	–	–	–	43	–	–	199	98	95
NCI-H460	40	–	nt	nt	–	100	–	–	189	89	50
NCI-H522	78	54	–	–	nt	nt	nt	nt	186	137	100
Colon cancer											
COLO 205	–	–	–	nt	–	155	–	–	190	155	98
HCC-2998	42	–	nt	nt	43	108	–	–	200	79	73
HCT-116	61	45	–	–	–	93	56	46	199	123	92
HCT-15	–	–	–	–	–	87	–	–	128	97	83
HT29	60	–	–	57	–	119	–	–	141	89	80
KM12	–	76	–	–	–	96	–	–	199	130	115
SW-620	–	–	–	–	–	87	–	–	164	nt	75
CNS cancer											
SF-268	53	48	–	–	–	84	79	–	146	94	80
SF-295	–	57	–	–	–	95	84	–	194	97	87
SF-539	49	55	nt	nt	–	89	61	–	191	120	102
SNB-19	–	–	–	–	–	67	58	–	189	98	85
SNB-75	74	86	–	–	–	71	78	–	197	127	103
U251	40	68	–	–	–	76	57	–	194	100	83
Melanoma											
LOX IMVI	nt	nt	–	–	–	88	–	–	95	123	86
MALME-3M	46	48	–	–	nt	nt	nt	nt	194	112	85
M14	–	–	–	45	–	101	–	–	197	86	74
SK-MEL-2	62	200	–	–	–	125	48	–	197	140	113
SK-MEL-28	–	–	–	–	–	76	–	–	192	94	62
SK-MEL-5	65	–	–	–	–	129	–	–	89	172	154
UACC-257	44	–	–	–	–	62	–	–	200	122	104
UACC-62	67	–	–	–	–	75	42	–	200	98	75
Ovarian cancer											
IGROV1	47	–	–	–	–	94	68	–	167	102	83
OVCAR-3	63	200	–	–	nt	nt	nt	nt	200	142	124
OVCAR-4	51	61	–	–	–	90	59	41	199	90	65
OVCAR-5	41	–	–	–	nt	nt	nt	nt	196	73	77
OVCAR-8	46	–	–	–	–	91	57	–	138	112	86
SK-OV-3	–	44	–	–	–	86	107	–	152	107	92
Renal cancer											
786-0	61	70	–	–	–	91	82	–	198	88	79
A498	62	–	–	43	–	122	53	–	200	105	83
ACHN	59	41	–	46	–	106	66	–	200	95	83
CAKI-1	59	83	–	–	–	76	63	–	190	110	105
RXF 393	70	52	–	–	–	130	109	–	197	136	126
SN12C	–	–	–	–	nt	nt	nt	nt	190	106	96
TK-10	57	–	–	–	–	45	46	–	199	86	68
UO-31	47	–	–	–	–	86	55	–	200	111	94

(continued)

Table 3 *(continued)*

Panel/cell-lines	**8**	**9**	**12**	**13**	**14**	**15**	**16**	**17**	**24**	**27**	**28**
Prostate cancer											
PC-3	58	200	–	–	–	109	–	–	198	124	102
DU-145	nt	–	–	–	65	94	–	–	199	98	83
Breast cancer											
MCF7	53	–	–	nt	–	96	–	–	154	109	70
MCF7/ADR-RES	64	67	–	–	nt	nt	nt	nt	nt	nt	nt
NCI/ADR-RES	nt	nt	nt	nt	41	113	60	–	138	119	87
MDA-MB-231/ATCC	57	–	–	–	116	93	109	–	199	101	99
HS 578T	111	92	–	46	–	115	97	–	134	nt	nt
MDA-MB-435	–	–	–	–	–	96	–	–	197	113	109
MDA-N	–	–	–	–	nt	nt	nt	–	195	85	89
BT-549	49	–	–	–	–	126	–	–	172	104	76
T-47D	46	–	–	–	–	90	73	44	124	131	86

[a] (–), below 40% growth inhibition; (nt), not tested at this molar concentration.

the activity of those compounds which showed percent growth inhibition $>40\%$ recorded on subpanel cell-lines at 10^{-4} molar concentration. Table 4 shows the response parameters of GI_{50}, TGI and LC_{50} expressed as mean graph midpoints. Table 5 reports the most significant data of percent growth inhibition of compound **24** at 10^{-5} and 10^{-6} molar concentrations.

4. Results and discussion

Among all new quinoxalinones **3–31** submitted to in vitro preliminary evaluation for antimicrobial activity, only compounds **23** and **24** exhibited interesting activity against *S. aureus* (MIC = 31.2 and 62.5 μg/ml, respectively), while the other derivatives were found to be moderately (**26**; MIC = 125 μg/ml) or poorly effective (**3–22**, **25**, **27**, **31**; MIC = 250–500 μg/ml). All com-

Table 4
$-\mathrm{Log}_{10}\,GI_{50}$, $-\log_{10}$ TGI and $-\log_{10}\,LC_{50}$ mean graph midpoints (MG-MID)[a] of in vitro inhibitory activity tests for compounds **8**, **9**, **12–17**, **24**, **27** and **28** against human tumor cell lines[b]

Compd.	$-\log_{10}\,GI_{50}$	$-\log_{10}$ TGI	$-\log_{10}\,LC_{50}$
8	4.19	4.00	4.00
9	4.12	4.03	4.02
12	4.00	4.00	4.00
13	4.07	4.01	4.00
14	4.03	4.01	4.00
15	4.47	4.06	4.00
16	4.16	4.01	4.00
17	4.03	4.00	4.00
24	5.08	4.57	4.23
27	4.62	4.12	4.00
28	4.47	4.03	4.00

[a] (MG-MID): mean graph midpoints, the average sensitivity of all cell-lines toward the test agent.
[b] From NCI.

Table 5
Percent tumor growth inhibition recorded on subpanel cell-lines at 10^{-5} and 10^{-6} M of compound **24**[a]

Panel/cell-lines	10^{-5}	10^{-6}	Panel/cell-lines	10^{-5}	10^{-6}
Leukemia			*Melanoma*		
CCRF-CEM	102	–	LOX IMVI	95	–
HL-60(TB)	112	–	MALME-3M	44	–
K-562	76	–	M14	49	–
MOLT-4	86	–	UACC-62	56	–
RPMI-8226	63	–	*Ovarian cancer*		
SR	90	–	IGROV1	53	–
Non-small cell lung cancer			OVCAR-3	98	–
HOP-62	56	50	OVCAR-8	50	–
HOP-92	125	44	*Renal cancer*		
NCI-H522	188	123	786-0	93	–
Colon cancer			CAKI-1	82	–
COLO 205	40	–	UO-31	81	–
KM12	40	–	*Breast cancer*		
SW-620	49	–	MCF7	70	–
CNS cancer			MDA-MB-435	47	–
U251	68	–	MDA-N	51	–

[a] (–), below 40% growth inhibition.

pounds tested, with the exception of **25** which showed a moderate activity against *E. coli* (MIC = 125 μg/ml), resulted inactive against Gram negative bacteria and their data are not reported.

All compounds were evaluated against *C. albicans* ATCC 90029 and various strains of *Candida* spp. (hospital isolated). The most active compounds in the series were also tested against various other strains of *Candida*. The results reported in Table 2 show a generally good antifungal activity of the selected compounds. In particular the nitro derivative **25** exhibited an interesting activity on all strains of *Candida* tested (MIC = 8–62.5 μg/ml), whereas compounds **27** and **28** showed high activity (MIC = 8 μg/ml) against *C. albicans* ATCC 90029. In addition compound **28** inhibited the growth of a strain of *Candida* spp[1]. and *C. parapsilosis* (MIC = 15.6 μg/ml). The other compounds reported (**23**, **24** and **26**) were found to be less effective although they exhibited a specific activity (MIC = 15.6–62.5 μg/ml) on some strains.

The quinoxalinones **23–28** were also submitted to in vitro screening for evaluation of the activity on *M. tuberculosis*. Only compound **25** showed a moderate growth inhibition activity (15% at 12.5 μg/ml).

The results of the in vitro anticancer activity concerning the quinoxalinones **8**, **9**, **12–17**, **24**, **27** and **28** are reported in Tables 3 and 4. In particular the data of Table 3 show a wide range of inhibitory activity recorded at 10^{-4} molar concentration. Among these, compounds **8**, **15**, **27** and **28** exhibited a good cytostatic or cytocidal activity on all subpanel cell-lines tested at this concentration, even if their average inhibitory activity, represented as mean graph midpoints (Table 4), falls in the range of 10^{-4}–10^{-5} molar concentrations. Compound **16** resulted generally less effective than the above-mentioned derivatives, but associated with a good selectivity against some CNS cancer, ovarian cancer and renal cancer subpanel cell-lines. Compound **24** was the most interesting, since its antiproliferative activity was very high in all subpanel cell-lines at 10^{-4} molar concentration. This compound maintains its cytotoxicity also at 10^{-5} molar concentration on all leukemia cell-lines and on various subpanel cell-lines of non-small cell lung cancer, colon cancer, CNS cancer, melanoma, ovarian cancer, renal cancer and breast cancer (Table 5). These results, along with data of mean graph midpoints (Table 4), induced the BEC/C (NCI) to test compound **24** in vivo preliminary assays. Finally none of the quinoxalinones tested exhibited a significative anti-HIV activity.

5. Conclusions

In conclusion, all of the biological data allowed us to make some observations on structure–activity relationships. The results of antibacterial and anticancer tests seem to confirm that for the biological activity an electron-withdrawing substituent in the benzene moiety is necessary and the highest activity is reached when a lipophilic side-chain at C-3 of the hetero ring is present. Replacement of the trifluoromethyl group with both di-fluoro and nitro-groups do not seem to affect substantially either antibacterial or anticancer activities. On the contrary, this replacement sensibly affects the activity against *Candida* strains. The nitro group seems also to determine a moderate (15% growth inhibition at 12.5 μg/ml) antimycobacterial activity. As regards to the modifications of the substituent at C-3, we have found that, in general, a second electronegative group (CH_2Br or CF_3) in this position determines both highest antifungal and the most selective anticancer activities.

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