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Synthesis and evaluation for biological activity of 3-alkyl and 3-halogenoalkyl-quinoxalin-2-ones variously substituted. Part 4

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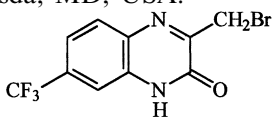
Abstract

A new series of 3-isopropyl-, 3-trifluoromethyl- and 3-bromomethylquinoxaline-2-ones variously substituted on the benzo-moiety were synthesized and submitted to a preliminary in vitro evaluation for antibacterial, antifungal and anti-HIV activities. Furthermore, all compounds were also tested for cytotoxicity. Results of the screening showed that compound **10** exhibits moderate antimicrobial activity against *Staphylococcus aureus* (MIC = 33 μM), and that **25** and **26** showed interesting cytotoxicity versus mock-infected MT-4 cells. All the other compounds were inactive.

Keywords: 2-Quinoxalinones; Antimicrobial activity; Cytotoxic activity

1. Introduction

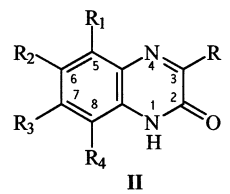
In previous reports we described the synthesis and antimicrobial and anticancer activities of various quinoxalin-2-one derivatives [1–3]. Among these, compounds with a CF_3 or NO_2 in C-6 or C-7 and a CH_2Br or CF_3 at C-3 showed good activity against various strains of *Candida* (*C. albicans*, *C. krusei*, *C. parapsilosis*, *C. tropicalis*, *C. glabrata* and various clinical isolated of *Candida* spp.), whereas derivatives bearing Cl or CF_3 at C-6 or C-7 and a carboxyethyl, ethyl or CH_2Br group at C-3 exhibited moderate antibacterial activity. Moreover, 3-bromomethyl-7-trifluoromethyl-2(1*H*)-quinoxalinone (Formula **I**) proved fairly active in vitro as an anticancer agent against about 60 human tumor cell lines and it is actually tested in vivo at the National Cancer Institute (NCI) of Bethesda, MD, USA.



I

These results prompted us to prepare a new series of quinoxalin-2-one derivatives in the hope of increasing the activity and obtaining additional data for structure–activity relationships. In this paper we describe the synthesis and microbiological and cytotoxic activities of 24 derivatives, the structure of which is summarized in Formula **II**.

R=CH_2Br, CF_3, $CH(CH_3)_2$;
R_1=H, Cl, NH_2;
R_2=H, Cl, CF_3, OCH_3;
R_3=H, Cl, CF_3, OCH_3;
R_4=H, Cl, NH_2



II

In these new compounds we introduced in the benzo-moiety alternatively one or two atoms of chlorine, one electron-releasing group (CH_3O), or one electron-withdrawing (Cl or CF_3) group together with an electron-releasing group (CH_3O or NH_2), whereas we introduced at the C-3 position an isopropyl, bromomethyl or trifluoromethyl group in order to evaluate the effect of electron-releasing and/or electron-withdrawing substituents on biological activities.

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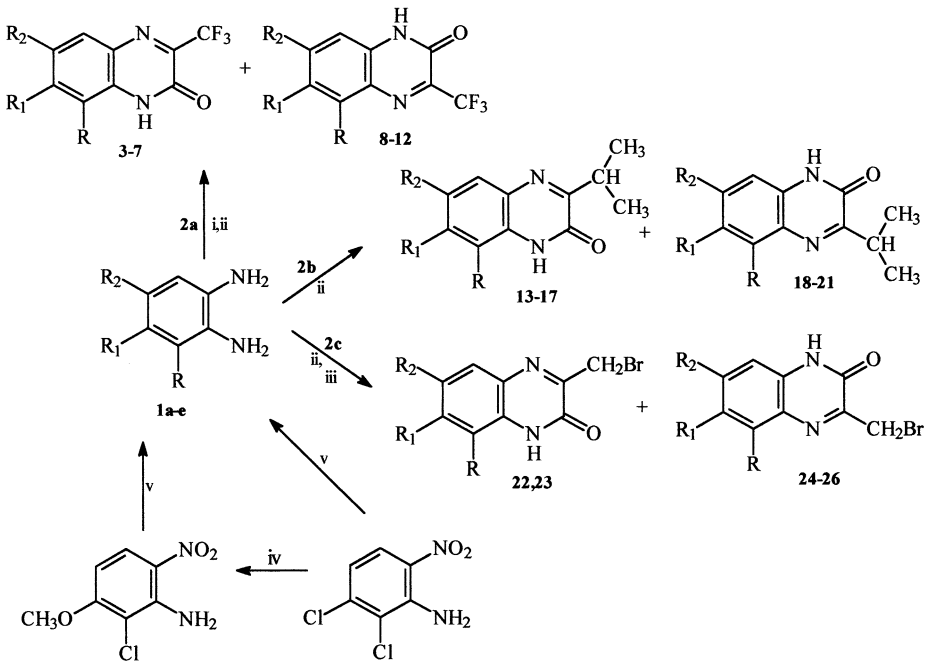
2. Chemistry

The quinoxalinones **3**–**26**, listed in Table 1, were prepared following the procedure depicted in Scheme 1. According to our previous report [3], the diamine **1a**–**d** was reacted with the suitable α-ketoester **2a**–**c** in 10% aqueous solution of sulfuric acid to give compounds **3**–**6**, **8**–**11**, **13**–**16**, **18**–**20**, **23**, **25** and **26** in good yields, while the condensation of **1a** with **2c**, to obtain compounds **22** and **24**, failed. The latter compounds were obtained by condensation in ethanol at room temperature for 1 h, while the isomeric pairs **7**/**12** and **17**/**21** were obtained by condensation in ethanol under reflux for 3 h of the diamine **1e** with the α-ketoesters **2a**,**b**, respectively.

It is important to note that when 1,2-diamino-4-methoxybenzene (**1b**) and 1,2-diamino-3-chloro-4-methoxybenzene (**1d**) were condensed with **2a** and **2c** under acidic conditions, we obtained prevalently (**9** > **4**, **11** > **6**) or exclusively (**26**) the 6-methoxy derivatives. This behavior is reversed in the case of the condensation of the same diamines **1b**,**d** with **2b**. In the former case only the 7-methoxy derivative **14** is obtained whereas in the latter case **16** is prevalent over **20**. These results show that protonation of the amino group on the *para* position of the methoxy group precedes the condensation, and that the steric hindrance of the isopropyl group in the case of **2b** promotes an inversion of reactivity in comparison with other α-ketoesters. Quinoxalinones **3** and **8** were already described by

Table 1
Compounds of Scheme 1

Comp.	R	R_1	R_2	Comp.	R	R_1	R_2	Comp.	R	R_1	R_2
1a	H	Cl	H	**8**	H	Cl	H	**18**	H	Cl	H
1b	H	OCH_3	H	**9**	H	OCH_3	H	**19**	Cl	Cl	H
1c	Cl	Cl	H	**10**	Cl	Cl	H	**20**	Cl	OCH_3	H
1d	Cl	OCH_3	H	**11**	Cl	OCH_3	H	**21**	NH_2	H	CF_3
1e	NH_2	H	CF_3	**12**	NH_2	H	CF_3	**22**	H	Cl	H
3	H	Cl	H	**13**	H	Cl	H	**23**	Cl	Cl	H
4	H	OCH_3	H	**14**	H	OCH_3	H	**24**	H	Cl	H
5	Cl	Cl	H	**15**	Cl	Cl	H	**25**	Cl	Cl	H
6	Cl	OCH_3	H	**16**	Cl	OCH_3	H	**26**	Cl	OCH_3	H
7	NH_2	H	CF_3	**17**	NH_2	H	CF_3				



Scheme 1. Preparation of substituted quinoxalin-2-ones (**3**–**26**). Reagents: (**2a**) CF_3COCO_2Et; (**2b**) $(CH_3)_2CHCOCO_2Et$; (**2c**) $CH_2BrCOCO_2Et$. Conditions: (i) EtOH, under reflux for 3 h; (ii) H_2SO_4 10% aqueous solution at 60–100 °C for 1–3 h; (iii) EtOH r.t. for 1 h; (iv) MeONa/MeOH, under reflux for 8 h; (v) H_2 on 10% Pd/C at 3 atm.

Mustafa et al. [4], but we reprepared and concluded on the basis of ^{1}H NMR data that the previous assignment of the structure to two isomers must be reversed. The structures of all quinoxalinones **3**–**26** have been supported by us by analytical and spectroscopic data (MS spectra, IR, UV, ^{1}H NMR) and are in agreement with those previously reported for similar compounds [1–3].

3. Experimental

3.1. Chemistry

Melting points were determined in a Kofler hot stage or Digital Electrothermal apparatus, and are uncorrected. Infrared spectra were recorded in Nujol mulls and were recorded using a Perkin–Elmer 781 spectrophotometer. Qualitative UV spectra (ethanol) were recorded in a Perkin–Elmer Lambda 5 spectrophotometer and are reported in nanometers. ^{1}H NMR spectra were recorded in a Varian XL-200 (200 MHz) instrument, using TMS as internal standard. The chemical shift values are reported in ppm (δ) and coupling constants (J) in Hertz (Hz). Signal multiplicities are represented by: s (singlet), d (doublet), dd (double doublet), m (multiplet), and br s (broad singlet). MS spectra were recorded in a combined HP 5790-HP 5970 GC/MS apparatus. Column chromatography was 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, when present, were within $\pm$0.4% of the theoretical values.

3.1.1. Intermediates

The diaminobenzene derivatives **1a**,**b** were commercially available, **1e** was prepared following the literature procedure [5]; **1c** [6] was reprepared by us following a new procedure by hydrogenation of the parent 2,3-dichloro-6-nitroaniline [7] using 10% Pd/C catalyst at 3 atm. Diamine **1d** is a new compound and was prepared in 80% yield by hydrogenation using 10% Pd/C catalyst at 3 atm, starting from the parent 2-chloro-3-methoxy-6-nitroaniline. The latter compound [8] was reprepared by us in 96% yield following a new procedure by treatment of the 2,3-dichloro-6-nitroaniline with MeONa/MeOH, under reflux for 8 h, followed by evaporation in vacuo of the solvent and treatment with water.

3.1.1.1. 1,2-Diamino-3-chloro-4-methoxybenzene ***(1d)***. M.p. 100–101 °C; IR: ν 3400, 3360, 3280, 3240, 1660, 1640, 1610 cm^{-1}; UV: λ 301, 221 nm; ^{1}H NMR ($CDCl_3$): δ 6.59 (1H, d, $J = 8.2$ Hz, H-6), 6.28 (1H, d, $J = 8.2$ Hz, H-5), 3.82 (1H, s, CH_3), 3.70–3.10 (4H, m, $2NH_2$).

3.1.2. General procedure for preparation of quinoxalinones *3–6*, *8–11*, *13–16*, *18–20*, *23*, *25* and *26*

A mixture of equimolar amounts (3.5 mmol) of the appropriate diamine **1a**–**d** and the suitable α-ketoester **2a**,**c** in 10% aqueous solution of sulfuric acid was stirred at 100 °C for 1 h in the case of **3**, **4**, **8**, **9**, **13**, **14**, **18** and **24** or at 60 °C for 3 h in the case of **5**, **6**, **10**, **11**, **15**, **16**, **19**, **20**, **23**, **25** and **26**. After cooling of the reaction mixture to room temperature (r.t.), the crude precipitate formed was collected by filtration and chromatographed on a silica gel column, eluting as reported below, in order to separate the mixture of isomers where present.

3.1.2.1. 7-Chloro-3-trifluoromethyl-2(1H)-quinoxalinone ***(3)***. This compound was obtained in 38% yield by eluting with a 8:2 mixture of light petroleum–ethyl acetate; m.p. 244–246 °C; IR: ν 1670, 1610 cm^{-1}; UV: λ 345, 276, 219, 194 nm; ^{1}H NMR ($CDCl_3$ + DMSO-d_6): δ 13.08 (1H, br s, NH), 7.84 (1H, d, $J = 8.8$ Hz, H-5), 7.43 (1H, d, $J = 2.4$ Hz, H-8), 7.31 (1H, dd, $J = 8.8$ and 2.4 Hz, H-6); MS: m/z 248 (M^+).

3.1.2.2. 6-Chloro-3-trifluoromethyl-2(1H)-quinoxalinone ***(8)***. This compound (42% yield) was eluted as described for **3**; m.p. 208–210 °C (EtOH); IR: ν 1570 cm^{-1}; UV: λ 352, 269, 218 nm; ^{1}H NMR ($CDCl_3$ + DMSO-d_6): δ 13.12 (1H, br s, NH), 7.69 (1H, d, $J = 2.0$ Hz, H-5), 7.57 (1H, dd, $J = 8.8$ and 2.0 Hz, H-7), 7.38 (1H, d, $J = 8.8$ Hz, H-8); MS: m/z 248 (M^+).

3.1.2.3. 7-Methoxy-3-trifluoromethyl-2(1H)-quinoxalinone ***(4)***. This compound was obtained in 10% yield by eluting with a 1:1 mixture of light petroleum–ethyl acetate; m.p. 260–262 °C; IR: ν 1660, 1620 cm^{-1}; UV: λ 358, 264, 232, 214 nm; ^{1}H NMR ($CDCl_3$ + DMSO-d_6): δ 12.75 (1H, br s, NH), 7.71 (1H, d, $J = 8.8$ Hz, H-5), 6.86 (1H, dd, $J = 8.8$ and 2.6 Hz, H-6), 6.72 (1H, d, $J = 2.6$ Hz, H-8), 3.83 (3H, s, CH_3); MS: m/z 244 (M^+).

3.1.2.4. 6-Methoxy-3-trifluoromethyl-2(1H)-quinoxalinone ***(9)***. This compound (57% yield) was eluted as described for **4**; m.p. 230–232 °C; IR: ν 1670 cm^{-1}; UV: λ 288, 237, 206 nm; ^{1}H NMR ($CDCl_3$ + DMSO-d_6): δ 12.93 (1H, br s, NH), 7.37 (1H, d, $J = 9.0$ Hz, H-8), 7.34 (1H, d, $J = 1.2$ Hz, H-5), 7.24 (1H, dd, $J = 9.0$ and 1.2 Hz, H-7), 3.88 (3H, s, CH_3); MS: m/z 244 (M^+).

3.1.2.5. 7,8-Dichloro-3-trifluoromethyl-2(1H)-quinoxalinone ***(5)***. This compound was obtained in 46% yield by eluting with diethyl ether; m.p. 242–244 °C; IR: ν 1680, 1595 cm^{-1}; UV: λ 379, 296, 253, 216 nm; ^{1}H NMR ($CDCl_3$ + DMSO-d_6): δ 7.82 (1H, d, $J = 8.8$ Hz, H-6), 7.49 (1H, d, $J = 8.8$ Hz, H-5); MS: m/z 281 (M^+), 282 (M^+).

*3.1.2.6. 5,6-Dichloro-3-trifluoromethyl-2(1H)-quinoxalinone (**10**).* This compound (28% yield) was eluted as described for **5**; m.p. 252–254 °C; IR: ν 1685, 1595, 1580 cm^{-1}; UV: λ 381, 300 sh, 256, 215 nm; ^1H NMR ($CDCl_3$ + DMSO-d_6): δ 13.32 (1H, br s, NH), 7.66 (1H, d, $J = 8.8$ Hz, H-7), 7.33 (1H, d, $J = 8.8$ Hz, H-8); MS: m/z 282 (M^+).

*3.1.2.7. 8-Chloro-7-methoxy-3-trifluoromethyl-2(1H)-quinoxalinone (**6**).* This compound was obtained in 25% yield by eluting with diethyl ether; m.p. 272–274 °C; IR: ν 1690, 1620, 1600 cm^{-1}; UV: λ 362, 253, 220 nm; ^1H NMR ($CDCl_3$ + DMSO-d_6): δ 12.40 (1H, br s, NH), 7.88 (1H, d, $J = 9.2$ Hz, H-5), 7.26 (1H, d, $J = 9.2$ Hz, H-6), 4.04 (3H, s, CH_3); MS: m/z 278 (M^+).

*3.1.2.8. 5-Chloro-6-methoxy-3-trifluoromethyl-2(1H)-quinoxalinone (**11**).* This compound (55% yield) was eluted as described for **6**; m.p. 288–290 °C; IR: ν 1680, 1610, 1600 cm^{-1}; UV: λ 396, 299, 240, 212 nm; ^1H NMR ($CDCl_3$ + DMSO-d_6): δ 13.45 (1H, br s, NH), 7.56 (1H, d, $J = 9.2$ Hz, H-8), 7.34 (1H, d, $J = 9.2$ Hz, H-7), 3.95 (3H, s, CH_3); MS: m/z 278 (M^+).

*3.1.2.9. 7-Chloro-3-isopropyl-2(1H)-quinoxalinone (**13**).* This compound was obtained in 24% yield by eluting with a 8:2 mixture of light petroleum–ethyl acetate; m.p. 207–209 °C; IR: ν 1670, 1610 cm^{-1}; UV: λ 339, 275, 232, 208 nm; ^1H NMR ($CDCl_3$ + DMSO-d_6): δ 12.32 (1H, br s, NH), 7.68 (1H, d, $J = 8.6$ Hz, H-5), 7.28 (1H, d, $J = 2.0$ Hz, H-8), 7.20 (1H, dd, $J = 8.6$ and 2.0 Hz, H-6), 3.52 (1H, m, CH), 1.29 (6H, d, $J = 6.8$ Hz, 2 CH_3); MS: m/z 222 (M^+).

*3.1.2.10. 6-Chloro-3-isopropyl-2(1H)-quinoxalinone (**18**).* This compound (16% yield) was eluted as described for **13**; m.p. 229–231 °C; IR: ν 1660 cm^{-1}; UV: λ 337, 280, 231, 211 nm; ^1H NMR ($CDCl_3$ + DMSO-d_6): δ 12.06 (1H, br s, NH), 7.79 (1H, d, $J = 2.6$ Hz, H-5), 7.35 (1H, dd, $J = 8.8$ and 2.6 Hz, H-7), 7.22 (1H, d, $J = 8.8$ Hz, H-8), 3.59 (1H, m, CH), 1.30 (6H, d, $J = 6.8$ Hz, $2CH_3$); MS: m/z 222 (M^+), 222 (M^+).

*3.1.2.11. 7-Methoxy-3-isopropyl-2(1H)-quinoxalinone (**14**).* This compound was obtained pure in 79% yield; m.p. 223–225 °C; IR: ν 1670, 1610, 1600 cm^{-1}; UV: λ 340, 265, 211 nm; ^1H NMR ($CDCl_3$ + DMSO-d_6): δ 12.06 (1H, br s, NH), 7.62 (1H, d, $J = 8.8$ Hz, H-5), 6.80 (1H, dd, $J = 8.6$ and 2.8 Hz, H-6), 6.74 (1H, d, $J = 2.8$ Hz, H-8), 3.85 (3H, s, CH_3O), 3.49 (1H, m, CH), 1.26 (6H, d, $J = 6.8$ Hz, 2 CH_3); MS: m/z 218 (M^+).

*3.1.2.12. 7,8-Dichloro-3-isopropyl-2(1H)-quinoxalinone (**15**).* This compound was obtained in 29% yield by eluting with a 1:1 mixture of light petroleum–diethyl ether; m.p. 236–237 °C; IR: ν 1670, 1600 cm^{-1}; UV: λ 350 infl, 340, 330 infl, 280 sh, 236, 211 nm; ^1H NMR ($CDCl_3$ + DMSO-d_6): δ 11.75 (1H, br s, NH), 7.67 (1H, d, $J = 8.6$ Hz, H-6), 7.40 (1H, d, $J = 8.6$ Hz, H-5), 3.54 (1H, m, CH), 1.26 (6H, d, $J = 6.8$ Hz, 2 CH_3); MS: m/z 256 (M^+).

*3.1.2.13. 5,6-Dichloro-3-isopropyl-2(1H)-quinoxalinone (**19**).* This compound (25% yield) was eluted as described for **15**; m.p. 247–248 °C; IR: ν 1665, 1565, 1580 cm^{-1}; UV: λ 360 infl, 348, 338 infl, 285, 241, 213 nm; ^1H NMR ($CDCl_3$ + DMSO-d_6): δ 12.58 (1H, br s, NH), 7.62 (1H, d, $J = 8.8$ Hz, H-7), 7.24 (1H, d, $J = 8.8$ Hz, H-8); 3.51 (1H, m, CH), 1.26 (6H, d, $J = 6.8$ Hz, $2CH_3$); MS: m/z 256 (M^+).

*3.1.2.14. 8-Chloro-7-methoxy-3-isopropyl-2(1H)-quinoxalinone (**16**).* This compound was obtained in 47% yield by eluting with diethyl ether; m.p. 244–245 °C; IR: ν 1665, 1620 cm^{-1}; UV: λ 348 infl, 338, 267, 260 sh, 228 sh, 220 nm; ^1H NMR (DMSO-d_6): δ 11.73 (1H, br s, NH), 7.72 (1H, d, $J = 9.4$ Hz, H-5), 7.17 (1H, d, $J = 9.4$ Hz, H-6), 3.97 (3H, s, OCH_3), 3.90 (1H, m, CH), 1.24 (6H, d, $J = 6.8$ Hz, $2CH_3$); MS: m/z 252 (M^+).

*3.1.2.15. 5-Chloro-6-methoxy-3-isopropyl-2(1H)-quinoxalinone (**20**).* This compound (3% yield) was eluted as described for **16**; m.p. 242–243 °C; IR: ν 1675, 1610, 1590 cm^{-1}; UV: λ 358, 289, 268, 240, 216 nm; ^1H NMR (DMSO-d_6): δ 12.36 (1H, br s, NH), 7.41 (1H, d, $J = 9.2$ Hz, H-8), 7.22 (1H, d, $J = 9.2$ Hz, H-7), 3.92 (1H, m, CH), 3.90 (3H, s, OCH_3), 1.24 (6H, d, $J = 6.6$ Hz, $2CH_3$).

*3.1.2.16. 3-Bromomethyl-7,8-dichloro-2(1H)-quinoxalinone (**23**).* This compound was obtained in 33% yield by eluting with a 7:3 mixture of diethyl ether–light petroleum; m.p. 215–216 °C; IR: ν 1670, 1595, 1580 cm^{-1}; UV: λ 371, 300, 255, 217 nm; ^1H NMR ($CDCl_3$): δ 9.60 (1H, br s, NH), 7.73 (1H, d, $J = 8.4$ Hz, H-6), 7.45 (1H, d, $J = 8.4$ Hz, H-5), 4.62 (2H, s, CH_2); MS: m/z 306 (M^+).

*3.1.2.17. 3-Bromomethyl-5,6-dichloro-2(1H)-quinoxalinone (**25**).* This compound (20% yield) was eluted as described for **23**; m.p. > 300 °C; IR: ν 1675, 1600, 1590 cm^{-1}; UV: λ 372, 296, 250, 216 nm; ^1H NMR ($CDCl_3$ + DMSO-d_6): δ 12.92 (1H, br s, NH), 7.65 (1H, d, $J = 8.6$ Hz, H-7), 7.30 (1H, d, $J = 8.6$ Hz, H-8), 4.67 (2H, s, CH_2).

*3.1.2.18. 3-Bromomethyl-5-chloro-methoxy-2(1H)-quinoxalinone (**26**).* This compound was obtained in 20% yield by eluting with diethyl ether; m.p. 226–228 °C; IR: ν 1665, 1610, 1590 cm^{-1}; UV: λ 386, 301, 260 sh,

242, 216 nm; ^{1}H NMR (DMSO-d_6): δ 12.71 (1H, br s, NH), 7.54 (1H, d, $J = 9.2$ Hz, H-8), 7.29 (1H, d, $J = 9.2$ Hz, H-7), 4.65 (2H, s, CH_2), 3.93 (3H, s, CH_3).

3.1.3. General procedure for preparation of quinoxalinones ***7***, ***12***, ***17***, ***21***, ***22*** *and* ***24***

A mixture of equimolar amounts (2.5 mmol) of the appropriate diamine and the suitable α-ketoester in ethanol (10 ml) was stirred at r.t. for 1 h (**1a** with **2c**) or at reflux for 3 h in the case of **1e** with **2a** and **2b**, respectively. After evaporation of the solvent, the crude solid residue was purified by chromatography on a silica gel column, eluting as reported below.

3.1.3.1. 8-Amino-3,6-bis(trifluoromethyl)-2(1H)-quinoxalinone ***(7)***. This compound was obtained in 37% yield by eluting with a 8:2 mixture of diethyl ether–light petroleum; m.p. 220–222 °C; IR: ν 3450, 3390, 1680, 1610 cm^{-1}; UV: λ 282, 250, 231 nm; ^{1}H NMR ($CDCl_3$ + DMSO-d_6): δ 12.50 (1H, br s, NH), 7.41 (1H, s, H-7), 7.21 (1H, s, H-5), 5.89 (2H, br s, NH_2); MS: m/z 297 (M^+).

3.1.3.2. 5-Amino-3,7-bis(trifluoromethyl)-2(1H)-quinoxalinone ***(12)***. This compound (37% yield) was eluted as described for **7**; m.p. 280–282 °C; IR: ν 3500, 3400, 1680, 1610 cm^{-1}; UV: λ 343, 284, 222 nm; ^{1}H NMR ($CDCl_3$ + DMSO-d_6): δ 12.50 (1H, br s, NH), 6.80 (1H, s, H-7), 6.68 (1H, s, H-5), 6.34 (2H, br s, NH_2); MS: m/z 297 (M^+).

3.1.3.3. 8-Amino-6-trifluoromethyl-3-isopropyl-2(1H)-quinoxalinone ***(17)***. This compound was obtained in 60% yield by eluting with a 8:2 mixture of diethyl ether–light petroleum; m.p. 270–272 °C; IR: ν 3480, 3400, 1660, 1630 cm^{-1}; UV: λ 277, 229, 211 nm; ^{1}H NMR ($CDCl_3$ + DMSO-d_6): δ 11.80 (1H, br s, NH), 7.36 (1H, s, H-7), 7.01 (1H, s, H-5), 3.56 (1H, m, CH), 1.30 (6H, d, $J = 6.8$ Hz, $2CH_3$); MS: m/z 271 (M^+).

3.1.3.4. 5-Amino-7-trifluoromethyl-3-isopropyl-2(1H)-quinoxalinone ***(21)***. This compound (21% yield) was eluted as described for **17**; m.p. 256–258 °C; IR: ν 3490, 3380, 1660, 1610 cm^{-1}; UV: λ 328, 280, 228, 212 nm; ^{1}H NMR ($CDCl_3$ + DMSO-d_6): δ 12.18 (1H, br s, NH), 6.75 (2H, s, H-6 + H-8), 3.49 (1H, m, CH), 1.28 (6H, d, $J = 6.8$ Hz, $2CH_3$); MS: m/z 271 (M^+).

3.1.3.5. 3-Bromomethyl-7-chloro-2(1H)-quinoxalinone ***(22)***. This compound was obtained in 32% yield by eluting with a 7:3 mixture of light petroleum–ethyl acetate; m.p. 216–218 °C; IR: ν 1670 cm^{-1}; UV: λ 336, 277, 219, 195 nm; ^{1}H NMR ($CDCl_3$ + DMSO-d_6): δ 12.62 (1H, br s, NH), 7.71 (1H, d, $J = 8.6$ Hz, H-5), 7.36 (1H, d, $J = 2.4$ Hz, H-8), 7.24 (1H, dd, $J = 8.6$ and 2.4 Hz, H-6), 4.59 (2H, s, CH_2); MS: m/z 272 (M^+).

3.1.3.6. 3-Bromomethyl-6-chloro-2(1H)-quinoxalinone ***(24)***. This compound (34% yield) was eluted as described for **22**; m.p. 223–225 °C; IR: ν 1660 cm^{-1}; UV: λ 341, 269, 222, 193 nm; ^{1}H NMR ($CDCl_3$ + DMSO-d_6): δ 12.66 (1H, br s, NH), 7.41 (1H, d, $J = 2.4$ Hz, H-5), 7.46 (1H, dd, $J = 8.6$ and 2.4 Hz, H-7), 7.34 (1H, d, $J = 8.6$ Hz, H-8), 4.59 (2H, s, CH_2); MS: m/z 272 (M^+).

3.2. Biological assays

All the compounds were evaluated in vitro for antimicrobial activity against yeast (*C. albicans*), Gram positive (*Staphylococcus aureus*) and Gram negative (*Salmonella* spp.) bacteria. Miconazole and streptomycin were used as reference drugs in antifungal and antibacterial assays, respectively. Test compounds were also evaluated for antiretroviral activity in MT-4 cells infected with HIV-1. Cytotoxicity for MT-4 cells, carried out in parallel with the anti-HIV-1 activity, was evaluated to determine whether the test compounds were endowed with selective antimicrobial/antiviral activity.

3.2.1. Material and method

3.2.1.1. Compounds. The test compounds were dissolved in DMSO at an initial concentration of 200 mM and then were serially diluted in culture medium.

3.2.1.2. Cells. Cells lines were from the American Type Culture Collection (ATCC). Bacterial and fungal strains were either clinical isolates (obtained from Clinica Dermosifilopatica, University of Cagliari) or collection strains form ATCC. H9/IIIB, MT-4 and C8166 cells [grown in RPMI 1640 containing 10% fetal calf serum (FCS), 100 UI/ml penicillin G and 100 μg/ml streptomycin] were used for anti-HIV-1 assays. Cell cultures were checked periodically for the absence of mycoplasma contamination with a MycoTect Kit (GIBCO).

3.2.1.3. Viruses. Human immunodeficiency virus type-1 (HIV-1, III_B strain) was obtained from supernatants of persistently infected H9/III_B cells. HIV-1 stock solutions had a titer of 5×10^7 cell culture infectious dose fifty ($CCID_{50}$)/ml.

3.2.1.4. Antiviral assay. Activity against the HIV-1 multiplication in acutely infected cells was based on inhibition of virus-induced cytopathogenicity in MT-4 cells [9]. Briefly, 50 μl of RPMI 10% FCS containing

Table 2
Compound dose (μM) required to reduce the viability of mock-infected MT-4 cells by 50%; (reference compound **I** $CC_{50} = 62$ μM)

Comp.	CC_{50}	Comp.	CC_{50}	Comp.	CC_{50}	Comp.	CC_{50}
3	>200	**9**	>200	**15**	>200	**21**	114 ± 5
4	>200	**10**	108 ± 5	**16**	>200	**22**	146 ± 2
5	119 ± 3	**11**	96 ± 1.5	**17**	>200	**23**	112 ± 3
6	>200	**12**	143 ± 1.5	**18**	>200	**24**	139 ± 3
7	173 ± 2.5	**13**	>200	**19**	>200	**25**	24 ± 2
8	161 ± 4	**14**	200	**20**	64 ± 2	**26**	21 ± 1.8

Data represent mean values of three separate experiments. Variation among triplicate samples was less than 15%.

1×10^4 cells were added to each well of flat-bottomed microtiter trays containing 50 μl of medium and serial dilutions of test compounds. Then, 20 μl of an HIV-1 suspension containing 100 $CCID_{50}$ were added. After a 4 day incubation at 37 °C, the number of viable cells was determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method [10,11]. Cytotoxicity of compounds, based on the viability of mock-infected cells, as monitored by the MTT method, was evaluated in parallel with antiviral activity.

3.2.1.5. Antibacterial assay. *S. aureus*, group D *Streptococcus*, *Salmonella* and *Shigella* spp. were recent clinical isolates. Assays were carried out in nutrient broth, pH 7.2, with an inoculum of 10^3 bacterial cells/tube. Minimum inhibitory concentrations (MIC) were determined after incubation at 37 °C for 18 h in the presence of serial dilutions of test compounds. The minimal bactericidal concentrations (MBC) were determined by subcultivating in Triptosio agar samples from cultures with no apparent growth.

3.2.1.6. Antimycotic assay. Yeast inocula were obtained by properly diluting cultures incubated at 37 °C for 30 h in Sabouraud dextrose broth to obtain 5×10^3 cells/ml. On the contrary, dermatophyte inocula were obtained from cultures grown at 37 °C for 5 days in Sabouraud dextrose broth by finely dispersing clumps with a glass homogenizer before diluting to 0.05 OD590/ml. Then, 20 μl of the above suspensions were added to each well of flat-bottomed microtiter trays containing 80 μl of medium with serial dilutions of test compounds, and were incubated at 37 °C. Growth controls were visually determined after 2 (yeasts) or 3 days (dermatophytes). MIC was defined as the compound concentration at which no macroscopic sign of fungal growth was detected. The minimal fungicidal concentrations (MFC) were determined by subcultivating in Sabouraud dextrose agar samples from cultures with no apparent growth.

3.2.1.7. Linear regression analysis. Viral and tumor cell growth at each drug concentration was expressed as percentage of untreated controls and the concentrations resulting in 50% (EC_{50}, IC_{50}) growth inhibition was determined by linear regression analysis.

4. Results and discussion

All the quinoxalinones **3**–**26** were tested for antibacterial and antifungal activities in vitro by the microdilution method. Compound **10** bearing CF_3 group in C-3 exhibited only a marginal activity against *S. aureus* (MIC = 33 μM, MBC = 200 μM), while none of the remaining compounds was found to be active at concentrations lower than 200 μM in antibacterial and antifungal assays. None of the compounds tested was capable of protecting MT-4 cells from the cytopathic effect induced by HIV-1, at least at concentrations that were lower than those cytotoxic.

The results of the in vitro cytotoxicity concerning all the new quinoxalinones are reported in Table 2.

These data show a generally low cytotoxicity for all compounds tested with the exception of **25** and **26** which exhibited a higher activity than **I** [3], which was used as reference compound.

These results seem to confirm our previous observations that a CH_2Br group at C-3 position promotes cytotoxic activity, especially when a chlorine atom is present in C-5 (compounds **25** and **26**), while shifting the chlorine atom in other positions (compounds **22**–**24**) were less effective.

Despite our expectations, quinoxalinones having a CF_3 group in C-3 (compounds **3**–**12**) were quite inactive in the antifungal assay, while showing only a moderate antibacterial activity when two chlorine atoms were present in both C-5 and C-6 positions (compound **10**). On the contrary, the presence of only one chlorine atom at C-6 or C-7 position, or introduction of electron-releasing groups (CH_3O or NH_2) in the benzo-moiety completely canceled the activity.

In conclusion, the results obtained with this series of quinoxalinones provide evidence that both pattern and position of substituents in the benzo-moiety is equally as important as the substituent in C-3 for biological activities.

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References

[1] 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.

[2] 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.

[3] 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.

[4] M. El-Said Mustafa, A. Takaoka, N. Ishikawa, Trifluoropyruvic acid hydrate in heterocyclic synthesis. Synthesis of trifluoromethylated five, six and seven membered heterocycles with two or more heteroatoms, Bull. Soc. Chim. Fr. 6 (1986) 944–954.

[5] R.E. Lyle, J.L. LaMattina, Selective hydrogenation of 2,6-dinitroanilines, Synthesis (1974) 726–727.

[6] S. Saluja, R. Zou, J.C. Drach, L.B. Townsend, Structure–activity relationships among 2-substituted 5,6-dichloro-, 4,6-dichloro-, and 4,5-dichloro-1-[(2-hydroxyethoxy)methyl]- and 1-[(1,3-dihydroxy-2-propoxy)methyl]benzimidazoles, J. Med. Chem. 39 (1996) 881–891.

[7] 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.

[8] F.B. Mallory, C.S. Wood, B.M. Hurwitz, Furazan oxides. IV. Extensions of the scope of the haloalkoxy substitution reaction, J. Org. Chem. 29 (1964) 2605–2606.

[9] A. May, M. Artico, G. Sbardella, S. Quartarone, S. Massa, A.G. Loi, A. De Montis, F. Scintu, M. Putzolu, P. La Colla, Dihydro(alkylthio)(naphthylmethyl)oxopyrimidines: novel non nucleoside reverse transcriptase inhibitors of the S-DABO series, J. Med. Chem. 40 (1997) 1447–1454.

[10] R. Pauwels, J. Balzarini, M. Baba, R. Snoeck, D. Herdewijin, J. Desmyster, E. De Clercq, Rapid and automated tetrazolium-based colorimetric assay for the detection of anti-HIV compounds, J. Virol. Methods 20 (1988) 309.

[11] F. Denizot, R. Lang, Rapid colorimetric assay for cell growth and survival, J. Immunol. Methods 89 (1986) 271–277.