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Synthesis of substituted 2-ethoxycarbonyl- and 2-carboxyquinoxalin-3-ones for evaluation of antimicrobial and anticancer activity

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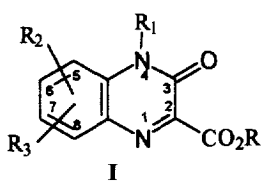
Abstract

A series of variously substituted quinoxalin-3-ones bearing an ethoxycarbonyl or carboxy group in the C-2 position has been prepared and their structures proved by ^{1}H NMR spectroscopy. The obtained compounds were investigated in vitro for antimicrobial and anticancer activities. Preliminary results showed a moderate activity against a few strains of bacteria but no significant anticancer and anti-HIV activity.

Keywords: Quinoxalinones; Antimicrobial activity; Anticancer activity

1. Introduction

Quinoxaline and quinoxalinone derivatives have received much attention in recent years owing to their biological properties [1–6]. As contributions in this field some of us have reported the preparation and in vitro antitumoral activity of a large series of quinoxaline derivatives considered as classical and non-classical analogues of the antifolic agents methotrexate and trimetrexate [7–9] as well as the antibacterial activity of quinoxaline-*N*-oxides [10]. In connection with these studies we have now prepared a series of 34 quinoxalinones of general structure **I** which were evaluated for both antimicrobial, anticancer and anti-HIV activities.



I

R, R_1 = H, Et
R_2, R_3 = H,CH_3,Cl, F, CF_3, NO_2, 4-methylpiperazine, morpholine

These compounds have not been studied much from this point of view, but further interest might arise from an in-depth screening of their biological activities in other fields. In this context we have considered those substituents that in other series (antibacterial quinolones and classical and non-classical antifolate agents) proved to be endowed with biological activity, also in the light of structural analogies between the quinoxalinone and quinolones nuclei.

Preliminary results on their antimicrobial activity have been previously communicated [11,12], whereas in this paper we report the overall results of in vitro antimicrobial, anticancer and anti-HIV activities.

2. Chemistry

The synthetic pathway for preparation of substituted quinoxalin-3-ones and quinoxalines listed in Table 1 is shown in Scheme 1. According to a general classical reaction, the 2-ethoxycarbonylquinoxalin-3-ones **2–19** were obtained in good yields by condensation of the appropriate 1,2-diaminobenzenes **1a–l** with diethyl ketomalonate in refluxing ethanol [1].

Formation of quinoxalinone isomers was observed in the case of the monosubstituted diaminobenzenes **1b–f** and of the non-symmetrical disubstituted compounds **1g**, **1i** and **1l**. However, owing to the different reactivity of the amino groups, in most cases the 6-substituted quinoxalin-3-one prevailed over the 7-isomer. The esters **2–6** and **9–11** underwent alkaline hydrolysis into the acids **20–27**. Compounds **2** [13], **4** [14], **6** and **23** [15], **9** and **10** [9], and **20** [16] were known and have been previously reported as indicated. Now they have been prepared for antimicrobial screening and a simpler characterization has been accomplished, avoiding the

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Table 1
Structure, physical and spectroscopic data of compounds **2–39**

Comp.	R^1	R^2	R^3	R^4	M.p. (°C)	Yield (%)	Purification method	IR(nujol), ν_{max} (cm^{-1})	^1H NMR(solvent), δ (ppm)
2	H	H	H	H	279–281 [a]	87	A	1740, 1730, 1650, 1610	($CDCl_3$) 12.96 (1H, s, NH), 7.95 (1H, dd, J=8.2 and 1.6 Hz, H-8), 7.64 (1H, dd, J=8.4 and 1.6 Hz, H-5), 7.51 (2H, m, H-6+H-7), 4.56 (2H, q, J=7.2 Hz, CH_2), 1.48 (3H, t, J=7.2 Hz, Me)
3	H	CH_3	H	H	183–185	9 (86 [b])	A	1730, 1670, 1630	($CDCl_3$) 7.84 (1H, d, J=8.2 Hz, H-8), 7.26 (1H, s, H-5), 7.23 (1H, d, J=8.2 Hz, H-7), 4.56 (2H, q, J=7.2 Hz, CH_2–Me), 2.51 (3H, s, CH_3), 1.49 (3H, t, J=7.2 Hz, Me–CH_2)
4	H	H	CH_3	H	188–190 [c]	7 (86 [b])	B	1740, 1710, 1660	($CDCl_3$) 12.92 (1H, d, br s, NH), 7.74 (1H, s, H-8), 7.46 (1H, d, J=8.4 Hz, H-5), 7.37 (1H, d, J=8.4 Hz, H-6), 4.55 (2H, q, J=7.2 Hz, CH_2), 2.47 (1H, s, CH_3), 1.48 (3H, t, J=7.2 Hz, Me–CH_2)
5	H	Cl	H	H	236–238	25	C+A	1740, 1730, 1675, 1630, 1610	($CDCl_3$) 12.81 (1H, br s, NH), 7.80 (1H, d, J=8.6 Hz, H-8), 7.37 (1H, d, J=2 Hz, H-5), 7.26 (1H, dd, J=8.6 and 2 Hz, H-7), 4.49 (2H, q, J=7 Hz, CH_2), 1.44 (3H, t, J=7 Hz, Me)
6	H	H	Cl	H	213–215 [d]	35	C	1730, 1670, 1600	($CDCl_3$+DMSO-d_6) 12.84 (1H, br s, NH), 7.84 (1H, d, J=2.2 Hz, H-8), 7.49 (1H, dd, J=8.8 and 2.2 Hz, H-6), 7.34 (1H, d, J=8.8 Hz, H-5), 4.48 (2H, q, J=7 Hz, CH_2), 1.44 (3H, t, J=7 Hz, Me)
7	H	F	H	H	205–206	4 (98 [e])	D	1730, 1670, 1620, 1600	($CDCl_3$) 12.85 (1H, br s, NH), 7.85 (1H, dd, J=8.4 and 5.6 Hz, H-8), 7.07 (1H, d, J=8.4 Hz, H-5), 7.05 (1H, dd, J=8.4 and 2.2 Hz, H-7), 4.46 (2H, q, J=7.2 Hz, CH_2), 1.43 (3H, t, J=7.2 Hz, Me)
8	H	H	F	H	179-181	3 (98 [e])	C	1735, 1670	($CDCl_3$) 13.03 (1H, br s, NH), 7.65 (1H, dd, J=8.4 and 2.6 Hz, H-8), 7.54–7.36 (2H, m, H-5+H-6), 4.55 (2H, q, J=7.2 Hz, CH_2), 1.48 (3H, t, J=7.2 Hz, Me)
9 [f]	H	CF_3	H	H	200–202	47	C	1730, 1670	($CDCl_3$) 12.66 (1H, br s, NH), 8.10 (1H, d, J=8.4 Hz, H-8), 7.72 (1H, d, J=1.8 Hz, H-5), 7.65 (1H, dd, J=8.4 and 1.8 Hz, H-7), 4.58 (2H, q, J=7 Hz, CH_2), 1.49 (3H, t, J=7 Hz, Me)
10 [f]	H	H	CF_3	H	161–163	31	C	3170, 1730, 1680, 1630	($CDCl_3$) 12.73 (1H, br s, NH), 8.24 (1H, d, J=1.8 Hz, H-8), 7.82 (1H, dd, J=8.4 and 1.8 Hz, H-6), 7.57 (1H, d, J=8.4 Hz, H-5), 4.35 (2H, q, J=7 Hz, CH_2), 1.48 (3H, t, J=7 Hz, Me)
11	H	NO_2	H	H	229–230	48	E	1700, 1640, 1600	($CDCl_3$+DMSO-d_6) 13.20 (1H, s, NH), 8.22 (1H, d, J=2.2 Hz, H-5), 8.11 (1H, dd, J=8.8 and 2.2 Hz, H-7), 8.02 (1H, d, J=8.8 Hz, H-8), 4.46 (2H, q, J=7 Hz, CH_2), 1.42 (3H, t, J=7 Hz, Me)
12	H	H	NO_2	H	198–200	4	E	1740, 1680, 1670, 1620, 1600	($CDCl_3$) 12.70 (1H, s, NH), 8.88 (1H, s, H-8), 8.50 (1H, d, J=7.8 Hz, H-6), 7.63 (1H, d, J=7.8 Hz, H-5), 4.57 (2H, q, J=7 Hz, CH_2), 1.50 (3H, t, J=7 Hz, Me)
13	F	H	F	H	201–202	12 (76 [g])	F	1740, 1660, 1610	($CDCl_3$) 11.73 (1H, s, NH), 7.51 (1H, ddd, J=8.2, 2.4 and 2 Hz, H-8), 7.30–7.24 (1H, m, H-6), 4.55 (2H, q, J=7.2 Hz, CH_2), 1.48 (3H, t, J=7.2 Hz, Me)
14	H	F	H	F	216–218	7 (76 [g])	F	1750, 1680, 1640, 1610	($CDCl_3$) 12.72 (1H, s, NH), 7.09 (1H, dd, J=8.4 and 2.4 Hz, H-5), 6.92 (1H, ddd, J=10.1, 7 and 2.4 Hz, H-7), 4.53 (2H, q, J=7.2 Hz, CH_2), 1.46 (3H, t, J=7.2 Hz, Me)
15	H	F	F	H	185–187	55	E	3200, 1750, 1730, 1670, 1650, 1630,	($CDCl_3$) 7.86 (1H, dd, J=9.8 and 8 Hz, H-8), 7.52 (1H, dd, J=10 and 7.2 Hz, H-5), 4.63 (2H, q, J=7 Hz, CH_2), 1.55 (3H, t, J=7 Hz, Me)
16	H	F	MPi	H	212–214	11 (61 [h])	E	1740, 1675, 1500	($CDCl_3$+DMSO-d_6) 7.42 (1H, d, J=8.4 Hz, H-8), 7.08 (1H, d, J=12.2 Hz, H-5), 4.47 (2H, q, J=7 Hz, CH_2–Me), 3.11 (4H, t, J=4.4 Hz, CH_2-2′+CH_2-6′), 2.62 (4H, t, J=4.4 Hz, CH_2-3′+CH_2-5′), 2.37 (3H, s, Me–N), 1.43 (3H, t, J=7 Hz, Me–CH_2)
17	H	MPi	F	H	204–205	9 (61 [h])	E	1730, 1650, 1630, 1510	($CDCl_3$) 7.55 (1H, d, J=13.4 Hz, H-8), 6.85 (1H, d, J=8 Hz, H-5), 4.51 (2H, q, J=7.2 Hz, CH_2–Me), 3.38 (4H, t, J=4.4 Hz, CH_2-2′+CH_2-6′), 2.69 (4H, t, J=4.4 Hz, CH_2-3′+CH_2-5′), 2.42 (3H, s, Me–N), 1.46 (3H, t, J=7.2 Hz, Me–CH_2)
18	H	F	Mor	H	165–168	45	E	1735, 1655, 1600	($CDCl_3$) 7.49 (1H, d, J=8.4 Hz, H-8), 7.28 (1H, d, J=12.2 Hz, H-5), 4.56 (2H, q, J=7.2 Hz, CH_2–Me), 3.92 (4H, t, J=4.4 Hz, CH_2-3′+CH_2-5′), 3.13 (4H, t, J=4.4 Hz, CH_2-2′+CH_2-6′), 1.48 (3H, t, J=7.2 Hz, Me–CH_2)

(*continued*)

Table 1 (*continued*)

Comp.	R^1	R^2	R^3	R^4	M.p. (°C)	Yield (%)	Purification method	IR(nujol), ν_{max} (cm^{-1})	^1H NMR(solvent), δ (ppm)
19	H	Mor	F	H	243–244	39	E	1740, 1650, 1630	($CDCl_3$) 7.49 (1H, d, J = 12.8 Hz, H-8), 6.75 (1H, d, J = 8 Hz, H-5), 4.43 (2H, q, J = 7.2 Hz, CH_2–Me), 3.82 (4H, t, J = 4.4 Hz, CH_2-2′ + CH_2-6′), 3.24 (4H, t, J = 4.4 Hz, CH_2-3′ + CH_2-5′), 1.38 (3H, t, J = 7.2 Hz, Me–CH_2)
20	H	H	H	H	276–277 [i]	63	A	3400, 1740, 1640	($CDCl_3$ + DMSO-d_6) 13.45 (1H, br s, NH), 7.99 (1H, d, J = 7.8 Hz, H-8), 7.67 (1H, dd, J = 8.2 and 7.2 Hz, H-7), 7.49 (2H, m, H-5 + H-6)
21	H	CH_3	H	H	280–282	80	–	3100, 1760, 1620, 1580	($CDCl_3$ + DMSO-d_6) 13.10 (1H, br s, NH), 7.81 (1H, d, J = 8 Hz, H-8), 7.24 (1H, d, J = 8 Hz, H-7), 7.22 (1H, s, H-4), 2.49 (3H, s, Me)
22	H	H	CH_3	H	278–280	89	–	3400, 1740, 1650, 1610	($CDCl_3$ + DMSO-d_6) 13.10 (1H, br s, NH), 7.70 (1H, s, H-8), 7.50 (1H, dd, J = 8.4 and 1.8 Hz, H-6), 7.34 (1H, d, J = 8.4 Hz, H-5), 2.45 (3H, s, Me)
23	H	Cl	H	H	269–271 [l]	90	A	3500, 3400, 1730, 1660, 1600	(DMSO-d_6) 13.20 (1H, br s, NH), 7.86 (1H, d, J = 8.6 Hz, H-8), 7.41 (1H, dd, J = 8.6 and 2.2 Hz, H-7), 7.36 (1H, d, J = 2.2 Hz, H-5)
24	H	H	Cl	H	295–296	89	–	3440, 1700, 1670	(DMSO-d_6) 13.40 (1H, br s, NH), 7.94 (1H, d, J = 2 Hz, H-8), 7.70 (1H, dd, J = 8.8 and 2 Hz, H-6), 7.37 (1H, d, J = 8.8 Hz, H-5)
25	H	CF_3	H	H	182–184	56	–	3500, 3400, 1740, 1710, 1640, 1590	(DMSO-d_6) 13.30 (1H, br s, NH), 8.05 (1H, d, J = 8.6 Hz, H-8), 7.67 (1H, d, J = 8.6 Hz, H-7), 7.64 (1H, s, H-5)
26	H	H	CF_3	H	195–196	82	–	3480, 1700, 1670, 1620	(DMSO-d_6) 13.30 (1H, br s, NH), 8.20 (1H, s, H-8), 7.96 (1H, d, J = 8.6 Hz, H-6), 7.52 (1H, d, J = 8.6 Hz, H-5)
27	H	NO_2	H	H	277–279	79	–	3200, 1750, 1650, 1610	($CDCl_3$ + DMSO-d_6) 13.20 (1H, br s, NH), 8.18 (1H, d, J = 8.6 Hz, H-8), 8.07 (2H, br m, H-5 + H-7)
28	H	H	H	H	70–72	44	–	1740, 1670, 1600	($CDCl_3$) 7.97 (1H, dd, J = 8.2 and 1.6 Hz, H-8), 7.69 (1H, dd, J = 8.2 and 1.6 Hz, H-5), 7.43 (2H, m, H-6 + H-7), 4.52 (2H, q, J = 7.2 Hz, CH_2–O), 4.36 (2H, q, J = 7.2 Hz, CH_2–N), 1.45 (3H, t, J = 7.2 Hz, Me–CH_2–O), 1.40 (3H, t, J = 7.2 Hz, Me–CH_2–N)
29	H	Cl	H	H	116–117	44	–	1730, 1650, 1600, 1540	($CDCl_3$) 7.89 (1H, d, J = 9.2 Hz, H-8), 7.36 (1H, s, H-5), 7.32 (1H, d, J = 9.2 Hz, H-7), 4.51 (2H, q, J = 7.2 Hz, CH_2–O), 4.30 (2H, q, J = 7.2 Hz, CH_2–N), 1.45 (3H, t, J = 7.2 Hz, Me–CH_2–O), 1.40 (3H, t, J = 7.2 Hz, Me–CH_2–N)
30	H	CF_3	H	H	91–92	23	–	1745, 1660, 1620, 1570	($CDCl_3$) 8.08 (1H, d, J = 8.4 Hz, H-8), 7.62 (1H, d, J = 8.4 Hz, H-7), 7.61 (1H, s, H-5), 4.53 (2H, q, J = 7 Hz, CH_2–O), 4.38 (2H, q, J = 7 Hz, CH_2–N), 1.46 (3H, t, J = 7 Hz, Me–CH_2–O), 1.43 (3H, t, J = 7 Hz, Me–CH_2–N)
31	H	H	CF_3	H	142–143	56	–	1735, 1660, 1620, 1590, 1560	($CDCl_3$) 8.25 (1H, s, H-8), 7.87 (1H, dd, J = 9 and 2 Hz, H-6), 7.48 (1H, d, J = 9 Hz, H-5), 4.52 (2H, q, J = 7.2 Hz, CH_2–O), 4.37 (2H, q, J = 7.2 Hz, CH_2–N), 1.45 (3H, t, J = 7.2 Hz, Me–CH_2O), 1.44 (3H, J = 7.2 Hz, Me–CH_2N)
32	H	H	H	H	yellow oil	35	–	1740, 1580 [m]	($CDCl_3$) 8.08 (1H, d, J = 8.4 Hz, H-8) 7.84 (1H, dd, J = 8.4 and 1.4 Hz, H-5), 7.72 (1H, dd, J = 8.4 and 1.4 Hz, H-6), 7.58 (1H, dd, J = 8.4 and 1.4 Hz, H-7), 4.61 (2H, q, J = 7.2 Hz, CH_2–O), 4.53 (2H, q, J = 7.2 Hz, CH_2–N), 1.49 (3H, t, J = 7.2 Hz, Me–CH_2–O), 1.46 (3H, t, J = 7.2 Hz, Me–CH_2–N)
33	H	Cl	H	H	79–80	48	G	1740, 1610, 1580, 1570	($CDCl_3$) 8.01 (1H, d, J = 8.8 Hz, H-8), 7.84 (1H, d, J = 2.2 Hz, H-5), 7.54 (1H, dd, J = 8.8 and 2.2 Hz, H-7), 4.59 (2H, q, J = 7.2 Hz, CH_2–CO_2), 4.53 (2H, q, J = 7.2 Hz, CH_2–O), 1.49 (3H, t, J = 7.2 Hz, Me–CH_2–CO_2), 1.46 (3H, t, J = 7.2 Hz, Me–CH_2–O)
34	H	CF_3	H	H	73–74	45	–	1740, 1695, 1630, 1590, 1570	($CDCl_3$) 8.18 (1H, d, J = 10.6 Hz, H-8), 8.15, (1H, s, H-5), 7.77 (1H, dd, J = 10.6 and 1.8 Hz, H-7), 4.62 (2H, q, J = 7 Hz, CH_2–CO_2), 4.55 (2H, q, J = 7.2 Hz, CH_2–O), 1.51 (3H, t, J = 7 Hz, Me–CH_2–CO_2), 1.49 (3H, t, J = 7.2 Hz, Me–CH_2–O)
35	H	H	CF_3	H	43–46	36	G	1745, 1630, 1580, 1570	($CDCl_3$) 8.39 (1H, d, J = 1.2 Hz, H-8), 7.95 (1H, d, J = 8.4 Hz, H-5), 7.89 (1H, dd, J = 8.4 and 1.2 Hz, H-6), 4.64 (2H, q, J = 7 Hz, CH_2–CO_2), 4.54 (2H, q, J = 7 Hz, CH_2–O), 1.50 (3H, J = 7 Hz, Me–CH_2–CO_2), 1.47 (3H, J = 7 Hz, Me–CH_2–O)

(*continued*)

Table 1 (*continued*)

Comp.	R^1	R^2	R^3	R^4	M.p. (°C)	Yield (%)	Purification method	IR(nujol), ν_{max} (cm^{-1})	^1H NMR(solvent), δ (ppm)
36	H	H	H	H	178–180	68	–	3400, 1765, 1720, 1620, 1580	(DMSO-d_6) 7.91 (1H, d, J=8.4 Hz, H-8), 7.74 (2H, m, H-5+H-6), 7.50 (1H, dd, J=8.4 and 2.4 Hz, H-7), 4.31 (2H, q, J=7.2 Hz, CH_2), 1.27 (3H, t, J=7.2 Hz, Me)
37	H	Cl	H	H	192–195	57	–	3400, 1765, 1710, 1585	($CDCl_3$) 14.10 (1H, br s, OH), 8.21 (1H, d, J=8.6 Hz, H-8), 7.54 (1H, d, J=8.6 Hz, H-7), 7.51 (1H, s, H-5), 4.43 (2H, q, J=7.2 Hz, CH_2), 1.49 (3H, t, J=7.2 Hz, Me)
38	H	CF_3	H	H	179–180	43	–	3420, 1770, 1740, 1640, 1620	($CDCl_3$+DMSO-d_6) 8.08 (1H, d, J=9 Hz, H-8), 7.99 (1H, s, H-5), 7.74 (1H, dd, J=9 and 2 Hz, H-7), 4.36 (2H, q, J=7.2 Hz, CH_2), 1.27 (3H, t, J=7.2 Hz, Me)
39	H	H	CF_3	H	144–146	50	B	3400, 1765, 1730, 1640, 1600	($CDCl_3$) 8.58 (1H, d, J=2 Hz, H-8), 8.04 (1H, dd, J=8.8 and 2 Hz, H-6), 7.66 (1H, d, J=8.8 Hz, H-5), 4.51 (2H, q, J=7 Hz, CH_2), 1.50 (3H, t, J=7 Hz, Me)

MPi = 4-methylpiperazine; Mor = morpholine.

[a] Ref. [13], 266°C.

[b] Overall yield for mixture of isomers **3** and **4**.

[c] Ref. [14], 171–172°C.

[d] Ref. [15], 206–207°C.

[e] Overall yield for mixture of isomers **7** and **8**.

[f] Ref. [9].

[g] Overall yield for mixture of isomers **13** and **14**.

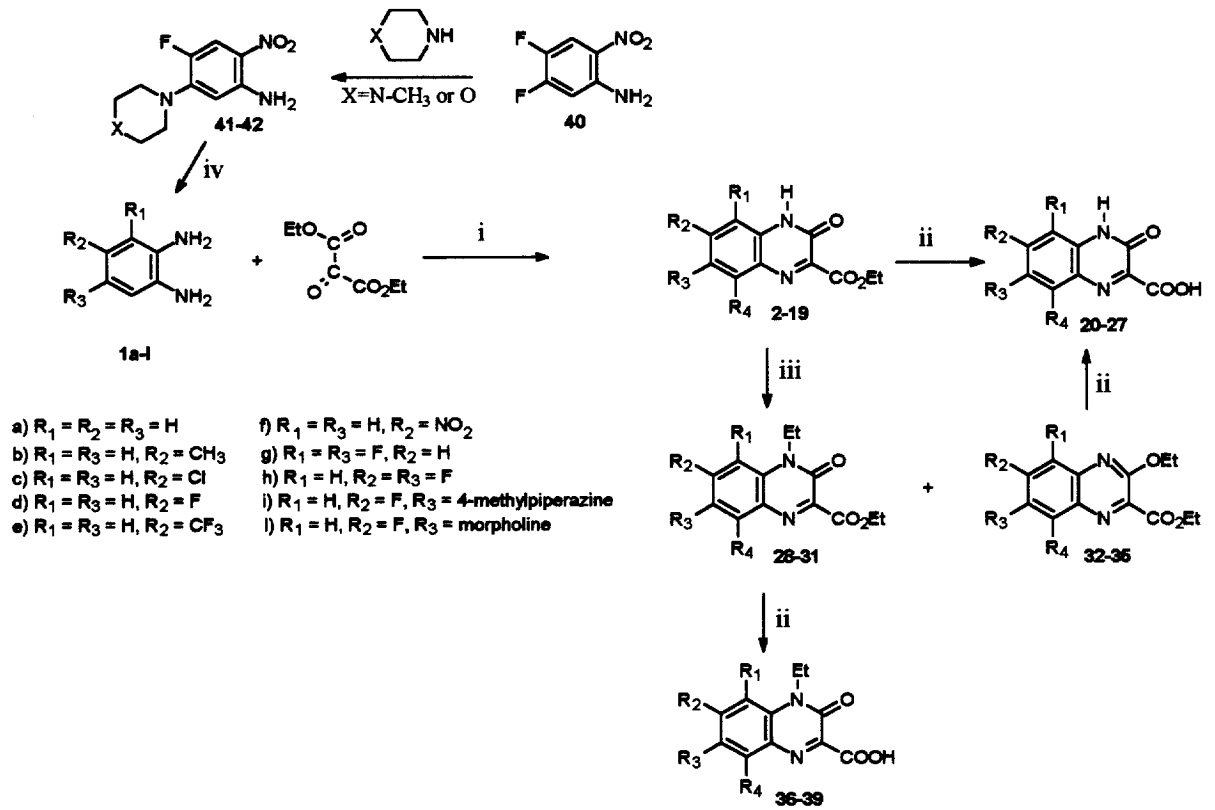
[h] Overall yield for mixture of isomers **16** and **17**.

[i] Ref. [16], 263–265°C.

[l] Ref. [15], 194–196°C.

[m] Film.

A: fractional crystallization from ethanol; B: fractional crystallization from chloroform/diethyl ether mixture, ratio 1:1; C: flash-chromatography on silica gel eluting with light petroleum/ethyl acetate mixtures with increasing percentage of ethyl acetate; D: fractional crystallization from diethyl ether/acetone mixture, ratio 8:2; E, chromatography on silica gel eluting with diethyl ether/acetone mixtures with increasing percentage of acetone; F: fractional crystallization from diethyl ether; G: crystallization from light petroleum.



Scheme 1. Preparation of substituted quinoxalin-3-ones and quinoxalines: (i) EtOH/reflux; (ii) aqueous OH/100–110°C; (iii) C_2H_5I/NaH; (iv) H_2N–NH_2 on 10% Pd/C or H_2/PtO_2.

tedious unambiguous synthesis reported for similar cases in the past [1,7,8,17].

Reaction of quinoxalinones **2**, **5**, **9** and **10** with ethyl iodide in the presence of sodium hydride gave, according to the observations of Katoh et al. [18], a mixture of *N*-ethyl derivatives **28–31** and *O*-ethyl products **32–35**. Hydrolysis of *N*-ethyl esters in alkaline medium yielded the expected acids **36–39**, whereas the same reaction in the case of 2-ethoxy derivatives afforded the above-mentioned acids **20**, **23**, **25** and **26**.

Assignment of the exact structure to 6- and 7-substituted quinoxalin-3-one isomers **2–19**, coming from monosubstituted diamines, has now been obtained by application of ^{1}H NMR spectroscopy to all new quinoxalinone isomers according to the previous observations reported by us for compounds **9** and **10** [9]. Data from ^{1}H NOESY experiments clearly exhibited a nuclear Overhauser effect (NOE) between the NH-4 and H-5 protons. In the case of 6-monosubstituted isomers **3**, **5**, **7**, **9** and **11** the ^{1}H NMR spectra showed that the H-5 signal appears as a singlet, whereas it resonated as a doublet in the 7-substituted isomers **4**, **6**, **8**, **10** and **12**. This is in accordance with the diamagnetic shift of the H-5 proton signal owing to the *peri* effect [19] determined from NH-4 in both cases (see Table 1). Similar reasoning was applied to the structure of disubstituted derivatives **13–19** which were unambiguously assigned.

3. Experimental

Melting points were determined by a Kofler hot stage or Digital Electrothermal apparatus, and were uncorrected. IR spectra were recorded using a Perkin-Elmer 781 spectrophotometer. ^{1}H NMR spectra were recorded on a Varian XL-200 (200 MHz) instrument, using tetramethylsilane (TMS) as internal standard. Column chromatographies were performed using 70–230 and 230–400 mesh silica gel (Merck silica gel 60) in the case of flash-chromatography. Elemental analyses were performed by the Laboratorio di Microanalisi, Dipartimento di Scienze Farmaceutiche, Università di Padova (Padua). Analytical results for C, H, N, and halogen, when present, were within $\pm 0.4\%$ of the theoretical values.

3.1. Intermediates

The diamines **1a,b,c** and **1f** were commercially available (Aldrich), while **1d**, **1e** and **1h** were prepared from the corresponding 2-nitroanilines by hydrogenation in a Parr apparatus, using 10% Pd/C as catalyst. The amine **1g** was prepared according to the method described by Finger et al. [20]. In this case, however, from nitration of the 2,4-difluoroacetanilide intermediate, besides the previously described 2,4-difluoro-6-nitroacetanilide, we were able to isolate a second product, in ~1:3 ratio, identified as 2,4-difluoro-5-nitroacetanilide. Although this compound has been reported in two

previous patents [21,22], its characteristics were not indicated, thus both analytical and spectroscopic data are now given: m.p. 146–148°C; yield 11%; IR (nujol): ν3240, 3200, 3130, 1675, 1640, 1610 cm^{-1}; NMR ($CDCl_3$): δ 9.15 (1H, dd, J=8.2 and 8.0 Hz, H-6), 7.52 (1H, s, NH), 7.09 (1H, dd, J=10.4 and 10.2 Hz, H-3), 2.27 (3H, s, CH_3). *Anal.* $C_8H_6F_2NO_3$ (202.1): C, H, F, N.

Diamines **1i** and **1l** were prepared by reduction of the parent nitroanilines **41** and **42**, respectively. In the case of **1i** previously isolated in 51% yield by El-Abadelah et al. [23], we were able to obtain an overall yield of 75% using an ethanolic solution of hydrazine in the presence of 10% Pd/C, while **1l** was obtained in 80% yield by hydrogenation in a Parr apparatus using PtO_2 as catalyst. Data for the unknown **1l** are given as follows: m.p. 124–127°C; IR (nujol): ν3350, 3230, 1630, 1525 cm^{-1}; NMR ($CDCl_3$): δ 6.47 (1H, d, J=12.8 Hz, H-3), 6.36 (1H, d, J=8.2 Hz, H-6), 3.85 (4H, t, J=4.6 Hz, CH_2-3′+CH_2-5′), 3.50 (4H, s, 2 NH_2), 2.96 (4H, t, J=4.6 Hz, CH_2-2′+CH_2-6′).

Intermediates **41** and **42** were in turn prepared by modifying the method described by El-Abadelah et al. [24] for the 4-fluoro-5-(4-methyl-1-piperazinyl)-2-nitroaniline (**41**), by straightforward condensation of the 4,5-difluoro-2-nitroaniline (**40**) with 4-methylpiperazine (95% yield) and morpholine (97% yield), respectively, and successive crystallization from diethyl ether. Data for the unknown 4-fluoro-5-morpholino-2-nitroaniline **42** are as follows: m.p. 182-183°C, IR (nujol): ν 3440, 3320, 1630, 1610, 1570 cm^{-1}; NMR ($CDCl_3$+DMSO-d_6): δ 7.61 (1H, d, J=14.2 Hz, H-3), 6.92 (2H, s, NH_2), 6.25 (1H, d, J=8 Hz, H-6), 3.76 (4H, t, J=4.4 Hz, CH_2-3′+CH_2-5′), 3.14 (4H, t, J=4.4 Hz, CH_2-2′+CH_2-6′).

3.2. General procedure for preparation of the substituted 2-ethoxycarbonylquinoxalin-3-ones 2–19

To a suspension of the appropriate diamine **1a–l** (2 g, 8.8–18.5 mmol) in ethanol (30 ml), diethyl ketomalonate (1.85–3.87 g, 10.6–22.2 mmol) was slowly added under stirring, and the mixture was refluxed for 2 h. After evaporation of the solvent, the crude residue was purified by chromatography or recrystallization. In Table 1 we report the structures, melting points, yields, purification methods used, and spectroscopic data of all compounds synthesized.

3.3. General procedure for preparation of the substituted 2-carboxyquinoxalin-3-one acids 20–27

A suspension of the appropriate ester **2–6** or **9–11** (0.5 g, 1.7–2.3 mmol) in 2 M NaOH aqueous solution (10 ml) was stirred under reflux for 1 h. On cooling, the solution was made acidic (pH 2–3) using 6 M HCl aqueous solution. The resulting precipitate was collected by filtration, washed with water and eventually dried. Structures, melting points, yields, purification methods used, and spectroscopic data of synthesized acids are reported in Table 1.

3.4. General procedure for preparation of N-ethyl-2-ethoxycarbonylquinoxalin-3-ones 28–31 and 3-ethoxy-2-ethoxycarbonylquinoxalines 32–35

A solution of the appropriate ester **2**, **5**, **9** or **10** (1 g, 3.5–4.4 mmol) in anhydrous *N*,*N*-dimethylformamide (DMF, 5 ml) was slowly added to a stirred suspension of sodium hydride (0.1–0.13 g, 4.2–5.3 mmol), as 60% dispersion in mineral oil, in 10 ml of anhydrous DMF. The reaction mixture was then heated under reflux for 2 h, ethyl iodide (1.9–2.4 g, 12.2–15.4 mmol) was added and the reflux continued for an additional 6 h. On cooling, the solvent was removed in vacuo and the residue was taken up with water and extracted with chloroform. The combined extracts were dried on anhydrous sodium sulfate and evaporated in vacuo to give a crude product which was purified by flash-chromatography on a silica gel column, using a mixture of diethyl ether/light petroleum in the ratio 60:40 as eluent. In Table 1 we report the structures, melting points, yields, purification methods used, and spectroscopic data of all synthesized compounds.

3.5. Hydrolysis of N-ethyl-2-ethoxycarbonylquinoxalin-3-ones 28–31 and 3-ethoxy-2-ethoxycarbonylquinoxalines 32–35

In an identical manner to that reported above for the preparation of the acids **20–27**, on hydrolysis of the *N*-ethyl derivatives **28–31** we obtained the acids **36–39**, whereas 2-ethoxy esters **32–35** gave the acids **20**, **23**, **25**, and **26**, identical to the compounds described above. Structures, melting points, yields, purification methods used, and spectroscopic data of the synthesized acids are reported in Table 1.

4. Microbiology

Antimicrobial activity was investigated in vitro at the Institute of Microbiology and Virology of Sassari University. The strains used were *Escherichia coli* ATCC 25922, *Escherichia coli* (hospital isolate), *Pseudomonas aeruginosa* ATCC 27922, *Staphylococcus aureus* ATCC 25923, *Candida spp.* (hospital isolate), *Trichomonas vaginalis*, *Leishmania major*, and *Acanthamoeba castellanii*.

The minimum inhibitory concentration (MIC) was determined according to the dilution method in broth with test-tubes. Each compound was dissolved in dimethyl sulfoxide (DMSO), then diluted in Lb broth (Luria broth, Difco). The range of concentration used for each compound was 500–0.5 μg/ml. The final concentration of the inoculum was 10^6 CFU/ml. After overnight incubation at 37°C, test organisms were diluted to the optical density of a 0.5 McFarland turbidity standard and measured at 450 nm. The MIC was determined as the lowest concentration of compound that completely inhibited bacteria growth.

5. In vitro antitumoral and anti-HIV activity

Some of the new compounds synthesized (**6**, **9**, **13**, **21**, **23**, **24**, **25**, **26** and **28**) were evaluated for anticancer and anti-

HIV activity at the National Cancer Institute (NCI), Bethesda, MD, USA, following the known in vitro disease-oriented antitumor screening program against a panel of ~60 human tumor cell lines and the anti-HIV drug testing system [25,26]. The activity of each compound tested was deduced from dose–response curves on the basis of the data provided by NCI.

6. Results and discussion

All described quinoxalinones and quinoxalines were tested in vitro for antibacterial activity against Gram positive (*S. aureus*) and Gram negative (*E. coli* and *Ps. aeruginosa*) bacteria. The results obtained generally indicate that most of these compounds were only poorly active or completely inactive (MIC ≥ 500 μg/ml). However, the esters **3**, **8** and **19** showed a moderate activity against *Ps. aeruginosa* (MIC = 250 μg/ml). The ester **10** was active against *E. coli* (MIC = 125 μg/ml) and against *S. aureus* (MIC = 250 μg/ml), while the ester **9** and the acid **23** exhibited activity against *E. coli* (MIC = 125 μg/ml). Compounds **3**, **4**, **6**, **9**, **10**, **13**, **14**, **15**, **17**, **19** and **21** were also tested against *Candida spp.* (hospital isolate). Only derivative **3** showed activity (MIC = 250 μg/ml). None of the compounds (**4**, **13**, **14**, **15**, **26** and **30**) tested against protozoa showed appreciable activity.

Finally, the results of both anticancer and anti-HIV activities were barely significant. Only a few compounds (**6**, **9**, **13**, **23** and **26**) exhibited modest growth inhibition on some subpanel cell lines at 10^{-4} molar concentration.

References

[1] G.W.H. Cheeseman, R.F. Cookson, Condensed Pyrazines, in: A. Weissberger, E.C. Taylor (Eds.), Heterocyclic Compounds, vol. 35, Wiley, New York, 1979, pp. 78–94.

[2] T. Honore, S.N. Davies, J. Drejer, E.J. Fletcher, P. Jacobsen, D. Lodge, F.E. Neilsen, Quinoxalinediones: Potent competitive non-NMDA glutamate receptor antagonist, Science 241 (1988) 701–703.

[3] P.D. Leeson, L.L. Iversen, The glycine site on the NMDA receptor: structure–activity relationships and therapeutic potential, J. Med. Chem. 37 (1994) 4053–4067.

[4] Fujisawa Pharmaceutical Co., Preparation of quinoxaline derivatives as aldose reductase inhibitors and a process for their preparation, Jpn. Kokai Tokkyo Koho JP 63 301 874 (1988) [Chem. Abstr. 111 (1989) 78027u].

[5] C.H. Beherens, B.A. Dusak, B.A. Harrison, M.J. Orwat, 2-[(Quinoxalinyloxy)phenoxy] propanoates and related derivatives as anticancer agents, PCT Int. Appl. WO 94 13 647 (1994) [Chem. Abstr. 121 (1994) 157671j].

[6] M. Yaso, Y. Suzuki, T. Saito, D. Mochizuki, Preparation of 2-(1-piperazinylaliloxy) quinoxaline derivatives for treatment of serotoninergic disorders, PCT Int. Appl. WO 93 12 792 (1993) [Chem. Abstr. 120 (1994) 8617y].

[7] M. Loriga, M. Fiore, P. Sanna, G. Paglietti, Quinoxaline chemistry, Part 4. 2-(*R*)-Anilinoquinoxalines as non classical antifolate agents. Synthesis, structure elucidation and evaluation of in vitro anticancer activity, Farmaco 50 (1995) 289–301.

[8] M. Loriga, M. Fiore, P. Sanna, G. Paglietti, Quinoxaline chemistry, Part 5. 2-(*R*)-Benzylaminoquinoxalines as non classical antifolate agents. Synthesis and evaluation of in vitro anticancer activity, Farmaco 51 (1996) 559–568.

[9] M. Loriga, S. Piras, P. Sanna, G. Paglietti, Quinoxaline chemistry, Part 7. 2-[Aminobenzoates]- and 2-[aminobenzoylglutamate]-quinoxalines as classical antifolate agents. Synthesis and evaluation of in vitro anticancer, anti-HIV and antifungal activity, Farmaco 52 (1997) 157–166.

[10] M. Loriga, A. Nuvole, G. Paglietti, S. Zanetti, G. Fadda, 2-Phenyl-6(7)-*R*-substituted quinoxalines *N*-oxides. Synthesis, structure elucidation and antimicrobial activity, Eur. J. Med. Chem. 25 (1990) 527–532.

[11] P. Sanna, A. Carta, S. Zanetti, Sintesi e valutazione biologica in vitro di carbossi- e carbossietilchinossaline e chinossalinoni variamente sostituiti, Atti II Congr. Congiunto Italiano-Spagnolo di Chimica Farmaceutica, Ferrara, Italy, 30 Aug.–3 Sept. 1995, P11.

[12] P. Sanna, A. Carta, M. Loriga, S. Zanetti, I. Duprè, Preparazione e valutazione microbiologica in vitro di nuovi derivati 3,4-diidrochinossalinonici variamente sostituiti, Atti XIII Convegno Nazionale della Divisione di Chimica Farmaceutica, Paestum, Italy, 23–27 Sept. 1996, Italian Chemical Society, Rome, p. 176.

[13] R.F. Abdulla, K.H. Fuhr, Azeto[1,2-a]quinoxaline-1,3-diones, a new class of bridgehead nitrogen β-lactams, J. Heterocycl. Chem. 13 (1976) 427–432.

[14] Y. Yamada, K. Kishi, H. Yasuda, The reaction of tetraethoxycarbonylethylene with aromatic 1,2-diamines, Utsunomiya Daigaku Kyoikugakubu Kiyo Dai-2-bu 37 (1987) 49–55 [Chem. Abstr. 107 (1987) 236662w].

[15] Y. Ahmad, M.S. Habib, M. Iqbal, M.I. Qureshi, B.B. Ziauddin, Quinoxaline derivatives, VII. The mechanism of the formation of 6-chloro-1,2,3,4,2′,3′-hexahydro-4,1′-dimethyl-3,2′-dioxoquinoxaline-2-spiro-3′-indole from a quinoxaline *N*-oxide derivative by nucleophilic chlorination, Bull. Chem. Soc. Jpn. 38 (1965) 1659–1663.

[16] A.H. Gowenlock, G.T. Newbold, F.S. Spring, Syntheses of 2-monosubstituted and 2,3-disubstituted quinoxalines, J. Chem. Soc. (1945) 622–625.

[17] R. Van Dusen, H.P. Schultz, Quinoxaline studies, IX. The preparation of 3-methyl-6- and -7-bromo-2-quinoxalinols, J. Org. Chem. 21 (1956) 1326–1327.

[18] A. Katoh, T. Yoshida, J. Ohkanda, T. Nishio, Structural analysis of *N*-(ω-phenylalkyl) substituted quinoxalin-2(1*H*)-ones and -thiones, Heterocycles 44 (1997) 357–366.

[19] M. Jackman, S. Sternhell, Application of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry, Pergamon, Oxford, 1969, pp. 204–208.

[20] G.C. Finger, F.H. Reed, J.L. Finnerty, Aromatic fluorine compounds, V. 1,3,5-Trifluoro benzene, J. Am. Chem. Soc. 73 (1951) 153–155.

[21] H.J. Braun, Fluoronitrophenylenediamine derivatives; their preparation and use in hair dyes, Ger. Offen. DE 3 707 273 (1988) [Chem. Abstr. 110 (1989) 28915r].

[22] Wella AG, Preparation of 3-mesylaminoanilines as coupling agents for oxidative hair dyes, Ger. Offen. DE 3 521 995 (1987) [Chem. Abstr. 111 (1989) 180459y].

[23] M.M. El-Abadelah, S.S. Sabri, M.H. Abu Zarga, R.J. Abdel-Jalil, Substituted benzimidazoles, Part I. Synthesis and properties of some 2-aryl-5-fluoro-6-(4-methyl-1-piperazinyl)-1*H*-benzimidazoles, Heterocycles 41 (1995) 2713–2728.

[24] M.M. El-Abadelah, M.Z. Nazer, N.S. El-Abadla, H. Meier, 6-Fluoro-7-(1-piperazinyl)quinoxaline 1,4-dioxides, Part I. 2-(*N*-2-Hydroxyalkylcarbamoyl) derivatives, Heterocycles 41 (1995) 2203–2219.

[25] M.R. Boyd, Status of the NCI Preclinical Antitumor Drug Discovery Screen, Princ. Pract. Oncol. 3 (10) (1989) 1–12.

[26] O.W. Weislow, R. Kiser, D. Fine, J. Bader, R.H. Shoemaker, M.R. Boyd, New soluble-formazan assay for HIV-1 cytopathic effects: application to high-flux screening of synthetic and natural products for AIDS antiviral activity, J. Natl. Cancer Inst. 81 (1989) 577–586.