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Il Farmaco 57 (2002) 63–69

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Synthesis and antibacterial activity of pyridazino[4,3-*b*]indole-4-carboxylic acids carrying different substituents at N-2

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Received 5 June 2001; received in revised form 9 August 2001; accepted 14 August 2001

Dedicated to Professor Giovanni Casini on the occasion of his retirement

Abstract

The synthesis and the in vitro evaluation of antibacterial activity of new pyridazino[4,3-*b*]indole-4-carboxylic acids **2**–**4**, **6** against some selected representative of Gram-positive and Gram-negative bacteria are reported. The role of the lipophilicity in the modulation of the antibacterial activity of the tested compounds is discussed. All the synthesized compounds appear quite weak against Gram-positive bacteria, whereas have no significant activity against Gram-negative bacteria. Only derivative **2g** possesses an interesting activity against Gram-positive bacteria. © 2002 Elsevier Science S.A. All rights reserved.

Keywords: Pyridazino[4,3-*b*]indole; Norfloxacin; Antibacterial activity; Isatin derivatives

1. Introduction

As a part of our ongoing research on the central benzodiazepine receptor ligands, we recently reported the synthesis, biological and pharmacological activities of a large series of 2-aryl-2,5-dihydro-pyridazino[4,3-*b*]indol-3(3H)-ones (PI) [1,2]. The synthetic pathway of PIs, led us to prepare, as intermediates, a number of 2-aryl-3,5-dihydro-3-oxo-2*H*-pyridazino[4,3-*b*]indole-4-carboxylic acids that were screened against some selected representative of Gram-positive and Gram-negative bacteria [3]. Some of the tested compounds showed an interesting antibacterial activity against Gram-positive bacteria whereas no activity was observed against Gram-negative microorganisms. A preliminary structure–antibacterial activity relationship study suggested the lipophilicity as an important property modulating the biological activity.

With the aim to confirm that preliminary observation and to better defining the main structural requirements for a more potent and extended antibacterial activity, we decided to enlarge our investigation by synthesizing new lipophilic 2-aryl-3,5-dihydro-3-oxo-2*H*-pyridazino-[4,3-*b*]indole-4-carboxylic acids **2e**, **h**, as well as a new series of simplified and less lipophilic N-2 alkylated analogs **3a**, **i**, **l**, **4a**, **i** or N-2 unsubstituted **6a**, **i**, **l** and their derivatives **8** and **10** (Schemes 1–3).

2. Chemistry

The synthetic pathway leading to compounds **2e**, **h**, **3a**, **i**, **l**, **4a**, **i**, **6a**, **i**, **l** is described in Scheme 1. The key intermediates diethyl-2-(3-oxo-1,3-dihydro-2*H*-indol-2-ylidene)malonates **1a**, **h**, **i**, **l** were prepared by refluxing the corresponding isatins with phosphorus pentachloride in anhydrous benzene to give 2-chloro-3*H*-indol-3-one derivatives which were then condensed with diethylmalonate sodium salt according to a method previously described by us [1,2].

The intermediates **1a**, **h** were condensed with *p*-OCF_3- and *p*-Br-phenylhydrazine hydrochloride respectively to afford compounds **2e**, **h**. Treatment of **1a**, **i**, **l**

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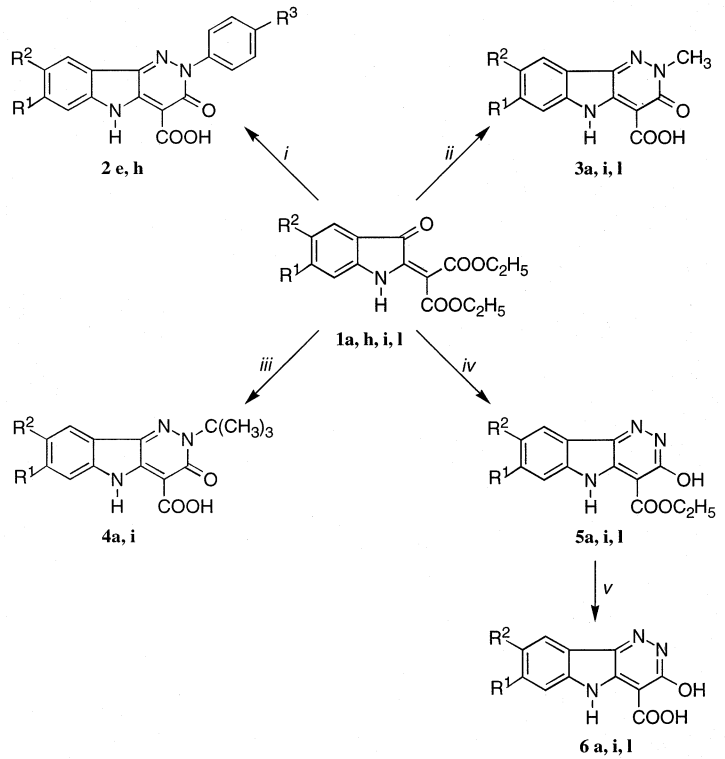
PII: S0014-827X(01)01173-9



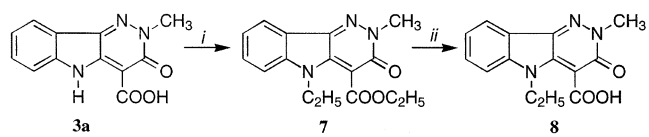
a: R^1=H, R^2=H; **e**: R^1=H, R^2=H, R^3=OCF_3; **h**: R^1=H, R^2= cyclohexyl, R^3=Br; **i**: R^1=H, R^2=F; **l**: R^1=Cl, R^2=F.

Scheme 1. Reagents: (i) R^3-C_6H_4-$NHNH_2$·HCl–EtOH, H_2O, reflux; (ii) CH_3-$NHNH_2$–HCl, EtOH, H_2O, reflux; (iii) $(CH_3)_3C$-$NHNH_2$·HCl–EtOH, H_2O, reflux; (iv) NH_2NH_2·HCl–EtOH, H_2O, reflux; (v) 1. NaOH–EtOH, reflux; 2. HCl, r.t.

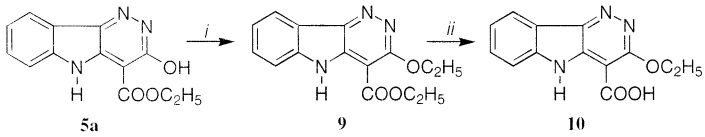
with methylhydrazine in the presence of HCl directly gave N-2 methyl derivatives **3a**, **i**, **l**. When *t*-butylhydrazine hydrochloride was reacted with **1a**, **i**, the N-2 *t*-butyl congeners **4a**, **i** were obtained. The N-2 unsubstituted derivatives **6a**, **i**, **l** were prepared through the hydrolysis of the corresponding ethyl esters **5a**, **i**, **l**, obtained in turn by reacting **1a**, **i**, **l** with hydrazine hydrochloride. Spectral data of compounds **5** and **6** indicated that they exist only in the tautomeric 3-hydroxypyridazine form. The N-5 and O-3 ethyl derivatives **8** and **10** were synthesized as depicted in Schemes 2 and 3, in order to make a proper comparison with the unsubstituded congeners **3a** and **6a**, respectively. The analytical and spectroscopic data of all the synthesized compounds are reported in Table 1.

3. Biological results and conclusions

The MIC values reported in Table 2 showed that compounds **2**, **3**, **4** and **6** are weakly active against tested Gram-positive bacteria and substantially inactive against Gram-negative bacteria. Compound **2g** elicited the highest activity against Gram-positive bacteria with MIC values ranging from 0.78 to 1.6 μg/ml. MIC data in Table 2 confirm that, with few exceptions, in the series of 2-aryl-3,5-dihydro-3-oxo-2*H*-pyridazino[4,3-*b*]indole-4-carboxylic acids **2a**–**g** the lipophilicity of the substituent in para position is directly related to the antibacterial activity. This relationship is more evident for the *Bacillus subtilis* ATCC 6633, *Enterococcus faecalis* ATCC 29212 and *Staphylococcus aureus* ATCC 29213 Gram-positive organisms. Interestingly, a linear relationship between log 1/MIC (MIC expressed in μM) and the hydrophobic substituent constant π was found ($n = 7$, $r^2 = 0.73$, $s = 0.31$). However, a further strong increase of the lipophilicity did not afford a higher activity (compare **2h** with **2d**). The effect of lipophilicity was further explored by eliminating the N-2 aryl substituent (derivatives **6**) or by replacing it with alkyl substituents of markedly different lipophilicity (derivatives **3** and **4**). Unexpectedly, the less lipophilic N-2 unsubstituted compound **6a** elicited an activity very close to that of the corresponding more lipophilic N-2



Scheme 2. Reagents: (i) 1. NaH–an DMF, r.t.; 2. CH_3CH_2I–an DMF, r.t.; (ii) 1. NaOH–EtOH, reflux; 2. HCl, r.t.



Scheme 3. Reagents: (i) 1. NaH–an DMF, r.t.; 2. CH_3CH_2I–an DMF, r.t.; (ii) 1. NaOH–EtOH, reflux; 2. HCl, r.t.

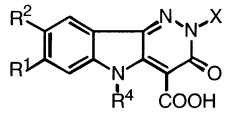
Table 1
Physical and spectroscopic data of compounds **2–10**

Comp	m.p. (°C) (crystallization solvent)	IR ν_{max}, (cm^{-1})	^1H NMR, δ (ppm), J (Hz) [a]
2e	323–325 dec (acetic acid)	3300, 1710, 1640, 1610	7.25–7.40 (m, 1H, Arom); 7.55–7.70 (m, 4H, Arom); 7.75–7.90 (m, 2H, Arom); 8.01 (d, 1H, Arom, $J = 7.9$); 12.20 (s, 1H, NH); 14.30 (s, 1H, COOH)
2h	260–261 (methanol)	3290, 1710, 1650, 1620	1.10–1.55 (m, 6H, $3CH_2$); 1.65–1.85 (m, 4H, $2CH_2$); 2.50–2.70 (m, 1H, CH); 7.45–7.55 (m, 2H, Arom); 7.60–7.70 (m, 2H, Arom); 7.75–7.80 (m, 2H, Arom); 7.82 (s, 1H, Arom); 12.09 (s, 1H, NH); 14.33 (s, 1H, COOH)
3a	305–310 dec (dioxane)	3310, 1710, 1650, 1615	3.94 (s, 3H, CH_3); 7.25–7.40 (m, 1H, Arom); 7.55–7.65 (m, 2H, Arom); 7.99 (d, 1H, Arom, $J = 7.6$); 12.06 (s, 1H, NH); 14.60 (s, 1H, COOH)
3i	326–328 dec (dioxane)	3315, 1700, 1650	3.94 (s, 3H, CH_3); 7.46 (dt, 1H, Arom, $J = 8.8$, $J = 2.6$); 7.58 (dd, 1H, Arom, $J = 8.8$, $J = 4.4$); 7.84 (dd, 1 H, Arom, $J = 8.2$, $J = 2.6$); 12.10 (s, 1H, NH)
3l	302–303 (dioxane)	3255, 1695, 1650, 1625	3.95 (s, 3H, CH_3); 7.70 (d, 1H, $J = 6.0$); 8.11 (d, 1H, $J = 8.5$); 12.10 (s, 1H, NH); 14.45 (s, 1H, COOH)
4a	304–305 (acetonitrile)	3305, 1710, 1650	1.74 (s, 9H, $3CH_3$); 7.31 (dd, 1H, Arom, $J = 7.0$, $J = 1.9$); 7.55–7.65 (m, 2H, Arom); 8.00 (d, 1H, Arom, $J = 7.8$); 12.00 (s, 1H, NH); 15.00 (s, 1H, COOH)
4i	314–316 (acetonitrile)	3280, 1710, 1630	1.79 (s, 9H, $3CH_3$); 7.26 (dt, 1H, $J = 8.7$, $J = 2.3$); 7.36 (dd, 1H, $J = 8.7$, $J = 4.1$); 7.71 (dd, 1H, $J = 7.8$, $J = 2.3$); 9.92 (s, 1H, NH); 14.78 (s, 1H, COOH)
5a	306–307 (ethanol)	3220, 1650, 1630	1.32 (t, 3H, CH_3, $J = 7.0$); 4.35 (q, 2H, CH_2, $J = 7.0$); 7.20–7.30 (m, 1H, Arom); 7.45–7.55 (m, 2H, Arom); 7.89 (d, 1H, Arom, $J = 7.8$); 11.46 (s, 1H, NH), 13.19 (s, 1H, OH)
5i	324–326 (acetic acid)	3240, 1690, 1650	1.31 (t, 3H, CH_3, $J = 7.2$); 4.35 (q, 2H, CH_2, $J = 7.2$); 7.35 (dt, 1H, Arom, $J = 8.8$, $J = 2.5$); 7.51 (dd, 1H, Arom, $J = 8.8$, $J = 4.4$); 7.73 (dd, 1H, Arom, $J = 8.2$, $J = 2.5$); 11.48 (s, 1H, NH); 13.30 (s, 1H, OH)
5l	343–347 dec (acetic acid)	3240, 1695, 1650	1.31 (t, 3H, CH_3, $J = 7.0$); 4.36 (q, 2H, CH_2, $J = 7.0$); 7.61 (d, 1H, Arom, $J = 6.0$); 7.97 (d, 1H, Arom, $J = 8.5$); 11.54 (s, 1H, NH); 13.40 (s, 1H, OH)
6a	336–345 dec (acetic acid)	3260, 1710, 1640	7.25–7.35 (m, 1H, Arom), 7.55–7.65 (m, 2H, Arom); 8.02 (d, 1H, Arom, $J = 7.7$); 12.08 (s, 1H, NH); 14.18 (s, 1H, OH), 14.80 (s, 1H, COOH)
6i	>350 (acetic acid)	3285, 1690, 1640	7.45 (dt, 1H, Arom, $J = 8.8$, $J = 2.5$); 7.59 (dd, 1H, Arom, $J = 8.8$, $J = 4.4$); 7.87 (dd, 1H, Arom, $J = 8.2$, $J = 2.5$); 12.08 (s, 1H, NH); 14.25 (s, 1H, OH); 14.74 (s, 1H, COOH)
6l	340–350 dec (acetic acid)	3230, 1710, 1655, 1625	7.69 (d, 1H, Arom, $J = 6.0$); 8.14 (d, 1H, Arom, $J = 8.6$); 12.13 (s, 1H, NH); 14.40 (s, 1H, OH), 14.80 (s, 1H, COOH)
7	130–133 (acetonitrile–water)	1730, 1640, 1610	1.38 (t, 3H, CH_3, $J = 7.2$); 1.48 (t, 3H, CH_3, $J = 7.2$); 3.98 (s, 3H, CH_3); 4.06 (q, 2H, CH_2, $J = 7.2$); 4.54 (q, 2H, CH_2, $J = 7.2$); 7.20–7.35 (m, 2H, Arom); 7.55 (dt, 1H, Arom, $J = 7.6$, $J = 1.2$); 8.02 (d, 1H, Arom, $J = 7.6$)
8	205–210 (dioxane)	3430, 1720, 1595	1.25 (t, 3H, CH_3, $J = 7.2$); 3.85 (s, 3H, CH_3); 4.30 (q, 2H, CH_2, $J = 7.2$); 7.25–7.35 (m, 1H, Arom); 7.55–7.65 (m, 2H, Arom); 7.98 (d, 1H, Arom, $J = 7.5$)
9	168–170 (acetonitrile–water)	3500, 1720, 1630, 1610	1.32 (t, 3H, CH_3, $J = 7.1$); 1.33 (t, 3H, CH_3, $J = 7.1$); 4.25 (q, 2H, CH_2, $J = 7.1$); 4.36 (q, 2H, CH_2, $J = 7.1$); 7.20–7.25 (m, 1H, Arom); 7.45–7.55 (m, 2H, Arom); 7.91 (d, 1H, Arom, $J = 7.7$); 11.45 (s, 1H, NH)
10	295–305 dec (acetic acid)	3300, 1700, 1640, 1605	1.39 (t, 3H, CH_3, $J = 7.1$); 4.38 (q, 2H, CH_2, $J = 7.1$); 7.30–7.35 (m, 1H, Arom); 7.55–7.65 (m, 2H, Arom); 8.03 (d, 1H, Arom, $J = 7.9$); 12.07 (s, 1H, NH); 14.75 (s, 1H, COOH)

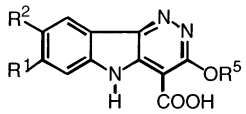
[a] ^1H NMR spectra were recorded in DMSO-d_6 with the exception of compound **4i** and **7** ($CDCl_3$).

alkyl derivatives **3a** and **4a** and even greater of that of the N-2 phenyl analog **2a**. As for the N-2 alkylated compounds, the N-2 CH_3 derivatives **3** showed an activity close to that of the corresponding more lipophilic N-2 *tert*-butyl derivatives **4**. The introduction of halogens in positions 7 and 8 afforded more active compounds only in the series of N-2 unsubstituted derivatives (compare compound **6l** with **6a**, **i**). Finally the alkylation of the indolic NH in the N-2 methyl, or phenolic OH in the N-2 unsubstituted series (derivatives **8** and **10** respectively), led to a drop of activity.

Table 2
Antibacterial activity of pyridazino[4,3-*b*]indole-4-carboxylic acids



2, 3, 4, 8



6, 10

Comp						**MIC** (μg/ml)								
	X	R^1	R^2	R^4	R^5	*B. su.*	*E. fa.*	*S. au. 1*	*S. au. 2*	*S. au. 3*	*E. co. 1*	*E. co. 2*	*P. ae.*	*Kl. ox.*
2a [a]	C_6H_5	H	H	H		>50	12.5	25	25	N.D.	>50	>50	>50	N.D.
2b [a]	C_6H_4-*p*-F	H	H	H		>50	25	>50	25	N.D.	25	>50	>50	N.D.
2c [a]	C_6H_4-*p*-Cl	H	H	H		6.2	5	5	25	N.D.	20	>50	>50	N.D.
2d [a]	C_6H_4-*p*-Br	H	H	H		6.2	6.2	>50	1.6	N.D.	>50	>50	25	N.D.
2e	C_6H_4-*p*-OCF_3	H	H	H		1.6	12.5	6.2	6.2	6.2	12.5	12.5	>50	>50
2f [a]	C_6H_4-*p*-I	H	H	H		1.6	3.1	>50	3.1	N.D.	>50	>50	>50	N.D.
2g [b]	C_6H_4-*p*-$CH(CH_3)_2$	H	H	H		0.78	0.78	0.78	0.78	1.6	>50	>50	>50	>50
2h	C_6H_4-*p*-Br	H	c C_6H_{11}	H		3.1	3.1	25	25	25	>50	>50	>50	>50
3a	CH_3	H	H	H		12.5	12.5	12.5	12.5	>50	>50	12.5	>50	>50
3i	CH_3	H	F	H		3.1	3.1	3.1	3.1	3.1	>50	>50	>50	>50
3l	CH_3	Cl	F	H		3.1	6.2	6.2	6.2	6.2	>50	>50	>50	>50
4a	$C(CH_3)_3$	H	H	H		3.1	3.1	6.2	6.2	6.2	>50	>50	>50	>50
4i	$C(CH_3)_3$	H	F	H		3.1	6.2	6.2	6.2	6.2	>50	>50	>50	>50
6a		H	H		H	12.5	12.5	12.5	12.5	>50	12.5	>50	>50	>50
6i		H	F		H	6.2	12.5	12.5	12.5	12.5	12.5	12.5	>50	>50
6l		Cl	F		H	1.6	1.6	6.2	6.2	6.2	12.5	>50	25	>50
8	CH_3	H	H	C_2H_5		>50	>50	>50	>50	>50	>50	>50	>50	>50
10		H	H		C_2H_5	>50	>50	>50	>50	>50	>50	>50	>50	>50
Norfloxacin						0.1	1.6	0.2	0.2	0.2	0.2	0.8	1.6	0.8

B. su., *B. subtilis ATCC 6633*; *E. fa.*, *E. faecalis ATCC 29212*; *S. au.1*, *S. aureus ATCC 6538*; *S. au. 2*, *S. aureus ATCC 29213*; *S. au. 3*, *S. aureus ATCC 25923*; *E. co. 1*, *Escherichia coli ATCC 8739*; *E. co. 2*, *Escherichia coli ATCC 25922*; *P. ae.*, *Pseudomonas aeruginosa ATCC 27853*; *Kl. ox.*, *Klebsiella oxytoca ATCC 4913*. N.D., not detected.

[a] The MIC values [3] were reported here in order to make a proper comparative evaluation of the antibacterial activity with that of **2e**, **g**, **h**.

[b] The synthesis was reported in Ref. [2].

From the above discussion it appears that the lipophilicity plays an interesting role in the modulation of the antibacterial activity of the tested compounds. Lipophilic substituents in *para* position of the N-2 phenyl ring definitely enhance the activity, whereas a marginal or no role is played by the lipophilicity of the alkyl groups at N-2.

Most likely, other physicochemical properties, besides lipophilicity, may influence the observed antibacterial activity, and this deserves further investigations.

4. Experimental

4.1. Chemistry

Melting points were taken on a Gallenkamp MFB 595 010 M apparatus and are uncorrected. Elemental analyses were performed on a Carlo Erba 1106 analyzer for C, H, N; experimental results agreed to within $\pm 0.40\%$ of the theoretical values. IR spectra were recorded using potassium bromide disks on a Perkin–Elmer 283 spectrophotometer, only the most significant and diagnostic absorption bands being reported. ^{1}H NMR spectra were recorded on a Bruker AM 300 WB 300 MHz or on a Varian 300 Mercury spectrometers. Chemical shifts are expressed in δ (ppm) and the coupling constants J in Hz. The following abbreviations were used: s, singlet; d, doublet; t, triplet; q, quadruplet; m, multiplet; dd, double doublet; dt, double triplet. Exchange with deuterium oxide was used to identify OH and NH protons, which in some cases gave broad signals spread widely on the base line, to be very difficult to detect. *Norfloxacin* reference antibacterial substance, reagents and solvents were purchased from Sigma–Aldrich Chemie. 5-Cyclohexyl and 5-fluoro-6-chloro isatins, not available commercially, were prepared by the method of Marvel and Hiers starting from the appropriate anilines with some modifications (see preparations) [4–6].

4.1.1. 5-Cyclohexyl-1*H*-indole-2,3-dione

A solution of cyclohexylaniline (7.0 g, 40 mmol) in HCl 1.5 N (28 ml) and dioxane (10 ml) at 90–100 °C was added to a water (96 ml) solution of chloral hydrate (14.6 g, 88 mmol) and sodium sulfate decahydrate (104.0 g, 324 mmol) heated at 90–100 °C. A solution of hydroxylamine hydrochloride (17.5 g, 252 mmol) in water (40 ml) was then added and the resulting mixture refluxed for 45 min. *N*-(4-cyclohexylphenyl)-2-hydroxymino-acetamide was obtained as a slurry which was decanted, washed with water and recrystallized from chloroform–hexane (2.6 g, 26% yield). m.p. 157–158 °C; IR, ν_{max} (cm^{-1}): 3360, 3330, 1660; ^{1}H NMR ($CDCl_3$) δ (ppm): 1.10–1.90 (m, 10H, $5CH_2$); 2.40–2.60 (m, 1H, CH); 7.18 (d, 2H, Arom, $J = 8.5$ Hz); 7.48 (d, 2H, Arom, $J = 8.5$ Hz); 7.59 (s, 1 H, CH); 8.23 (s, 1H, NH); 8.45 (s, 1H, OH).

The *N*-(4-cyclohexylphenyl)-2-hydroxymino-acetamide (2.45 g, 10 mmol) was added portion-wise with stirring at room temperature (r.t.) to methylsulphonic acid (8.85 ml). The resulting mixture was refluxed for 2 h and, after cooling, poured on ice (160 ml). The orange precipitate was filtered, washed with cold water, dried and recrystallized from chloroform–hexane to give 5-cyclohexyl-1*H*-indole-2,3-dione. m.p. 175–177 °C; IR, ν_{max} (cm^{-1}): 3120, 1765, 1735, 1710, 1625; ^{1}H NMR (DMSO-d_6) δ (ppm): 1.10–1.90 (m, 10H, 5 CH_2); 2.40–2.50 (m, 1H, CH, partially masked from DMSO signals); 6.81 (d, 1H, Arom, $J = 8.1$ Hz); 7.32 (d, 1H, Arom, $J = 1.5$ Hz); 7.44 (dd, 1H, Arom, $J = 8.1$ Hz, $J = 1.5$ Hz); 10.92 (s, 1H, NH).

4.1.2. 6-Chloro-5-fluoro-1*H*-indole-2,3-dione

A mixture of 3-chloro-4-fluoroaniline (7.27 g, 50 mmol) in HCl 1.8 N (35 ml) was added to a water solution (120 ml) of chloral hydrate (9 g, 54 mmol) and sodium sulfate decahydrate (130.0 g, 404 mmol). A water solution (50 ml) of hydroxylamine hydrochloride (11.0 g, 158 mmol) was then added and the resulting mixture refluxed for 45 min. The solid obtained after cooling was filtered, washed with water and recrystallized from water to yield *N*-(3-chloro-4-fluorophenyl)-2-hydroxymino-acetamide (2.16 g, 20% yield). m.p. 176–180 °C; IR, ν_{max} (cm^{-1}): 3400, 3350, 1660; ^{1}H NMR (DMSO-d_6) δ (ppm): 7.38 (t, 1H, Arom, $J = 8.8$ Hz), 7.58–7.64 (m, 1H, Arom); 7.61 (s, 1H, CH, overlapped to aromatic signal); 7.99 (dd, 1H, Arom, $J =$ 6.8, $J = 2.4$ Hz); 10.38 (s, 1H, NH); 12.3 (s, 1H, OH).

The *N*-(3-chloro-4-fluorophenyl)-2-hydroxymino-acetamide (2.14 g, 10 mmol) was added portion-wise and with stirring to sulfuric acid (6.8 ml), the temperature being kept at 60–70 °C, then raised to 80 °C for 10 min. After cooling the reaction mixture was poured on ice (120 ml) and the resulting precipitate was filtered, washed with cold water, dried and chromatographed by the flash technique using ethyl acetate:chloroform mixture (4:6) to obtain 6-chloro-5-fluoro-1*H*-indole-2,3-dione ($R_f = 0.82$, 0.499 g, 25% yield) and 4-chloro-5-fluoro-1*H*-indole-2,3-dione ($R_f =$ 0.47, 0.898 g, 45% yield).

6-Chloro-5-fluoro-1*H*-indole-2,3-dione was recrystallized from ethanol. m.p. 277–278 °C; IR, ν_{max} (cm^{-1}): 3170, 1750, 1710, 1620; ^{1}H NMR (DMSO-d_6) δ (ppm): 7.06 (d, 1H, Arom, $J = 5.8$ Hz), 7.65 (d, 1H, Arom, $J = 7.6$ Hz), 11.14 (s, 1H, NH).

4-Chloro-5-fluoro-1*H*-indole-2,3-dione was recrystallized from ethanol. m.p. 243–246 °C; IR, ν_{max} (cm^{-1}): 3160, 1740, 1710, 1620 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ (ppm): 6.84 (dd, 1H, Arom, $J = 8.7$ Hz, $J = 3.6$ Hz), 7.59 (dd, 1H, Arom, $J = 9.8$ Hz, $J = 8.7$ Hz), 11.14 (s, 1H, NH).

*4.1.3. Diethyl-2-(5-cyclohexyl-3-oxo-1,3-dihydro-2H-indol-2-ylidene)malonate (**1h**) and diethyl-2-(6-chloro-5-fluoro-3-oxo-1,3-dihydro-2H-indol-2-ylidene)-malonate (**1l**)*

A mixture of appropriately substituted 1*H*-indole-2,3-dione (6 mmol), phosphorus (V) chloride (1.32 g, 6.3 mmol) and anhydrous benzene (2.7 ml) was refluxed for 30 min (**1h**) or 2 h (**1l**). The solution was allowed to cool to r.t. and the intermediate imidoylchloride was obtained as a solid residue after the evaporation of the solvent in vacuo (**1h**) or as a precipitate, which was collected by filtration under dry nitrogen (**1l**). After washing with petroleum ether, the solid was immediately dissolved in anhydrous dioxane (11 ml) and an equimolecular solution of diethylmalonate sodium salt in anhydrous dioxane (13 ml) was then added dropwise. The reaction mixture was stirred at r.t. overnight. The precipitate of sodium chloride was filtered off and the dioxane solution evaporated in vacuo. The residue was chromatographed on silica gel to furnish **1h** (eluent ethyl acetate–petroleum ether 3:7, $R_f = 0.55$, 0.162 g, 7.3% yield) or recrystallized from cyclohexane to afford **1l** (1.52 g, 74% yield).

*4.1.4. 3-Oxo-2-[4-(trifluoromethoxy)phenyl]-3,5-dihydro-2H-pyridazino[4,3-b]indole-4-carboxylic acid (**2e**)*

4-Trifluomethoxyphenylhydrazine hydrochloride (0.685 g, 3 mmol) dissolved in ethanol:water (1:1, 15 ml) was added to a solution of **1a** (0.722 g, 2.5 mmol) in ethanol (5 ml) and the resulting mixture refluxed for 14 h. The precipitate was collected and recrystallized from acetic acid to afford the carboxylic acid **2e** (0.390 g, 39% yield). The reaction mother liquor was concentrated in vacuo to afford a precipitate which was collected and chromatographed on silica gel, using ethyl acetate:petroleum ether (4:6) as the eluent, to give 1*H*-indole-2,3-dione 3-{*N*-[4-(trifluoromethoxy)-phenyl]hydrazone} ($R_f = 0.65$, 0.158 g, 20% yield) and ethyl 3-oxo-2-[4-(trifluoromethoxy)phenyl]-3,5-dihydro-2*H*-pyridazino[4,3-*b*]indole-4-carboxylate ($R_f = 0.27$, 0.227 g, 22% yield).

1*H*-Indole-2,3-dione 3-{*N*-[4-(trifluoromethoxy)-phenyl]hydrazone} was recrystallized from lygroin. m.p. 219–221 °C; IR, ν_{max} (cm^{-1}): 3160, 1675, 1670, 1615; ^1H NMR (DMSO-d_6) δ (ppm): 6.91 (d, 1H, Arom, $J = 7.6$ Hz); 7.04 (t, 1H, Arom, $J = 7.2$ Hz); 7.25 (dt, 1H, Arom, $J = 7.5$ Hz, $J = 1.1$ Hz); 7.34 (d, 2H, Arom, $J = 8.5$ Hz); 7.50–7.60 (m, 3H, Arom); 11.04 (s, 1H, NH), 12.70 (s, 1H, NH).

Ethyl 3-oxo-2-[4-(trifluoromethoxy)phenyl]-3,5-dihydro-2*H*-pyridazino[4,3-*b*]indole-4-carboxylate was recrystallized from lygroin. m.p. 225–227 °C; IR, ν_{max} (cm^{-1}): 3370, 1670, 1645, 1610; ^1H NMR ($CDCl_3$) δ (ppm): 1.43 (t, 3H, CH_3, $J = 7.1$ Hz); 4.46 (q, 2H, CH_2, $J = 7.1$ Hz); 7.25–7.35 (m, 4H, Arom); 7.45–7.55 (m, 1H, Arom); 7.65–7.70 (m, 2H, Arom); 7.98 (d, 1H, Arom, $J = 7.3$ Hz); 9.86 (s, 1H, NH).

The hydrolysis with ethanolic NaOH furnished the acid **2e** in almost quantitative yield.

*4.1.5. 8-Cyclohexyl-2-(4-bromophenyl)-3-oxo-3,5-dihydro-2H-pyridazino[4,3-b]indole-4-carboxylic acid (**2h**)*

4-Bromophenylhydrazine hydrochloride (0.670 g, 3 mmol) dissolved in ethanol:water (1:1, 15 ml) was added to a solution of **1h** (0.865 g, 2.5 mmol) in ethanol (20 ml) and the resulting mixture refluxed for 8 h. The precipitate was collected and recrystallized from methanol to afford the carboxylic acid **2h** (0.116 g, 10% yield). The recrystallization mother liquor was evaporated in vacuo and the residue chromatographed on silica gel to give the corresponding ethyl ester (eluent ethyl acetate–petroleum ether, 4:6, $R_f = 0.42$, 0.865 g, 70% yield). The solid was then recrystallized from acetonitrile. m.p. 250–252 °C; IR, ν_{max} (cm^{-1}): 3375, 1675; ^1H NMR ($CDCl_3$) δ (ppm): 1.45 (t, 3H, CH_3, $J = 7.1$ Hz); 1.20–1.65 (m, 6H, $3CH_2$, overlapped to the CH_3 signals); 1.70–2.00 (m, 4H, $2CH_2$); 2.50–2.70 (m, 1H, CH); 4.48 (q, 2H, CH_2, $J = 7.1$ Hz); 7.18 (d, 1H, Arom, $J = 8.4$ Hz); 7.36 (dd, 1H, Arom, $J = 8.4$ Hz, $J = 1.3$ Hz); 7.49–7.54 (m, 2H, Arom), 7.56–7.64 (m, 2H, Arom); 7.84 (d, 1H, Arom, $J = 1.3$ Hz); 9.78 (s, 1H, NH).

The hydrolysis with ethanolic NaOH furnished the acid **2h** in almost quantitative yield.

*4.1.6. 2-Methyl-3-oxo-3,5-dihydro-2H-pyridazino[4,3-b]indole-4-carboxylic acids (**3a**, **i**, **l**)*

HCl 6 N (1.65 ml) and methylhydrazine (0.525 ml, 10 mmol) were added to a stirred solution of **1** (0.5 mmol) in ethanol (10 ml) cooled at 0 °C. The mixture was then refluxed for 22 h. The precipitate was collected, washed with ethanol–water (1:1) and recrystallized from dioxane to afford the carboxylic acid **3** (**3a**: 0.057 g, 47% yield; **3i**: 0.026 g, 20% yield; **3l**: 0.030 g, 20% yield).

*4.1.7. 2-(tert-Butyl)-3-oxo-3,5-dihydro-2H-pyridazino[4,3-b]indole-4-carboxylic acids (**4a**, **i**)*

To a stirred solution of **1a** (0.246 g, 0.85 mmol) or **1i** (0.261 g, 0.85 mmol) in ethanol–water (5:1, 18 ml), *tert*-butylhydrazine hydrochloride (0.530 g, 4.25 mmol, for **1a** or 0.106 g, 0.85 mmol for **1i**) was added. The mixture was refluxed for 40 h. The precipitate was collected, washed with ethanol–water and recrystallized from acetonitrile to afford the carboxylic acid **4** (**4a**: 0.082 g, 34% yield; **4i**: 0.051 g, 18% yield).

*4.1.8. Ethyl 3-hydroxy-5H-pyridazino[4,3-b]indole-4-carboxylates (**5a**, **i**, **l**)*

To a stirred solution of **1a** (0.434 g, 1.5 mmol) or **1i**

(0.461 g, 1.5 mmol) or **1l** (0.512 g, 1.5 mmol) in anhydrous ethanol (30 ml), hydrazine hydrochloride (0.123 g, 1.8 mmol for **1a** and **1i** or 0.307 g, 4.5 mmol for **1l**) was added. The mixture was refluxed for 22 h and, after cooling to r.t., the precipitate was collected, washed with ethanol–water and recrystallized from ethanol (**5a,** 0.274 g, 71% yield) or from acetic acid (**5i**, 0.231 g, 56% yield; **5l**, 0.237 g, 51% yield).

*4.1.9. 3-Hydroxy-5H-pyridazino[4,3-b]indole-4-carboxylic acids (**6a**, **i**, **l**)*

A solution of NaOH (0.200 g, 5 mmol) in ethanol (20 ml) was added with stirring to a suspension of **5** (0.5 mmol) in ethanol (5 ml) and the resulting mixture refluxed for 1 h. The residue obtained after the evaporation of the solvent in vacuo was suspended in water (30 ml). The suspension was acidified with conc. HCl to pH 1 and then stirred at r.t. for 1 h. The formed yellow solid was filtered, washed with water, and dried. Recrystallization from acetic acid gave pure carboxylic acids **6a**, **i**, **l** in 75–83% yield.

*4.1.10. Ethyl 5-ethyl-2-methyl-3-oxo-3,5-dihydro-2H-pyridazino[4,3-b]indole-4-carboxylate (**7**)*

Sodium hydride 97% (0.025 g, 1 mmol) was added with stirring to a suspension of **4a** (0.050 g, 0.2 mmol) in dry DMF (2 ml) under nitrogen. After 20 min iodoethane (0.080 ml, 1 mmol) was added and stirring was continued for 8 h. The mixture was then poured on ice (30 ml). The mixture was extracted twice with 30 ml of chloroform and the organic phases washed with water, dried on anhydrous sodium sulfate and evaporated in vacuo. The obtained residue was recrystallized from acetonitrile–water to give compound **7** (0.022 g, 36% yield).

*4.1.11. 5-Ethyl-2-methyl-3-oxo-3,5-dihydro-2H-pyridazino[4,3-b]indole-4-carboxylic acid (**8**)*

The carboxylic acid **8** was prepared (66% yield) by hydrolysis of the corresponding ethyl ester **7** by the method reported for the synthesis of acids **6**.

*4.1.12. Ethyl 3-ethoxy-5H-pyridazino[4,3-b]indole-4-carboxylate (**9**)*

Sodium hydride 97% (0.010 g, 0.4 mmol) was added to a stirred suspension of **5a** (0.064 g, 0.25 mmol) in dry DMF (3 ml) under nitrogen. After 20 min iodoethane (0.028 ml, 0.35 mmol) was added and stirring was continued for 90 min. The mixture was then poured on ice (25 ml) and the precipitate was filtered, washed with water, dried and recrystallized from acetonitrile–water to give compound **9** (0.052 g, 76% yield).

*4.1.13. 3-Ethoxy-5H-pyridazino[4,3-b]indole-4-carboxylic acid (**10**)*

The carboxylic acid **10** was prepared in 77% yield from the corresponding ethyl ester **9** by the method described for the synthesis of acids **6**.

4.2. Microbiology

Tests of antibacterial activity were carried out by the microdilution method according to NCCLS approved standard [7,8] against the following American Type Culture Collection (ATCC) microorganisms: *B. subtilis* ATCC 6633, *E. faecalis* ATCC 29212, *S. aureus* ATCC 6538, *S. aureus* ATCC 29213, *S. aureus* ATCC 25923, *Escherichia coli* ATCC 8739, *Escherichia coli* ATCC 25922, *Klebsiella oxytoca* ATCC 49131 and *Pseudomonas aeruginosa* ATCC 27853. The minimum inhibitory concentration (MIC), that is the lowest concentration of compound that completely inhibited bacteria growth, was determined by the method of two-fold serial dilution technique in liquid media [7,8] using disposable microplates. Mueller Hinton Broth (MHB) was used as medium to prepare microdilution samples (Difco laboratories, Accumedia, and Oxoid, Basingstoke, UK). The tested substances were dissolved in dimethyl sulfoxide and diluted with MHB to a final 5% dimethyl sulfoxide solution. The antibacterial activity was measured after 24 h of incubation at 37 °C.

Quinolone *norfloxacin* was used as reference substance in order to make a proper comparative evaluation of the antibacterial activity [8,9].

References

[1] F. Campagna, A. Carotti, G. Casini, F. Palluotto, G. Genchi, G.B. De Sarro, 2-Aryl-2,5-dihydropyridazino[4,3-*b*]indol-3(3H)-ones: Novel rigid planar benzodiazepine receptor ligands, J. Bioorg. Med. Chem. 1 (1993) 437–446.

[2] F. Palluotto, A. Carotti, G. Casini, F. Campagna, G. Genchi, M. Rizzo, G.B. De Sarro, Structure–activity relationships of 2-aryl-2,5-dihydropyridazino[4,3-*b*]indol-3(3*H*)-ones at the benzodiazepine receptor, J. Bioorg. Med. Chem. 4 (1996) 2091–2104.

[3] F. Palluotto, A. Carotti, G. Casini, M. Ferappi, A. Rosato, C. Vitali, F. Campagna, Synthesis and antibacterial activity of 2-aryl-2,5-dihydro-3(3*H*)-oxo-pyridazino[4,3-*b*]indole-4-carboxylic acids, Il Farmaco 54 (1999) 191–194.

[4] T. Sandmeyer, Sandmeyer isonitrosoacetanilide isatin synthesis, Helv. Chim. Acta 2 (1919) 234.

[5] C.S. Marvel, G.S. Hiers, Isatin, Organic Synthesis, coll, vol. I, 2nd ed., 1941, pp. 327–330.

[6] R. Krishnan, S.A. Lang, M.M. Siegel, Synthesis of aminoquinolone derivatives, J. Heterocycl. Chem. 23 (1986) 1801–1804.

[7] Antibacterial National Committee for Clinical Laboratory Standards. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard. M7-A4, Villanova, PA (1997).

[8] Antibacterial National Committee for Clinical Laboratory Standards. Performance standards for antimicrobial susceptibility testing. Eighth informational supplement. M100-S8, Villanova, PA (1998).

[9] Braj. B. Lohray, S. Baskaran, Bonthu Srinivasa Rao, B. Mallesham, K.S.N. Bharath, B. Yadi Reddy, S. Venkateswarlu, Ashok K. Sadhuklan, M. Sitaram Kumar, Hemant M. Sarnaik, Novel quinolone derivatives as potent antibacterials, Bioorg. Med. Chem. Lett. 8 (1998) 525–528.