Quinolone Analogues 6 [1-5]. Synthesis of 3-Halogeno-1-methylpyridazino[3,4-*b*]quinoxalin-4(1*H*)-ones

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The reaction of the quinoxaline *N*-oxides **7a,b** with diethyl ethoxymethylenemalonate gave the 1-methylpyridazino[3,4-*b*]quinoxaline-4,4-dicarboxylates **8a,b**, whose reaction with *N*-bromosuccinimide or *N*-chlorosuccinimide afforded the 3-halogeno-1-methylpyridazino[3,4-*b*]quinoxaline-4,4-dicarboxylates **9a-d**. The reaction of compounds **9a-d** with hydrazine hydrate resulted in hydrolysis and decarboxylation to provide the 3-halogeno-1-methylpyridazino[3,4-*b*]quinoxaline-4-carboxylates **10a-d**, whose reaction with nitrous acid effected oxidation to furnish the 3-halogeno-4-hydroxy-1-methylpyridazino[3,4-*b*]quinoxaline-4-carboxylates **11a-d**, respectively. The reaction of compounds **11a-d** with hydrazine hydrate afforded the 3-halogeno-1-methylpyridazino[3,4-*b*]quinoxalin-4-ols **12a-d**, whose oxidation provided the 3-halogeno-1-methylpyridazino[3,4-*b*]quinoxalin-4(1*H*)-ones **6a-d**, respectively. Compounds **6a-d** had antifungal activities *in vitro*.

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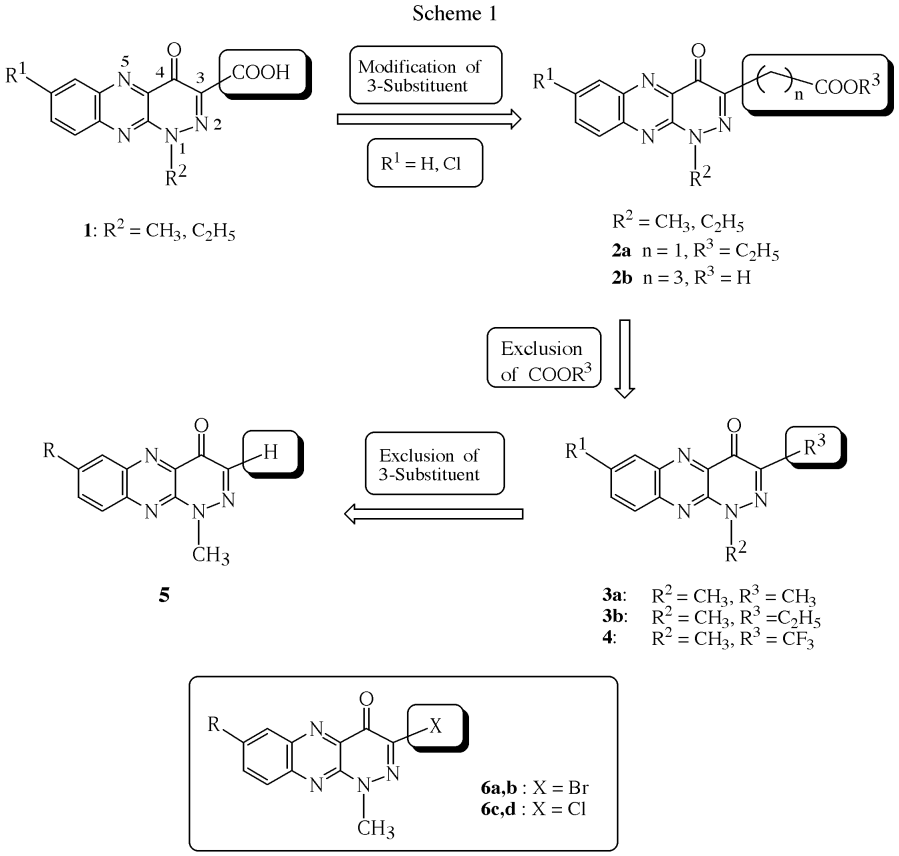
Introduction.

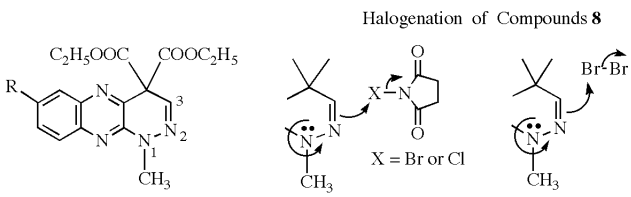
In previous papers [1-5], we have reported the synthesis of the 1-alkylpyridazino[3,4-*b*]quinoxalin-4-ones **1-5** (Scheme 1) as candidates of antimicrobial quinolone analogues. Since the 3-carboxylic acid derivatives **1** [1] showed only weak antibacterial activities, we produced the methylene-inserted carboxylate and carboxylic acid derivatives **2a,b** [2] and then the 3-alkyl derivatives **3** [3] and **4** [4]. In these modifications, we found that compounds **1** and **2** had similar antibacterial activities, but compounds **3** and **4** exhibited better antibacterial activity [5] than those of compounds **1** and **2**.

Scheme 1



Moreover, compounds **3** and **4** showed good antifungal activities [5-7]. Thus, the exclusion of a carboxyl function from the 3-substituent was found to confer antifungal activities for our quinolone analogues with the pyridazino[3,4-*b*]quinoxalin-4(1*H*)-one ring system. Accordingly, we further planed to exclude the 3-alkyl function of compounds **3** and **4**, leading to the production of the 3-H derivatives **5** [5], which also showed good antifungal activities [6-8]. In continuation of these studies, we undertook the synthesis of the 3-halogeno-1-methylpyridazino[3,4-*b*]quinoxalin-4(1*H*)-ones **6a-d** in order to search for more potent compounds than compounds **3** and **4**. In this paper, we report the synthesis and antifungal activities of the 3-halogeno homologues **6a-d**.

Chart 1



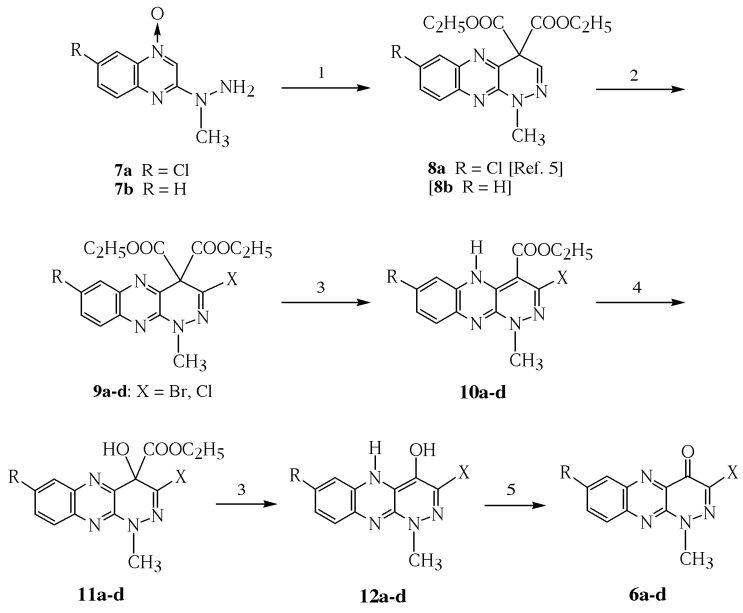
Methods for The Synthesis of Compounds **6a-d**.

While there might be other ways to synthesize the 3-halogenoquinolone analogues **6a-d**, we selected the route shown in Scheme 2. Namely, the halogenation was conveniently accomplished for the 1,4-dihydro-1-methylpyridazino[3,4-*b*]quinoxaline-4,4-dicarboxylates **8a** [5] and **8b** using *N*-bromosuccinimide, bromine, or *N*-chlorosuccinimide, since compounds **8a,b** include a hydrazone moiety [9,10] in the N_1-N_2-C_3 of the pyridazine ring (Chart 1).

Synthesis of Compounds **6a-d**.

The synthesis of compound **8a** has already been reported in a previous paper [5]. The 3-halogeno-1,4-dihydro-1-methylpyridazino[3,4-*b*]quinoxaline-4,4-dicarboxylate **9a** or **9c** (R = Cl) was synthesized by the reaction of compound **8a** with *N*-bromosuccinimide or *N*-chlorosuccinimide, respectively. The use of bromine as a brominating agent gave compound **9a** in a better yield. When compounds **9b** and **9d** (R = H) were synthesized from compound **7b**, compound **8b** was not purified in order to raise overall yields. The reaction of compounds **9a-d** with hydrazine hydrate resulted in hydrolysis and decarboxylation [5] to afford the 3-halogeno-1,5-dihydro-1-methylpyridazino[3,4-*b*]quinoxaline-4-carboxylates **10a-d** [5,12-15], whose reaction with nitrous acid effected oxidation [1,2,4,5,11] to provide the 3-halogeno-1,4-dihydro-4-hydroxy-1-methylpyridazino[3,4-*b*]quinoxaline-4-carboxylates **11a-d**, respectively. The reaction of compounds **11a-d** with hydrazine hydrate also resulted in hydrolysis and decarboxylation [5] to furnish the 3-halogeno-1,5-

Scheme 2



Reagents: (1) diethyl ethoxymethylenemalonate in CH_3COOH; (2) Br_2, *N*-bromosuccinimide, or *N*-chlorosuccinimide in CH_3COOH; (3) $NH_2NH_2 \cdot H_2O$ in C_2H_5OH; (4) $NaNO_2$ in H_2O/CH_3COOH; (5) *N*-bromosuccinimide or *N*-chlorosuccinimide in H_2O/CH_3COOH

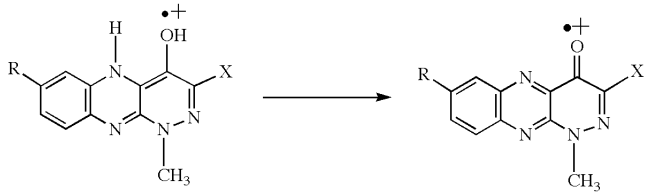
dihydro-1-methylpyridazino[3,4-*b*]quinoxalin-4-ols **12a-d** [5,12-15], respectively. Oxidation of compounds **12a,b** with *N*-bromosuccinimide/water or compounds **12c,d** with *N*-chlorosuccinimide/water gave compounds **6a,b** or **6c,d**, respectively.

Spectral Data for Compounds **12**.

The structural assignment of new compounds **6** and **9-12** was based on the analytical and spectral data, but attention had to be paid to the interpretation of the mass and nmr spectral data for compounds **12** because of susceptibility to oxidation (Schemes 3 and 4). Compounds **13a,b** (Scheme 5) already reported in our previous paper [4] also showed a similar susceptibility to oxidation in the mass and nmr spectroscopy [16]. In the mass spectra, compounds **12b-d** showed the (M^+ - 2) ion peaks corresponding to compounds **6b-d**, lacking the M^+ ion peaks corresponding to compounds **12b-d** (Scheme 3). The nmr spectra of compounds **12a-d** exhibited two groups of the aromatic proton signals in higher magnetic field (δ 6.65 - 6.34) and in lower magnetic field (δ 8.27 - 7.89) (Scheme 4), which would correspond to the aromatic proton signals of the 4-hydroxy derivatives **12a-d** and the 4-oxo derivatives **6a-d**, respectively [4,17]. In previous papers [1,2,4], we reported that the aromatic proton signals with the 1,5-dihydro form (δ 7.23 - 6.61) were observed in higher magnetic field than those with the 1,4-dihydro form (δ 8.50 - 7.72) in the pyridazino[3,4-*b*]quinoxaline ring system. Similarly, the aromatic proton signals of compounds **12a-d** with the 1,5-dihydro form (δ 6.65 - 6.34) were observed apparently in higher magnetic field than those of compounds **6a-d** with the 1,4-dihydro form (δ 8.27 - 7.89) [17]. The aromatic proton signals of compounds **10a-d** with the 1,5-dihydro form (δ 7.05 - 6.67) were also observed in higher magnetic field than those of compounds **6a-d** with the 1,4-dihydro form (δ 8.34 - 7.88) (Chart 2). The oxidation of compounds **13a,b** into compounds **4a,b** observed by nmr spectroscopy (Scheme 5) [4] also supports the above nmr spectral interpretation for compounds **12a-d**.

Scheme 3

Oxidation of Compounds **12b-d** into Compounds **6b-d** observed in Mass Spectroscopy.



X = Br, Cl

Species Corresponding to Compounds **12b-d**

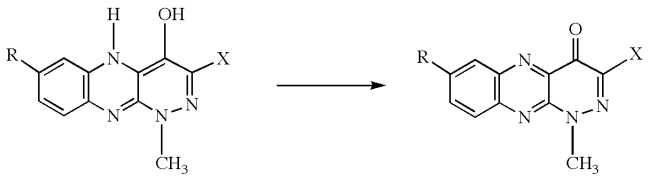
X = Br, Cl

Species Corresponding to Compounds **6b-d**

Compounds **12b-d** were oxidized into **6b-d** when mass spectra were measured.

Scheme 4

Oxidation of Compounds **12a-d** into Compounds **6a-d** observed in NMR Spectroscopy [a].



12a-d

1,5-Dihydro Form

Aromatic Proton Signals
δ 6.65 - 6.34

6a-d

1,4-Dihydro Form

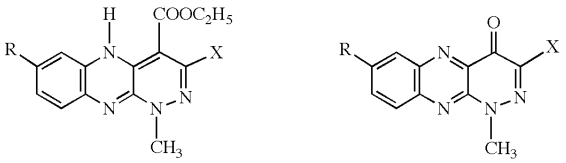
Aromatic Proton Signals
δ 8.27 - 7.89

Compounds **12a-d** were oxidized into **6a-d** when nmr spectra were measured. Accordingly, there were two kinds of signals due to compounds **12** and **6** in the nmr spectra.

[a] Measured in deuteriotrifluoroacetic acid.

Chart 2

Aromatic Proton Signals for Compounds **6a-d** and **10a-d**.



10 a - d

1,5-Dihydro Form

Aromatic Proton Signals [a]
δ 7.05 - 6.67

6 a - d

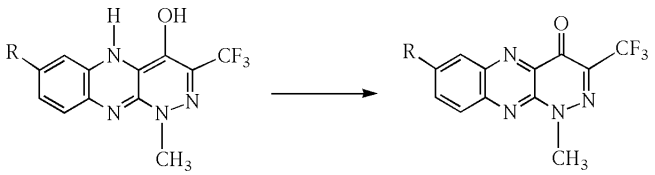
1,4-Dihydro Form

Aromatic Proton Signals [b]
δ 8.34 - 7.88

[a] Measured in deuteriotrifluoroacetic acid or deuteriodimethyl sullfoxide.
[b] Measured in deuteriotrifluoroacetic acid.

Scheme 5 [Ref. 4]

Oxidation of Compounds **13a,b** into Compounds **4a,b** observed in NMR Spectroscopy [a].



13a,b (R = Cl, H)

1,5-Dihydro Form

Aromatic Proton Signals
δ 6.99 - 6.61

4a,b (R = Cl, H)

1,4-Dihydro Form

Aromatic Proton Signals
δ 8.50 - 8.01

[Ref. 4] Compounds **13a,b** were oxidized into **4a,b** when nmr spectra were measured. Accordingly, there were two kinds of signals due to compounds **13** and **4**.

[a] Measured in deuteriodimethyl sulfoxide.

Screening Data.

Compounds **6a-d** showed antifungal activities *in vitro* against *Trichophyton mentagrophytes* at 1 - 2 ppm and *Trichophyton rubrum* at 0.5 - 1 ppm.

EXPERIMENTAL

All melting points were determined on a Yazawa micro melting point BY-2 apparatus and are uncorrected. The ir spectra (potassium bromide) were recorded with a JASCO FT/IR-200 spectrophotometer. The nmr spectra were measured with a Varian XL-400 spectrometer at 400 MHz. The chemical shifts are given in the δ scale. The mass spectra (ms) were determined with a JEOL JMS-01S spectrometer. Elemental analyses were performed on a Perkin-Elmer 240B instrument.

Diethyl 3-Bromo-7-chloro-1,4-dihydro-1-methylpyridazino-[3,4-*b*]quinoxaline-4,4-dicarboxylate (**9a**).

Method A.

Bromine (6 ml) was added to a suspension of compound **8a** (10 g) in acetic acid (300 ml) under stirring at room temperature to give a clear solution, which was further stirred for 4 hours. A solution of sodium acetate (22 g) in water (100 ml), a solution of potassium iodide (45 g) in water (100 ml), and a solution of sodium thiosulfate (35 g) in water (100 ml) were added successively to the above reaction mixture to precipitate yellow needles of compound **9a**, which were collected by filtration and then washed with water to give an analytically pure sample (14.66 g, 75%), mp 97-98°; ir: ν cm^{-1} 1755, 1740; ms: m/z 454 (M^+), 456 ($M^+ + 2$); ^1H nmr (deuteriodimethyl sulfoxide): δ 8.10 (d, J = 2.5 Hz, 1H, C_6-H), 7.95 (d, J = 9.5 Hz, 1H, C_9-H), 7.85 (dd, J = 9.5, 2.5 Hz, 1H, C_8-H), 4.25 (q, J = 7.0 Hz, 4H, $2CH_2$), 3.64 (s, 3H, NCH_3), 1.18 (t, J = 7.0 Hz, 6H, $2CH_3$).

Anal. Calcd. for $C_{17}H_{16}BrClN_4O_4$: C, 44.81; H, 3.54; N, 12.29. Found: C, 44.84; H, 3.56; N, 12.41.

Method B.

A solution of compound **8a** (5 g, 13.3 mmols) and *N*-bromosuccinimide (3.54 g, 19.9 mmols) in acetic acid (100 ml) was refluxed for 1 hour. Evaporation of the solvent *in vacuo* gave an oily substance, whose crystallization from ethanol/water afforded yellow needles of compound **9a** (3.77 g, 62%).

Ethyl 3-Bromo-7-chloro-1,5-dihydro-1-methylpyridazino-[3,4-*b*]quinoxaline-4-carboxylate (**10a**).

A solution of compound **9a** (10 g) and hydrazine hydrate (100% purity, 5 ml) in ethanol (300 ml) was refluxed for 1 hour to precipitate orange needles of compound **10a**, which were collected by filtration and then washed with ethanol to give an analytically pure sample (7.77 g, 92%), mp 185-186°; ir: ν cm^{-1} 1650; ms: m/z 382 (M^+), 384 ($M^+ + 2$); ^1H nmr (deuteriodimethyl sulfoxide): δ 9.60 (s, 1H, NH), 7.05 (s, 1H, C_6-H), 6.79 (d, J = 8.0 Hz, 1H, C_9-H), 6.67 (d, J = 8.0 Hz, 1H, C_8-H), 4.21 (q, J = 7.0 Hz, 2H, CH_2), 3.09 (s, 3H, NCH_3), 1.26 (t, J = 7.0 Hz, 3H, CH_3).

Anal. Calcd. for $C_{14}H_{12}BrClN_4O_2$: C, 43.83; H, 3.15; N, 14.60. Found: C, 43.85; H, 3.30; N, 14.90.

Ethyl 3-Bromo-7-chloro-1,4-dihydro-4-hydroxy-1-methylpyridazino[3,4-*b*]quinoxaline-4-carboxylate (**11a**).

A solution of sodium nitrite (2.70 g, 39.2 mmols) in water (50 ml) was added to a suspension of compound **10a** (10 g, 26.1 mmols) in acetic acid (200 ml)/water (100 ml) with stirring at room temperature. The mixture was further stirred under heating at 90-100° for 1 hour to precipitate yellow needles of compound **11a**, which were collected by filtration and then washed with water to give an analytically pure sample (10.10 g, 93%), mp 174-175°; ir: ν cm^{-1} 3420, 1740; ms: m/z 398 (M^+), 400 ($M^+ + 2$); ^1H nmr (deuteriodimethyl sulfoxide): δ 8.07 (d, J = 2.5 Hz, 1H, C_6-H), 7.93 (d, J = 9.0 Hz, 1H, C_9-H), 7.83 (dd, J = 9.0, 2.5 Hz, 1H, C_8-H), 7.69 (s, 1H, OH), 4.23 (dq, J = 10.0, 7.0 Hz, 1H, methylene CH), 4.14 (dq, J = 10.0, 7.0, 1H, methylene CH), 3,71 (s, 3H, NCH_3), 1.10 (dd, J = 7.0, 7.0 Hz, 3H, CH_3).

Anal. Calcd. for $C_{14}H_{12}BrClN_4O_3$: C, 42.08; H, 3.03; N, 14.02. Found: C, 42.09; H, 3.05; N, 14.07.

3-Bromo-7-chloro-1,5-dihydro-1-methylpyridazino[3,4-*b*]-quinoxalin-4-ol (**12a**).

A solution of hydrazine hydrate (100% purity, 3 ml) and compound **11a** (5 g) in ethanol (250 ml) was refluxed for 2 hours to precipitate greenish yellow needles of compound **12a**, which were collected by filtration and washed with ethanol to afford an analytically pure sample (3.54 g, 86%), mp 295-297°; ir: ν cm^{-1} 3220, 1640, 1605; ms: m/z 324 ($M^+ - 1$), 325 (M^+), 327 ($M^+ + 2$); ^1H nmr (deuteriotrifluoroacetic acid): δ (signals corresponding to 4-hydroxy derivative **12a**) (12%) 6.48 (s, aromatic H), 6.36 (s, aromatic H), 6.34 (s, aromatic H), 3.55 (s, NCH_3); (signals corresponding to 4-oxo derivative **6a**) (88%) 8.15 (s, C_6-H), 8.06 (d, J = 7.0 Hz, aromatic H), 7.89 (d, J = 7.0 Hz, aromatic H), 4.32 (s, NCH_3); ^1H nmr (deuteriodimethyl sulfoxide): δ (signals corresponding to 4-oxo derivative **6a**) (100%): 8.45 (s, 1H, C_6-H), 8.18 (s, 1H, C_9-H) , 8.10 (s, 1H, C_8-H) , 4.15 (s, 3H, NCH_3).

Anal. Calcd. for $C_{11}H_8BrClN_4O$: C, 40.33; H, 2.46; N, 17.10. Found: C, 40.57; H, 2.55; N, 17.29.

3-Bromo-7-chloro-1-methylpyridazino[3,4-*b*]quinoxalin-4(1*H*)-one (**6a**).

A solution of compound **12a** (10 g, 30.5 mmols) and *N*-bromosuccinimide (8.15 g, 45.8 mmols) in acetic acid (250 ml) and water (50 ml) was refluxed for 1 hour. The solution was allowed to stand overnight at room temperature to precipitate yellow scaly crystals of compound **6a**, which were collected by filtration and then washed with *n*-hexane to provide an analytically pure sample (6.94 g). Evaporation of the filtrate *in vacuo* gave yellow crystals of compound **6a**, which were collected by filtration and then washed with *n*-hexane (1.20 g). Total yield: 8.14 g (82%). Compound **6a** had mp 267-268°; ir: ν cm^{-1} 1655, 1600; ms: m/z 323 (M^+), 325 ($M^+ + 2$); ^1H nmr (deuteriotrifluoroacetic acid): δ 8.13 (d, J = 2.0 Hz, 1H, C_6-H); 8.07 (d, J = 9.5 Hz, 1H, C_9-H), 7.89 (dd, J = 9.5, 2.0 Hz, 1H, C_8-H), 4.32 (s, 3H, NCH_3), ^1H nmr (deuteriochloroform): δ 8.34 (dd, J = 2.0, 0.5 Hz, 1H, C_6-H); 8.06 (dd, J = 9.5, 0.5 Hz, 1H, C_9-H), 7.89 (dd, J = 9.5, 2.0 Hz, 1H, C_8-H) , 4.29 (s, 3H, NCH_3).

Anal. Calcd. for $C_{11}H_6BrClN_4O$: C, 40.58; H, 1.86; N, 17.21. Found: C, 40.52; H, 1.94; N, 17.13.

Diethyl 3-Bromo-1,4-dihydro-1-methylpyridazino[3,4-*b*]quinoxaline-4,4-dicarboxylate (**9b**).

A solution of compound **7b** (10 g, 52.6 mmols) and diethyl ethoxymethylenemalonate (17.04 g, 78.9 mmols) in acetic acid (200 ml) was refluxed for 3 hours. Evaporation of the solvent *in*

vacuo gave an oily residue, which was washed with hot *n*-hexane (30 ml) three times (*n*-hexane layer is not necessary). Further evaporation of the above oily residue afforded brown crystals of compound **8b** [ms: m/z 304 (M^+)], which were dissolved in acetic acid (150 ml). Bromine (4 ml) was added to the acetic acid solution with stirring at room temperature, and stirring was continued for 2 hours. Solutions of sodium acetate (22 g) in water (100 ml), potassium iodide (45 g) in water (100 ml), and sodium thiosulfate (22 g) in water (100 ml) were successively added to the above acetic acid solution with stirring at room temperature to precipitate yellow crystals of compound **9b**, which were collected by filtration (10.88 g, 49%). Recrystallization from ethanol/water provided yellow needles, mp 118-119°; ir: ν cm^{-1} 1765, 1740; ms: m/z 420 (M^+), 422 (M^+ + 2); ^{1}H nmr (deuteriodimethyl sulfoxide): δ 7.99 (dd, J = 8.0 ,1.5 Hz, 1H, aromatic H), 7.93 (dd, J = 8.0, 1.5 Hz, 1H, aromatic H), 7.83 (ddd, J = 8.0, 8.0, 1.5 Hz, 1H, aromatic H), 7.67 (ddd, J = 8.0, 8.0, 1.5 Hz, 1H, aromatic H), 4.25 (q, J = 7.0 Hz, 4H, $2CH_2$), 3.64 (s, 3H, NCH_3), 1.18 (t, J = 7.0 Hz, 6H, $2CH_3$).

Anal. Calcd. for $C_{17}H_{17}BrN_4O_4$, 48.47; H, 4.07; N, 13.30. Found: C, 48.17; H, 4.11; N, 13.33.

Ethyl 3-Bromo-1,5-dihydro-1-methylpyridazino[3,4-*b*]quinoxaline-4-carboxylate (**10b**).

A solution of compound **9b** (10 g) and hydrazine hydrate (100% purity, 5 ml) in ethanol (200 ml) was refluxed for 1 hour. The solution was allowed to stand at room temperature to precipitate orange needles of compound **10b**, which were collected by filtration and washed with ethanol to give an analytically pure sample (6.03 g). Evaporation of the filtrate *in vacuo* afforded orange crystals of compound **10b**, which were triturated with ethanol and collected by filtration (0.45 g). Total yield: 6.48 g (78%). Compound **10b** had mp 151-152°; ir: ν cm^{-1} 1640, 1620; ms: m/z 348 (M^+), 350 (M^+ + 2); ^{1}H nmr (deuteriotrifluoroacetic acid): δ 6.93, (ddd, J = 7.5, 7.5, 2.0 Hz, 1H, aromatic H), 6.87 (ddd, J = 7.5, 7.5, 2.0 Hz, 1H, aromatic H), 6.82 (dd, J = 7.5, 2.0 Hz, 1H, aromatic H), 6.79 (dd, J = 7.5, 2.0 Hz, 1H, aromatic H), 4.28 (q, J = 7.0 Hz, 2H, CH_2), 3.57 (s, 3H, NCH_3), 1.26 (t, J = 7.0 Hz, 3H, CH_3).

Anal. Calcd. for $C_{14}H_{13}$ BrN_4O_2: C, 48.16; H, 3.75; N, 16.04. Found: C, 47.86; H, 3.77; N, 16.07.

Ethyl 3-Bromo-1,4-dihydro-4-hydroxy-1-methylpyridazino-[3,4-*b*]quinoxaline-4-carboxylate (**11b**).

A solution of sodium nitrite (1.78 g, 25.8 mmols) in water (30 ml) was added to a suspension of compound **10b** (6 g, 17.2 mmols) in acetic acid (150 ml)/water (70 ml) with stirring at room temperature. The suspension was heated at 90-100° with stirring for 1 hour to precipitate yellow needles of compound **11b**, which were collected by filtration and washed with ethanol/water (1:1) to give an analytically pure sample (5.02 g, 80%), mp 158-159°; ir: ν cm^{-1} 1760; ms: m/z 364 (M^+), 366 (M^+ + 2); ^{1}H nmr (deuteriodimethyl sulfoxide): δ 7.97 (ddd, J = 7.5, 1.5, 1.0 Hz, 1H, aromatic H), 7.92 (ddd, J = 7.5, 1.5, 1.0 Hz, 1H, aromatic H), 7.82 (ddd, J = 7.5, 7.5, 1.5 Hz, 1H, aromatic H), 7.66 (ddd, J = 7.5, 7.5, 1.5 Hz, 1H, aromatic H), 7.61 (s, 1H, OH), 4.23 (dq, J = 10.0, 7.0 Hz, 1H, methylene CH), 4.14 (dq, J = 10.0, 7.0 Hz, 1H, methylene CH), 3.72 (s, 3H, NCH_3), 1.09 (dd, J = 7.0, 7.0 Hz, 3H, CH_3).

Anal. Calcd. for $C_{14}H_{13}BrN_4O_3$: C, 46.05; H, 3.59; N, 15.34. Found: C, 45.79; H, 3.57; N, 15.18.

3-Bromo-1,5-dihydro-1-methylpyridazino[3,4-*b*]quinoxalin-4-ol (**12b**).

A solution of compound **11b** (5 g) and hydrazine hydrate (100% purity, 5 ml) in ethanol (200 ml) was refluxed for 2 hours to precipitate greenish yellow needles of compound **12b**, which were collected by filtration and then washed with ethanol/water (1:1) to give an analytically pure sample (3.34 g, 83%) , mp 265-266°; ir: ν cm^{-1} 3215, 1610; ms: m/z 290 [M^+ - 2 (2H)], 292 [(M^+ + 2) - 2 (2H)]; ^{1}H nmr (deuteriotrifluoroacetic acid): δ (signals corresponding to 4-hydroxy derivative **12b**) (89%) 6.52 (s, aromatic H), 6.44 (s, aromatic H), 3.50 (s, NCH_3); (signals corresponding to 4-oxo derivative **6b**) (11%) 8.22 (s, aromatic H), 8.06 (s, aromatic H), 4.34 (s, NCH_3); ^{1}H nmr (deuteriodimethyl sulfoxide): δ (signals corresponding to 4-hydroxy derivative **12b**) (29%) 10.20 (s, NH), 7.55 (s, OH), 6.95 (dd, J = 7.5, 1.5 Hz, aromatic H), 6.90 (ddd, J = 7.5, 7.5, 1.5 Hz, aromatic H), 6.78 (dd, J = 7.5, 1.5 Hz, aromatic H), 6.60 (ddd, J = 7.5, 7.5, 1.5 Hz, aromatic H), 3.49 (s, NCH_3); (signals corresponding to 4-oxo derivative **6b**) (62%) 8.30 (ddd, J = 8.0, 1.5, 1.0 Hz, aromatic H), 8.15 (ddd, J = 8.0, 1.5, 1.0 Hz, aromatic H), 8.09, (ddd, J = 8.0, 8.0, 1.5 Hz, aromatic H), 7.96 (ddd, J = 8.0, 8.0, 1.5 Hz, aromatic H), 4.16 (s, NCH_3).

Anal. Calcd. for $C_{11}H_9BrN_4O$: C, 45.07; H, 3.09; N, 19.11. Found: C, 45.06; H, 2.99; N, 19.02.

3-Bromo-1-methylpyridazino[3,4-*b*]quinoxalin-4(1*H*)-one (**6b**).

A suspension of compound **12b** (2 g, 6.83 mmols) and *N*-bromosuccinimide (1.82 g, 10.2 mmols) in acetic acid (80 ml)/water(10 ml) was refluxed for 1 hour to give a clear solution. The solvent was evaporated *in vacuo* to give yellow crystals of compound **6b**, whose recrystallization from acetic acd/water afforded yellow needles (1.62 g). Evaporation of the filtrate *in vacuo* gave yellow crystals, which were triturated with hot ethanol/water (1:1) to afford yellow needles of compound **6b** (0.24 g). Total yield: 1.86 g (93%). Compound **6b** had mp 268-269°; ir: ν cm^{-1} 1655; ms: m/z 290 (M^+), 292 (M^+ + 2); ^{1}H nmr (deuteriotrifluoroacetic acid): δ 8.34 (dd, J = 7.5, 2.0 Hz, 1H, aromatic H), 8.30 (dd, J = 7.5, 2.0 Hz, 1H, aromatic H), 8.16, (ddd, J = 7.5, 7.5, 2.0 Hz, 1H, aromatic H), 8.13 (ddd, J = 7.5, 7.5, 2.0 Hz, 1H, aromatic H), 4.43 (s, 3H, NCH_3).

Anal. Calcd. for $C_{11}H_7BrN_4O$: C, 45.39; H, 2.42; N, 19.25. Found: C, 45.24; H, 2.52; N, 19.13.

Diethyl 3,7-Dichloro-1,4-dihydro-1-methylpyridazino[3,4-*b*]-quinoxaline-4,4-dicarboxylate (**9c**).

A solution of compound **8a** (10 g, 26.6 mmols) and *N*-chlorosuccinimide (5.33 g, 39.9 mmols) in acetic acid (200 ml) was refluxed for 1 hour. Evaporation of the solvent *in vacuo* gave an oily substance, whose crystallization from ethanol/water afforded yellow needles of compound **9c**. The yellow needles were collected by filtration and then washed with ethanol/water (1:1) (6.62 g, 61%), mp 99-100°; ir: ν cm^{-1} 1760, 1740; ms: m/z 410 (M^+), 412 (M^+ + 2); ^{1}H nmr (deuteriodimethyl sulfoxide): δ 8.85 (dd, J = 2.5, 1.0 Hz, 1H, C_6-H), 7.92 (dd, J = 9.0, 1.0 Hz, 1H, C_9-H), 7.82 (dd, J = 9.0, 2.5 Hz, 1H, C_8-H), 4.26 (q, J = 7.0 Hz, 4H, $2CH_2$), 3.62 (s, 3H, NCH_3), 1.17 (t, J = 7.0 Hz, 6H, $2CH_3$).

Anal. Calcd. for $C_{17}H_{16}Cl_2N_4O_4$: C, 49.65; H, 3.92; N, 13.62. Found: C, 49.58; H, 3.97; N, 13.73.

Ethyl 3,7-Dichloro-1,5-dihydro-1-methylpyridazino[3,4-*b*]-quinoxaline-4-carboxylate (**10c**).

A solution of compound **9c** (12 g) and hydrazine hydrate (100% purity, 6 ml) in ethanol (300 ml) was refluxed for 1 hour to

precipitate orange needles of compound **10c**. After cooling to room temperature, compound **10c** was collected by filtration and then washed with ethanol to give an analytically pure sample (9.39 g, 95%), mp 198-199°; ir: ν cm^{-1} 1650; ms: m/z 338 (M^+), 340 ($M^+ + 2$); ^1H nmr (deuteriotrifluoroacetic acid): δ 6.79 (d, J = 2.0 Hz, 1H, C_6-H), 6.79 (dd, J = 9.0, 2.0 Hz, 1H, C_8-H), 6.72 (d, J = 9.0 Hz, 1H, C_9-H), 4.23 (q, J = 7.0 Hz, 2H, CH_2), 3.54 (s, 3H , NCH_3), 1.20 (t, J = 7.0 Hz, 3H, CH_3).

Anal. Calcd. for $C_{14}H_{12}Cl_2N_4O_2$: C, 49.58; H, 3.57; N, 16.52. Found: C, 49.67; H, 3.68; N, 16,57.

Ethyl 3,7-Dichloro-1,4-dihydro-4-hydroxy-1-methylpyridazino[3,4-*b*]quinoxaline-4-carboxylate (**11c**).

A solution of sodium nitrite (2.44 g, 35.4 mmols) in water (30 ml) was added to a suspension of compound **10c** (8 g, 23.6 mmols) in acetic acid (240 ml)/water (50 ml) at room temperature. The reaction mixture was heated at 90-100° with stirring for 1 hour to precipitate yellow needles of compound **11c**, which were collected by filtration after cooling to room temperature and washed with ethanol/water (1:1) to give an analytically pure sample (7.84 g, 94%), mp 173-174°; ir: ν cm^{-1} 3270, 1760; ms: m/z 354 (M^+), 356 ($M^+ + 2$), 281 [M^+ - 73 (COOEt)], 283 [($M^+ + 2$) - 73 (COOEt)]; ^1H nmr (deuteriodimethyl sulfoxide): δ 8.06 (d, J = 2.5 Hz, 1H, C_6-H), 7.93 (d, J = 9.0 Hz, 1H, C_9-H), 7.83 (dd, J = 9.0, 2.5 Hz, 1H, C_8-H), 7.70 (s, 1H, OH), 4.24 (dq , J = 10.5, 7.0 Hz, 1H, methylene CH), 4.16 (dq, J = 10.5, 7.0 Hz, 1H, methylene CH), 3.71 (s, 3H, NCH_3), 1.09 (dd, J = 7.0, 7.0 Hz, 3H, CH_3).

Anal. Calcd. for $C_{14}H_{12}Cl_2N_4O_3$: C, 47.34; H, 3.41; N, 15.77. Found: C, 47.44; H, 3.45; N, 15.75.

3,7-Dichloro-1,5-dihydro-1-methylpyridazino[3,4-*b*]quinoxalin-4-ol (**12c**).

A solution of compound **11c** (6 g) and hydrazine hydrate (100% purity, 3 ml) was refluxed for 1 hour to precipitate greenish yellow needles of compound **12c**, which were collected by filtration and washed with ethanol to give an analytically pure sample (4.63 g, 97%), mp 300-302°; ir: ν cm^{-1} 3220, 1640, 1610; ms: m/z 280 [M^+ - 2 (2H)], 282 [($M^+ + 2$) - 2 (2H)]; ^1H nmr (deuteriotrifluoroacetic acid): δ (signals corresponding to 4-hydroxy derivative **12c**) (72%) 6.65 (d, J = 8.0 Hz, aromatic H), 6.53 (s, aromatic H), 6.52 (d, J = 8.0 Hz, aromatic H), 3.67 (s, NCH_3); (signals corresponding to 4-oxo derivative **6c**) (28%) 8.27 (s, C_6-H), 8.19 (d, J = 9.0 Hz, aromatic H), 8.01 (d, J = 9.0 Hz, aromatic H), 3.67 (s, NCH_3).

Anal. Calcd. for $C_{11}H_8Cl_2N_4O$: C, 46.67; H, 2.85; N, 19.79. Found: C, 46.56; H, 2.81; N, 19.85.

3,7-Dichloro-1-methylpyridazino[3,4-*b*]quinoxalin-4(1*H*)-one (**6c**).

A suspension of compound **12c** (2 g, 7.07 mmols) and *N*-chlorosuccinimide (1.42 g, 10.6 mmols) in acetic acid (50 ml)/water (10 ml) was refluxed for 1 hour to give a clear solution. The solution was allowed to stand overnight at room temperature to precipitate yellow needles of compound **6c**, which were collected by filtration and then washed with ethanol/water (3:1) to provide an analytically pure sample (1.53 g, 77%). Evaporation of the filtrate *in vacuo* gave yellow crystals of compound **6c**, which were collected by filtration (0.21 g). Total yield: 1.74 g (87%). Compound **6c** had mp 260-261°; ir: ν cm^{-1} 1655, 1605; ms: m/z 280 (M^+), 282 ($M^+ + 2$); ^1H nmr (deuteriotrifluoroacetic acid): δ 8.13 (d, J = 2.0 Hz, 1H, C_6-H), 8.07 (d, J = 9.0 Hz, 1H, C_9-H), 7.88 (dd, J = 9.0, 2.0 Hz, 1H, C_8-H), 4.32 (s, 3H, NCH_3); ^1H nmr (deuteriodimethyl sulfoxide): δ 8.44 (d, J = 2.5 Hz, 1H, C_6-H), 8.18 (d, J = 9.0 Hz, 1H, C_9-H), 8.09 (dd, J = 9.0, 2.5 Hz, 1H, C_8-H), 4.14 (s, 3H, NCH_3).

Anal. Calcd. for $C_{11}H_6Cl_2N_4O$: C, 46.85; H, 2.23; N, 19.99. Found: C, 47.00; H, 2.15; N, 19.93.

Diethyl 3-Chloro-1,4-dihydro-1-methylpyridazino[3,4-*b*]quinoxaline-4,4-dicarboxylate (**9d**).

A solution of compound **7b** (10 g, 52.6 mmols) and diethyl ethoxymethylenemalonate (17.04 g, 78.9 mmols) in acetic acid (200 ml) was refluxed for 3 hours. Evaporation of the solvent *in vacuo* gave an oily residue, which was washed with hot *n*-hexane three times (*n*-hexane layer is not necessary). Further evaporation of the oily substance afforded yellow crystals of compound **8b** [ms: m/z 304 (M^+)]. Subsequent reflux of compound **8b** with *N*-chlorosuccinimide (10.53 g, 78.9 mmols) in acetic acid (200 ml) for 1 hour and then evaporation of the solvent *in vacuo* provided an oily substance, which was crystallized from ethanol/water to give yellow needles of compound **9d** (5.70 g, 29%); mp 115 - 116°; ir: ν cm^{-1} 2980, 1750, 1735; ms: m/z 376 (M^+), 378 ($M^+ + 2$); ^1H nmr (deuteriodimethyl sulfoxide): δ 8.00 (ddd, J = 8.0, 1.5, 0.5 Hz, 1H, aromatic H), 7.93 (ddd, J = 8.0, 1.5, 0.5 Hz, 1H, aromatic H), 7.83 (ddd, J = 8.0, 8.0, 1.5 Hz, 1H, aromatic H), 7.63 (ddd, J = 8.0, 8.0, 1.5 Hz, 1H, aromatic H), 4.26 (q, J = 7.0 Hz, 4H, $2CH_2$), 3.63 (s, 3H, NCH_3), 1.17 (t, J = 7.0 Hz, 6H, $2CH_3$).

Anal. Calcd. for $C_{17}H_{17}ClN_4O$: C, 54.19, H, 4.55; N, 14.87. Found: C, 54.33; H, 4.61; N, 14.95.

Ethyl 3-Chloro-1,5-dihydro-1-methylpyridazino[3,4-*b*]quinoxaline-4-carboxylate (**10d**).

A solution of compound **9d** (5 g) and hydrazine hydrate (100% purity, 2.5 ml) in ethanol (100 ml) was refluxed for 1 hour. The solution was allowed to stand at room temperature to precipitate orange needles of compound **10d**, which were collected by filtration and then washed with ethanol to give an analytically pure sample (3.18 g, 79 %); mp 165-166°; ir: ν cm^{-1} 1638; ms: m/z 304 (M^+), 306 ($M^+ + 2$); ^1H nmr (deuteriotrifluoroacetic acid): δ 6.89 (ddd, J = 7.5, 7.5, 2.0 Hz, 1H, aromatic H), 6.84 (ddd, J = 7.5, 7.5, 2.0 Hz, 1H, aromatic H), 6.78 (dd, J = 7.5, 2.0 Hz, 1H, aromatic H), 6.76 (dd, J = 7.5, 2.0 Hz, 1H, aromatic H), 4.22 (q, J = 7.0 Hz, 2H, CH_2), 3.53 (s, 3H, NCH_3), 1.20 (t, J = 7.0 Hz, 3H, CH_3).

Anal. Calcd. for $C_{14}H_{13}ClN_4O_2$: C, 55.18, H, 4.30; N, 18.39. Found: C, 54.89; H, 4.23; N, 18.22.

Ethyl 3-Chloro-1,4-dihydro-4-hydroxy-1-methylpyridazino-[3,4-*b*]quinoxaline-4-carboxylate (**11d**).

A solution of sodium nitrite (1.70 g, 24.6 mmols) in water (20 ml) was added to a suspension of compound **10d** (5 g, 16.4 mmols) in acetic acid (150 ml)/water (30 ml), and this mixture was heated at 90-100° for 1 hour to give a clear solution. The solution was allowed to stand overnight at room temperature to precipitate yellow needles of compound **11d**, which were collected by filtration and then washed with ethanol/water (1:1) to afford an analytically pure sample (2.82 g). Evaporation of the filtrate *in vacuo* provided yellow crystals of compound **11d**, which were triturated with ethanol/water (1:1) and then collected by filtration (2.0 g). Total yield: 4.82 g (92 %). Compound **11d** had mp 155-156°; ir: ν cm^{-1} 3260, 1762; ms: m/z 320 (M^+), 322

($M^+ + 2$); ^1H nmr (deuteriodimethyl sulfoxide): δ 7.98 (dd, J = 8.0, 1.5 Hz, 1H, aromatic H), 7.92 (dd, J = 8.0, 1.5 Hz, 1H, aromatic H), 7.82 (ddd, J = 8.0, 8.0, 1.5 Hz, 1H, aromatic H), 7.66 (ddd, J = 8.0, 8.0, 1.5 Hz, 1H, aromatic H), 7.64 (s, 1H, OH), 4.24 (dq, J = 10.0, 7.0 Hz, 1H, methylene CH), 4.16 (dq, J = 10.0, 7.0 Hz, 1H, methylene CH), 3.72 (s, 3H, NCH_3), 1.09 (dd, J = 7.0, 7.0 Hz, 3H, CH_3).

Anal. Calcd. for $C_{14}H_{13}ClN_4O_3$: C, 52.43, H, 4.09; N, 17.47. Found: C, 52.23; H, 4.12, N, 17.32.

3-Chloro-1,5-dihydro-1-methylpyridazino[3,4-*b*]quinoxalin-4-ol (**12d**).

A solution of compound **11d** (4 g) and hydrazine hydrate (100% purity, 2 ml) in ethanol (100 ml) was refluxed for 1 hour to precipitate yellowish green crystals of compound **12d**, which were collected by filtration and then washed with ethanol to give an analytically pure sample (2.41 g). Evaporation of the filtrate afforded yellowish green crystals (0.50 g). Total yield: 2.91 g (94 %). Compound **12d** had mp 278-279°; ir: ν cm^{-1} 3200, 1620; ms: m/z 248 (M^+), 250 ($M^+ + 2$); 246 [$M^+ - 2$ (2H)], 248 [($M^+ + 2$) - 2 (2H)]; ^1H nmr (deuteriotrifluoroacetic acid): δ (signals corresponding to 4-hydroxy derivative **12d**) (96 %) 6.57 (d, J = 3.5 Hz, aromatic H), 6.39 (d, J = 3.5 Hz, aromatic H), 3.51 (s, NCH_3); (signals corresponding to 4-oxo derivative **6d**) (4 %) 8.21 (s, aromatic H), 8.04 (s, aromatic H), 4.33 (s, NCH_3).

Anal. Calcd. for $C_{11}H_9ClN_4O$: C, 53.13; H, 3.65; N, 22.53. Found: C, 52.87, H, 3.62, N, 22.43.

3-Chloro-1-methylpyridazino[3,4-*b*]quinoxalin-4(1*H*)-one (**6d**).

A suspension of compound **12d** (2 g, 8.05 mmols) and *N*-chlorosuccinimide (1.62 g, 12.1 mmols) in acetic acid (40 ml)/water (10 ml) was refluxed for 1 hour to precipitate yellow needles of compound **6d**, which were collected by filtration (1.73 g). Evaporation of the filtrate afforded yellow crystals of compound **6d** (0.19 g). Total yield: 1.92 g (97 %). Recrystallization from acetic acid provided yellow needles, mp 282-283°; ir: ν cm^{-1} 1665, 1662 (branching); ms: m/z 280 (M^+), 282 ($M^+ + 2$); ^1H nmr (deuteriotrifluoroacetic acid): δ 8.18 (ddd, J = 8.0, 1.5, 1.0 Hz, 1H, aromatic H), 8.16 (ddd, J = 8.0, 1.5, 1.0 Hz, 1H, aromatic H), 8.02 (ddd, J = 8.0, 8.0, 1.5 Hz, 1H, aromatic H), 7.98 (ddd, J = 8.0, 8.0, 1.5 Hz, 1H, aromatic H), 4.29 (s, 3H, NCH_3); ^1H nmr (deuteriodimethyl sulfoxide): δ 8.31 (ddd, J = 8.0, 1.5, 0.5 Hz, 1H, aromatic H), 8.16 (ddd, J = 8.0, 1.5, 0.5 Hz, 1H, aromatic H), 8.09 (ddd, J = 8.0, 8.0, 1.5 Hz, 1H, aromatic H), 7.96 (ddd, J = 8.0, 8.0, 1.5 Hz, 1H, aromatic H), 4.16 (s, 3H, NCH_3).

Anal. Calcd. for $C_{11}H_7ClN_4O$: C, 53.56, H, 2.86; N, 22.71. Found: C, 53.75; H, 2.96; N, 22.43.

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[6] The *in vitro* minimum inhibitory concentrations of compounds **3** were between 1.0 and 2.0 ppm against *Bacillus subtilis* (bacteria), and those of compounds **3** and **5** were between 0.5 and 2.0 ppm against *Trichophyton mentagrophytes* (fungi).

[7] In compounds **3**, the antimicrobial activities of the 1-methyl derivatives were similar to those of the 1- ethyl derivatives. Consequently, the 1-methyl derivatives were synthesized for compounds **4** - **6**.

[8] The detailed screening data will be reported elsewhere.

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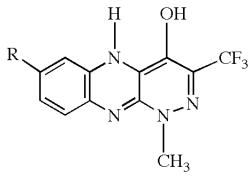
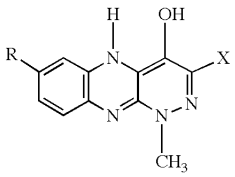
[15] This type of compounds were clarified by us to exist as the 1,5-dihydropyridazino[3,4-*b*]quinoxaline form, but not the 1,4-dihydropyridazino[3,4-*b*]quinoxaline form, in solution and solid state [12], while dihydropyridazine [13] and dihydrocinnolines [14] were reported to predominate as the 1,4-dihydro form.





[16] The 3-trifluoromethyl derivatives [4] also showed susceptibility to oxidation and similar spectral data to those of compounds **12a-d**.

[17] The aromatic proton signals of the 1,5-dihydropyridazino[3,4-*b*]quinoxalines (4-hydroxy derivatives **12a-d** and 4-carboxylates **10a-d**) are observed in higher magnetic field than those of the 1,4-dihydropyridazino[3,4-*b*]quinoxalines (4-oxo derivatives **6a-d**). These results have already been confirmed for the 3-trifluoromethyl derivatives [4].