DOI 10.1007/s10600-019-02728-1
Chemistry of Natural Compounds, Vol. 55, No. 3, May, 2019

SYNTHESIS OF NATURAL AND BIOLOGICALLY ACTIVE QUINOXALINE ANALOGS

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Reactions of quinoxalines and quinoxalin-2-ones with C*-nucleophiles under acid-catalysis conditions gave products from nucleophilic substitution of hydrogen. Substitution of F atoms in the aromatic core of quinoxalines was studied. Antibacterial and fungistatic activity of the synthesized compounds was studied.*

Keywords: quinoxalines, 6,7-difluoroquinoxalines, *C*-, *N*-, *O*-nucleophiles, antibacterial and fungistatic activity.

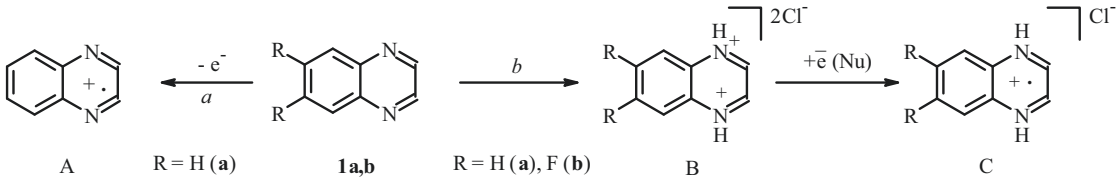
The pyrazine heterocycle appears in various biologically active and natural compounds. Benzoannelated pyrazine (quinoxaline) occurs in vitamin B_2 and the antibiotics echinomycin (natural peptide antibiotic) and actinoleutin, which inhibit growth of Gram-positive bacteria and are active against various tumors [1, 2]. Currently, preparations of quinoxidine and dioxidine, which incorporate quinoxaline into their structures [3], are used in medical practice for serious microbial infections.

In the present work, quinoxalines were functionalized on the heterocyclic core using environmentally and chemically benign nucleophilic substitution of hydrogen [4].

Electron paramagnetic resonance (EPR) studies of the reactivity of quinoxaline in the presence of electron donors or acceptors found two different types of radicals. Previously, quinoxalines were reported to form cation-radicals in a couple of instances [5, 6].

Quinoxaline cation-radical A was detected in an MeCN (or C_6H_6) solution of **1a** (Scheme 1, Fig. 1*a*). The triplet EPR spectrum with intensity distribution 1:1:1 (Fig. 1*a*) was characteristic of splitting by one ^{14}N nucleus, which confirmed the structure was quinoxaline cation-radical A. The coupling constant of the unpaired electron with the ^{14}N nucleus was $\alpha_N = 1.58$ mT in the hyperfine structure of the spectrum. An EPR study of the reaction mixture from **1a** or **1b** and a nucleophile (dimedone) in DMSO in the presence of acid detected stable cation-radical C of a diprotic quinoxalinium salt (Fig. 1*b* and 1*c*) [7].

The coupling constants of the unpaired electron with the two equivalent N nuclei ($\alpha_N^{NH} = 0.67$ mT) and the protons bonded to them ($\alpha_H^{NH} = 0.37$ mT) were similar for a solution of 6,7-difluoroquinoxaline **1b** and dimedone in the presence of HCl (Fig. 1*c*).



a. CH_3CN/C_6H_6 (+TBAF); *b*. HCl, DMSO

Scheme 1

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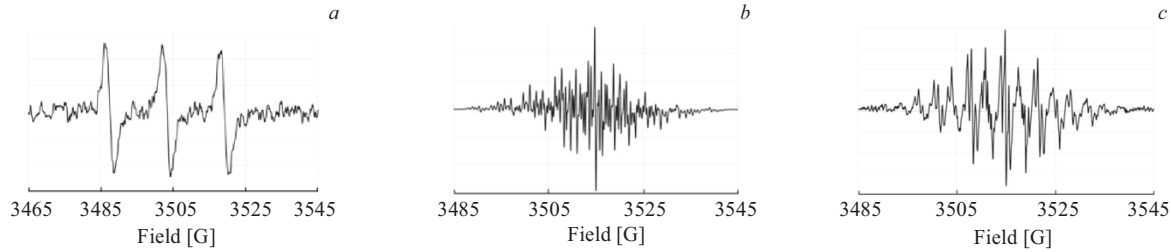
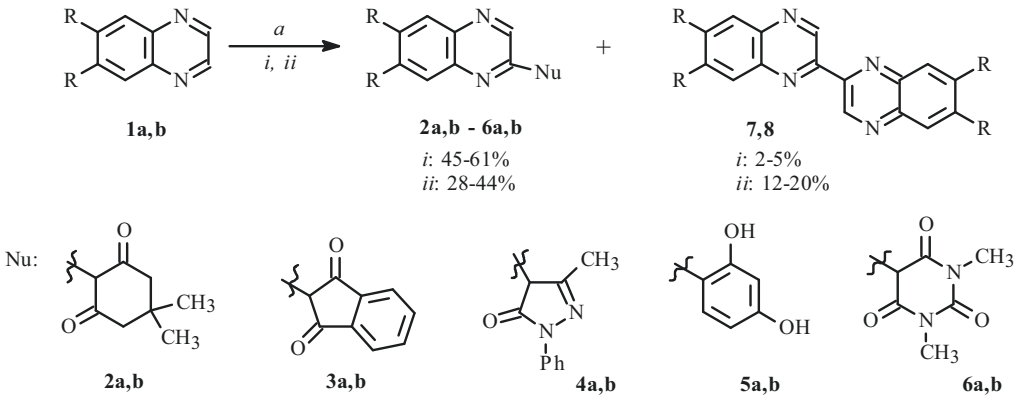


Fig. 1. EPR spectra of quinoxaline radicals: cation-radical **1a** in MeCN or C_6H_6 (*a*); quinoxaline **1a** in DMSO with added HCl (*b*); quinoxaline **1b** in DMSO with added HCl (*c*).

Obviously, the tendency of quinoxalines **1a**,**b** to form radicals in the presence of weak electron acceptors (C_6H_6, MeCN) indicated that the compounds had pronounced electron-donating properties (reactions with electrophiles, e.g., acids). However, the formation of protonated quinoxaline salt radicals under acid catalysis conditions was indicative of pronounced electron-accepting properties (reactions with nucleophiles).

Quinoxaline reacted smoothly under mild conditions with *C*-nucleophiles in our previous work [8, 9]. A more detailed study of these reactions detected quinoxaline dimers **7** and **8** in 3–5% yields. The present work showed that purging air through the mixture of quinoxaline (**1a** or **1b**) and nucleophile (Scheme 2) increased significantly (by 10–15%) the yield of target product whereas the yields of dimers **7** and **8** were 2–5%. The yields of dimers **7** and **8** increased to 12–20% and the yields of target compounds **2a**,**b**–**6a**,**b** decreased by 7–10% if the reaction was performed under N_2.

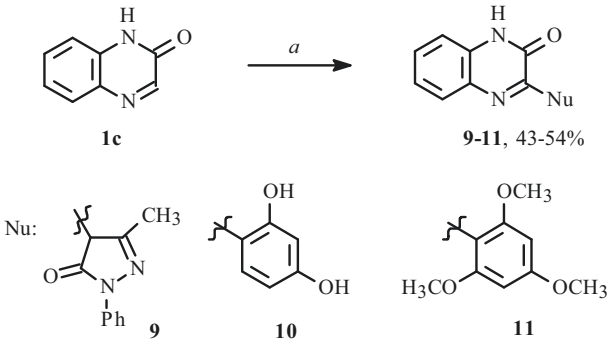


a. HNu, HCl, DMSO, 20°C, 2 h, (*i*) air; (*ii*) N_2

Scheme 2

Obviously, products from monosubstitution of hydrogen during the reaction under N_2 (or with an O_2 deficiency) were formed by addition of nucleophiles to quinoxaline C^2 and oxidation of the resulting σ-adducts by starting quinoxalines **1a**,**b** or their protonated salts. In turn, the intermediates were oxidized and reduced the starting protonated quinoxaline salts to cation-radicals. The reaction of cation-radicals with the starting quinoxalines was a typical hetarylation reaction that produced dimers **7** and **8**.

Thus, the dimerization products confirmed that radicals formed in the reaction mixture and underwent nucleophilic substitution of hydrogen.



a. HNu, AcOH, Δ, 40 h

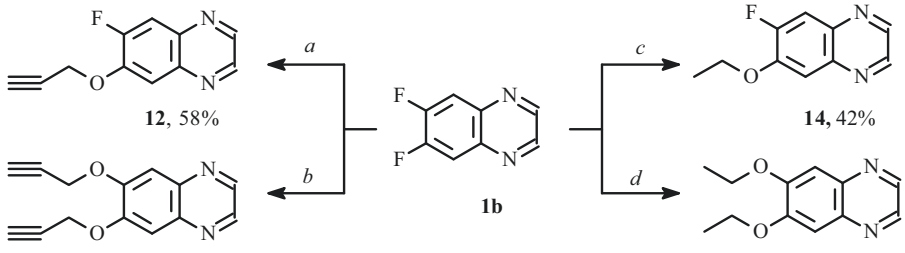
Scheme 3

Quinoxalones, i.e., quinoxaline derivatives with a 2-oxo group, are more stable in nucleophilic substitution of hydrogen and do not form radical species. So, the yields of target compounds in reactions of quinoxalones are greater than those of quinoxalines. The products from reactions of quinoxalones with several nucleophiles were previously reported [10, 11].

New examples of reactions of quinoxalones with *C*-nucleophiles were obtained by us. The reactions were carried out with heating in AcOH and formed hydrogen substitution products **9**–**11** in good yields (Scheme 3).

Additional structural modifications were made possible by introducing F atoms into the quinoxaline aromatic fragment. The conditions for monosubstitution of F atoms were especially interesting for 6,7-difluorinated quinoxalines. It was found earlier that substitution of F atoms in polyfluorinated quinoxaline derivatives can occur stepwise in reactions with sodium methoxide and dimethylamine [12, 13].

Our results for *F*-substitution of 6,7-difluoroquinoxaline (**1b**) by propargyl alcohol indicated that the reaction occurred stepwise at 40–50°C with substitution of one F atom to give **12**. Both F atoms were replaced at 90–100°C to produce disubstituted derivative **13** (Scheme 4).



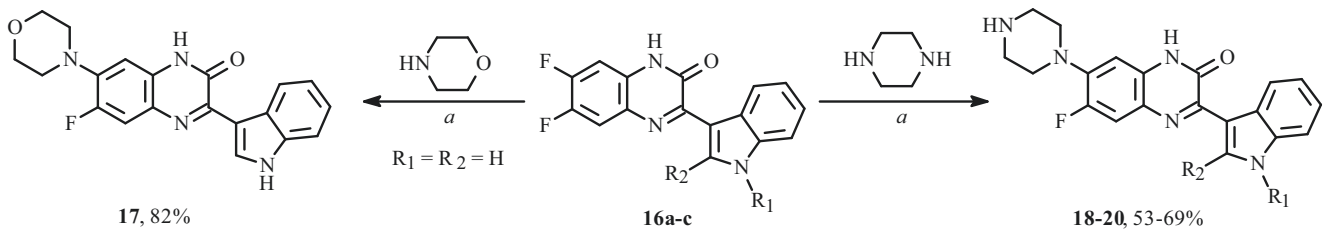
a. CH≡C-CH_2OH, 40–50°C, 1 h; *b*. CH≡C-CH_2OH, 90–100°C, 1 h; *c*. EtOH, NaOH, 40–50°C, 1h; *d*. EtONa, Δ, 1 h

Scheme 4

Heating **1b** in EtOH at 40–50°C in the presence of base gave the product from monosubstitution of F (**14**). Quinoxaline **1b** reacted with NaOEt in refluxing EtOH to form the product from disubstitution of F (**15**).

Selective monosubstitution of F in 6,7-difluoroquinoxalines by amines was especially significant because it allowed pharmacophores analogous to those in fluoroquinolone antibiotics to be incorporated into the molecular structure [14].

Previously, reactions of 6,7-difluoroquinoxalines with *N*-methylpiperazine were shown to form products corresponding monosubstitution of the 7-F [9, 15]. We expanded the series of used nucleophiles and produced previously unknown quinoxalones **17**–**20** (Scheme 5). The reactions of the quinoxalones with amines occurred smoothly in good yields upon heating in DMSO.



16a,18: $R_1 = R_2 = H$; **16b,19:** $R_1 = CH_3$, $R_2 = H$; **16c,20:** $R_1 = H$, $R_2 = CH_3$
a. DMSO, 135°C, 2 h

Scheme 5

The obtained quinoxalines were screened for antimicrobial and fungistatic activity. The biological activity tests also included quinoxalines **21**–**25** that were reported by us earlier [7, 15] (Table 1). The tested compounds were screened preliminarily (*in vitro*) for microbial activity against bacteria strains of *Escherichia coli*, *Klebsiella pneumoniae*, *Citrobacter braakii*, *Proteus vulgaris*, *Serratia marcescens*, *Shigella flexneri*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Streptococcus pyogenes*. Spectinomycin was used as a control. Table 1 presents the compounds with the highest activity parameters.

The development of new antimicrobial agents for treating diseases caused by *Neisseria gonorrhoeae* is critical because the resistance of this strain to cephalosporins, the latest line of antibiotics [16], is increasing globally. Studied quinoxalines that were active against *N. gonorrhoeae* included **4a**, **18**, **19**, **21**, and **25**, which were comparable to spectinomycin. Compounds **18**, **19**, and **23** were highly active against an antibiotic-resistant strain of MRSA (*S. aureus*).

TABLE 1. Antimicrobial Activity of Synthesized Quinoxalines (MIC,* μg/mL)

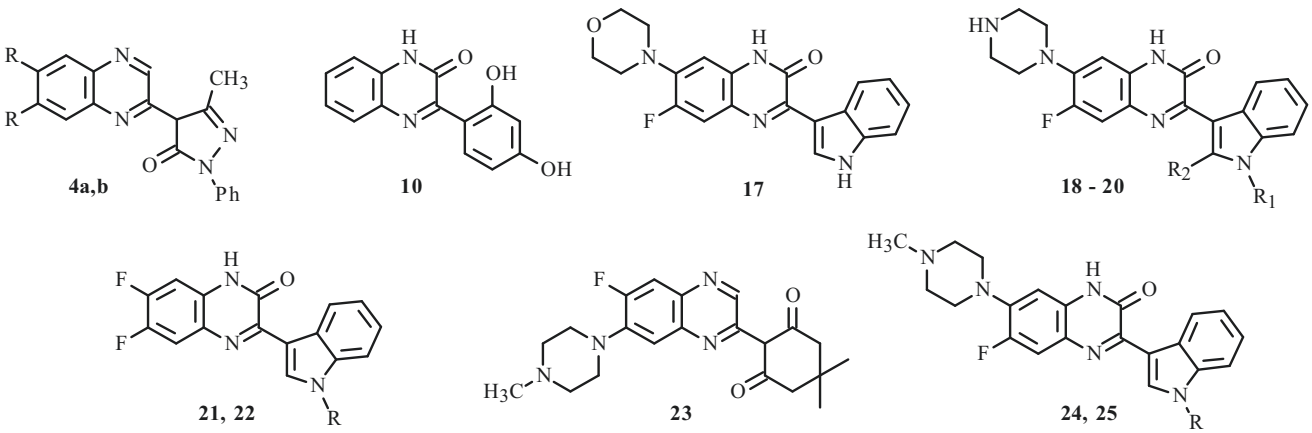
Compound	*Escherichia coli*	*Shigella flexneri*	*Staphylococcus aureus*	*Streptococcus piogenes*	*Staphylococcus aureus* (MRSA)	*Neisseria gonorrhoeae*
4a	> 250	> 250	**31.2**	> 250	**15.6**	31.2
4b	> 250	> 250	62.5	> 250	**31.2**	125
10	> 250	–	**31.2**	–	> 250	> 250
17	> 250	–	125	–	**62.5**	250
18	**15.6**	**15.6**	**7.8**	**3.9**	**7.8**	**31.2**
19	31.2	> 250	**7.8**	**3.9**	**7.8**	**15.6**
20	> 250	> 250	> 250	> 250	**31.2**	**62.5**
21	> 250	> 250	> 250	> 250	> 250	**31.2**
22	> 250	–	31.2	–	> 250	> 250
23	> 250	–	62.5	–	**15.6**	> 250
24	> 250	> 250	**31.2**	**15.6**	**31.2**	62.5
25	125	> 250	**31.2**	125	**31.2**	**15.6**
Spectinomycin	15.6–31.2	15.6	31.2–62.5	15.6	> 250	16–32

*MIC = minimum inhibitory concentration.

TABLE 2. Antifungal Activity of Synthesized Quinoxalines (MIC, μg/mL)

Compound	*Trichophyton rubrum*	*Trichophyton violaceum*	*Trichophyton mentagrophytes* var. *interdigitale*	*Epidermophyton floccosum*	*Microsporum canis*
17	50	> 100	> 100	> 100	> 100
18	**25**	**25**	50	100	100
19	100	50	100	> 100	100
20	> 100	50	> 100	> 100	100
25	**25**	**25**	50	50	50
Fluconazole	6.25	100	> 100	3.12	100

*MIC = minimum inhibitory concentration.



4a: R = H; **4b:** R = F; **18:** $R_1 = R_2 = H$; **19:** $R_1 = CH_3$, $R_2 = H$; **20:** $R_1 = H$, $R_2 = CH_3$; **21:** R = H; **22:** $R_2 = CH_3$; **24:** R = CH_3; **25:** R = H

The synthesized compounds were tested for antifungal activity against strains of *Trichophyton rubrum*, *T. mentagrophytes* var. *gypseum*, *T. tonsurans*, *T. violaceum*, *T. mentagrophytes* var. *interdigitale*, *Epidermophyton floccosum*, *Microsporum canis*, and *Candida albicans*. The antifungal drug fluconazole was used as a positive control. The studied quinoxalines included **18** and **25**, which possessed moderate fungicidal activity against two of the six studied strains (Table 2).

EXPERIMENTAL

All reagents were commercially available and were used without further purification (Sigma Aldrich, Merck). The course of reactions was monitored by TLC on silica gel plates (Merck). PMR and ^{13}C NMR spectra were recorded in DMSO-d_6 with TMS internal standard on an Avance-400 instrument. Electron-impact (70 eV) mass spectra were obtained on a MicrOTOF-Q instrument (Bruker Daltonics) at average ionizing potential 70 eV and heating to 250°C. EPR spectra were recorded on a Bruker Elexsys E 500 EPR spectrometer.

The synthetic methods for **2a**,**b**–**6a**,**b** were improved as compared to the previously reported ones [4]. Thus, the yields of target products **2a**,**b**–**6a**,**b** increased by 10–15% if the reaction mixture was purged with air. The reactions of **1a**,**b** with the used nucleophiles under N_2 gave 12–20% yields of dimers **7** and **8**.

General Method for the Reactions of Quinoxalin-2-one with Nucleophiles. A mixture of **1c** (0.3 mmol) and the appropriate nucleophile (0.4 mmol) was heated in AcOH (1.5 mL) for 40 h at 110°C and cooled. When the reaction was finished (TLC), the resulting precipitate was filtered off and rinsed with H_2O.

3-(3-Methyl-5-oxo-1-phenyl-4,5-dihydro-1*H*-pyrazol-4-yl)quinoxalin-2(1*H*)-one (9). Yield 54%, mp > 300°C. ^1H NMR spectrum (400 MHz, DMSO-d_6, δ, ppm, J/Hz): 2.60 (3H, s, CH_3), 7.22 (1H, t, J = 7.6, CH), 7.32–7.34 (1H, m, CH), 7.38–7.45 (4H, m, CH), 7.67 (1H, d, J = 7.6, CH), 7.91 (2H, d, J = 8, CH), 13.08 (1H, s, NH), 15.44 (1H, br.s, NH). ^{13}C NMR spectrum (100 MHz, DMSO-d_6, δ, ppm): 18.52, 98.29, 115.66, 119.90, 123.24, 124.56, 125.25, 127.67, 128.25, 128.84, 138.26, 147.69, 148.66, 155.80, 159.60. Mass spectrum (EI, 70 eV), *m/z* (I_{rel}, %): 318 (M^+, 100), 226 (45), 157 (29), 77 (28).

3-(2,4-Dihydroxyphenyl)quinoxalin-2(1*H*)-one (10). Yield 51%, mp > 300°C. ^1H NMR spectrum (400 MHz, DMSO-d_6, δ, ppm, J/Hz): 6.33 (1H, s, CH), 6.38 (1H, d, J = 10.0, CH), 7.27–7.34 (2H, m, CH), 7.50 (1H, t, J = 7.6, CH), 7.74 (1H, d, J = 7.6, CH), 9.01 (1H, d, J = 10.0, CH), 10.16 (1H, s, OH), 12.65 (1H, br.s, OH), 14.16 (1H, s, NH). ^{13}C NMR spectrum (100 MHz, DMSO-d_6, δ, ppm): 102.98, 106.16, 107.17, 110.53, 115.01, 123.67, 126.25, 129.23, 129.33, 131.08, 132.97, 153.53, 154.83, 161.89, 163.10. Mass spectrum (EI, 70 eV), *m/z* (I_{rel}, %): 254 (M^+, 100), 226 (57), 169 (39).

3-(2,4,6-Trimethoxyphenyl)quinoxalin-2(1*H*)-one (11). Yield 43%, mp > 300°C. ^1H NMR spectrum (400 MHz, DMSO-d_6, δ, ppm, J/Hz): 3.71 (6H, s, $2CH_3$), 3.86 (3H, s, CH_3), 6.24 (2H, s, 2CH), 7.22 (1H, t, J = 8.0, CH), 7.31 (1H, d, J = 8.0, CH), 7.43 (1H, t, J = 8.0, CH), 7.69 (1H, d, J = 8.0, CH), 12.17 (1H, s, NH). ^{13}C NMR spectrum (100 MHz, DMSO-d_6, δ, ppm): 55.40, 55.69, 90.86, 107.10, 115.16, 123.01, 128.54, 130.09, 131.89, 132.01, 154.39, 156.11, 158.76, 161.57. Mass spectrum (EI, 70 eV), *m/z* (I_{rel}, %): 312 (M^+, 100), 283 (73), 269 (50).

6-Fluoro-7-(prop-2-yn-1-yloxy)quinoxaline (12). 6,7-Difluoroquinoxaline (**1b**, 0.166 g, 1.0 mmol) in propargyl alcohol (3.0 mL) in the presence of NaOH (0.128 g, 3.2 mmol) was held at 60–70°C for 1 h. When the reaction was finished (TLC), the mixture was cooled and diluted with H_2O (2.0 mL). The precipitate of **12** was filtered off and rinsed with H_2O. Yield 0.090 g (58%), mp 128–130°C. ^1H NMR spectrum (400 MHz, DMSO-d_6, δ, ppm, J/Hz): 3.43 (1H, t, J = 2.4, CH), 5.06 (2H, d, J = 2.4, CH_2), 7.70 (1H, d, J = 8.8, CH), 7.76 (1H, d, J = 11.2, CH), 8.76 (1H, d, J = 2.0, CH), 8.81 (1H, d, J = 2.0, CH). ^{13}C NMR spectrum (100 MHz, DMSO-d_6, δ, ppm): 56.95, 79.54, 110.88, 113.22, 138.19, 140.52, 144.15, 145.12, 148.00, 152.42, 154.94. Mass spectrum (EI, 70 eV), *m/z* (I_{rel}, %): 201 (M^+, 50), 173 (88), 135 (69), 38 (100).

6,7-*bis*(Prop-2-yn-1-yloxy)quinoxaline (13). 6,7-Difluoroquinoxaline (**1b**, 0.166 g, 1.0 mmol) in propargyl alcohol (3.0 mL) in the presence of NaOH (0.128 g, 3.2 mmol) was held at 100–110°C for 1 h. When the reaction was finished (TLC), the mixture was cooled and diluted with H_2O (10.0 mL). The precipitate of **13** was filtered off, rinsed with H_2O, and recrystallized from aqueous EtOH. Yield 0.070 r (55%), mp 144–145°C. ^1H NMR spectrum (400 MHz, DMSO-d_6, δ, ppm, J/Hz): 3.41 (2H, t, J = 2.4, CH), 5.06 (4H, d, J = 2.4, CH_2), 7.51 (2H, s, CH), 8.68 (2H, s, CH). ^{13}C NMR spectrum (100 MHz, DMSO-d_6, δ, ppm): 56.37, 79.12, 108.97, 139.25, 143.24, 150.02. Mass spectrum (EI, 70 eV), *m/z* (I_{rel}, %): 238 (M^+, 15), 199 (29), 171 (70), 143 (27), 38 (100).

6-Ethoxy-7-fluoroquinoxaline (14). 6,7-Difluoroquinoxaline (**1b**, 0.160 g, 0.96 mmol) in anhydrous EtOH (1.0 mL) in the presence of NaOH (0.1 g, 2.5 mmol) was held at 45–50°C for 1.0–1.5 h. When the reaction was finished (TLC), the solvent was evaporated. The solid was worked up with H_2O (8.0 mL). The precipitate of **14** was filtered off, rinsed with H_2O, and recrystallized from aqueous EtOH. Yield 42%, mp 108–110°C. The spectral characteristics were analogous to those published earlier [17].

6,7-Diethoxyquinoxaline (15). 6,7-Difluoroquinoxaline (**1b**, 0.083 g, 0.50 mmol) in NaOEt solution (0.05 g, 2.2 mmol) in anhydrous EtOH (5.0 mL) was refluxed for 2.0 h and cooled. The precipitate of **15** was filtered off and rinsed with H_2O. Yield 0.045 g (54%), mp 130–131°C. The spectral characteristics were analogous to those published earlier [14].

Reaction of 6,7-Difluoroquinoxalin-2-ones 16a-c with Piperazine and Morpholine (general method). A mixture of **16a–c** (0.2 mmol) and piperazine or morpholine (1.5 mmol) in DMSO (1.0 mL) was heated at 130–135°C for 24 h.

When the reaction was finished (TLC), the mixture was cooled and diluted (3×) with H_2O. The precipitate was filtered off and rinsed with H_2O.

6-Fluoro-3-(1*H*-indol-3-yl)-7-morpholinoquinoxalin-2(1*H*)-one (17). Yield 82%, mp 287–288°C. ^{1}H NMR spectrum (400 MHz, DMSO-d_6, δ, ppm, J/Hz): 3.11–3.14 (4H, m, $2CH_2$), 3.81–3.85 (4H, m, $2CH_2$), 6.82 (1H, d, J = 8.4, CH), 7.14–7.20 (2H, m, CH), 7.45–7.47 (1H, m, CH), 7.53 (1H, d, J = 13.2, CH), 8.78–8.80 (1H, m, CH), 8.85 (1H, d, J = 4.0, CH), 11.57 (1H, s, NH), 12.22 (1H, s, NH). ^{13}C NMR spectrum (100 MHz, DMSO-d_6, δ, ppm): 50.33 (2C), 66.01 (2C), 103.22, 111.32, 111.74, 113.37, 120.79, 122.41, 122.98, 126.05, 132.43, 136.20, 140.34, 150.26, 154.32. Mass spectrum (EI, 70 eV), *m/z* (I_{rel}, %): 364 (M^+, 100), 306 (37), 278 (40).

6-Fluoro-3-(1*H*-indol-3-yl)-7-(piperazin-1-yl)quinoxalin-2(1*H*)-one (18). Yield 68%, mp 258–259°C. ^{1}H NMR spectrum (400 MHz, DMSO-d_6, δ, ppm, J/Hz): 2.95–3.17 (8H, m, $4CH_2$), 6.79 (1H, d, J = 8.4, CH), 7.14–7.19 (4H, m, CH), 7.43–7.50 (2H, m, CH), 8.77–8.97 (1H, m, CH), 8.85 (1H, d, J = 2.8, CH), 11.49 (1H, s, NH). ^{13}C NMR spectrum (100 MHz, DMSO-d_6, δ, ppm): 45.32 (2C), 51.03 (2C), 103.33, 111.34, 111.74, 113.27, 113.40, 120.77, 122.40, 122.98, 126.06, 127.27, 127.57, 132.35, 136.20, 140.58, 150.13, 152.56, 154.34. Mass spectrum (EI, 70 eV), *m/z* (I_{rel}, %): 363 (M^+, 83), 321 (100), 306 (21).

6-Fluoro-3-(1-methyl-1*H*-indol-3-yl)-7-(piperazin-1-yl)quinoxalin-2(1*H*)-one (19). Yield 53%, mp 289–291°C. ^{1}H NMR spectrum (400 MHz, DMSO-d_6, δ, ppm, J/Hz): 2.94–2.98 (4H, m, $2CH_2$), 3.00–3.10 (4H, m, $2CH_2$), 3.93 (3H, s, CH_3), 6.80 (1H, d, J = 8.0, CH), 7.21–7.27 (2H, m, CH), 7.47 (1H, m, CH), 7.50 (1H, d, J = 12.0, CH), 8.81 (1H, m, CH), 8.83 (1H, s, CH). ^{13}C NMR spectrum (100 MHz, DMSO-d_6, δ, ppm): 32.93, 45.48 (2C), 51.32 (2C), 103.24, 110.06, 113.35, 121.07, 122.50, 123.14, 126.54, 127.17, 127.57, 136.10, 136.77, 141.15, 149.67, 150.19, 152.59, 154.28. Mass spectrum (EI, 70 eV), *m/z* (I_{rel}, %): 377 (M^+, 97), 335 (100).

6-Fluoro-3-(2-methyl-1*H*-indol-3-yl)-7-(piperazin-1-yl)quinoxalin-2(1*H*)-one (20). Yield 69%, mp 269–270°C. ^{1}H NMR spectrum (400 MHz, DMSO-d_6, δ, ppm, J/Hz): 2.53 (3H, s, CH_3), 3.27–3.32 (8H, m, $4CH_2$), 6.92 (1H, d, J = 8.0, CH), 7.00 (1H, t, J = 8.0, CH), 7.03 (1H, t, J = 8.0, CH), 7.31 (1H, d, J = 8.0, CH), 7.50 (1H, d, J = 12.0, CH), 7.74 (1H, d, J = 8.0, CH), 9.37 (1H, br.s, NH), 11.44 (1H, s, NH), 12.26 (1H, br.s, NH). ^{13}C NMR spectrum (100 MHz, DMSO-d_6, δ, ppm): 14.23, 42.69 (2C), 46.86 (2C), 103.86, 109.02, 110.53, 113.72, 113.94, 119.42, 120.77, 127.84, 128.71, 135.09, 139.09, 139.80, 149.79, 152.19, 153.16, 154.40. Mass spectrum (EI, 70 eV), *m/z* (I_{rel}, %): 377 (M^+, 87), 335 (100).

Antimicrobial Activity. Antibacterial activity of the compounds was studied using standard bacteria strains from the American Type Culture Collection (ATCC), National Collection of Type Cultures (NCTC, England), and Russian Collection of Pathogenic Microorganisms (RCPM). Strains *N. gonorrhoeae* (ATCC 49226/NCTC 12700), *E. coli* (ATCC 8739), *C. braakii* (ATCC 101/57), *Shigella flexneri* (RCPM 1a8516), *Proteus vulgaris* [RCPM 160125 (222)], *Serratia marcescens* (ATCC 13880), *K. pneumoniae* (ATCC 13883), *P. aeruginosa* (ATCC 9027), *S. pyogenes* (ATCC 19615), *S. aureus* [ATCC 25923/NCTC 12981 (F-49)], and methicillin-resistant *S. aureus* (MRSA) (NCTC 12493) were used. One-day microorganism cultures were identified on a VITEK® MS analyzer (bioMerieux).

Antibacterial activity of the compounds against obligate pathogen *N. gonorrhoeae* was determined using double serial dilutions in agar (gold standard). At least 12 points with dilutions 250–0.06 μg/mL were prepared for each chemical compound. The solvent was DMSO; diluents, distilled sterile H_2O (for injection) and agar-based growth medium. The inoculation dose (final concentration) of the aliquot of one-day *N. gonorrhoeae* culture was 10^5 CFU/mL. Plates were incubated at 37°C. Results were estimated after 18–24 h [18].

Antibacterial activity of the chemical compounds against the other pathogenic microorganisms was tested using sequential microdilutions in Mueller—Hinton broth in sterile 96-well plates [19]. The solvent was DMSO; diluents, distilled sterile H_2O (for injection) and Mueller–Hinton growth broth. Aliquots of control strains were prepared according to a 0.5 MacFarland standard ($1.5 \cdot 10^8$ CFU/mL) and diluted 100× to a concentration of 10^6 CFU/mL. Each well of a horizontal row was charged with an aliquot of the appropriate strain (50.0 μL). Plates were incubated in a thermostat at 37°C for 18–24 h [18]. The control drug was spectinomycin (Sigma-Aldrich, USA).

Antifungal Activity. The fungistatic activity was studied using test cultures of dermatophytes from the Russian Collection of Pathogenic Fungi (RCPF) including *Trichophyton rubrum* (RCPF 1408), *T. mentagrophytes* var. *gypseum* (RCPF 1425), *T. tonsurans* (RCPF 1458), *T. violaceum* (RCPF 1393/658), *T. mentagrophytes* var. *interdigitale* (RCPF 1229), *T. schoenleinii* (RCPF 235/25), *Epidermophyton floccosum* (RCPF 1174), *Microsporum canis* (RCPF 1403), and *C. albicans* (RCPF 401/NCTC-885-653).

Double serial dilutions and Sabouraud broth (Russia) were used [20]. Fungi were cultivated at 27°C. Test compounds and the standard were dissolved in DMSO (1:10) and used at various concentrations (from 250 to 1.9 μg/mL). The diluents were distilled sterile H_2O (for injection) and growth medium. DMSO was used as a negative control; fluconazole antifungal preparation, as a positive control. Samples were incubated at 27°C for 14 d.

ACKNOWLEDGMENT

We thank the RFBR (Grants 18-33-00727 mol_a and 18-03-00715 A) for sponsoring the research.

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