Synthesis and Antimicrobial Activity of Novel Quinoxaline Derivatives

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In this study, certain 3-substituted styrylquinoxalin-2(1H)-ones (**2a-d**) and their 2-chloro (**3a-d**) and 2-piperazinyl derivatives (**4a-g**) were synthesized from 3-methylquinoxalin-2(1H)-one (**1**). In addition, a series of 1-alkyl-3-substituted styrylquinoxalin-2(1H)-ones (**5a-d**) was also prepared. Moreover, 3-(N^2-arylidenehydrazinocarbonyl)quinoxalin-2(1H)-ones (**8a-c**) as well as their cyclized oxadiazolinyl derivatives (**9a-c**) were prepared from 3-hydrazinocarbonylquinoxalin-2(1H)-one (**7**). Furthermore, 3-(5-substituted thio-1,3,4-oxadiazol-2-yl)quinoxalin-2(1H)-ones (**11a-c**) and (**12a-c**) were obtained from the intermediate compound (**10**) - previously obtained via cyclization of (**7**) with CS_2. Likewise, 3-(5-oxo-4,5-dihydro-(1,3,4-oxadiazol-2-yl)quinoxalin-2(1H)-one (**13**), 3-[5-(4-nitrophenyl)-1,3,4-oxadiazol-2-yl]-quinoxalin-2(1H)-one (**14**) and its 2-chloro derivative (**15**) were prepared from 3-hydrazinocarbonyl-quinoxalin-2(1H)-one (**7**). Some of these derivatives were evaluated for antimicrobial activity *in vitro* and some of the tested compounds showed antibacterial or antifungal activity.

Keywords: 2-Piperazinyl-3-styrylquinoxalines; 3-Oxadiazolylquinoxalin-2(1H)-one; Antimicrobial activity.

INTRODUCTION

A wide variety of pharmacological properties has been associated with quinoxaline derivatives. These include antidepressant,[1] anticancer,[2] antidiabetic,[3] antiinflammatory,[4,5] antimicrobial[6,7] and antiviral[8,9] activities. Of particular interest are the styryl,[10] N-alkyl,[10] arylhydrazinocarbonyl,[9-12] oxadiazolyl,[12-15] and thioalkyl[16,17] quinoxalines which are reported to possess antibacterial,[10,13-16] antifungal[11,12,15,17] and antiviral[9] activity. In view of these observations and in continuation of our research in this field,[15,17] it was of interest to synthesize certain new quinoxaline analogues to be evaluated for antimicrobial activity.

RESULTS AND DISCUSSION

The starting material, 3-methylquinoxalin-2(1H)-one (**1**), was prepared via the reaction of 1,2-phenylenediamine and ethyl pyruvate adopting a reported procedure.[18] Reaction of **1** with certain aromatic aldehydes using acetic anhydride as a solvent in the presence of a few drops of piperidine, led to the formation of easily separated and high yield products of 3-substituted styrylquinoxalin-2(1H)-ones (**2a-d**). The ^{1}H-NMR spectra of compounds **2a-d** showed two doublet signals at δ 7.19-7.71 and δ 7.81-8.16 ppm, corresponding to the two styryl protons. Chlorination of compounds **2a-d** with phosphorus oxychloride gave 2-chloro-3-substituted styrylquinoxalines (**3a-d**). The mass spectrum of compound **3c** demonstrated a distinct peak due to the loss of chlorine radical from the molecular ion peak. It is worth mentioning that chlorination prior to styryl formation resulted in very poor yield due to the formation of resinous materials.

Amination of compounds **3a-d** with certain substituted piperazines in n-butanol, gave 2-(4-substituted piperazin-1-yl)-3-substituted styrylquinoxalines (**4a-g**).

It was reported that alkylation of quinoxalin-2-ones resulted in the formation of N-alkylated quinoxalinone derivatives as the sole product.[10,19] Thus the required 1-(alkyl or allyl)-3-substituted styrylquinoxalin-2(1H)-ones (**5a-d**) were prepared by heating a mixture of compounds **2a**, **2c** or **2d**, the halocompound and anhydrous potassium carbonate in dry acetone. The IR spectra of the compounds **5a-d** showed the presence of strong absorption bands at 1660-1640 characteristic for C=O, while they lacked NH absorption. In addition, the ^{1}H-NMR proton signals and the mass

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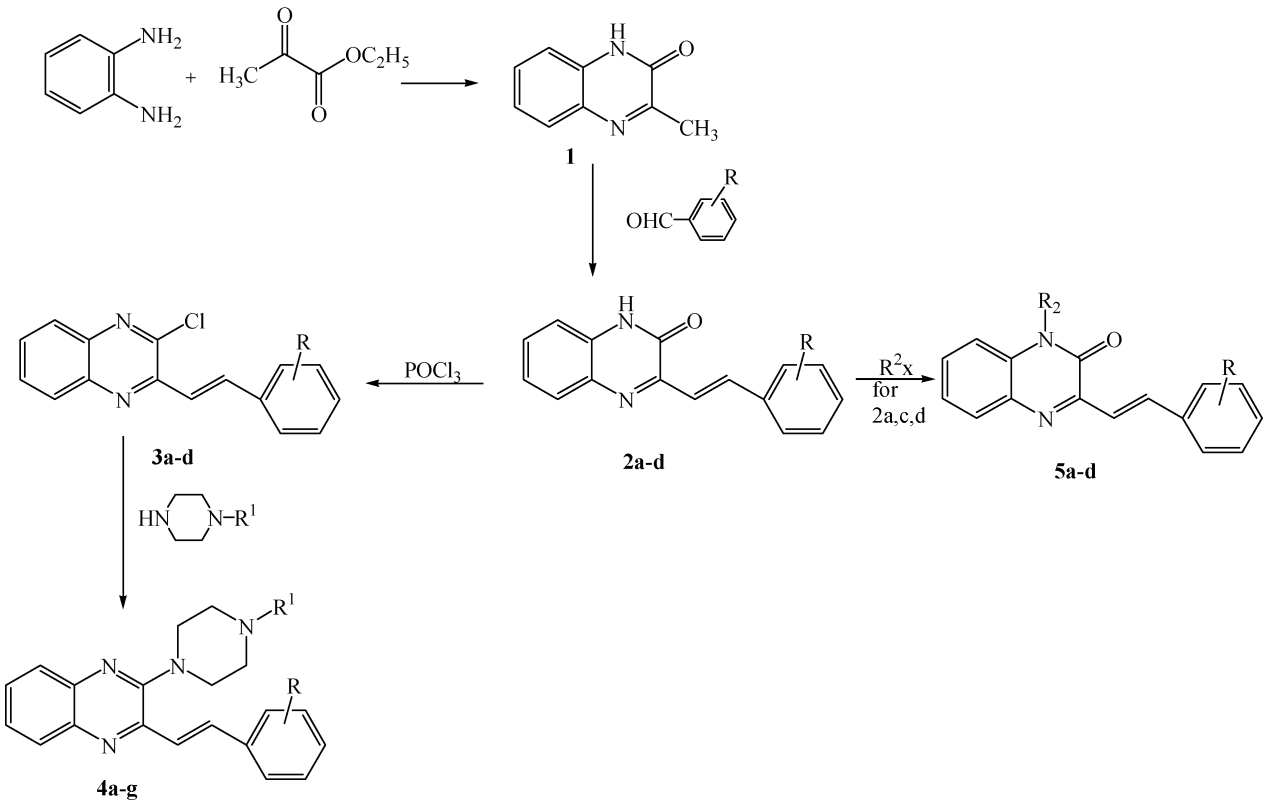
Scheme I



2a,3a	R=2-F	4e	R=3-Br, R^1=CH_3
2b, 3b	R = 2-Cl	4f	R=4-Br, R^1=CH_3
2c, 3c	R = 3-Br	4g	R=4-Br, R^1=4-Cl-C_6H_4
2d, 3d	R = 4-Br	5a	R=2-F, R^2= CH_2-CH=CH_2
4a	R=2-F, R^1 =4-CH_3	5b	R=2-Cl, R^2=-CH_3
4b	R=2-F, R^1=2-OC_2H_5-C_6H_4	5c	R=2-Cl, R^2=-CH_2-CH_3
4c	R=2-Cl, R^1=2-OCH_3-C_6H_4	5d	R=3-Br, R^2=-CH_3
4d	R=2-Cl, R^1=4-Cl-C_6H_4		

spectrum of **5b** are in agreement with N-alkylation.

It is suggested that compounds **2a-d**, **3a-d**, **4a-g** and **5a-d** are of trans configuration, since a strong absorption band appeared in the region of 980-975 cm^{-1}, in the IR spectra of all the above compounds, characteristic of trans olefinic compounds.[20-22] Also, the coupling constants of the styryl protons, in ^{1}H-NMR spectra, are 15-16 Hz, confirming its trans configuration .[10,21,22]

On the other hand, 3-ethoxycarbonyl-2(1H)-one (**6**) was prepared[23] and treated with hydrazine hydrate to afford 3-hydrazinocarbonyl quinoxalin-2(1H)-one (**7**).[24] The latter was used as a versatile compound for the building of other new heterocyclic systems. Thus, treatment of **7** with certain aromatic aldehydes gave 3-(N^2-arylidenehydrazinocarbonyl)quinoxalin-2(1H)-ones (**8a-c**) which upon cyclization with acetic anhydride yielded the corresponding 3-(4-acetyl-5-aryl-1,3,4-oxadiazolin-2-yl)quinoxalin-2(1H)-ones (**9a-c**).

The formation of compounds **9a-c** was substantiated by spectral evidence. Thus, the ^{1}H-NMR spectra revealed the absence of the two signals corresponding to azomethine (CH=N) and (CO-NH) protons of its precursors. Moreover, the appearance of a signal at 2.32 ppm corresponding to an acetyl group, in addition to a singlet signal at δ 7.2 ppm cor-

responding to the proton at position 5 of oxadiazoline ring, were indicative of successful formation of the title compounds **9a-c**.

Conversely, compound **7** was allowed to react with carbon disulphide in alcoholic potassium hydroxide to afford 3-(5-mercapto-1,3,4-oxadiazol-2-yl)quinoxalin-2(1H)-one (**10**).[25]

S-Alkylation of **10** with certain alkylhalides furnished the respective 3-(5-substituted thio-1,3,4-oxadiazol-2-yl)quinoxalin-2(1H)-ones (**11a-c**). The ^{1}H-NMR spectrum of compound **11c** showed the appearance of a singlet signal at δ 4.5 integrating for two protons of SCH_2. Also, the mass spectrum of compound **11b** showed (M-alkyl) peak, in addition to the molecular ion peak.

The alkylating synthons, 1-(4-substituted piperazin-1-yl)-2-chloroethan-1-ones, needed for the synthesis of **12a-c** were prepared via the reaction of N-substituted piperazines with chloroacetyl chloride in dry acetone.[26] 3-[5-(4-Substituted piperazin-1-ylcarbonylmethylthio)-1,3,4-oxadiazol-2-yl]quinoxalin-2(1H)-ones (**12a-c**) were prepared by the reaction of **10** with certain 1-(4-substituted piperazin-1-yl)-2-chloroethan-1-ones in dry acetone containing anhydrous potassium carbonate. Furthermore, cyclization of **7** with 1,1′-carbonyl diimidazole in the presence of triethyl amine furnished 3-(5-oxo-4,5-dihydro-1,3,4-oxadiazol-2-yl)quinoxalin-2(1H)-one (**13**). The IR spectrum showed two NH peaks at 3400, 3150 cm^{-1} and two carbonyl peaks at 1780, 1670 cm^{-1} corresponding to the oxadiazole and the quinoxaline rings, whereas the ^{1}H-NMR revealed two exchangeable singlet signals at δ 9.24 and 10.17 ppm corresponding to two NH groups.

Finally, treatment of **7** with 4-nitrobenzoic acid in phosphorus oxychloride afforded 2-chloro 3-[5-(4-nitrophenyl)-1,3,4-oxadiazol-2-yl]quinoxaline (**15**). In this one-pot reaction, the acid was first converted to its acid chloride, which subsequently reacted with the parent hydrazinocarbonyl quinoxaline **7** to give the corresponding N^2-acylhydrazinocarbonyl derivative *in situ*. The latter underwent cyclodehydration followed by chlorination to yield the oxadiazolyl quinoxaline **15**. The progress of the reaction was monitored by TLC, and it was observed that after 3 hours, 3-[5-(4-nitrophenyl)-1,3,4-oxadiazol-2-yl]-quinoxalin-2(1H)-one (**14**) was the main separated product as confirmed by its mass spectrum. The chloro derivative (**15**) can also be prepared by chlorination of (**14**) with phosphorus oxychloride.

EXPERIMENTAL

Melting points were obtained on a Graffin apparatus and are uncorrected. Microanalyses for C, H and N were carried out at the Microanalytical Center, Cairo University. IR spectra were recorded on a Shimadzu 435 Spectrometer, using KBr discs. ^{1}H-NMR spectra were performed an a Jeol NMR FXQ-200 MHz Spectrometer, Varian Gemini 300 MHz spectrometer and Jeol 90 MHz spectrometer, using TMS as internal standard. Mass spectra were recorded on a GCMS-QP 1000 EX Mass Spectrometer. Progress of the reactions was monitored by TLC using precoated aluminum sheets silica gel MERCK 60 F 254 and was visualized by UV lamp. Compounds **2b** and **3b** were reported as patented.[4]

General procedure for the synthesis of 2a-d

A mixture of 3-methyl quinoxaline (**1**) (0.5 g, 0.003 mol), the appropriate aromatic aldehyde (0.006 mol), acetic anhydride (1 mL) and piperidine (3 drops) was refluxed for 24 hours. After cooling, the precipitated solid was filtered off, washed with water, dried and crystallized from dimethylformamide.

3-(2-(2-Fluorophenyl)ethenyl)quinoxalin-2(1H)-one 2a

Yield: 80.0%; mp: 260-262 °C; IR: 3300 (NH), 1660 (C=O), 1620 (C=N), 1590 (CH=CH), 980 (trans CH=CH); ^{1}H-NMR (DMSO-d_6) (90 MHz): 7.11-8.17 (m, 10H, 8ArH and 2 ethenyl-H); Anal. Calcd. for $C_{16}H_{11}FN_2O$: C, 72.17; H, 4.16; N, 10.52. Found: C, 72.35; H, 4.25; N, 10.40.

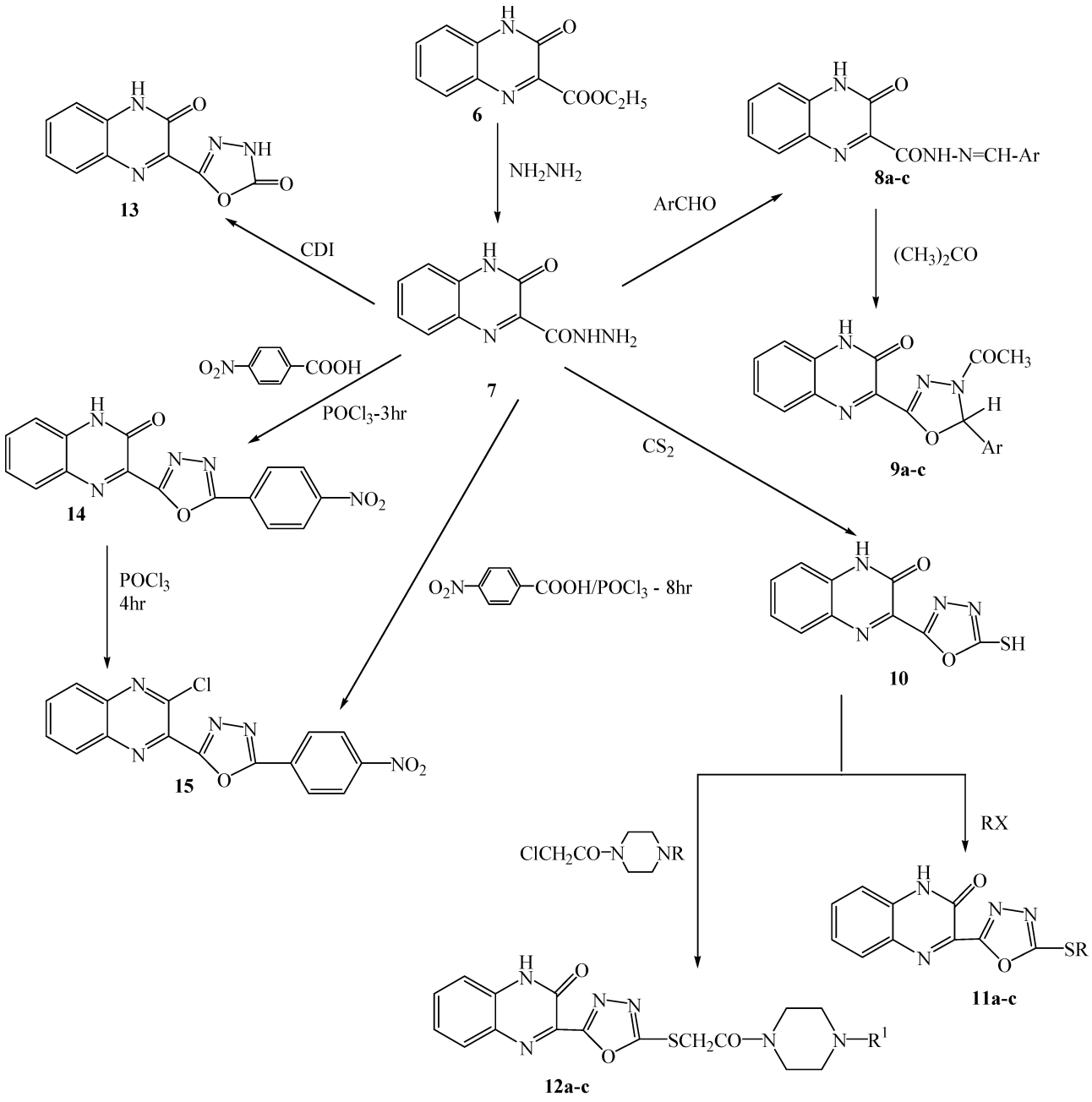
3-(2-(2-Chlorophenyl)ethenyl)quinoxalin-2(1H)-one 2b

Yield: 70.0%; mp: 242-244 °C; IR: 3450 (NH), 1660 (C=O), 1620 (C=N), 1590 (CH=CH), 975 (trans CH=CH); ^{1}H-NMR (DMSO-d_6) (300 MHz): 7.36-7.59 (m, 5H, ArH), 7.68 (d, 1H, J = 15 Hz, ethenyl H), 7.81-7.84 (m, 3H, ArH), 8.10 (d, 1H, J = 15 Hz, ethenyl H), 12.61 (s, 1H, NH, D_2O exchangeable); MS: *m/z* 284 (M+2, 33.37%), 283 (M+1, 46.58%), 282 (M^+, 93.56%), 254 (M + -CO; 21.05%), 253 (100%). Anal. Calcd. for $C_{16}H_{11}ClN_2O$: C, 67.97; H, 3.92; N, 9.91. Found: C, 67.85; H, 4.25; N, 9.92.

3-(2-(3-Bromophenyl)ethenyl)quinoxalin-2(1H)-one 2c

Yield: 50.0%; mp: 240-242 °C; IR: 3400 (NH), 1660 (C=O), 1620 (C=N), 1590 (CH=CH), 975 (trans CH=CH); Anal. Calcd. for $C_{16}H_{11}BrN_2O$: C, 58.73; H, 3.39; N, 8.56.

Scheme II



8a,9a	Ar=4-Cl-C_6H_4	12a	R^1 =-CH_3
8b, 9b	Ar = 2-Br-C_6H_4	12b	R^1 =-CH_2-C_6H_5
8c, 9c	Ar = 2-thienyl	12c	R^1 =-4-OCH_3-C_6H_4
11a	R=-CH_2CH_3		
11b	R=-$CH_2CH_2CH_3$		
11c	R=-CH_2-C_6H_5		

Found: C, 58.36; H, 3.49; N, 8.44.

3-(2-(4-Bromophenyl)ethenyl)quinoxalin-2(1H)-one 2d

Yield: 78.0%; mp: 298-300 °C; IR: 3400 (NH), 1660 (C=O), 1620 (C=N); 1590 (CH=CH), 975 (trans CH=CH); ^{1}H-NMR (DMSO-d_6) (90 MHz): 7.19-8.16 (m, 10H, 8ArH and 2 ethenyl H), 12.40 (s, 1H, NH, D_2O exchangeable). Anal. Calcd. for $C_{16}H_{11}BrN_2O$: C, 58.73; H, 3.39; N, 8.56. Found: C, 58.33; H, 3.72; N, 8.33.

General procedure for the synthesis of 3a-d

Phosphorus oxychloride (15 mL) was added to the corresponding styrylquinoxaline **2a-d** (0.013 mol), and the mixture was refluxed for 2 hours. Excess phosphorus oxychloride was distilled under reduced pressure, and the resultant residue was treated with cold water (200 mL). The precipitate formed was filtered off, washed with water, air dried and crystallized from ethanol.

2-Chloro-3-(2-(2-fluorophenyl)ethenyl)quinoxaline 3a

Yield: 70.0%; mp: 170-172 °C; IR: 1620 (C=N), 1570 (CH=CH), 980 (trans CH=CH), ^{1}H-NMR ($CDCl_3$-d_1) (90 MHz): 7.09-8.16 (m, 10H, 8ArH and 2 ethenyl H as 2d at 7.21, 8.03, J = 15 Hz); Anal. Calcd. for $C_{16}H_{10}ClFN_2$: C, 67.49; H, 3.54; N, 9.84. Found: C, 67.43; H, 3.77; N, 9.85.

2-Chloro-3-(2-(2-chlorophenyl)ethenyl)quinoxaline 3b

Yield: 75.0%; mp: 208-210 °C; IR: 1640 (C=N), 1570 (CH=CH), 975 (trans CH=CH); MS: *m/z* 303 (M+3, 11.6%), 302 (M+2, 38.1%), 301 (M+1, 46.4%), 300 (M^+, 59.4%), 265 (M^+-Cl, 100%); Anal. Calcd. for $C_{16}H_{10}Cl_2N_2$: C, 63.80; H, 3.35; N, 9.30. Found: C, 63.86; H, 3.34; H, 9.38.

3-(2-(3-Bromophenyl)ethenyl)-2-chloro quinoxaline 3c

Yield: 50.0%; mp: 236-238 °C; IR: 1620 (C=N), 1590 (CH=CH), 980 (trans CH=CH); ^{1}H-NMR ($CDCl_3$-d_1) (90 MHz): 7.39-7.79 (m, 10H, 8ArH and 2 ethenyl H); Anal. Calcd. for $C_{16}H_{10}BrClN_2$: C, 55.60; H, 2.92; N, 8.11. Found: C, 55.71; H, 3.28; N, 8.25.

3-(2-(4-Bromophenyl)ethenyl)-2-chloro quinoxaline 3d

Yield: 65.0%; mp: 194-196 °C; IR: 1625 (C=N), 1590 (CH=CH), 975 (trans CH=CH); Anal. Calcd. for $C_{16}H_{10}BrClN_2$: C, 55.60; H, 2.92; N, 8.11. Found: C, 55.42; H, 2.84; N, 8.09.

General procedure for the synthesis of 4a-g

To a solution of the corresponding styrylquinoxalines (**3a-d**) (0.002 mol) in n-butanol (10 mL), the respective N-substituted piperazine (0.004 mol) and triethyl amine (4 drops) were added. The mixture was heated under reflux for 12-24 hours (monitored by TLC). Upon cooling, the solid that separated was collected and crystallized from ethanol.

3-(2-(2-Fluorophenyl)ethenyl)-2-(4-methylpiperazin-1-yl)quinoxaline 4a

Yield: 60.0%; mp: 196-198 °C; IR: 2850 (CH aliph.), 1630 (C=N), 1600 (CH=CH), 975 (trans CH=CH); Anal. Calcd. for $C_{21}H_{21}FN_4$: C, 72.39; H, 6.08; N, 16.08. Found: C, 72.30; H, 6.10; N, 16.40.

2-(4-(2-Ethoxyphenyl)piperazin-1-yl)-3-(2-(2-fluorophenyl)ethenyl)quinoxaline 4b

Yield: 40.0%; mp: 144-146 °C; IR: 2950 (CH aliph.), 1630 (C=N), 1610 (CH=CH), 980 (trans CH=CH); ^{1}H-NMR ($CDCl_3$-d_1) (300 MHz): 1.46 (t, 3H, $OCH_2\underline{CH_3}$, J = 6.6 Hz), 3.37 (brs, 4H, $2CH_2$ piperazine), 3.70 (brs, 4H, $2CH_2$ piperazine), 4.09 (q, 2H, $\underline{OCH_2}CH_3$, J = 6.6 Hz), 7.09-8.26 (m, 14H, 12ArH and 2 ethenyl H as 2d at 7.83, 8.23, J = 15.9 Hz, 2ethenyl H); Anal. Calcd. for $C_{28}H_{27}FN_4O$: C, 73.98; H, 5.99; N, 12.33. Found: C, 73.88; H, 5.80; N, 12.34.

3-(2-(2-Chlorophenyl)ethenyl)-2-(4-(2-methoxyphenyl)-piperazin-1-yl)quinoxaline 4c

Yield: 70.0%; mp: 190-192 °C; IR: 2850 (CH aliph.), 1630 (C=N), 1590 (CH=CH), 980 (trans CH=CH); MS: *m/z* 457 (M+1, 3.8%), 456 (M+, 3.1), 247 (100%). Anal. Calcd. for $C_{27}H_{25}ClN_4O$: C, 70.96; H, 5.51; N, 12.26. Found: C, 70.90; H, 5.80; N, 12.60.

3-(2-(2-Clorophenyl)ethenyl)-2-(4-(4-chlorophenyl)-piperazin-1-yl)quinoxaline 4d

Yield: 70.0%; mp: 222-224 °C; IR: 2900 (CH aliph.), 1620 (C=N), 1590 (CH=CH), 975 (trans CH=CH); ^{1}H-NMR ($CDCl_3$-d_1) (90 MHz): 3.20-3.38 (m, 4H, $2CH_2$ piperazine), 3.48-3.62 (m, 4H, $2CH_2$ piperazine), 6.84-8.28 (m, 14H, 12ArH and 2 ethenyl H); Anal. Calcd. for $C_{26}H_{22}Cl_2N_4$: C, 67.68; H, 4.81; N, 12.14. Found: C, 67.70; H, 4.80; N, 12.50.

3-(2-(3-Bromophenyl)ethenyl)-2-(4-methyl piperazin-1-yl)quinoxaline 4e

Yield: 50.0%; mp: 246-248 °C; IR: 2800 (CH aliph.), 1620 (C=N), 1590 (CH=CH), 980 (trans CH=CH); Anal. Calcd. for $C_{21}H_{21}BrN_4$: C, 61.62; H, 5.17; N, 13.69. Found: C, 61.84; H, 5.20; N, 13.45.

3-(2-(4-Bromophenyl)ethenyl)-2-(4-methylpiperazin-1-yl)quinoxaline 4f

Yield: 63.0%; mp: 160-162 °C; IR: 2800 (CH aliph.), 1630 (C=N), 1590 (CH=CH), 980 (trans CH=CH); MS: *m/z* 410 (M+2, 14.9%), 408 (M^+, 13.5%), 70 (100%); Anal. Calcd. for $C_{21}H_{21}BrN_4$: C, 61.62; H, 5.17; N, 13.69. Found: C, 61.70; H, 5.10; N, 13.80.

3-(2-(4-Bromophenyl)ethenyl)-2-(4-(4-chlorophenyl)-piperazin-1-yl)quinoxaline 4g

Yield: 70.0%, mp: 210-212 °C; IR: 2850 (CH aliph.), 1630 (C=N), 1590 (CH=CH), 980 (trans CH=CH); ^{1}H-NMR ($CDCl_3$-d_1) (90 MHz): 3.25-3.42 (m, 4H, $2CH_2$ piperazine), 3.51-3.81 (m, 4H, $2CH_2$, piperazine), 6.85-8.21 (m, 14H, 12ArH and 2 ethenyl H); Anal. Calcd. for $C_{26}H_{22}BrClN_4$: C, 61.73; H, 4.38; N, 11.08. Found: C, 61.77; H, 4.40; N, 11.11.

General procedure for the synthesis of 5

A mixture of compound **2a**, **2c** or **2d** (0.01 mol), alkyl or allyl halides (0.012 mol) and anhydrous potassium carbonate (1.3 g, 0.01 mol) in dry acetone (30 mL) was refluxed for 24 hours. After cooling, the solid that separated was filtered off and crystallized from ethanol.

1-Allyl-3-(2-(2-fluorophenyl)ethenyl)quinoxalin-2(1H)-one 5a

Yield: 95.0%, mp: 88-90 °C, IR: 2950 (CH aliph.), 1640 (C=O), 1600 (C=N), 1575 (CH=CH), 980 (trans CH=CH); ^{1}H-NMR (DMSO-d_6) (300 MHz): 4.95-4.97 (d, 2H, -NCH_2), 5.23-5.31 (m, 2H, CH=$\underline{CH_2}$), 5.95-6.15 (m, 1H, $\underline{CH}$=CH_2), 7.18-8.33 (m, 10H, 8ArH and 2 ethenyl H as 2d at 7.83, 8.31, J = 16 Hz); Anal. Calcd. for $C_{19}H_{15}FN_2O$: C, 74.49; H, 4.93; N, 9.15. Found: C, 74.36; H, 5.26; N, 9.03.

3-(2-(2-Chlorophenyl)ethenyl)-1-methylquinoxalin-2(1H)-one 5b

Yield: 95%; mp: 156-158 °C; IR: 2900 (CH aliph.), 1660 (C=O), 1600 (C=N), 1580 (CH=CH), 975 (trans CH=CH); ^{1}H-NMR ($CDCl_3$-d_1) (200 MHz): 3.75 (s, 3H, CH_3), 7.26-8.11 (m, 10H, 8ArH and 2 ethenyl H as 2d at 7.76, 8.07, J = 16 Hz); MS: *m/z* 298 (M+2, 34.9%), 297 (M+1, 43.5%), 296 (M^+, 100%), 283 (62.9%), 281 (M-CH_3, 21.9%); Anal. Calcd. for $C_{17}H_{13}ClN_2O$: C, 68.80; H, 4.41; N, 9.44. Found: C, 68.54; H, 4.68; N, 9.54.

3-(2-(2-Chlorophenyl)ethenyl)-1-ethylquinoxalin-2(1H)-one 5c

Yield: 80.0%, mp: 103-105 °C ; IR: 2950 (CH aliph.), 1660 (C=O), 1600 (C=N), 1575 (CH=CH), 975 (trans CH=CH); Anal. Calcd. for $C_{18}H_{15}ClN_2O$: C, 69.56; H, 4.86; N, 9.02. Found: C, 69.46; H, 4.91; N, 8.77.

3-(2-(3-Bromophenyl)ethenyl)-1-methylquinoxalin-2(1H)-one 5d

Yield: 85.0%; mp: 84-85 °C; IR: 2950 (CH aliph.), 1660 (C=O), 1600 (C=N), 1580 (CH=CH), 980 (trans CH=CH); ^{1}H-NMR ($CDCl_3$-d_1) (300 MHz): 3.83 (s, 3H, CH_3), 7.33-8.16 (m, 10H, 8ArH and 2 ethenyl H as 2d at 7.83, 8.31, J = 16 Hz), Anal. Cald. for $C_{17}H_{13}BrN_2O$: C, 59.84; H, 3.84; N, 8.21. Found: C, 59.50; H, 4.01; N, 8.49.

General procedure for the synthesis of 8

A mixture of 3-hydrazinocarbonylquinoxalin-2-(1H)-one (**7**) (0.6 g, 0.003 mol) and the respective aromatic aldehyde (0.003 mol) in ethanol (7 mL) was refluxed for 1 hour. Upon cooling, the precipitate that formed was filtered off, dried and crystallized from ethanol.

3-(N^2-4-Chlorobenzylidenehydrazinocarbonyl)quinoxalin-2(1H)-one 8a

Yield: 95.0%, mp: > 300 °C; IR: 3600-3400 (2NH), 1695, 1640 (2C=O), 1615 (C=N); ^{1}H-NMR (DMSO-d_6) (90 MHz): 7.14-7.87 (m, 8H, ArH), 8.08, 8.39 (2s, 1H, Syn and anti isomers of benzylidene H), 12.23, 12.36 (2s, 1H, NH, OH tautomers of –CONH–, D_2O exchangeable), 12.50, 12.63 (2s, 1H, NH, OH tautomers of quinoxaline, D_2O exchangeable); Anal. Calcd. for $C_{16}H_{11}ClN_4O_2$: C, 58.81; H, 3.39; N, 17.15. Found C, 58.62; H, 3.52; N, 16.96.

3-(N^2-2-Bromobenzylidenehydrazinocarbonyl)quinoxalin-(1H)-one 8b

Yield: 83.0%; mp: 294-296 °C; IR: 3500-3400 (2NH), 1700, 1640 (2C=O), 1595 (C=N), ^{1}H-NMR (DMSO-d_6) (200 MHz): 7.26-8.01 (m, 8H, ArH), 8.40, 8.68 (2s, 1H, Syn and anti isomers of benzylidene H) 12.42, 12.47 (2s,

1H, NH, OH tautomers of -CONH-D_2O exchangeable) 12.79, 12.95 (2s, 1H, NH, OH tautomers of quinoxaline, D_2O exchangeable); Anal. Calcd. for $C_{16}H_{11}Br\ N_4O_2$: C, 5l.77; H, 2.99; N, 15. 09. Found: C, 51.39; H, 2.98; N, 14.93.

3-(N^2-2-Thenylidenehydrazinocarbonyl)quinoxalin-2(1H)-one 8c

Yield: 80.0%; mp: 281-283 °C; IR: 3200-3250 (2NH), 1690, 1640 (2C=O), 1595 (C=N); MS: *m/z* 299 (M+1, 2.3%), 298 (M^+, 12.5%), 90 (100%); Anal. Calcd. for $C_{14}H_{10}N_4O_2S$: C, 56.36; H, 3.38; N, 18.78. Found: C, 56. 36; H, 3.75; N, 18.75.

General procedure for the synthesis of 9

The corresponding arylidenehydrazide (**8a-c**) (0.003 mol) in acetic anhydride (3 mL) was refluxed for 1 hour and then allowed to cool to room temperature. The mixture was neutralized with ammonium hydroxide. The solid obtained was collected by filtration, washed with water and crystallized from ethanol.

3-(4-Acetyl-5-(4-chlorophenyl)-1,3,4-oxadiazolin-2-yl)-quinoxalin-2(1H)-one 9a

Yield: 99.0%, mp 258-260 °C; IR: 3500-3350 (NH), 2900 (CH aliph.), 1680, 1660 (2C=O), 1620 (C=N); ^{1}H-NMR (DMSO-d_6) (200 MHz): 2.34 (s, 3H, CH_3), 7.18 (s, 1H, oxadiazoline), 7.37-8.02 (m, 8H, ArH), 12.79, 12.95 (2s, 1H, NH, OH tautomers of quinoxaline D_2O exchangeable); Anal. Calcd. for $C_{18}H_{13}ClN_4O_3$: C, 58.62; H, 3.55; N, 15.19. Found: C, 58.76; H, 3.59; N, 15.10.

3-(4-Acetyl-5-(2-bromophenyl)-1,3,4-oxadiazolin-2-yl)-quinoxalin-2(1H)-one 9b

Yield: 76.0%; mp: 206-208 °C; IR: 3450-3400 (NH), 2900 (CH aliph.), 1740, 1690 (2C=O), 1610 (C=N), ^{1}H-NMR (DMSO-d_6) (300 MHz): 2.32 (s, 3H, CH_3), 7.28 (s, 1H, oxadiazoline), 7.33-8.12 (m, 8H, ArH), 12.55, 12.71 (2s, 1H, NH, OH tautomers) of quinoxaline, D_2O exchangeable); Anal. Calcd. for $C_{18}H_{13}BrN_4O_3$: C, 52.32; H, 3.17; N, 13.56. Found: C, 52.21; H, 3.27; N, 13.54.

3-(4-Acetyl-5-(2-thienyl)-1,3,4-oxadiazolin-2-yl)quinoxalin-2(1H)-one 9c

Yield: 60.0%; mp 204-206 °C; IR: 3500-3400 (NH), 2800 (CH aliph.), 1740, 1670 (2C=O), 1620 (C=N); MS: *m/z* 341 (M+1, 0.8%), 340 (M^+, 29%), 145 (100%); Anal. Calcd. for $C_{16}H_{12}N_4O_3S$: C, 56.46; H, 3.56; N, 16.46. Found: C, 56.26; H, 4.10; N, 16.49.

3-(5-Ethylthio-1,3,4-oxadiazol-2-yl)quinoxalin-2(1H)-one 11a

A mixture of ethyliodide (1.6 mL, 0.01 mol) and compound (**10**) (1.23 g,0.005 mol) in pyridine (10 mL) was stirred at room temperature for 12 hours, then refluxed for 3 hours, cooled to room temperature and water (20 mL) was added. The formed precipitate was filtered off and crystallized from n-butanol. Yield: 80.0%; mp: 258-260 °C; IR: 3400 (NH), 2950 (CH aliph.), 1660 (C=O), 1590 (C=N); Anal. Calcd. for $C_{12}H_{10}N_4O_2S$: C, 52.54; H, 3. 67; N, 20.43. Found: C, 52.55; H, 3.65; N, 20.31.

General procedure for the synthesis of 11b and 11c

A mixture of compound (**10**) (3.69 g, 0.915 mol), the respective halogeno compound (0.018 mol), and sodium acetate (0.02 mol) in ethanol (30 mL) was heated under reflux for 3 hours, then allowed to cool and poured onto cold water (50 mL). The solid product separated was collected and crystallized from ethanol.

3-(5-Propylthio-1,3,4-oxadiazol-2-yl)quinoxalin-2(1H)-one 11b

Yield: 70.0%; mp: 288-290 °C; IR: 3450 (NH), 2900 (CH aliph.), 1685 (C=O); MS: *m/z* 288 (M^+, 17.95%), 246 (42.18%), 213 (29.93%), 145 (100%); Anal. Calcd. for $C_{13}H_{12}N_4O_2S$: C, 54.15; H, 4.20; N, 19.43. Found: C, 53.75; H, 4.20; N, 19.25.

3-(5-Benzylthio-1,3,4-oxadiazol-2-yl)quinoxalin-2(1H)-one 11c

Yield: 60.0%; mp > 300 °C; IR: 3200 (NH), 2900 (CH aliph.), 1660, 1640 (2C=O); ^{1}H-NMR (DMSO-d_6) (200 MHz): 4.5 (s, 2H, CH_2), 7.10-7.67 (m, 9H, ArH), 10.01 (s, 1H, NH, D_2O exchangeable). Anal. Calcd. for $C_{17}H_{12}N_4O_2S$: C, 60.70; H, 3.60; N, 16.66. Found: C, 60.60; H, 3.60; N, 16.55.

General procedure for the synthesis of 12

Compound (**10**) (1.23 g, 0.005 mol), the corresponding substituted piperazinylcarbonylmethyl chloride (0.005 mol) and anhydrous potassium carbonate (1 g, 0.007 mol) in dry acetone (30 mL) were refluxed on a water bath for 4 hours, then cooled to room temperature and poured onto crushed ice (50 g). The precipitated product was filtered off

and crystallized from ethanol.

3-[5-(4-Methylpiperazin-1-ylcarbonylmethylthio)-1,3,4-oxadiazol-2-yl]quinoxalin-2(1H)-one 12a

Yield: 55.0%; mp: > 300 °C; IR: 3400 (NH), 2900 (CH aliph.), 1670, 1650 (2C=O), 1610 (C=N), ^{1}H-NMR (DMSO-d_6) (300 MHz): 2.61 (s, 3H, CH_3), 3.58-3.82 (m, 4H, $2CH_2$ piperazine), 4.18-4.38 (m, 4H, $2CH_2$ piperazine), 4.60 (s, 2H, CH_2), 7.40-7.95 (m, 4H, ArH), 12.97 (s, 1H, NH, D_2O exchangeable); Anal. Calcd. for $C_{17}H_{18}N_6O_3S$: C, 52.84; H, 4.69; N, 12.75. Found: C, 52.92; H, 4.40; N, 12.73.

3-[5-(4-Benzylpiperazin-1-ylcarbonylmethylthio)-1,3,4-oxadiazol-2-yl]quinoxalin-2(1H)-one 12b

Yield: 60.0%; mp: 238-240 °C; IR: 3500 (NH), 2900 (CH aliph.), 1670, 1650 (2C=O), 1630 (C=N), ^{1}H-NMR (DMSO-d_6) (300 MHz): 3.58-3.80 (m, 4H, $2CH_2$ piperazine), 3.83-4.15 (m, 6H, $2CH_2$ piperazine + CH_2), 4.61 (s, 2H, CH_2), 6.60-7.46 (m, 9H, ArH), 12.95 (s, 1H, NH, D_2O exchangeable); Anal. Calcd. for $C_{23}H_{22}N_6O_3S$: C, 59.72; H, 4.79; N, 18.17. Found: C, 59.50; H, 4.80; N, 18.1.

3-[5-(4-Methoxyphenylpiperazin-1-ylcarbonylmethylthio)-1,3,4-oxadiazol-2-yl]quinoxalin-2(1H)-one 12c

Yield: 63.0%; mp: 94-95 °C; IR: 3400 (NH), 2950 (CH aliph.), 1670, 1650 (2C=O), 1595 (C=N); MS: *m/z* 478 (M^+, 1.9%), 205 (100%), 191 (23.6%); Anal. Calcd. for $C_{23}H_{22}N_6O_4S$: C, 57.72; H, 4.63; N, 17.56. Found: C, 58.09; H, 4.97; N, 17.77.

3-(5-Oxo-4,5-dihydro-1,3,4-oxadiazol-2-yl)quinoxalin-2(1H)-one 13

To a 0 °C solution of compound **7** (0.8 g, 0.004 mol) in tetrahydrofuran (12 mL) was added triethylamine (0.4 g, 0.005 mol) and 1,1′-carbonyl diimidazole (0.8 g, 0.005 mol). The resulting mixture was stirred for 18 hours at room temperature. The mixture was then concentrated in vaccuo and the residue dissolved in ether. The organic layer was washed successively with 2M HCl, saturated sodium bicarbonate solution, saturated sodium chloride solution and dried over magnesium sulphate. The solid produced was crystallized from dimethyl formamide to afford 0.7 g, yield: 78.09%; mp > 300 °C; IR: 3450, 3150 (2NH) 1780, 1670 (2C=O), 1640 (C=N); ^{1}H-NMR (DMSO-d_6) (200 MHz): 7.34-7.95 (m, 4H, ArH), 9.24, 10.17 (2s, 2NH, D_2O exchangeable); Anal. Calcd. for $C_{10}H_6N_4O_3$: C, 52.18; H, 2.63; N, 24.34. Found: C, 51.89; H, 2.35; N, 24.21.

3-[5-(4-Nitrophenyl)-1,3,4-oxadiazol-2-yl]quinoxalin-2(1H)-one 14

A mixture of the hydrazide (**7**) (0.4 g, 0.002 mol) and 4-nitrobenzoic acid (0.33 g, 0.002 mol) in phosphorus oxychloride (10 mL) was refluxed for 3 hours. After cooling, the reaction mixture was poured onto crushed ice (30 g). The formed precipitate was dried and crystallized from ethanol. Yield: 0.46 g, 70.0%; mp: 277-279 °C; IR: 3450-3200 (NH), 1665 (C=O), 1600 (C=N), 1520, 1340 (NO_2); MS: *m/z* 335 (M^+, 4.6%), 145 (11.7%), 76 (100%); Anal. Calcd. for $C_{16}H_9N_5O_4$: C, 57.31; H, 2.71; N, 20.89. Found: C, 57.70; H, 2.99; N, 20.70.

2-Chloro-3-[5-(4-nitrophenyl)-1,3,4-oxadiazol-2-yl)-quinoxaline 15

Method A

Compound (**14**) (0.33 g, 0.001 mol) was refluxed with phosphorus oxychloride (10 mL) for 4 hours. After cooling, the reaction mixture was poured onto crushed ice (30 g). The formed precipitate was dried and crystallized from ethanol. Yield: 0.31 g, 90.0%, mp: 225-227 °C; IR: 1600 (C=N), 1520, 1340 (NO_2); MS: *m/z* 353, 355 (M^+, 91.6%, 31.4%), 354, 356 (M+1, 18.95%, 6.24%), 163, 165 ($C_8H_4N_2Cl$, 100%, 33.4). Anal. Calcd. for $C_{16}H_8ClN_5O_3$: C, 54.31; H, 2.26; N, 19.80. Found: C, 54.29; H, 2.30; N, 19.82.

Method B

The same method described for the synthesis of **14** was used, but the reflux time is 8 hours.

Antimicrobial Activity

Test organisms

The selected compounds were tested for antimicrobial activity against various types of bacteria and fungi, namely:

1. *Staphylococcus aureus* (Gram positive bacteria).
2. *Bacillus subtilis* (Gram positive bacteria).
3. *Escherichia coli* (Gram negative bacteria).
4. *Pseudomonas aeruginosa* (very resistant Gram negative bacteria).
5. *Candida albicans* (a representative of fungi).

Culture media

Nutrient broth, Sabouraud's broth and nutrient agar

Table 1. Results of antimicrobial activity, zones of inhibition (mean ± standard deviation)

Compound No.	*S. aureus*	*B. subtilis*	*E. coli*	*Ps. aeruginosa*	*C. albicans*
2c	-	-	-	-	-
3a	-	-	-	-	-
4d	-	-	-	-	-
4h	-	-	-	-	-
5a	-	-	-	-	-
8b	-	-	-	-	-
9a	11.17 ± 0.764	-	-	-	-
9b	10.33 ± 1.041	9 ± 1.323	-	-	-
9c	-	-	-	-	-
11a	-	-	-	-	8.5 ± 1.0
11b	-	-	-	-	13.16 ± 1.756
11c	-	-	-	-	11.33 ± 1.258
12a	-	-	-	-	10.5 ± 1.323
12b	-	-	-	-	14.66 ± 1.527
12c	-	-	-	-	-
13	-	-	-	-	-
PRL	10	15	10	-	-
AML	8	10	-	-	-
N	-	-	-	-	26

PRL = Piperacillin standard disc. AML = Amoxicillin standard disc. N = Nystatin standard disc. - = inactive.

were the products of Oxoid Ltd., England.

Methodology

The agar plate disc diffusion technique[27]

Sterilized filter paper discs (6 mm in diameter) were wetted each with 10 mL of a solution of the tested compound containing 10 mg/mL in DMF, and the discs were allowed to air dry. The discs were then placed onto the surface of agar plates (nutrient agar for bacteria and Sabouraud's dextrose agar for fungi) seeded with the test organism. Each plate contained 15 mL of the agar medium, previously seeded with 0.2 mL of 18 hours broth culture of each organism. The inoculated plates were incubated at 37 °C for 48 hours, and the inhibition zones were measured in mm. Discs impregnated with DMF were used as control. The antibacterial references of piperacillin, amoxicillin, and the antifungal reference nystatin were tested concurrently as standards.

RESULTS AND DISCUSSION

The obtained results are presented in Table 1. The tests were done in triplicate and the results were given as mean ± standard deviation. Compounds **9a** & **9b** showed remarkable activity against Gram positive bacterial strains. These compounds exhibited activity comparable to that of piperacillin against *S. aureus*, and compound **9b** showed lower activity than amoxicillin against the sporeforming bacteria *B. subtilis.*

Of all the tested compounds, compounds **11a-c** and **12a,b** showed weak activity against Candida species.

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