Synthesis and Evaluation of 1*H*-Pyrazolo[3,4-*b*]quinoxalines and 1-Aryl-3-quinoxalinyl-1,2,4-triazole as Antibacterial Agent

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Novel 1*H*-pyrazolo[3,4-*b*]quinoxalines (flavazoles) **14**, **15** and 1-aryl-3-quinoxalinyl-1,2,4-triazole **17** were synthesized from the ester **11** *via* various hydrazones **12, 13** and **16**. In the antibacterial screening test of compounds **12-17**, compound **14** showed a relatively high antibacterial activity, wherein the MIC value was 25 μg/ml against *Bacillus licheniformis* KTCC 21425 and *Cellulomonas* sp. and 50 μg/ml against *Salmonella typhimurium* KCTC 1925 and *Flavobacterium devolans*.

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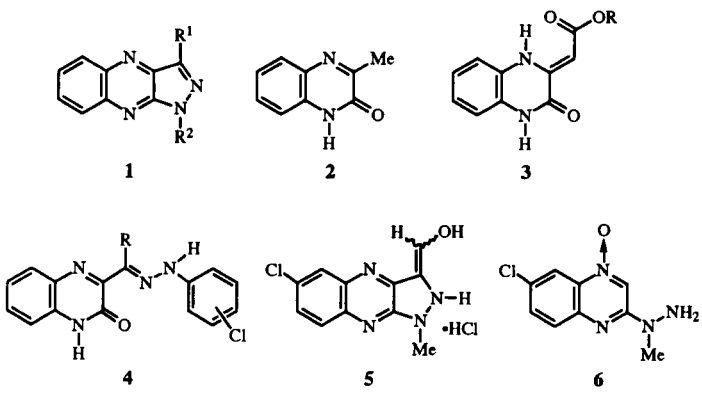
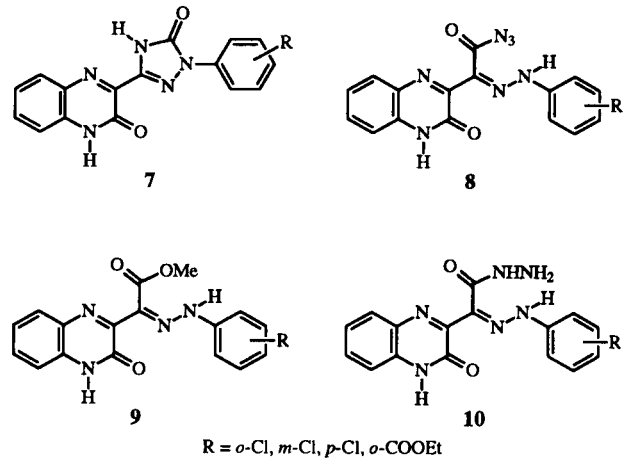
In previous papers [2-6], we reported the synthesis of various 1*H*-pyrazolo[3,4-*b*]quinoxalines (flavazoles) **1** from the quinoxalines **2** and **3** *via* the hydrazones **4** utilizing aryl diazonium salts. Moreover, the 1*H*-pyrazolo[3,4-*b*]quinoxaline hydrochloride **5** was synthesized as an additional flavazole derivative by the 1,3-dipolar cycloaddition reaction of 6-chloro-2-(1-methylhydrazino)quinoxaline 4-oxide **6** with 2-chloroacrylonitrile (Chart 1) [7]. We also reported the synthesis of the 1-aryl-3-quinoxalinyl-1,2,4-triazoles **7** by the Curtius rearrangement of the α-arylhydrazonoacylazides **8** obtained *via* the quinoxalines **9** and **10** (Chart 2) [8,9].

Chart 1



Concerning the biological activity of the flavazoles **1**, only weak antifungal activity was exhibited against *Pythium debaryanum*, *Pyricularia oryzae*, and *Rhizoctonia solani*. In order to improve the biological activity of the flavazoles **1**, we have produced the flavazoles **1** having various substituents at C_3 (R^1) and N_1 (R^2). As a result, we found that the introduction of the *p*-nitrophenyl group into N_1 (R^2) of the flavazole nucleus manifested antibacterial activity. Especially, the flavazole **14** (Scheme 1) showed a relatively high antibacterial activity against some Gram-positive and Gram-negative bacteria in the MIC value of 25-50 μg/ml.

Chart 2

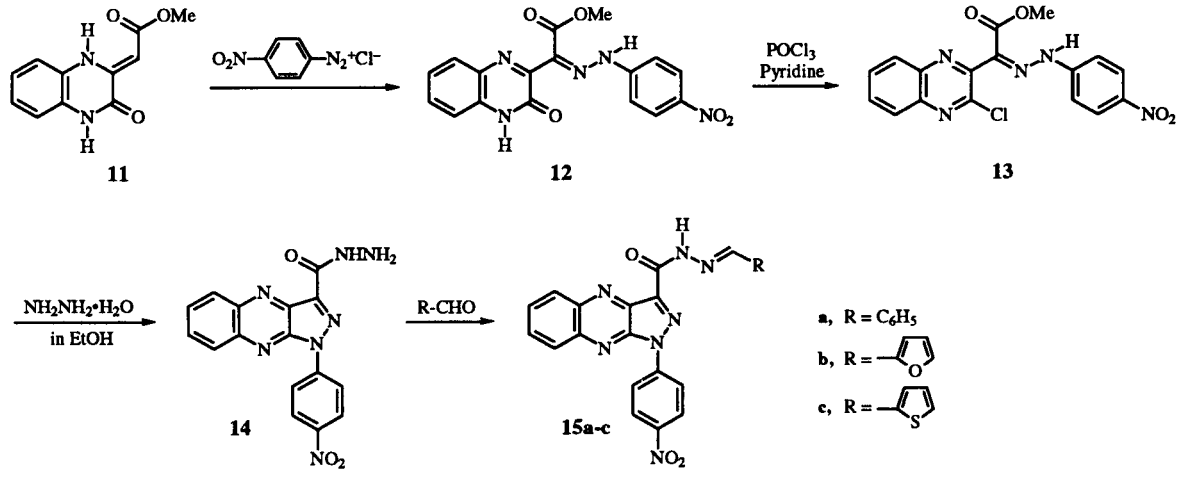


R = *o*-Cl, *m*-Cl, *p*-Cl, *o*-COOEt

This paper describes the synthesis of the 1*H*-pyrazolo-[3,4-*b*]quinoxalines **14, 15** (Scheme 1) and 1-aryl-3-quinoxalinyl-1,2,4-triazole **17** (Scheme 2) together with the antibacterial activity.

The synthesis of compound **12** has already been reported in a previous paper [10]. The chlorination of compound **12** with phosphoryl chloride afforded 2-chloro-3-[α-(*p*-nitrophenylhydrazono)methoxycarbonylmethyl]quinoxaline **13**, whose reaction with hydrazine hydrate provided 3-hydrazinocarbonyl-1-(*p*-nitrophenyl)-1*H*-pyrazolo[3,4-*b*]quinoxaline **14**. The reaction of compound **14** with benzaldehyde, furfural and 2-thiophenecarbaldehyde gave 3-(benzilidenehydrazinocarbonyl)-1-(*p*-nitrophenyl)-1*H*-pyrazolo-[3,4-*b*]quinoxaline **15a**, 3-(2-furylmethylenehydrazinocarbonyl)-1-(*p*-nitrophenyl)-1*H*-pyrazolo[3,4-*b*]quinoxaline **15b** and 1-(*p*-nitrophenyl)-3-(2-thienylmethylenehydrazinocarbonyl)-1*H*-pyrazolo[3,4-*b*]quinoxaline **15c**, respectively (Scheme 1).

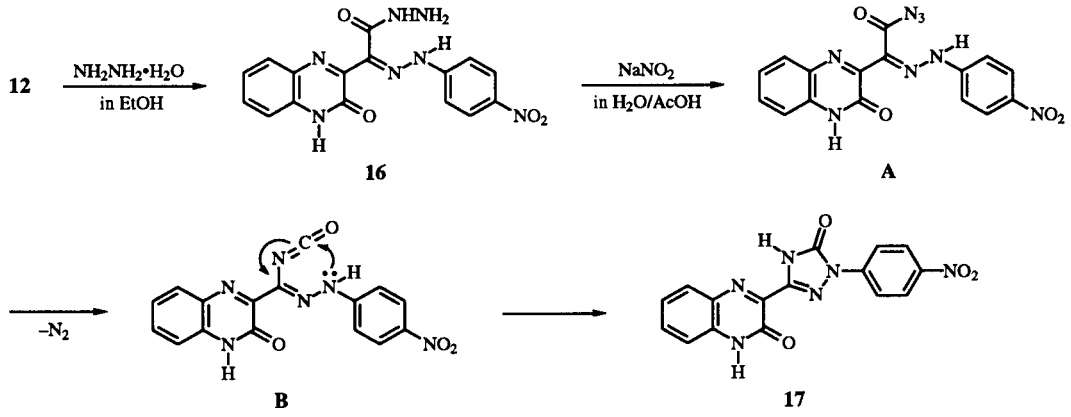
Scheme 1



On the other hand, the reaction of compound **12** with hydrazine hydrate afforded 3-[α-(*p*-nitrophenylhydrazono)hydrazinocarbonylmethyl]-2-oxo-1,2-dihydroquinoxaline **16**, whose reaction with nitrous acid effected the Curtius rearrangement to provide 1-(*p*-nitrophenyl)-3-(3-oxo-3,4-dihydroquinoxalin-2-yl)-4,5-dihydro-1*H*-1,2,4-triazol-5-one **17**, presumably *via* intermediates **A** and **B** (Scheme 2) [8,9]. The formation of compound **A** was checked by the ir spectrum, exhibiting the characteristic azide absorption band at 2144 cm^{-1}.

and 50 μg/ml against *Salmonella typhimurium* KCTC 1925 and *Flavobacterium devolans*. In expectation of the reinforcement of the antibacterial activity, compound **14** was modified into compounds **15a-c**, but these derivatives **15a-c** had no antibacterial activity against the above four Gram-positive and Gram-negative bacteria. These data suggest that the C_3-acylhydrazide moiety had better to be reserved for the maintenance of the antibacterial activity in the above flavazoles. Accordingly, the modification should be directed toward other sites such as the benzene ring

Scheme 2



Compounds **12-17** were tested for their antibacterial activity against Gram-positive (*Bacillus licheniformis* KTCC 21425 and *Cellulomonas* sp.) and Gram-negative (*Salmonella typhimurium* KCTC 1925 and *Flavobacterium devolans*) bacteria by an *in vitro* agar dilution method. Among compounds **12-17**, compound **14** showed the antibacterial activity against the above four bacteria, wherein the MIC value was found to be 25 μg/ml against *Bacillus licheniformis* KTCC 21425 and *Cellulomonas* sp. [(C_5-C_8) or ($C_{2'}$, $C_{3'}$, $C_{5'}$, $C_{6'}$)] of compound **14** in order to augment the antibacterial activity.

EXPERIMENTAL

All melting points were determined on a Haake Buchler melting point apparatus and are uncorrected. The ir spectra (potassium bromide) were recorded with a Mattson Polaris FT-IR spectrophotometer. The mass spectra (ms) were determined with

a Shimadzu GC/MS QP-1000 spectrometer. The nmr spectra were measured in deuteriodimethyl sulfoxide with a Bruker AM-300 spectrometer. Chemical shifts are given in the δ scale. Elemental analyses were performed on a Perkin Elmer 240B instrument.

2-Chloro-3-[α-(*p*-nitrophenylhydrazono)methoxycarbonylmethyl]quinoxaline **13**.

A solution of compound **12** (5 g, 14 mmoles) in phosphoryl chloride (50 ml) and pyridine (5 ml) was refluxed in an oil bath for 2 hours. The solution was evaporated *in vacuo* to give yellow crystals, to which ethanol was added. The mixture was poured onto crushed ice to precipitate yellow crystals, which were collected by suction filtration (4.9 g, 91%). Recrystallization from *N,N*-dimethylformamide/water gave bright yellow needles **13**, mp 84-85°; ir: ν cm^{-1} 1732, 1598, 1339, 853; ms: m/z 385 (M^+), 387 (M^++2); pmr: 11.36 (s, 1H, NH), 8.24-7.36 (m, 8H, aromatic), 3.78 (s, 3H, CH_3).

Anal. Calcd. for $C_{17}H_{12}ClN_5O_4$: C, 52.93; H, 3.14; Cl, 9.19; N, 18.16. Found: C, 53.12; H, 3.11; Cl, 9.25; N, 18.13.

3-Hydrazinocarbonyl-1-(*p*-nitrophenyl)-1*H*-pyrazolo[3,4-*b*]-quinoxaline **14**.

A suspension of compound **13** (2 g, 5.2 mmoles) and hydrazine hydrate (5 g, 100 mmoles) in ethanol (200 ml) was refluxed on a boiling water bath for 3 hours to precipitate sap-green crystals, which were collected by suction filtration. Trituration with ethanol gave an analytically pure sample of **14** (1.6 g, 86%), mp 250-251°; ir: ν cm^{-1} 3257, 1657, 1596, 1503, 849; ms: m/z 349 (M^+); pmr: 9.99 (brs, 1H, NH), 8.85 (d, J = 9.0 Hz, 2H, aromatic), 8.55 (d, J = 9.0 Hz, 2H, aromatic), 8.43-8.01 (m, 4H, aromatic), 4.82 (brs, 2H, NH_2).

Anal. Calcd. for $C_{16}H_{11}N_7O_3$: C, 55.02; H, 3.17; N, 28.07. Found: C, 55.15; H, 3.16; N, 28.17.

3-(Benzilidenehydrazinocarbonyl)-1-(*p*-nitrophenyl)-1*H*-pyrazolo[3,4-*b*]quinoxaline **15a**.

A solution of compound **14** (2 g, 5.7 mmoles) and benzaldehyde (0.91 g, 8.6 mmoles) in *N,N*-dimethylformamide (50 ml) was refluxed in an oil bath for 1 hour, and evaporation of the solvent *in vacuo* gave brown crystals of **15a** (1.9 g, 76%). Recrystallization from *N,N*-dimethylformamide/ethanol afforded brown needles, mp 261-262°; ir: ν cm^{-1} 3459, 1679, 1597, 1520, 1341, 846; ms: m/ z 437 (M^+); pmr: 12.23 (s, 1H, NH), 8.84 (d, J = 9.0 Hz, 2H, aromatic), 8.67 (s, 1H, hydrazone CH), 8.54 (d, J = 9.0 Hz, 2H, aromatic), 8.44-7.50 (m, 9H, aromatic).

Anal. Calcd. for $C_{23}H_{15}N_7O_3$: C, 63.16; H, 3.46; N, 22.42. Found: C, 63.32; H, 3.48; N, 22.36.

3-(2-Furylmethylenehydrazinocarbonyl)-1-(*p*-nitrophenyl)-1*H*-pyrazolo[3,4-*b*]quinoxaline **15b**.

A solution of compound **14** (2 g, 5.7 mmoles) and furfural (0.83 g, 8.6 mmoles) in *N,N*-dimethylformamide (50 ml) was refluxed in an oil bath for 1 hour. Cooling of the solution to room temperature precipitated yellow needles **15b**, which were collected by suction filtration and then washed with ethanol to give an analytically pure sample (1.6 g, 65%), mp 285-286°; ir: ν cm^{-1} 3420, 1702, 1596, 1515, 1341, 850; ms: m/z 427 (M^+); pmr: 12.25 (s, 1H, NH), 8.87 (d, J = 9.0 Hz, 2H, aromatic), 8.57 (d, J = 9.0 Hz, 2H, aromatic), 8.46 (d, J = 2.0 Hz, 1H, furan C_5-H), 8.35-8.02 (m, 4H, aromatic), 7.93 (s, 1H, hydrazone CH), 7.05 (d, J = 3.0 Hz, 1H furan C_3-H), 6.71 (dd, J =2.0, 3.0 Hz, 1H, furan C_4-H).

Anal. Calcd. for $C_{21}H_{13}N_7O_4$: C, 59.02; H, 3.07; N, 22.94. Found: C, 59.18; H, 3.09; N, 23.03.

1-(*p*-Nitrophenyl)-3-(2-thienylmethylenehydrazinocarbonyl)-1*H*-pyrazolo[3,4-*b*]quinoxaline **15c**.

A solution of compound **14** (2 g, 5.7 mmoles) and 2-thiophenecarbaldehyde (0.96 g, 8.6 mmoles) in *N,N*-dimethylformamide (50 ml) was refluxed in an oil bath for 1 hour. Evaporation of the solvent *in vacuo* gave brown needles **15c**, which were washed with ethanol and then collected by suction filtration to provide an analytically pure sample (2.1 g, 83%), mp 268-269°; ir: ν cm^{-1} 3468, 1681, 1596, 1519, 1341, 851; ms: m/z 443 (M^+); pmr: 12.26 (s, 1 H, NH), 8.88 (d, J = 9.0 Hz, 2H, aromatic), 8.57 (d, J = 9.0 Hz, 2H, aromatic), 8.46 (d, J =5.0 Hz, 1H, thiophene C_5-H), 8.34-7.96 (m, 4H, aromatic), 7.57 (s, 1H, hydrazone CH), 7.76 (d, J = 3.5 Hz, 1H, thiophene C_3-H), 7.22 (dd, J = 5.0, 3.5 Hz, 1H, thiophene C_4-H).

Anal. Calcd. for $C_{21}H_{13}N_7O_3S$: C, 56.88; H, 2.96; N, 22.11; S, 7.23. Found: C, 56.71; H, 2.94; N, 22.07; S, 7.16.

3-[α-(*p*-Nitrophenylhydrazono)hydrazinocarbonylmethyl]-2-oxo-1,2-dihydroquinoxaline **16**.

A suspension of compound **12** (2 g, 5.4 mmoles) and hydrazine hydrate (6.8 g, 136 mmoles) in ethanol (200 ml) was refluxed on a boiling water bath for 4 hours to precipitate yellow crystals, which were collected by suction filtration. Trituration with ethanol gave an analytically pure sample of **16** (1.8 g, 91%), mp 318-319°; ir: ν cm^{-1} 3492, 3254, 1684, 1630, 1597, 1328, 825; ms: m/z 367 (M^+); pmr: 10.82 (br, 2H, CONH, N_1-H), 9.66 (s, 1H, =N-NH), 8.24-7.34 (m, 8H, aromatic), 4.37 (br, 2H, NH_2).

Anal. Calcd. for $C_{16}H_{13}N_7O_4$: C, 52.32; H, 3.57; N, 26.70. Found: C, 52.45; H, 3.55; N, 26.65.

1-(*p*-Nitrophenyl)-3-(3-oxo-3,4-dihydroquinoxalin-2-yl)-4,5-dihydro-1*H*-1,2,4-triazol-5-one **17**.

A solution of sodium nitrite (1.9 g, 28 mmoles) in water (25 ml) was added dropwise to a suspension of compound **16** (2 g, 5.4 mmoles) in acetic acid (150 ml)/concentrated hydrochloric acid (3 ml)/water (15 ml) with stirring in an ice-water bath to precipitate the α-arylhydrazonoacylazide **A**. Without isolation of unstable compound **A**, the reaction mixture was heated on a boiling water bath with stirring until it gave a clear solution. The solvent was evaporated *in vacuo* to afford orange crystals, which were triturated with ethanol. The orange crystals were collected by suction filtration and washed with water (1.6 g, 84%). Recrystallization from *N,N*-dimethylformamide/water provided orange needles **17**, mp 310-311°; ir: ν cm^{-1} 1710, 1667, 1600, 853; ms: m/z 350 (M^+); pmr: 12.78 (brs, 1H, NH), 12.52 (s, 1H, NH), 8.38 (d, J = 9.0 Hz, 2H, aromatic), 8.30 (d, J = 9.0 Hz, 2H, aromatic), 7.89-7.37 (m, 4H, aromatic).

Anal. Calcd. for $C_{16}H_{10}N_6O_4$: C, 54.86; H, 2.88; N, 23.99. Found: C, 54.97; H, 2.87; N, 23.93.

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REFERENCES AND NOTES

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