New Quinoxaline and Pyrimido[4,5-*b*]quinoxaline Derivatives. Potential Antihypertensive and Blood Platelet Antiaggregating Agents

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Starting with 3-amino-2-quinoxalinecarbonitrile 1,4-dioxide **1**, a new series of quinoxaline derivatives **2-12** was synthetized through chemical modification of the 3-amino group, the 2-cyano group and selective monodeoxygenation of the 1-oxide or 4-oxide groups. On the other hand, two 2,4-diaminopyrimido[4,5-*b*]quinoxaline derivatives **13**, **14** were obtained condensing 3-amino-2-quinoxaline carbonitriles with quanidine. Some of the new compounds were studied as inhibitors of blood platelet aggregation as well as antihypertensive agents.

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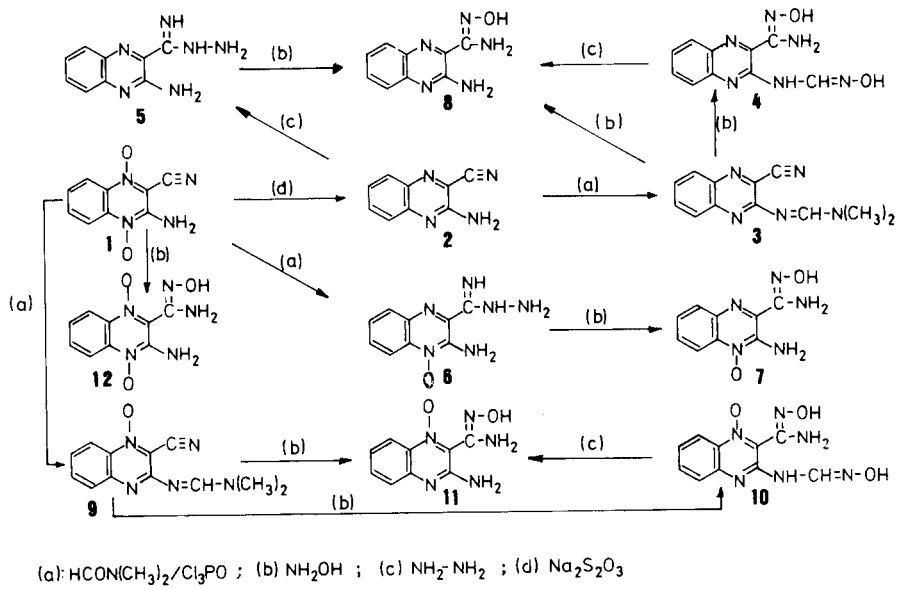
As a continuation to our previous work [1,2] on the synthesis of new quinoxaline derivatives and the study of their potential biological activities, we report in this paper the synthesis of a new series of hydroxyiminoquinoxaline and pyrimido[4,5-*b*]quinoxaline derivatives and some preliminary results on their potentiality as blood platelet antiaggregating agents, as well as antihypertensive agents.

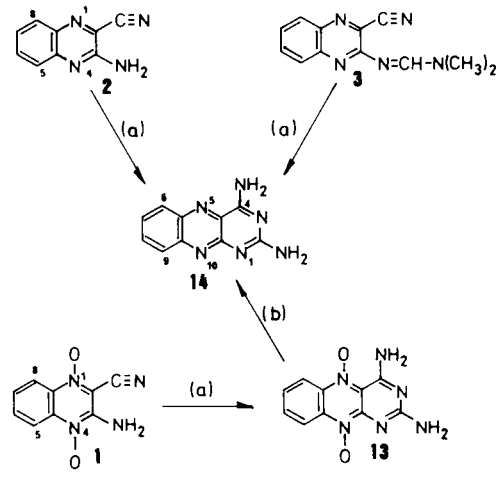
Compounds were obtained, as illustrated in Schemes 1 and 2, starting with 3-amino-2-quinoxalinecarbonitrile 1,4-dioxide **1** [2] and 3-aminoquinoxalinecarbonitrile **2**, obtained from **1** as previously reported [2]. Reaction of **2** with dimethylformamide and phosphorus oxychloride gave **3** (68%). Treating **3** with a boiling solution of hydroxylamine in methanol the dioxime **4** was obtained (54%). On the other hand, treating **2** with boiling hydrazine hydrate we obtained **5** (68%), mp = 196-198°. The ^{1}H-nmr spectra (DMSO-d_6) of **5** shows broad signal for two =NH groups at about δ = 7.20-7.40 (1H) and δ = 8.70-9.00 (1H), indicating that **5** is 3-amino-2-quinoxaline carbohydrazide imide; its tautomer, 3-amino-2-quinoxalinecarboxamide hydrazone, mp = 296-297°, has been previously reported by us [1] and its ^{1}H-nmr spectra (DMSO-d_6) shows broad signals for three -NH_2 groups at δ = 5.89 (2H), 6.09 (2H) and 7.72 (2H). Both compounds show very similar ir spectra, but quite different ^{1}H-nmr spectra.

In a similar way, we have reported [1] that treating **1** with boiling hydrazine hydrate, we obtained 3-amino-2-quinoxalinecarboxamide hydrazone 4-oxide, mp = 213-217°. The ir spectra (potassium bromide) of this compound showed the characteristic band at about 1350 cm^{-1}

SCHEME 1



SCHEME 2



(a): NH_2-C(=NH)-NH_2

(b): $Na_2S_2O_3$

for the 4-oxide in these compounds [1], and the ^{1}H-nmr spectra (DMSO-d_6) of these compounds showed, as expected, three characteristic broad signals for three $-NH_2$ group at about δ = 6.00 (2H), 6.08 (2H) and 7.90 (2H). However, when this reaction was repeated, apparently under the same conditions, some time later, we obtained a new compound, with the same mp = 214-216°, practically the same ir spectra, including the above mentioned band for 4-oxide, but rather different ^{1}H-nmr spectra. This spectra (DMSO-d_6) shows two characteristically broad signals for two =NH groups at about δ = 6.70 (1H) and 9.20 (1H); on the basis of these results, this compound is 3-amino-2-quinoxalinecarbohydrazide imide 4-oxide, **6**, the tautomer of the compound previously reported [1]. We do not know the reasons why we obtained one or the other tautomer apparently under the same experimental conditions.

Reactions of **6**, on the one hand, and **2**, **3**, **4** or **5**, on the other hand, with hydroxylamine under different conditions, gave **7** (42%) and **8** (38-56%), respectively. The reaction of **1** with dimethylformamide and phosphorus oxychloride, under similar conditions, as reported above for **2** → **3**, gave **9** (66%). The ir spectra of this compound show a characteristic band at about 1220 cm^{-1} for the 1-oxide group, but not any other band at about 1350 cm^{-1} for a 4-oxide group, as characteristic of these compounds [1]. Therefore, the reaction brings out a selective monodesoxygenation of the 4-oxide group. Selective monodeoxygenation of quinoxaline 1,4-dioxide derivatives on 1-oxide or 4-oxide groups are well known [1,3,4].

Reaction of **9** with two moles of hydroxylamine in boiling methanol gave **10** (58%). On the other hand, reaction of **9** with one mole of hydroxylamine in pyridine/2-propanol/water gave **11** (56%). In this case, hydrolysis of the dimethylaminomethylene group took place. Compound **11** was also obtained treating **10** with one mole of hydrazine hydrate. The ir spectra of both compounds shows the characteristic band for 1-oxide in this type of compounds at about 1220 cm^{-1} for **10** and 1200 cm^{-1} for **11**.

Condensation of **1** with quanidine in boiling methanol gave **13** (80%); its ir spectra show two bands at about 1350 and 1200 cm^{-1} characteristics for the 4- and 1-oxide groups, respectively, and the ^{1}H-nmr spectra show two singlets at about δ = 7.58 (2H) and 8.12 (2H) for two $-NH_2$ groups. Reduction of **13** with sodium dithionite gave **14** (52%). This compound was also obtained by the reactions of **2** and **3** with guanidine with yields of 88% and 42% respectively.

Compounds **4**, **7**, **8**, **11**, **12** to a concentration of 0.5 mmole and compound **14** to a concentration of 0.25 mmole were tested as inhibitors of the blood platelet aggregation induced by arachidonic acid (AA), adenosine-5'-diphosphate (ADP) or collagen. In these experiments whole blood from the guinea pig was used and the assays were carried out according to a previously reported method [6], using a Crono-Log aggregometer. No significant activity was found in any case with the exception of compound **7** and **14**, which inhibited 42% and 33% respectively the platelet aggregation induce by AA, and ADP, respectively.

On the other hand, compounds **4**, **5**, **6**, **10**, **13** and **14** were studied to a dose of 30 mg/Kg as antihypertensive agents using male Kyoto-Wistar spontaneously hypertensive rats (SHR), according to the previously reported procedures [7]. The percentage drops in arterial pressure was measured in the tail of the animals, using a W + W Electronic Register. The following compounds were significantly active, and the percent of the drop in the arterial pressure observed is given in brackets for 1, 4, 5 and 24 hours after dosing: **5** (39, 29, 32, 34%); **6** (40, 37, 35, 29%); **13** (38, 29, 26, 30%). The rest of the compounds were inactive in the test. More detailed studies on the antihypertensive activity of these compounds are being performed.

EXPERIMENTAL

Melting points were determined with a Reichert Microscope on a hot plate and they are uncorrected. Elemental analyses were obtained from vacuum-dried samples (over phosphorus pentoxide at 1-2 mm Hg, 12 hours, at about 50-60°. Infrared spectra were recorded on a Perkin-Elmer 681 apparatus, using potassium bromide discs and the frequencies are expressed in cm^{-1}. The ^{1}H (200 MHz) and ^{13}C (50 MHz) spectra were obtained on a Bruker AC-200E instrument, at a concentration of about 0.1 g/ml and dimethyl sulfoxide-d_6 as the solvent; the chemical shifts are reported in ppm from tetramethylsilane and are given in δ units. The abbreviations are the usual.

Thin-layer chromatography (tlc) was carried out on silica gel

(HF, 254-366, Merck or DSF-5, Cammaga, 0.3 mm thickness) with toluene:dioxane:acetic acid (90:25:4 v/v) as the solvent and the plates were scanned under ultraviolet light, λ = 254 and 366 nm. Solvents were usually removed under vaccum in a rotatory evaporator.

The following compounds were obtained according to previously reported methods:

3-Amino-2-quinoxalinecarbonitrile 1,4-Dioxide **1**.

mp, 253° dec [1,5]; and 3-amino-2-quinoxalinecarbonitrile, **2** mp, 201-203° [1].

3-(Dimethylaminomethylene)amino-2-quinoxalinecarbonitrile, **3**.

Phosphorus oxychloride (5 ml) was added dropwise to ice-cooled and stirred dimethylformamide (20 ml). The ice-bath was removed and the solution allowed to reach room temperature. Then this solution was added dropwise to a stirred and ice-cooled solution of **2** (2.0 g, 10 mmoles) in dimethylformamide (10 ml). The mixture was stirred for 2 hours and then poured cautiously on crushed ice and neutralized with ammonium hydroxide. The solid material was collected, washed with water and recrystallized, mp 158-160° (green coloured brillant crystals, ethanol), yield 1.53 g (68%); ir: 2220, 1620, 1570, 770; ^{1}H-nmr: 3.25 (s, 3H, CH_3), 3.33 (s, 3H, CH_3), 7.57 (m, 1H, H-6), 7.80 (m, 2H, H-7, H-8), 8.22 (m, 1H, H-5), 8.76 (s, 1H, -N=CH-).

Anal. Calcd. for $C_{12}H_{11}N_5$: C, 64.00; H, 4.89; N, 31.11. Found: C, 63.69; H, 4.83; N, 30.91.

3-(Hydroxyiminomethyl)amino-2-quinoxalinecarboxamide Oxime, **4**.

Method A.

To a solution of sodium methoxide, obtained dissolving sodium (0.23 g, 10 mmoles) in dried methanol (25 ml), hydroxylamine hydrochloride (0.70 g, 10 mmoles) was added in small portions and under stirring. The precipitate of sodium chloride was removed by filtration and the filtrate added to a solution of **3** (1.0 g, 5 mmoles) in methanol (25 ml). The mixture was boiled for 4 hours, then cooled, and the precipitate collected, washed with water and recrystallized, mp 212-214° (dark yellow coloured crystals, formamide/water, 1:1, v/v), yield 1.33 g (54%); ir: 3480-3320, 3200-2800, 1550, 760; ^{1}H-nmr: 7.60-7.80 (bs, 2H, NH_2), 8.15 (d, 1H, CH=N-O), 7.60-8.31 (m, 4H, H-5, H-6, H-7, H-8), 10.61 (s, 1H, N-OH), 10.81 (s, 1H, N-OH), 11.42 (d, 1H, NH).

Anal. Calcd. for $C_{10}H_{10}N_6O_2$: C, 48.78, H, 4.06; N, 34.15. Found: C, 48.88; H, 4.18; N, 33.89.

3-Amino-2-quinoxalinecarbohydrazide Imide, **5**.

A mixture of **2** (1.70 g, 10 mmoles) and 100% hydrazine hydrate (1.10 g, 22 mmoles) was boiled for 5 hours under stirring. The mixture was cooled and the solid material collected and washed with ethanol and recrystallized, mp 196-198° (dark yellow coloured needles, ethanol), yield 1.37 g (68%); ir: 3390, 3280-3200, 1640-1610; ^{1}H-nmr: 5.91 (s, 2H, N-NH_2), 6.08 (s, 2H, C-NH_2), 7.20-7.40 (bs, 1H, NH), 7.40 (m, 1H, H-6); 7.55 (m, 2H, H-7, H-8), 7.86 (m, 1H, H-5); 8.70-9.00 (bs, 1H, NH).

Anal. Calcd. for $C_9H_{10}N_6$: C, 53.46; H, 4.95; N, 41.58. Found: C, 53.84; H, 4.88; N, 41.25.

3-Amino-2-quinoxalinecarbohydrazine Imide 4-Oxide, **6**.

A mixture of **1** (2.0 g, 1 mmole) and 100% hydrazine hydrate (1.10 g, 22 mmoles) was boiled for 5 hours under stirring. The mixture was cooled and the solid material collected, washed with ethanol and recrystallized, mp = 214-216° (yellow needles, ethanol), yield 1.35 g (62%); ir: 3410, 3300-3100, 1630-1600, 1350; ^{1}H-nmr: 6.10 (s, 2H, N-NH_2), 6.20 (s, 2H, C-NH_2), 6.50-6.80 (bs, 1H, NH), 9.20 (s, 1H, NH), 7.59 (c, 1H, H-7), 7.70 (c, 1H, H-6), 8.00 (d, 1H, H-8), 8.25 (d, 1H, H-5).

Anal. Calcd. for $C_9H_{10}N_6O$: C, 49.54; H, 4.59; N, 38.53. Found: C, 49.12; H, 4.73; N, 38.10.

3-Amino-2-quinoxalinecarboxamide Oxime 4-Oxide, **7**.

To a stirred solution of **6** (2.20 g, 10 mmoles) and pyridine (2.0 ml) in ethanol (25 ml), a solution of hydroxylamine hydrochloride (0.70 g, 10 mmoles) in water (20 ml) was added dropwise and the mixture boiled for 2 hours, and then cooled. The precipitate was collected, washed with water and ethyl ether and recrystallized, mp 252-254° (orange coloured crystalline powder, ethanol), yield 0.92 g (42%); ir: 3380-3350, 3300-3100, 1370, 1600-1590; ^{1}H-nmr: 6.35 (s, 2H, NH_2), 7.60-8.00 (bs, 2H, NH_2), 7.60 (m, 1H, H-7), 7.76 (m, 1H, H-6), 8.02 (m, 1H, H-8), 8.28 (m, 1H, H-5), 10.73 (s, 1H, N-OH).

Anal. Calcd. for $C_9H_9N_5O_2$: C, 49.31; H, 4.11; N, 31.96. Found: C, 49.66; H, 4.14; N, 31.78.

3-Amino-2-quinoxalinecarboxamide Oxime, **8**.

Method A.

To a stirred solution of **2** (1.70 g, 10 mmoles) and pyridine (2.0 ml) in methanol (50 ml), heated at about 70°, a solution of hydroxylamine hydrochloride (0.70 g, 10 mmoles) in water (20 ml) was added dropwise, and then the mixture boiled for 6 hours. Most of the solvent (about 2/3) was removed in vacuum and the residual solution was cooled. The precipitate was collected, washed with water and ethyl ether and recrystallized, mp 263-265° (cottony dark yellow coloured crystals, ethanol/2-propanol, 1:1 v/v), yield 1.05 g (52%); ir: 3490-3390, 3200-2800, 1650-1580; ^{1}H-nmr: 6.20 (s, 2H, NH_2), 7.53 (s, 2H, NH_2), 7.40 (m, 1H, H-6), 7.50-7.70 (m, 2H, H-7, H-8), 8.88 (m, 1H, H-5), 10.57 (s, 1H, N-OH).

Anal. Calcd. for $C_9H_9N_5O$: C, 53.20; H, 4.43; N, 34.48. Found: C, 52.93; H, 4.53; N, 34.28.

Method B.

To a boiling solution of **5** (2.0 g, 10 mmoles) in ethanol (50 ml) a solution of hydroxylamine hydrochloride (0.70 g, 10 mmoles) in water (30 ml) was added dropwise. Further, the mixture was boiled for 4 hours and then cooled. The precipitate was collected and washed and recrystallized as above described in Method A, yield 1.07 g (56%).

Method C.

From **3** (1.1 g, 5 mmoles), pyridine (2 ml), ethanol (25 ml), hydroxylamine hydrochloride (0.70 g, 10 mmoles) and water (20 ml) in a similar way as above described from **2** in Method A, yield 0.87 g (43%).

Method D.

To a solution of **2** (1.70 g, 10 mmoles) in dried methanol (25 ml) a solution of sodium methoxide, prepared dissolving sodium (0.46 g, 20 mmoles) in dried methanol (40 ml), was added. To this boiling mixture, a solution of hydroxilamine hydrochloride (0.70 g, 10 mmcles) was added dropwise. Subsequently the mixture was boiled for 3 hours and then cooled, and the precipitate collected and washed with water, then with 0.1*M* sodium hydroxide, and

finally with water again, and recrystallized as in Method A, yield 0.77 g (38%).

Method E.

A mixture of **4** (1.23 g, 5 mmoles) and 100% hydrazine hydrate (0.30 g, 6 mmoles) was boiled under stirring for 4 hours. The mixture was cooled, the solid material collected and washed with cold ethanol and recrystallized as in Method A, yield 0.95 g (47%).

3-(Dimethylaminomethylene)amino-2-quinoxalinecarbonitrile 1-Oxide, **9**.

From **1** in a similar way as above described to obtain **3** from **2**, mp 139-141° (orange coloured crystals, ethanol/2-propanol, 1:1, v/v), yield 1.60 g (66%); ir: 2220, 1620, 1570, 1220, 750; ^{1}H-nmr: 2.96 (s, 3H, CH_3), 3.08 (s, 3H, CH_3), 8.57 (s, 1H, N=CH), 7.39-8.06 (m, 4H, H-5, H-6, H-7, H-8).

Anal. Calcd. for $C_{12}H_{11}N_5O$: C, 59.75; H, 4.56; N, 29.05. Found: C, 59.46; H, 4.47; N, 28.75.

3-(Hydroxyiminomethyl)amino-2-quinoxalinecarboxamide Oxime 1-Oxide, **10**.

This compound was obtained from **9** (1.20 g, 5 mmoles) in a similar way as above reported to obtain **4** from **3**, mp 203-205° (yellow coloured crystals, dimethylformamide/water; 1:1; v/v), yield 1.52 g (58%); ir: 3420-3300, 3100-2800, 1520, 1220, 770; ^{1}H-nmr: 6.40-6.80 (bs, 1H, NH_2); 8.35 (d, 1H, CH=N-O), 7.69-8.02 (m, 4H, H-5, H-6, H-7, H-8), 10.0 (d, 1H, NH), 10.64 (s, 1H, N-OH), 10.75 (s, 1H, N-OH).

Anal. Calcd. for $C_{10}H_{10}N_6O_3$: C, 45.80; H, 3.82; N, 32.06. Found: C, 45.32; H, 3.71; N, 31.87.

3-Amino-2-quinoxalinecarboxamide Oxime 1-Oxide, **11**.

Method A.

To a stirred and boiling solution of **9** (2.41 g, 10 mmoles) and pyridine (2.0 ml) in 2-propanol (25 ml), a solution of hydroxylamine hydrochloride (0.70 g, 10 mmoles) in water (10 ml) was added dropwise. Subsequently, the mixture was boiled for 4 hours, cooled and diluted with water (20 ml), and 0.2*M* hydrochloric acid (5 ml) was added. The precipitate was collected and washed with water and then with ethyl ether and recrystallized, mp 237-239° (orange coloured brillant crystals, ethanol), yield 1.23 g (56%); ir: 3460-3400, 3300-2800; 1640-1590; 1200; ^{1}H-nmr: 6.33 (s, 2H, NH_2), 7.60-7.90 (bs, 2H, NH_2), 7.63 (m, 1H, H-6), 7.75 (m, 1H, H-7), 8.00 (d, 1H, H-8), 8.26 (s, 1H, H-5), 10.70 (s, 1H, N-OH).

Anal. Calcd. for $C_9H_9N_5O_2$: C, 49.31; H, 4.11; N, 31.96. Found: C, 49.38; H, 4.19; N, 31.69.

Method B.

From **10** (1.31 g, 5 mmoles) and hydrazine hydrate (0.30 g, 6 mmoles) as above reported to obtain **8** from **4**, yield 0.72 g (36%).

3-Amino-2-quinoxalinecarboxamide Oxime 1,4-Dioxide, **12**.

Method A.

From **1** (2.0 g, 10 mmoles) and hydroxylamine hydrochloride (0.70 g, 10 mmoles) in a similar way as above reported to obtain **8** from **2** in Method A. However, the mixture was not boiled, but heated at about 70° for 8 hours. After heating the precipitate was collected washed with water and recrystallized, mp 222-224° (yellow colored needles, ethanol), yield 1.31 g (56%); ir: 3410-3300, 3100-2700, 1630-1590, 1340, 1250; ^{1}H-nmr: 6.40-6.80 (bs, 2H, NH_2), 7.65 (s, 2H, NH_2), 7.70 (m, 1H, H-6), 7.90 (m, 1H, H-7), 8.32 (m, 1H, H-8), 8.40 (m, 1H, H-5), 10.46 (s, 1H, N-OH).

Anal. Calcd. for $C_9H_9N_5O_3$: C, 45.96; H, 3.83; N, 29.78. Found: C, 46.08; H, 3.88; N, 30.02.

Method B.

From **1** (2.0 g, 10 mmoles) and hydroxylamine hydrochloride (1.40 g, 20 mmoles) in a similar way as above reported to obtain **8** from **2** in Method D. However, the reaction mixture was not boiled, but stirred at room temperature for 2 hours, yield 1.07 g (46%).

2,4-Diaminopyrimido[5,4-*b*]quinoxaline 5,10-Dioxide, **13**.

Guanidine hydrochloride (2.10 g, 21.5 mmoles) was added to a stirred solution of sodium methoxide, obtained dissolving sodium (1.36 g, 59 mmoles) in dried methanol (90 ml). The precipitate of sodium chloride was removed by filtration. To the solution **1** (1.5 g, 9 mmoles) was added, and the mixture boiled for 9 hours. After cooling, the solid material was collected, washed with water and ethyl ether and recrystallized, mp >300° (pale dark yellow coloured crystals, dimethylformamide), yield 2.05 g (84%); ir: 3300-3050, 1650, 1350, 1220, 760; ^{1}H-nmr: 7.58 (s, 2H, NH_2), 8.12 (s, 2H, NH_2), 7.58 (m, 1H, H-8), 7.87 (m, 1H, H-7), 8.41 (m, 1H) and 8.43 (m, 1H) for H-6, H-9.

Anal. Calcd. for $C_{10}H_8N_6O_2$: C, 49.18; H, 3.28; N, 34.43. Found: C, 48.91; H, 3.39; N, 34.78.

2,4-Diaminopyrimido[4,5-*b*]quinoxaline, **14**.

Method A.

From **2** (1.81 g, 9 mmoles) in a similar way to that above reported to obtain **13** from **1**. However, the reaction mixture was boiled for 10 hours. The solvents were removed under vacuum and the residue recrystallized, mp >300° (yellow coloured brillant crystals, dimethylformamide), yield 1.86 g (88%); ir: 3480-3100, 1640, 750; ^{1}H-nmr: 6.80-7.15 (bs, 2H, NH_2), 8.10 (s, 2H, NH_2), 7.64 (m, 1H, H-8), 7.84 (m, 1H, H-7), 8.02 (m, 1H) and 8.06 (m, 1H) for H-6, H-9.

Anal. Calcd. for $C_{10}H_8N_6$: C, 56.60; H, 3.77; N, 39.62. Found: C, 56.21; H, 3.82; N, 39.36.

Method B.

From **3** (2.2 g, 10 mmoles) in a similar way to that above reported to obtain **13** from **1**. However the reaction mixture was boiled for 15 hours, yield 0.89 g (42%).

Method C.

To a boiling solution of **13** (1.2 g, 5 mmoles) in methanol, a solution of sodium dithionite (2.5 g, 15 mmoles) in water (10 ml) was added dropwise. Subsequently the reaction mixture was boiled for 4 hours. Solvents were removed in vacuum and the residual material washed with water and with ethyl ether, yield 1.10 g (52%).

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