CONDENSED *as*-TRIAZINE III : SYNTHESIS OF 2-AMINO-6-AZAPURINE (3-AMINOIMIDAZO[4,5-*e*]-*as*-TRIAZINE) AS A POTENTIAL ANTITUMOR AGENT

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Abstract- 2-Amino-6-azapurine, an aza analogue of guanine, has been prepared from its 3,5,6-triamino-*as*-triazine precursor and triethyl orthoformate under acidic conditions. 3-Amino-6-bromo-*as*-triazin-5-one was chosen as a proper starting material for the synthesis of this precursor. Thus, reaction of 3-amino-6-bromo-*as*-triazin-5-one with liquid ammonia and a catalytic amount of fine copper powder gave 3,6-diamino-*as*-triazin-5-one which was then thiated with phosphorous pentasulfide in hexamethylphosphoric triamide to yield 3,6-diamino-*as*-triazin-5-thione. Methylation of 3,6-diamino-*as*-triazin-5-thione with methyl iodide under basic conditions gave 3,6-diamino-5-methylthio-*as*-triazine which was treated with methanolic ammonia to afford 3,5,6-triamino-*as*-triazine.

INTRODUCTION

Aza analogues of purine have been of interest in recent years because such agents

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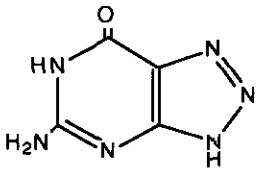
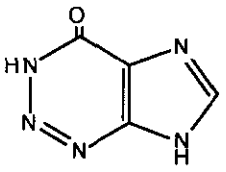
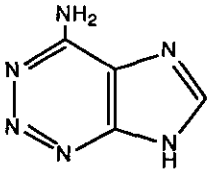
have shown a wide spectrum of chemotherapeutic and biological properties.[1] For example, 8-azaguanine, an analogue of guanine in which the C8 carbon has been replaced by a nitrogen atom, was one of the first modified purine to display notable carcinostatic effects against murine malignancies.[2] Other azapurines such as 2-azaadenine and 2-azahypoxanthine were also proved to be the growth inhibitors of both microbial and mammalian cells.[3]



8-azaguanine



2-azahypoxanthine



2-azaadenine

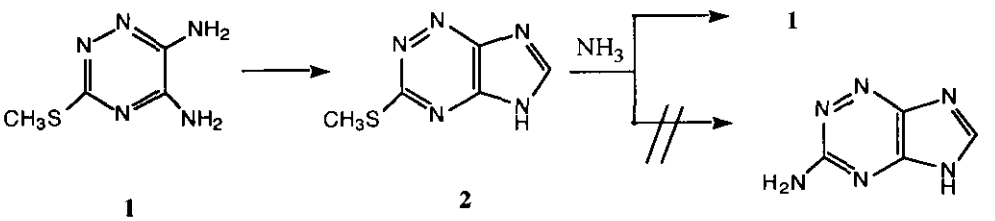
Though the isomeric 6-azapurine (imidazo[4,5-*e*]-*as*-triazine) ring system is of interest from a chemical and biological point of view, only few reports on this system appeared in the literature. Kaji and Kawase described the synthesis of *N*-substituted 6-azapurines by the cyclization of 5,6-diamino-*as*-triazin-3-(2*H*)-ones with one-carbon delivering reagents.[4] Yoneda *et al.* have prepared *N*-alkyl-6-azapurines *via* a benzilic acid type rearrangement of certain 7-azalumazines (pyrimido[4,5-*e*]-*as*-triazines).[5] To synthesize parent 6-azapurine ring free from any *N*-substitution, we explored an efficient, practical route for the preparation of 5,6-diamino-*as*-triazines[6] and their cyclization with triethyl orthoformate.[7] 2-Methylthio-6-azapurine and its analogues were unambiguously obtained in a good yield. According to Skinner *et al.*, the group substituted in the 6-position may be modified considerably with retention of biological activity, other changes in the purine nucleus have resulted in compounds which are inactive against the growth of most microorganisms.[8] Lateron, a number of the more effective 6-substituted purine analogues were prepared by introducing an amino group in the 2-position and found to possess activity in the pteridine-inhibited bacterial system.[9] A study on the structure-activity relationships has also revealed that the substitution on the purine ring at position C2 with other than hydrogen, amino, or substituted amino

group usually resulted in a loss of antitumor activity.[10] These results prompted us to prepare 2-amino-6-azapurine and provide for an aza analogue of guanine which maintained the integrity of the purine nucleus except the changeable 6-position.

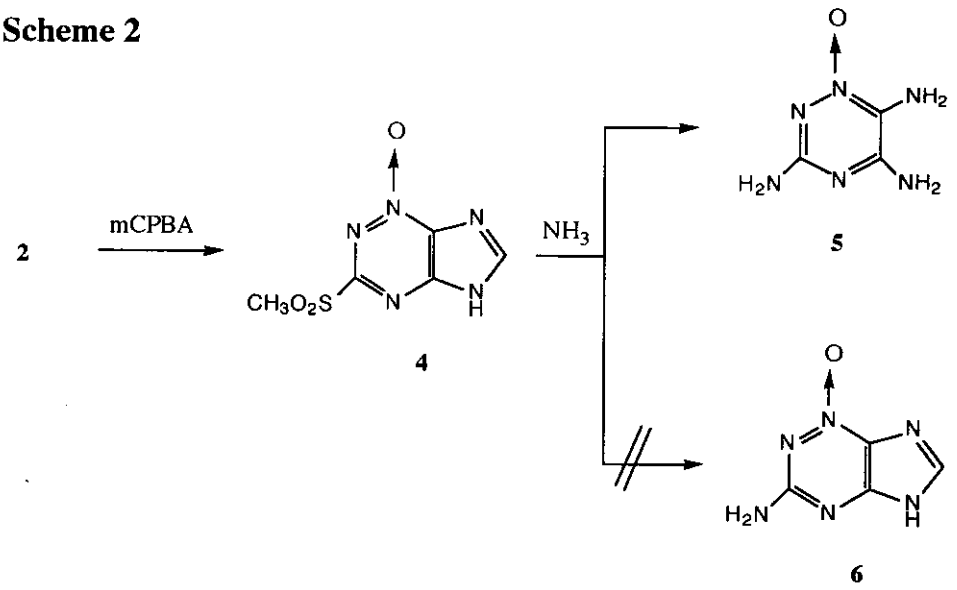
RESULTS AND DISCUSSION

The most straightforward synthesis of the target compound is the ammonolysis of 3-methylthioimidazo[4,5-*e*]-*as*-triazine (2) which was synthesized *via* ring closure of 5,6-diamino-3-methylthio-*as*-triazine (1) with triethyl orthoformate (TEOF).[7] Reaction of 2 with liquid ammonia at 90 °C for 24 h furnished a solid mass which was identified (tlc, nmr) as ring opening compound instead of the desired product (Scheme 1). The rupture of imidazole ring must be proceeded in preference to the replacement of methylthio group, leading to the formation of **1**. To enhance the replacement, 2 was oxidized with *m*-chloroperoxybenzoic acid (mCPBA) to give 3-methylsulfonylimidazo[4,5-*e*]-*as*-triazine (4) which has a very good leaving group, methylsulfonyl, and an electron-withdrawing *N*-oxide to facilitate nucleophilic substitution (Scheme 2). Reacting 4 with liquid ammonia gave 3,5,6-triamino-*as*-triazine *N*-oxide (5), with no 3-aminoimidazo[4,5-*e*]-*as*-triazine *N*-oxide (6) was detected. The alterative pathway for a more efficient preparation of 4 and 5 is depicted in Scheme 3. Oxidation of 1 with mCPBA in acetonitrile gave 5,6-diamino-3-methylsulfonyl-*as*-triazine *N*-oxide(7) in 73% yield. The site of *N*-oxidation was assigned by the comparison of ^{1}H and ^{13}C nmr spectra of 7 and its precursor. The ^{1}H and ^{13}C nmr spectra of certain *as*-triazine *N*-oxides when compared with those of the starting *as*-triazines to determine the site of *N*-oxidation were previously

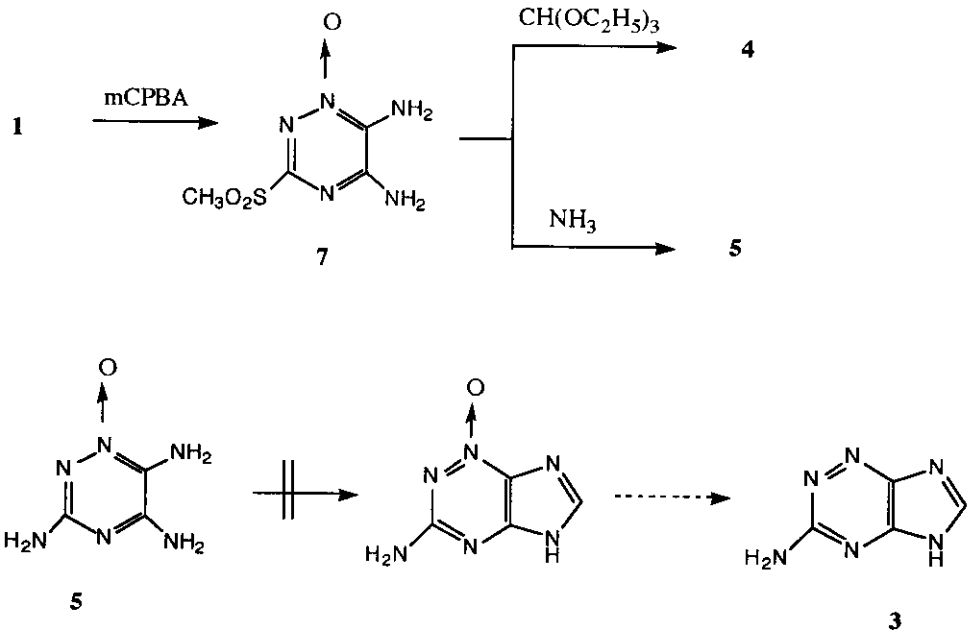
Scheme 1



Scheme 2



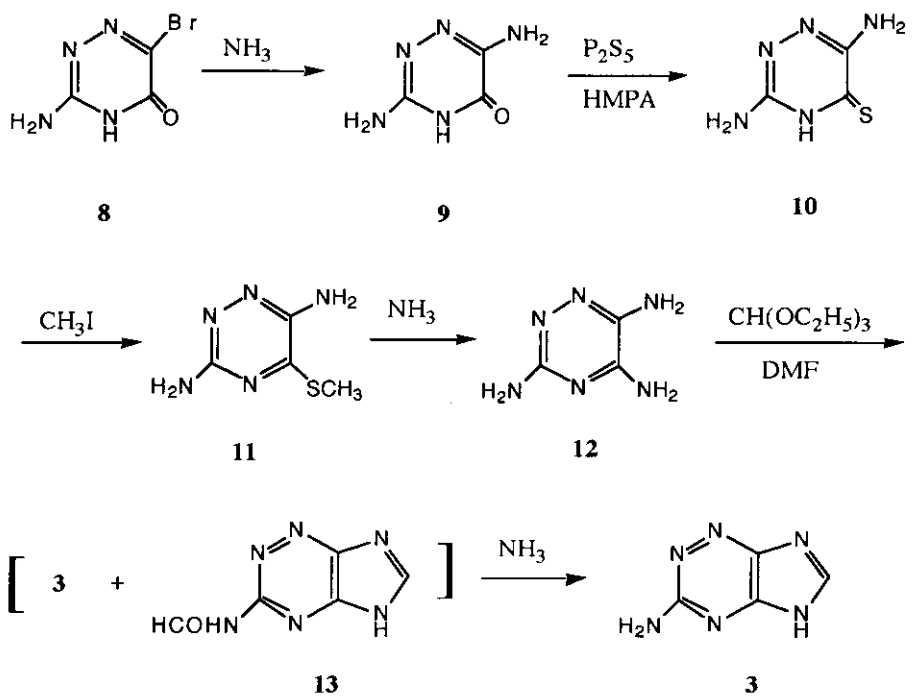
Scheme 3



described.[11] Ring closure of 7 with TEOF afforded compound (4) in 65% yield. Compound (5) was obtained by reacting 7 with liquid ammonia in 72% yield. Attempts to achieve the ring closure of 5 with TEOF to give 6 under different

reaction conditions were not successful. Since the replacement of methylthio substituent by amino group at C3 position of *as*-triazines seems to be very difficult and due to the fact that 3,5,6-triamino-*as*-triazine was a key intermediate for the synthesis of the target compound, we decided to explore another synthetic route. From the literature survey,[12] we found 3-amino-6-bromo-*as*-triazin-5-one (8), bearing an amino group at C3 position of *as*-triazine, a proper starting material for this research project. Thus, 8 was treated with liquid ammonia and fine copper powder in a high pressure autoclave at 80 °C for two days to afford 3,6-diamino-*as*-triazin-5-one (9) in 82% yield. Thiation of 9 with phosphorous pentasulfide in pyridine as described in the literature[6] led to the formation of a complicated mixture. Recently, Smolder *et al.*[13] reported the preparation of some 9-acridonethiones by treatment of the corresponding 9-oxo analogues with phosphorous pentasulfide in hexamethyl phosphoric triamide (HMPA). Shridhar *et al.*[14] applied sodium hydrogen carbonate as a basic additive in Smolders' procedure

Scheme 4



resulted in both rapid and selective thiation of the lactam carbonyl function to give the desired thiolactams. Following Shridhar's procedure, we were able to obtain 3,6-diamino-*as*-triazin-5-thione (**10**) from **9** in 87% yield (Scheme 4). Methylation of **10** with methyl iodide gave 3,6-diamino-5-methylthio-*as*-triazine (**11**) which was then treated with methanolic ammonia to give 3,5,6-triamino-*as*-triazine (**12**) in a good overall yield. Ring closure of **12** with TEOF under acidic conditions as described in the literature[7] was not successful due to the poor solubility of **12** in TEOF. To circumvent this situation, a mixed solvent of TEOF and dimethylformamide (DMF) in equal volume was used in stead of a neat TEOF. The cyclization product thus obtained was a mixture of **3** and 2-formamido-6-azapurine (**13**) [^{1}H nmr: 8.86 (s, 1H, C_8H), 9.40(dd, J=2.0 and 7.0 Hz ,1H, HCO), 11.29 (dd, J=2.0 and 7.0 Hz, 1H, C_2-NH)] which were very difficult to be separated and purified. Therefore, the initial product was treated with methanolic ammonia to cleave the formamide bond furnished pure **3** in 38% yield.

EXPERIMENTAL

Melting points were determined with a Thomas-Hoover apparatus and are uncorrected. The ultraviolet spectra were determined in 0.1 N HCl (pH 1), methanol and 0.1 N NaOH (pH 13) with a Hitachi-U-2000 spectrophotometer. Nuclear magnetic resonance (^{1}H and ^{13}C) spectra were recorded on a Varian VSR-300S spectrometer. All the samples were dissolved in deuteric dimethyl sulfoxide (DMSO-d_6) and the chemical shifts are expressed in parts per million with respect to tetramethylsilane (TMS) as an internal standard. The progress of reaction was followed by thin layer chromatography(tlc) on silica gel 60 F-254 plates purchased from E. Merck. Mass spectra were determined with a Quattro VG-5022 mass spectrometer in the electron-impact(EI) mode. Elemental analyses were preformed by Heraeus CHN-O-Rapid elemental analyzer.

3-Methylsulfonylimidazo[4,5-*e*]-*as*-triazine *N*-oxide(**4**)

Method A: mCPBA (0.73 g, 4.2 mmol) in acetonitrile (8 ml) was added slowly to a

suspension of 2 (0.17 g, 1.0 mmol) in acetonitrile (12 ml). The mixture was stirred at room temperature for 30 min to give the crystalline solid which was collected and recrystallized from methanol to afford 4 (0.14 g) in 65% yield. mp >300 °C, ^{1}H nmr: δ 3.08 (s, 3H, SO_2CH_3), 9.21 (s, 1H, CH). Anal. Calcd for $C_5H_5N_5O_3S$: C,27.91; H, 2.34; N, 32.54; S,14.90. Found: C, 28.03; H, 2.37; N, 32.46; S, 14.82.

Method B: To a well stirred suspension of 7 (0.21 g, 1.0 mmol) in triethyl orthformate (10 ml, 60 mmol) was added concentrated hydrochloric acid (0.4 ml). The reaction mixture was then heated at reflux (oil bath, 130 °C) for 4 h. The reaction was allowed to cool to room temperature and the resulting precipitate was collected and crystallized from methanol to give 4 (0.14 g) in 65% yield.

3,5,6-Triamino-*as*-triazine *N*-oxide (5)

Method A: Compound (7) (0.4 g, 1.95 mmol) and liquid ammonia (12 ml) were heated at 70 °C in an autoclave for 2 days. After the reaction cooled, excess ammonia was vented off to give a residual solid which was collected by filtration, washed with cold, absolute ethanol, and recrystallized from H_2O to give 5 (0.2 g) in 72 % yield. mp>300 °C, ^{1}H nmr: δ 5.52(s, 2H, C3-NH_2), 5.80 (s, 2H, C6-NH_2), 7.21(s, 2H, C5-NH_2); ^{13}C nmr: δ 124.93 (C-6), 151.96 (C-5), 156.60 (C-3). Anal. Calcd for $C_3H_6N_6O$: C,25.35; H, 4.26; N, 59.13. Found: C, 25.17; H, 4.43; N, 58.89.

Method B: The same reaction conditions were applied except that the starting heterocycle was compound (4) (0.22 g, 1.0 mmol) to give 5 (0.1 g, 70% yield) after a work-up procedure as described above.

5,6-Diamino-3-methylsulfonyl-*as*-triazine *N*-oxide (7)

mCPBA (3.7 g, 22 mmol) in acetonitrile (40 ml) was added slowly to a suspension of 1 (0.78 g, 5 mmol) in acetonitrile (60 ml). The mixture was stirred at room temperature for 30 min and the resulting precipitate was collected and crystallized from methanol to give 7 (0.74 g) in 73% yield. mp >300 °C, ^{1}H nmr: δ 3.20 (s, 3H, SO_2CH_3), 6.92 (s, 2H, C6-NH_2), 7.79 (br s, 2H, C5-NH_2); ^{13}C nmr: δ 132.37 (C-6),

150.47 (C-5), 154.09 (C-3). Anal. Calcd for $C_4H_7N_5O_3S$: C,23.41; H, 3.44; N, 34.13; S,15.63. Found: C, 23.32; H, 3.68; N, 33.98; S, 15.37.

3,6-Diamino-*as*-triazin-5-one (9).

A mixture of 3-amino-6-bromo-*as*-triazin-5-one (**8**) (57.3 g, 0.30 mol), copper powder (3 g) and liquid ammonia (150 ml) was heated in a glass-lined, stainless steel reaction vessel at 80 °C for 2 days. After the reaction was cooled, the excess ammonia was vented off, and the residual solid was suspended in distilled water (1000 ml). To which was added 0.1 N NaOH till clear solution obtained and then filtered. The filtrate was acidified to pH 7-8 with concentrated HCl. The resulting precipitate was collected and crystallized from H_2O to furnish 31.2 g (82%) of **9**. mp > 300 °C; uv: (pH 1) λ max 298 (ε 4100), 249 (ε 4400), sh 210 (ε 7500); (MeOH) λ max 328 (ε 4700), sh 246 (ε 5000), 222 (ε 14900); (pH 13) λ max 304 (ε 3900), sh 238 (ε 5000); ^{1}H nmr: δ 5.74 (s, 2H, C3-NH_2), 6.23 (s, 2H, C6-NH_2), 10.90 (br s, 1H, NH); ^{13}C nmr: δ 146.84 (C-6), 155.22 (C-3), 160.01 (C-5). Anal. Calcd for $C_3H_5N_5O$: C, 28.34; H, 3.96; N, 55.10. Found: C, 28.25; H, 3.90; N, 55.02.

3,6-Diamino-*as*-triazin-5-thione(10).

Compound (**9**) (6.35 g , 50 mmol) was added to a suspension of P_2S_5 (13.32 g, 60.0 mmol) and $NaHCO_3$ (12.6 g, 150 mmol) in HMPA (50 ml) and the resulting mixture was vigorously stirred with mechanical stirrer at 100 °C for 4 h. It was then cooled to room temperature and added to ice cold water (1000 ml) when the required product separated as a solid. The solid was collected and crystallized from water to give 6.2 g (87 %) of **10** , mp > 300 °C; uv: (pH 1) λ max 299 (ε 8400), 257 (ε 9000), 215 (ε 18500); (MeOH) λ max 328 (ε 6900), sh 255 (ε 17700), 222 (ε 28600); (pH 13) λ max 354 (ε 12500), 250 (ε 10300); ^{1}H nmr: δ 6.204 (s, 2H, C6-NH_2), 6.778 (s, 2H, C3-NH_2), 11.80 (br s, 1H, N2-H); ^{13}C nmr: δ 149.30 (C-6), 149.65 (C-3), 184.49 (C-5). Ms: m/z 143 (M^+). Anal. Calcd for $C_3H_5N_5S$: C, 25.17; H, 3.52; N, 48.92. Found: C, 25.29; H, 3.54; N, 48.52.

3,6-Diamino-5-methylthio-*as*-triazine(11).

To a stirred suspension of **10** (3.58 g, 25 mmol) in absolute ethanol (200 ml) was added 1N sodium hydroxide solution (25 ml). After stirring at room temperature for 20 min, methyl iodide (6 ml, 96 mmol) was added. The reaction mixture was allowed to stir at room temperature for 4 h (monitored by tlc) and then excess solvent was removed under diminished pressure. The residue was triturated with cold water (10 ml), and extracted with chloroform (5 x 100 ml). The organic layer was dried over anhydrous Na_2SO_4, filtered, and the filtrate concentrated. The yellow solid thus obtained was purified by column chromatography on silica gel with chloroform: methanol (10: 1) as an eluent to yield 2.62 g (67%) of **11**. mp> 300 °C; uv: (pH 1) λ max 294 (ε 8000), 245 (ε 13400); (MeOH) λ max sh 361(ε 4300), 245 (ε 13500); (pH 13) λ max 240 (ε 14000); ^{1}H nmr: δ 2.47 (s, 3H, SCH_3), 5.47 (s, 2H, C6-NH_2), 5.99 (s , 2H, C3-NH_2); ^{13}C *nmr*: δ 10.78 (*SCH*$_3$), 149.80 (C-6), 154.29 (C-5), 157.66 (C-3). Ms: m/z 157 (M^+). Anal. Calcd for $C_4H_7N_5S$: C, 30.56; H, 4.49; N, 44.55. Found: C, 30.44; H, 4.56; N, 44.41.

3,5,6-Triamino-*as*-triazine (12).

Compound (11) (0.47 g, 3 mmol) and methanolic ammonia (30 ml, saturated at 0 °C) were heated in a glass-lined, stainless steel reaction vessel at 70 °C for 24 h, then the solution was evaporated under diminished pressure (to *ca.* 10 ml). The residual solid was collected by filtration and recrystallized from water to give **12** (310 mg, 82%). mp >300 °C; uv: (pH 1) λ max 302 (ε 2400), 229 (ε 15500); (MeOH) λ max 319 (ε 3900), 229 (ε 15100); (pH 13) λ max 319 (ε 4500), sh 240 (7600); ^{1}H nmr: δ 5.22(s, 2H, C6-NH_2), 5.24 (s, 2H, C3-NH_2), 6.75 (br s, 2H, C5-NH_2); ^{13}C nmr: δ 142.54 (C-6), 148.73 (C-5), 157.85 (C-3). HRms m/z : Calcd for $C_3H_6N_6$: 126.0656. Found: 126.0651.

2-Amino-6-azapurine(3)

To a well stirred suspension of **12** (126 mg, 1.0 mmol) in a mixed solvent of triethyl orthoformate (10 ml, 60 mmol) and DMF (10 ml) was added concentrated

hydrochloric acid (0.3 ml). The mixture was then heated at reflux (oil bath) for 4 h. After this period, the solution was filtered and the filtrate was concentrated in vacuo to obtain an orange syrup which was dissolved in methanolic ammonia (25 ml, saturated at 0 °C) and stirred at 40 °C for 16 h to hydrolyze the *N*-formyl group. The resulting solid was removed by filtration and the filtrate was evaporated in vacuo to afford a residue which was purified by flash column chromatography on silica gel using chloroform: methanol (5:1) as an eluent to give **3** (52 mg, 38%). mp >300 °C; uv: (pH 1) λ max 223 (ε 18800); (MeOH) λ max 297 (ε 6500), 218 (ε 15400); (pH 13) λ max 297 (ε 5800); ^{1}H nmr: δ 6.87 (s, 2H, C2-NH_2), 8.47 (s, 1H, CH); ^{13}C nmr: 145.85 (C-5), 147.54 (C-8), 149.25 (C-4), 161.67 (C-2). HRms m/z : Calcd for $C_4H_4N_6$: 136.0499. Found: 136.0493.

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