

The Synthesis and Antimicrobial Screening of Some Novel Aza-Imidoxy Compounds as Potential Chemotherapeutic Agents

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Some novel azaimidoxy compounds viz. 2-[(4-chlorophenyl)diazenyl]oxy-1H-isoindole-1,3-(2H)-dione (Va), and 1-[[1-naphthyl]diazenyl]oxy]pyrrolidine-2,5-dione (IVc), etc. have been synthesized by a simple diazotization reaction followed by a coupling with 2-hydroxy-1H-isoindole-1,3(2H)-dione (III) / 1-hydroxypyrrolidine-2,5-dione (II) of corresponding aromatic primary amine derivatives at a suitable pH. A similar reaction with a [1,3]thiazolo[4,5-b]pyridin-2-amine (VIII) lead us to some interesting results variable with a pH. The structure of all synthesized compounds has been established by IR, ¹H NMR, and mass studies. These compounds have been screened for antimicrobial activities in order to evaluate the possibility of the derivatives to be used as potential chemotherapeutic agents.

Keywords Antimicrobial activities; azaimidoxy; coupling; diazotization; pH; spectral studies

INTRODUCTION

Azoxy derivatives form a very interesting class of compounds because of their significant biological (antibacterial, anticancer, antituberculous, and nematocidal) activities,¹ and their chemistry is consequently now receiving considerable attention. Azoxybacilin,² an unusual amino acid with an azoxy moiety, is active against a broad spectrum of fungi, especially against mycelial fungi, such as *Aspergillus*. Similarly,

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valanimycin, which is a naturally occurring azoxy compound, shows good antibiotic and antibacterial activity. In addition to this, valanimycin also exhibits potent antitumor activity against *in vitro* cell cultures of mouse leukemia L1210, P388/S, and P388/ADR.^{3–9} As part of our studies aimed at the simple and efficient synthesis of heterocyclic compounds possessing phthalimidoxy, imidoxy, or aminoxy moieties, and in continuation of our work,^{10–13} it seemed interesting to synthesize some novel azoxy compounds associated with a imidoxy moiety. In this article, we report the synthesis of novel azaimidoxy compounds of aromatic amines, and sulfur-containing five-membered fused heterocyclic system, and the confirmation of structures of resulting products by spectroscopic data. The newly synthesized azaimidoxy compounds appear to be a promising substrate for biological activity evaluation.

RESULTS AND DISCUSSION

All the synthesized compounds are well supported by their spectral data.

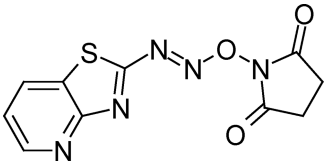
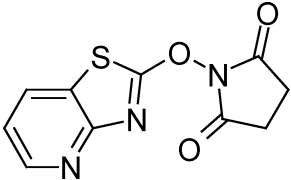
In the present investigation, the diazotization of primary aromatic amines (**I**) was carried out with NaNO_2 and HCl at $0\text{--}5^\circ\text{C}$, then a diazotized product was coupled with imidoxy [2-hydroxy-1*H*-isindole-1,3(2*H*)-dione (**III**)/1-hydroxypyrrolidine-2,5-dione (**II**)] compounds between 4–5 pH. The coupled compound was characterized by the absence of a NH_2 peak at $3450\text{--}3560\text{ cm}^{-1}$ and a OH peak at $2700\text{--}3300\text{ cm}^{-1}$ in IR spectra, which was present in a precursor primary aromatic amine and imidoxy compound, respectively. The product was also confirmed by the absence of a NH_2 peak at δ 4–5 in ^1H NMR spectra (Scheme 1).

In order to synthesize sulfur-containing five-membered fused aromatic heterocyclic azaimidoxy compounds, the diazotization and coupling reaction of [1,3]thiazolo[4,5-*b*]pyridine-2-amine (**VIII**) has been carried out (Scheme 2). It was prepared by a cyclization reaction of 1-pyridin-2-ylthiourea (**VII**) in the presence of a Br_2 and CHCl_3 and was confirmed by the presence of a $\text{C}=\text{N}$ peak at 1646 cm^{-1} in its IR spectrum. [1,3]thiazolo[4,5-*b*]pyridin-2-amine (**VIII**) was also diazotized by its reaction with NaNO_2 and HCl at $0\text{--}5^\circ\text{C}$, but unlike primary aromatic amines, its coupling reaction did not occur in an acidic pH. During its diazotization and coupling reaction with imidoxy compounds, many interesting and important product variations have been obtained (Table 1) when the pH of a reaction was modified. Various results at different pH are discussed:

1. A coupled product was never obtained in an acidic pH.
2. A coupled azaimidoxy product was obtained when the pH was maintained between 8–10.



TABLE I Spectral Characterization

| Coupled product obtained at pH 8–10 | Product obtained at pH 10–12 |
|--|--|
|  <ol style="list-style-type: none"> 1. Due to the chromophoric effect of N=N group, UV obtain at higher wavelength, i.e., 435 nm. 2. An IR peak of N=N group was obtain at 1566 cm^{-1}. 3. A diazonium fragment peak at 163 was obtained in mass spectra. |  <ol style="list-style-type: none"> 1. A UV band was obtained at lower wavelength, i.e., 340 nm. 2. An IR peak of N=N was not obtained. 3. A diazonium fragment peak was absent. |

3. Above pH 8–10, a normal coupled aza compound was obtained with the evolution of N_2 .
4. In a highly basic medium, the thiazole ring breaks with the evolution of N_2 , and colloidal sulfur precipitates out.

Lastly, the presence of a $\text{C}=\text{O}$ peak at $1700\text{--}1711\text{ cm}^{-1}$ and the absence of an OH peak at $2700\text{--}3300\text{ cm}^{-1}$ also confirmed the formation of the final compounds. 2-hydroxy-1*H*-isoindole-1,3(2*H*)-dione has been prepared by the reported method,¹⁴ while 1-hydroxypyrrolidine-2,5-dione has been purchased by commercial resources.

ANTIMICROBIAL SCREENING

All synthesized azaimidoxy compounds were screened for their antibacterial and antifungal activities using ampicillin and fluconazole, respectively, as reference compounds. The test organisms used for antibacterial studies were *Escherichia coli*, *Proteus vulgaris*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. *Aspergillus fumigatus* and *Candida albicans* were used for antifungal studies. The cup or well method developed by Collee and colleagues¹⁵ has been followed. The results have been tabulated in the form of inhibition zones and activity index (Table II). Although all the compounds show less

TABLE II The Results of Antimicrobial Studies of Azaimidoxy Compounds. Zone of Growth Inhibition (mm) (Activity Index)[#]

| Compound no. | Antibacterial activity (300 µg/mL) | | | | | Antifungal activity | | | |
|--------------|------------------------------------|----------|----------|----------|----------|---------------------|----------|----------|----------|
| | | | | | | Org. I | | Org. II | |
| | Org. I | Org. II | Org. III | Org. IV | Org. V | 50 ppm | 100 ppm | 50 ppm | 100 ppm |
| IVa | 13(0.61) | 20(1.00) | 19(0.82) | 13(0.61) | 8(0.34) | 18(1.28) | 26(1.18) | 16(1.33) | 20(1.25) |
| IVb | 12(0.57) | 12(0.60) | 14(0.60) | 15(0.71) | 13(0.56) | 14(1.00) | 19(0.86) | 12(1.00) | 17(1.06) |
| IVc | 15(0.71) | 5(0.25) | 15(0.65) | 4(0.19) | 4(0.17) | 13(0.92) | 23(1.04) | 12(1.00) | 16(1.00) |
| IVd | 10(0.47) | 15(0.75) | 8(0.34) | 16(0.76) | 15(0.65) | 16(1.14) | 21(0.95) | 10(0.83) | 12(0.75) |
| Va | 12(0.57) | 15(0.75) | 9(0.39) | 18(0.85) | 7(0.30) | 16(1.14) | 23(1.04) | 12(1.00) | 14(0.87) |
| Vb | 13(0.61) | 18(0.90) | 17(0.73) | 17(0.80) | 6(0.26) | 18(1.28) | 20(0.90) | 11(0.91) | 15(0.93) |
| Vc | 12(0.57) | 9(0.45) | 15(0.65) | 6(0.28) | 14(0.60) | 17(1.21) | 23(1.04) | 10(0.83) | 14(0.87) |
| Vd | 14(0.66) | 13(0.65) | 14(0.60) | 9(0.42) | 15(0.65) | 15(1.07) | 19(0.86) | 12(1.00) | 17(1.06) |
| IX | 9(0.42) | 6(0.30) | 8(0.34) | 8(0.38) | 18(0.78) | 18(1.28) | 23(1.04) | 14(1.16) | 16(1.00) |
| X | 13(0.61) | 12(0.60) | 7(0.30) | 8(0.38) | 16(0.69) | 17(1.21) | 22(1.00) | 16(1.33) | 20(1.25) |
| Standard | 21 | 20 | 23 | 21 | 23 | 14 | 22 | 12 | 16 |

[#](Activity index)-Inhibition zone of the sample/Inhibition zone of the standard.

For antibacterial activity, Org. I = *Escherichia coli*, Org. II = *Staphylococcus aureus*, Org. III = *Proteus vulgaris*, Org. IV = *Klebsiella pneumonia*, and Org. V = *Pseudomonas aeruginosa*. Standard used = Amicacin.

For antifungal activity, Org. I = *Aspergillus fumigatus* and Org. II = *Candida albicans*. Standard used = Fluconazole.

to moderate activity against bacteria but possess very strong activity against fungi, which reveals that azaimidoxy compounds are better antifungal agents as compared to antibacterial.

EXPERIMENTAL

Melting points of all synthesized compounds were determined in an open capillary tube and are uncorrected. IR spectra (KBr) and ¹H NMR spectra (CDCl₃) were recorded on a Perkin-Elmer 1800 spectrophotometer and DRX-300 (300 MHz) spectrophotometer using TMS as an internal standard, respectively, and mass spectra were recorded on a Jeol SX-102 (FAB) spectrometer. The purity of compounds was checked by elemental analysis and also by TLC using silica gel "G" as an adsorbent, and visualization was accomplished by iodine.

The Synthesis of 2-[(4-Chlorophenyl)diazenyl]oxy}-1*H*-isoindole-1,3-(2*H*)-dione (Va)

4-chloroaniline (0.01 mole) was dissolved in 5*N* HCl and cooled. An ice-cold solution of NaNO₂ (0.01 mole) in water was added gradually,

maintaining a temperature between 0–5°C. A cooled solution of 2-hydroxy-1*H*-isoindole-1,3(2*H*)-dione in diluted NaOH was added to a diazotized reaction mixture between 0–5°C and a pH between 4–5. The reaction mixture was stirred on a water bath for some time, and a resulting solid was filtered, washed with ethanol, and recrystallised from benzene. Yield (80%), m.p. 214°C (found: N, 13.80 C₁₄H₈N₃O₃Cl requires: 13.93; ν_{\max} : 3104 (Ar–H), 1736 (CO–N–CO), 1133 (N–O), 1539 (N=N), 710 (C–Cl) cm^{–1}; δ (ppm): 7.2–7.4 (8H, m, ArH); m/z (%): 301 (27.3) [M⁺], 162 (12.2), 155 (100), 146 (25.5), 139 (45.1), 111 (59.0).

Other compounds (**Vb–Vd**) were also synthesized in a similar way with a slight variation in the concentration of HCl required to dissolve the substituted aniline.

2-{[(4-Methylphenyl)diazenyl]oxy}-1*H*-isoindole-1,3-(2*H*)-dione (**Vb**)

Yield (75%), m.p. 162°C (found: N, 14.80 C₁₅H₁₁N₃O₃ requires: 14.94%); ν_{\max} : 3090 (Ar–H), 2973 (CH₃), 1730 (CO–N–CO), 1120 (N–O), 1520 (N=N) cm^{–1}; δ : 7.0–7.3 (8H, m, ArH), 2.4 (3H, s, CH₃); m/z (%): 281 (22.7) [M⁺], 162 (11.9), 146 (20.8), 135 (100), 119 (32.5), 91 (39.9).

2-{[1-Naphthyl]diazenyl]oxy}-1*H*-isoindole-1,3-(2*H*)-dione (**Vc**)

Yield (62%), m.p. 181°C (found: N, 13.15 C₁₈H₁₁N₃O₃ requires: 13.24%); ν_{\max} : 3088 (Ar–H), 1725 (CO–N–CO), 1123 (N–O), 1524 (N=N) cm^{–1}; δ : 7.0–7.2 (8H, m, ArH); m/z (%): 317 (30.2) [M⁺], 171 (100), 162 (13.4), 155 (32.2), 146 (22.7), 127 (52.3).

2-{[(4-Nitrophenyl)diazenyl]oxy}-1*H*-isoindole-1,3-(2*H*)-dione (**Vd**)

Yield (64%), m.p. 155°C (found: N, 17.88 C₁₄H₈N₄O₅ requires: 17.92%); ν_{\max} : 3106 (Ar–H), 1740 (CO–N–CO), 1540 and 1350 (NO₂), 1140 (N–O), 1542 (N=N) cm^{–1}; δ (8.1 (2H, d, Ar–H near NO₂), 7.2 (6H, m, ArH); m/z (%): 312 (35.9) [M⁺], 166 (100), 162 (16.6), 150 (41.6), 146 (20.9), 122 (60.1).

Compounds (**IVa–IVd**) were synthesized similarly like (**Va**), but 1-hydroxypyrrolidine-2,5-dione(**II**) was used instead of 2-hydroxy-1*H*-isoindole-1,3(2*H*)-dione(**III**).

1-{[(4-Chlorophenyl)diazenyl]oxy}pyrrolidine-2,5-dione (**IVa**)

Yield (74%), m.p. 194°C (found: N, 14.82 C₁₀H₈N₃O₃Cl requires: 14.92%); ν_{\max} : 3020 (Ar–H), 2890 (CH₂), 1705 (CO–N–CO), 1072

(N—O), 1560 (N=N), 720 (C—Cl) cm^{-1} ; δ : 7.2–7.4 (4H, m, ArH), 2.2 (4H, s, CH_2); m/z (%): 253 (32.9) $[\text{M}^+]$, 155 (100), 139 (41.9), 114 (15.7), 111 (64.2), 98 (20.2).

1-{[(4-Methylphenyl)diazenyl]oxy}pyrrolidine-2,5-dione (IVb)

Yield (70%), m.p. 178°C (found: N, 17.85 $\text{C}_{11}\text{H}_{11}\text{N}_3\text{O}_3$ requires: 18.02%); ν_{max} : 3010 (Ar—H), 2970 (CH_3), 2880 (CH_2), 1689 (CO—N—CO), 1020 (N—O), 1540 (N=N) cm^{-1} ; δ : 7.1 (4H, m, ArH), 2.6 (3H, s, CH_3), 2.2 (4H, s, CH_2); m/z (%): 233 (27.6) $[\text{M}^+]$, 135 (100), 119 (41.4), 114 (20.2), 98 (26.2), 91 (44.9).

1-{[1-Naphthyldiazenyl]oxy}pyrrolidine-2,5-dione (IVc)

Yield (62%), m.p. 220°C (found: N, 14.02 $\text{C}_{14}\text{H}_{11}\text{N}_3\text{O}_3$ requires: 14.12%); ν_{max} : 3015 (Ar—H), 2885 (CH_2), 1700 (CO—N—CO), 1040 (N—O), 1550 (N=N) cm^{-1} ; δ : 7.1–7.2 (7H, m, ArH), 2.1 (4H, s, CH_2); m/z (%): 269 (34.3) $[\text{M}^+]$, 171 (100), 155 (21.5), 127 (39.7), 114 (12.8), 98 (20.9).

1-{[(4-Nitrophenyl)diazenyl]oxy}pyrrolidine-2,5-dione (IVd)

Yield (60%), m.p. 174°C (found: N, 20.04 $\text{C}_{10}\text{H}_8\text{N}_4\text{O}_5$ requires: 21.21%); ν_{max} : 3040 (Ar—H), 2890 (CH_2), 1720 (CO—N—CO), 1520 and 1310 (NO_2), 1060 (N—O), 1560 (N=N) cm^{-1} ; δ (ppm): 8.0 (4H, m, ArH), 2.3 (4H, s, CH_2); m/z (%): 264 (22.3) $[\text{M}^+]$, 166 (100), 150 (35.6), 122 (57.9), 114 (11.4), 98 (21.9).

The Synthesis of 1-Pyridin-2-ylthiourea (VII)

2-Amino pyridine (0.01 mole) was dissolved in 1N HCl, and ammonium thiocyanate was added with stirring. The reaction mixture was refluxed for 6 h, and an excess of solvent was removed by reduced pressure. A yellow brown solid obtained was recrystallised from alcohol. Yield (64%), m.p. 182°C (found: N, 27.12 $\text{C}_6\text{H}_7\text{N}_3\text{S}$ requires: 27.43%); ν_{max} : 3380 (NH_2), 3312 (NH), 3010 (Ar—H), 1664 (C=N), 1580, 1500 (C=C), 1262 (C—N), 1212 (C=S) cm^{-1} ; δ (ppm): 7.3 (4H, m, ArH), 7.8 (1H, s, NH), 7.2 (2H, s, NH_2); m/z (%): 153 (42.3) $[\text{M}^+]$, 136 (100), 78 (35.9).

The Synthesis of [1,3]thiazolo[4,5-*b*]pyridin-2-amine (VIII)

1-Pyridine-2-ylthiourea (0.01 mole) in CHCl_3 was cyclized in the presence of Br_2 in CHCl_3 via refluxing on a water bath for 8–10 h. The solvent was removed under reduced pressure, thus, a yellow solid obtained was recrystallized from a chloroform methanol mixture. Yield (57%), m.p. 228°C (found: N, 27.62 $\text{C}_5\text{H}_5\text{N}_3\text{S}$ requires: 27.79%); ν_{max} :

3410 (NH₂), 3016 (Ar—H), 1646 (C=N), 1586 (C=C), 1268 (C—N), 850 (C—S—C) cm⁻¹; δ : 7.7 (2H, s, NH₂), 7.2 (3H, m, ArH); m/z (%): 151 (31.4) [M⁺], 135 (100), 103 (65.8), 77 (42.7).

The Synthesis of 2-{[[1,3]thiazolo[4,5-*b*]pyridin-2-ylldiazenyl]oxy}-1*H*-isoindole-1,3(2*H*)-dione (IX)

[1,3]thiazolo[5,4-*b*]pyridine-2-amine (0.01 mole) was dissolved in dil HCl. An ice-cold solution of NaNO₂ (0.01 mole) in water was added gradually, maintaining temperature between 0–5°C. A cooled solution of 2-hydroxy-1*H*-isoindole-1,3(2*H*)-dione in diluted NaOH was added to a diazotized reaction mixture between 0–5°C and pH between 8–10. The reaction mixture was kept for some time, and a resulting solid was filtered. An orange solid obtained was recrystallized from CHCl₃. Yield (52%), m.p. > 300°C (found: N, 21.42 C₁₄H₇N₅O₃S requires: 21.53%); ν_{\max} : 3069 (Ar—H), 1711 (C=O), 1646 (C=N), 1566 (N=N), 1088 (N—O) cm⁻¹; δ : 7.2–7.4 (7H, m, ArH); m/z (%): 325 (15.9) [M⁺], 179 (100), 163 (35.6), 146 (22.3), 135 (52.4), 77 (15.7).

1-{[[1,3]Thiazolo[4,5-*b*]pyridin-2-ylldiazenyl]oxy}-Pyrrolidine-2,5-dione (X)

It is synthesized in similar manner in which compound (IX) was synthesized but 1-hydroxypyrrolidine-2,5-dione(II) was used for coupling instead of 2-hydroxy-1*H*-isoindole-1,3(2*H*)-dione(III). Yield (55%), m.p. > 300°C (found: N, 25.90 C₁₀H₇N₅O₃S requires: 25.23%); ν_{\max} : 3062 (Ar—H), 2880 (CH₂), 1700 (C=O), 1675 (C=N), 1565 (N=N), 1020 (N—O) cm⁻¹; δ : 7.3 (3H, m, ArH), 2.3 (4H, s, CH₂); m/z (%): 277 (28.1) [M⁺], 179 (100), 163 (31.8), 135 (47.3), 98 (25.7), 77 (20.7).

2-([1,3]Thiazolo[4,5-*b*]pyridin-2-yloxy)-1*H*-isoindole-1,3(2*H*)-dione (XI)

Prepared similarly as compound (IX) with a slight change in pH, i.e., between pH 10–12. Yield (42%), m.p. 273°C (found: N, 13.80 C₁₄H₇N₃O₃S requires: 14.13%); ν_{\max} : 3060 (Ar—H), 1710 (C=O), 1640 (C=N), 1082 (N—O) cm⁻¹; δ : 7.2 (7H, m, ArH); m/z (%): 297 (32.9) [M⁺], 151 (100), 135 (58.6), 146 (32.9), 77 (18.9).

1-([1,3]Thiazolo[4,5-*b*]pyridin-2-yloxy)pyrrolidine-2,5-dione (XII)

Prepared similarly as compound (X) with a slight change in pH, i.e. between pH 10–12. Yield (51%), m.p. 254°C (found: N, 16.65 C₁₀H₇N₃O₃S requires: 16.86%); ν_{\max} : 3052 (Ar—H), 2884 (CH₂), 1710 (C=O), 1645

(C=N), 1073 (N—O) cm^{-1} ; δ : 7.2 (3H, m, ArH), 2.2 (4H, s, CH_2); m/z (%): 249 (25.8) [M^+], 151 (100), 135 (40.9), 98 (28.7), 77 (19.6).

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