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Synthesis of a series of new Schiff bases having heterocyclic moiety and their microbial activity

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Heterocyclic compounds were synthesized using water based method. These were synthesized by reacting different aldehydes namely benzaldehyde, 2-chloro benzaldehyde, 4-chloro benzaldehyde, 2-nitro benzaldehyde and 3-nitro benzaldehyde with different heterocyclic amines like 4-amino phenazone and 4-amino-1,2,4-trizole. This method constitute an energy-efficient and environmentally benign greener chemistry version of the classical condensation reactions for Schiff base formation. These compounds were characterized by IR, ^{1}H NMR and ^{13}C NMR spectroscopic techniques. The colored Schiff bases (**1**, **3**, **5**, **7** and **9**) were also characterized by UV spectra. Synthesized anils were screened for their microbial activity against *Mesorhizobium* sp. (SB 271), *S. aureus, E. coli* and *Pseudomonas* sp. (PGPR 3). Maximum growth was inhibited by compounds **6** and **8** against *Mesorhizobium* sp. Compounds **1**, **3**, **4** and **10** showed maximum inhibited growth for *S. aureus.* Maximum *Pseudomonas* sp. growth was inhibited by compounds **2**, **4** and **9**. Maximum *E. coli* growth was inhibited by the compounds **2**, **5** and **10**. However, these compounds showed less microbial activity as compared to streptomycin except the compound **9** recorded higher microbial activity than streptomycin against *Pseudomonas* at 5000 ug/ml. All other anils including this **9** exhibited less activity than streptomycin at all the concentrations.

Keywords: Anils, heterocyclic moiety, water based method, UV, IR, ^{1}H NMR, ^{13}C NMR, microbial activity.

Introduction

Schiff bases are used as substrate in the preparation of a large amount of bioactive and industrial compounds. It has been suggested that azomethine linkage (C=N) might be responsible for their biological activities[1]. Schiff base approach is one of the most promising. These are associated with diverse biological activities like antibacterial, antifungal, antitubercular, anticancer and herbicidal properties[2–4]. Heterocyclic chemistry is one of the most interesting and rapidly growing areas of chemical research. These compounds are organic compounds that have a ring structure containing hetero atoms such as nitrogen, oxygen or sulphur in addition to carbon.

Carbon-nitrogen double bond is widely distributed in nature, in the form of biological compounds which are important for the vital metabolism of the living organisms and in various synthetic compounds of agricultural, industrial and pharmaceutical importance. Imine formation is of major importance in biological processes as many of these involve the initial binding of carbonyl compounds to an enzyme through imine formation. Anils are the condensation products of primary amines and aldehydes. Imines due to presence of carbon-nitrogen double bond in their molecules provide a potential site for both chemical and biological activities. This method constitutes an energy-efficient and environmentally benign greener chemistry version of the classical condensation reactions for Schiff bases formation. Water is used as a solvent for the synthesis of Schiff bases[5] because it offers several advantages like inexpensiveness, noninflammable, nontoxic, easily available and safe to use and also its unique physical and chemical properties which increase the reactivity or selectivity[6]. Moreover use of water as solvent is undoubtedly the best alternative as there are generally no harsh reaction conditions and no need of vigorous drying of the solvent[7–12].

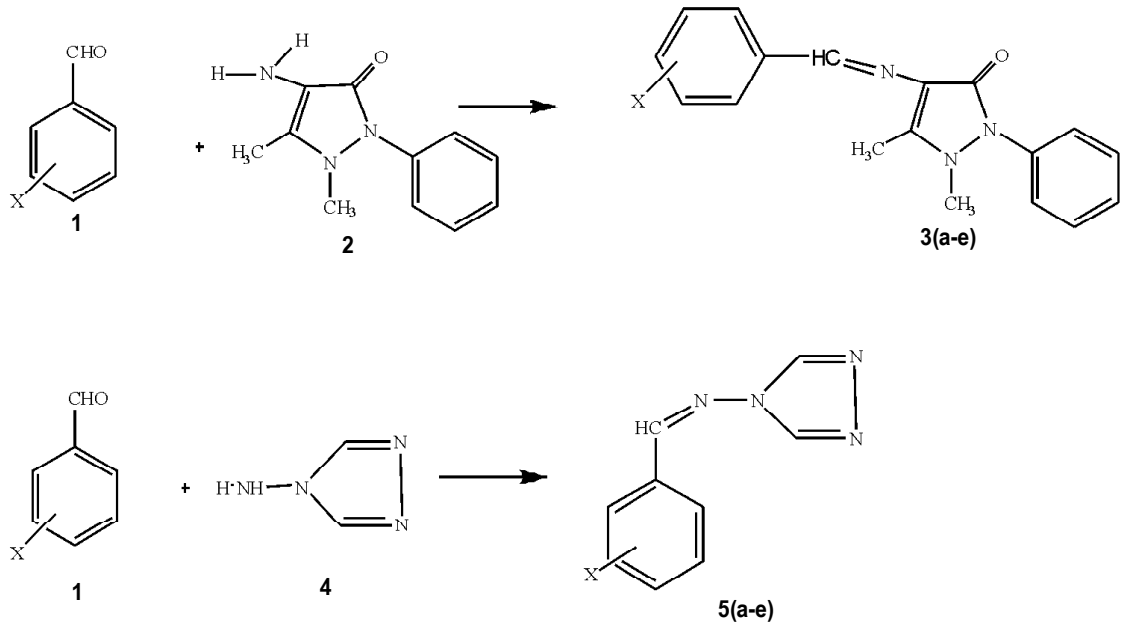
Results and discussion

Different substituted benzaldehydes were made to react with different heterocyclic amines by water based method (Scheme 1) to yield corresponding anils **3(a-e)**, **5(a-e)** whose structures were confirmed by IR, ^{1}H NMR and ^{13}C NMR spectra. Anils **3(a-e)** having different colors were also characterized by UV spectra. The UV absorption was found in the range of 284–354 nm (HC=N-). In IR spectra an absorption was found in the range of 1670–1685 (C=N stretching), 2962–3038 (C-H stretching), 1654–1704 (C=O stretching) and 1591–1630 cm^{-1} (C=C stretching). In H^{1} NMR, two singlets appeared in the range of 7.26–7.54 due to azomethine hyrdrogens (CH=NH). In ^{13}C NMR the signal of azomethines carbons (CH=NH) of all the anils were appeared in the range of 159.45–167.34 C_1 (-*C*H=N). Physical data (yield, melting point, state and colour) of anils was determined.

Microbial testing:

The synthesized anils viz. **3(a-e)** and **5(a-e)** were screened for their inhibitory effect on the growth of four bacterial species viz. *Mesorhizobium* sp. (SB 271), *E. coli*, *S. aureus* and *Pseudomonas* sp. (PGPR 3) at various concentrations.

The results of screening of anils **3(a-e)** and **5(a-e)** against bacteria are shown in Tables 3, 4, 5 and 6. Maximum *Mesorhizobium* sp. growth was inhibited by compounds **3b, 5a, 5c** and **5d** showing inhibition zones at 13 and 15 mm. Maximum *E. coli* growth was inhibited by compounds **5a, 3c** and **5e** showing inhibition zones at 19, 16 and 14 mm. Maximum *S. aureus* growth was inhibited by compounds **3a, 3b** and **5e** showing inhibition zone 15 mm. Maximum *Pseudomonas* sp. growth was inhibited by compounds **5a** and **3e** showing inhibition zone 17 mm. This inhibition may be attributed to substitution of nitro and chloro group on phenyl ring. So overall it was observed that the compounds having substitution of nitro and chloro groups on phenyl ring exhibited more inhibition of bacteria as compared to compounds with no substitution.



X = H, 2-Cl, 4-Cl, 2-NO_2, 3-NO_2

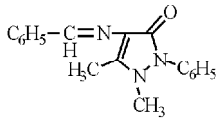
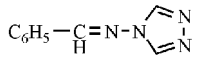
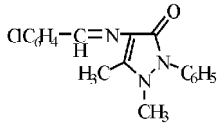
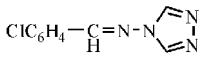
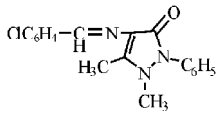
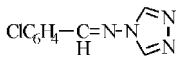
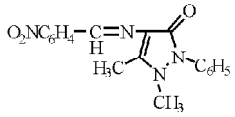
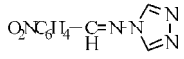
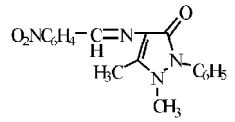
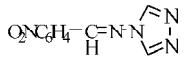
Scheme I

Experimental

General:

Open capillaries methods were used to determine the melting points and are uncorrected. IR, ^{1}H NMR and ^{13}C NMR spectra were got scanned from Sophisticated Analytical Instrumentation Facility (SAIF), Central Instrument Laboratory (CIL), Panjab University, Chandigarh. IR Spectra were

Table 1. Physical data of different anils

Sr. No.	Structure of compound	Molecular formula	Color	Melting point (°C)	Time (min)	Yield (%)	R_f
3a		$C_{18}H_{17}N_3O$	Yellowish brown	180–183	37	78	0.69
5a		$C_9H_8N_4$	Off white	166–167	39	88	0.80
3b		$C_{18}H_{17}N_3ClO$	Light yellow	180–182	30	74	0.62
5b		$C_9H_8N_4Cl$	White	120–122	36	82	0.75
3c		$C_{18}H_{17}N_3ClO$	Light yellow	210–212	33	94	0.68
5c		$C_9H_8N_4Cl$	White	160–163	31	80	0.79
3d		$C_{18}H_{17}N_4O_3$	Yellow	215–217	32	95	0.70
5d		$C_9H_8N_5O_2$	White	119–121	38	80	0.76
3e		$C_{18}H_{17}N_4O_3$	Orange	215–218	35	97	0.73
5e		$C_9H_8N_5O_2$	Off white	130–132	37	80	0.78

*Physical state of all the synthesized products is solid.

recorded on Perkin-Elmer FTIR spectrophotometer with λ_{max} in cm^{-1}. ^1H NMR and ^{13}C NMR spectra were recorded on Bruker Avance II 400 spectrophotometer using TMS as internal reference. UV spectra of coloured compounds were recorded on UV 2600 spectrophotometer Techcomp using chloroform as solvent. The chemical shifts were expressed in δ (ppm) values and the abbreviations 's' and 'm' stand for singlet and multiplet respectively. The purity of the compound was checked on silica gel G coated TLC plates and the visualization was done in iodine chamber.

General procedure for the preparation of anils:

Different substituted benzaldehydes (0.005 mol) were made to react with various heterocyclic amines (0.005 mol) in water (10 ml) and stirred for various intervals of times (30–39 min) at room temperature. The crude product was filtered, washed with water and dried. The formation and purity of the compound was checked by thin layer chromatography (Scheme I).

Table 2. Microbial activity of different anils on the growth of *Mesorhizobium* sp. at different concentrations (ug/ml)

Compounds	Diameter of growth inhibition zone (mm)							
	5000	4000	3000	2000	1000	500	250	125
3a	12	12	7	7	6	0	0	0
5a	13	12	8	7	6	0	0	0
3b	13	8	7	6	0	0	0	0
5b	10	9	8	7	6	0	0	0
3c	10	13	8	7	7	0	0	0
5c	15	13	12	9	6	0	0	0
3d	12	13	8	7	7	0	0	0
5d	15	14	13	9	8	0	0	0
3e	9	8	7	7	6	0	0	0
5e	10	9	8	7	7	0	0	0
Streptomycin	21	19	18	17	16	15	13	12

Table 3. Microbial activity of different anils on the growth of *E. coli* at different concentrations (ug/ml)

Compounds	Diameter of growth inhibition zone (mm)							
	5000	4000	3000s	2000	1000	500	250	125
3a	10	11	11	10	8	0	0	0
5a	19	19	11	10	9	0	0	0
3b	10	12	12	9	0	0	0	0
5b	11	13	11	10	11	0	0	0
3c	16	12	10	11	9	0	0	0
5c	8	10	10	10	8	0	0	0
3d	9	10	10	10	10	0	0	0
5d	13	12	13	12	10	0	0	0
3e	12	12	11	10	10	0	0	0
5e	14	12	11	11	9	0	0	0
Streptomycin	21	19	18	17	16	15	13	12

Table 4. Microbial activity of different anils on the growth of *S. aureus* at different concentrations (ug/ml)

Compounds	Diameter of growth inhibition zone (mm)							
	5000	4000	3000	2000	1000	500	250	125
3a	15	10	10	9	8	7	0	0
5a	10	9	9	8	7	7	0	0
3b	15	14	15	9	8	8	0	0
5b	14	13	12	11	9	11	0	0
3c	9	9	9	8	8	7	0	0
5c	9	8	8	9	8	6	0	0
3d	12	10	9	8	7	0	0	0
5d	13	11	10	9	9	8	0	0
3e	9	7	7	6.5	0	0	0	0
5e	15	12	11	10	9	8	0	0
Streptomycin	21	19	18	17	16	15	13	12

Table 5. Microbial activity of different anils on the growth of *Pseudomonas* sp. (PGPR 3) at different concentrations (ug/ml)

Compounds	Diameter of growth inhibition zone (mm)							
	5000	4000	3000	2000	1000	500	250	125
3a	0	0	0	0	0	0	0	0
5a	17	15	10	9	8	0	0	0
3b	0	0	0	0	0	0	0	0
5b	15	11	7	6.5	6	0	0	0
3c	14	12	10	9	7	0	0	0
5c	12	10	9	8	0	0	0	0
3d	0	0	0	0	0	0	0	0
5d	0	0	0	0	0	0	0	0
3e	17	12	9	7	0	0	0	0
5e	13	9	8	7	0	0	0	0
Streptomycin	16	15	15	14	13	13	12	11

Biological study:

The newly synthesized Schiff bases were screened for their microbial activity against different bacteria viz. *Mesorhizobium* sp., *S. aureus*, *E. coli* and *Pseudomonas* sp.

Bacterial activity:

The synthesized compounds **3(a-e)**, **5(a-e)** were tested for their effect on growth of selected bacteria viz. *Mesorhizobium* sp. (SB 271), *E. coli*, *S. aureus* and *Pseudomonas* sp. (PGPR 3). Method of screening for bacteria was filter paper disc method. Nutrient agar medium was used for *E. coli*, *S. aureus*[13], King's B medium for *Pseudomonas* sp.[14], Congo red yeast extract mannitol agar (CRYEMA) for *Mesorhizobium* sp. The medium was sterilized by autoclaving at 15 psi and 121°C for 20 min. The plates were prepared by pouring 15–20 ml of the sterilized media on sterilized petri plates and were allowed to solidify at room temperature. After keeping the plates for two days to ensure sterility, 3–4 days old broth of test organism was spread on specific media plates. The stock solutions of test compounds were prepared by dissolving in minimum quantity of acetone and then in water. It was further diluted with water to make the desired concentrations. Sterile filter paper discs moistened with test compound solution (in water) of different concentration were placed on the inoculated plates under sterile conditions. Disc moistened with water only served as control. The plates were incubated at 28±1°C for 24 h and the diameter of growth inhibition zone (mm) was measured.

4-(Benzylideneamino)-1,5-dimethyl-2-phenyl-1H-pyrazol-3(2H)-one ***(3a)****:*

Yield: 78%; m.p. 180–183°C; UV-Visible (λ_{max}): 284 and 332 nm (HC=N-); IR (KBr): 3037 (C-H aromatic stretching), 2923 (C-H aliphatic stretching), 1675 (C=N stretching), 1669 (C=O stretching), 1650 (C=C stretching), 1378 (C-N stretching), 904 (=C-H bending), 757 (C-H bending) and 693 cm^{-1} (C=C bending); ^{1}H NMR (DMSO): δ 2.49 (3H, s, C*H*$_3$), 3.15 (3H, s, C*H*$_3$), 9.75 (1H, s, N=C-*H*), 7.26–7.50 (10H, m, Ar-*H*); ^{13}C NMR (DMSO); δ 9.81 (CH_3), 35.31 (-N-CH_3), 167.59 (-*C*H=N), 159.75 (O=*C*-C-N-), 151.99 (CH_3-*C*=C), 116.89 (-*C*-N=CH), 121.84–134.49 (m, *C*-Ar).

N-Benzylidene-4H-1,2,4-trizol-4-amine ***(5a)****:*

Yield: 88%; m.p. 166–167°C; IR (KBr): 3192 (C-H aromatic stretching), 3088 (C-H aliphatic stretching), 1555 (C=N stretching), 1516 (C=C stretching), 1335 (C-H stretching), 1189 (N-N stretching), 1061 (C-N stretching), 977 (=C-H bending), 716 (C-H bending) and 637 cm^{-1} (C=C bending); ^{1}H NMR (DMSO): δ 7.97 (2H, s, CH_2), 7.19–7.48 (5H, m, Ar-H), 8.12 (1H, s, N =C-H); ^{13}C NMR (DMSO): 149.08 (N=C-N), 189.10 (-*C*H=N), 128.02–133.94 (m, *C*-Ar).

4-(2-Chlorobenzylideneamino)-1,5-dimethyl-2-phenyl-1H-pyrazol-3(2H)-one ***(3b)****:*

Yield: 74%; m.p.180–182°C; UV-Visible (λ_{max}): 288 and 333 nm (HC=N-); IR (KBr): 3053 (C-H aromatic stretching), 2935 (C-H stretching of aliphatic group), 1672 (C=N stretching), 1668 (C=O stretching), 1648 (C=C stretching), 1380 (C-N stretching), 1132 (C-Cl stretching), 905 (=C-H bending), 762 (C-Cl bending), 750 (C-H bending) and 698 cm^{-1} (C=C bending); ^{1}H NMR (DMSO): δ 2.50 (3H, s, CH_3), 3.18 (3H, s, CH_3), 10.16 (1H, s, N=C-H), 7.26–7.49 (9H, m, Ar-H); ^{13}C NMR (DMSO): δ 9.61 (CH_3), 35.10 (-N-CH_3), 159.45 (-*C*H=N), 152.28 (O=*C*-C-N-), 150.44 (CH_3-*C*=C), 116.77 (-*C*-N=CH), 124.54-136.60 (m, *C*-Ar).

N-(2-Chlorobenzylidene)-4H-1,2,4-trizol-4-amine ***(5b)****:*

Yield: 82%; m.p.120-122°C; IR (KBr): 3107 (C-H aromatic stretching), 2352 (C-H stretching of aliphatic group), 1663 (C=N stretching), 1507 (C=C stretching), 1338 (C-H stretching), 1211 (N-N stretching), 1054 (C-N stretching), 1130 (C-Cl stretching), 969 (=C-H bending), 755 (C-Cl bending), 716 (C-H bending) and 682 cm^{-1} (C=C bending); ^{1}H NMR (DMSO): δ 7.46 (2H, s, CH_2), 7.19–7.24 (4H, m, Ar-H), 7.86 (1H, s, N=C-H); ^{13}C NMR (DMSO): 143.87 (N=C-N), 153.44 (-*C*H=N), 133.3 (-C-Cl), 127.02–130.01 (m, *C*-Ar).

4-(4-Chlorobenzylideneamino)-1,5-dimethyl-2-phenyl-1H-pyrazol-3(2H)-one ***(3c)****:*

Yield: 94%; m.p. 210–220°C; UV-Visible (λ_{max}): 289 and 333 nm (HC=N-); IR (KBr): 3059 (C-H aromatic stretching), 2938 (C-H aliphatic stretching), 1676 (C=N stretching), 1670 (C=O stretching), 1649 (C=C stretching), 1378 (C-N stretching), 1164 (C-Cl stretching), 946 (=C-H bending), 767 (C-Cl bending), 748 (C-H bending) and 698 cm^{-1} (C=C bending); ^{1}H NMR (DMSO): δ 2.48 (3H, s, CH_3), 3.16 (3H, s, CH_3), 9.72 (1H, s, N=C-H), 7.26–7.50 (9H, m, Ar-H); ^{13}C NMR (DMSO): δ 9.63 (CH_3), 35.22 (-N-CH_3), 161.37 (-*C*H=N), 152.28 (O=*C*-C-N-), 151.97 (CH_3-*C*=C), 116.63 (-*C*-N=CH), 121.85–136.26 (m, *C*-Ar).

N-(4-Chlorobenzylidene)-4H-1,2,4-trizol-4-amine ***(5c)****:*

Yield: 80%; m.p. 160–163°C; IR (KBr): 3120 (C-H aromatic stretching), 2934 (C-H stretching of aliphatic group), 1582 (C=N stretching), 1520 (C=C stretching), 1353 (C-H stretching), 1203 (N-N stretching), 1056 (C-N stretching), 1171 (C-Cl stretching), 953 (=C-H bending), 759 (C-Cl bending), 728 (C-H bending) and 673 cm^{-1} (C=C bending); ^{1}H NMR (DMSO): δ 8.0 (2H, s,CH_2), 7.33–7.58 (4H, m, Ar-H), 8.21 (1H, s, N =C-H); ^{13}C NMR (DMSO): 148.23 (N=C-N), 191.22 (-*C*H=N), 137.13 (-C-Cl), 128.30–130.70 (m, *C*-Ar).

1,5-Dimethyl-4-(3-nitrobenzylideneamino)-2-phenyl-1H-pyrazol-3(2H)-one ***(3d)****:*

Yield: 95%; m.p. 215–217°C; UV-Visible (λ_{max}): 284 and 335 nm (HC=N-); IR (KBr): 3064 (C-H aromatic stretching), 2944 (C-H stretching aliphatic), 1670 (C=N stretching), 1705 (C=O stretching), 1647 (C=C stretching), 1381 (C-N stretching), 1522 (N=O stretching), 1491 (N⎓O stretching), 955 (=C-H bending), 735 (C-H bending) and 699 cm^{-1} (C=C bending); ^{1}H NMR (DMSO): δ 2.53 (3H, s, CH_3), 3.21 (3H, s, CH_3), 9.81 (1H, s, N=C-H), 7.26–7.59 (9H, m, Ar-H); ^{13}C NMR (DMSO): δ 9.84 (CH_3), 35.05 (-N-CH_3), 161.44 (-*C*H=N), 159.31 (O=*C*-C-N-), 148.31 (CH_3-*C*=C), 119.97 (-*C*-N=CH), 123.95–134.29 (m, *C*-Ar).

N-(3-Nitrobenzylidene)-4H-1,2,4-trizol-4-amine ***(5d)****:*

Yield: 80%; m.p.119–121°C; IR (KBr): 3090 (C-H aromatic stretching), 2970 (C-H aliphatic stretching), 1593 (C=N stretching), 1490 (C=C stretching), 1393 (C-H stretching),

1215 (N-N stretching), 1053 (C-N stretching), 1515 (N=O stretching), 1464 (N⎓O stretching), 977 (=C-H bending), 763 (C-H bending) and 662 cm^{-1} (C=C bending); ^1H NMR (DMSO): δ 7.24 (2H, s, CH_2), 6.88–7.22 (4H, m, Ar-H), 7.92 (1H, s, N=C-H); ^{13}C NMR (DMSO); 138.80 (N=C-N), 156.44 (-*C*H=N), 137.10 (-C-NO_2), 128.43–137.01 (m, *C*-Ar).

1,5-Dimethyl-4-(2-nitrobenzylideneamino)-2-phenyl-1H-pyrazol-3(2H)-one ***(3e)****:*

Yield: 97%; m.p. 215–218°C; UV-Visible (λ_{max}): 284 and 354 nm (HC=N-); IR (KBr): 3072 (C-H aromatic stretching), 2917 (C-H aliphatic stretching), 1672 (C=N stretching), 1700 (C=O stretching), 1649 (C=C stretching), 1385 (C-N stretching), 1520 (N=O stretching), 1486 (N⎓O stretching), 944 (=C-H bending), 758 (C-H bending) and 698 cm^{-1} (C=C bending); ^1H NMR (DMSO): δ 2.47 (3H, s, CH_3), 3.20 (3H, s, CH_3), 10.02 (1H, s, N=C-H), 7.21–7.86 (9H, m, Ar-H); ^{13}C NMR (DMSO): δ 9.81 (CH_3), 34.94 (-N-CH_3), 161.38 (-*C*H=N), 159.16 (O=*C*-C-N-), 152.39 (CH_3-*C*=C), 116.26 (-*C*-N=CH), 119.78–134.15 (m, *C*-Ar).

N-(2-Nitrobenzylidene)-4H-1,2,4-trizol-4-amine ***(5e)****:*

Yield: 80%; m.p. 130–132°C; IR (KBr): 3101(C-H aromatic stretching), 2955 (C-H aliphatic stretching), 1678 (C=N stretching), 1502 (C=C stretching), 1450 (C-H stretching), 1216 (N-N stretching), 1058 (C-N stretching), 1518 (N=O stretching), 1462 (N⎓O stretching), 976 (=C-H bending), 751 (C-H bending) and 687 cm^{-1} (C=C bending); ^1H NMR (DMSO): δ 7.99 (2H, s, CH_2), 7.21–7.59 (4H, m, Ar-H), 7.92 (1H, s, N=C-H); ^{13}C NMR (DMSO); 157.64 (N=C-N), 167.34 (-*C*H=N), 143.96 (-C-NO_2), 128.21–132.51 (m, *C*-Ar).

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