Synthesis, Spectroscopic and Cytotoxic Studies of Biologically Active New Schiff Bases Derived from *p*-Nitrobenzaldehyde

Amjid IQBAL,[a] Hamid Latif SIDDIQUI,*[,a] Chaudhary Muhammad ASHRAF,[b] Mujahid Hussain BUKHARI,[a] and Chaudhary Muhammad AKRAM[a]

[a] *Institute of Chemistry, University of the Punjab; Lahore–54590, Pakistan: and* [b] *Department of Chemistry, Forman Christian College (A Chartered University); Lahore–54600, Pakistan.*

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Thirteen new Schiff bases derived from *p*-nitrobenzaldehyde were synthesized by condensation with the appropriate amines. An unusual reduction of the *p*-nitrobenzaldehyde to the corresponding alcohol was also observed in one of the reactions. The structures of the compounds were identified using spectroscopic techniques. Cytotoxicity for the titled compounds was studied against Brine Shrimp, used as the test animal.

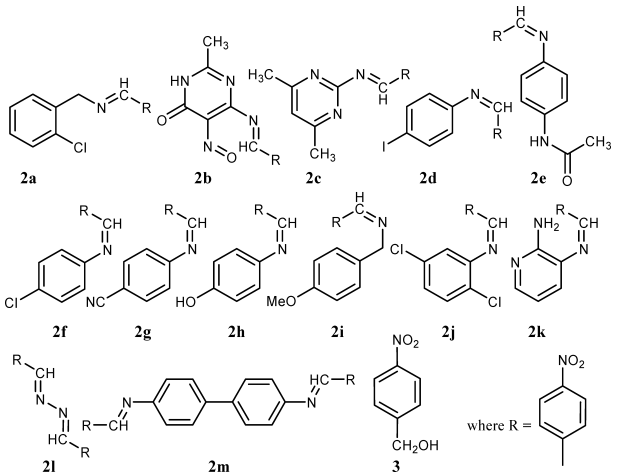
Key words *p*-nitrobenzadehyde; primary amine; unusual aldehyde reduction; biological activity

Schiff bases are potentially biologically active compounds and have been reported to possess antifungal,[1,3] anticancer,[4—7] anticonvulsant[8] and diuretic[9] activities. Schiff bases derived from various heterocycles were reported to possess cytotoxic activity.[10] It is believed that the presence of a nitro group in the *p*-position appears to be an important condition for the development of bacteriostatic activity.[11] Nitroaromatic compounds are widely used in medicine, industry and agriculture.[12] The anti-malarial activity of nitroaromatic compounds was attributed to the formation of reactive oxygen species during flavoenzyme catalyzed redox cycling reactions and/or oxyhemoglobin oxidation.[13,14] With this envision in mind, we synthesized several new Schiff bases derived from *p*-nitrobenzaldehyde by condensation with the appropriate amines. An unusual reduction of the *p*-nitrobenzaldehyde to the corresponding alcohol was also observed in one of the reactions. The structures of the compounds were identified using spectroscopic techniques. Cytotoxicity for the titled compounds was studied against the brine shrimp, used as the test animal. The mortality rate was observed to increase with increasing conc. of the sample.

Results and Discussion

The Schiff bases **2a**—**m** were synthesized by the condensation of *p*-nitrobenzaldehyde with various amines by reaction in hot ethanol using molecular sieves as dehydrating agent. It is known that condensation of amines with aldehydes is favoured by a polar medium.[15] The Schiff bases **2a**—**m** were identified by IR, ^{1}H-NMR and mass spectroscopy. Compounds **2a**—**m** showed in the IR spectra an absorption band at 1618—1690 cm^{-1}, typical of the stretching vibrations of the double C=N bond. The absence of absorption in the regions of 1720 and 3400 cm^{-1} indicates complete transformation of the C=O and NH_2 groups respectively. However, compound **2b** showed absorption at 3404 cm^{-1} due to NH/OH group (different tautomeric forms possible) in the pyrimidine nucleus of the Schiff base. While compounds **2e** and **2h** show absorption at 3438 and 3443 indicating NH group and OH group respectively. Compound **2k** indicates absorption at 3466 showing one amino group unreacted. The ^{1}H-NMR spectra of **2a**—**m** contained multiplet signals due to aromatic protons in the region δ 7.17—8.31 ppm, and singlets at δ 8.15—8.91 ppm from the C–H protons of the CH=N groups.

While condensing 4-nitrobenzaldehyde with 2-amino-4,6-dimethylpyrimidine, an unusual reduction reaction occurred, in addition to the formation of a Schiff base (**2c**), *p*-nitrobenzyl alcohol **3** was isolated from the product mixture in 30% yield. It was identified by IR, NMR and FAB-MS spectroscopy. It is not clear how this compound was formed in the absence of any reducing reagents in the reaction mixture, but possibly a Cannizzaro-type reaction may have occurred. However, no *p*-nitrobenzoic acid, the expected co-product for a Cannizzaro reaction was detected. Possibly hydride transfer from the hemi-aminal intermediate **4** to another molecule of the aldehyde could have occurred as shown in Chart 1, but none of the amide **5** expected as a by-product of this process was detected. The proposed mechanistic aspects have not been fully explored. However, further investigations on the



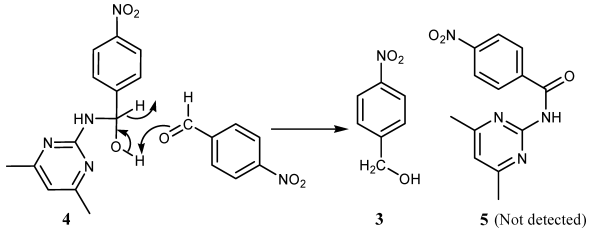


Chart 1. Probable Mechanism for the Formation of *p*-Nitrobenzyl Alcohol

* To whom correspondence should be addressed. e-mail: drhamidlatif@yahoo.com

Table 1. Correlation between Concentration and LC_{50} Value

Compounds	LC_{50} of title compounds at various concentrations		
	50 μg/ml	100 μg/ml	150 μg/ml
2a	1.30	1.25	1.20
2b	1.20	1.10	1.05
2c	2.45	2.40	2.30
2d	1.80	1.65	1.50
2e	2.35	2.35	2.30
2f	2.15	2.05	2.00
2g	2.50	2.45	2.35
2h	2.50	2.50	2.45
2i	2.45	2.45	2.40
2j	2.30	2.25	2.10
2k	1.30	1.20	1.10
2l	2.35	2.30	2.20
2m	2.10	2.00	2.00

mechanism of this reaction are in progress.

Cytotoxic Activity. Test Animal Brine shrimp (*Artemia salina*) was used as the test animal for the investigation of cytotoxicity.

Brine shrimp was kept at ambient temperature and salinity of 30—35 ppm, a pH of 8.0—9.0 and strong aeration under a continuous light regime. Subsequently, about one teaspoon of brine shrimp eggs were added into the systems and approximately after 12 h hatching the phototropic nauplii were collected with a pipette from the lighted side and concentrated in a small beaker and applied for testing.

Preparation of Test Sample For cytotoxicity study, dimethyl sulfoxide (DMSO) was used as a solvent and mortality was observed almost zero. Different concentrations (150, 100, 50 μg/ml) of the test samples were prepared and taken into the separated test tubes, so that each test tube contained not more than 50 μl of DMSO. Now three brine shrimps were transferred to each test tube using micro pipettes.

Counting of Nauplii The test tubes containing different concentrations of test samples were observed and the number of survived nauplii in each test tube was counted. From this the percentage of lethality of brine shrimp nauplii was counted at each concentration for each sample. An approximate linear correlation was observed between the logarithm of concentration and percentage of mortality while the values of LC_{50} were calculated using a simple PC program. Although there was no mortality in the control group, the test samples shown different mortality rate at different concentrations, which was found to increase with increasing concentration of the sample.

Table 1 indicates that compounds **2a**, **2b**, **2d** and **2k** were found to be more effective as compared to others.

Experimental

General All the chemicals were obtained commercially from Lancaster Research Chemicals. ^{1}H-NMR spectra were recorded on a Bruker DPX-400 instrument at 400 MHz. Chemical shifts are reported in ppm referenced to the residual solvent signal. Mass spectra were recorded on a Jeol SX-102 instrument and IR spectra were recorded on a Perkin Elmer Paragon 1000 spectrometer. Elemental analysis was carried out using a Perkin-Elmer 2400-CHN analyzer. Melting points were recorded on a Stuart Scientific-SMP3 apparatus and are uncorrected.

General Procedure for the Synthesis of 2a—k *p*-Nitrobenzaldehyde (1.0 mmol, 0.15 g) was added to respective primary amine (1.0 mmol) in EtOH (absolute, 10 ml) in addition to molecular sieves and refluxed (oil bath at 90 °C) for 4 h under N_2 (g). After filtration, evaporation and recrystallization from EtOH, the yield of the title Schiff base was calculated.

4′-Nitrobenzylidene-(2-chlorobenzyl)amine 2a The yield of the title Schiff base was found to be 80%, mp 76—77 °C, HR-MS (FAB, MH^+): Calcd for $C_{14}H_{11}N_2O_2Cl$ 275.0587, Found 275.0592; IR (ν_{max}, KBr, cm^{-1}): 1647, 1601, 1518, 1345; *Anal.* Calcd for $C_{14}H_{11}N_2O_2Cl$: C, 61.21; H, 4.04; N, 10.19. Found: C, 61.16; H, 4.16; N, 9.93; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.48 (1H, s, CH=N), 8.27—7.17 (8H, m, ArH), 4.95 (2H, s, CH_2N).

4′-Nitrobenzylidene-(6-hydroxy-2-methyl-5-nitrosopyrimidin-4-yl)-amine (2b) Compound **2b** was synthesized following the method described above. The yield of the compound was found to be 60% and mp 115 °C; HR-MS (FAB, MH^+): Calcd for $C_{12}H_9N_5O_4$ 288.0732, Found 288.0726; IR (ν_{max}, DCM, cm^{-1}) 3404 (br), 1689, 1598, 1461, 1409, 1273, 1101; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.32 (1H, s, CH=N), 8.31—8.08 (4H, m, ArH), 3.38 (3H, s, CH_3).

4′-Nitrobenzylidene-(4,6-dimethylpyrimidin-2-yl)amine 2c Compound (**2c**) was synthesized by the method described above. However, in the reaction mixture, two spots were observed on TLC with *Rf* values 0.68 and 0.29 (after 3 elutions of the plate), unlike and otherwise different, from those of the reactants, using the solvent system pet. ether/EtOAc 7 : 3. The two components as Fr. 1 and Fr. 2 were separated by column chromatography in the solvent system pet. ether/EtOAc 4 : 1. Fraction 1 was determined as the title compound **2c** with an *Rf* value 0.69 and Fr. 2 as 4-nitrobenzyl alcohol on the basis of spectroscopic data. The title compound was oil and was obtained in 40% yield; HR-MS (FAB, MH^+): Calcd for $C_{13}H_{12}N_4O_2$ 257.1038, Found 257.1035; IR (ν_{max}, DCM, cm^{-1}): 1682, 1590, 1566, 1450, 1347; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.15 (1H, s, CH=N), 8.12—7.62 (4H, m, ArH), 6.38 (1H, s, ArH), 2.25 (6H, s, 2×CH_3).

The yield of nitrobenzyl alcohol in Fr. 2 was 30% with mp 90 °C (lit.[16] 91—92 °C) the molecular ion peak in the FAB-MS of Fr. 2 showed the MH^+ at *m/z* 153.0426 in agreement with the molecular formula $C_7H_7NO_3$ (Calcd 153.0425); IR (ν_{max}, DCM, cm^{-1}): 3519 (br), 1685, 1602, 1509, 1458, 1402, 1342; ^{1}H-NMR (400 MHz, $CDCl_3$): 8.16—7.59 (4H, m, ArH), 4.77 (2H, s, CH_2).

4-Iodo-*N*-(4-nitrobenzylidene)aniline (2d) Yield of title compound was found to be 83%, mp 204 °C. MS (M^+): Calcd for $C_{13}H_9IN_2O_2$ 351.9709, Found 352.0. IR (ν_{max}, KBr, cm^{-1}); 1624, 1594, 1508, 1470, 1350. Elemental analysis Calcd: C, 44.34; H, 2.58; N, 7.96. Found C, 44.15; H, 2.61; N, 7.90. ^{1}H-NMR (400 MHz): 7.16 (2H, d, *J*=8.6 Hz), 7.79 (2H, d, *J*=8.6 Hz), 8.18 (2H, d, *J*=8.7 Hz), 8.36 (2H, d, *J*=8.8 Hz), and 8.81 (1H, s).

***N*-(4-(4-Nitrobenzylideneamino)phenyl)acetamide (2e)** Yield was found to be 92%, mp 156 °C. MS (M^+): Calcd for $C_{15}H_{13}N_3O_3$ 283.282, Found 283.1. IR (ν_{max}, KBr, cm^{-1}): 3438, 1681, 1597, 1407, 1346. Elemental analysis Calcd: C, 63.60; H, 4.63; N, 14.83. Found C, 63.30; H, 4.69; N, 14.70. ^{1}H-NMR (400 MHz): 3.31 (3H, s), 7.36 (2H, d, *J*=8.7 Hz), 7.70 (2H, d, *J*=8.7 Hz), 8.16 (2H, d, *J*=8.7 Hz), 8.34 (2H, d, *J*=8.6 Hz), and 8.83 (1H, s).

4-Chloro-*N*-(4-nitrobenzylidene)aniline (2f) Yield was found to be 87%, mp 128 °C. MS (M^+): Calcd for $C_{13}H_9ClN_2O_2$ 260.0353, Found 260.1. IR (ν_{max}, KBr, cm^{-1}): 1628, 1598, 1516, 1483, 1343. Elemental analysis Calcd: C, 59.90; H, 3.48; N, 10.75. Found C, 59.60; H, 3.69; N, 10.70. ^{1}H-NMR (400 MHz): 7.37 (2H, d, *J*=8.4 Hz), 7.51 (2H, d, *J*=8.4 Hz), 8.18 (2H, d, *J*=8.8 Hz), 8.36 (2H, d, *J*=8.4 Hz), 8.82 (1H, s).

4-(4-Nitrobenzylideneamino)benzonitirle (2g) Yield was found to be 60%, mp 169 °C. MS (M^+): Calcd for $C_{14}H_9N_3O_2$ 251.0695, Found 251.1. IR (ν_{max}, KBr, cm^{-1}): 1629, 1595, 1489, 1412, 1340; Elemental analysis Calcd: C, 66.93; H, 3.61; N, 16.73. Found C, 66.60; H, 3.69; N, 16.70; ^{1}H-NMR (400 MHz): 7.46 (2H, d, *J*=8.4 Hz), 7.92 (2H, d, *J*=8.4 Hz), 8.20 (2H, d, *J*=8.4 Hz), 8.38 (2H, d, *J*=8.4 Hz), 8.81 (1H, s).

4-(4-Nitrobenzylideneamino)phenol (2h) Yield was found to be 65%, mp 176 °C. MS (M^+): Calcd for $C_{13}H_{10}N_2O_3$ 242.0691, Found 242.1. IR (ν_{max}, KBr, cm^{-1}): 3443, 1624, 1596, 1509, 1440; Elemental analysis Calcd: C, 64.46; H, 4.16; N, 11.56. Found C, 64.60; H, 4.30; N, 11.70; ^{1}H-NMR (400 MHz): 6.83 (2H, d, *J*=1.6 Hz), 7.30 (2H, d, *J*=8.8 Hz), 8.13 (2H, d, *J*=8.8 Hz), 8.33 (2H, d, *J*=8.3 Hz), and 8.80 (1H, s).

1-(4-Methoxyphenyl)-*N*-(4-nitrobenzylidene)methanamine (2i) Yield was found to be 71%, mp 161 °C. MS (M^+): Calcd for $C_{15}H_{14}N_2O_3$ 270.1004, Found 270.0. IR (ν_{max}, KBr, cm^{-1}): 1639, 1608, 1411, 1343; Elemental analysis Calcd: C, 66.66; H, 5.22; N, 10.36. Found C, 66.60; H, 5.30; N, 10.70; ^{1}H-NMR (400 MHz): 3.72 (3H, s), 4.76 (2H, s), 6.87 (2H, d, *J*=8.5 Hz), 7.25 (2H, d, *J*=8.5 Hz), 8.01 (2H, d, *J*=8.8 Hz), 8.29 (2H, d, *J*=8.7 Hz), 8.62 (1H, s).

2,5-Dichloro-*N*-(4-nitrobenzylidene)aniline (2j) Yield was found to be 70%, mp 132 °C. MS (M^+): Calcd for $C_{13}H_8Cl_2N_2O_2$ 293.9963, Found 294.0. IR (ν_{max}, KBr, cm^{-1}): 1627, 1599, 1520, 1468, 1380; Elemental

analysis Calcd: C, 52.91; H, 2.73; N, 9.49. Found C, 52.60; H, 2.90; N, 9.70. ^{1}H-NMR (400 MHz): 7.35 (1H, dd, J=2.3, 8.5 Hz), 7.51 (1H, d, J=2.3 Hz), 7.58 (1H, d, J=6.5 Hz), 8.20 (2H, d, J=8.7 Hz), 8.39 (2H, d, J=8.7 Hz), 8.80 (1H, s).

N^3-(4-Nitrobenzylidene)pyridine-2,3-diamine (2k) Yield was found to be 80%, mp 220 °C. MS (M^+): Calcd for $C_{12}H_{10}N_4O_2$ 242.0804, Found 242.1. IR (ν_{max}, KBr, cm^{-1}): 3466, 1618, 1593, 1508, 1472; Elemental analysis: Calcd: C, 59.50; H, 4.16; N, 23.13. Found C, 59.40; H, 4.60; N, 23.70. ^{1}H-NMR (400 MHz): 6.59 (1H, dd, J=4.9, 7.6 Hz), 7.89 (1H, dd, J=1.4, 4.8 Hz), 8.32 (4H, q, J=8.8 Hz), 8.87 (1H, s).

1,2-Bis(4-nitrobenzylidene)hydrazine (2l) *p*-Nitrobenzaldehyde (2.0 mmol, 0.30 g) was added to hydrazine hydrate (1.0 mmol, 0.049 ml) in EtOH (absolute, 10 ml) in addition to molecular sieves and stirred at room temperature for 5 min under N_2 (g). After filtration and recrystallization from EtOH, the yield of title compound was found to be 89%, mp 178 °C. MS (M^+): Calcd for $C_{14}H_{10}N_4O_4$ 298.2536, Found 298.0. IR (ν_{max}, KBr, cm^{-1}): 1630, 1596, 1522, 1382. Elemental analysis Calcd: C, 56.38; H, 3.38; N, 18.78. Found C, 56.15; H, 3.61; N, 18.90. ^{1}H-NMR (400 MHz): 8.15 (2H, d, J=8.7 Hz), 8.38 (2H, d, J=8.7 Hz), 8.86 (1H, s).

N^4,$N^{4'}$-Bis(4-nitrobenzylidene)biphenyl-4,4′-diamine (2m) *p*-Nitrobenzaldehyde (2.0 mmol, 0.30 g) was added to benzidine (1.0 mmol, 0.184 g) in EtOH (absolute, 10 ml) in addition to molecular sieves and stirred at room temperature for 10 min under N_2 (g). After filtration and recrystallization from EtOH, the yield of title compound was found to be 90%, mp 250 °C; MS (M^+): Calcd for $C_{26}H_{18}N_4O_4$ 450.1328, Found 450.1. IR (ν_{max}, KBr, cm^{-1}): 1628, 1597, 1515, 1341. Elemental analysis Calcd: C, 69.33; H, 4.03; N, 12.44. Found C, 69.15; H, 4.21; N, 12.90. ^{1}H-NMR (400 MHz): 7.48 (2H, d, J=8.1 Hz), 7.83 (2H, d, J=8.2 Hz), 8.21 (2H, d, J=8.4 Hz), 8.38 (2H, d, J=8.3 Hz), 8.91 (1H, s).

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References

1) Safwat H. M., Ragab F. A., Eid N. M., Abdel Gawad. M., *Egyptian J. Pharm. Sci.*, **29**, 99—110 (1988).
2) Metri R., Yadawe M., Patil S. A., *Orient. J. Chem.*, **12**, 101—102 (1996).
3) Hossain M. E., Allam M. N., Begum J., Akbar M. A., Uddin M. N., Smith F. E., Hynes R. C., *Inorg. Chim. Acta*, **249**, 207—213 (1996).
4) Kuz'min V. E., Artemenko A. G., Lozytska R. N., Fedtchouk A. S., Lozitsky V. P., Muratov E. N., Mescheriakov A. K., *SAR and QSAR in Environmental Research*, **16**, 219—230 (2005).
5) Hodnett E. M., Dunn W. J., *J. Med. Chem.*, **13**, 768—770 (1970).
6) Shingare M. S., Ingle D. B., *J. Indian Chem. Soc.*, **53**, 1036 (1976).
7) Jo Y. S., *Hwahak Kwa Kongop Ui Chinbo*, **12**, 432—436 (1972).
8) Tewari S. S., Husain M. I., Srivastava G. C., *J. Indian Chem. Soc.*, **58**, 214—216 (1981).
9) Mishra P., Gupta P. N., Shakya A. K., Shukla R., Srimal R. C., *Indian J. Physiol. Pharmacol.*, **39**, 169—171 (1995).
10) Tarafder M. T., Kasbollah A., Saravan N., Crouse K. A., Ali A. M., Tin O. K., *J. Biochem., Mol. Biol. Biophys.*, **6**, 85—91 (2002).
11) Ma Y., Fan Y., Wang D., Chen Y., Jinan J. D. X., *Ziran Kexueban*, **17**, 292—294 (2003).
12) Purohit V., Basu A. K., *Chem. Res. Toxicol.*, **13**, 673—692 (2000).
13) Vennerstrom L. J., Eaton J. W., *J. Med. Chem.* **31**, 1269—1277 (1988).
14) Docampo R., *Chem. Biol. Interact.* **73**, 1—27 (1990).
15) Reichardt C., "Solvents and Solvent Effects in Organic Chemistry," 2nd ed., Weinheim VCH, 1988.
16) Kotsuki H., Ushio Y., Yoshimura N., Ochi M., *Bull. Chem. Soc. Jpn.*, **61**, 2684—2686 (1988).