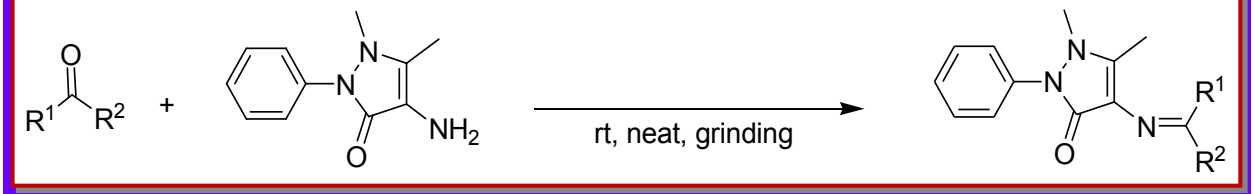

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Synthesis of 4–aminoantipyrine derived Schiff bases and their evaluation for antibacterial, cytotoxic and free radical scavenging activity

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Abstract

The work was aimed to synthesize 4-Aminoantipyrine derived Schiff bases in economical way and to screen it for study the effect of nitro group on its antibacterial potential, conduct anti-tumor preliminary study, the effects of group presence in benzylidene phenyl ring on the cytotoxic potentials and study the effects of electron withdrawing and donating group on anti-oxidant potential. We used green method with 75% reduction in general synthesis time of Schiff bases. Synthesized compound possess antibacterial potentials and nitro group presence enhances this potential. **G2, G3, G4, G5, G6, G7** and **G8** have significant cytotoxic and no significant anti-oxidant activity.

Introduction

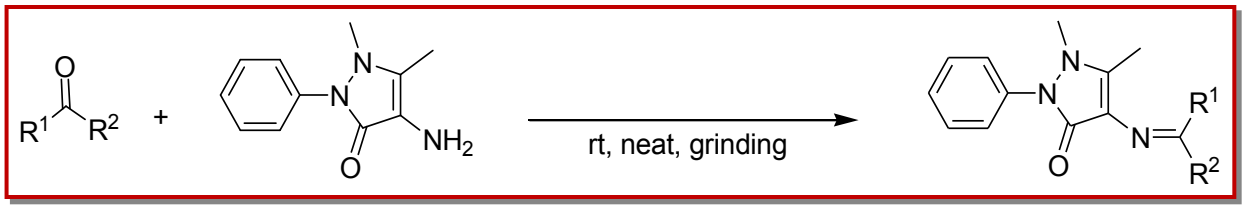
Irrational use of medicine has resulted in resistance to available antimicrobial agents. The resistant microorganisms have resulted in high morbidity and mortality. Schiff bases, the easy synthesis, wide range of pharmacological activities and its azomethane functional group –CN– have tilted research in pharmaceutical and medicinal chemistry (Malladi et al., 2013). They are reported for various pharmacological activities such as bacteriostatic (Karthikeyan et al., 2006), bactericidal (Metzler et al., 1980), antimalarial (Harpstrite et al., 2008), anti-oxidant activities (Thorat et al., 2012), antiviral (Maity et al., 2012), anti-pyretic (Kalaivani et al., 2012), antifungal (Cinarli et al., 2011), insecticidal (Patil et al., 2012), anti-tumor (Venkatesan et al., 2012) and anti-inflammatory (Shivarama et al., 2003). Schiff bases synthesized from aromatic reactants have variety of applications in biological, inorganic and analytical chemistry (Kabak et al., 2000).

4-aminoantipyrine is a pyrazolone derivative, it has shown wide range of biological activities such as antimicrobial activity (Awad et al., 2007), analgesic (Burdulene et al., 1999), antiviral (Evstropov et al., 1992) and also used as precursors in the synthesis of bioactive compounds i.e. β-lactams (Raman et al., 2009).

Schiff bases are reported for antimicrobial, anti-tumor and anti-oxidant potentials herein we report the synthesis and antibacterial, cytotoxic and anti-oxidant screening of Schiff bases derived from 4-aminoantipyrene and aromatic carbonyl compounds. The Schiff bases were synthesized by grinding equimolar quantities of 4-aminoantipyrene and carbonyl compounds at room temperature in mortar using pestle (Scheme 1).

Materials and Methods

Chemistry



Scheme 1: Synthesis of Schiff bases (G1-G8)

All the solvents and chemicals were Sigma-Aldrich brand. On Barnstead electrothermal melting point apparatus melting points (mp) were determined in open capillaries and are uncorrected. Thin-layer chromatography (TLC) on Merck silica gel 60 F254 aluminum sheets (Merck; Darmstadt, Germany) was used to monitor the reaction progress, using various developing system in various ratios and observed under UV light (λ= 254/365 nm). Bruker AV spectrometer was used for recording ^{1}H-NMR spectra at 300 MHz and tetramethyl silane (TMS) was used as internal standard. Chemical shift values (δ) were mentioned in ppm. FTS 3000 MX, Bio-Rad Merlin Fourier Transform Infra Red spectrophotometer was used to record IR spectra. KBr pellets were used for recording their spectra.

General procedure for synthesis of compounds G1-G8

Solid carbonyl compounds (2 mmol) were first grinded in a mortar with a pestle to fine powder then 4-aminoanti-pyrene (2 mmol, 0.4 g) was added and grinded up to 30 min at room temperature without the use of any solvent and catalyst. The progress of the reaction was monitored by thin layer chromatography (TLC). Upon completion of reaction the product was crystallized in absolute ethanol.

(Z)-4-(benzylideneamino)-2, 3-dimethyl-1-phenyl-1, 2-dihy-dropyrazol-5-one (G1)

Cream color crystalline solid, Yield: 75%, mp 178°C, Rf 0.70, ^{1}H-NMR (300 MHz, $CDCl_3$), δ: 2.51 (s, 3H, =C-CH_3), 3.17 (S, 3H, -N-CH_3),7.27-7.57 (m, 8H, ArH), 7.81-7.93 (m, 2H, ArH), 9.79 (s, 1H, -N=CH) IR (KBr) 1646 (>C=O), 1594.2 (C=N) cm^{-1}.

(Z)-4-(4-hydroxy-3-methoxybenzylideneamino)-2, 3-dimethyl-1-phenyl-1, 2dihydropyrazol-5-one (G2)

Cream color crystalline solid, Yield: 73%, mp 207°C, Rf 0.61, ^{1}H-NMR (300 MHz, $CDCl_3$), δ: 2.48 (s, 3H, =C-CH_3), 3.16 (s, 3H, -N-CH_3), 3.95 (s, 3H, OCH_3), 6.10 (s, 1H, OH), 6.94 (s,1H,ArH),7.44 (m, 7H, ArH), 9.68 (s,1H, -N=C-H); IR (KBr) 1624 (>C=O), 1576 (C=N)cm^{-1}.

(Z)-4-(2-nitrobenzylideneamino)-2, 3-dimethyl-1-phenyl-1-2-dihydropyrazol-5-one (G3)

Deep yellow color crystalline solid, Yield: 76%, mp 212°C, Rf 0.5, ^{1}H-NMR (300 MHz, $CDCl_3$), δ: 2.49 (s, 3H, =C-CH_3), 3.22 (s, 3H, -N-CH_3), 6.96-8.44 (m, 9H, ArH), 10.04 (s, 1H, -N=CH); IR (KBr) 1646 (>C=O), 1567 (C=N) cm^{-1}.

(Z)-4-(2-hydroxybenzylideneamino)-2, 3-dimethyl-1-phenyl-1-2-dihydropyrazol-5-one (G4)

Light yellow crystalline solid, Yield, 72%, mp 200°C, Rf 0.14, ^{1}H-NMR (300 MHz, $CDCl_3$), δ: 2.43 (s, 3H, =C-CH_3), 3.19 (s, 3H, -N-CH_3), 6.85-7.02 (m, 5H, ArH), 7.46-7.57 (m, 2H, ArH), 9.85 (s, 1H, -N=CH), 13.37 (s, 1H, ArOH); IR (KBr) 1652 (>C=O), 1589(C=N) cm^{-1}.

(Z)-4-(3-nitrobenzylideneamino)-2, 3-dimethyl-1-phenyl-1-2-dihydropyrazol-5-one (G5)

Yellow color crystalline solid, Yield: 73%, mp 218°C, Rf 0.75, ^{1}H-NMR (300 MHz, $CDCl_3$), δ: 2.54 (s, 3H, =C-H_3), 3.23 (s, 3H, -N-CH_3), 7.31-7.65 (m, 6H, ArH), 7.83-8.40 (m, 2H, ArH), 8.77 (s, 1H, -CH-NO_2), 9.83 (s, 1H, -N=CH); IR (KBr) 1645 (>C=O), 1592 (C=N) cm^{-1}.

(Z)-4-(4-nitrobenzylideneamino)-2, 3-dimethyl-1-phenyl-1-2-dihydropyrazol-5-one (G6)

Golden color crystalline solid, Yield: 71%, mp 254°C, Rf 0.6, ^{1}H-NMR (300 MHz, $CDCl_3$), δ: 2.54 (s, 3H, =C-CH_3), 3.25 (s, 3H, -N-CH_3), 7.23-7.58 (m, 5H, ArH), 7.99 (d, J=8.3Hz, 2H, ArH), 8.27 (d, J=8.3Hz, 2H, ArH), 9.82 (s, 1H, -N=CH); IR (KBr) 1640 (>C=O), 1572 (C=N) cm^{-1}.

(Z)-4-(4-dimethylamino)benzylideneamino)-2, 3-dimethyl-1-phenyl-1-2-dihydropyrazol-5 one (G7)

Yellow color crystalline solid, Yield: 75%, mp 219°C, Rf 0.8, ^{1}H-NMR (300 MHz, $CDCl_3$), δ: 2.48 (s, 3H, =C-CH_3), 3.04 (s, 6H, -N-$(CH_3)_2$), 3.11 (s, 3H, -N-CH3), 6.68-6.79 (m, 2H, ArH), 7.15-7.92 (m, 7H, ArH), 9.68 (s, 1H, -N=CH); IR (KBr) 1645 (>C=O), 1577 (C=N) cm^{-1};

(Z)-4-(1-(2 hydroxy phenyl) ethylilideneamino)-2, 3-dimethyl-1-phenyl-1-2-dihydropyrazol-5-one (G8)

Yellow color crystalline solid, Yield: 71%, mp 166, Rf 0.37, ^{1}H-NMR (300 MHz, $CDCl_3$), δ: 2.32 (s, 3H, N=C-CH_3), 2.54 (s, 3H, =C-CH_3), 3.14 (s, 3H, N$(CH_3)_2$), 6.82-7.05 (m, 2H, ArH), 7.26-7.56 (m, 6H, ArH), 7.68 (dd, J=8.1, 1.6Hz, 1H, ArH), 1.5 (s, 1H, OH); IR (KBr) 1658 (>C=O), 1592 (C=N) cm^{-1}.

Pharmacological evaluation

Antibacterial activity

G1-G8 were evaluated for antimicrobial activity by well diffusion method (Kumar et al., 2013) against two Gram negative bacteria (*Escherichia coli* 739, *Proteus mirabilis*

13315) and two Gram positive (*Staph aureus* 29213 and *Bacillus cereus* locally collected). Each petri plate was filled with 20 mL of agar medium then swabbed with 10 µg/mL test microorganism and kept for 15 min for adsorption. Wells were bored into the seeded agar plates through sterile cork borer of 5 mm diameter and loaded with a 10 mg/mL of test compounds. Ceftriaxone was used as positive controls for bacteria. All the plates were incubated at 37°C for 24 hours. The antimicrobial activity of G1-G8 was evaluated by measuring the zone of inhibition against the test microorganisms with scale.

Cytotoxicity

The cytotoxicity of **G1, G2, G3, G4, G5, G6, G7** and **G8** was carried out on active nauplii of brine shrimp (*Artemia salina*). Eggs were hatched in sterile artificial seawater having composition of sea salt 38.0 g/L and pH 8 then left in continuous aeration for 48 hours. Different concentrations (1, 5, 10, 50, 100, 250, 500 and 750 ppm) of compounds (**G1, G2, G3, G4, G5, G6, G7** and **G8)** were prepared in dimethyl sulfoxide (DMSO). After evaporation of DMSO, 10 brine shrimp larvae were added to each concentration (Ali et al., 2011). The percent mortality was determined after 24 hours after counting survived and dead shrimps. The readings were taken in triplicate. LC_{50} value was calculated using graph pad prism software version V.

Anti-oxidant activity

DPPH free radical scavenging assay of the test sample and standard was accessed as described in protocols with a slight modification (Ilahi et al., 2013). Briefly, 6 mg of each **G1, G2, G3, G4, G5, G6, G7** and **G8** was dissolved in 600 µL of methanol to prepare stock solutions (10,000 ppm). The stock solutions were then serially diluted to get a concentration of 20, 40, 60, 80 and 100 ppm. Ascorbic acid, a standard, was also prepared in same concentrations. DPPH (0.002%) was dissolved in methanol. 1 mL stable 2, 2- diphenyl-2-picrylhydrazyl (DPPH) of was added to each concentration. The solution mixture was then incubated for 30 min in dark area of the laboratory at room temperature. Methanol containing DPPH was used as blank. Ascorbic acid was also accessed as that of test sample. After required time the absorption of the compounds were measured at 520 nm on spectrophotometer (Shimadzu UV-1700).

The % absorption was then calculated by using the following formula:

$$\% \text{ of inhibition of DPPH activity} = \frac{A-B}{A} \times 100$$

A is absorption in blank and B is absorption of test sample.

Table I

Antibacterial activity of G1-G8

Zone of inhibition	Zone of Inhibition			
Com-pounds	*Proteus mirabilis*	*Staph aureus*	*Escherichia coli*	*Bacillus cereus*
G1	10	26	10	10
G2	30	20	15	25
G3	15	18	15	15
G4	32	20	20	25
G5	38	20	21	18
G6	25	25	15	5
G7	5	25	20	8
G8	20	22	20	7
Ceftriaxone	45	42	30	45
Inoculums control	Growth in all concentrations			

Results and Discussion

Proteus mirabilis 13315 was found susceptible to **G2**, **G4**, **G5**, **G6** and **G8**, intermediate to **G3** and resistant to **G1** and **G7**. *Staphylococcus aureus* 29213 was found susceptible to all test compounds **G1-G8**. *Escherichia coli* was found susceptible to **G4**, **G5**, **G7** and **G8**, intermediate to **G2, G3** and **G6** and resistant to **G1**. *Bacillus cereus* (locally collected) was found susceptible to **G2**, **G4** and **G5**, intermediate to **G3** and resistant to **G1**, **G6**, **G7** and **G8** (Colwell et al., 1968) as shown in Table I.

$$\% \text{ Lethality} = \frac{\text{NSN Control} - \text{NSN Test}}{\text{NSN Control}} \times 100$$

NSN control - Number of surviving napulii in control; NSN Test - Number of surviving napulii in test

G2 to **G8** all showed significant cytotoxicity because their LC_{50} values were below 20 µg/mL (Table II). We can speculate that test compounds show better activity when a group is attached to benzylidene phenyl ring. These results not only tell us about toxicity of test compound but it also gives us preliminary data about anti-tumor (Khafagi et al., 2004), enzyme inhibition and iron regulation activities (Venugopal et al., 2011).

DPPH scavenging activity (Table III) of synthesized compounds **G1, G2, G3, G4, G5, G6, G7** and **G8** was carried out at concentration of 20, 40, 60, 80 and 100 ppm giving IC_{50} at 31.26[a], 1.13[a], 133, >500, >500, 285, 2.4[a], 118 µg/mL respectively. The anti-oxidant capability was evaluated through IC_{50}, the effective concentration at which 50% of the radicals were scavenged. A compound is potent anti-oxidant if its IC_{50} value is less than 10 µg/mL (Zhang et al., 2013).

G1 benzylidene phenyl ring is unsubstituted, gives IC_{50} at 31.3 µg/mL and shows no significant anti-oxidant

Table II

Percent death and LC_{50} values for brine shrimp assay of synthesized compounds

Compounds	Percent lethality at 24 hours									Total Exposed	LC_{50}
	Negative control	1 ppm	5 ppm	10 ppm	50 ppm	100 ppm	250 ppm	500 ppm	750 ppm		
G1	0	0	30	30	50	60	80	90	100	10	50 ppm
G2	0	30	50	50	60	70	70	80	100	10	5 pm
G3	0	20	30	50	70	80	90	100	100	10	10 ppm
G4	0	30	50	50	70	80	90	100	100	10	5 ppm
G5	0	50	50	60	70	70	70	80	100	10	1 ppm
G6	0	30	30	50	70	80	80	80	100	10	10 ppm
G7	0	0	30	30	70	70	70	100	100	10	<50 ppm
G8	0	20	30	50	70	80	80	80	100	10	10 ppm

Table III

DPPH scavenging activity of synthesized shiff bases of 4-aminoantipyrene

Compounds	IC_{50}	Compounds	IC_{50}
G1	31.3[a]	G5	>500
G2	1.1[a]	G6	285
G3	133.0	G7	2.4[a]
G4	>500	G8	118

activity. Anti-oxidant data of electron donating groups (OH, OMe) at various positions in benzylidene phenyl ring has been reported (Alam and Lee, 2012). We introduced strong electron withdrawing group (NO_2) at various positions and didn't find any significant activity. The comparative study shows that introducetion of electron donating groups to benzylidene phenyl ring of the Schiff base analogues of 4-aminoantipyrine are important for anti-oxidant activity.

In conclusion, 4-aminoantipyrine derived Schiff bases have antibacterial potentials and cytotoxic potentials which can be enhanced in presence of nitro group and when a group is attached to benzylidene phenyl ring respectively. 4-Aminoantipyrine derived Schiff bases have anti-tumor potentials and when substituted with electron donating group have positive effect on its anti-oxidant activity.

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References

Alam MS, Lee DU. Synthesis, molecular structure and antioxidant activity of (E)-4[benzylideneamino]-1, 5-dimethyl-2-phenyl-1H-pyrazol-3 (2H)-one, a Schiff base ligand of 4-aminoantipyrine. J Chem Crystallogr. 2012; 42: 93-102.

Ali N, Ali Shah S, Ahmad B. Calcium channel blocking activity of fruits of *Callistemon citrinus*. 2011; 33: 245-48.

Awad L, Ibrahim E, Bdeewy OK. Synthesis of antipyrine derivatives derived from dimedone. China J Chem. 2007; 25: 570-73.

Burdulene D, Palaima A, Stumbryavichyute Z, Talaikite Z. Synthesis and anti-inflammatory activity of 4-aminoantipyrine derivatives of succinamides. Pharm Chem J. 1999; 33: 191-93.

Cinarli A, Gürbüz D, Tavman A, Birteksöz AS. Synthesis, spectral characterizations and antimicrobial activity of some Schiff bases of 4-chloro-2-aminophenol. Bull Chem Soc Ethiop. 2011; 25: 407-17.

Colwell WT, Lange JH, Henry DW. Chemotherapeutic nitroheterocycles. Nitropyrrole-2-carboxaldehyde derivatives. J Med Chem. 1968; 11: 282-85.

Evstropov A, Yavorovskaya V, Vorob'ev E, et al. Synthesis and antiviral activity of antipyrine derivatives. Pharm Chem J. 1992; 26: 426-30.

Harpstrite SE, Collins SD, Oksman A, Goldberg DE, Sharma V. Synthesis, characterization, and antimalarial activity of novel Schiff-base-phenol and naphthalene-amine ligands. Med Chem. 2008; 4: 392-95.

Ilahi I, Samar S, Khan I, Ahmad I. *In vitro* anti-oxidant activities of four medicinal plants on the basis of DPPH free radical scavenging. Pak J Pharm Sci. 2013; 26: 949-52.

Kabak M, Elmali A, Elerman Y, Durlu T. Conformational study and structure of bis-N,N'-p-bromo-salicylideneamine-1, 2-diaminobenzene. J Mol Struct. 2000; 553: 187-92.

Kalaivani S, Priya NP, Arunachalam S. Schiff bases: Facile synthesis, spectral characterization and biocidal studies. Int J App Bio Pharm Tech. 2012; 3: 219-23.

Karthikeyan MS, Prasad DJ, Poojary B, Subrahmanya Bhat K, Holla BS, Kumari NS. Synthesis and biological activity of Schiff and Mannich bases bearing 2, 4-dichloro-5-fluoro-phenyl moiety. Bioorg Med Chem. 2006; 14: 7482-89.

Khafagi I, Dewedar A, Farouk S. *In vitro* cytotoxicity and antimicrobial activities of some common essential oils. Egypt J Bio. 2004; 2: 20-27.

Kumar D, Chadda S, Sharma J, Surain P. Syntheses, spectral characterization, and antimicrobial studies on the coordination compounds of metal ions with Schiff base containing both aliphatic and aromatic hydrazide moieties. Bioinorg Chem App. 2013; 2013: 1-10.

Maity S, Khan SA, Ahmad S. Synthesis, characterization, antimicrobial and anti-oxidant activity of some novel Schiff bases derived from 8-hydroxy quinoline. IJBPS. 2012; 3: 90-98.

Malladi S, Isloor AM, Isloor S, Akhila D, Fun H-K. Synthesis, characterization and antibacterial activity of some new pyrazole based Schiff bases. Arabian J Chem. 2013; 6: 335-40.

Metzler CM, Cahill A, Metzler DE. Equilibriums and absorption spectra of Schiff bases. J Am Chem Soc. 1980; 102: 6075-82.

Patil S, Jhadav S, Patil U. Natural acid catalyzed synthesis of Schiff base under solvent-free condition: As a green approach. Arch Ap Sci Res. 2012; 4: 1074-78.

Raman N, Mitu L, Sakthivel A, Pandi M. Studies on DNA cleavage and antimicrobial screening of transition metal complexes of 4-aminoantipyrine derivatives of N_2O_2 type. J Iran Chem Soc. 2009; 6: 738-48.

Shivarama HB, Veerendra B, Shivananda M, Poojary B. Synthesis characterization and anti-cancer activity studies on some Mannich bases derived from 1, 2, 4-triazoles. Eu J Med Chem. 2003; 38: 759-67.

Thorat B, Mandewale M, Shelke S, et al. Synthesis of novel Schiff bases of 4-hydroxy-3-methoxy-5-nitrobenzaldehyde and development of HPLC chromatographic method for their analysis. J Chem Pharm Res. 2012; 4: 14-17.

Venkatesan K, Satyanarayana V, Sivakumar A. Microwave-assisted synthesis and evaluation of antibacterial activity of 2, 2'-(naph-thalene-2, 7-diylbis (oxy)) bis (N'-substituted acetohydrazide) derivatives. Bull Chem Soc Ethiop. 2012; 26: 257-65.

Venugopal T, Swathi D, Suchitha Y, et al. Mineral composition, cytotoxic and anticariogenic activity of *Scleropyrum pentandrum* (Dennst.) Mabb. Int J Drug Develop Res. 2011; 3: 344-50.

Zhang Y, Fang Y, Liang H. Synthesis and anti-oxidant activities of 2-oxo-quinoline-3-carbaldehyde Schiff-base derivatives. Bioorg Med Chem lett. 2013; 23: 107-11.

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