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

## Synthesis of 4-aminoantipyrene 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-aminoantipyrene 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 antioxidant 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 antioxidant 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), antioxidant 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-aminoantipyrene 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.  $\beta$ -lactams (Raman et al., 2009).

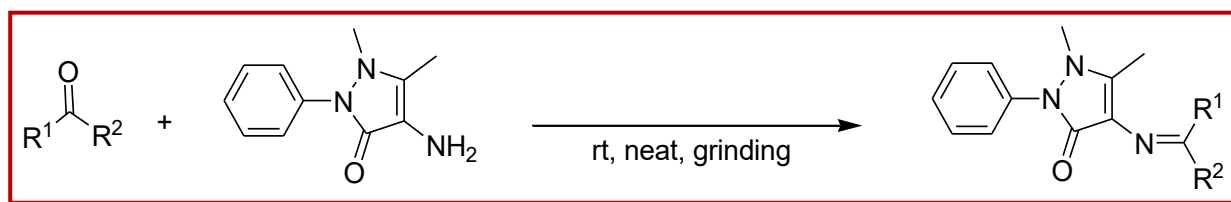
Schiff bases are reported for antimicrobial, anti-tumor and antioxidant potentials herein we report the synthesis and antibacterial, cytotoxic and antioxidant screening of Schiff bases derived from 4-aminoantipyrene and aromatic carbonyl compounds. The Schiff bases were synthesized by grinding equimolar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

### Materials and Methods

#### Chemistry

All the solvents and chemicals were Sigma-Aldrich





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

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 ( $\lambda = 254/365$  nm). Bruker AV spectrometer was used for recording  $^1\text{H-NMR}$  spectra at 300 MHz and tetramethyl silane (TMS) was used as internal standard. Chemical shift values ( $\delta$ ) 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-dihydro-pyrazol-5-one (G1)

Cream color crystalline solid, Yield: 75%, mp 178°C, Rf 0.70,  $^1\text{H-NMR}$  (300 MHz,  $\text{CDCl}_3$ ),  $\delta$ : 2.51 (s, 3H, =C-CH<sub>3</sub>), 3.17 (s, 3H, -N-CH<sub>3</sub>), 7.27-7.57 (m, 8H, ArH), 7.81-7.93 (m, 2H, ArH), 9.79 (s, 1H, -N=CH) IR (KBr) 1646 ( $>\text{C}=\text{O}$ ), 1594.2 (C=N)  $\text{cm}^{-1}$ .

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

Cream color crystalline solid, Yield: 73%, mp 207°C, Rf 0.61,  $^1\text{H-NMR}$  (300 MHz,  $\text{CDCl}_3$ ),  $\delta$ : 2.48 (s, 3H, =C-CH<sub>3</sub>), 3.16 (s, 3H, -N-CH<sub>3</sub>), 3.95 (s, 3H, OCH<sub>3</sub>), 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 ( $>\text{C}=\text{O}$ ), 1576 (C=N)  $\text{cm}^{-1}$ .

#### (Z)-4-(2-nitrobenzylideneamino)-2, 3-dimethyl-1-phenyl-1-2-dihydro-pyrazol-5-one (G3)

Deep yellow color crystalline solid, Yield: 76%, mp 212°C, Rf 0.5,  $^1\text{H-NMR}$  (300 MHz,  $\text{CDCl}_3$ ),  $\delta$ : 2.49 (s, 3H, =C-CH<sub>3</sub>), 3.22 (s, 3H, -N-CH<sub>3</sub>), 6.96-8.44 (m, 9H, ArH), 10.04 (s, 1H, -N=CH); IR (KBr) 1646 ( $>\text{C}=\text{O}$ ), 1567 (C=N)  $\text{cm}^{-1}$ .

#### (Z)-4-(2-hydroxybenzylideneamino)-2, 3-dimethyl-1-phenyl-1-2-dihydro-pyrazol-5-one (G4)

Light yellow crystalline solid, Yield, 72%, mp 200°C, Rf 0.14,  $^1\text{H-NMR}$  (300 MHz,  $\text{CDCl}_3$ ),  $\delta$ : 2.43 (s, 3H, =C-CH<sub>3</sub>), 3.19 (s, 3H, -N-CH<sub>3</sub>), 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 ( $>\text{C}=\text{O}$ ), 1589 (C=N)  $\text{cm}^{-1}$ .

#### (Z)-4-(3-nitrobenzylideneamino)-2, 3-dimethyl-1-phenyl-1-2-dihydro-pyrazol-5-one (G5)

Yellow color crystalline solid, Yield: 73%, mp 218°C, Rf 0.75,  $^1\text{H-NMR}$  (300 MHz,  $\text{CDCl}_3$ ),  $\delta$ : 2.54 (s, 3H, =C-CH<sub>3</sub>), 3.23 (s, 3H, -N-CH<sub>3</sub>), 7.31-7.65 (m, 6H, ArH), 7.83-8.40 (m, 2H, ArH), 8.77 (s, 1H, -CH-NO<sub>2</sub>), 9.83 (s, 1H, -N=CH); IR (KBr) 1645 ( $>\text{C}=\text{O}$ ), 1592 (C=N)  $\text{cm}^{-1}$ .

#### (Z)-4-(4-nitrobenzylideneamino)-2, 3-dimethyl-1-phenyl-1-2-dihydro-pyrazol-5-one (G6)

Golden color crystalline solid, Yield: 71%, mp 254°C, Rf 0.6,  $^1\text{H-NMR}$  (300 MHz,  $\text{CDCl}_3$ ),  $\delta$ : 2.54 (s, 3H, =C-CH<sub>3</sub>), 3.25 (s, 3H, -N-CH<sub>3</sub>), 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 ( $>\text{C}=\text{O}$ ), 1572 (C=N)  $\text{cm}^{-1}$ .

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

Yellow color crystalline solid, Yield: 75%, mp 219°C, Rf 0.8,  $^1\text{H-NMR}$  (300 MHz,  $\text{CDCl}_3$ ),  $\delta$ : 2.48 (s, 3H, =C-CH<sub>3</sub>), 3.04 (s, 6H, -N-(CH<sub>3</sub>)<sub>2</sub>), 3.11 (s, 3H, -N-CH<sub>3</sub>), 6.68-6.79 (m, 2H, ArH), 7.15-7.92 (m, 7H, ArH), 9.68 (s, 1H, -N=CH); IR (KBr) 1645 ( $>\text{C}=\text{O}$ ), 1577 (C=N)  $\text{cm}^{-1}$ ;

#### (Z)-4-(1-(2 hydroxy phenyl) ethylideneamino)-2, 3-dimethyl-1-phenyl-1-2-dihydro-pyrazol-5-one (G8)

Yellow color crystalline solid, Yield: 71%, mp 166, Rf 0.37,  $^1\text{H-NMR}$  (300 MHz,  $\text{CDCl}_3$ ),  $\delta$ : 2.32 (s, 3H, =C-CH<sub>3</sub>), 2.54 (s, 3H, =C-CH<sub>3</sub>), 3.14 (s, 3H, N(CH<sub>3</sub>)<sub>2</sub>), 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 ( $>\text{C}=\text{O}$ ), 1592 (C=N)  $\text{cm}^{-1}$ .

#### 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<sub>50</sub> value was calculated using graph pad prism software version V.

#### Antioxidant 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.

## 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 nauplii in control; NSN Test - Number of surviving nauplii in test

G2 to G8 all showed significant cytotoxicity because their LC<sub>50</sub> 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<sub>50</sub> at 31.26<sup>a</sup>, 1.13<sup>a</sup>, 133, >500, >500, 285, 2.4<sup>a</sup>, 118 µg/mL respectively. The antioxidant capability was evaluated through IC<sub>50</sub>, the effective concentration at which 50% of the radicals were scavenged. A compound is potent antioxidant if its IC<sub>50</sub> value is less than 10 µg/mL (Zhang et al., 2013).

G1 benzylidene phenyl ring is unsubstituted, gives IC<sub>50</sub> at 31.3 µg/mL and shows no significant antioxidant activity. Antioxidant data of electron donating groups (OH, OMe) at various positions in benzylidene phenyl

Table I

Antibacterial activity of G1-G8				
Zone of inhibition	Zone of Inhibition			
	Com-pounds	<i>Proteus mirabilis</i>	<i>Staph aureus</i>	<i>Escherichia coli</i>
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			

Table II

Percent death and LC<sub>50</sub> values for brine shrimp assay of synthesized compounds

Compounds	Percent lethality at 24 hours									Total Exposed	LC <sub>50</sub>
	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 ppm
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 Schiff bases of 4-aminoantipyrene

Compounds	IC <sub>50</sub>	Compounds	IC <sub>50</sub>
G1	31.3 <sup>a</sup>	G5	>500
G2	1.1 <sup>a</sup>	G6	285
G3	133.0	G7	2.4 <sup>a</sup>
G4	>500	G8	118

ring has been reported (Alam and Lee, 2012). We introduced strong electron withdrawing group (NO<sub>2</sub>) at various positions and didn't find any significant activity. The comparative study shows that introduction of electron donating groups to benzylidene phenyl ring of the Schiff base analogues of 4-aminoantipyrene are important for antioxidant activity.

## Conclusion

4-Aminoantipyrene 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-Aminoantipyrene derived Schiff bases have anti-tumor potentials and when substituted with electron donating group have positive effect on its antioxidant activity.

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## Conflict of Interest

Authors declare no conflict of interest

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