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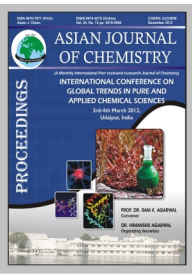

Synthesis, Characterization and Antimicrobial Activity of Some New Schiff's Bases Derived from 5-Acetyl-2,6-dimethylpyrimidin-4(3*H*)-one and Primary Aromatic Amines†

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2,6-Dimethyl-5-[1-(arylimino)ethyl]pyrimidin-4(3*H*)-one were synthesized from primary aromatic amines and 5-acetyl-2,6-dimethylpyrimidin-4(3*H*)-one. These were characterized by IR, ^{1}H NMR, ^{13}C NMR and mass spectral analysis. *In vitro* biological screening effects of the investigated compounds were tested against the bacterial species *Staphylococcus aureus, Escherichia coli, Salmonella typhi* and *Bacillus subtilis* by Agar cup method. Fungal species *Aspergillus niger, Penicillium chrysogenum, Fusarium moneliforme* and *Aspergillus flavus* by the poison plate method.

Key Words: 5-Acetyl-2,6-dimethylpyrimidin-4(3*H*)-one, Aromatic amines, Schiff base, Spectra, Antibacterial, Antifungal.

INTRODUCTION

The biological significance of the pyrimidine derivatives has led us to the synthesis of substituted pyrimidine. As pyrimidine is a basic nucleus in DNA and RNA, it has been found to be associated with diverse biological activities[1]. The synthesis of substituted pyrimidine and many detailed reviews have been appeared[2,3]. Pyrimidines and their derivatives are considered to be important for drugs and agricultural chemicals. Pyrimidine derivatives possess several interesting biological activities such as antimicrobial[4], antitumour[5] and antifungal activities[6]. Many pyrimidine derivatives are used for thyroid drugs and leukemia.

In addition, Schiff's bases perform important role in biological systems, where the > C=N- linkage is an essential structural requirement for biological activity[7]. Many Schiff bases exhibited remarkable antibacterial[8,9], antifungal[10,11], anticancer[12], diuretic activities[13] and can also be regarded as mimetic systems for enzyme models[14]. Some substituted Schiff bases, such as *N*-4-arylideneaminotriazole derivatives, exhibited anti-HIV activity[15].

No attempt has been made on the synthesis of Schiff bases derived from 5-acetyl-2,6-dimethylpyrimidin-4(3*H*)-one and primary aromatic amine. In views of the above facts, we herein report the preparation of 2,6-dimethyl-5-[1-(arylimino)-ethyl]pyrimidin-4-(3*H*)-one by condensation-5-acetyl-2,6-dimethylpyrimidin-4(3*H*)-one with aromatic amines such as aniline, 4-toluidine, 4-chloroaniline, 4-bromoaniline, 4-iodoaniline, 4-methoxyaniline, 4-ethoxyaniline to synthesize 2,6-dimethyl-5-(1-(arylimino)ethyl)pyrimidin-4(3*H*)-one. These were characterized by IR, ^{1}H NMR, ^{13}C NMR and mass spectral analysis.

EXPERIMENTAL

All the chemical and solvents used were of A.R. grade. All the chemicals used were of E-Merck and S.D. Fine Ltd. Melting points were determined in an open capillary tube and are uncorrected. The purity of the compound has been checked by TLC. IR spectra were recorded in $CHCl_3$ on a Shimadzu FTIR-8300 spectrophotometer. The ^{1}H NMR (300 MHz) and ^{13}C NMR (70 MHz) were run on a Bruker Avance DPX-250 spectrometer in $CDCl_3$ using tetramethylsilane as an internal standard. Chemical shift values are given in δ scale. Mass spectra were recorded on Finnigan Mat LCQ Mass spectrometer using methanol as mobile phase. The *in vitro* biological screenings of the investigated compounds were tested against the bacterial species by agar cup method and fungal species by the poison plate method.

Synthesis of 2,6-dimethyl-5-[1-(arylimino)ethyl]-pyrimidin-4-(3*H*)-one: The Schiff bases(**2a-2h**, Fig. 1) were prepared by adding 5-acetyl-2,6-dimethylpyrimidin-4-(3*H*)-one (0.01 mol) and the corresponding aromatic amine (0.01 mol) in ethanol (50 mL) and refluxing the mixture for 4 h. After cooling, the product was crystallized from ethanol. The

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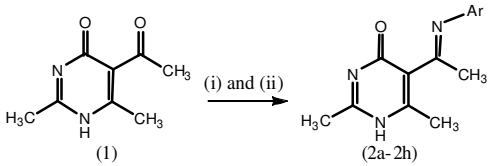
purity of the ligands was checked by m.p. and TLC. These are characterized by IR, ^{1}H NMR, ^{13}C NMR and mass spectral studies.



(i) Ar-NH_2 and (ii) EtOH

Ar = phenyl (a), toluyl (b), 2,4-dimethylphenyl (c), 4-chlorophenyl (d), 4-bromophenyl (e), 4-iodophenyl (f), 4-methoxyphenyl (g) and 4-ethoxyphenyl (h)

Fig. 1. 2,6-Dimethyl-5-(1-(phenylimino)ethyl)pyrimidin-4(3*H*)-one (**2a**)

Characterization of synthesized Schiff's bases (2a-h)

2,6-Dimethyl-5-(1(phenylimino)-ethyl)pyrimidin-4-(3*H*)-one (2a): Colour: yellow; yield: 85 %; m.p. 173-175 ºC; IR (KBr, ν_{max}, cm^{-1}): 3332 ν(NH), 1680 ν(C=O) ofpyrimidone, 1618 ν(C=N) of imine, 1564 and 1483 aromatic ν(C=C); ^{1}H NMR: δ 1.97 and δ 2.36 (S, 3H, CH_3) of 2,6-dimethylpyridone and δ 2.70 (S, 3H, imine -CH_3), 6.66 (S,1H, N-H), 7.5-7.1 (m, 5H, Ph-H); ^{13}C NMR: δ 18.05, 21.32 (for 2,6, dimethyl carbons) and 27.02 (imine-CH_3 carbon), 170.62, 168.62 and 154 for C^6, C^4, C^2 ring carbon of pyrimidone, 150-120 for aromatic carbons and 181.7 for imine carbon. Mass spectra: $[M^+]$ = 241.08.

2,6-Dimethyl-5-(1-(*p*-tolylimino)-ethyl)pyrimidin-4(3H)-one (2b): Colour: yellow; yield: 72 %; m.p. 182-183 ºC; IR (KBr, ν_{max}, cm^{-1}): 3350 ν (NH), 1684 ν(C=O) of pyrimidone, 1620 ν(C=N) of imine, 1567, 1485 aromatic ν(C=C); ^{1}H NMR: δ 2.07 and δ 2.43 (S, 3H, -CH_3) of 2,6-dimethyl pyridone, 2.5 (S, 3H, of *p*-CH_3 of aryl) and δ 2.66 (S, 3H, imine -CH_3), 6.64 (S, 1H, N-H), 7.19 and 7.47dd, 4H, (-C_6H_4-, *p*-substituted); ^{13}C NMR: δ 17.65, 20.39 (for 2,6 methyl carbons) and 25.26 (imine -CH_3 carbon), 170.62,168.62 and 154 for C^6, C^4, C^2 ring carbon of pyrimidine, 130 to 122 for aromatic carbons and 180.8 for imine carbon. Mass spectra: $[M^+]$ = 255.02.

2,6-Dimethyl-5-(1-(2,4-dimethyl-phenylimino)ethyl)-pyrimidin-4(3*H*)-one(2c): Colour: pale yellow; yield: 76 %; m.p. 190-192 ºC; IR (KBr, ν_{max}, cm^{-1}): 3342 ν(NH), 1688 ν(C=O) of pyrimidone, 1626 ν(C=N) of imine, 1565, 1487 aromatic ν(C=C); ^{1}H NMR: 2.52, 2.4, 2.24 and 2.2 for (four S, 3H each for *o,p*-CH_3 of aryl and 2,6 -CH_3 of pyrimidone), δ 2.7 (S, 3H, imine -CH_3), 7.28 to 7.06 (m, 3H, Ph-H), 6.54 (S,1H, N-H); ^{13}C NMR: δ 20.38 (imine-CH_3 carbon), 18.6 and 21.04 (2,4-CH_3), 99.16 for C^3, 170.1, 166.36 and 153.5 for C^6, C^4, C^2 ring carbon of pyrimidone, 140 and 120 for aromatic carbons and 181.08 for imine carbon. Mass Spectra: $[M^+]$ = 270.04.

2,6-Dimethyl-5-(1-(*p*-clorophenylimino)ethyl)-pyrimidin-4(3*H*)-one(2d): Colour: yellowish green; yield: 82 %; m.p. 206-208 ºC; IR (KBr, ν_{max}, cm^{-1}): 3347 ν(NH), 1680 ν(C=O) of pyrimidone, 1623 ν(C=N) of imine, 1559, 1481 aromatic ν(C=C); ^{1}H NMR: δ 1.97 and δ 2.33 (S, 3H, -CH_3) of 2,6-dimethyl pyridone and δ 2.63 (S, 3H, imine -CH_3), 6.62 (S, 1H, N-H), 7.22 and 7.46 dd, 4H, (-C_6H_4-, *p*-substituted); ^{13}C NMR: δ 17.82, 20.44 (for 2,6-dimethyl carbons) and 26.8 (imine-CH_3 carbon), 170.6, 168.66 and 153 for C^6, C^4, C^2 ring carbon of pyrimidine, 134 and 126 for aromatic carbons and 178.9 for imine carbon. Mass spectra: $[M^+]$ = 275.12 and 277.19 (in isotopic ratio of chlorine).

2,6-Dimethyl-5-(1-(p-bromophenylimino)ethyl)-pyrimidin-4(3*H*)-one(2e): Colour: greenish yellow; yield: 68 %; m.p. 236-237 ºC; IR (KBr, ν_{max}, cm^{-1}): 3344 ν(NH), 1678 ν(C=O) of pyrimidone, 1629 ν(C=N) of imine, 1555, 1475 aromatic ν(C=C); ^{1}H NMR: δ 1.96 and δ 2.3 (S, 3H, CH_3) of 2,6-dimethyl pyridone and δ 2.67 (S, 3H, imine -CH_3), 6.6 (S, 1H, N-H), 7.2 and 7.4 dd, 4H, (-C_6H_4-, *p*-substituted); ^{13}C NMR: δ 18.2, 20.4 (for 2,6 methyl carbons) and 27.8 (imine-CH_3 carbon), 171.6, 168.6 and 150 for C_6, C_4, C_2 ring carbon of pyrimidine, 144 and 122 for aromatic carbons and 180.3 for imine carbon. Mass spectra: $[M^+]$ = 319.58 and 321.60 (in isotopic ratio of bromine).

2,6-Dimethyl-5-(1-(*p*-iodophenylimino)ethyl)-pyrimidin-4(3*H*)-one(2f): Colour: green; yield: 72 %; m.p. 248-250 ºC; IR (KBr, ν_{max}, cm^{-1}): 3349 ν(NH), 1683 ν(C=O) of pyrimidone, 1622 ν(C=N) of imine, 1553, 1483 aromatic ν(C=C); ^{1}H NMR: δ 2.06 and δ 2.42 (S, 3H, CH_3) of 2,6-dimethyl pyridone and δ 2.78 (S, 3H, imine -CH_3), 6.66 (S,1H, N-H), 7.2 and 7.4 dd, 4H, (-C_6H_4-, *p*-substituted); ^{13}C NMR: δ 20.2, 22.4 (for 2,6-dimethyl carbons) and 26.7 (imine-CH_3 carbon), 174.6, 168.6 and 156 for C^6, C^4, C^2 ring carbon of pyrimidine, 147 and 123 for aromatic carbons and 181.02 for imine carbon. Mass spectra: $[M^+]$ = 367.06.

2,6-Dimethyl-5-(1-(*p*-methoxyphenylimino)ethyl)-pyrimidin-4(3*H*)-one (2g): Colour: light green; yield: 82 %; m.p. 204-205 ºC; IR (KBr, ν_{max}, cm^{-1}): 3343 ν(NH), 1680 ν(C=O) of pyrimidone, 1620 ν(C=N) of imine, 1550, 1480 aromatic ν(C=C); ^{1}H NMR: δ 2.0 and δ 2.4 (S, 3H, CH_3) of 2,6-dimethyl pyridone, δ 2.7 (S, 3H, imine-CH_3), δ 4.02 (S, 3H, *p*-OCH_3), 6.72 (S, 1H, N-H), 7.42 and 7.7 dd, 4H, (-C_6H_4-, *p*-substituted); ^{13}C NMR: δ 20.28, 23.02 (for 2,6-dimethyl carbons) and 27.6 (imine-CH_3 carbon), 56.3 (-OCH_3 carbon), 172.4, 166 and 153.4 for C^6, C^4, C^2 ring carbon of pyrimidine, 140 and 120 for aromatic carbons and 180.82 for imine carbon. Mass spectra: $[M^+]$ = 272.16.

2,6-Dimethyl-5-(1-(*p*-ethoxyphenylimino)ethyl)-pyrimidin-4(3*H*)-one(2h): Colour: green; yield: 74 %; m.p. 210-212 ºC ; IR (KBr, ν_{max}, cm^{-1}): 3336 ν(NH), 1682 ν(C=O) of pyrimidone,1625 ν(C=N) of imine, 1557, 1489 aromatic ν(C=C); ^{1}H NMR: δ 2.08 and δ 2.51 (S, 3H, -CH_3) of 2,6-dimethyl pyridone, δ 2.64 (S, 3H, imine-CH_3), δ 1.12 (t, 3H, *p*-OCH_2CH_3), δ 4.24 (q, 2H, *p*-OCH_3), 6.68 (S, 1H, N-H), 7.42 and 7.7 dd, 4H, (-C_6H_4-, *p*-substituted); ^{13}C NMR: δ 21.38, 24.2 (for 2,6-dimethyl carbons) and 25.8 (imine-CH_3 carbon), 56.3 and 14.2 (-OCH_2- and CH_3 carbon), 171.9, 167.7 and 150.44 for C^6, C^4, C^2 ring carbon of pyrimidine, 135 and 118 for aromatic carbons and 181.8 for imine carbon. Mass spectra: $[M^+]$ = 286.3.

Antibacterial activity: The antibacterial activity was measured by agar cup method[16] using nutrient agar (Himedia) prepared and sterilized at 15 Psi for 15 min in the autoclave. It was allowed to cool below 45 ºC and seeded with turbid suspension of test bacteria separately, prepared from 24 h old slant cultures. 3 % Inoculate were used every time. The bacterial cultures selected were, two gram negative cultures *viz. Escherichia coli, Salmonella typhi* and two Gram positive

cultures *viz. Staphylococcus aureu, Bacillus subtilis*. This seeded preparation was then poured separately in sterile petri plate under aseptic condition and allowed it to solidify.

Cups of 10 mm diameter were made in the agar plate with sterile cork borer. 100 mL of compound solution prepared in ethanol (0.1 %) was added in the cups under aseptic condition with the help of micropipette. 100 mL of ethanol was placed in separate cups as blank (negative control). 100 mL of solution of penicillin in ethanol (0.1 %) was also placed on the seeded nutrient agar surface as standard reference antibiotic (positive control).

The plates were kept in refrigerator for 15 min to allow diffusion of the compound from agar cup into the medium. Then the plates were shifted to incubator at 37 ºC and incubated for 24 h.

After incubation plates were observed for the zone of inhibition of bacterial growth around the agar cup. Results were recorded by measuring the zone of inhibition in millimeter (mm) using zone reader (Table-1).

TABLE-1
ANTI BACTERIAL ACTIVITY

Compound	Zone of inhibition (diameter in mm)			
	E. coli	*S. typhi*	*S.aureus*	*B. subtilis*
Penicillin	24	18	21	14
(2a)	12	-	11	6
(2b)	13	-	10	7
(2c)	9	-	8	6
(2d)	22	8	15	10
(2e)	20	8	14	10
(2f)	19	9	14	9
(2g)	14	-	10	6
(2h)	13	-	9	6

Antifungal activity: Antifungal activity was performed by Poison plate method[16]. Using potato dextrose agar (Himedia) as medium for fungi growth. The medium was prepared and sterilized at 10 Psi in autoclave for 15 min. The compound to be tested is added to the sterile medium in aseptic condition so as to get final concentration as 1 %. A plate with ethanol was prepared as blank (negative control) similarly a plate with 1 % Gresiofulvin was prepared as standard reference plate (positive control).

Aspergillus niger, Penicillium chrysogenum, Fusarium moneliforme and *Aspergillus flavus* were selected as test fungal cultures. These were allowed to grow on slant for 48 h so as to get profuse sporulation. 5 mL of 1:100 aqueous solution of Tween 80 was added to the slant and spores were scraped with the help of nichrome wire loop to form suspension.

The fungal suspension was inoculated on the plate prepared using compound with the help of nicrome wire loop. The plates were incubated at room temperature for 48 h.

After incubation plates were observed for the growth of inoculated fungi. Results were recorded (Table-2) as moderate growth of fungi (++), reduced growth of fungi (+) and no growth of inoculated fungi (-) antifungal activity.

RESULTS AND DISCUSSION

All the reactions were carried out under conventional methods. 2,6-Dimethyl-5-[1-(arylimino)ethyl]pyrimidin-4(3*H*)-one (**2a-2h**) were obtained by refluxing-5-acetyl-2,6-dimethylpyrimidin-4(3*H*)-one and aromatic amines (**a-h**) in ethanol for 4 h. Increase in the time of refluxing did not improve the yield of product.

TABLE-2
ANTI FUNGAL ACTIVITY

Compound	Growth of fungi			
	A. niger	*P. chrysogenum*	*F. moneliforme*	*A. flavus*
Gresiofulvin	-	-	-	-
(2a)	+	+	++	+
(2b)	+	+	++	+
(2c)	+	+	++	+
(2d)	-	-	+	-
(2e)	-	-	+	-
(2f)	-	-	+	-
(2g)	+	+	++	+
(2h)	+	+	++	+

Moderate growth (++), reduced growth (+) and no growth (-) of fungi

Assignment of significant peaks observed in IR, ^{1}H NMR, ^{13}C NMR spectra of the compounds **2a-2h** is clarified in the analytical data. The IR spectra of compound **2a-2h** showed high intensity band observed at 1629-1618 cm^{-1} is assigned to ν(C=N) vibration suggesting the formation of Schiff base. Band around 3356-3332 cm^{-1} is assigned to -NH in the Schiff bases. The band at 1567-1550 and 1489-1480 cm^{-1} is assigned to the ν(C=C) of the aromatic ring. A high intensity band in the region 1688-1678 cm^{-1} are assigned to pyridone carbonyl.

Each one of the ^{1}H NMR spectra of **2a-2h** revealed singlet for 3*H* between 2.63-2.70 ppm assigned to imino methyl group. Peaks between 7.7-7.1 ppm are assigned to aromatic protons. All ^{1}H NMR spectra of compounds **2b-2h** showed double doublet confirming *para*-substitution at aryl moiety bonded to imino nitrogen. A broad singlet at 6.72-6.54 ppm confirms the presence of -NH- group. Compound **2g** revealed a peak at 4.02 ppm assigned to $-OCH_3$. Methylene proton of $-OC_2H_5$ revealed a peak at 4.24 ppm. ^{13}C NMR of **2a-2h** showed peaks between 181.8-178.9 ppm for imine carbon. Assignment given to other peaks observed in ^{1}H NMR, ^{13}C NMR spectra and also molecular ion peaks in mass spectra justifies the structures of compounds **2a-2h**.

The synthesized Schiff's bases were studied for antibacterial and anti-fungal activity with different strains of bacteria and fungi. Results are shown in Tables 1 and 2. All imines have shown lesser activity against *E. coli, S. aureus and B. subtilis* compared with penicillin taken as standard. The activity of compounds **2d-2f** against bacteria and fungi was higher in comparison with other and shown activity against *S. typhi* and fungi. Antifungal activity observed against *Aspergillus species* and *Penicillium chrysogenum* was encouraging in comparison with *Fusarium moneliforme*. However, compounds **2d-2f** has reduced the growth of these organisms.

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