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Synthesis and Biological Applications of (*E*)-*N*-Benzylidene-5-bromo-2-chloropyrimidin-4-amine Derivatives

H.B. Vasanth Patil[1], V. Lakshmi Ranganatha[2], T. Prashanth[3] and C. Mallikarjunaswamy[3,✉]

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ABSTRACT

Schiff bases are aldehyde-like compounds in which an imine group replaces the carbonyl group. They are widely used for industrial purposes and also exhibit a broad range of biological activities. This study represents the synthesis of a new series of (*E*)-*N*-benzylidene-5-bromo-2-chloropyrimidin-4-amine derivatives (**6a-l**). The newly synthesized compounds were characterized by different spectral studies. All these compounds are screened for their anti-inflammatory, antimicrobial and *in vitro* antioxidant activities. The structure-activity relationship analysis demonstrates that hydroxyl groups on the aromatic ring contribute critically to the antioxidant activity. Compounds **6k**, **6j**, **6d** and **6e** showed significant radical scavenging and compounds **6d**, **6e** and **6f** showed good antimicrobial and anti-inflammatory activities.

KEYWORDS

Schiff base, Aldehydes, Antimicrobial activity, Antioxidant activity, Anti-inflammatory.

INTRODUCTION

Reactive oxygen species (ROS) readily attack and induce oxidative damage to various biomolecules including proteins, lipids, lipoproteins and DNA [1] and thus may lead to various diseases such as carcinogens, drug-associated toxicity, inflammation, atherogenesis and ageing in aerobic organisms [2-4]. Therefore, the significance of free radicals and ROS in the pathogenesis of multifarious diseases has attracted consi-derable attention. Antioxidants are currently fabricated as the drug candidates to counter these diseases. Minor dietary compositions have been seriously considered to combat the ill effects of free radicals and ROS. Based on growing interest in free radical biology and the lack of effective therapies for most chronic diseases, the usefulness of antioxidants in protecting against these diseases is warranted. Antioxidants are chemical substances that reduce or prevent oxidation. They have the ability to counteract the damaging effects of free radicals in tissues and are thus believed to protect against cancer, heart disease and several other diseases [5]. Many studies have shown that phenolic compounds display the antioxidant activity because of their capacity to scavenge free radicals [6]. The naturally occurring polyphenols are widely distributed in

Author affiliations:

[1]PG Department of Studies and Research in Bio-Technology, JSS College, Ooty Road, Mysuru-25, India
[2]Department of Chemistry, The National Institute of Engineering, Manandavadi Road, Mysuru-08, India
[3]PG Department of Chemistry, JSS College of Arts, Commerce and Science, Ooty Road, Mysore-25, India

✉To whom correspondence to be addressed:

E-mail: mallik.aanekere@gmail.com

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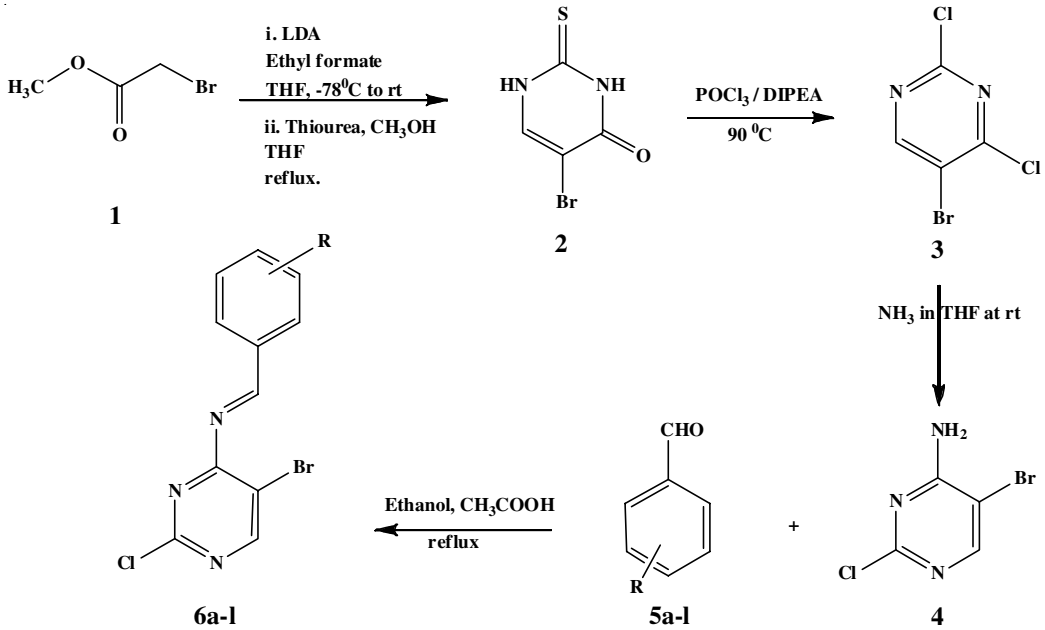
nature [7]. Liu and coworkers have reported the protective effects of hydroxyl-substituted Schiff bases against free radical-induced peroxidation of triolein in micelles, hemolysis of human red cells and oxidation of DNA [8]. Pyrimidine, being an integral part of DNA and RNA, has imparted diverse pharmacological properties as effective bactericide and fungicide [9-11]. Certain pyrimidine derivatives were also known to exhibit anti-inflammatory [12], antioxidant [13,14], antimicrobial [15,16], anthelmintic [17] and anti-HIV activities [18]. In addition to the diverse biological activities of pyrimidine, other heterocycles in association with pyrimidines play an essential role in several biological processes and have a considerable chemical and pharmacological importance. Pyrimidines in association with Schiff base have occupied a prominent place in medicinal chemistry because of their significant properties as therapeutics in clinical application. Schiff bases have also been shown to exhibit a broad range of biological activities, including antifungal, antibacterial, antimalarial, antiproliferative, anti-inflammatory, antiviral and antipyretic properties [19,20].

On the other hand, hydroxyl-substituted Schiff bases obtained from the reaction between the corresponding aromatic aldehyde and amines have a similar structure of *trans*-stilbene skeleton of resveratrol (3,5,42-trihydroxy-trans-stilbene), a well-characterized antioxidant and cancer chemopreventive molecule found in grapes and a variety of medicinal plants [21]. Their structural differences exist only in the connection of two aromatic rings, one is carbon–nitrogen double bond and the other is a carbon–carbon double bond. Although many studies have investigated the antioxidant properties of resveratrol [22]. There have been only a few reports of the antioxidant effects of hydroxyl-substituted Schiff bases. It was previously found that simple structural modification of resveratrol could significantly enhance its antioxidative activity [23]. Encouraged by the aforementioned information and in an attempt to better understand the structure–activity relationship (SAR) of hydroxyl-substituted Schiff bases as antioxidants and cancer chemopreventive agents, we synthesized hydroxyl-substituted Schiff bases with the different substitutions and investigated their antioxidant, antimicrobial activity and anti-inflammatory effects.

EXPERIMENTAL

All solvents and reagents were purchased from Sigma Aldrich Chemicals. Melting points were determined on an electrically heated VMPIII melting point apparatus. The elemental analyses of the compounds were performed on a Perkin Elmer 2400 Elemental Analyzer. The FTIR spectra were recorded using KBr discs on FTIR 4100 Infrared spectrophotometer. The NMR spectra were recorded using Bruker DRX 400 spectrometer at 400 MHz for ^{1}H NMR with tetramethylsilane as the internal standard. Mass spectral data were obtained by LC/MSD Trap XCT. Silica gel for column chromatography was performed using Merck 7734 silica gel and Merck made TLC plates.

Synthesis of 5-bromo-2-chloropyrimidin-4-amine (4): The compound **2** was synthesized by treatment of the appropriate ester enolate with ethyl formate followed by condensation with thiourea in one pot, which gave 5-bromo-2,3-dihydro-2-thioxopyrimidin-4 (1*H*)-one, which was converted into 5-bromo-2,4-dichloropyrimidine **3** with $POCl_3$/N,N-diisopropylethylamine (DIPEA). Then the compound **3** was treated with ammonia in THF at room temperature for 10 min to produce 5-bromo-2-chloropyrimidin-4-amine **4** in > 95 % yield. The formation of compound **4** was confirmed by single-crystal X-ray diffraction [24] and IR spectra. Further, the compound **4** for treatment with different substituted aldehydes in the presence of ethanol and a few drops of acetic acid yielded the title compounds **6a-l** in good yield (**Scheme-I**). The structures of newly synthesized compounds **6a-l** were confirmed based on ^{1}H NMR, mass, elemental analysis and FT-IR spectral analysis. The formation of compounds (**6a-l**) was conrmed by IR spectra which showed characteristic absorption bands in the range between 1592–1581 cm^{-1} and 1691–1681 cm^{-1} due



Scheme-I: Synthetic route for pyrimidine derivatives

to C=N and C=C stretching, respectively. The ^{1}H NMR spectral data showed singlets in the range between δ 7.01 and 7.78 ppm for CH groups, respectively. The compound **6k** shows peaks at δ 3.71–3.81 ppm for –OCH_3 and these spectral data have provided support for the conformation of the structures of synthesized compounds. ^{13}C NMR spectral data and the mass spectrum of all the compounds showed molecular ion peak at M^{+1} corresponding to its molecular formula, which confirmed its chemical structure (Table-1).

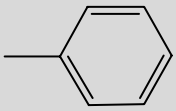
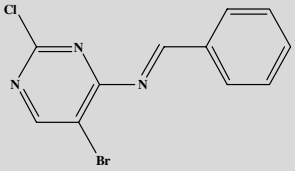
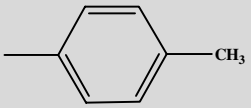
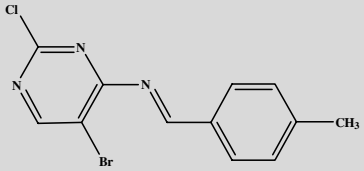
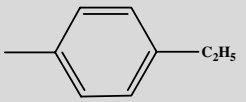
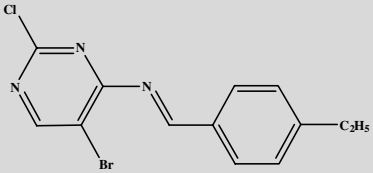
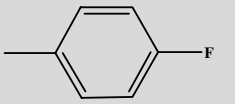
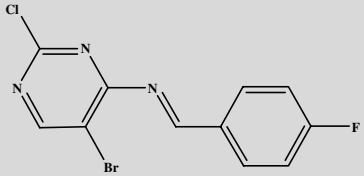
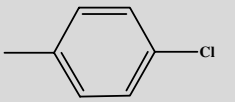
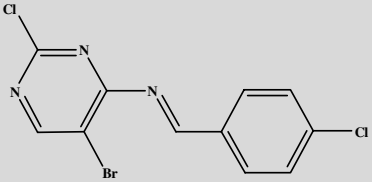
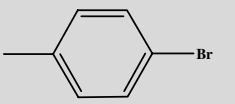
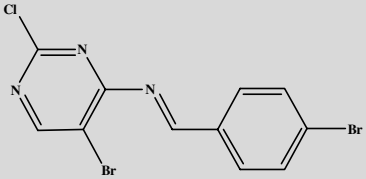
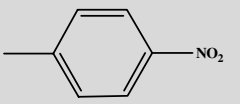
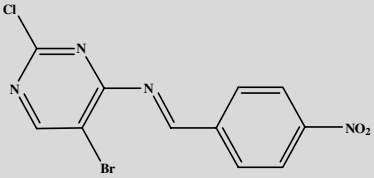
Pharmacological screening

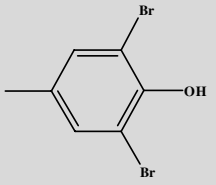
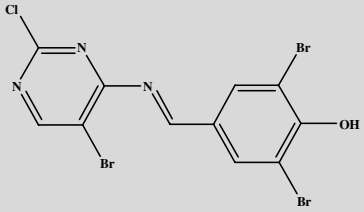
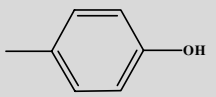
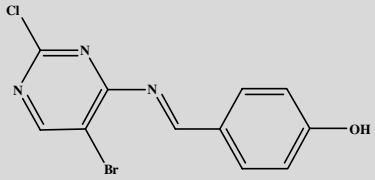
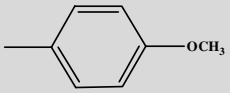
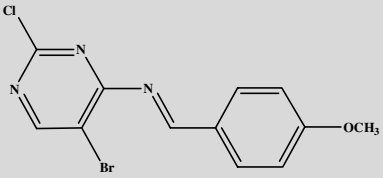
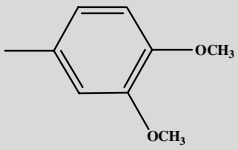
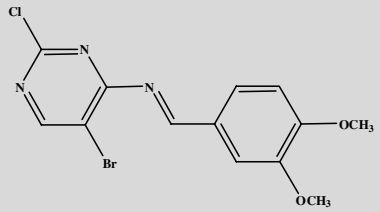
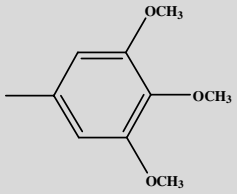
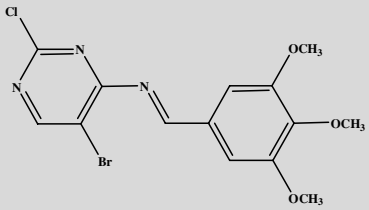
Antioxidant screening: Compounds **6a-l** are tested for antioxidant property by 1,1-diphenyl-1-picrylhydrazyl (DPPH) [25,26], nitric oxide [27,28] and hydrogen peroxide (H_2O_2) [29] methods.

DPPH radical scavenging activity: The hydrogen atom- or electron-donating ability of the compounds was measured from the bleaching of the purple-coloured methanol solution of DPPH. The spectrophotometric assay uses the stable radical DPPH as a reagent. 1 mL of various concentrations of the test compounds (25, 50 and 75 µg/mL) in methanol was added to 4 mL of 0.004 % (w/v) methanol solution of DPPH. After 30 min incubation period at room temperature, the absorbance was read against blank at 517 nm. The percentage of inhibition (%) of free radical production from DPPH was calculated by the following equation:

$$\text{Scavenging}\ (\%) = \frac{A_{control} - A_{sample}}{A_{blank}} \times 100 \qquad (1)$$

TABLE-1
CHEMICAL STRUCTURE AND MELTING RANGE OF
(*E*)-*N*-BENZYLIDENE-5-BROMO-2-CHLOROPYRIMIDIN-4-AMINEDERIVATIVES (**6a-l**)

Compounds	R	Structure	m.p. (°C)	Yield (%)
6a			210-213	67
6b			215-218	75
6c			197-200	72
6d			225-228	81
6e			221-223	76
6f			215-218	86
6g			209-211	82

6h			231-233	74
6i			218-221	73
6j			196-199	83
6k			247-250	85
6l			242-245	84

where $A_{control}$ is the absorbance of the control reaction (containing all reagents except the test compound) and A_{sample} is the absorbance of the test compound. The tests were carried in triplicate.

Nitric oxide (NO) scavenging activity: The nitric oxide (NO) scavenging activity was measured by slightly modified methods of Green *et al.* [27] and Marcocci *et al.* [28]. The procedure is based on the principle that sodium nitroprusside in aqueous solution at physiological pH spontaneously generates NO, which interacts with oxygen to produce nitrite ions that can be estimated using the Griess reagent [1 % sulfanilamide, 2 % H_3PO_4 and 0.1 % *N*-(1-naphthyl)ethylenediamine dihydrochloride]. Scavengers of NO compete with oxygen, leading to reduced production of nitrite ions. Approximately 1 mL of sodium nitroprusside (10 mm) and 1.5 mL of phosphate buffer saline (0.2 M, pH 7.4) were added to different concentrations (25, 50 and 75 µg/mL) of the test compounds and incubated for 150 min at 25 °C and 1 mL of the reaction mixture was treated with 1 mL of the Griess reagent. The absorbance of the chromatophore was measured at 546 nm. The NO scavenging activity was calculated using eqn. 1.

H_2O_2 scavenging activity: The H_2O_2 scavenging ability of the test compound was determined according to the method of Ruch *et al.* [29]. A solution of H_2O_2 (40 mm) was prepared in phosphate buffer (pH 7.4). A total of 25, 50 and 75 µg/mL concentrations of the test compounds in 3.4 mL phosphate buffer were added to H_2O_2 solution (0.6 mL, 40 mm). The absorbance value of the reaction mixture was recorded at 230 nm. The percentage of scavenging of H_2O_2 was calculated using eqn. 1.

Anti-inflammatory screening: The synthesized compounds screened for anti-inflammatory activity by using inhibition of albumin denaturation technique, which was studied according to Mizushima and Kabayashi [30] with slight modification. The standard drug and test compounds were dissolved in a minimum amount of DMF and diluted with phosphate buffer saline (pH 7.4) in such a way that the concentration of DMF in all solutions was less than 2.5 %. Test solution (1 mL, 100 mg/mL) was mixed with 1 mL of 1 % albumin solution in phosphate buffer saline and incubated at 27 °C for 15 min. Denaturation was induced by keeping the reaction mixture at 60 °C in a water bath for 10 min. After cooling, the turbidity was measured at 660 nm with UV-visible spectrophotometer. The percentage of inhibition of denaturation was calculated from the control where no drug was added. Each experiment was done in triplicate and the average was taken. The diclofenac was used as a standard drug. The percentage of inhibition was calculated using the formula:

$$\text{Inhibition of denaturation (\%)} = \left(\frac{V_t}{V_c} - 1\right) \times 100 \qquad (2)$$

where V_t is the absorption of test compound and V_c is the absorption of control.

Antimicrobial activity: By using the agar plate diffusion technique [31], all of the synthesized compounds were screened *in vitro* for antibacterial activity against *Escheria coli*, *Pseudomonas aeruginosa* (Gram-negative), *Staphylococcus aureus* and *Bacillus subtilis* (Gram-positive) at 50 mg/mL and 100 mg/mL concentrations, respectively. Streptomycin was chosen as a standard drug [32]. Similarly, the antifungal screening of the compounds was carried out *in vitro* by the disc diffusion method against two fungi *Aspergillus niger* and *Candida albicans* by using amphotericin-B as a standard drug [32-34].

Synthesis of (*E*)-*N*-benzylidene-5-bromo-2-chloropyrimidin-4-amine derivatives (6a-l): The Schiff base was prepared by reaction of equimole of **5a-l** and 5-bromo-2-chloropyrimidin-4-amine. Each reactant was dissolved in a minimum amount of ethanol, then mixed together and followed by the addition of 2 mL glacial acetic acid. The solution was refluxed for 8 h, then cooled to room temperature and poured into ice-cold water. The solid product was collected through filtration and then dried using drying oven at 80 °C. The product was redissolved in ethanol for recrystallization and then dried to give a product.

Synthesis of (*E*)-*N*-benzylidene-5-bromo-2-chloropyrimidin-4-amine (6a): The general experimental procedure described above afforded **6a** and the product was obtained from 5-bromo-2-chloropyrimidin-4-amine (**4**) (2.08 g, 0.01 mol) and benzaldehyde (**5a**) (1.06 g, 0.01 mol). FT-IR (KBr, ν_{max}, cm^{-1}): 1689 (C=C), 1586 (C=N). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 7.06–7.26 (m, 5H, Ar–H), 7.37 (s, 1H, CH–N), 7.51 (s, 1H, Ar–H). ^{13}C NMR (DMSO-d_6, 125 MHz) (δ ppm): 111.1, 128.9, 129.2, 131.1, 133.8, 160.1, 161.1, 163.5, 183.8. MS (ESI) *m/z*: 296.55. Anal. calcd. for $C_{11}H_7N_3BrCl$ (%): C, 44.55; H, 2.38; N, 14.17. Found C, 44.41; H, 2.22; N, 14.03.

Synthesis of (5-bromo-2-chloro-pyrimidine-4-yl)-(4-methyl-benzylidine)amine (6b): The general experimental procedure described above afforded **6b** and the product was obtained from 5-bromo-2-chloropyrimidin-4-amine (**4**) (2.08 g, 0.01 mol) and 4-methyl benzaldehyde (**5b**) (1.20 g, 0.01 mol). FT-IR (KBr, ν_{max}, cm^{-1}): 1690 (C=C), 1582 (C=N). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 2.41 (s, 3H, Ar–CH_3), 7.02–7.31 (m, 4H, Ar–H), 7.43 (s, 1H, CH–N), 7.56 (s, 1H, Ar–H). ^{13}C NMR (DMSO-d_6, 125 MHz) (δ ppm): 24.6, 111.5, 129.1, 129.2, 133.8, 140.7, 160.5, 161.3, 163.2, 183.5. MS (ESI) *m/z*: 310.58. Anal. calcd. for $C_{12}H_9N_3BrCl$ (%): C, 46.41; H, 2.92; N, 13.53. Found C, 46.35; H, 2.86; N, 13.48.

Synthesis of (*E*)-*N*-(4-ethylbenzylidene)-5-bromo-2-chloropyrimidin-4-amine (6c): The general experimental procedure described above afforded **6c** and the product was obtained from 5-bromo-2-chloropyrimidin-4-amine (**4**) (2.08 g, 0.01 mol) and 4-ethylbenzaldehyde (**5c**) (1.34 g, 0.01 mol). FT-IR (KBr, ν_{max}, cm^{-1}): 1684 (C=C), 1588 (C=N). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 1.31 (t, 3H, Ar–CH_3), 2.41 (q, 2H, Ar–CH_2), 7.06–7.26 (m, 4H, Ar–H), 7.37 (s, 1H, CH–N), 7.51 (s, 1H, Ar–H). ^{13}C NMR (DMSO-d_6, 125 MHz) (δ ppm): 24.6, 32.6, 111.3, 127.5, 129.2, 131.6, 141.2, 160.2, 161.1, 163.3, 183.2. MS (ESI) *m/z*: 324.6. Anal. calcd. for $C_{13}H_{11}N_3BrCl$ (%): C, 48.10; H, 3.42; N, 12.95. Found C, 48.06; H, 3.38; N, 12.81.

Synthesis of (*E*)-*N*-(4-fluorobenzylidene)-5-bromo-2-chloropyrimidin-4-amine (6d): The general experimental procedure described above afforded **6d** and the product was obtained from 5-bromo-2-chloropyrimidin-4-amine (**4**) (2.08 g, 0.01 mol) and 4-fluorobenzaldehyde (**5d**) (1.24 g, 0.01 mol). FT-IR (KBr, ν_{max}, cm^{-1}): 1687 (C=C), 1588 (C=N). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 7.02–7.31 (m, 4H, Ar–H), 7.39 (s, 1H, CH–N), 7.56 (s, 1H, Ar–H). ^{13}C NMR (DMSO-d_6, 125 MHz) (δ ppm): 110.5, 115.2, 129.2, 130.5, 160.5, 161.7, 163.1, 165.6, 184.1. MS (ESI) *m/z*: 314.54. Anal. calcd. for $C_{11}H_6N_3BrClF$ (%): C, 42.00; H, 1.92; N, 13.36. Found C, 41.97; H, 1.84; N, 13.22.

Synthesis of (*E*)-*N*-(4-chlorobenzylidene)-5-bromo-2-chloropyrimidin-4-amine (6e): The general experimental procedure described above afforded **6e** and the product was obtained from 5-bromo-2-chloropyrimidin-4-amine (**4**) (2.08 g, 0.01 mol) and 4-chlorobenzaldehyde (**5e**) (1.40 g, 0.01 mol). FT-IR (KBr, ν_{max}, cm^{-1}): 1687 (C=C), 1582 (C=N). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 7.11–7.29 (m, 4H, Ar–H), 7.31 (s, 1H, CH–N), 7.41 (s, 1H, Ar–H). ^{13}C NMR (DMSO-d_6, 125 MHz) (δ ppm): 111.1, 129.1, 130.9, 136.1, 160.6, 161.8, 163.7, 165.6, 183.9. MS (ESI) *m/z*: 331.00. Anal. calcd. for $C_{11}H_6N_3BrCl_2$ (%): C, 39.92; H, 1.83; N, 12.70. Found C, 39.86; H, 1.78; N, 12.65.

Synthesis of (*E*)-*N*-(4-bromobenzylidene)-5-bromo-2-chloropyrimidin-4-amine (6f): The general experimental procedure described above afforded **6f** and the product was obtained from 5-bromo-2-chloropyrimidin-4-amine (**4**) (2.08 g, 0.01 mol) and 4-bromobenzaldehyde (**5f**) (1.84 g, 0.01 mol). FT-IR (KBr, ν_{max}, cm^{-1}): 1690 (C=C), 1588 (C=N). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 7.04–7.34 (m, 4H, Ar–H), 7.42 (s, 1H, CH–N), 7.56 (s, 1H, Ar–H). ^{13}C NMR (DMSO-d_6, 125 MHz) (δ ppm): 110.5, 124.2, 131.1, 131.9, 132.8, 160.2, 161.2, 163.1, 183.1. MS (ESI) *m/z*: 375.45. Anal. calcd. for $C_{11}H_6N_3Br_2Cl$ (%): C, 35.19; H, 1.61; N, 11.19. Found C, 35.01; H, 1.55; N, 11.06.

Synthesis of (*E*)-*N*-(4-nitrobenzylidene)-5-bromo-2-chloropyrimidin-4-amine (6g): The general experimental procedure described above afforded **6g** and the product was obtained from 5-bromo-2-chloropyrimidin-4-amine (**4**) (2.08 g, 0.01 mol) and 4-nitrobenzaldehyde (**5g**) (1.51 g, 0.01 mol). FT-IR (KBr, ν_{max}, cm^{-1}): 1688 (C=C), 1586 (C=N). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 7.11–7.41 (m, 4H, Ar–H), 7.57 (s, 1H, CH–N), 7.77 (s, 1H, Ar–H). ^{13}C NMR (DMSO-d_6, 125 MHz) (δ ppm): 110.9, 122.1, 130.9, 139.2, 150.1, 160.6, 161.5, 163.8, 183.6. MS (ESI) *m/z*: 341.55. Anal. calcd. for $C_{11}H_6N_4O_2BrCl$ (%): C, 38.68; H, 1.77; N, 16.40. Found C, 38.51; H, 1.62; N, 16.36.

Synthesis of 4-[(*E*)-(5-bromo-2-chloropyrimidin-4-ylimino)methyl]-2,6-dibromophenol (6h): The general experimental procedure described above afforded **6h** and the product was obtained from 5-bromo-2-chloropyrimidin-4-amine (**4**) (2.08 g, 0.01 mol) and 3,5-dibromo-4-hydroxybenzaldehyde (**5h**) (2.77 g, 0.01 mol). FT-IR (KBr, ν_{max}, cm^{-1}): 1690 (C=C), 1582 (C=N). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 5.25 (bs, 1H, C–OH), 7.06–7.26 (s, 2H, Ar–H), 7.37 (s, 1H, CH–N), 7.51 (s, 1H, Ar–H). ^{13}C NMR (DMSO-d_6, 125 MHz) (δ ppm): 111.3, 115.6, 130.5, 132.9, 133.9, 160.6, 161.2, 163.2,

184.1. MS (ESI) *m/z*: 470.34. Anal. calcd. for $C_{11}H_5N_3OBr_3Cl$ (%): C, 28.09; H, 1.07; N, 8.93. Found C, 28.12; H, 1.15; N, 8.87.

Synthesis of 4-[(*E*)-(5-bromo-2-chloropyrimidin-4-ylimino)methyl]phenol (6i): The general experimental procedure described above afforded **6i** and the product was obtained from 5-bromo-2-chloropyrimidin-4-amine (**4**) (2.08 g, 0.01 mol) and 4-hydroxybenzaldehyde (**5i**) (1.22 g, 0.01 mol). FT-IR (KBr, ν_{max}, cm^{-1}): 1687 (C=C), 1586 (C=N). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 5.35 (bs, 1H, C–OH), 6.86–7.36 (m, 4H, Ar–H), 7.41 (s, 1H, CH–N), 7.61 (s, 1H, Ar–H). ^{13}C NMR (DMSO-d_6, 125 MHz) (δ ppm): 110.6, 116.6, 125.9, 131.5, 160.1, 160.7, 161.9, 163.7, 183.5. MS (ESI) *m/z*: 312.55. Anal. calcd. for $C_{11}H_7N_3OBrCl$ (%): C, 42.27; H, 2.26; N, 13.44. Found C, 42.17; H, 2.32; N, 13.31.

Synthesis of (*E*)-*N*-(4-methoxybenzylidene)-5-bromo-2-chloropyrimidin-4-amine (6j): The general experimental procedure described above afforded **6j** and the product was obtained from 5-bromo-2-chloropyrimidin-4-amine (**4**) (2.08 g, 0.01 mol) and 4-methoxybenzaldehyde (**5j**) (1.36 g, 0.01 mol). FT-IR (KBr, ν_{max}, cm^{-1}): 1689 (C=C), 1581 (C=N). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 3.65 (s, 3H, O–CH_3), 6.86–7.36 (m, 4H, Ar–H), 7.57 (s, 1H, CH–N), 7.71 (s, 1H, Ar–H). ^{13}C NMR (DMSO-d_6, 125 MHz) (δ ppm): 111.2, 115.1, 126.1, 130.5, 160.3, 160.2, 161.2, 163.2, 184.5. ^{13}C NMR (DMSO-d_6, 125 MHz) (δ ppm): 111.2, 115.1, 126.1, 130.5, 160.3, 160.2, 161.2, 163.2, 184.5. MS (ESI) *m/z*: 326.58. Anal. calcd. for $C_{12}H_9N_3OBrCl$ (%): C, 44.13; H, 2.78; N, 12.87. Found C, 44.24; H, 2.67; N, 12.75.

Synthesis of (*E*)-*N*-(3,4-dimethoxybenzylidene)-5-bromo-2-chloropyrimidin-4-amine (6k): The general experimental procedure described above afforded **6k** and the product was obtained from 5-bromo-2-chloropyrimidin-4-amine (**4**) (2.08 g, 0.01 mol) and 3,4-dimethoxybenzaldehyde (**5k**) (1.66 g, 0.01 mol). FT-IR (KBr, ν_{max}, cm^{-1}): 1682 (C=C), 1589 (C=N). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 3.78 (s, 6H, O–CH_3), 7.01–7.26 (m, 3H, Ar–H), 7.57 (s, 1H, CH–N), 7.61 (s, 1H, Ar–H). ^{13}C NMR (DMSO-d_6, 125 MHz) (δ ppm): 55.9 (OMe), 110.5, 113.5, 115.9, 122.9, 127.6, 149.1, 151.5, 160.1, 161.2, 163.2, 183.1. MS (ESI) *m/z*: 356.60. Anal. calcd. for $C_{13}H_{11}N_3O_2BrCl$ (%): C, 43.79; H, 3.11; N, 11.78. Found C, 43.82; H, 3.22; N, 11.64.

Synthesis of (*E*)-*N*-(3,4,5-trimethoxybenzylidene)-5-bromo-2-chloropyrimidin-4-amine (6l): The general experimental procedure described above afforded **6l** and the product was obtained from 5-bromo-2-chloropyrimidin-4-amine (**4**) (2.08 g, 0.01 mol) and 3,4,5-trimethoxybenzaldehyde (**5l**) (1.96 g, 0.01 mol). FT-IR (KBr, ν_{max}, cm^{-1}): 1684 (C=C), 1586 (C=N). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 3.71 (s, 9H, O–CH_3), 7.06–7.26 (s, 2H, Ar–H), 7.37 (s, 1H, CH–N), 7.51 (s, 1H, Ar–H). ^{13}C NMR (DMSO-d_6, 125 MHz) (δ ppm): 56.1 (OMe), 56.9, 111.2, 105.9, 128.1, 127.6, 142.2, 150.8, 160.5, 161.8, 163.9, 183.9. MS (ESI) *m/z*: 386.63. Anal. calcd. for $C_{14}H_{13}N_3O_3BrCl$ (%): C, 43.49; H, 3.39; N, 10.77. Found C, 43.34; H, 3.23; N, 10.65.

The compound **2** was synthesized from methyl-2-bromoacetate, ethyl formate and thiourea and it was converted into **3** with $POCl_3$ and DIPEA. The compound **3** was then treated with ammonia in THF at room temperature for 10 min to produce compound **4** [35,36].

RESULTS AND DISCUSSION

Biological activity: Compounds **6a-l** are tested for *in vitro* antioxidant property by 1,1-diphenylpicrylhydrazyl (DPPH), NO and H_2O_2 methods, which were summarized in Tables 2-4, respectively. It is well known that one of the main characters responsible for the antioxidant activity of a phenolic compound is its ability to scavenge free radicals. DPPH$^\bullet$ is a relatively stable oxygen radical and has been widely used for evaluating the antioxidant activity. Consequently, study of the scavenging reaction of **6a-l** towards DPPH$^\bullet$ at 25 °C was performed in methanol by UV-visible spectroscopy by recording the decay of the DPPH$^\bullet$ visible absorbance (at 517 nm). Litwinienko and Ingold [37] observed an abnormal increase of rate constants of the (DPPH$^\bullet$) radical scavenging reaction in alcoholic media, which was attributed to partial ionization of the phenolic and a very fast electron transfer from phenolate anion to DPPH$^\bullet$. These studies suggest that in alcoholic media the sequential proton loss electron transfer (SPLET mechanism) predominates over the direct hydrogen atom transfer (HAT mechanism) for hydroxyl-substituted Schiff bases. Both SPLET and HAT mechanisms ultimately result in the formation of same phenoxyl radical PhO; therefore, the stabilization of this free radical finally decides the effect of different substitution on the antioxidant activity. Electron-donating groups on the *ortho* or *para* position of the benzene ring enhance the activity by stabilization of the free radical, while electron-withdrawing groups decrease the antioxidant activity.

To study the SAR of the antioxidant activity, Schiff bases containing strong and weak electron-donating or -withdrawing substituents were synthesized (**6a-6l**). The investigation of antioxidant screening data revealed that some of the tested compounds showed moderate-to-good antioxidant activity. Particularly, compounds having an –OH group at the *para* position (**6h** and **6i**) showed more promising antioxidant

TABLE-2
in vitro ANTIOXIDANT ACTIVITY OF COMPOUNDS **6a-l** IN DPPH METHOD

Compound	Concentration (µg/mL)			
	25	50	75	IC_{50}
6a	68.13±1.07	71.43±0.65	76.52±1.12	17.01±1.15
6b	66.13±0.27	72.23±0.35	77.22±1.02	18.10±1.05
6c	65.71±1.47	67.44±1.24	72.84±1.56	18.35±1.55
6d	49.60±0.61	51.33±1.14	55.13±0.35	17.42±0.15
6e	53.71±1.52	57.25±1.10	60.31±0.82	19.55±1.21
6f	58.72±0.51	63.12±1.16	68.94±0.76	16.72±1.42
6g	68.43±1.20	71.61±1.35	74.93±1.18	15.25±1.15
6h	74.53±0.70	75.25±0.22	77.85±0.65	25.14±0.72
6i	76.41±0.41	77.81±0.51	78.36±0.70	23.11±0.96
6j	65.21±1.27	67.24±1.14	72.24±1.26	18.15±1.25
6k	64.81±0.62	66.31±1.19	70.28±1.23	16.02±0.43
6l	63.80±0.20	67.12±0.25	69.63±0.25	16.92±0.61
Ascorbic acid	82.15±0.22	83.12±0.28	86.12±0.24	15.25±0.43
Blank	–	–	–	–

'–' no scavenging activity. Values were the mean of three replicates ± SD.

TABLE-3
in vitro ANTIOXIDANT ACTIVITY OF
COMPOUNDS **6a-l** IN NITRIC OXIDE METHOD

Compound	Concentration (μg/mL)			
	25	50	75	IC_{50}
6a	73.21±0.25	75.06±0.24	76.15±1.11	17.14±0.26
6b	70.24±0.26	72.51±0.17	79.34±0.17	16.65±0.60
6c	73.40±0.65	75.16±0.64	76.25±1.10	17.24±0.16
6d	60.27±1.18	64.22±1.45	68.61±1.23	16.25±1.16
6e	54.14±1.39	57.45±1.24	59.13±0.25	14.15±1.24
6f	64.02±1.41	67.88±1.42	69.12±0.38	15.25±0.25
6g	69.35±1.15	70.23±1.32	74.56±1.32	16.29±0.14
6h	80.13±0.33	83.14±0.25	84.34±0.62	22.16±0.55
6i	79.84±0.17	82.29±0.25	83.25±0.14	23.19±1.25
6j	68.34±0.95	70.61±1.39	74.18±0.95	17.37±1.25
6k	64.21±0.65	67.65±0.68	70.19±0.13	16.45±0.46
6l	64.11±0.25	67.25±0.38	70.09±0.23	16.25±0.26
Ascorbic acid	84.22±0.28	85.16±0.25	88.12±0.45	14.51±0.14
Blank	–	–	–	–

'–' no scavenging activity. Values were the mean of three replicates ± SD.

TABLE-4
in vitro ANTIOXIDANT ACTIVITY OF COMPOUNDS
6a-l IN HYDROGEN PEROXIDE METHOD

Compound	Concentration (μg/mL)			
	25	50	75	IC_{50}
6a	62.01±0.85	64.31±1.58	69.12±1.07	17.47±1.23
6b	62.14±1.32	66.32±1.34	69.21±1.01	20.15±0.75
6c	64.12±0.89	68.31±1.19	71.15±0.58	21.54±0.42
6d	58.25±1.17	62.31±1.17	65.74±1.47	21.22±1.07
6e	55.12±0.88	57.18±1.17	60.14±1.07	27.75±0.65
6f	60.26±1.06	63.48±1.27	67.84±1.57	17.24±0.25
6g	63.17±1.16	67.23±0.86	70.32±0.17	20.33±1.04
6h	71.25±0.27	74.25±0.64	77.11±0.49	24.21±0.24
6i	70.94±1.05	73.23±1.25	78.14±0.62	23.15±0.42
6j	62.17±0.32	64.23±0.31	67.87±0.34	16.17±1.01
6k	61.16±1.06	62.38±1.27	68.84±1.37	17.14±0.15
6l	60.16±0.16	63.28±1.17	67.64±1.17	17.20±0.20
Ascorbic acid	75.21±0.08	77.61±0.13	81.21±0.21	15.21±0.21
Blank	–	–	–	–

'–' no scavenging activity. Values were the mean of three replicates ± SD.

activity as compared with that of standard ascorbic acid. Compounds with the methoxy substituent exhibited slightly lower activity than the hydroxyl group containing compounds. For example, the compound having the methoxy group in the *para* position (**6j**) showed a good level of activity (IC_{50} = 12–14 μg/mL). Introducing another methoxy group at 3-position (**6k**) makes the compounds slightly less active. Again, the compound with 3,4,5-OMe (**6l**) was found to be less active than 4-methoxy. Compounds having halogens at the *para* position of the benzene ring (**6d**, **6e**, **6f**) showed mild activity due to their negative inductive effect, which destabilizes the free radical. Whereas alkyl group containing compound (**6b**) showed mild activity but better than the halogen-containing compounds due to their positive inductive effect, they stabilize the radical to some extent, which causes an increase in the antioxidant activity in comparison to halogen derivatives.

All of the compounds **6a-l** were tested for *in vitro* anti-inflammatory activity. Compared to the standard diclofenac sodium, they have shown an acceptable anti-inflammatory activity. *In vitro* anti-inflammatory activity of compounds is summarized in Table-5. The results revealed that the compounds **6d**, **6e** and **6f** exhibited moderate anti-inflammatory activities. Among all the tested compounds, **6e** was found to be more potent. All the other compounds presented weak-to-moderate activities.

TABLE-5
in vitro ANTI-INFLAMMATORY
ACTIVITY OF COMPOUNDS **6a-l**

Compound	Mean absorbance ± SD	Inhibition of denaturation (%)
Control	0.1880 ± 0.025	–
6a	0.2315 ± 0.016	67.02
6b	0.2624 ± 0.020	55.61
6c	0.3011 ± 0.002	45.12
6d	0.3451 ± 0.003	78.23
6e	0.3525 ± 0.007	79.92
6f	0.3215 ± 0.011	77.21
6g	0.2621 ± 0.009	65.21
6h	0.3112 ± 0.023	66.54
6i	0.2432 ± 0.012	52.22
6j	0.2925 ± 0.009	55.23
6k	0.2335 ± 0.026	67.12
6l	0.2531 ± 0.021	51.12
Diclofenac sodium	0.3625 ± 0.004	83.12

SD = Standard deviation (average of three determination)

The antimicrobial activity of the compounds **6a-l** was tested against *E. coli*, *P. aeruginosa* (Gram-negative bacteria), *B. subtilis* and *S. aureus* (Gram-positive bacteria) and two fungi, *C. albicans* and *A. niger* and the results were reported as a zone of inhibition. The results of the preliminary antibacterial testing of compounds **6a-l** are shown in Table-6. The results revealed that all the derivatives of pyrimidines (**6a-l**) were showing good-to-potent antibacterial activity against all the tested strains of bacteria. While the entire derivatives showed moderate-to-potent activity against *B. subtilis*, the halogenated derivatives of **6d**, **6e** and **6f** exhibited potent antibacterial activity. The pyrimidine ring may responsible for the good activity against *B. subtilis*. Moreover, the other compounds were weakly active against the tested organism. The results of preliminary antifungal testing of the compounds **6a-l** are shown in Table-7. Compounds **6e** and **6f** exhibited potent activity against *C. albicans* and *A. niger*, while the other compounds exhibited moderate-to-good activity.

Conclusion

In conclusion, a new class of (*E*)-*N*-benzylidene-5-bromo-2-chloropyrimidin-4-amine derivatives were prepared from simple starting material, substituted by aldehydes in good yields and studied for their antioxidant, anti-inflammatory and antimicrobial activities. It was observed that the compounds having the hydroxyl group exhibited greater antioxidant activity and halogenated compounds showed good antimicrobial and anti-inflammatory activities. The investigation of the antioxidant screening data reveals that among the 12 compounds screened, compounds **6h, 6i** and **6j** showed excellent, almost equivalent to that of standard remaining compounds that showed moderate-to-mild inhibition activity. The presence of

TABLE-6
ANTIBACTERIAL ACTIVITY OF THE COMPOUNDS **6a-l**

Compounds	Zone of inhibition (mm)							
	E. coli		*P. aeruginosa*		*B. subtilis*		*S. aureus*	
	50 µg/mL	100 µg/mL	50 µg/mL	100 µg/mL	50 µg/mL	100 µg/mL	50 µg/mL	100 µg/mL
6a	10	13	09	15	09	14	11	13
6b	09	12	08	14	10	13	10	13
6c	12	14	14	16	11	15	11	14
6d	19	26	16	28	18	29	20	27
6e	19	27	17	28	17	28	20	27
6f	18	26	16	27	18	28	19	26
6g	16	21	14	23	15	20	17	22
6h	17	22	13	25	16	21	16	24
6i	13	20	11	23	12	16	14	22
6j	14	21	14	24	13	17	15	23
6k	17	12	13	22	14	21	17	21
6l	13	20	10	22	13	17	15	23
Standard	21	28	18	30	20	31	22	29

TABLE-7
ANTIFUNGAL ACTIVITY OF COMPOUNDS **(6a-l)**

Compound	Zone of inhibition (mm)			
	C. albicans		*A. niger*	
	50 µg/mL	100 µg/mL	50 µg/mL	100 µg/mL
6a	10.25	18.12	12.12	21.12
6b	10.12	20.24	13.42	22.14
6c	11.25	21.21	13.12	22.33
6d	11.14	19.12	15.42	23.25
6e	15.15	22.93	16.92	25.62
6f	14.21	22.56	16.52	25.91
6g	12.12	20.12	14.12	23.25
6h	11.14	19.16	13.92	23.15
6i	13.14	21.12	12.12	21.32
6j	12.42	20.16	12.45	23.12
6k	12.22	20.11	14.10	23.15
6l	11.21	21.11	13.22	22.23
Amphotericin-B	15.36	23.15	17.16	26.24

the electron-donating substituent on ring enhances the activity and electron-withdrawing groups like nitro group decrease. Many research models have been established in chemical and/or biological systems for studying the mechanisms of action of antioxidants and for identifying new antioxidants. Ten substituted Schiff bases were synthesized and bio-evaluated for their antioxidant, antimicrobial and anti-inflammatory activities in pursuit of the more active compound.

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