Novel Pyrimidine Schiff bases: Synthesis and pharmacological screening

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 new compounds screened for their anti-inflammatory, antimicrobial and *in vitro* antioxidant activities. 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.

1. 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 aging in aerobic organisms [2-4]. So the significance of free radicals and ROS in the pathogenesis of multifarious diseases has attracted considerable 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 thus believed to protect against cancer, heart disease and several other diseases [5]. Many studies have shown that phenolic compounds display antioxidant activity because of their capacity to scavenge free radicals [6]. The naturally occurring polyphenols are widely distributed in nature [7]. Recently, 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, have imparted diverse pharmacological

properties as effective bactericide and fungicide [9-11]. Certain pyrimidine derivatives were also known to exhibit anti-inflammatory [12] anti-oxidant [13,14], antibmicrobial [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 (3,5,4′-trihydroxy-trans-stilbene), a well-characterized antioxidant and cancer chemo-preventive 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. 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 of hydroxyl-substituted Schiff bases as antioxidants and cancer chemo preventive agents, we synthesized here in hydroxyl-substituted Schiff bases with the different substitutions, and investigated their antioxidant, antimicrobial activity and anti-inflammatory effects.

2. Procedure

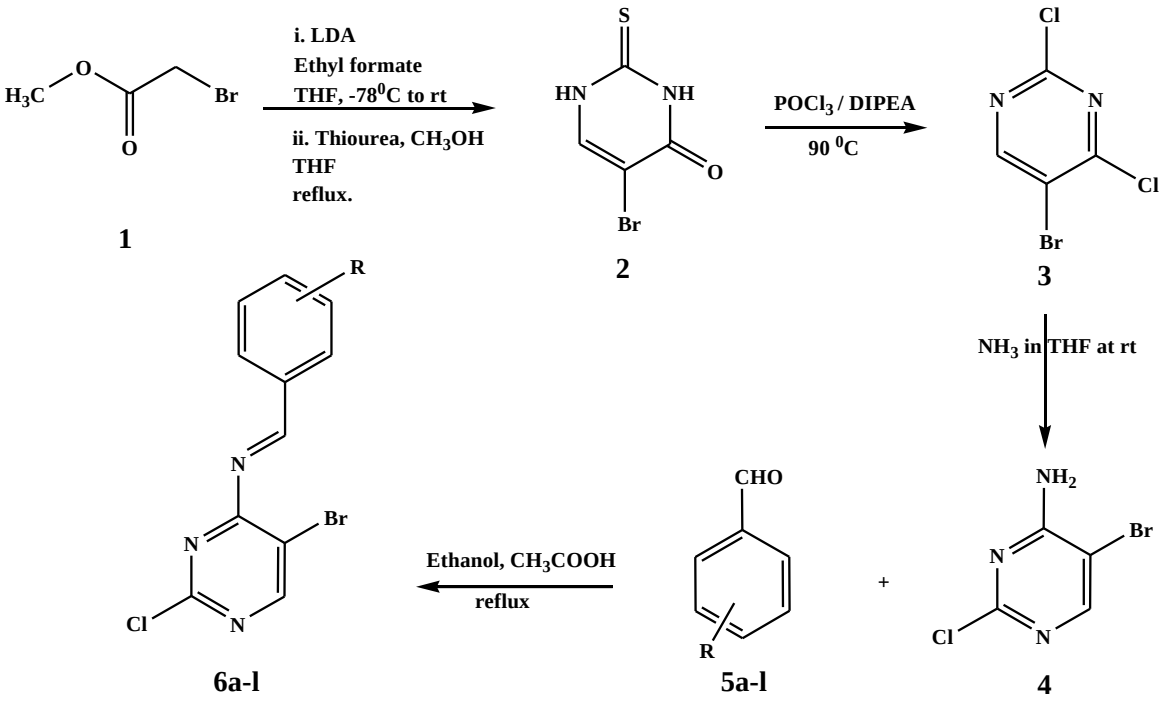
2.1 Materials and Method

All solvents and reagents purchased from Sigma Aldrich Chemicals. Melting points were determined on an electrically heated VMP-III melting point apparatus. The elemental analyses of the compounds were performed on a Perkin Elmer 2400 Elemental Analyzer. The FT-IR spectra were recorded using KBr discs on FT-IR 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.

2.2 Synthesis

General procedure for the synthesis of 5-bromo-2-chloropyrimidin-4-amine 4: The compound **2** was synthesized by treatment of the appropriate ester enolate with ethylformate followed by condensation with thiourea in one pot gave 5-bromo-2,3-dihydro-2-thioxopyrimidin-4 (1H)-one, which was converted to 5-bromo-2,4-dichloropyrimidine **3** with $POCl_3$/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** 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 1). 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 confirmed by IR spectra which showed characteristic absorption bands in the range between 1581-1592 cm^{-1} and 1681-1691 cm^{-1}due to C=N and C=Cstretching.The ^{1}H NMR spectral data showed singlets in the range between at δ 7.01-7.78 ppm for CH groups respectively. The compound **6k** shows peaks at δ 3.71-3.81 ppm for -OCH_3, 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).



Scheme 1: synthetic route for pyrimidine derivatives

2.3 Pharmacological screening

2.3.1 Antioxidant screening

Compounds **6a-l is** tested for antioxidant property by DPPH [25, 26], NO [27, 28] and H_2O_2 [29] methods.

2.3.1.1 DPPH radical scavenging activity

The hydrogen atom or electron donating ability of the compounds was measured from the bleaching of the purple colored methanol solution of 1,1-diphenyl-1-picrylhydrazyl (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 a 30 min incubation period at room temperature, the absorbance was read against blank at 517 nm. The percent of inhibition (I %) of free radical production from DPPH was calculated by the following equation

% of scavenging = [(A control - A sample) / A blank] × 100 ---------(1)

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. Tests were carried at in triplicate.

2.3.1.2 Nitric oxide (NO) scavenging activity

Nitric oxide scavenging activity was measured by slightly modified methods of Green *et al*. and Marcocci *et al*. The procedure is based on the principle that, sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide which interacts with oxygen to produce nitrite ions that can be estimated using Griess reagent (1% sulfanilamide, 2% H_3PO_4 and 0.1% *N*-(1-naphthyl) ethylenediamine dihydrochloride). Scavengers of nitric oxide compete with oxygen, leading to reduced production of nitrite ions. 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 ^{0}C and 1 ml of the reaction mixture was treated with 1 ml of Griess reagent. The absorbance of the chromatophore was measured at 546 nm. Nitric oxide scavenging activity was calculated using Eq. (1).

2.3.1.3 Hydrogen peroxide (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*. A solution of H_2O_2 (40 mm) was prepared in phosphate buffer (pH 7.4). 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 percent of scavenging of H_2O_2 was calculated using Equation (1).

2.3.2 Anti-inflammatory screening

The synthesized compounds screened for anti-inflammatory activity by using inhibition of albumin denaturation technique, which studied according to Muzushima and Kabayashi[30] with slight modification. The standard drug and test compounds dissolved in a minimum amount of DMF and diluted with phosphate buffer saline (pH 7.4) in such a way that 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 ^{0}C in an incubated for 15 min. Denaturation was induced by keeping the reaction mixture at 60 ^{0}C in a water bath for 10 min. After cooling, the turbidity measured at 660 nm with UV-vis spectrophotometer. Percentage of inhibition of denaturation calculated from the control where no drug added. Each experizentdone in triplicate and the average taken. The diclofenac used as standard drug. The percentage of inhibition calculated using the formula,

% Inhibition of denaturation = $[(Vt/ V_c) - 1] \times 100$ ----------(2)

Where, V_t=absorption of test compound, V_c=absorption of control.

2.3.3 Antimicrobial activity

Applying the agar plate diffusion technique [31] all of the newly synthesized compounds were screened *in vitro* for antibacterial activity against *E. coli*, *P. Aeruginosa* (Gram-negative), *S. aureus*, *B. Subtilis* (Gram-positive) at 50 and 100 mg/ml concentrations, respectively. Streptomycin was chosen as a standard drug [32]. Streptomycin is an antibiotic that inhibits both gram-positive and gram-negative bacteria, and is therefore a useful broad spectrum antibiotic. Similarly, the antifungal screening of the compounds was carried out *in vitro* by disc diffusion method against two fungi *A. Niger* and *C. Albicans* by using Amphotericin-B as a standard [32, 33].

3 Data, Value and Validation

3.1 Chemistry

General procedure for the 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 hours, 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 80ºC. The product was redissolved in ethanol for recrystalliziation and then dried to give a product.

3.1.1 Synthesis of (*E*)-N-benzylidene-5-bromo-2-chloropyrimidin-4-amine (6a): The general experimental procedure described above afforded **6a**, and the product 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, cm^{-1}) *v*: 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$C_{11}H_7BrClN_3$ (in %): C, 44.55; H, 2.38; N, 14.17. Found C, 44.41; H, 2.22; N, 14.03.

3.1.2 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 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, cm^{-1}) *v*: 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 $C_{12}H_9BrClN_3$ (in %): C, 46.41; H, 2.92; N, 13.53. Found C, 46.35; H, 2.86; N, 13.48.

3.1.3 Synthesis of (*E*)-N-(4-ethylbenzylidene)-5-bromo-2-chloropyrimidin-4-amine (**6c**): The general experimental procedure described above afforded **6c**, and the product 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, cm^{-1}) *v*: 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$C_{13}H_{11}BrClN_3$ (in %): C, 48.10; H, 3.42; N, 12.95. Found C, 48.06; H, 3.38; N, 12.81.

3.1.4 Synthesis of (*E*)-N-(4-fluorobenzylidene)-5-bromo-2-chloropyrimidin-4-amine (**6d**): The general experimental procedure described above afforded **6d**, and the product 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, cm^{-1}) *v*: 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 $C_{11}H_6BrClFN_3$ (in %): C, 42.00; H, 1.92; N, 13.36. Found 41.97; H, 1.84; N, 13.22.

3.1.5 Synthesis of (*E*)-N-(4-chlorobenzylidene)-5-bromo-2-chloropyrimidin-4-amine (**6e**): The general experimental procedure described above afforded **6e**, and the product 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, cm^{-1}) *v*: 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 $C_{11}H_6BrCl_2N_3$(in %): C, 39.92; H, 1.83; N, 12.70. Found C, 39.86; H, 1.78; N, 12.65.

3.1.6 Synthesis of (*E*)-N-(4-bromobenzylidene)-5-bromo-2-chloropyrimidin-4-amine (**6f**): The general experimental procedure described above afforded **6f**, and the product 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, cm^{-1}) *v*: 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 $C_{11}H_6Br_2ClN_3$(in %): C, 35.19; H, 1.61; N, 11.19. Found C, 35.01; H, 1.55; N, 11.06.

3.1.7 Synthesis of (*E*)-N-(4-nitrobenzylidene)-5-bromo-2-chloropyrimidin-4-amine (**6g**): The general experimental procedure described above afforded **6g**, and the product 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, cm^{-1}) *v*: 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 $C_{11}H_6BrClN_4O_2$ (in %): C, 38.68; H, 1.77; N, 16.40. Found C, 38.51; H, 1.62; N, 16.36.

3.1.8 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 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, cm^{-1}) *v*: 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 $C_{11}H_5Br_3ClN_3O$ (in %): C, 28.09; H, 1.07; N, 8.93. Found C, 28.12; H, 1.15; N, 8.87.

3.1.9 Synthesis of 4-((*E*)-(5-bromo-2-chloropyrimidin-4-ylimino)methyl)phenol (**6i**): The general experimental procedure described above afforded **6i**, and the product 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, cm^{-1}) *v*: 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 $C_{11}H_7BrClN_3O$ (in %): C, 42.27; H, 2.26; N, 13.44. Found 42.17; H, 2.32; N, 13.31.

3.1.10 Synthesis of (*E*)-N-(4-methoxybenzylidene)-5-bromo-2-chloropyrimidin-4-amine (**6j**): The general experimental procedure described above afforded **6j**, and the product 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, cm^{-1}) *v*: 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 $C_{12}H_9BrClN_3O$ (in %): C, 44.13; H, 2.78; N, 12.87. Found C, 44.24; H, 2.67; N, 12.75.

3.1.11 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 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, cm^{-1}) *v*: 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 $C_{13}H_{11}BrClN_3O_2$ (in %): C, 43.79; H, 3.11; N, 11.78. Found C, 43.82; H, 3.22; N, 11.64.

3.1.12 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 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, cm^{-1}) *v*: 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 $C_{14}H1_3BrClN_3O_3$ (in %): 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 N, N-Diisopropylethylamine (DIPEA). Then the compound **3** was treated with ammonia in THF at room temperature for 10 min to produce compound **4** [35, 36].

3.2 Biological activity

Compounds **6a-l** is tested for *in vitro* antioxidant property by 1,1-diphenylpicrylhydrazyl (DPPH), nitric oxide (NO) and hydrogen peroxide (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$^{\cdot}$ is a relatively stable oxygen radical and has been widely used for evaluating antioxidant activity. Consequently, study of the scavenging reaction of **6a-l** toward DPPH$^{\cdot}$ at 25^0C was performed in methanol by UV-vis spectroscopy by recording the decay of the DPPH$^{\cdot}$ Visible absorbance (at 517 NM). Ingold [37] has observed previously an abnormal increase of rate constants of (DPPH$^{\cdot}$) 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$^{\cdot}$. These studies, together with our recent results 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. SPLET or HAT mechanism both ultimately results in the formation of same phenoxyl radical PhO$^{\cdot}$, 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 structure-activity relationship (SAR) of antioxidant activity, Schiff bases containing strong and weak electron donating or withdrawing substituent's were synthesized (**6a-6l**). The investigation of antioxidant screening revealed that some of the tested compounds showed moderate to good antioxidant activity. Particularly, compounds having an OH group at para-position (**6h** and **6i**) showed more promising antioxidant activity as compared to that of standard, ascorbic acid. Compounds with methoxy substituent exhibited slightly lower activity than the hydroxyl group containing compounds. For example 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 slight less active. Again compound with 3,4,5-OMe (**6l**) 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 cause an increase in antioxidant activity in comparison to halogen derivatives.

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

The antimicrobial activity of the compounds **6a-l** were tested against *Escheria coli*, *Pseudomonas aeruginosa* (gram-negative bacteria), *Bacillus subtillis* and *Staphylococcus aureus* (gram-positive bacteria), two fungi, *Candida albicans*, *Aspergillus Niger*, and the results were reported as a zone of inhibition. The results of 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 Bacillus subtilis. The halogenated derivatives of **6d**, **6e** and **6f** was exhibited potent antibacterial activity. While 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** is 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 and substituted aldehydes in good yields and studied for their antioxidant activity, anti-inflammatory and antimicrobial activity. It was observed that the compounds having hydroxyl group exhibited greater antioxidant activity and halogenated compounds shows good antimicrobial and anti-inflammatory activity. The investigation of antioxidant screening data reveals that among the twelve compounds screened, compounds **6h, 6i** and **6j** showed excellent, almost equivalent to that of standards the remaining compounds showed moderate to mild inhibition activity. The presence of the electron donating substituent on ring enhances the activity and electron withdrawing groups like Nitro 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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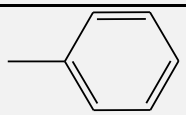
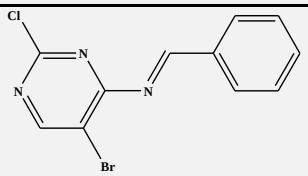
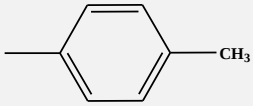
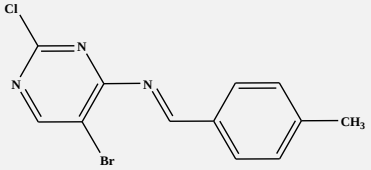
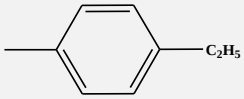
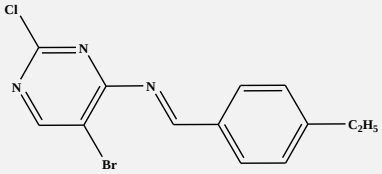
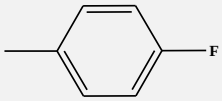
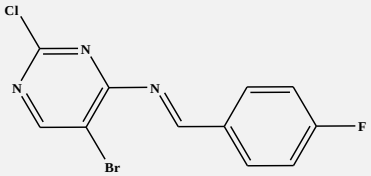
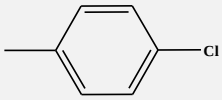
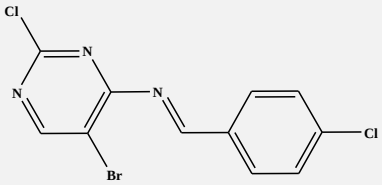
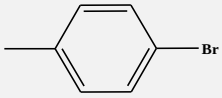
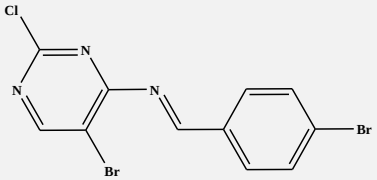
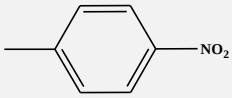
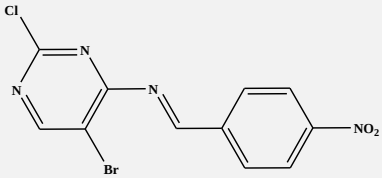
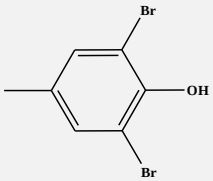
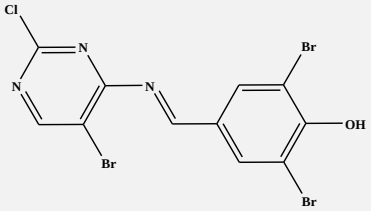
Table 1: Chemical structure and melting range of (*E*)-*N*-benzylidene-5-bromo-2-chloropyrimidin-4-aminederivatives (**6a-l**).

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

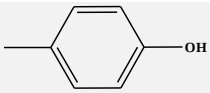
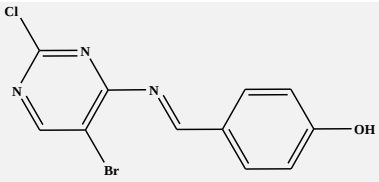
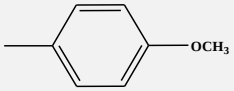
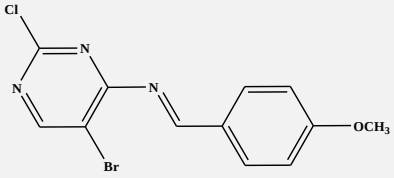
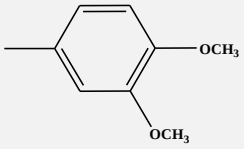
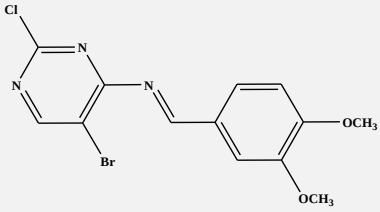
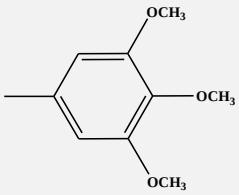
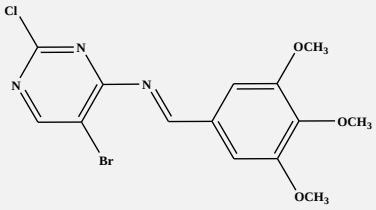
6i			218-221	73
6j			196-199	83
6k			247-250	85
6l			242-245	84

Table 2: The *in vitro* antioxidant activity of **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	-	-	-	-

(-) Showed no scavenging activity. Values were the means of three replicates ± SD.

Table 3: The *in vitro* antioxidant activity of **6(a-l)** in nitric oxide (NO) 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	-	-	-	-

(-) Showed no scavenging activity. Values were the means of three replicates ± SD.

Table 4: The *in vitro* antioxidant activity of **6(a-l)** in hydrogen peroxide (H_2O_2) 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	-	-	-	-

(-) Showed no scavenging activity. Values were the means of three replicates ± SD.

Table 5: *In vitro* anti-inflammatory activity of compounds (**6a-l**)

Compound	Mean obsorbance ± 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).

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	50µg/ml	100µg/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	*C.albicans*	*A.niger*
	Zone of inhibition (mm)	

	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

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