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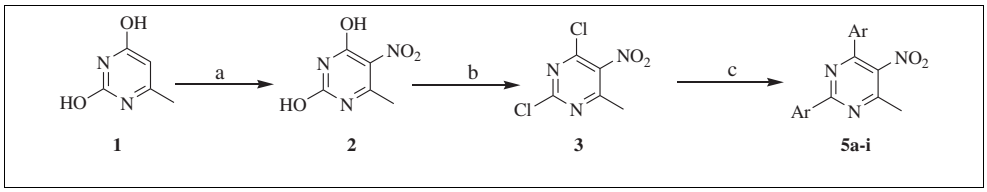

A new series of 2,4-diaryl-6-methyl-5-nitropyrimidines (**5a–i**) were synthesized in good yields by Suzuki–Miyaura coupling of 2,4-dichloro-6-methyl-5-nitropyrimidine (**3**) with various aryl boronic esters (**4a–i**) in the presence of 1,1′- bis(diphenylphosphino)ferrocene dichloropalladium(II) ($Pd(dppf)_2Cl_2$). Further, antibacterial and antioxidant properties were screened for the title compounds **5a–i**. Most of the compounds possessed significant activity against Gram-positive bacteria *Staphylococcus aureus* and *Bacillus subtilis* and Gram-negative bacteria *Escherichia coli* and *Klebsiella pneumoniae*. The antioxidant activity of the title compounds showed significant antioxidant activity when compared with vitamin C.

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INTRODUCTION

The development of novel synthetic methods leading to pyrimidine derivatives has attracted much attention in recent years because of their prevalence and wide utility in organic synthesis. Furthermore, they play an important role in several biological and pharmacological active substances such as antimicrobial and antitumor agents [1–4] and agrochemical and veterinary products [5–7]. Some of the pyrimidinone derivatives are also used as potent and orally bioavailable HIV-1 integrase inhibitors [8] and anti-inflammatory and analgesic agents [9].

The Suzuki cross-coupling reaction is a powerful method for carbon–carbon bond formation that has been applied in a variety of settings, ranging from natural-product synthesis to materials chemistry, including large-scale production [10]. Among the attractive features of the Suzuki reaction are the wide availability, stability to air and moisture, and low toxicity of boronic acids, as well as the facile removal of the boron-containing side products of the coupling process. Catalyst systems based on palladium precatalysts to generate biaryls have also proven to be highly effective [11,12]. Herein, we report the synthesis of 2,4-diaryl pyrimidine derivatives by using the catalytic system 1,1′- bis(diphenylphosphino)ferrocene dichloropalladium(II) (Scheme 1) for the Suzuki–Miyaura reaction of aryl boronic esters and 2,4-dichloro-6-methyl-5-nitropyrimidine.

In view of the biological significance of pyrimidine derivatives, we are interested in obtaining some new compounds derived by aryl substitution at the 2- and 4-positions of 2,4-dichloro-6-methyl-5-nitropyrimidine (Scheme 1) and tested their antibacterial and antioxidant activities.

RESULTS AND DISCUSSION

The synthesis of **5a–i** was carried out according to Scheme 1. Briefly, 6-methylpyrimidine-2,4-diol (**1**) was prepared by the reaction of ethylacetoacetate with urea in the presence of NaOMe in methanol at reflux temperature [13]. Nitration of **1** in the presence of H_2SO_4 and fuming nitric acid obtained 6-methyl-5-nitropyrimidine (**2**) [1]. It reacted with $POCl_3$ in the presence of *N*,*N*′-dimethylaniline and tetrabutylammoniumchloride to afford 2,4-dichloro-6-methyl-5-nitropyrimidine (**3**) [2]. Finally, diarylation of compound **3** with various aryl boronic esters (**4a–i**) in the presence of 1,1′-bis(diphenylphosphino)ferrocenedichloropalladium(II) and Na_2CO_3 in 1,4-dioxane/water by Suzuki–Miyaura coupling [10,14] furnished the title compounds as 2,4-diaryl-6-methyl-5-nitropyrimidines (**5a–i**). We determined the of synthesized derivatives by IR, ^{1}H NMR, and ^{13}C NMR, and their molecular masses were determined by mass spectrometry (Table 1).

The IR spectra of **5a–i** exhibited characteristic absorption bands in the regions 1548–1559 and 1335–1375 cm^{-1} for NO_2 antisymmetric and symmetric stretching bands, aromatic C=C stretching absorption bands showed at 1575–1592 cm^{-1}, and C–H antisymmetric and symmetric stretching absorption bands of methyl group exhibited at 2935–2956 and 2840–2877 cm^{-1}.

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Scheme 1. Reaction conditions for analog synthesis: (a) HNO_3, H_2SO_4; (b) Bu_4NCI, $PhN(Me)_2$, $POCI_3$; (c) aryl boronic esters **4a–i**, $Pd(dppf)_2CI_2$/Na_2CO_3, 1,4-dioxane/H_2O, 75–85%.

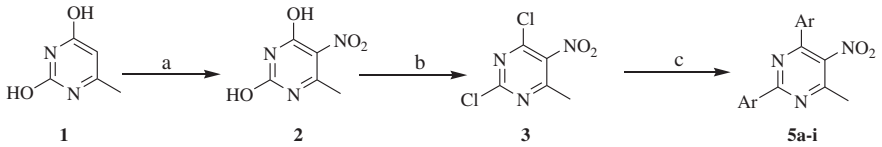
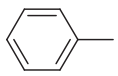
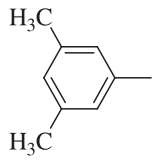
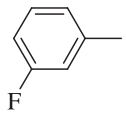
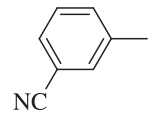
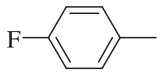
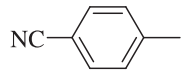
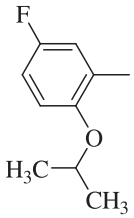
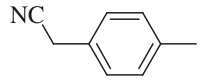
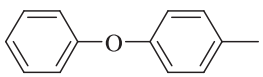


Table 1

2,4-Diaryl-6-methyl-5-nitropyrimidines **5a–i**.

Entry	Ar	Entry	Ar
5a		**5f**	
5b		**5g**	
5c		**5h**	
5d		**5i**	
5e			

The ^{1}H NMR spectra of **5a** showed four doublets at δ 8.56 (J = 8.0 Hz, 1H, Ar–H), 8.55 (J = 8.4 Hz, 1H, Ar–H) and 7.81 (J = 7.6 Hz, 1H, Ar–H), 7.77 (J = 8.0 Hz, 1H, Ar–H), a multiplet at δ 7.57–7.49 (m, 6H, Ar–H) for aromatic protons, and the protons in methyl group appeared as singlet at δ 2.69 (s, 3H, CH_3). In compound **5d**, the aromatic protons resonated as doublet of doublets at δ 7.61, 7.58 (J = 8.8, 8.7 Hz, 1H) and 7.47, 7.44 (J = 8.7, 8.7 Hz, 1H) and multiplets at δ 7.16–7.09 (2H), 7.02–6.98 (1H), 6.85–6.80 (1H). The OCH protons appeared as multiplet in the region δ 4.61–4.43 (2H, 2OCH), the methyl protons of pyrimidine ring resonated as singlet at δ 2.78 (3H, CH_3), and the two methyl groups of the isopropyl moiety attributed as doublets at δ 1.35 (J = 6.0 Hz, 6H, 2×CH_3) and 1.23 (J = 5.7 Hz, 6H, 2×CH_3). Similarly, the other compounds **5b**, **5c**, and **5e–i** were also characterized by the ^{1}H NMR spectra, and their data are given in the Experimental section.

The ^{13}C NMR spectra of **5a** showed 11 peaks in the aromatic region at δ 163.68, 160.01, 157.53, 143.52, 136.07, 134.16, 131.76, 131.18, 129.00, 128.66, and 128.10 and methyl carbon signal found at δ 20.72. The other compounds (**5b–i**) were also characterized by the ^{13}C NMR spectra, and their data are given in the Experimental section. Chemical ionization mass spectra of **5a–i**

gave molecular ion peaks at their respective expected *m*/*z* values.

We monitored the compounds **5a–i** for antibacterial activity against both Gram-positive bacteria and Gram-negative bacteria by the literature method [6,15]. Assays of Gram-positive bacteria *Staphylococcus aureus* and *Bacillus subtilis* and Gram-negative bacteria *Escherichia coli* and *Klebsiella pneumoniae* by the disc diffusion method in nutrient agar medium at two concentrations (50, 100 μg/mL) in DMSO were carried out. The compounds were diluted in DMSO for biological assays. The samples were tested in triplicate, and average results were recorded. The results were presented in Table 2 and compared with ampicillin (50 μg/mL). The results revealed that the compounds **5b**, **5c**, and **5d** confer the highest antibacterial activity against all tested bacterial strains in both 50- and 100-μg concentrations. This is due to fluorine and oxygen showing highly negative-inductive effect, and NO_2 is a highly electron-withdrawing moiety. However, the other compounds **5a** and **5e–i** exhibited a moderate activity.

Antioxidants help organisms deal with oxidative stress, and its main characteristic is its ability to trap free radicals. The free radical scavenging capacity of **5a–i** compounds were evaluated by 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) [16,17] and nitric oxide radical scavenging activity [18,19] methods (Table 3). The results confirmed that the compound **5e** showed high DPPH scavenging activity with 32 μg/mL; it is due to the electron-donating phenoxyphenyl group and $–NO_2$ substituent's role in **5e**, which affect the electron-donating capacitates appearing to be useful in inducing antioxidant activity. The other compounds **5b** and **5c** also exhibit the high activity because fluorine exhibits highly negative-inductive effect and $–NO_2$ is a highly electron-withdrawing moiety; therefore, electron density around nitrogen heterocycle decreases and increases affinity towards aromatic ring derived from free radicals and mobilizes reactive oxygen species to be scavenged out of a living organism. The compounds **5b–5e** also showed the highest NO scavenging activity compared with other compounds. The title compounds **5a–i**, containing nitrogen heterocycle, are expected to be more active because of the presence of hetero atoms containing non-bonded electron pairs that serve as binding sites in the bio-matrix. The compounds **5a**, **5d**, and **5f–i** displayed appreciable antioxidant activity because these compounds contain $–NO_2$ group in the parent pyrimidine moiety.

Table 2
Antibacterial activity (diameter of zone of inhibition of compounds **5a–i**, μg/mL).[a]

Bacteria	**5a**		**5b**		**5c**		**5d**		**5e**		**5f**		**5g**		**5h**		**5i**		Ampicillin[b]
	50	100	50	100	50	100	50	100	50	100	50	100	50	100	50	100	50	100	50
S. aureus	8	10	10	14	14	15	15	18	15	17	7	10	9	12	7	10	8	11	16
B. subtilis	9	10	11	13	13	15	14	17	16	19	9	12	8	10	8	11	7	9	19
E. coli	10	12	12	15	16	20	14	18	17	20	8	11	10	11	7	12	9	10	21
K. pneumoniae	9	12	11	13	14	18	15	18	15	19	11	13	9	12	10	13	10	13	19

[a]Two concentrations (50, 100 μg/mL) in DMSO.
[b]Reference compound.

EXPERIMENTAL

All reactions were carried out under nitrogen atmosphere. Glassware was dried in an oven prior to use. Reactions were monitored by TLC with Merck silica gel 60 F_{254} plates or with neutral aluminum oxide 60 F_{254} plates (Merck, India). Flash

Table 3

DPPH radical and nitric oxide radical scavenging activity of **5a–i** compounds.

Compound	DPPH assay IC_{50} (μg/mL)	NO scavenging activity IC_{50} (μg/mL)
5a	44	50
5b	34	31
5c	36	34
5d	42	35
5e	32	32
5f	40	42
5g	41	43
5h	40	44
5i	43	45
Ascorbic acid (vitamin C)	68.25	65.35

column chromatography was performed on silica gel 60 (70–230 mesh ASTM) from Merck. All other chemicals such as Pd(dppf)Cl_2 and aryl boronic esters, in which its preparation is not described in the succeeding texts, were brought from Aldrich, Acros, Merck, or Combi-blocks. Melting points were determined on a Buchi R-535 apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer FTIR 240-c spectrophotometer with the use of KBr optics. ^{1}H and ^{13}C NMR spectra were recorded on an AMX 400 MHz NMR or 300 MHz NMR spectrometer from Bruker in $CDCl_3$ unless otherwise stated. Mass spectra were recorded on a Finnigan MAT 1020/Micro-Mass Q-T of micro AMPS MAX 10/6A, Hz 60/50 system fitted with a built-in inlet system. Standard strains of Gram-positive bacteria *S. aureus* and *B. subtilis* and Gram-negative bacteria *E. coli* and *K. pneumonia*e were obtained from IMTECH, Chandigarh, India.

The starting material 2,4-dichloro-6-methyl-5-nitropyrimidine was synthesized by the procedure reported in the literature[1,2,13].

General procedure for the preparation of 2,4-diaryl-6-methyl-5-nitropyrimidines.

Preparation of 4-methyl-5-nitro-2,6-diphenylpyrimidine 5a. Phenyl boronic ester (4,4,5,5-tetramethyl-2-phenyl-1,3,2-dioxaborolane) **4a** (2.04 g, 0.01 mol, 4 eq) and sodium carbonate (0.94 g, 0.01 mol, 4 eq) were added to a stirred solution of 2,4-dichloro-6-methyl-5-nitropyrimidine **3** (0.520 g, 0.0025 mol, 1 eq) in 1,4-dioxane (20 mL) and water (5 mL). The reaction mixture was degassed with nitrogen gas and stirred at room temperature for 30 min. To this reaction mixture, Pd(dppf)$_2$Cl$_2$ (0.365 g, 0.0005 mol, 0.2 eq, 20 mol%) was added and heated at 110 °C for overnight. After cooling to room temperature, solvent was removed under reduced pressure. Water was then added, and the impure product was extracted into ethyl acetate. The combined organic layers were dried over anhydrous Na_2SO_4, and the solvent was removed under vacuum. The crude product was purified by FCC (3% MeOH/CH_2Cl_2) to give a pure compound **5a** as an amorphous solid (0.62 g, 2.5 mmol, 85%); mp 131–133 °C; IR (KBr) 3123, 3021, 2943, 2857, 1584, 1550, 1362 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.56 (d, J = 8.0 Hz, 1H, Ar–H), 8.55 (d, J = 8.4 Hz, 1H, Ar–H), 7.81 (d, J = 7.6 Hz, 1H, Ar–H), 7.77 (d, J = 8.0 Hz, 1H, Ar–H), 7.57–7.49 (m, 6H, Ar–H), 2.69 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 163.68, 160.01, 157.53, 143.52, 136.07, 134.16, 131.76, 131.18, 129.00, 128.66, 128.10, 20.72; MS (ESI, 0 V), *m/z* 292 (M + 1, 100%). *Anal*. Calcd for $C_{17}H_{13}N_3O_2$: C, 70.09; H, 4.50; N, 14.42. Found: C, 70.17; H, 4.64; N, 14.28.

2,4-Bis(3-fluorophenyl)-6-methyl-5-nitropyrimidine 5b. Amorphous solid (0.63 g, 2.5 mmol, 77%); mp 135–137 °C; IR (KBr) 3112, 3016, 2952, 2840, 1586, 1556, 1335 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.35 (d, J = 7.9 Hz, 1H, Ar–H), 8.25 (d, J = 9.9 Hz, 1H, Ar–H), 7.54–7.44 (m, 4H, Ar–H), 7.28 (s, 2H, Ar–H), 2.65 (s, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ 164.31, 162.56, 160.49, 156.15, 143.49, 138.11, 135.88, 130.93, 124.66, 123.76, 119.08, 115.87, 20.78; MS (ESI, 0 V), *m/z* 328 (M + 1, 100%). *Anal*. Calcd for $C_{17}H_{11}F_2N_3O_2$: C, 62.39; H, 3.39; N, 12.84. Found: C, 62.30; H, 3.48; N, 12.69.

2,4-Bis(4-fluorophenyl)-6-methyl-5-nitropyrimidine 5c. Amorphous solid (0.65 g, 2.5 mmol, 80%); mp 142–144 °C; IR (KBr) 3121, 3019, 2949, 2866, 1587, 1548, 1337 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.29 (d, J = 8.4 Hz, 2H, Ar–H), 8.15 (d, J = 9.0 Hz, 2H, Ar–H), 7.46–7.49 (m, 4H, Ar–H), 2.68 (s, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ 164.10, 162.42, 159.87, 154.41, 142.89, 137.41, 135.32, 130.26, 123.96, 123.46, 118.88, 115.51, 20.45; MS (ESI, 0 V), *m/z* 328 (M + 1, 100%). *Anal*. Calcd for $C_{17}H_{11}F_2N_3O_2$: C, 62.39; H, 3.39; N, 12.84. Found: C, 62.24; H, 3.50; N, 12.71.

2,4-Bis(5-fluoro-2-isopropoxyphenyl)-6-methyl-5-nitropyrimidine 5d. Amorphous solid (0.86 g, 2.5 mmol, 78%); mp 118–120 °C; IR (KBr) 3122, 3018, 2934, 2870, 1575, 1553, 1360, 1120 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.60 (dd, J = 8.7 Hz, 1H, Ar–H), 7.45 (dd, J = 8.7, 1H, Ar–H), 7.16–7.09 (m, 2H, Ar–H), 7.02–6.98 (m, 1H, Ar–H), 6.85–6.80 (m, 1H, Ar–H), 4.61–4.52 (m, 1H, 2OCH), 4.52–4.41 (m, 1H, 2OCH), 2.78 (s, 3H, CH_3); 1.35 (d, J = 6.0 Hz, 6H, $(CH_3)_2$), 1.23 (d, J = 5.7 Hz, 6H, $(CH_3)_2$); ^{13}C NMR (75 MHz, $CDCl_3$): δ 163.80, 160.65, 157.59, 156.05, 154.44, 152.56, 150.69, 142.89, 128.75, 125.98, 118.61, 117.63, 114.87, 71.56, 21.94, 20.81; MS (ESI, 0 V), *m/z* 444 (M + 1, 100%). *Anal*. Calcd for $C_{23}H_{23}F_2N_3O_4$: C, 62.30; H, 5.23; N, 9.48. Found: C, 62.21; H, 5.37; N, 9.56.

2,4-Bis(4-phenoxyphenyl)-6-methyl-5-nitropyrimidine 5e. Amorphous solid (0.90 g, 2.5 mmol, 76%); mp 122–124 °C; IR (KBr) 3100, 3018, 2947, 2856, 1591, 1559, 1461, 1375, 1151 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.51 (d, J = 9.6 Hz, 2H, Ar–H), 7.75 (d, J = 7.2 Hz, 2H, Ar–H), 7.41–7.37 (m, 4H, Ar–H), 7.21–7.16 (m, 2H, Ar–H), 7.10–7.05 (m, 8H, Ar–H), 2.65 (s, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ 162.14, 160.38, 159.83, 156.54, 155.32, 142.35, 130.85, 130.30, 128.19, 124.62, 119.83, 118.02, 20.67; MS (ESI, 0 V), *m/z* 476 (M + 1, 100%). *Anal.* Calcd for $C_{29}H_{21}N_3O_4$: C, 73.25; H, 4.45; N, 8.84. Found: C, 73.36; H, 4.54; N, 8.73.

4-Methyl-2,6-bis(3,5-dimethylphenyl)-5-nitropyrimidine 5f. Amorphous solid (0.70 g, 2.5 mmol, 80%); mp 134–136 °C; IR (KBr) 3132, 3021, 2943, 2863, 1589, 1554, 1427, 1372 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.12 (s, 2H, Ar–H), 7.78 (s, 2H, Ar–H), 7.52 (s, 2H, Ar–H), 2.78 (s, 3H, CH_3); 2.35 (s, 6H, 2 (CH_3)), 2.31 (s, 6H, 2(CH_3)); ^{13}C NMR (100 MHz, $CDCl_3$): δ 163.87, 161.07, 158.30, 142.85, 136.41, 134.65, 131.56, 131.32, 129.48, 128.19, 128.40, 23.78, 20.45; MS (ESI, 0 V), *m/z* 348 (M + 1, 100%). *Anal*. Calcd for $C_{21}H_{21}N_3O_2$: C, 72.60; H, 6.09; N, 12.10. Found: C, 72.44; H, 6.27; N, 12.28.

2,4-Bis(3-cyanophenyl)-6-methyl-5-nitropyrimidine 5g. Amorphous solid (0.64 g, 2.5 mmol, 75%); mp 140–142 °C; IR (KBr) 3124, 3026, 2942, 2877, 2228, 1590, 1565, 1421, 1348 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.02 (d, J = 8.2 Hz, 1H, Ar–H), 7.91 (d, J = 8.6 Hz, 1H, Ar–H), 7.50–7.41 (m, 4H, Ar–H), 7.35 (s, 2H, Ar–H), 2.63 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$):

δ 163.75, 161.32, 158.03, 143.65, 136.87, 133.91, 131.43, 131.21, 129.80, 128.41, 128.10, 115.32, 21.13; MS (ESI, 0 V), *m/z* 342 (M + 1, 100%). *Anal*. Calcd for $C_{19}H_{11}N_5O_2$: C, 66.86; H, 3.25; N, 20.52. Found: C, 66.68; H, 3.38; N, 20.69.

2,4-Bis(4-cyanophenyl)-6-methyl-5-nitropyrimidine 5h. Amorsolid (0.66 g, 2.5 mmol, 78%); mp 134–136 °C; IR (KBr) 3124, 3031, 2935, 2864, 2226, 1579, 1553, 1411, 1343 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 8.13 (d, J = 8.0 Hz, 2H, Ar–H), 7.86 (d, J = 8.3 Hz, 2H, Ar–H), 7.54–7.36 (m, 4H, Ar–H), 2.67 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 162.96, 160.67, 158.32, 144.19, 138.09, 135.48, 131.39, 130.95, 129.22, 128.66, 127.78, 115.34, 20.98; MS (ESI, 0 V), *m/z* 342 (M + 1, 100%). *Anal*. Calcd for $C_{19}H_{11}N_5O_2$: C, 66.86; H, 3.25; N, 20.52. Found: C, 66.74; H, 3.30; N, 20.59.

2,4-Bis(4-cyanobenzyl)-6-methyl-5-nitropyrimidine 5i. Amorsolid (0.77 g, 2.5 mmol, 83%); mp 154–156 °C; IR (KBr) 3107, 3020, 2956, 2869, 2216, 1592, 1545, 1432, 1360 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 8.57 (d, J = 8.4 Hz, 2H, Ar–H), 7.80 (d, J = 8.4 Hz, 2H, Ar–H), 7.51–7.48 (m, 4H, Ar–H), 3.80 (s, 4H, $2CH_2$), 2.70 (s, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ 163.14, 162.67, 157.48, 145.94, 136.58, 134.36, 131.69, 130.56, 129.36, 128.71, 128.45, 118.54, 26.43, 20.24 MS (ESI, 0 V), *m/z* 370 (M + 1, 100%). *Anal*. Calcd for $C_{21}H_{15}N_5O_2$: C, 68.28; H, 4.09; N, 18.96. Found: C, 68.42; H, 3.93; N, 18.91.

Antibacterial activity. Impregnated discs of each chemical compound (**5a–i**) were prepared in separate vials with the use of Hi Media sterilized discs (Hi Media Labs, Mumbai, India). The title compounds were dissolved in DMSO, and these solutions were added drop by drop to the respective disc and air dried. The final concentration of the compound in disc was set to 50 or 100 µg of dry matter. Ampicillin was used as a positive control. We also maintained DMSO as control, and its inhibitory zone was set to zero.

We carried out bactericidal activity assays by disc diffusion method by standard method of Bauer [15], placing discs on agar media, which were lawn cultured with the required bacterial strain (10^5 CFU/mL). All plates were incubated at 37 °C for 24 h, and the zone of inhibition was recorded in m*M* for each compound along with positive control ampicillin disc.

Antioxidant activity

2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. The free radical scavenging activity of **5a–i** and ascorbic acid were measured with the use of the method of Blois [17], and the data are presented in Table 3. One milliliter of various concentrations of the title compounds (20, 40, 60, 80, and 100 µg/mL) in methanol was added to 4 mL of 0.004% DPPH in methanol. After a 30-min incubation period at room temperature, the absorbance was measured against blank at 517 nm. The antioxidant activity of these compounds was expressed as inhibitory concentration 50% (IC_{50}). The percent of inhibition of free radical production from DPPH was calculated with the use of the following equation, using ascorbic acid (vitamin C) as a standard control. The percent of inhibition of free radical production from DPPH was calculated with the use of the following equation.

$$\%\,\text{DPPH radical scavenging} = \frac{[Absorbance\ of\ control - Absorbance\ of\ test\ sample]}{Absorbance\ of\ control} 100$$

Nitric oxide scavenging activity. Nitric oxide scavenging activity was measured for the title compounds **5 a–i** with the use of slightly modified methods of Green et al. [18] and Marcocci et al. [19]. Nitric oxide radicals were generated from sodium nitroprusside. One milliliter of sodium nitroprusside (m*M*) and 1.5 mL of phosphate buffer saline (0.2 *M*, pH 7.4) were added to the different concentrations (20, 40, 60, 80, and 100 µg) of the compounds and incubated for 150 min at 25 °C. After incubation, 1 mL of the reaction mixture was treated with 1 mL of Griess reagent (1% sulfanilamide, 2% of H_3PO_4, and 0.1% naphthylethylene diamine dihydrochloride). Absorbance was measured at 546 nm. The percent of inhibition of free radical production was calculated with the use of the following equation, using ascorbic acid (vitamin C) as a standard control.

$$\%\,\text{Nitric oxide scavenging} = \frac{[Absorbance\ of\ control - Absorbance\ of\ test\ sample]}{Absorbance\ of\ control} 100$$

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REFERENCES AND NOTES

[1] Talukdar, A.; Breen, M.; Bacher, A.; Illarionov, B.; Fischer, M.; Georg, G.; Ye, Q.-Z.; Cushman, M. J. Org Chem 2009, 74, 5123.

[2] Chen, X.; Adrian, J.; Cushing, T.; DiMaio, H.; Liang, L.; Mayorga, V.; Miao, S.; Peterson, M. G.; Powers, J. P.; Spector, F.; Stein, C.; Wright, M.; Xu, D.; Ye, Q.; Jaen, J. Bio Org Med Chem Lett 2007, 17, 2188.

[3] Oliveira-Campos, A. M. F.; Salaheldin, A. M.; Rodrigues K. M. Arkivoc 2007, XVI, 92.

[4] Razik, H. H. A.; Said, S. B. Chem Pap 2004, 58, 209.

[5] Quintero, A.; Pelcastre, A.; Solano J. D. J Pharm Pharmceut Sci 1999, 2, 108.

[6] Fathalla, O. A.; Zeid, I. F.; Haiba, M. E.; Soliman, A. M.; Abd-Elmoez, S. I.; El-Serwy, W. S. World J Chem 2009, 4, 127.

[7] Raic´-Malic´, S.; Svedruz˘ic´, D.; Gazivoda, T.; Marunovic´, A.; Hergold-Brundic´, A.; Balzarini, A. J.; De Clercq, E.; Mintas M. J Med Chem 2000, 43, 4806.

[8] Gardelli, C.; Nizi, E.; Muraglia, E., Crescenzi, B.; Ferrara, M.,; Orvieto, F.; Pace, P.; Pescatore, G.; Poma, M.; Ferreira, M. R. R.; Scarpelli, R.; Homnick, C. F.; Ikemoto, N.; Alfieri, A.; Verdirame, M.; Bonelli, F.; Gonzalez Paz, O.; Taliani, M.; Monteagudo, E.; Pesci, S.; Laufer, R.; Felock, P.; Stillmock, K. A.; Hazuda, D.; Rowley, M.; Summa, V. J Med Chem 2007, 50, 4953.

[9] Rajendra Prasad, Y.; Srinivasa Rao, A. Chem Pharm Bull 2011, 59, 1079.

[10] Molnar, A. Chem Rev 2011, 111, 2251.

[11] Netherton, M. R.; Dai, C.; Neuschuta, K.; Fu, G. J Am Chem Soc 2001, 123, 10099.

[12] Hadei, N.; Kantchev, E. A. B.; O'Brien, C. J.; Organ, M. G. Org Lett 2005, 7, 1991.

[13] Foster, H. M.; Synder, H. R.; Org Synthesis Coll 1963, 4, 638.

[14] Thompson, A. E.; Hughes, G.; Batsanov, A. S.; Bryce, M. R.; Parry, P. R.; Tarbit, B. J Org Chem 2005, 70, 388.

[15] Bauer, A. W.; Truck, H.; Sherries, J. C. Am J Clin Pathol 1990, 45, 493.

[16] Okhawa, H.; Ohishi, N.; Yagi, K. Anal Bio Chem 1979, 95, 351.

[17] Blois, P. Nature 1958, 181, 1199.

[18] Green, L. C.; Wagner, D. A.; Glogowski, J.; Skipper, P. L.; Tannenbaum, S. R. Anal Bio Chem 1982, 126, 131.

[19] Marcocci, L.; Maguire, J. J.; Droy-Lefaix, M. T.; Packer, L. Biochem Biophys Res Commun 1994, 201, 748.