

Research Article

Syntheses and Antibacterial Studies of Some 1-Phenyl-3-(4-(2-ethanoloxyl) phenyl)-5-aryl-1*H*-pyrazoles

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A series of 1-phenyl-3-(4-(2-ethanoloxyl) phenyl)-5-aryl-1*H*-pyrazoles were synthesized from chalcones, that is, 3-aryl-1-(4-hydroxyphenyl) prop-2-en-1-ones and studied for their *in vitro* antibacterial activity. Chalcones **1** on reaction with phenylhydrazine in the presence of acetic acid and few drops of hydrochloric acid yielded the corresponding 1-phenyl-3-(4-hydroxyphenyl)-5-aryl-1*H*-pyrazoles **2** which on further reaction with 2-chloroethanol furnished the title compounds **3**. These compounds were characterized by CHN analyses, IR, mass and ¹H NMR spectral data. All the compounds were evaluated for their *in vitro* antibacterial activity against two Gram positive strains (*Bacillus subtilis* and *Staphylococcus aureus*) and two Gram negative strains (*Escherichia coli* and *Pseudomonas aeruginosa*), and their minimum inhibitory concentration (MIC) was determined.

1. Introduction

The extensive utilization of chemotherapeutic agents for the management of infectious diseases leads to the development of microbial resistance to existing drugs. The appearance of resistance to the major classes of antibacterial drugs is recognized as a major health concern of world population. This opens the gate for the medicinal chemists for the development of novel antimicrobial drugs having a different mechanism of action to combat the problem of multidrug resistance [1]. Heterocyclic compounds continue to attract considerable interest due to their diverse biological activities. Amongst them five membered heterocyclic compounds occupy a unique place in the field of natural and synthetic organic chemistry. Five membered heterocycles like pyrazoles have been found to display wide application as pharmaceutical and agrochemical agents. In recent years, attention has increasingly been given to the synthesis of pyrazole derivatives as a source of new antibacterial agents. Pyrazole derivatives have been reported to possess diverse biological activities such as antibacterial [2, 3], antifungal [4, 5], herbicidal [6], insecticidal [7], anti-inflammatory [8, 9] anticonvulsant [10], antitumor [11], anti-oxidant [12] and so

forth. Further, 1*H*-pyrazoles containing phenoxy propanol [13] and phenoxy butanol [14] side chain have been reported to possess antibacterial activity by us as a part of our ongoing research program in the field of synthesis and antimicrobial activity of medicinally important heterocyclic compounds [15, 16]. These reports inspired us to undertake the synthesis of some 1*H*-pyrazoles bearing phenoxy ethanol moiety. The synthesized compounds were characterized on the basis of elemental analysis, IR, ¹H NMR and mass spectral data. All the compounds were screened for their *in vitro* antibacterial activity against two Gram positive strains (*Bacillus subtilis* and *Staphylococcus aureus*) and two Gram negative strains (*Escherichia coli* and *Pseudomonas aeruginosa*), respectively.

2. Experimental

2.1. Chemistry. The purity of all the synthesized compounds was checked by thin-layer chromatography on silica gel G as a stationary phase and different solvent systems as a mobile phase using iodine vapors as detecting agent. Melting points were determined by the Tempo melting point determination apparatus in open capillary tubes and are

uncorrected. Elemental analyses were carried out on Perkin Elmer 2400 CHN Elemental Analyser. Infrared spectra were recorded on Shimadzu 8000 FTIR Spectrophotometer in KBr phase. Proton NMR spectra were done on Bruker Avance II 400 NMR Spectrometer using tetramethyl silane as internal standard. Mass spectra of the compounds were carried out on Waters Micromass Q-ToF Micro Mass Spectrometer using electrospray ionization (ESI) technique. Chalcones **1a-g** were synthesized by a base-catalyzed Claisen-Schmidt condensation reaction of appropriately substituted benzaldehydes and *p*-hydroxy acetophenone [17], and 1-phenyl-3-(4-hydroxyphenyl)-5-aryl-1*H*-pyrazoles **2a-g** were prepared from the chalcones **1a-g** following the procedure described in the literature [18].

2.2. General Procedure for the Synthesis of 1-phenyl-3-(4-(2-ethanoloxy)phenyl)-5-aryl-1*H*-pyrazoles (3a-g). 1-Phenyl-3-(4-hydroxyphenyl)-5-aryl-1*H*-pyrazoles (2a-g 0.01 M) and 2-chloroethanol (0.01 M) were refluxed in acetone (50 mL) in the presence of triethylamine (0.01 M) for about four hours. Excess of solvent was removed under reduced pressure. The residue thus obtained was washed thoroughly with cold distilled water, dried, and then recrystallized from ethanol. The physical and analytical data of the synthesized title compounds are given as follows.

1,5-Diphenyl-3-(4-(2-ethanoloxy) phenyl)-1*H*-pyrazole (3a): Yield: 69%; m.p.: 74–76°C; IR (KBr, cm^{-1}): 3340 (O-H), 3063 (aromatic C-H str), 2917 (C-H), 1465 (CH₂), 1256 (C-O-C), 1065 (C-O), 830, 732, and 691 (aromatic C-H def); ¹HNMR (CDCl₃): δ (ppm) 8.06–7.08 (m, 14H, ArH), 7.02 (s, 1H, =CH-), 4.33–4.31 (t, 2H, HO-CH₂-CH₂-O-Ar), 3.59–3.57 (t, 2H, HO-CH₂-CH₂-O-Ar), 3.65 (s, 1H, O-H); MS, m/z (%): 357 [M+H]⁺ (100%). Anal.: Calcd. For C₂₃H₂₀N₂O₂: C, 77.51; H, 5.66; N, 7.86. Found: C, 77.56; H, 5.61; N, 7.80.

1-Phenyl-3-(4-(2-ethanoloxy) phenyl)-5-(4-methyl-phenyl)-1*H*-pyrazole (3b): Yield: 72%; m.p.: 66–68°C; IR (KBr, cm^{-1}): 3342 (O-H), 3065 (aromatic C-H str), 2916 (C-H), 1465 (CH₂), 1255 (C-O-C), 1065 (C-O), 830, 730, and 692 (aromatic C-H def); ¹HNMR (CDCl₃): δ (ppm) 7.68–7.09 (m, 13H, ArH), 7.02 (s, 1H, =CH-), 4.32–4.30 (t, 2H, HO-CH₂-CH₂-O-Ar), 3.58–3.56 (t, 2H, HO-CH₂-CH₂-O-Ar), 3.65 (s, 1H, O-H), 2.34 (s, 3H, CH₃-Ar); MS, m/z (%): 371 [M+H]⁺ (100%). Anal.: Calcd. For C₂₄H₂₂N₂O₂: C, 77.81; H, 5.99; N, 7.56. Found: C, 77.87; H, 5.92; N, 7.50.

1-Phenyl-3-(4-(2-ethanoloxy) phenyl)-5-(4-methoxy-phenyl)-1*H*-pyrazole (3c): Yield: 64%; m.p.: 95–97°C; IR (KBr, cm^{-1}): 3340 (O-H), 3063 (aromatic C-H str), 2919 (C-H), 1465 (CH₂), 1255 (C-O-C), 1066 (C-O), 831, 732, and 690 (aromatic C-H def); ¹HNMR (CDCl₃): δ (ppm) 7.63–7.08 (m, 13H, ArH), 7.01 (s, 1H, =CH-), 4.33–4.31 (t, 2H, HO-CH₂-CH₂-O-Ar), 3.83 (s, 3H, CH₃O-Ar), 3.59–3.57 (t, 2H, HO-CH₂-CH₂-O-Ar), 3.65 (s, 1H, O-H); MS, m/z (%): 387 [M+H]⁺ (100%). Anal.: Calcd. For C₂₄H₂₂N₂O₃: C, 74.59; H, 5.74; N, 7.25. Found: C, 74.52; H, 5.79, N, 7.31.

1-Phenyl-3-(4-(2-ethanoloxy) phenyl)-5-(4-chlorophenyl)-1*H*-pyrazole (3d): Yield: 71%; m.p.: 78–80°C; IR (KBr,

cm^{-1}): 3342 (O-H), 3068 (aromatic C-H str), 2919 (C-H), 1460 (CH₂), 1253 (C-O-C), 1066 (C-O), 833, 733, and 690 (aromatic C-H def); ¹HNMR (CDCl₃): δ (ppm) 8.03–7.08 (m, 13H, ArH), 7.02 (s, 1H, =CH-), 4.32–4.30 (t, 2H, HO-CH₂-CH₂-O-Ar), 3.56–3.54 (t, 2H, HO-CH₂-CH₂-O-Ar), 3.65 (s, 1H, O-H); MS, m/z (%): 391 [M+H]⁺ (100%), 393 [M+2+H]⁺ (35%). Anal.: Calcd. For C₂₃H₁₉ClN₂O₂: C, 70.68; H, 4.90; N, 7.17. Found: C, 70.61; H, 4.97; N, 7.12.

1-Phenyl-3-(4-(2-ethanoloxy) phenyl)-5-(4-bromo-phenyl)-1*H*-pyrazole (3e): Yield: 76%; m.p.: 87–89°C; IR (KBr, cm^{-1}): 3340 (O-H), 3065 (aromatic C-H str), 2916 (C-H), 1463 (CH₂), 1255 (C-O-C), 1065 (C-O), 831, 731, and 692 (aromatic C-H def); ¹HNMR (CDCl₃): δ (ppm) 7.79–7.09 (m, 13H, ArH), 7.02 (s, 1H, =CH-), 4.32–4.30 (t, 2H, HO-CH₂-CH₂-O-Ar), 3.56–3.54 (t, 2H, HO-CH₂-CH₂-O-Ar), 3.65 (s, 1H, O-H); MS, m/z (%): 435 [M+H]⁺ (100%), 437 [M+2+H]⁺ (98%). Anal.: Calcd. For C₂₃H₁₉BrN₂O₂: C, 63.46; H, 4.40; N, 6.44. Found: C, 63.41; H, 4.46; N, 4.49.

1-Phenyl-3-(4-(2-ethanoloxy) phenyl)-5-(4-fluorophenyl)-1*H*-pyrazole (3f): Yield: 69%; m.p.: 78–80°C; IR (KBr, cm^{-1}): 3344 (O-H), 3066 (aromatic C-H str), 2918 (C-H), 1465 (CH₂), 1255 (C-O-C), 1065 (C-O), 832, 730, and 692 (aromatic C-H def); ¹HNMR (CDCl₃): δ (ppm) 8.16–7.08 (m, 13H, ArH), 7.01 (s, 1H, =CH-), 4.33–4.31 (t, 2H, HO-CH₂-CH₂-O-Ar), 3.57–3.55 (t, 2H, HO-CH₂-CH₂-O-Ar), 3.65 (s, 1H, O-H); MS, m/z (%): 375 [M+H]⁺ (100%). Anal.: Calcd. for C₂₃H₁₉FN₂O₂: C, 73.78; H, 5.11; N, 7.48. Found: C, 73.72; H, 5.17; N, 7.41.

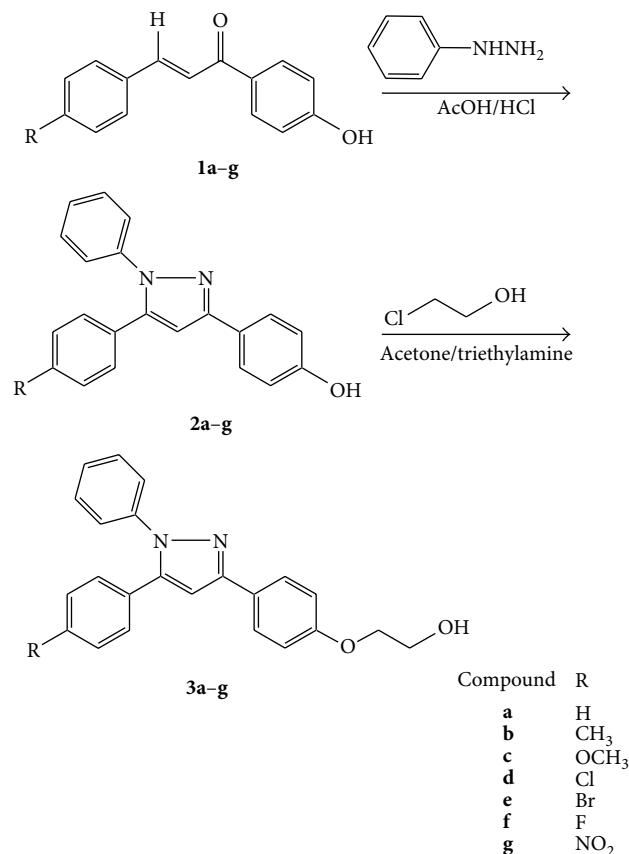
1-Phenyl-3-(4-(2-ethanoloxy) phenyl)-5-(4-nitrophenyl)-1*H*-pyrazole (3g): Yield: 68%; m.p.: 61–63°C; IR (KBr, cm^{-1}): 3340 (O-H), 3060 (aromatic C-H str), 2916 (C-H), 1465 (CH₂), 1256 (C-O-C), 1070 (C-O), 830, 732, and 691 (aromatic C-H def); ¹HNMR (CDCl₃): δ (ppm) 8.32–7.08 (m, 13H, ArH), 7.02 (s, 1H, =CH-), 4.33–4.31 (t, 2H, HO-CH₂-CH₂-O-Ar), 3.58–3.56 (t, 2H, HO-CH₂-CH₂-O-Ar), 3.65 (s, 1H, O-H); MS, m/z (%): 402 [M+H]⁺ (100%). Anal.: Calcd. For C₂₃H₁₉N₃O₄: C, 68.82; H, 4.77; N, 10.47. Found: C, 68.87; H, 4.71; N, 10.42.

2.3. Antibacterial Activity. All the title compounds were screened for their *in vitro* antibacterial activity against two Gram positive strains, that is, *Bacillus subtilis* (MTCC 121) and *Staphylococcus aureus* (MTCC 96) and two Gram negative strains, that is, *Escherichia coli* (MTCC 40) and *Pseudomonas aeruginosa* (MTCC 2453), respectively. Ciprofloxacin was used as the standard drug for the present study. Serial two-fold dilution technique was used for the study of antibacterial activity [19]. A stock solution (10 $\mu\text{g}/\text{mL}$) of all the title compounds and standard drug was prepared in dimethyl sulfoxide. Sterilized double-strength nutrient broth (DSNB) was used as a growth media. The stock solution was serially diluted by DSNB aseptically to give concentrations of 5.0–0.01 $\mu\text{g}/\text{mL}$ into a series of sterilized culture tubes. All the tubes were inoculated by bacterial strain. The inoculum's size was approximately 10⁶

colony forming units (CFU/mL). The inoculated tubes were incubated for 24 h at $37(\pm 1)^\circ\text{C}$. After 24 h, the inoculated culture tubes were macroscopically examined for turbidity. The culture tube showing turbidity (lower concentration) and the culture tube showing no turbidity (higher concentration) gave the minimum inhibitory concentration (MIC) for the compound. The MIC for the title compounds and the standard drug, that is, ciprofloxacin are presented in Table 1.

3. Result and Discussion

3.1. Chemistry. The syntheses of 1-phenyl-3-(4-(2-ethanol oxy) phenyl)-5-aryl-1*H*-pyrazoles were achieved following the steps outlined in Scheme 1. Chalcones, that is, 3-aryl-1-(4-hydroxyphenyl) prop-2-en-1-ones, **1** were prepared by the reaction of *p*-hydroxy acetophenone with substituted benzaldehydes following the Claisen-Schmidt reaction. The chalcones **1** then on refluxing with phenyl hydrazine in the presence of acetic acid and few drops of hydrochloric acid furnished 1-phenyl-3-(4-hydroxyphenyl)-5-aryl-1*H*-pyrazoles **2**. Reaction of 2-chloroethanol with **2** in the presence of triethyl amine yielded the title compounds **3**. All the compounds were obtained in good yield. These compounds were characterized on the basis of elemental and spectral analyses. IR spectra of each compound showed a band for O-H stretching vibrations for intermolecular hydrogen bonding near 3340 cm^{-1} , while the C-O stretching vibrations for primary alcohols were observed in the range of $1085\text{--}1050\text{ cm}^{-1}$. The C-O-C stretching vibrations for aryl alkyl ethers appeared near 1255 cm^{-1} . The C-H stretching vibrations for methylene groups appeared in the range of $2916\text{--}2919\text{ cm}^{-1}$, whereas bending vibrations for methylene scissoring were observed constantly at 1465 cm^{-1} . Aromatic C-H stretching vibrations were observed in the range of $3100\text{--}3050\text{ cm}^{-1}$, whereas aromatic C-H bending vibrations appeared below 900 cm^{-1} . In case of ^1H NMR, the chemical shift value for the O-H group was observed in the range of $3.70\text{--}3.65 \delta$ (ppm) and appeared as singlet (s). Aromatic protons appeared as multiplet (m) in the range of $8.33\text{--}7.08 \delta$ (ppm). The methine proton of the pyrazole nucleus absorbed at $7.03\text{--}7.01 \delta$ (ppm) and appeared as singlet (s). The methylene protons adjacent to the O-H group [HO-CH₂-CH₂-O-Ar] appeared as triplet (t) in the range of $3.59\text{--}3.51 \delta$ (ppm), whereas the methylene protons adjacent to the O-Ar group [HO-CH₂-CH₂-O-Ar] observed at $4.33\text{--}4.06 \delta$ (ppm) and also appeared as triplet (t). Aromatic methyl and methoxy protons were observed at $2.36\text{--}2.34 \delta$ (ppm) and $3.85\text{--}3.83 \delta$ (ppm), respectively as singlet (s). All the title compounds showed $[\text{M}+\text{H}]^+$ of 100% intensity as the molecular ion peak. Compound containing chlorine showed isotopic peak at $[\text{M}+2+\text{H}]^+$ of about 35% intensity to that of parent ion peak, whereas bromo derivative showed isotopic peak at $[\text{M}+2+\text{H}]^+$ of about equal intensity. The results of elemental analyses were found in good agreement with the calculated values.



SCHEME 1: Synthesis of 1-Phenyl-3-(4-(2-ethanol oxy) phenyl)-5-aryl-1*H*-pyrazoles.

3.2. Antibacterial Activity. All the synthesized title compounds were screened for their *in vitro* antibacterial activity against two Gram positive bacterial strains, that is, *Bacillus subtilis* (MTCC 121) and *Staphylococcus aureus* (MTCC 96) and two Gram negative bacterial strains, that is, *Escherichia coli* (MTCC 40) and *Pseudomonas aeruginosa* (MTCC 2453), respectively, and their minimum inhibitory concentration (MIC) was determined. A perusal of the Table 1 shows that all the title compounds were found to be active against all the bacterial strains used in this study. However, they showed more activity against the Gram negative than the Gram positive bacterial strains. Out of the two Gram negative bacterial strains, *E. coli* (MTCC 40) was found to be more susceptible than *P. aeruginosa* (MTCC 2453) against all the title compounds. The minimum inhibitory concentration (MIC) of the title compounds **3a-g** was found to be $0.45\text{--}0.30\text{ }\mu\text{g/mL}$, $0.50\text{--}0.35\text{ }\mu\text{g/mL}$, $0.55\text{--}0.40\text{ }\mu\text{g/mL}$, and $0.55\text{--}0.45\text{ }\mu\text{g/mL}$ against *E. coli* (MTCC 40), *P. aeruginosa* (MTCC 2453), *B. subtilis* (MTCC 121) and *S. aureus* (MTCC 96), respectively. The MICs of the title compounds containing electron withdrawing groups like fluoro, chloro, bromo, or nitro were found somewhat less than the compounds containing electron releasing groups like methyl and methoxy. The reference standard ciprofloxacin inhibited

TABLE 1: *In vitro* antibacterial activity of 1-phenyl-3-(4-(2-ethanolxy) phenyl)-5-aryl-1*H*-pyrazoles (**3a–g**).

Compound	<i>B. subtilis</i> (MTCC 121)	Minimum inhibitory concentration ($\mu\text{g/mL}$)		
		<i>S. aureus</i> (MTCC 96)	<i>E. coli</i> (MTCC 40)	<i>P. aeruginosa</i> (MTCC 2453)
3a	0.50	0.50	0.40	0.45
3b	0.50	0.50	0.40	0.45
3c	0.55	0.55	0.45	0.50
3d	0.45	0.45	0.30	0.40
3e	0.45	0.45	0.30	0.40
3f	0.45	0.45	0.30	0.40
3g	0.40	0.45	0.30	0.35
Ciprofloxacin (standard drug)	0.12	0.15	0.01	0.25

Gram negative bacteria namely, *E. coli* and *P. aeruginosa* at a MIC of $0.01 \mu\text{g/mL}$ and $0.25 \mu\text{g/mL}$, respectively, whereas against Gram positive bacteria namely, *S. aureus* and *B. subtilis* MIC was found to be $0.15 \mu\text{g/mL}$ and $0.12 \mu\text{g/mL}$, respectively. The results of the MIC for the standard drug, ciprofloxacin, against the bacterial strains used were found to be within the range as reported in the literature [20–22].

4. Conclusion

The present study describes the synthesis of a series of 1-phenyl-3-(4-(alkanolxy) phenyl)-5-aryl-1*H*-pyrazoles starting from the chalcones. The compounds were characterized by modern analytical techniques such as CHN analyses, IR, mass and proton NMR spectra. All the title compounds were screened for their *in vitro* antibacterial activity against *Bacillus subtilis*; *Staphylococcus aureus* (Gram positive) and *Escherichia coli*; *Pseudomonas aeruginosa* (Gram negative), and their minimum inhibitory concentration (MIC) was determined. The results of antibacterial activity showed that compounds containing electron withdrawing groups for example, chloro, bromo, fluoro, or nitro were found to be more active than the compounds containing electron releasing groups such as methyl and methoxy. These results suggest that some more compounds using different aromatic or heteroaromatic aldehydes, ketones, and haloalkanols should be synthesized and screened for their antibacterial activity to explore the possibility of 1-phenyl-3-(4-(alkanolxy) phenyl)-5-aryl-1*H*-pyrazoles as a novel series of antibacterials.

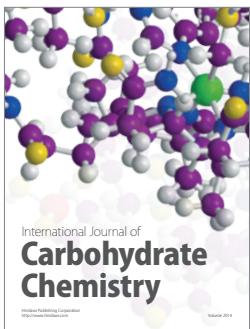
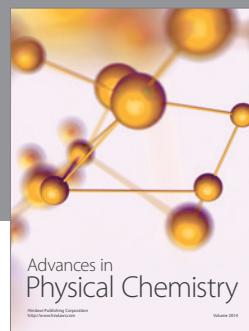
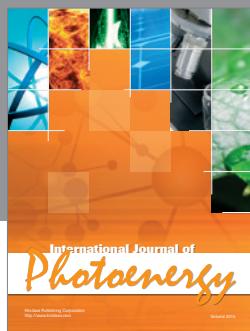
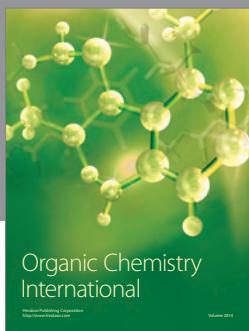
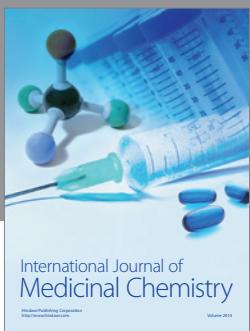
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