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Lemon juice catalyzed efficient one-pot synthesis, antioxidant and antimicrobial evaluation of bispyrazolyl methanes

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Abstract: A multicomponent one potnovel efficient and green protocol for the synthesis of a series of bis-pyrazolylmethanesas a potential antimicrobial and antioxidant agents*via* one-pot multi-component condensation of aldehydes, ethyl acetoacetate and phenyl hydrazine using lemon juice as an efficient and eco-friendly catalyst.Compared to other methods, the advantageous features of this methodologyare environmentally friendly and operational simplicity, including excellent yields, short reaction time, mild reaction conditions and environmentally benign catalyst. The synthesized bis-pyrazolylmethanes were evaluated for antimicrobial and antioxidant activity and also analyzed for ADME properties.

Keywords: Multicomponent, Green catalyst, Lemon juice,Antioxidant, Antimicrobial, ADME.

Introduction

Combinatorial chemistry is extensively useful for the discovery of novel biologically active compounds [1]. In this framework, multicomponent reactions (MCRs) are an effective tool in the current drug discovery process in terms of lead finding and optimization, but the range of easily accessible and functionalized small heterocycles is rather limited [2]. These strategies have emerged as flexible approaches in organic synthesis due to their advantages over the conventional multistep synthesis. In addition, they are ecofriendly, have superior atom economy, require less time and low-cost purification processes and without protection-deprotection steps. Therefore, the design and development of novel, efficient and green MCRs focused on a target product is one of the most important challenges in organic synthesis.

Pyrazole and its derivatives have drawn considerable attention of the researchers in the

past few decades owing to their high therapeutic values. Some of the drugs, possessing pyrazole as basic moiety, like celecoxib**1**, deracoxib**2** (**Figure1**), etoricoxiband atorivodineare already booming in the market.As pyrazole derivatives do not exist in nature, probably, due to the difficulty in the construction of *N*-*N* bond by living organisms, their availability depends on the synthetic methods. Pyrazole derivatives proved to possess different bioactivities such as, anti-inflammatory [3]**3**,p56 Lck inhibitor [4]**4**,anticancer [5]**5**, antidepressant [6]**6**,corticotrophin releasing factor-1 (CRF-1) receptor antagonist [7]**7**, antimalarial [8]**8**,GABA inhibitor **9** with selectivity towards insect versus mammalian receptors [9], antifungal [10]**10**, antibacterial [11]**11** and NPY5 antagonist [12]**12**(**Figure1**). Nowadays, the pyrazolone derivatives paid much attention for their various biological activities such as antitumor [13], selective COX-2 inhibitor [14], cytokine inhibitors [15], agrochemicals, dyes and pigments. Moreover, they are capable of prototropic tautomerism [16]. Compounds that contain two pyrazolone rings can be used as extractant for some metal ions [17] and ligands [18].

2,4-Dihydro-3*H*-pyrazol-3-one derivatives including 4,4'-(arylmethylene)bis(3-methyl-1-phenyl-1*H*-pyrazol-5-ols) are being used as gastric secretion stimulatory [19], antidepressant [20], antibacterial [21] and antifilarial agents [22]. Moreover, the corresponding 4,4'-(arylmethylene)bis(1*H*-pyrazol-5-ols) are applied as fungicides [23], pesticides [24], insecticides [25] and dyestuffs [26].

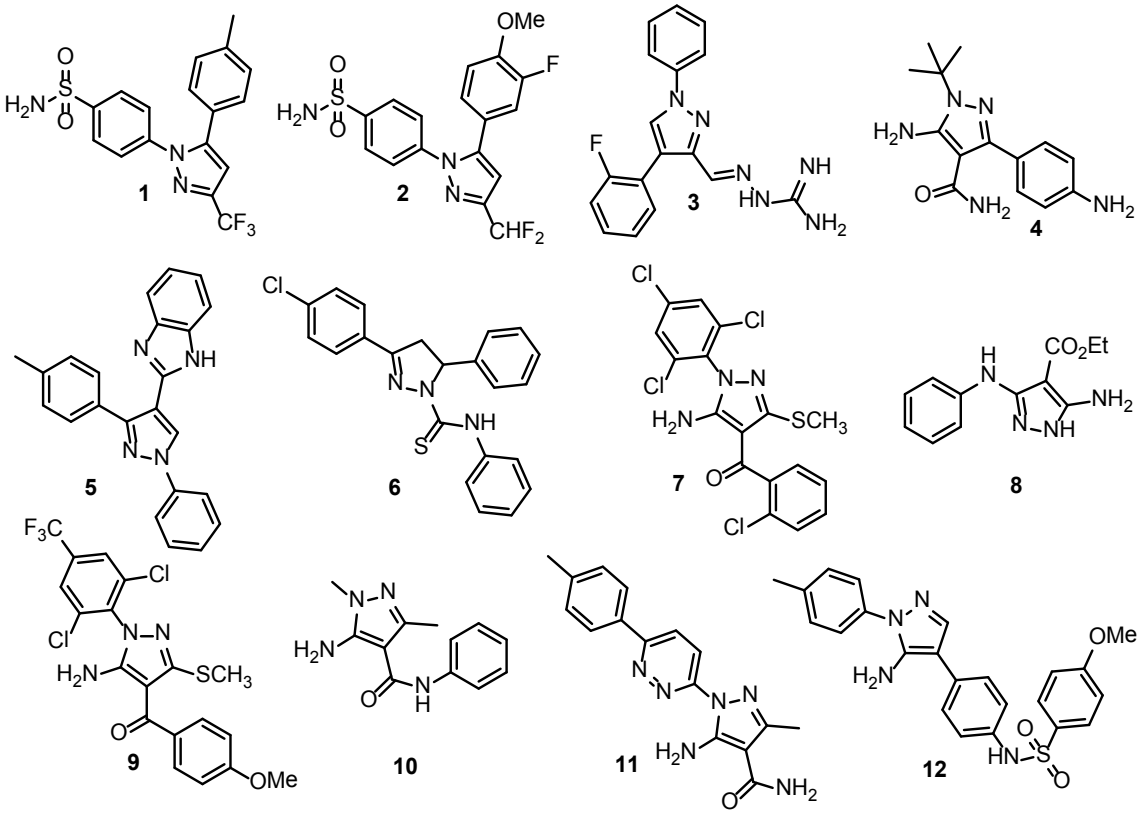
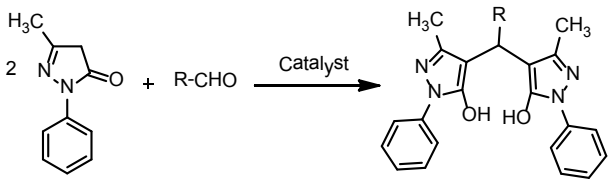


Figure1. Structure of pyrazole containing biological active molecules

The literature survey reveals that the synthesis of 4,4'-(arylmethylene)bis(3-methyl-1-phenyl-1*H*-pyrazol-5-ols) can be accomplished by two methods: (i) Knoevenagel reaction of 3-methyl-1-phenyl-1*H*-pyrazol-5(4*H*)-one with aldehydes to form the corresponding arylidenepyrazolones followed by base promoted Michael reaction with second equivalent of 3-methyl-1-phenyl-1*H*-pyrazol-5(4*H*)-one [27], and (ii) one-pot tandem Knoevenagel-Michael reaction of aldehydes with two equivalents of 3-methyl-1-phenyl-1*H*pyrazol-5(4*H*)-one under various reaction conditions. The most common method for the synthesis of 4,4′-(arylmethylene)bis(1*H*-pyrazol-5-ol)s is the one-pot pseudo three-component condensation of aldehydes with 3-methyl-1-phenyl-5-pyrazolone (**Scheme1**).



Scheme 1

Various catalysts have been used for this transformation including acetic acid or piperidine [28a], silica-bonded S-sulfonic acid [28b], PEG-SO_3H [28c], sodium dodecyl sulfate [28d], PEG-400 [28e], ETBA [28f], CAN [28g], an electrocatalysis [28h], ionic liquids [28i], $Ce(SO_4)_2.4H_2O$ [28j], LiOH [28k], silica sulfuric acid [28l], phosphomolybdic acid [28m], xanthan sulfuric acid [28n], 3-aminopropylated silica gel [28o], $[Cu(3,4\text{-tmtppa})](MeSO_4)_4$ [28p], cellulose sulfuric acid [28q], $LiOH.H_2O$ [28r], 1,3,5-tris(hydrogensulfato) benzene [28s], sulfuric acid([3-(3-silicapropyl)sulfanyl]propyl) ester [28t], ethylenediammoniumdiacetate [28u], $[Sipmim]HSO_4$ [28v], TEBA [28w], 2-hydroxyethylammoniumacetate [28x]and 1-sulfopyridinium chloride [28y].

Fruit juice is naturally occurring which was used as a biocatalyst in organic synthesis. Fruit juice is now being routinely used in organic synthesis as homogeneous catalysts for various selective transformations of simple and complex molecules. In recent years, chemical reactions using plant cell cultures and part of plants as biocatalysts have received great attention [29]. This crescent interest is due to the wide biotechnological potential of the enzymatic reactions. The bio catalytical transformations using edible plants [30], plant root [31] plant tubers [32] and plant leave [33] extract can be applied in many organic reactions. Lemon juice [34] obtained from lemon is sour in taste. The main ingredients of the extract of Citrus limonium species of lemon are moisture (85%), carbohydrates (11.2%), citric acid (5-7%), protein (1%), ascorbic acid or vitamin-C (0.5%), fat (0.9%), minerals (0.3%), fibres (1.6%) and some other organic acids [34]. The juice is soluble in water. Due to presence of citric acid and ascorbic acid, lemon juice is acidic (pH= 2-3) in nature, and thus it works as acid catalyst in organic reactions.Lemon juice used in many organic reactions like the Knoevenagel condensation [35], in Biginelli type synthesis of dihydropyrimidinone [36]. A three component one-pot clean biocyclocondensation reaction was reported by Sachdeva *et al*. using biocatalyst lemon juice of Citrus limonium species of lemon [37]. Pal and coworkers observed that lemon juice can be utilized for the biocondensation of indoles and aldehydes for the synthesis of bis-, and tris (indolyl) methanes [38].

All of the aforementioned procedures include two main steps: (i) 3-methyl-1-phenyl-5-pyrazolone should be synthesized from phenylhydrazine and ethyl acetoacetate,then (ii) 3-methyl-1-phenyl-5-pyrazolone reacts with aldehyde. Even though, 4,4'-(arylmethylene) bis(1*H*-pyrazol-5-ols) could be synthesized by these methods, most of the methods suffer from limitations such as long reaction time, use of expensive catalysts, the requirement of special apparatus, tedious work-up procedures

and noncompliance with green chemistry protocols. Therefore, finding an efficient and eco-friendly protocol for the preparation of 4,4'-(arylmethylene)bis(1*H*-pyrazol-5-ols) is of obvious importance. Recently, Hasaninejed et. al. [39] have reported the five-component synthesis under catalyst-free and ultrasound irradiation conditions which show better results when compared to the other reported reactions. However, Lewis acids are moisture sensitive; they lose their catalytic activity in the presence of water. Therefore, we decided to study the effect of water-soluble environmentally benign green catalysts lemon juice for the synthesis of 4,4'-(arylmethylene)bis(3-methyl-1-phenyl-1*H*-pyrazol-5-ols).

Experimental section

Chemistry

All the reagents and solvents were purchased from commercial suppliers Spectrochem, Rankem, Alfa Aesar, Sigma Aldrich and are used without further purification. Reaction time and purity of the products were observed by thin layer chromatography (TLC) aluminum sheets, silica gel 60-F_{254} precoated, Merck, Germany and spots were locating by using UV light or Iodine vapors as the visualizing agent. All the melting points were find out in open capillary method and are uncorrected. ^{1}H NMR spectra were recorded on Jeol 400 MHz and ^{13}C NMR on 100 MHz spectrometer using residual solvent as internal standard (DMSO &$CDCl_3$). The chemical shifts (δ) were reported in ppm and are given in parts per million (ppm). The splitting pattern abbreviations are designed as singlet (s); doublet (d); double doublet (dd); triplet (t); quartet (q) and multiplet (m). Mass spectra were recorded on micrOTOF-Q II spectrometer in the ESI (Electrospray Ionization) modes.

*General procedure for the synthesis of 4,4-(arylmethylene)-bis(3-methyl-1-phenyl-1H-pyrazol-5-ols)***(16a-n)**: A mixture of phenyl hydrazine **13** (2 mmol), ethyl acetoacetate **14** (2 mmol) in lemon juice was stirred in H_2O:EtOH mixture at 80 °C for 10 min and then aromatic aldehyde **15a-n** (1 mmol) was added to the reaction mixture and continued for the required time. Progress of the reaction was monitored by TLC. After completion of the reaction, the residue was filtered and was crystallized from ethanol.

Spectral data of the products:

4,4′-(Phenylmethylene)bis(3-methyl-1-phenyl-1*H*-pyrazol-5-ol) (16a)*:* ^{1}H NMR (DMSO-d_6, 400 MHz): δ2.13 (s, 6H, $2CH_3$), 4.79 (s, 1H, CH), 7.09-7.24 (m, 8H, ArH), 7.28-7.30 (m, 3H, ArH), 7.59 (d, 4H, *J* = 8 Hz, ArH), 12.78 (s, 1H, br., OH). ^{13}C NMR (DMSO-d_6, 100 MHz): δ99.99, 105.31, 121.15, 125.85, 126.21, 127.36, 128.31, 128.87, 137.50, 146.24, 157.66. MS (ESI) *m/z* $(M+H)^+$ Calcd. for $C_{27}H_{24}N_4O_2$: 436.19. Found: 437.3.

4,4′-[(4-Methylphenyl)methylene]bis(3-methyl-1-phenyl-1*H*-pyrazol-5-ol) (16b)*:* ^{1}H NMR (DMSO-d_6, 400 MHz): δ2.24 (s, 3H, $ArCH_3$), 2.30 (s, 6H, $2CH_3$), 3.69 (s, 3H, OCH_3), 4.92 (s, 1H, CH), 7.06 (d, 2H, *J* = 8 Hz, ArH), 7.06 (d, 2H, *J* = 7.6 Hz, ArH), 7.24 (m, 2H, ArH), 7.43 (t, 4H, *J* = 7.6 Hz, ArH), 7.70 (d, 4H, *J* = 8 Hz, ArH), 12.39 (s, 1H, br., OH), 13.96 (s, 1H, br., OH). MS (ESI) *m/z* $(M+H)^+$ Calcd. for $C_{28}H_{26}N_4O_2$: 450.21. Found: 451.35.

4,4′-[(4-Chlorophenyl)methylene]bis(3-methyl-1-phenyl-1*H*-pyrazol-5-ol) (16c)*:* ^{1}H NMR (DMSO-d_6, 400 MHz): δ2.31 (s, 6H, $2CH_3$), 4.96 (s, 1H, CH), 7.25 (d, 4H, *J* = 8 Hz, ArH), 7.33 (d, 2H, *J* = 8.4 Hz, ArH), 7.43 (t, 4H, *J* = 7.6 Hz, ArH), 7.69 (d, 4H, *J* = 8 Hz, ArH), 12.57 (s, 1H, br., OH), 13.83 (s, 1H, br., OH). MS (ESI) *m/z* $(M+H)^+$ Calcd. for $C_{27}H_{23}ClN_4O_3$: 470.15. Found: 471.3.

4,4′-[(4-Hydroxyphenyl)methylene]bis(3-methyl-1-phenyl-1*H*-pyrazol-5-ol) (16d). IR (KBr): 3420, 3150, 3090, 2920, 1593, 1492, 1410, 1270, 744, 690 cm-1. 1H NMR (300 MHz, DMSO-d6): δ (ppm) 2.30 (s, 6H), 4.85 (s, 1H), 6.67 (d, 2H, J= 7.72 Hz), 7.05 (d, 2H, J= 7.16 Hz), 7.24 (t, 2H, J= 5.0 Hz), 7.42-7.45 (m, 4H), 7.66-7.77 (m, 4H), 9.19 (s, 1H), 13.96 (brs, 2H). 13C NMR (75MHz, DMSO-d6): δ (ppm) 18.55, 32.39, 114.85, 120.47, 125.49, 128.08, 128.89, 132.27, 137.39, 146.18, 155.49.

4,4′-[(3-Nitrophenyl)methylene]bis(3-methyl-1-phenyl-1*H*-pyrazol-5-ol) (16f):^{1}H NMR ($CDCl_3$, 400 MHz): δ2.30 (s, 6H, $2CH_3$), 4.95 (s, 1H, CH), 7.2 (t, 2H, J = 7.2 Hz, ArH), 7.37 (t, 2H, J = 8 Hz, ArH), 7.44 (t, 2H, J = 8.8 Hz, ArH), 7.67 (d, 4H, J = 8 Hz, ArH), 8.07 (d, 4H, J = 6.4 Hz, ArH), 12.38 (s, 1H, br., OH).^{13}C NMR ($CDCl_3$100 MHz): δ 30.98, 33.37, 104.89, 121.58, 121.79, 122.21, 126.77, 129.04, 129.41, 133.68, 136.31, 142.85, 146.08, 148.37, 157.63. MS (ESI) *m/z* $(M+H)^+$ Calcd. for $C_{27}H_{23}N_5O_4$: 481.18. Found: 482.3.

4,4′-[(4-Nitrophenyl)methylene]bis(3-methyl-1-phenyl-1*H*-pyrazol-5-ol) (16g). IR (KBr): 3440, 3090, 2920, 1595, 1495, 1410, 1340, 744, 689 cm-1. 1H NMR (400 MHz, DMSO-d6): δ (ppm) 2.28 (s, 6H), 5.06 (s, 1H), 7.18 (t, 2H, J= 7.06 Hz), 7.38 (t, 4H, J= 7.31 Hz), 7.45 (d, 2H, J= 8.32 Hz), 7.64 (d, 4H, J= 7.82 Hz), 8.10 (d, 2H, J= 8.58 Hz), 13.81 (brs, 2H). ^{13}C NMR (100MHz, DMSO-d6): δ (ppm) 34.45, 121.91, 124.65, 127.03, 129.92, 130.25, 147.20, 147.58, 151.63.

4,4'-((3-chlorophenyl)methylene)bis(3-methyl-1-phenyl-1*H*-pyrazol-5-ol) (16h): ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 2.25 (*s*, 6*H*), 4.97 (*s*, 1*H*), 7.21-7.30 (*m*, 5*H*, Ar*H*), 7.32 (*s*, 1*H*, Ar*H*), 7.40-7.42 (*m*, 4*H*, Ar*H*), 7.70-7.76 (*m*, 4*H*, Ar*H*), 12.6 (*bs*, 1*H*, OH), 13.9 (*bs*, 1*H*, OH). ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 14.5, 40, 104.4, 121.4, 125.1, 126.3, 128, 128.2, 129.1, 129.6, 131, 140.1, 144, 148 and 148.4. Mass (LC-MS)m/z: 470; (M^-) observed at 469 and (M^+) observed at 471.

4,4'-((2-chlorophenyl)methylene)bis(3-methyl-1-phenyl-1*H*-pyrazol-5-ol) (16i). IR (KBr): 3450, 3070, 2910, 1610, 1555, 1495, 1395, 1360, 1300, 835, 740, 690 cm-1. 1H NMR (400 MHz, DMSO-d6): δ (ppm) 2.29 (s, 6H), 5.14 (s, 1H), 7.22-7.33 (m, 4H), 7.40 (d, 1H, J= 7.82 Hz), 7.44 (t, 4H, J= 7.57 Hz), 7.70 (d, 4H, J= 7.57 Hz), 7.80 (d, 1H, J= 7.06 Hz), 13.92 (brs, 2H). ^{13}C NMR (100 MHz, DMSO-d6): δ (ppm) 32.41, 120.67, 123.62, 126.92, 128.05, 128.93, 129.45, 130.32, 135.94, 137.36, 140.60, 141.18.

4,4′-[(4-Methoxyphenyl)methylene]bis(3-methyl-1-phenyl-1*H*-pyrazol-5-ol) (16j):^{1}H NMR (DMSO-d_6, 400 MHz): δ2.30 (s, 6H, $2CH_3$), 3.69 (s, 3H, OCH_3), 4.89 (s, 1H, CH), 6.83 (d, 2H, J = 8.4 Hz, ArH), 7.14-7.25 (m, 4H, ArH), 7.43 (t, 4H, J = 7.6 Hz, ArH), 7.70 (d, 4H, J = 8 Hz, ArH), 12.40 (s, 1H, br., OH), 13.91 (s, 1H, br., OH). ^{13}C NMR (DMSO-d_6, 100 MHz): δ13.56, 26.03, 28.07, 50.26, 52.51, 100.73, 108.70, 116.26, 120.89, 123.38, 123.89, 128.61, 132.50, 141.17, 153.00. MS (ESI) *m/z* $(M+H)^+$ Calcd. for $C_{28}H_{26}N_4O_3$: 466.2. Found: 467.35.

4,4′-[(4-Bromophenyl)methylene]bis(3-methyl-1-phenyl-1*H*-pyrazol-5-ol) (16l):^{1}H NMR (DMSO-d_6, 400 MHz): δ2.33 (s, 6H, $2CH_3$), 4.97 (s, 1H, CH), 7.18-7.24 (m, 4H, ArH), 7.41-7.47 (m, 6H, ArH), 7.69 (d, 4H, J = 8 Hz, ArH), 12.37 (s, 1H, br., OH), 13.81 (s, 1H, br., OH).MS (ESI) *m/z* $(M+H)^+$ Calcd. for $C_{27}H_{23}BrN_4O_2$: 516.1. Found: 517.3.

4,4'-(Thiophen-2-ylmethylene)bis(3-methyl-1-phenyl-1*H*-pyrazol-5-ol) (16n) IR (KBr): 3420, 3080, 2920, 1595, 1490, 1410, 1284, 779, 690 cm-1. 1H NMR (400 MHz, DMSO-d6): δ (ppm) 2.32 (s, 6H), 5.13 (s, 1H), 6.75-6.77

(m, 1H), 6.90-6.92 (m, 1H), 7.24-7.30 (m, 3H), 7.45 (t, 4H, J= 7.82 Hz), 7.71 (d, 4H, J= 7.82 Hz) 14.01 (brs, 2H). ^{13}C NMR (100MHz, DMSO-d6): δ (ppm) 29.43, 120.58, 124.05, 124.15, 126.75, 128.94, 132.99, 134.13, 147.73. Anal. Calcd for C25H22N4O2S: C, 67.85; H, 5.01; N, 12.66. found: C, 67.38; H, 4.99; N, 12.25.

Biological activity

Antibacterial activity

Minimum inhibitory concentration (MIC) values for bacteria determined according to the two-fold broth micro-dilution method using Muller-Hinton broth in 96-well micro-test plates recommended by National Committee for Clinical Laboratory Standards (NCCLS) guidelines [40].The antimicrobial susceptibility testing of newly synthesized compounds was performed *In Vitro* against bacterial strains *viz*., Gram-positive *Staphylococcus Aureus* (ATCC No. 29737), *Micrococcus Luteus* (ATCC No. 398) and Gram negative *Escherichia Coli* (NCIM No. 2256) and *Pseudomonas Fluorescens* (NCIM No. 2173), respectively, to find out minimum inhibitory concentration (MIC). The MIC was defined as the lowest concentrations of compound that completely inhibit the growth of each strain. Serial twofold dilutions of all samples were prepared in triplicate in micro titer plates and inoculated with suitably prepared cell suspension to achieve the required initial concentration. Serial dilutions were prepared for screening. Dimethylsulfoxide (DMSO) was used as solvent control. Ampicilin & kanamycin were used as a standard antibacterial drug. The concentration range of tested compounds and standard was 128-0.5 µg/mL. The plates were incubated at 37 °C for all micro-organisms; absorbance at 595 nm was recorded to assess the inhibition of cell growth after 24 h. The compounds which are showing promising antibacterial activity were selected for MIC studies. The MIC was determined by assaying at 128, 64, 32, 16, 8, 4, 2, 1 and 0.5 µg/mL concentrations along with standards at the same concentrations.

Antifungal activity

The antifungal activity was evaluated against five human pathogenic fungal strains, such as *Candida albicans* (NCIM 3471), *Fusarium oxysporum* (NCIM 1332) and *Aspergillus flavus* (NCIM 539), which are often encountered clinically and were compared with standard drug fluconazole & miconazole. Minimum inhibitory concentration (MIC) values were determined using standard agar method as per CLSI (formerly, NCCLS) guidelines (Approved Standard M7-A6, vol. 23. 2003) [41]. The standards used in the study were dissolved in a suitable solvent. The primary solutions were further diluted to the final strength using test medium. The medium yeast nitrogen base (Himedia, India) was dissolved in Phosphate buffer pH 7 and it was autoclaved at 110 °C for 10 minutes. The suitable concentration of standards was incorporated in the medium. The fungal strains were freshly subcultered on to Sabouraud dextrose agar (SDA) and incubated at 25 °C for 72 h. The fungal cells were suspended in sterile distilled water and diluted to get 105 cells/mL. 10 µL of standardized suspension was inoculated onto the control plates and the media incorporated with the antifungal agents. The inoculated plates were incubated at 25 °C for 48 h. The readings were taken at the end of 48 and 72 h.

Antioxidant activity

Antioxidant activities of the synthesized compounds **13-19** were measured using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay [42]. The hydrogen atom or electron donation ability of some compounds were measured from the bleaching of the

purple colored methanol solution of DPPH. The spectrophotometric assay uses the stable radical DPPH as a reagent. 1 mL of various concentrations of the test compounds (5, 10, 25, 50 and 100 µg/mL) in methanol was added to 4 mL of 0.004% (w/v) methanol solution of DPPH. The reaction mixture was incubated at 37 °C. The scavenging activity on DPPH was determined by measuring the absorbance at 517 nm after 30 min. All tests were performed in triplicate and the mean values were entered. The percent of inhibition (I %) of free radical production from DPPH was calculated by the following equation % of scavenging = [(*A*control – *A*sample)/(*A*sample × 100)] Where, *A*control is the absorbance of the control (DPPH radical without test sample) *A*sample is the absorbance of the test sample (DPPH radical with test sample). The control contains all reagents except the test samples.A lower IC_{50} value indicates the greater antioxidant activity. The IC_{50} (concentration required to scavenge 50% of the radicals) were calculated to evaluate the potential antioxidant activities. Butylated hydroxytoluene (BHT) has been used as a standard drug for the comparison of antioxidant activity and the observed results are summarized in **Table 4**.

Computational Study

ADME Properties

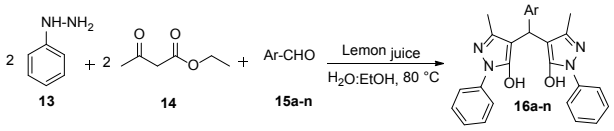
The success of a drug is determined not only by good efficacy but also by an acceptable ADME (absorption, distribution, metabolism and excretion) profile. In the present study, we have calculated molecular volume (MV), molecular weight (MW), logarithm of partition coefficient (miLog *P*), number of hydrogen bond acceptors (n-ON), number of hydrogen bonds donors (n-OHNH), topological polar surface area (TPSA), number of rotatable bonds (n-ROTB) and Lipinski's rule of five [43] using Molinspiration online property calculation toolkit [44]. Absorption (% ABS) was calculated by: % ABS = 109-(0.345×TPSA) [45] Drug-likeness model score (a collective property of physic-chemical properties, pharmacokinetics and pharmacodynamics of a compound is represented by a numerical value) was computed by MolSoft [46] software.

Result and discussion

Chemistry

Considering the significance of bis-pyrazolylmethanes and utility of lemon juice for various organic transformations, it has been planned to explore the catalytic activity of this catalyst for the synthesis of 4,4'-(arylmethylene) bis(3-methyl-1-phenyl-1*H*-pyrazol-5-ols) at 80 °C **Scheme2** using lemon juice as catalyst and further evaluation of their antimicrobial and antioxidant activity.

In present work, a facile, economic and green protocol for one-pot multicomponent condensation of phenyl hydrazine **13** (2 mmol) and ethyl acetoacetate **14** (2 mmol) with aromatic aldehydes **15a-n** (1 mmol) using lemon juice at 80 °C in ethanol solvent has been achieved (**Scheme 2**).



Scheme 2

In search of the best experimental reaction conditions, the model reaction of phenylhydrazine **13** (2 mmol), ethyl acetoacetate **14** (2 mmol) and benzaldehyde **15a** (1 mmol) was performed under catalyst-free conditions at reflux condition. Unfortunately, even after long reaction time the reaction did not proceed. Therefore, it was thought that for initiation of the reaction, intervention of catalyst is necessary.

In the next step, we have screened 0.25 mL of lemon juice at 80 °C as catalysts for the model reaction. Surprisingly, the product **16a** was obtained in 70% yield for 1 h (**Table1**, entry 2). Encouraged by this result, we have changed the amount of lemon juice from 0.25 mL to 3 mL and the results are summarized in **Table1**. Hence, the 1 mL of lemon juice is sufficient to carry out the reaction smoothly (**Table1**, entry 5). Excess amount of catalyst did not increase the yield of product neither reduces the time (**Table1**, entry 6, 7 and 8)

Table1. Effect of catalyst concentration[a]

Entry	Catalyst	Time(min)	Yield[b] (%)
1	0	4h	0
2	0.25 mL	60	70
3	0.50 mL	60	80
4	0.75 mL	45	84
5	1.0 mL	30	92
6	1.5 mL	30	92
7	2 mL	30	92
8	3 mL	30	92

[a]Reaction conditions: phenyl hydrazine (**13**) (2 mmol), ethyl acetoacetate (**14**) (2 mmol), benzaldehyde (**15a**) (1 mmol) and lemon juice at 80 °C. [b]Isolated yield.

The excess amount of lemon juice could not increase the yields of the reaction significantly. In order to evaluate the effect of solvent, various solvents such asMeOH, EtOH, aq. EtOH (50%), CH_3CN, THF and H_2O were used in the presence of lemon juice (**Table 2**). Reaction in EtOH, aq. EtOH and H_2O resulted in moderate yields (85, 92 and 60%, respectively). It has been observed that the use of other solvents retards the rate of reaction and affords the desired product in lower yields than that of ethanol-water solvent (**Table2**, entry 3).

Table 2. Screening of solvents[a]

Entry	Solvent	Time (min)	Yield[b] (%)
1	Methanol	75	80
2	Ethanol	60	85
3	Ethanol-water (1:1)	60	92
4	Acetonitrile	60	70
5	Tetrahydrofuran	60	67
6	Water	60	60

[a]Reaction conditions: Phenyl hydrazine **13** (2 mmol), ethyl acetoacetate **14** (2 mmol), benzaldehyde. **15a** (1 mmol) and solvent at reflux condition. [b]Isolated yield. [c]1 mL of lemon juice.

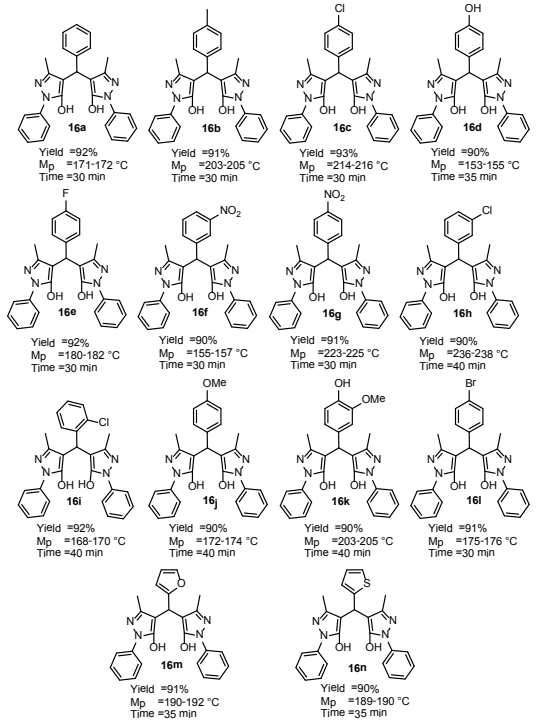


Figure2. Structures of 4,4'-(arylmethylene) bis(3-methyl-1-phenyl-1*H*-pyrazol-5-ols)**16a-n**

With these optimized reaction conditions for model reaction i.e. 1 mL lemon juiceas a catalyst at 80 °C and water:ethanol as a solvent, we have synthesized a series of 4,4'-(arylmethylene) bis(3-methyl-1-phenyl-1*H*-pyrazol-5-ols) **16a-n** by reacting phenylhydrazine **13**, ethyl acetoacetate**14** and aldehyde **15a-n** (with electron donating and withdrawing groups) in excellent yields (**Scheme 2**, **Figure 2**). The melting points of the synthesized compounds were compared with the reported methods.

In a plausible mechanism that is shown in **Figure3**, at first, 3-methyl-1-phenyl-5-pyrazolone I convert to II after tautomerisation. Then, II attacks to the carbonyl group of aldehyde that is activated by the lemon juice *via* hydrogen bonds and affords to intermediate III after removing one molecule of H_2O. III acts as a Michael acceptor and is activated by lemon juice. In this step, another molecule of 3-methyl-1-phenyl-5-pyrazolone in II tautomer form, attacks to III to give Intermediate IV. Finally, IV converts to V after tautomerisation and aromatization as product.

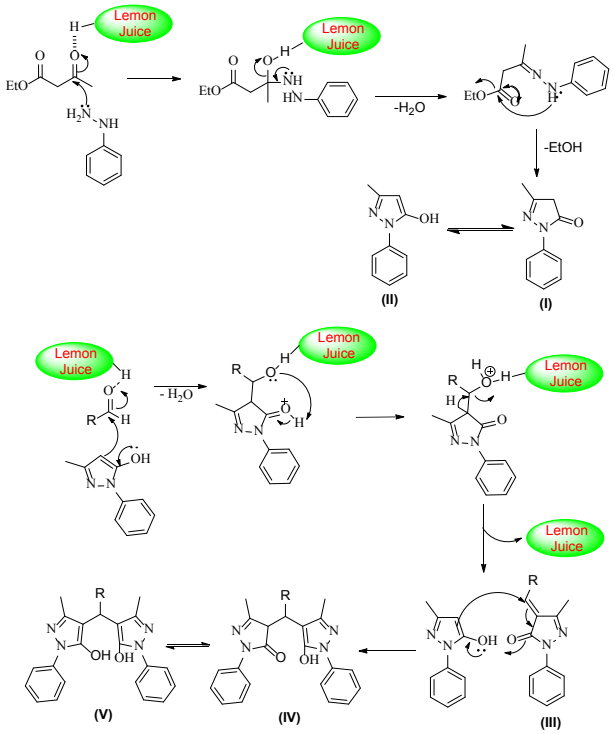


Figure3. Plausible mechanism

Biology

Antibacterial activity

The synthesized compounds**16a-n** were screened for antibacterial activity against the two Gram positive and two Gram negative bacterial strains and results are shown in **Table4**.

For bacterial strain *S. aureus*, it can be seen that, the compounds **16b**, **16d**, **16e**, **16j**, **16l** and **16m** showed excellent inhibitory activity with MIC value 4 µg/mL, which is equivalent toampicilin (MIC 4 µg/mL).For bacterial strain *M.luteus*, compounds **16c**, **16h**, **16i** and **16k** exhibited four-foldantibacterial activity with MIC value 4 µg/mLand compounds **16a**, **16d**, **16j**, **16l** and **16m**with MIC value 8 µg/mL exhibited two-foldmore activity as compared to the clinical drug ampicilin (MIC 16 µg/mL). For bacterial strain *E. coli* and *P. fluorescens*, all the synthesized compounds exhibited moderate antibacterial activity compared to the standard drug.

Antifungal activity

All the synthesized compounds**16a-n** showed good to moderate activity against all the tested fungal strains (**Table4**).Compounds **16b**, **16c**, **16h**, **16k** and **16l** with MIC value 4 µg/mL exhibited four-fold more activity compared with the standard drug miconazole and compounds **16d**, **16f** and **16m**with MIC value 8 µg/mL exhibited two-fold more activity compared to the miconazole against the fungicidal strain *C. albicans*. Compounds **16a**, **16e**, **16i**, **16j** and **16n** with MIC value 16 µg/mL exhibited equivalent activity compared with the standard drug miconazole.

Compounds **16c**, **16f** and **16l** with MIC value 4 µg/mL exhibited four-fold more activity compared with the standard drug miconazole and compounds **16a**, **16b**, **16h**, **16j** and **16k** with MIC value 8 µg/mL exhibited two-fold more activity compared to the miconazole for the fungicidal strain *F. oxysporum*. Compounds **16d**, **16e**, **16g**, **16m** and **16n** with MIC value 16 µg/mL exhibited equivalent activity compared with the standard drug miconazole. Compounds **16b** and **16k** with MIC value 4 µg/mL exhibited four-fold more activity compared with the standard drug miconazole and compounds

16a, **16c**, **16d**, **16f**, **16h**, **16j**, **16l** and **16m** with MIC value 8 µg/mL exhibited two-fold more activity compared to the miconazole against the fungicidal strain *A. flavus*. Compounds **16e**, **16i** and **16n** with MIC value 16 µg/mL exhibited equivalent activity compared with the standard drug miconazole.

Table4. *In vitro* antimicrobial and antioxidant activities of compounds **16a-n** (µg/mL)

Compounds	Gram +ve bacteria		Gram –ve bacteria		Antifungal activity			DPPH IC_{50}
	SA	*ML*	*EC*	*PF*	*CA*	*FO*	*AF*	
16a	8	8	16	16	16	8	8	21.1
16b	4	16	16	16	4	8	4	24.3
16c	16	4	8	4	4	4	8	23.1
16d	4	8	8	4	8	16	8	10.1
16e	4	32	8	4	16	16	16	18.3
16f	16	32	16	4	8	4	8	16.9
16g	32	16	8	4	16	16	32	12.1
16h	16	4	16	4	4	8	8	16.3
16i	8	4	16	16	16	32	16	17.3
16j	4	8	16	16	16	8	8	15.3
16k	16	4	8	4	4	8	4	10.2
16l	4	8	8	4	4	4	8	21.3
16m	4	8	8	4	8	16	8	24.1
16n	16	16	16	4	16	16	16	23.3
Ampicilin	4	16	4	2	-	-	-	-
Kanamycin	2	2	2	2	-	-	-	-
Miconazole	-	-	-	-	16	16	16	-
Fluconazole	-	-	-	-	2	2	4	-
BHT	-	-	-	-	-	-	-	16.5

Antioxidant activity

All the synthesized compounds **16a-n** shows good to moderate antioxidant activity as compared to the standard drug BHT (**Table4**). The compounds **16d**(10.1 µg/mL) and **16k**(10.2 µg/mL) with *hydroxy*- substituent on phenyl ring have shown excellent activity as compared to standard drug. Again, the compound **16g** (12.1 µg/mL) with *nitro*- group and**16j**(15.3 µg/mL) with *methoxy*- group showed excellent antioxidant activity as compared to the BHT. Remaining compounds exhibitgood to moderateantioxidant activity as compared to standard drug BHT.

Computational study

In silico ADME prediction

A computational study of all the synthesized 4,4'-(arylmethylene)bis(3-methyl-1-phenyl-1*H*-pyrazol-5-ols) **16a-n** was performed for prediction of ADME properties and the value obtained is presented in **Table 5**. It is observed that, the compounds exhibited a good % ABS (% absorption) ranging from 66.93 to 82.74%. Furthermore, only compounds **16l** violated Lipinski's rule of five (miLog $P \leq 5$). A molecule likely to be developed as an orally active drug candidate should show no more than one violation of the following four criteria: miLog *P* (octanol-water partition coefficient) ≤ 5, molecular weight ≤ 500, number of hydrogen bond acceptors ≤ 10 and number of hydrogen bond donors ≤ 5 [47]. The larger the value of the drug likeness model score, the higher is also probability that the particular molecule will be active. All the tested compounds followed the criteria for orally active drug and therefore, these compounds may have a good potential for eventual development as oral agents.

Conclusions

In conclusion, for the first time combination of *in vitro*antimicrobial and antioxidant screening of 4,4'-(arylmethylene)bis(3-methyl-1-phenyl-1*H*-pyrazol-5-ols). The advantages of the present procedure are shorter reaction time, no chromatographic separation, higher yield and attractive from an environmental friendly of view lemon juice as a catalyst, as it requires only simple and readily available starting materials and an inexpensive and nontoxic solvent (ethanol) making it an useful route for the synthesis of 4,4'-(arylmethylene) bis(3-methyl-1-phenyl-1*H*-pyrazol-5-ol)s. Furthermore, analysis of the ADME parameters

Table5. Pharmacokinetic parameters important for good oral bioavailability

Cpd	% ABS	TPSA (A^2)	n-ROTB	MV	MW	miLog *P*	n-ON	n-OHNH	Lipinski violation	Drug-likeness model score
Rule	-	-	-	-	< 500	≤ 5	< 10	< 5	≤ 1	-
16a	82.74	76.11	5	398.08	436.51	4.65	6	2	0	-0.21
16b	82.74	76.11	5	414.65	450.54	5.10	6	2	1	-0.00
16c	82.74	76.11	5	411.62	470.96	5.33	6	2	1	0.55
16d	75.76	96.34	5	406.10	452.51	4.17	7	3	0	0.43
16e	82.74	76.11	5	403.02	454.50	4.82	6	2	0	0.36
16f	66.93	121.93	6	421.42	481.51	4.59	9	2	0	0.10
16g	66.93	121.93	6	421.42	481.51	4.61	9	2	0	0.26
16h	82.74	76.11	5	411.62	470.96	5.31	6	2	1	0.13
16i	82.74	76.11	5	411.62	470.96	5.28	6	2	1	0.34
16j	79.55	85.34	6	423.636	466.54	4.71	7	2	0	0.23
16k	72.57	105.57	6	431.65	482.54	3.99	8	3	0	0.70
16l	82.74	76.11	5	415.97	515.41	5.46	6	2	2	0.17
16m	78.20	89.25	5	379.65	426.48	3.91	7	2	0	-0.12
16n	82.74	76.11	5	388.80	442.54	4.55	6	2	0	-0.03

for synthesized compounds showed good drug like properties and can be developed as oral drug candidate.

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Conflicts of interest

The authors declare no competing financial interest.

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