

Synthesis and biological evaluation of new 1-(4,6-dimethylpyrimidin-2-yl)-1'-aryl/heteroaryl-3,3'-dimethyl-(4,5'-bipyrazol)-5-ols as antimicrobial agents

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Abstract: 5-Hydroxy-3-methyl-1-(4,6-dimethylpyrimidin-2-yl)pyrazol-4-yl-1,3-butanedione 4, obtained by the rearrangement of 2-(4,6-dimethylpyrimidin-2-yl)-1-[1-(4-hydroxy-6-methylpyran-2-one-3-yl)ethylidene]hydrazine 3, afforded 1-(4,6-dimethylpyrimidin-2-yl)-1'-aryl/heteroaryl-3,3'-dimethyl-(4,5'-bipyrazol)-5-ols (6) in excellent yields after treatment with various aryl/heteroaryl hydrazines. The structures of the compounds were established by nuclear magnetic resonance (¹H and ¹³C), infrared, and mass spectral data, and by elemental analysis. All the compounds synthesized were screened for their antibacterial activity against two Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) and two Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*), and their antifungal activity against two yeasts (*Candida albicans* and *Saccharomyces cerevisiae*).

Keywords: 4,5'-bipyrazol-5-ol derivatives, antibacterial activity, antifungal activity

Introduction

The pyrazole nucleus represents a very attractive scaffold for obtaining novel molecules endowed with diverse biological activity, including anti-inflammatory, antidepressant, anticonvulsant, anticancer, analgesic, anthelmintic, antioxidant, and herbicidal properties.¹⁻⁶ Celecoxib, a selective cyclo-oxygenase-2 inhibitor, is used for the treatment of rheumatoid arthritis and osteoarthritis.⁷ Many other drugs, including lonazolac, aminopyrine, muzolimine, sulfaphenazole, and betazole, also contain a pyrazole nucleus. Moreno et al have recently described the synthesis of compounds containing a pyrazole moiety which exhibit in vitro and in vivo trypanosomicidal activity.⁸ Some bipyrazolyl derivatives have also proven to be useful as potential antitumor, anti-inflammatory, antibacterial, and cytotoxic agents.⁹⁻¹² Smaail et al synthesized bipyrazole derivatives which have shown promising antibacterial and antifungal activity.¹³ Bipyrazoles, in particular, have been reported to be active components in capturing active oxygen and free radicals in vivo. They are also useful agents for preventing and treating various diseases induced by active oxygen. Some bipyrazoles have found application as agents for detecting singlet oxygen,¹⁴ and others have been synthesized and used as agents with central nervous system activity.¹⁵

Many classes of chemotherapeutic agents containing a pyrimidine nucleus are in clinical use, including sulfadiazine, sulfamerazine, sulfamethoxydiazine, and brodimoprim (antibacterials), flucytosine and hexitidine (antifungals), 5-fluorouracil, ftorafur, and gemcitabine (antineoplastic agents), stavudine, idoxuridine, trifluridine, and

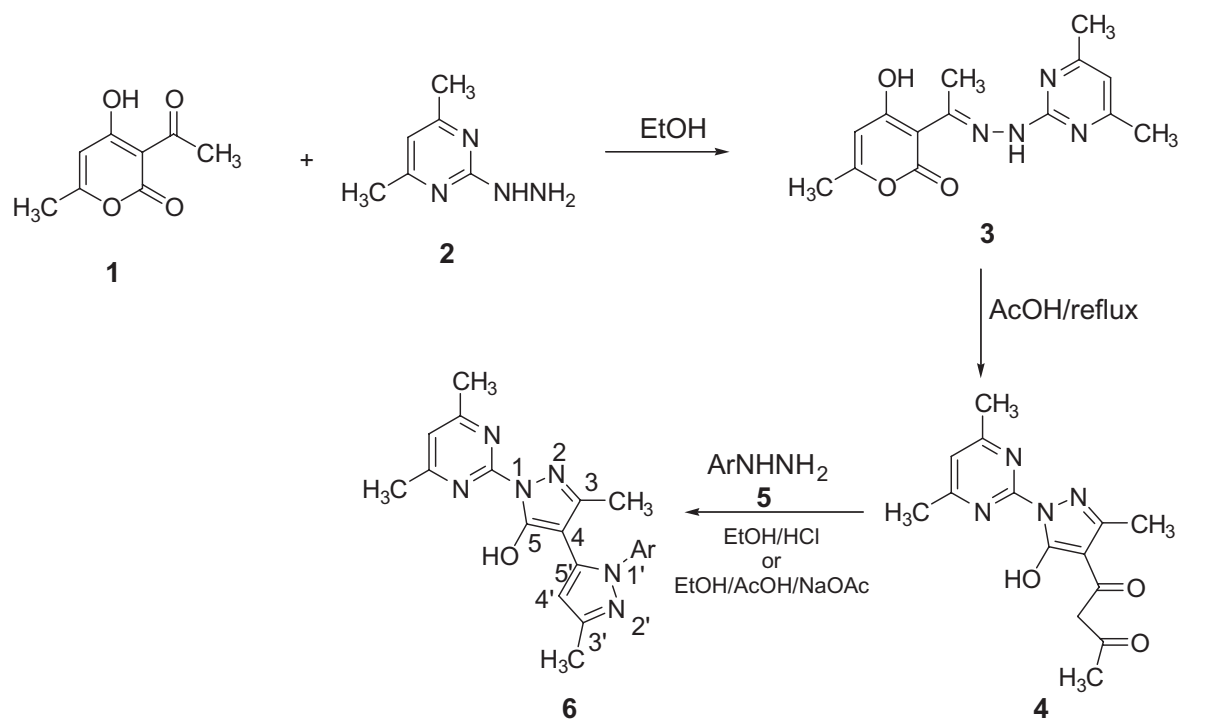
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zidovudine (antivirals), and pyrimethamine (an antimalarial). The most common method used for synthesis of pyrazoles and their derivatives involves the reaction of hydrazines with 1,3-dicarbonyl compounds or α , β -unsaturated aldehydes or ketones.¹⁶⁻²² Recently, Zora et al reported the synthesis of some pyrazoles via electrophilic cyclization of α , β -alkynic hydrazones.^{23,24} Synthesis of pyrazoles has also been accomplished by intermolecular 1,3-dipolar cycloaddition of diazoalkanes or nitrilimines with alkenes or alkynes.²⁵⁻²⁷ Reaction of dehydroacetic acid with phenylhydrazine to generate 4-(acylacetyl)-1-phenylpyrazolin-5-one and its synthetic applications to bipyrazol-5-ols and pyranopyrazoles has been studied by Gelin et al.²⁸ In view of these observations and in continuation of our research program on the synthesis of bipyrazoles,²⁹⁻³¹ we herein report the synthesis of some novel 4,5'-bipyrazol-5-ols containing a pyrimidine

ring which have been found to have interesting antibacterial and antifungal activities.

Materials and methods

2-(4,6-Dimethylpyrimidin-2-yl)-1-[1-(4-hydroxy-6-methylpyran-2-one-3-yl)ethylidene]hydrazine (3), obtained by the condensation of dehydroacetic acid (1) and 2-hydrazino-4,6-dimethylpyrimidine (2) in ethanol, on refluxing in acetic acid underwent rearrangement to yield the key intermediate, 5-hydroxy-3-methyl-1-(4,6-dimethylpyrimidin-2-yl)pyrazol-4-yl-1,3-butanedione (4). Further, synthesis of title compounds, 1-(4,6-dimethylpyrimidin-2-yl)-1'-aryl/heteroaryl-3,3'-dimethyl-(4,5'-bipyrazol)-5-ols (6), was achieved by the reaction of (4) with various aryl/heteroaryl hydrazines (5) in the presence of ethanol and concentrated HCl or ethanol/AcOH/sodium acetate (Figure 1).



5, 6	a	b	c	d	e	f	g	h
Ar								

Figure 1 Synthesis of 1-(4,6-dimethylpyrimidin-2-yl)-1'-aryl/heteroaryl-3,3'-dimethyl-(4,5'-bipyrazol)-5-ols (6)

The plausible mechanism for these reaction steps is outlined in Figure 2. Initial nucleophilic attack of the amino group of hydrazine 2 on the carbonyl of the acetyl side chain at the 3 position of dehydroacetic acid 1 followed by loss of one molecule of water yielded hydrazone 3. This hydrazone 3 then underwent rearrangement, involving a nitrogen nucleophilic attack at the C₂ lactone carbonyl with ring opening, thus generating 4. In the last step, this bielectrophilic pyrazolyl diketone 4 reacts with binucleophilic aryl/heteroaryl hydrazines to give 4,5'-bipyrazol-5-ols 6 (Figure 2). The structure of all the newly synthesized compounds was established

on the basis of infrared, mass, nuclear magnetic resonance (¹H and ¹³C) spectral data and elemental analysis. The infrared spectrum of 4 displayed absorption bands at 1551 cm⁻¹ (C=N) and 1713 cm⁻¹ (C=O). The ¹H NMR spectrum of 4 indicated its existence in two forms, ie, a keto form (42%) and an enol form (58%), as shown in Figure 3. In the keto form, two methyl groups attached to the pyrimidine ring of 4a resonated as a singlet at about δ 2.54 ppm. Two singlets were also observed at about δ 2.04 and δ 2.51 ppm, which correspond to a methyl attached to carbonyl and the other due to a C₃-CH₃ in the pyrazole ring, respectively. One singlet at

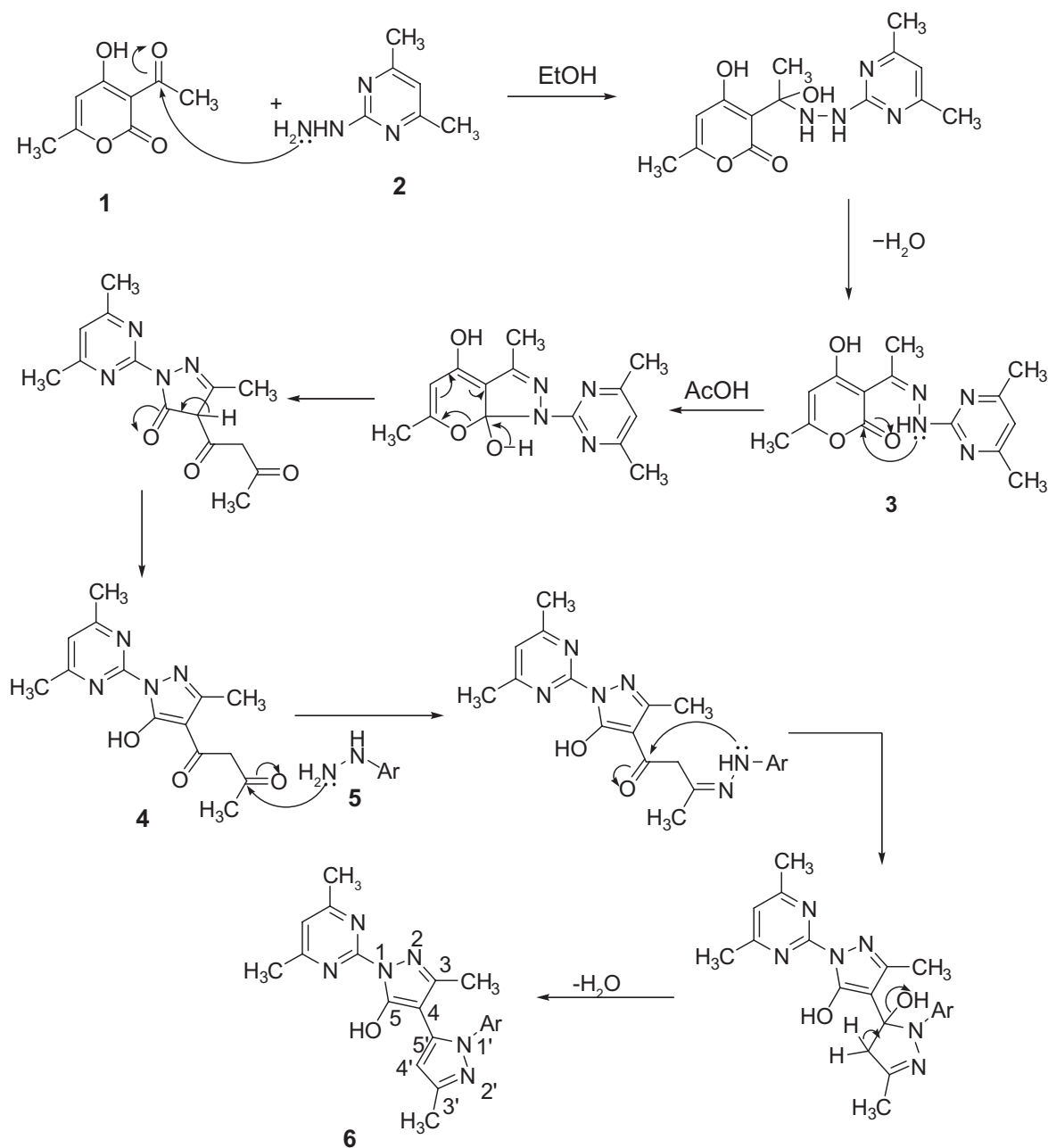


Figure 2 Plausible mechanism of formation of 1-(4,6-dimethylpyrimidin-2-yl)-1'-aryl/heteroaryl-3,3'-dimethyl-(4,5'-bipyrazol)-5-ols (6)

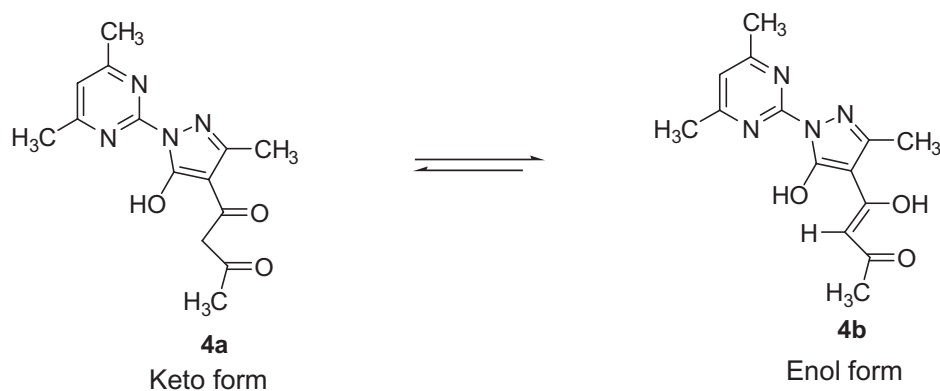


Figure 3 Keto-enol tautomerism of 5-hydroxy-3-methyl-1-(4,6-dimethylpyrimidin-2-yl)pyrazol-4-yl-1,3-butanedione (4)

about δ 3.92 ppm corresponding to methylene protons was also seen. One singlet was observed at about δ 6.95 ppm, corresponding to pyrimidine-5H. In enol form, one singlet at about δ 2.54 ppm corresponding to two methyl groups attached to pyrimidine ring was observed. Two singlets at about δ 2.25 and δ 2.48 ppm, corresponding to methyl attached to carbonyl and the other due to C_3 -CH₃ of pyrazole ring, respectively, were seen. Two more singlets were also observed, one signal at about δ 6.16 ppm, due to a vinylic proton of enol form, and another at about δ 6.94 ppm, corresponding to pyrimidine-5H. One broad singlet was observed at about δ 16.19 ppm due to enolic OH.

The ¹³C NMR of 4 also supports the existence of both a keto and an enol form. One signal at δ 30.62 ppm due to a carbon on the methyl group attached to a carbonyl of keto form was observed. One more signal at δ 56.55 ppm confirmed the presence of carbon attached to two carbonyl groups in keto form. One signal for vinylic carbon at δ 100.60 ppm also confirmed the enol form. A signal at about δ 203.18 ppm confirmed the presence of a carbonyl group attached to a methyl group in the keto form which shifted to 187.37 ppm in the enol form. A signal at about δ 187.02 ppm corresponding to another carbonyl group was seen, which shifted to 182.78 ppm in the enol form.

The infrared spectrum of compound 6a displayed absorption bands at 3140 cm⁻¹ (OH stretching), 1551 cm⁻¹ (C=N), 1651 cm⁻¹, and 1597 cm⁻¹ (aromatic region). The ¹H NMR spectra of 6 (a-h) displayed one singlet of six proton intensity at δ 2.57–2.60 ppm due to two methyl groups at the pyrimidine nucleus. Two singlets of one proton intensity each also appeared at δ 6.29–6.36 ppm and δ 6.93–6.97 ppm, corresponding to pyrazole-4H and pyrimidine-5H, respectively, which is in accordance with the value reported in the literature.^{31,32} Two more singlets of three proton intensity were also observed, one at δ 2.42–2.46 ppm, and the position

of the other methyl group was variable, ranging from δ 1.93 to δ 2.44 ppm in 6a–h, depending on the nature of the aryl/heteroaryl group located on the pyrazole moiety. In compound 6a, one methyl group resonated at about δ 1.93 and the other at about δ 2.42 ppm. It has earlier been observed, using nuclear overhauser enhancement techniques, that the upfield signal belongs to the pyrazole of the methyl group of pyrazolone (C_3 -CH₃), while the downfield signal is due to the methyl group located on the pyrazole moiety (C_3' -CH₃).³¹ This unexpected shielding of C_3 -CH₃ may be attributed to the fact that these protons fall in the shielding zone of the phenyl ring.^{29,31} As we go from 6a to 6c, with an increase in the withdrawing nature of electrons and bulk of the aryl group, the C_3 -CH₃ protons which fall in the shielding zone of the aryl ring become somewhat deshielded and resonate at about δ 2.13 and 2.21 ppm in 6b and 6c, respectively. However, the signal due to C_3' -CH₃ remains almost constant. Similarly, in 6d, a marked shift in the position of the signal due to C_3 -CH₃ was observed with δ 2.42 ppm, but the position of the signal due to C_3' -CH₃ was not altered as compared with 6a. When the phenyl group at position-1, of 6a is replaced by a bulkier and electron-withdrawing benzothiazole moiety, as in compounds 6e–h, the C_3 -CH₃ and C_3' -CH₃ protons resonated at δ 2.21–2.22 ppm and δ 2.44–2.45 ppm, respectively. Thus, it was confirmed that the presence of a phenyl group at position-1, of 6a shields the protons of the methyl group at position-3, but when the phenyl group is replaced by a bulkier and electron-withdrawing substituent, deshielding occurs. An increase in the bulk at position-1, of 6 helps the molecule to attain planarity, thus reducing the shielding of the methyl group considerably. The bulkier the group, the more planar the molecule, and the less the variation in chemical shifts of C_3 -CH₃ and C_3' -CH₃. Further, the ¹³C NMR spectra of 6a confirmed the presence of two carbons from methyl groups attached to the pyrimidine nucleus, by exhibiting signals at

about δ 23.96 ppm and two more signals at about δ 13.65 and 13.52 ppm for two carbons from methyl groups attached to pyrazole rings, respectively. A signal at δ 116.91 ppm was assigned to C₅ of the pyrimidine nucleus, as reported in literature.³³ Further, the C₄ of pyrazolone and the C_{4'} of pyrazole nuclei resonated at about δ 93.65 and 109.06 ppm, which is also in agreement with the literature.³¹ The complete assignment of carbon signals of the compounds 6 are given in Table 1.

Antimicrobial activity

All the compounds synthesized (6a–h) were assayed in vitro for their antibacterial and antifungal activity against two Gram-positive bacteria (*Staphylococcus aureus* MTCC 96 and *Bacillus subtilis* MTCC 121), two Gram-negative bacteria (*Escherichia coli* MTCC 1652 and *Pseudomonas aeruginosa* MTCC 741), and two yeasts, *Candida albicans* (MTCC 227) and *Saccharomyces cerevisiae* (MTCC 170).

Ciprofloxacin and amphotericin B were used as the standard drugs for evaluating antibacterial and antifungal activity, respectively. The zones of inhibition of antimicrobial activity are summarized in Table 2. The observed minimum inhibitory concentrations (MIC) presented in Table 3

were in accordance with the results obtained in the primary screening. All the tested compounds (6a–h) have shown moderate to good activity against the Gram-positive bacteria and the yeast, *S. cerevisiae*. However, only four compounds (6a, 6e, 6f, and 6g) were found to be active against Gram-negative *E. coli* bacteria. None of the compounds exhibited any activity against Gram-negative *P. aeruginosa* bacteria and the yeast *C. albicans*. On the basis of the maximum inhibitory activity shown against Gram-positive bacteria and yeast, compound 6f was found to be the most effective against *B. subtilis*, *S. aureus*, and *S. cerevisiae*, with zones of inhibition of 23.6 mm, 21.6 mm, and 15.6 mm, respectively (Table 2 and Figure 4), which are comparable with that of the commercial drugs, ciprofloxacin and amphotericin B. Compound 6f, which was found to be the most active, contains a benzothiazole moiety substituted at the 6-position by a methyl group. As we go from 6f to 6g, both the position and nature of the substituent at the benzothiazole moiety changes from 6-methyl to 5-methoxy, and somewhat of a decrease in activity was observed (Table 2). When the substituent at the benzothiazole moiety was removed, as in 6e, a further decrease in activity was observed. However, in the case of 6h, where a benzothiazole moiety is substituted by

Table 1 ¹³C NMR data of compounds 6a–h

Carbon	6a	6b	6c	6d	6e	6f	6g	6h
Pyrazole								
C ₃	149.49	149.70	150.89	150.80	151.06	149.00	149.38	147.59
C ₄	93.65	93.59	91.33	94.82	92.85	92.91	92.73	92.74
C ₅	154.02	154.04	153.85	153.31	154.72	154.68	154.84	154.71
3–CH ₃	13.52	13.54	13.29	13.66	13.82	13.80	13.76	13.78
C _{3'}	151.78	151.77	152.40	151.72	152.49	152.28	152.48	152.59
C _{4'}	109.06	109.05	110.15	110.89	112.42	112.19	112.45	112.47
C _{5'}	133.22	133.28	135.12	134.85	133.25	133.38	133.48	134.14
3'–CH ₃	13.65	13.65	13.53	13.79	13.99	13.98	13.95	13.96
Pyrimidine								
C ₂	156.72	156.69	156.26	156.68	156.84	156.84	156.82	156.83
C _{4'} C ₆	168.73	168.77	168.73	168.69	168.76	168.75	168.74	168.78
C ₅	116.91	116.97	117.35	116.71	116.95	116.93	116.95	116.97
CH ₃	23.96	23.99	23.82	23.78	24.01	24.01	24.01	24.00
Aryl/heteroaryl								
C ₁	140.57	140.46	144.12	–	–	–	–	–
C ₂	123.74	126.76	138.34	156.58	159.27	158.44	159.65	158.91
C ₃	128.87	128.92	120.68	–	–	–	–	–
C ₄	126.68	143.52	145.41	168.51	121.04	120.87	120.34	123.67
C ₅	–	–	127.18	117.38	124.52	127.55	154.65	107.31
C ₆	123.74	126.76	128.75	168.51	126.09	134.61	119.56	134.28
C ₇	–	–	–	–	122.89	122.46	129.76	114.59
C ₈	–	–	–	–	152.71	152.71	152.74	152.65
C ₉	–	–	–	–	134.69	134.56	134.67	134.63
Others								
CH ₃	–	–	–	23.78	–	21.47	–	–
OCH ₃	–	–	–	–	–	–	55.34	–

Table 2 In vitro antimicrobial activity of compounds 6a–h using the agar diffusion assay technique

Compound	Diameter of zone of growth inhibition (mm) ^a					
	Gram-positive bacteria		Gram-negative bacteria		Yeasts	
	<i>S. aureus</i>	<i>B. subtilis</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>S. cerevisiae</i>	<i>C. albicans</i>
6a	17.6	19.3	13.6	–	12.3	–
6b	15.6	17.6	–	–	12.6	–
6c	16.3	17.3	–	–	13.6	–
6d	15.6	17.6	–	–	12.6	–
6e	17.3	19.3	12.3	–	13.0	–
6f	21.3	23.6	16.3	–	15.6	–
6g	20.6	22.6	15.6	–	14.3	–
6h	18.3	19.3	–	–	12.3	–
Ciprofloxacin	26.6	24.0	25.0	22.0	–	–
Amphotericin B	–	–	–	–	19.3	16.6

Notes: –, no activity; ^avalues, including diameter of the well (8 mm), are means of three replicates.

Abbreviations: *S. aureus*, *Staphylococcus aureus*; *E. coli*, *Escherichia coli*; *P. aeruginosa*, *Pseudomonas aeruginosa*; *C. albicans*, *Candida albicans*; *S. cerevisiae*, *Saccharomyces cerevisiae*; *B. subtilis*, *Bacillus subtilis*.

a fluoro group at the 6-position, a complete loss of activity against Gram-negative bacteria and a decrease in activity against Gram-positive bacteria and yeast was observed when compared with 6f. Compound 6a containing a phenyl group showed moderate activity. However, in compounds 6b–d, the activity further decreased on changing the group from phenyl to *p*-nitrophenyl, 2,4-dinitrophenyl, and 4,6-dimethylpyrimidyl (Table 2).

The MIC of the various chemical compounds tested was in the range of 16–256 µg/mL against bacteria and 64–128 µg/mL against yeasts. Compound 6f exhibited the most promising results, showing the lowest MIC of 32 µg/mL against *S. aureus*, 16 µg/mL against *B. subtilis*, and 64 µg/mL against *S. cerevisiae* (Table 3). In conclusion, compounds having a phenyl group (6a) and a benzothiazole moiety (6e, 6f and 6g) showed activity against both Gram-positive bacteria, Gram-negative bacteria, and the yeast, *S. cerevisiae*. Among

Table 3 Minimum inhibitory concentration (µg/mL) values for compounds 6a–h and reference drugs against the study microorganisms

Compound	<i>S. aureus</i>	<i>B. subtilis</i>	<i>E. coli</i>	<i>S. cerevisiae</i>
6a	128	64	256	128
6b	128	128	–	128
6c	128	128	–	128
6d	128	128	–	128
6e	128	64	512	128
6f	32	16	128	64
6g	64	32	256	128
6h	64	64	–	128
Ciprofloxacin	6.25	6.25	–	–
Amphotericin B	–	–	6.25	12.5

Abbreviations: *S. aureus*, *Staphylococcus aureus*; *E. coli*, *Escherichia coli*; *S. cerevisiae*, *Saccharomyces cerevisiae*; *B. subtilis*, *Bacillus subtilis*.

all the tested chemical compounds, compound 6f containing a 6-methyl-substituted benzothiazole moiety, was found to be the most effective for inhibiting the growth of Gram-positive bacteria, Gram-negative bacteria, and yeasts, so this compound could be probed further as an antimicrobial agent with application in the pharmaceutical industry, after testing its toxicity in humans.

Experimental studies

Melting points were determined in open capillaries and are uncorrected. Infrared spectra were recorded on a spectrophotometer (IR M-500, Buck Scientific Inc, Norwalk, CT) in KBr pellets (ν_{\max} in cm^{-1}). ¹H and ¹³C NMR spectra for analytical purposes were recorded in CDCl₃ on a Bruker instrument (Billerica, MA) at 300 MHz and 75 MHz, respectively. Tetramethylsilane was taken as an internal standard. Chemical shifts (δ) were measured in ppm. Coupling constants (J) are given in Hertz (Hz).

2-Hydrazino-4,6-dimethylpyrimidine, 2-hydrazino-6-substituted benzothiazole, and 2-hydrazino-5-substituted benzothiazole were prepared following a method already reported in the literature.^{34,35} Dehydroacetic acid, phenyl hydrazine, *p*-nitrophenyl hydrazine, and 2,4-dinitrophenyl hydrazine were commercially available.

Preparation of 2-(4,6-dimethylpyrimidin-2-yl)-1-[1-(4-hydroxy-6-methylpyran-2-one-3-yl)ethylidene]hydrazine (3)

2-hydrazino-4,6-dimethylpyrimidine (1.38 g, 0.01 mol) was added to an ethanolic solution of dehydroacetic acid (1.68 g, 0.01 mol), and the contents were allowed to reflux for 6 hours. The progress of the reaction was monitored by

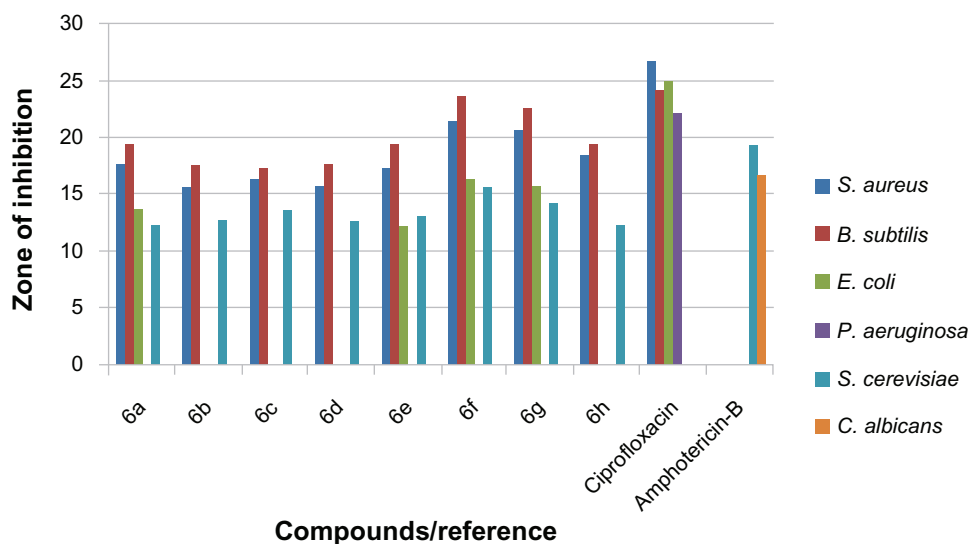


Figure 4 In vitro antimicrobial assay of test compounds/reference at concentration 4 mg/mL.

Abbreviations: *S. aureus*, *Staphylococcus aureus*; *E. coli*, *Escherichia coli*; *P. aeruginosa*, *Pseudomonas aeruginosa*; *C. albicans*, *Candida albicans*; *S. cerevisiae*, *Saccharomyces cerevisiae*; *B. subtilis*, *Bacillus subtilis*.

thin layer chromatography. On completion of the reaction, the reaction mixture was allowed to cool. A yellow solid separated out which was filtered and washed with cold ethanol.

Yield 88%; melting point 178°C–180°C (literature melting point 180°C);³⁶ IR (KBr, cm⁻¹): 1705 (C=O), 1643, 1597, 1551, 1443, 1358; ¹H NMR (300 MHz, CDCl₃) δ: 2.22 (s, 3H, -CH₃), 2.42 (s, 6H, C₄-CH₃, C₆-CH₃), 2.65 (s, 3H, -CH₃), 5.91 (s, 1H, pyrone-5-H), 6.62 (s, 1H, pyrimidine-5-H).

Preparation of 5-hydroxy-3-methyl-1-(4,6-dimethylpyrimidin-2-yl)pyrazol-4-yl-1,3-butanedione (4)

Hydrazone of dehydroacetic acid 3 (2.88 g, 0.01 mol) was dissolved in about 15 mL of acetic acid and the solution was allowed to reflux for about 45 minutes. The reaction mixture was poured in cold water, extracted with dichloromethane, and the solvent was distilled off to obtain a solid which was recrystallized from ethanol.

Yield 74%; melting point 195°C–198°C; IR (KBr, cm⁻¹): 1713 (C=O), 1551, 1427, 1373; ¹H NMR (300 MHz, CDCl₃) δ: keto form; 2.04 (s, -CH₃), 2.51 (s, 3-CH₃), 2.54 (s, pyrimidine-4, 6-CH₃), 3.92 (s, COCH₂CO), 6.95 (s, 1H, pyrimidine-5-H), enol form; 2.25 (s, -CH₃), 2.48 (s, 3-CH₃), 2.54 (s, pyrimidine-4, 6-CH₃), 6.16 (s, 1H, vinylic), 16.19 (bs, enolic-OH); ¹³C NMR (75 MHz, CDCl₃) δ: 15.47, 15.87, 23.85, 23.99, 30.62, 56.55, 97.97, 100.66,

104.28, 117.48, 117.63, 153.57, 154.06, 156.23, 156.32, 157.91, 159.49, 168.81, 182.78, 187.02, 187.37, 203.18; MS (m/z): 289.17 ([M+H]⁺); Analysis calculated for C₁₄H₁₆N₄O₃: C, 58.32; H, 5.59; N, 19.43. Found: C, 58.42; H, 5.55; N, 19.40.

Preparation of 1-(4,6-dimethylpyrimidin-2-yl)-1'-aryl-3,3'-dimethyl-(4,5'-bipyrazol)-5-ols (6)

To an ethanolic solution of 4 (2.88 g, 0.01 mol), appropriately substituted aryl/heteroaryl hydrazine 5 (0.01 mol) was added, followed by 4–5 drops of concentrated HCl or AcOH/sodium acetate (0.01 mol). The contents were allowed to reflux for 5–6 hours. The progress of the reaction was monitored by thin layer chromatography. The solid obtained after concentrating and cooling the reaction mixture was filtered and recrystallized from ethanol.

1-(4,6-dimethylpyrimidin-2-yl)-1'-phenyl-3,3'-dimethyl-(4,5'-bipyrazol)-5-ols (6a)

Yield 82%; melting point 152°C–154°C; IR (KBr, cm⁻¹): 3140 (OH), 3055, 1651, 1558, 1504, 1458, 1335; ¹H NMR (300 MHz, CDCl₃) δ: 1.93 (s, 3H, C₃-CH₃), 2.42 (s, 3H, C₃'-CH₃), 2.57 (s, 6H, pyrimidine-4, 6-CH₃), 6.29 (s, 1H, C₄'-H), 6.93 (s, 1H, pyrimidine-5-H), 7.21–7.34 (m, 3H, Ph 3, 4, 5-H), 7.41–7.44 (m, 2H, Ph 2, 6-H); MS (m/z): 361.23 ([M+H]⁺); analysis calculated for C₂₀H₂₀N₆O: C, 66.65; H, 5.59; N, 23.32. Found: C, 66.67; H, 5.55; N, 23.30.

1-(4,6-dimethylpyrimidin-2-yl)-1'-(4-nitrophenyl)-3,3'-dimethyl-(4,5'-bipyrazol)-5-ol (6b)

Yield 65%; melting point 220°C (d); IR (KBr, cm^{-1}): 3148 (OH), 1651, 1597, 1551, 1504, 1466, 1381, 1342; ^1H NMR (300 MHz, CDCl_3) δ : 2.13 (s, 3H, $\text{C}_3\text{-CH}_3$), 2.42 (s, 3H, $\text{C}_3'\text{-CH}_3$), 2.58 (s, 6H, pyrimidine-4, 6- CH_3), 6.33 (s, 1H, $\text{C}_4\text{-H}$), 6.97 (s, 1H, pyrimidine-5-H), 7.65 (d, 2H, $J = 7.2$ Hz, Ph-2, 6-H), 8.19 (d, 2H, $J = 7.2$ Hz, Ph-3, 5-H); MS (m/z): 406.22 ($[\text{M}+\text{H}]^+$); analysis calculated for $\text{C}_{20}\text{H}_{19}\text{N}_7\text{O}_3$: C, 59.25; H, 4.72; N, 24.18. Found: C, 59.23; H, 4.76; N, 24.23.

1-(4,6-dimethylpyrimidin-2-yl)-1'-(2,4-dinitrophenyl)-3,3'-dimethyl-(4,5'-bipyrazol)-5-ol (6c)

Yield 78%; melting point 228°C–232°C; IR (KBr, cm^{-1}): 3141 (OH), 1651, 1597, 1528, 1458, 1335; ^1H NMR (300 MHz, CDCl_3) δ : 2.21 (s, 3H, $\text{C}_3\text{-CH}_3$), 2.46 (s, 3H, $\text{C}_3'\text{-CH}_3$), 2.57 (s, 6H, pyrimidine-4, 6- CH_3), 6.34 (s, 1H, $\text{C}_4\text{-H}$), 6.95 (s, 1H, pyrimidine-5-H), 7.76 (d, 1H, $J = 8.7$ Hz, Ph-6-H), 8.40 (d, 1H, $J = 8.7$ Hz, Ph-5-H), 8.69 (s, 1H, Ph-3-H); MS (m/z): 451.23 ($[\text{M}+\text{H}]^+$); analysis calculated for $\text{C}_{20}\text{H}_{18}\text{N}_8\text{O}_5$: C, 53.33; H, 4.03; N, 24.88. Found: C, 53.28; H, 4.10; N, 24.85.

1-(4,6-dimethylpyrimidin-2-yl)-1'-(4,6-dimethylpyrimidin-2-yl)-3,3'-dimethyl-(4,5'-bipyrazol)-5-ol (6d)

Yield 71%; melting point 168°C–170°C; IR (KBr, cm^{-1}): 3271 (OH), 2932, 1643, 1597, 1551, 1504, 1427, 1372; ^1H NMR (300 MHz, CDCl_3) δ : 2.42 (s, 6H, $\text{C}_3\text{-CH}_3$, $\text{C}_3'\text{-CH}_3$), 2.57 (s, 12H, pyrimidine-4, 6- CH_3), 6.32 (s, 1H, $\text{C}_4\text{-H}$), 6.86 (s, 1H, pyrimidine-5-H), 6.91 (s, 1H, pyrimidine-5-H); MS (m/z): 391.24 ($[\text{M}+\text{H}]^+$); analysis calculated for $\text{C}_{20}\text{H}_{22}\text{N}_8\text{O}$: C, 61.52; H, 5.68; N, 28.70. Found: C, 61.57; H, 5.62; N, 28.68.

1-(4,6-dimethylpyrimidin-2-yl)-1'-(benzothiazol-2-yl)-3,3'-dimethyl-(4,5'-bipyrazol)-5-ol (6e)

Yield 84%; melting point 234°C–236°C; IR (KBr, cm^{-1}): 1720 (C=O), 1643, 1605, 1543, 1435, 1373; ^1H NMR (300 MHz, CDCl_3) δ : 2.22 (s, 3H, $\text{C}_3\text{-CH}_3$), 2.45 (s, 3H, $\text{C}_3'\text{-CH}_3$), 2.60 (s, 6H, pyrimidine-4, 6- CH_3), 6.36 (s, 1H, $\text{C}_4\text{-H}$), 6.96 (s, 1H, pyrimidine-5-H), 7.29–7.32 (m, 1H, benzothiazole-5-H), 7.36–7.41 (m, 1H, benzothiazole-6-H), 7.75–7.82 (m, 2H, benzothiazole-4, 7-H); MS (m/z): 418.18 ($[\text{M}+\text{H}]^+$); analysis calculated for $\text{C}_{21}\text{H}_{19}\text{N}_7\text{OS}$: C, 60.42; H, 4.59; N, 23.49. Found: C, 60.39; H, 4.62; N, 23.50.

1-(4,6-dimethylpyrimidin-2-yl)-1'-(6-methylbenzothiazol-2-yl)-3,3'-dimethyl-(4,5'-bipyrazol)-5-ol (6f)

Yield 88%; melting point 118°C–120°C; IR (KBr, cm^{-1}): 1713 (C=O), 1651, 1605, 1543, 1458, 1435, 1373; ^1H NMR (300 MHz, CDCl_3) δ : 2.21 (s, 3H, $\text{C}_3\text{-CH}_3$), 2.44 (s, 3H, benzothiazole- $\text{C}_6\text{-CH}_3$), 2.45 (s, 3H, $\text{C}_3'\text{-CH}_3$), 2.60 (s, 6H, pyrimidine-4, 6- CH_3), 6.36 (s, 1H, $\text{C}_4\text{-H}$), 6.96 (s, 1H, pyrimidine-5-H), 7.18–7.20 (m, 1H, benzothiazole-5-H), 7.55 (s, 1H, benzothiazole-7-H), 7.67–7.70 (m, 1H, benzothiazole-4-H); MS (m/z): 432.23 ($[\text{M}+\text{H}]^+$); analysis calculated for $\text{C}_{22}\text{H}_{21}\text{N}_7\text{OS}$: C, 61.23; H, 4.91; N, 22.72. Found: C, 61.28; H, 4.89; N, 22.68.

1-(4,6-dimethylpyrimidin-2-yl)-1'-(5-methoxybenzothiazol-2-yl)-3,3'-dimethyl-(4,5'-bipyrazol)-5-ol (6g)

Yield 74%; melting point 125°C–128°C; IR (KBr, cm^{-1}): 1712 (C=O), 1642, 1602, 1537, 1458, 1373; ^1H NMR (300 MHz, CDCl_3) δ : 2.29 (s, 3H, $\text{C}_3\text{-CH}_3$), 2.44 (s, 3H, $\text{C}_3'\text{-CH}_3$), 2.60 (s, 6H, pyrimidine-4, 6- CH_3), 3.89 (s, 3H, OCH_3), 6.35 (s, 1H, $\text{C}_4\text{-H}$), 6.97 (s, 1H, pyrimidine-5-H), 7.03–7.11 (m, 1H, benzothiazole-6-H), 7.72–7.75 (m, 1H, benzothiazole-4-H), 7.85–7.87 (m, 1H, benzothiazole-7-H); MS (m/z): 448.19 ($[\text{M}+\text{H}]^+$); analysis calculated for $\text{C}_{22}\text{H}_{21}\text{N}_7\text{OS}$: C, 59.05; H, 4.73; N, 21.91. Found: C, 58.99; H, 4.71; N, 21.89.

1-(4,6-dimethylpyrimidin-2-yl)-1'-(6-fluorobenzothiazol-2-yl)-3,3'-dimethyl-(4,5'-bipyrazol)-5-ol (6h)

Yield 86%; melting point 138°C–140°C; IR (KBr, cm^{-1}): 1713 (C=O), 1651, 1605, 1551, 1458, 1381; ^1H NMR (300 MHz, CDCl_3) δ : 2.22 (s, 3H, $\text{C}_3\text{-CH}_3$), 2.44 (s, 3H, $\text{C}_3'\text{-CH}_3$), 2.59 (s, 6H, pyrimidine-4, 6- CH_3), 6.35 (s, 1H, $\text{C}_4\text{-H}$), 6.96 (s, 1H, pyrimidine-5-H), 7.08–7.20 (dt, 1H, $J = 2.7$ Hz, $J = 9.0$ Hz, benzothiazole-5-H), 7.43–7.47 (dd, 1H, $J = 2.7$ Hz, $J = 8.1$ Hz, benzothiazole-7-H), 7.70–7.75 (dd, 1H, $J = 4.8$ Hz, $J = 9.0$ Hz, benzothiazole-4-H); MS (m/z): 436.18 ($[\text{M}+\text{H}]^+$); analysis calculated for $\text{C}_{21}\text{H}_{18}\text{FN}_7\text{OS}$: C, 57.92; H, 4.17; N, 22.51. Found: C, 57.89; H, 4.12; N, 22.57.

Antimicrobial assay

All the microbial cultures were sourced from the Microbial Type Culture Collection (MTCC, Imtech, Chandigarh). The microbial isolates representing Gram-negative and

Gram-positive bacteria were subcultured on nutrient agar, whereas the yeast was subcultured on malt yeast agar. The screening of eight compounds (6 a–h) was done in vitro using the agar well diffusion method.³⁷ The stock solutions (4 mg/mL) of the test compounds were prepared by dissolving 4 mg of test compound in 1 mL of dimethyl sulfoxide. All samples were sterilized through a 0.2 mm membrane filter and stored at 4°C until further use. Microbial inoculums were prepared from 24-hour-old cultures and turbidity was adjusted equivalent to the 0.5 McFarland turbidity standard, which is visually comparable with a microbial suspension of approximately 1.5×10^8 cfu/mL. By inoculating 100 μ L of each test bacterial culture in 20 mL of warm, melted, autoclaved Mueller Hinton agar, seed layers were prepared (separate flasks were used for each bacterial culture). After mixing, these were poured into sterilized labeled Petri plates (150 mm \times 20 mm). The 8 mm wells were punched in the solidified Petri plates with the help of a sterile cork borer. Using a micropipette, 100 μ L of each test compound (stock 4 mg/mL) was added aseptically to the individual wells. The loaded plates were incubated in an upright position at $37^\circ\text{C} \pm 1^\circ\text{C}$ for 24 hours. The diameter of the zone of growth inhibition around each well after incubation was measured in millimeters using a zone reader (HiAntibiotic zone scale, Table 2). Ciprofloxacin 4 mg/mL was used as the standard antibiotic for bacteria and amphotericin B for yeasts, with dimethyl sulfoxide as a negative control under similar conditions for comparison. This procedure was performed in three replicate plates for each organism.

Determination of minimum inhibitory concentration

The MIC is the lowest concentration of an antimicrobial compound that will inhibit visible growth of a microorganism after overnight incubation. The MIC of the various compounds against bacterial strains was tested using a modified agar well diffusion method. MICs were determined using the broth microdilution method, according to National Committee for Clinical Laboratory Standards recommendations.³⁸ Testing was performed at pH 7.4 ± 0.1 . The inoculums were prepared using a 16-hour broth culture of each bacterial strain adjusted to a turbidity equivalent to a 0.5 McFarland standard, and diluted in Mueller Hinton agar broth medium to give a concentration of 1×10^6 cfu/mL for bacteria. The test compounds were dissolved in dimethyl sulfoxide to obtain 4 mg/mL stock solutions. To ensure that the solvent

had no effect on microbial growth, a control test was performed using test medium supplemented with dimethyl sulfoxide at the same dilution as used in the experiments. A positive control (containing inoculum but no compound) and a negative control (medium only, without inoculum) were also prepared. Using this method, a two-fold serial dilution of each chemically synthesized compound was prepared by first reconstituting the compound in dimethyl sulfoxide followed by dilution in sterile distilled water to achieve a decreasing concentration range of 512 to 1 μ g/mL. A 100 μ L volume of each dilution was introduced into wells (in triplicate) in the agar plates already seeded with 100 μ L of standardized inoculum (10^6 cfu/mL) of the test microbial strain. All test plates were incubated aerobically at 37°C for 24 hours and observed for the inhibition zones. MIC, taken as the lowest concentration of the chemical compound that completely inhibited the growth of bacteria, shown by a clear zone of inhibition, was recorded for each test organism. Ciprofloxacin was used as a positive control for bacteria and amphotericin B for yeast, with dimethylsulfoxide as a negative control.

Conclusion

We have synthesized novel 4,5'-bipyrazol-5-ols 6 (a–h), which has an interesting profile of antibacterial activity against Gram-positive and Gram-negative bacteria, as well as antifungal activity against yeasts.

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Disclosure

The authors report no conflicts of interest in this work.

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