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ORIGINAL PAPER

Synthesis and antimicrobial/antioxidant evaluation of novel pyrimidine-based derivatives with pendant pyrazoles using vinamidinum salts

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Abstract

In the present study, synthesis of a variety of 5-bromo-4-methyl-2-substituted pyrimidines endowed with pyrazolyl substituent appended in C^4 position **(7a-f)** is described. These products are generated via treatment of 5-bromo-4-hydrazineyl-6-methyl-2-substituted pyrimidines **(6a–f)** and vinamidinum salt of (*E*)-*N*-(3-(dimethylamino)-2-phenylallylidene)-*N*-methylmethanaminium perchlorate **(5)** in good to excellent yields. The inhibitory and hydrogen-atom donating abilities of the synthesized products **(7a-f)** were assessed against nine pathogens including six bacterial strains (both Gram-negative and Gram-positive), three fungal strains and DPPH free radicals. Notable antioxidant properties were not observed with the products. IC_{50} values were in the range of 107.49–929.16 μg ml^{-1}. The inhibition zone diameters of products at 10 mg ml^{-1} concentrations were recorded in the range of 10.51–18.44 mm via disk diffusion method. 5-Bromo-4-methyl-6-pyrazolylpyrimidine (**7e**) containing 2-(4-methylpiperazin) substituent showed better antioxidant and antimicrobial effects than other products. It was effective on all the tested microbial strains except *Staphylococcus epidermidis*. All the synthesized pyrazolyl pyrimidines, especially (**7e**) can be used to disinfect the environment and treat infectious diseases.

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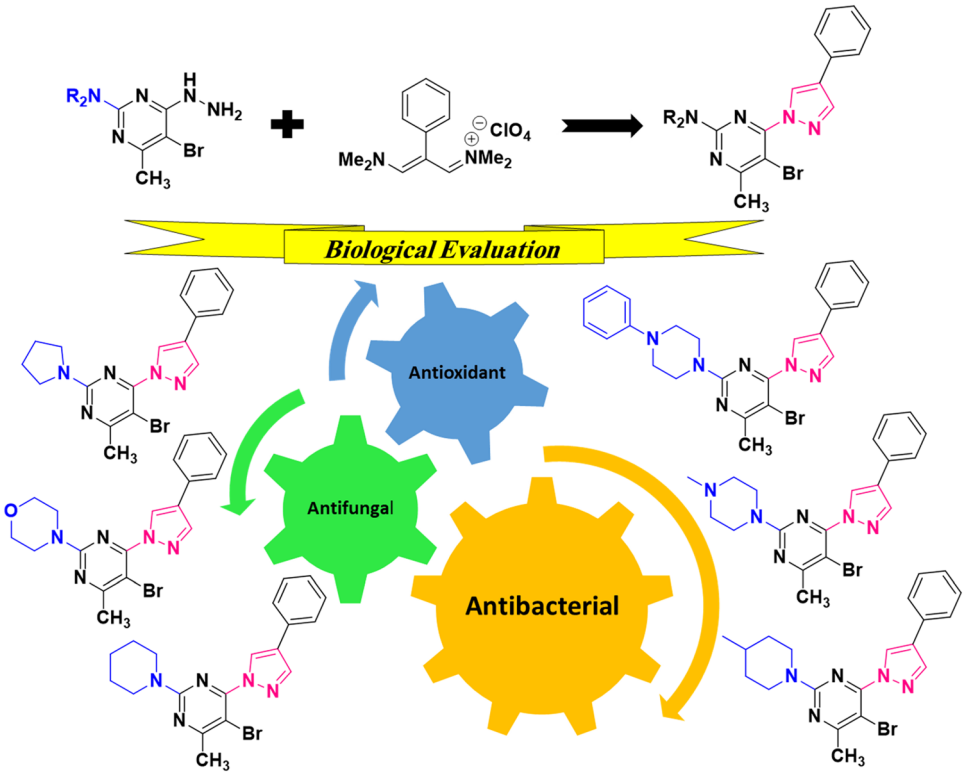
Graphical Abstract



Keywords Vinamidinum salts · Pyrazolyl pyrimidine · Antibacterial · Antifungal · Antioxidant

Introduction

1,5-diazapentadienium salts known as vinamidinium salts are vinylogous of amidinium compounds. They are kind of "push–pull alkene" which their stability spring from 'push–pull' or resonance effects between the electron-donating amino group and the electron-withdrawing ammonium group which are located at either end of the alkene chain, and are susceptible of substitution rather than addition reactions. Vinamidinium salts due to an alternation of electron density on different positions within their structures, represent specific reactivities towards both nucleophiles and electrophiles on the electron poor α-carbons and electron rich β-carbon, respectively [1–6]. From the viewpoint of their unique properties, these salts have practically obtained synthetic utility, so that many diversely functionalized vinamidinium salts have hitherto been developed and employed as the potent three-carbon skeletons in organic synthesis [4, 7, 8]. For instance, they are successfully utilized for the synthesis of heterocyclic compounds via the reaction with amidines, hydroxylamines, and hydrazines as bifunctional hetero-nucleophiles [9–14]. These salts are also reported to react with nucleophilic carbons such as enolates or related carbanions. Their reactions with enolates are particularly applied as the ready access pathway to dienaminone derivatives which can be valuable intermediates in natural product synthesis [1, 15–21].

Vinamidinium salts are commonly prepared with counter ions hexafluorophosphate or perchlorate. Despite of their chloride salts, these salts are stable and do not absorb moisture. Both ease of synthesis and their stability, make vinamidinium salts suitable synthons for the synthesis of heterocycles [7, 22–24].

On the other hand, infectious diseases including tuberculosis, lower respiratory infections and diarrheal diseases have always been one of the 10 causes of human death [25]. They have significantly increased the mortality rate in cancer patients [26]. The design of new disinfectant and antimicrobial agents is still one of the scientific research priorities.

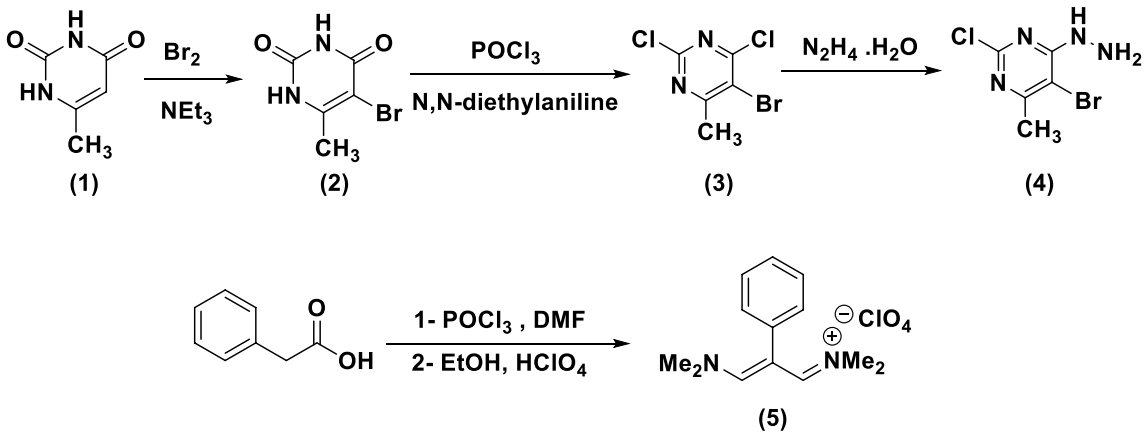
On the basis of the ease of access to vinamidinium salts as fascinating precursors and due to our abiding interest in synthesis of potentially bioactive heterocyclic scaffolds [27–35], we have continued to explore the synthetic utility of these salts for the synthesis of novel 5-bromo-4-methyl-6-pyrazolylpyrimidines appended with various secondary amine substituents. To diverse

antimicrobial libraries, their antibacterial and antifungal capacities were assessed against nine pathogenic strains. In addition, they evaluated for their possible antioxidant properties on DPPH (2,2-diphenyl-1-picrylhydrazyl) free radicals.

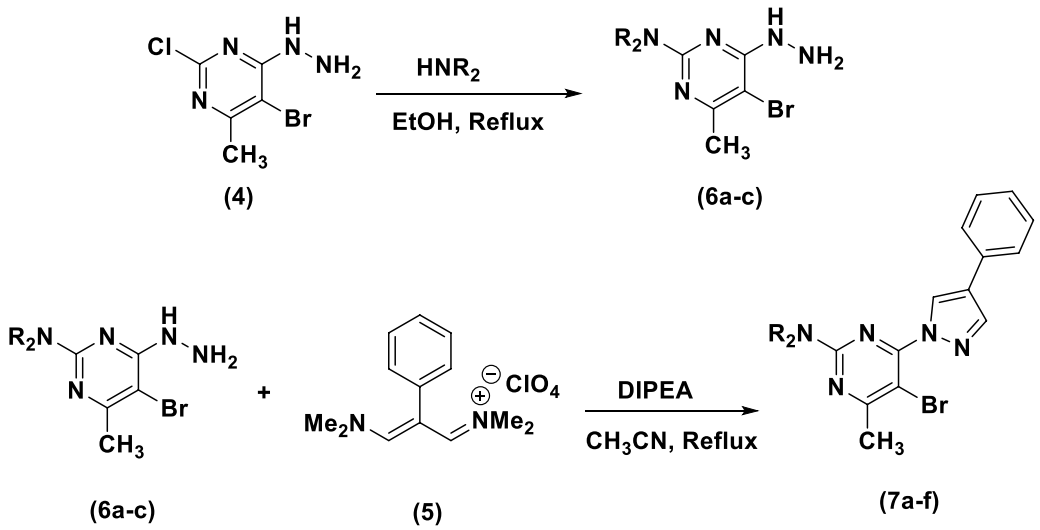
Results and discussion

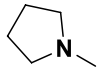
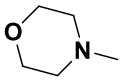
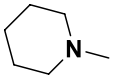
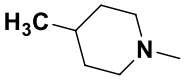
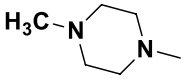
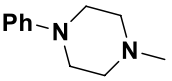
Chemistry

The synthetic pathways to assemble the desired 5-bromo-4-methyl-6-(4-phenyl-1*H*-pyrazol-1-yl)- 2-substituted-pyrimidine (**7a-f**) are outlined in Schemes 1 and 2. Initially, the required starting materials namely



Scheme 1 Synthesis of starting materials (**4**) and (**5**)



Entry	(7a)	(7b)	(7c)	(7d)	(7e)	(7f)
R_2N-						

Scheme 2 Synthesis of 5-bromo-4-methyl-2-substituted-6-(4-phenyl-1*H*-pyrazol-1-yl) pyrimidines (**7a-f**)

5-bromo-2-chloro-4-hydrazineyl-6-methylpyrimidine **(4)** and (*E*)-*N*-(3-(dimethylamino)-2-phenylallylidene)-*N*-methylmethanaminium perchlorate **(5)** were prepared in good overall yields according to the published methods [9, 36], as depicted in Scheme 1.

Subsequently, the reaction of compound **(4)** with excess quantities of various secondary amines in boiling EtOH underwent S_NAr substitution of Cl-2 on pyrimidine core to give new derivatives of 5-bromo-4-hydrazineyl-6-methyl-2-substituted pyrimidine **(6a–f)** in good yields. In continuation, the reaction of each of compounds **(6a-f)** as a nucleophile with **(5)** as an electrophile gave **(7a-f)** through a simple two-step inter- and intramolecular reactions in the presence of diisopropylethylamine (DIPEA) in CH_3CN, as depicted in Scheme 2.

The structural elucidation of all the synthesized compounds **(7a–f)** was accomplished using their physical, chemical and spectral data. As an example, the ^{1}H NMR spectrum of compound **(7a)** exhibited a multiple signal around δ_H 2.02 ppm and a triplet signal at δ_H 3.62 ppm due to methylene groups of pyrrolidine substituent and a singlet signal at δ_H 2.64 ppm corresponding to the methyl group. Aromatic protons of phenyl moiety were identified by a triplet signal at δ_H 7.31 ppm (J = 7.3 Hz), a triplet signal at δ_H 7.43 ppm (J = 7.6 Hz) and a doublet signal at δ_H 7.60 ppm (J = 7.0 Hz). Tow singlet signals at δ_H 8.11 ppm and 8.50 ppm was assigned to the hydrogens of the pyrazole moiety, as well. ^{13}C NMR spectrum of **(7a)** showed fourteen distinguished signals for the corresponding carbons ranged from δ_C 25.5–1702.3 ppm. According to the Fig. 1, in the IR spectrum of compound **(7a)**, the disappearance of NH stretching band and the symmetric & asymmetric stretching NH_2 bands of compound **(6a)** from $\bar{\upsilon}$ = 3125.07–3295.23 cm^{-1} strongly supports the possibility of the heterocyclization of pyrazole in C^4 position of 5-bromo-4-methyl-2-pyrrolidinyl pyrimidine leading to compound **(7a).**

Additionally, the observation of the molecular ion peak at *m/z* 384 in the mass spectrum together with the complimentary results of the elemental analysis of **(7a)** substantiated the substitution of phenyl pyrazolyl on the C^4 position of pyrimidine core endowed with 2-pyrrolidinyl pendant.

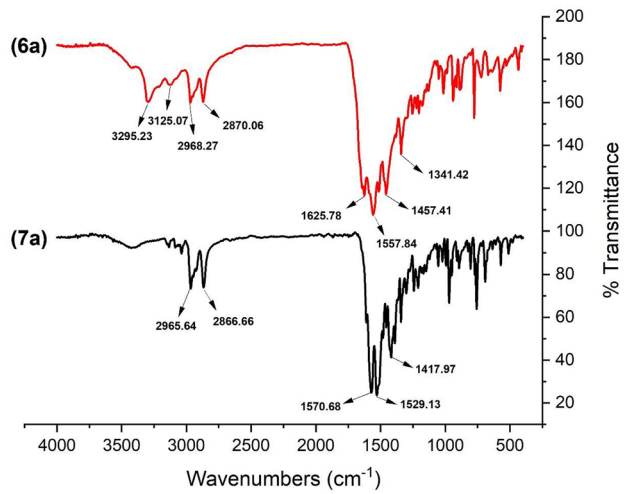


Fig. 1 The IR spectra of compounds **(6a)** and **(7a)**

Biological evaluation

Antibacterial and antifungal potentials of pyrazolylpyrimidines **(7a-f)** were investigated on pathogenic strains and compared with those of ceftriaxone (CFX) and ketoconazole (KTZ) (Fig. 2).

All the synthesized pyrazolylpyrimidines **(7a-f)** were effective in inhibiting the growth of *K. pneumoniae*, *S. pyogenes*, *C. albicans* and *A. fumigatus* strains. No inhibitory activity was observed with them against *S. epidermidis*. 5-Bromo-4-methyl-2-(4-methylpiperazin-1-yl)-6-(4-phenyl-1H-pyrazol-1-yl)pyrimidine **(7e)** displayed the best antimicrobial activities and it was the only effective pyrazolylpyrimidine on *F. oxysporum*. Pyrazolylpyrimidine **(7e)** containing 2-(4-methylpiperazin) substituent had better and wider antimicrobial effects than analogue **(7f)** containing 2-(4-phenylpiperazin) substituent.

Antibacterial, antifungal, antimalarial and antituberculosis activities of some *N*-thiomide analogues of ethyl 5-methyl-1-(6-(piperazin-1-yl)pyrimidin-4-yl)-1*H*-pyrazole-4-carboxylate were studied against a variety of microorganisms [37]. The minimum inhibitory concentrations (MICs) were in the range of 25–1000 μg ml^{-1}. Some of them showed better antibacterial effects than ciprofloxacin, ampicillin and chloramphenicol antibiotics. Inhibitory activity of 4-(3,5-dimethylpyrazolyl)-5,6,7,8-tetrahydrobenzothieno[2,3-d]pyrimidine was studied against some pathogenic bacteria and fungi [38]. IZD values at 1 mg ml^{-1} concentrations were ranged from 28 to 31 mm. Mycelial growth inhibitions of 48.3–65.6% were recorded with it against four tested fungal strains. Some pyrazolylhydrazonopyrimidine derivatives were evaluated for their antimicrobial potentials against *E. coli*, *P. aeruginosa*, *B. subtilis*, *S. aureus*, *A. flavus* and *C. albicans* strains [39]. MICs of 32–125 μg ml^{-1} were recorded with them. All derivatives showed antibacterial effects against *P. aeruginosa* compared to ampicillin.

Interaction of all pyrazolylpyrimidines (**7a-f**) with DPPH radicals were evaluated to determine their antioxidant activities as IC_{50} values (Fig. 3).

No significant scavenging activities were observed with the synthesized products against DPPH free radicals. Resembling to the antimicrobial activities, pyrazolylpyrimidine **(7e)** showed better antioxidant effects, too.

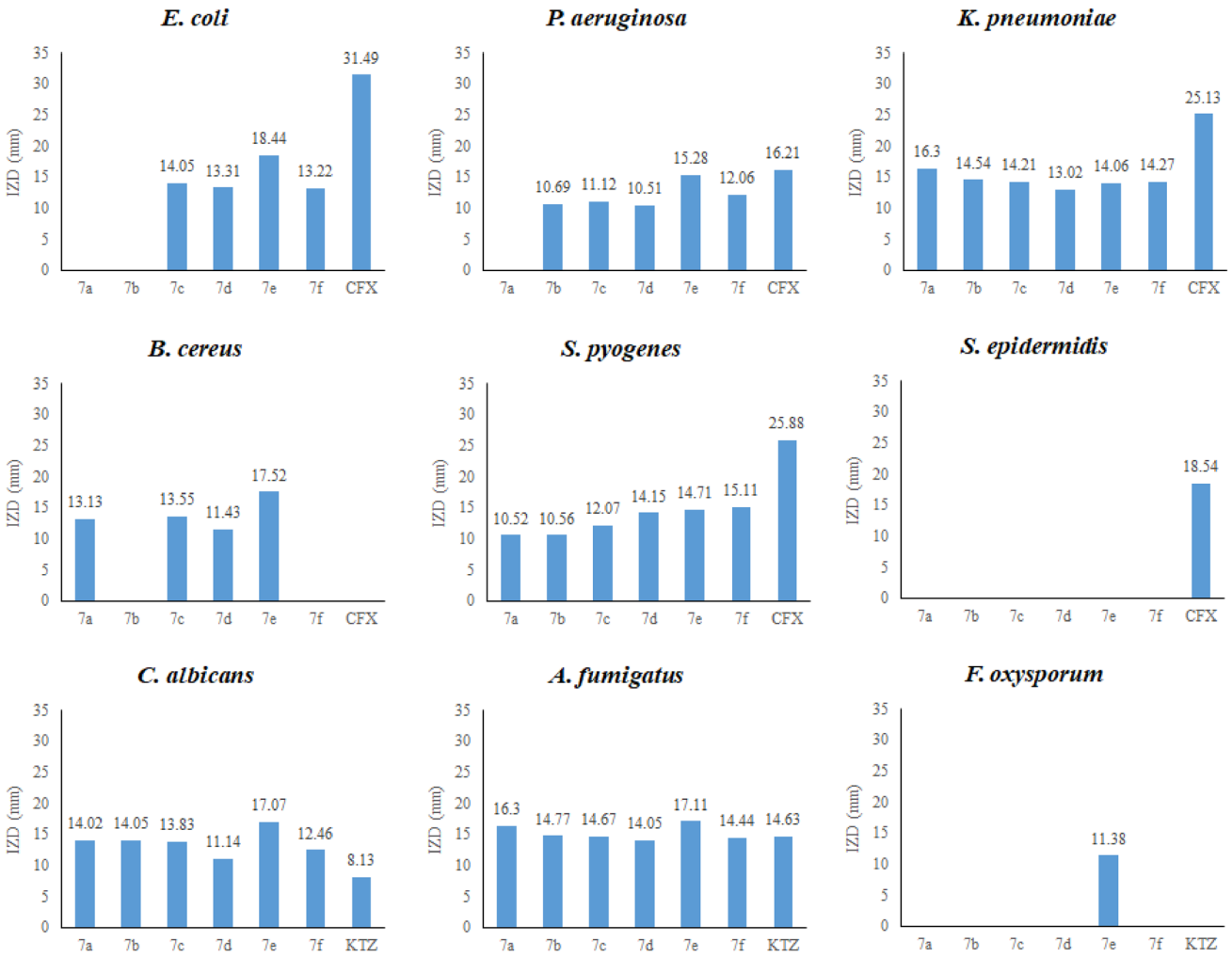


Fig. 2 Inhibition Zone Diameter(IZD) values of the synthesized pyrazolylpyrimidines and drugs

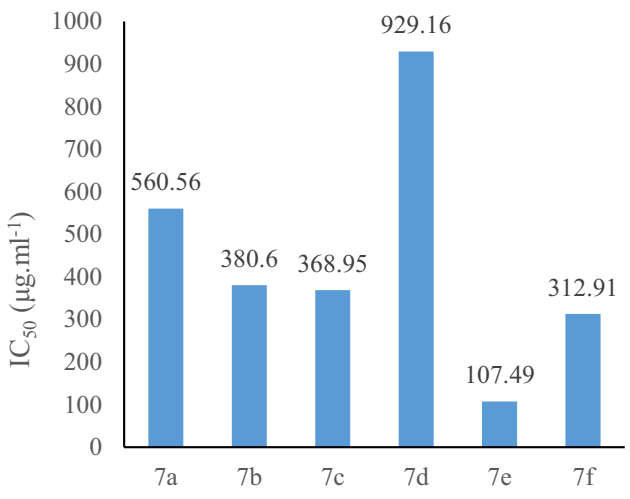


Fig. 3 IC_{50} values of pyrazolylpyrimidines (**7a-f**)

Experimental

Chemicals

Melting points were recorded on an electrothermal type 9200 melting point apparatus. The IR spectra were obtained on Avatar 370 FT-IR Thermo Nicolet and only noteworthy absorptions are listed. The ^{1}H NMR (300 MHz) and the ^{13}C NMR (75 MHz) spectra were recorded on a Bruker Avance-III 300 NMR Fourier transformer spectrometer. The mass spectra were scanned on a Varian Mat CH-7 at 70 eV. Elemental analyses were performed on a Thermo Finnigan Flash EA 1112 microanalyzer.

Synthesis of 5-bromo-4-hydrazineyl-6-methyl-2-substituted pyrimidine (6a-f); general procedure

A mixture of 5-bromo-2-chloro-4-hydrazineyl-6-methylpyrimidine (**4**) (1 mmol, 0.24 g) and the appropriate secondary

amin (3 mmol) in EtOH (5 mL) was refluxed for 12 h. After reaction completion, which was monitored by TLC using $CHCl_3$:MeOH (30:1), The solvent was evaporated and the crude product was purified by chromatography to give 5-bromo-4-hydrazineyl-6-methyl-2-substituted pyrimidine (**6a-f**).

5-Bromo-4-hydrazineyl-6-methyl-2-(pyrrolidin-1-yl) pyrimidine (6a)

White solid; yield 60%; m.p.:120–122 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.94–2.04 (m, 4H, 2 CH_2), 2.47 (s, 3H, CH_3–pyrimidine), 3.60–3.64 (m, 4H, 2 NCH_2), 6.68 (s, 2H, NH_2), 8.23 (s, 1H, NH) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ = 24.4, 25.5, 47.2, 89.7, 158.0, 159.5, 165.4 ppm; IR (KBr): $\bar{\upsilon}$ = 3295, 3125, 2986, 2870, 1625, 1557, 1510, 1457, 1341, 1222, 1014 cm^{-1}; MS: *m/z* = 272. Anal. calcd for C_9HBrN_5 (%): C, 39.72; H, 5.19; N, 25.73; Found: C, 39.69; H, 5.18; N, 25.71%.

4-(5-Bromo-4-hydrazineyl-6-methylpyrimidin-2-yl) morpholine (6b)

White solid; yield 57%; m.p.:128–130 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 2.27 (s, 3H, CH_3), 3.67 (s, 8H, 4 CH_2), 6.45 (s, 2H, NH_2), 7.99 (s, 1H, NH) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ = 24.1, 44.3, 44.4, 66.8, 66.9, 90.5, 159.4, 159.9, 162.9 ppm; IR (KBr): $\bar{\upsilon}$ = 3329, 3244, 2959, 2892, 2850, 1555, 1475, 1449, 1352, 1303, 1275, 1248, 1111 cm^{-1}; MS: *m/z* = 288. Anal. calcd for $C_9H_{14}BrN_5O$ (%): C, 37.51; H, 4.90; N, 24.31; Found: C, 37.49; H, 4.89; N, 24.28%.

5-Bromo-4-hydrazineyl-6-methyl-2-(piperidin-1-yl) pyrimidine (6c)

White solid, yield 56%; m.p.: 118–120 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.60–1.77 (m, 6H, 3 CH_2), 2.58 (s, 3H, CH_3), 3.89–4.06 (m, 4H, 2 NCH_2), 6.66 (s, 2H, NH2), 8.22 (s, 1H, NH) ppm; IR (KBr): $\bar{\upsilon}$ = 3398, 3305, 3186, 2933, 2854, 1613, 1556, 1442, 1397, 1287, 1252, 1022 cm^{-1}; MS: *m/z* = 286. Anal. calcd for $C_{10}H_{16}BrN_5$ (%): C, 41.97; H, 5.64; N, 24.47; Found: C, 41.96; H, 5.63; N, 24.45%.

5-Bromo-4-hydrazineyl-6-methyl-2-(4-methylpiperidin-1-yl) pyrimidine (6d)

White solid; yield 80%; m.p.: 142- 144 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.88 (d, *J* = 6.4 Hz, 3H, CH_3–piperidine), 0.99–1.12 (m, 2H, CH_2), 1.47- 1.54 (m, 1H, CH), 1.57–1.63 (m, 2H, CH_2), 2.26 (s, 3H, CH_3–pyrimidine), 2.71 (td, *J* = 12.7, 2.8 Hz, 2H, NCH_2), 4.63 (d, *J* = 8.2 Hz, 2H, NCH_2), 6.31 (s, 2H, NH2) ppm; 13C NMR (75 MHz, $CDCl_3$): δ = 21.9, 22.0, 24.2, 31.3, 34.1, 44.2, 90.0, 159.4, 159.9, 162.9 ppm; IR (KBr): $\bar{\upsilon}$ = 3317, 3277, 2948, 2917, 2842, 1562, 1455, 1309, 1273, 1247, 1122, 1082 cm^{-1}; MS: *m/z* = 300. Anal. calcd for $C_{11}H_{18}BrN_5$ (%): C, 44.01; H, 6.04; N, 23.33; Found: C, 44.00; H, 6.02; N, 23.31%.

5-Bromo-4-hydrazineyl-6-methyl-2-(4-methylpiperazin-1-yl) pyrimidine (6e)

White solid; yield 65%; m.p.: 104–106 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ = 2.29 (s, 3H, CH_3–pyrimidine), 2.47–2.57 (m, 4H, 2 NCH_2), 2.76 (s, 3H, CH_3–piperazine), 3.94–4.01 (m, 4H, 2 NCH_2), 6.39 (s, 2H, NH2) ppm; IR (KBr): $\bar{\upsilon}$ = 3313, 3276, 2923, 2843, 2799, 1570, 1548, 1478, 1442, 1301, 1273, 1249, 1002 cm^{-1}; MS: *m/z* = 304. Anal. calcd for $C_{10}H_{17}BrN_6$ (%): C, 39.88; H, 5.69; N, 27.90; Found: C, 39.86; H, 5.68; N, 27.88%.

5-Bromo-4-hydrazineyl-6-methyl-2-(4-phenylpiperazin-1-yl) pyrimidine (6f)

White solid; yield 70%; m.p.: 102–104 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 2.50 (s, 3H, CH_3), 3.18–3.36 (m, 4H, 2 NCH_2), 3.97–4.27 (m, 4H, 2 NCH_2), 6.58 (s, 2H, NH2), $\bar{\upsilon}\bar{\upsilon}$7.20–7.50 (m, 5H, aromatic) ppm; IR (KBr): $\bar{\upsilon}$ = 3380, 2950, 2925, 2843, 2814, 1646, 1598, 1570, 1552, 1493, 1444, 1375, 1280, 1230, 1155 cm^{-1}; MS: *m/z* = 363. Anal. calcd for $C_{15}H_{19}BrN_6$ (%): C, 49.60; H, 5.27; N, 23.14; Found: C, 49.58; H, 5.26; N, 23.11%.

General procedure for the synthesis of 5-bromo-4-methyl-2-substituted-6-(4-phenyl-1*H*-pyrazol-1-yl) pyrimidine (7a-f);

Vinamidinium salt (**5**) (1.0 mmol) was dissolved in CH_3CN (5 ml) and the mixture was added dropwise to the stirred solution of 5-bromo-4-hydrazineyl-6-methyl-2-substituted pyrimidine **(6a-f)** (1.0 mmol) and *N*, *N*- Diisopropylethylamine (2 mmol, 0.28 mL) in CH_3CN (5 mL). The solution was heated to reflux for 8–12 h. TLC was used to monitor the progress of the reactions. After the completion of reaction, the solvent was evaporated and the crude product was purified by chromatography to give 5-bromo-4-methyl-2-substituted-6-(4-phenyl-1*H*-pyrazol-1-yl) pyrimidines (**7a-f**), quantitively.

5-Bromo-4-methyl-6-(4-phenyl-1*H*-pyrazol-1-yl)-2-(pyrrolidin-1-yl)pyrimidine (7a)

White solid; yield 75%; m.p.: 130–132 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 2.02 (p, *J* = 3.3 Hz, 4H, 2 CH_2), 2.65 (s, 3H, CH_3), 3.62 (m, 4H, 2 N CH_2); 7.31 (t, *J* = 7.3,

1H, aromatic), 7.43 (t, $J=7.6$ Hz, 2H, aromatic), 7.60 (d, $J=7.0$ Hz, 2H, aromatic), 8.11 (s, 1H, Pyrazole), 8.50 (s, 1H, Pyrazole) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ = 25.5, 26.2, 46.9, 95.1, 124.2, 125.9, 126.7, 127.0, 128.9, 131.7, 139.7, 154.2, 157.9, 170.3 ppm; IR (KBr): $\bar{\upsilon}=3137$, 3043, 2965, 2866, 1570, 1529, 1455, 1417, 1342, 1242, 1210 cm^{-1}; MS: $m/z=384$. Anal. calcd for $C_{18}H_{18}BrN_5$ (%): C, 56.26; H, 4.72; N, 18.22; Found: C, 56.25; H, 4.70; N, 18.21%.

4-(5-Bromo-4-methyl-6-(4-phenyl-1*H*-pyrazol-1-yl)pyrimidin-2-yl)morpholine (7b)

White solid; yield 81%; m.p.:149–151 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 2.46 (s, 3H, CH_3), 3.83–3.86 (m, 4H, 2 NCH_2), 3.91–3.94 (m, 4H, 2 OCH_2), 7.30–7.35 (m, 1H, aromatic),7.44 (t, $J=7.5$ Hz, 2H, aromatic),7.63 (d, $J=7.0$ Hz, 2H, aromatic), 8.06 (s, 1H, Pyrazole), 8.74 (s, 1H, Pyrazole) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ = 24.6, 44.3, 66.9, 96.6, 123.3, 125.0, 125.8, 127.2, 128.9, 131.6, 140.6, 157.6, 161.3, 170.4 ppm; IR (KBr): $\bar{\upsilon}=3088$, 2994, 2969, 2855, 1600, 1561, 1428, 1361, 1263, 1240, 1175 cm^{-1}; MS: $m/z=400$. Anal. calcd for $C_{18}H_{18}BrN_5O$ (%): C, 54.01; H, 19.96; N, 17.50; Found: C, 53.98; H, 19.95; N, 17.48%.

5-Bromo-4-methyl-6-(4-phenyl-1*H*-pyrazol-1-yl)-2-(piperidin-1-yl)pyrimidine (7c)

White solid; yield 75%; m.p.: 108–110 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.69 (dt, $J=9.0$, 4.7 Hz, 6H, 3 CH_2), 2.69 (s, 3H, CH_3), 3.87 (t, $J=5.3$ Hz, 4H, 2 NCH_2), 7.33 (m, 1H, aromatic), 7.45 (t, $J=7.6$ Hz, 2H, aromatic), 7.62 (d, $J=7.0$ Hz, 2H, aromatic), 8.12 (s, 1H, Pyrazole), 8.47 (s, 1H, Pyrazole) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ = 24.6, 25.7, 25.8, 45.3, 94.8, 124.5, 126.0, 126.7, 127.7, 128.9, 131.6, 140.0, 169.9 ppm; IR (KBr): $\bar{\upsilon}=2998$, 2930, 2851, 1573, 1520, 1428, 1402, 1392, 1291, 1257 cm^{-1}; MS: $m/z=398$. Anal. calcd for $C_{19}H_{20}BrN_5$ (%): C, 57.29; H, 5.06; N, 17.58; Found: C, 57.26; H, 5.05; N, 17.56%.

5-Bromo-4-methyl-2-(4-methylpiperidin-1-yl)-6-(4-phenyl-1*H*-pyrazol-1-yl)pyrimidine (7d)

White solid; yield 78%; m.p.: 82- 84 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.01 (d, $J=6.4$ Hz, 3H, CH_3-piperidine), 1.23 (m, 2H, CH_2), 1.63–1.79 (m, 3H, CH_2 and CH), 2.63 (s, 3H, CH_3–pyrimidine), 2.91 (td, $J=12.7$, 2.8 Hz, 2H, N CH_2), 4.78 (dt, $J=13.4$, 2.7 Hz, 2H, N CH_2), 7.31 (t, $J=7.4$ Hz, 1H, aromatic), 7.44 (t, $J=7.4$ Hz, 2H, aromatic), 7.61 (d, $J=7.0$ Hz, 2H, aromatic), 8.11(s, 1H, Pyrazole), 8.46 (s, 1H, Pyrazole) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ = 21.9, 26.2, 31.1, 34.0, 44.4, 95.0, 124.0, 126.0, 126.7, 127.1, 128.9, 131.7, 139.7, 154.3, 159.0, 170.4 ppm; IR (KBr): $\bar{\upsilon}=2949$, 2917, 2842, 1571, 1521, 1430, 1253 cm^{-1}; MS: $m/z=411$. Anal. calcd for $C_{20}H_{22}BrN_5$ (%): C, 58.26; H, 5.38; N, 16.98; Found: C, 58.24; H, 5.35; N, 16.97%.

5-Bromo-4-methyl-2-(4-methylpiperazin-1-yl)-6-(4-phenyl-1*H*-pyrazol-1-yl)pyrimidine(7e)

White solid; yield 75%; m.p.: 105–107 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 2.38 (s, 3H, CH_3–piperazine), 2.49–2.53 (m, 4H, 2 NCH_2), 2.64 (s, 3H, CH_3-pyrimidine), 3.90 (t, $J=5.2$ Hz, 4H, 2 NCH_2), 7.31 (m, 1H, aromatic), 7.43 (t, $J=7.5$ Hz, 2H, aromatic), 7.60 (d, $J=7.0$ Hz, 2H, aromatic), 8.11 (s, 1H, Pyrazole), 8.45 (s, 1H, Pyrazole) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ = 26.2, 43.8, 46.2, 54.8, 95.9, 124.4, 126,126.1, 126.7, 128.9, 131.64, 139.9, 154.4, 159.0, 170.6 ppm; IR (KBr): $\bar{\upsilon}=2937$, 2847, 2789, 2736, 1578, 1528, 1431, 1369, 1287 cm^{-1}; MS: $m/z=413$.

Anal. calcd for $C_{19}H_{21}BrN_6$ (%): C, 55.21; H, 5.12; N, 20.33; Found: 55.20; H, 5.10; N, 20.31%.

5-Bromo-4-methyl-2-(4-phenylpiperazin-1-yl)-6-(4-phenyl-1*H*-pyrazol-1-yl)pyrimidine(7f)

White solid; yield 84%; m.p.: 142–144 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 2.57 (s, 3H, CH_3), 3.30 (br s, 4H, 2 NCH_2), 4.13 (br s, 4H, 2 NCH_2), 7.20–7.52 (m, 10H, aromatic), 8.02 (s, 1H, Pyrazole), 8.36 (s, 1H, Pyrazole) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ = 26.2, 43.8, 46.2, 54.8, 95.9, 124.4, 126,126.1, 126.7, 128.9, 131.64, 139.9, 154.4, 159.0, 170.6 ppm; IR (KBr): $\bar{\upsilon}=3101$, 3011, 2978, 2847, 2814, 2757, 1597, 1566, 1530, 1489, 1426, 1373, 1230, 1152 cm^{-1}; MS: $m/z=476$. Anal. calcd for $C_{24}H_{23}BrN_6$ (%): C, 60.64; H, 4.88; N, 17.68; Found: C, 60.62; H, 4.87; N, 17.65%.

Biological testing

DPPH, ceftriaxone and ketoconazole were prepared from Sigma-Aldrich company. *Staphylococcus epidermidis* (PTCC 1435, ATCC 14990), *Bacillus cereus* (PTCC 1665, ATCC 14579) and *Streptococcus pyogenes* (PTCC 1447, ATCC 12204) as Gram-positive bacterial strains, *Pseudomonas aeruginosa* (PTCC 1310, ATCC 10145), *Escherichia coli* (PTCC 1399, ATCC 25922) and *Klebsiella pneumoniae* (PTCC 1290, NCTC 5056) as Gram-negative bacterial strains and *Candida albicans* (PTCC 5027, ATCC 10231), *Fusarium oxysporum* (PTCC 5115, CBS 620.87) and *Aspergillus fumigatus* (PTCC 5009) as fungal strains were purchased from the Persian Type Culture Collection (PTCC), Karaj, Iran. The results of biological tests were expressed as the average of three independent experiments.

Disk diffusion procedure

The inhibition zone diameter (IZD) values were determined via disk diffusion susceptibility test [40]. The synthesized products and drugs were respectively dissolved in DMSO and distilled water to give initial concentrations of 10 and 0.02 mg ml^{-1}. 100 µl of microbial suspensions with 0.5 McFarland concentration was spread with swab on agar surface of petri dishes. The blank disks were placed on it at appropriate intervals. 10 µl of derivatives or drugs were poured on disks. Petri dishes were incubated for 24 h at 37 °C. The IZDs were determined as the area around the disks where there were lack of microbial growth.

Free radical scavenging testing

The half maximal inhibitory concentration (IC_{50}) values were determined via DPPH free radical scavenging assay [41]. 1 ml of each derivative (concentrations of 25, 50, and 100 µg ml^{-1}) was mixed with 3 ml of freshly prepared methanolic solution of DPPH (0.004% w/v). The mixtures were rested in the dark for 30 min. Then, the absorbance of the solutions was read at 517 nm. The inhibition percentage of samples were calculated according the equation: $I\% = [(A\ sample - A\ blank)/(A\ blank)] \times 100$, where A is the absorbance at 517 nm. Finally, the equation of the straight line was obtained and the IC_{50} value was quantified where y equals 50.

Conclusion

In this study, a novel series of 5-bromo-4-methyl-6-pyrazolylpyrimidines **(7a-f)** which are 2-substituted with pyrrolidine, morpholine, piperidine, 4-methyl piperidine, *N*-methyl piperazine and *N*-phenyl piperazine were synthesized in good to excellent yields via an inter- and intramolecular cyclization reaction of 5-bromo-4-hydrazineyl-6-methyl-2-substituted pyrimidine **(6a–f)** and (*E*)-*N*-(3-(dimethylamino)-2-phenylallylidene)-*N*-methylmethanaminium perchlorate **(5)** in refluxing CH_3CN. Their antimicrobial and antioxidant efficiencies were investigated on bacterial and fungal strains as well as DPPH free radicals. They could inhibit the growth of a variety of pathogenic microorganisms. Compound **(7e)** was recognized as a new wide-spectrum antimicrobial agent. It is predicted that *N*-alkylation and thioamidation of piperazine substituent will intensify its antimicrobial effects.

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Declarations

Conflict of interest No conflict of interest has been declared by the authors.

References

1. D. Lloyd, H. McNab, Angew. Chemie Int. Ed. English **15**, 459 (1976)
2. X. Shi, T. Ishihara, H. Yamanaka, J.T. Gupton, Tetrahedron Lett. **36**, 1527 (1995)
3. K. Kase, M. Katayama, T. Ishihara, H. Yamanaka, J.T. Gupton, J. Fluor. Chem. **90**, 29 (1998)
4. H. Yamanaka, T. Ishihara, J. Fluor. Chem. **105**, 295 (2000)
5. R. Jasiński, B. Mirosław, O.M. Demchuk, D. Babyuk, A. Łapczuk-Krygier, J. Mol. Struct. **1108**, 689 (2016)
6. F. Bureš, Rsc Adv. **4**, 58826 (2014)
7. A.P. Mityuk, S.V. Ryabukhin, Chem. Heterocycl. Compd. **56**, 1408 (2020)
8. I.W. Davies, D.M. Tellers, C.S. Shultz, F.J. Fleitz, D. Cai, Y. Sun, Org. Lett. **4**, 2969 (2002)
9. J.T. Gupton, D.A. Krolikowski, R.H. Yu, S.W. Riesinger, J.A. Sikorski, J. Org. Chem. **55**, 4735 (1990)
10. J.T. Gupton, J.E. Gall, S.W. Riesinger, J. Heterocycl. Chem. **28**, 58 (1991). https://doi.org/10.1002/jhet.5570280520
11. J.T. Gupton, D.A. Krolikowski, R.H. Yu, V. Phong, J.A. Sikorski, M.L. Dahl, C.R. Jones, J. Org. Chem. **57**, 5480 (1992)
12. S.N. Balasubrahmanyam, B. Jeyashri, I.N.N. Namboothiri, Tetrahedron **50**, 8127 (1994)
13. S.A. Petrich, F.A. Hicks, D.R. Wilkinson, J.G. Tarrant, S.M. Bruno, M. Vargas, K.N. Hosein, J.T. Gupton, J.A. Sikorski, Tetrahedron **51**, 1575 (1995)
14. S.A. Petrich, Z. Qian, L.M. Santiago, J.T. Gupton, J.A. Sikorski, Heterocycles **2**, 729 (1995)
15. C. Jutz, R.M. Wagner, Angew. Chemie Int. Ed. English **11**, 315 (1972)
16. C. Jutz, R.M. Wagner, H. Löbering, Angew. Chemie Int. Ed. English **13**, 737 (1974)
17. P. Bamfield, P.F. Gordon, Chem. Soc. Rev. **13**, 441 (1984)
18. V. Nair, C.S. Cooper, Tetrahedron Lett. **21**, 3155 (1980)
19. V. Nair, C.S. Cooper, J. Org. Chem. **46**, 4759 (1981)
20. V. Nair, T.S. Jahnke, Carbon elongations with 1, 5-diazapentadienium salts: reaction with aralkyl ketones. Synthesis (Stuttgart) **5**, 424–426 (1984)
21. V. Nair, T.S. Jahnke, Tetrahedron Lett. **25**, 3547 (1984)
22. F. Azamifar, M.R. Naimi-Jamal, O.M. Demchuk, Dye. Pigment. **161**, 438 (2019)
23. R.P. Frutos, X. Wei, N.D. Patel, T.G. Tampone, J.A. Mulder, C.A. Busacca, C.H. Senanayake, J. Org. Chem. **78**, 5800 (2013)
24. D.J. Paymode, L. Chang, D. Chen, B. Wang, K. Kashinath, V. Gopalsamuthiram, D.T. McQuade, N. Vasudevan, S. Ahmad, D.R. Snead, Org. Lett. **23**, 5400 (2021)
25. Q. Liu, W. Jing, M. Liu, J. Liu, J. Glob. Health **12**, 04028 (2022)
26. M. Elhadi, A. Khaled, A. Msherghi, Infect. Agent Cancer **16**, 1 (2021)
27. S. Sheikhi-mohammareh, A. Shiri, M. Bakavoli, J. Chem. Res. **39**, 403 (2015)
28. S. Sheikhi-Mohammareh, A. Shiri, M. Bakavoli, J. Mague, J. Heterocycl. Chem. **53**, 1231 (2016)

29. S. Sheikhi-Mohammareh, A. Shiri, J. Heterocycl. Chem. **55**, 2055 (2018)
30. S. Sheikhi-Mohammareh, A. Shiri, H. Beyzaei, E. Yarmohammadi, Monatshefte Für Chemie - Chem. Mon. **151**, 963 (2020)
31. S. Sheikhi-Mohammareh, A. Shiri, E.H. Maleki, M.M. Matin, H. Beyzaei, P. Baranipour, F. Oroojalian, T. Memariani, ChemistrySelect **5**, 10060 (2020)
32. S. Sheikhi-Mohammareh, M. Mashreghi, A. Shiri, J. Iran. Chem. Soc. **17**, 1555 (2020)
33. O. Kohandel, S. Sheikhi, M. Fatemeh, T. Memariani, J. Mague, Mol. Divers. (2021). https://doi.org/10.1007/s11030-021-10290-8
34. S. Sheikhi-Mohammareh, A. Shiri, J. Mague, Mol. Divers. **26**, 923 (2022)
35. S. Bigonah-Rasti, S. Sheikhi-Mohammareh, K. Saadat, A. Shiri, Novel Tricyclic 2-Alkoxy-8-methyl-6-(pyrrolidin-1-yl)-4 H-[1, 2, 4] triazolo [5, 1-f] purine Derivatives: Synthesis and Characterization. Polycyclic Aromatic Compounds. **42**(5), 2644–2654 (2022)
36. M. Bakavoli, M. Nikpour, M. Rahimizadeh, J. Heterocycl. Chem. **43**, 1327 (2006)
37. M.K. Vekariya, D.B. Patel, P.A. Pandya, R.H. Vekariya, P.U. Shah, D.P. Rajani, N.K. Shah, J. Mol. Struct. **1175**, 551 (2019)
38. M.H. Bhuiyan, K.M. Rahman, K. Hossain, A. Rahim, M.I. Hossain, M.A. Naser, ACTA Pharm. **56**, 441 (2006)
39. R.A. Alolayan, N. Ali Ahmed Elkanzi, H. Hrichi, and R. B. Bakr, Chem. Biodivers. (2023). https://doi.org/10.1002/cbdv.202201209
40. H. Beyzaei, M.K. Deljoo, R. Aryan, B. Ghasemi, M.M. Zahedi, M. Moghaddam-Manesh, Chem. Cent. J. **12**, 1 (2018)
41. H. Beyzaei, M. Moghaddam-Manesh, R. Aryan, B. Ghasemi, A. Samzadeh-Kermani, Chem. Pap. **71**, 1685 (2017)