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Design, synthesis and antimicrobial evaluation of pyrimidin-2-ol/thiol/amine analogues

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Abstract

Background: Pyrimidine is an aromatic heterocyclic moiety containing nitrogen atom at 1st and 3rd positions and play an important role to forms the central core for different necessity of biological active compounds, from this facts, we have designed and synthesized a new class of pyrimidin-2-ol/thiol/amine derivatives and screened for its in vitro antimicrobial activity.

Results and discussion: The synthesized pyrimidine derivatives were confirmed by IR, $^1\text{H}/^{13}\text{C}$ -NMR, Mass spectral studies and evaluated for their in vitro antimicrobial potential against Gram positive (*S. aureus* and *B. subtilis*), Gram negative (*E. coli*, *P. aeruginosa* and *S. enterica*) bacterial strains and fungal strain (*C. albicans* and *A. niger*) by tube dilution method and recorded minimum inhibitory concentration in $\mu\text{M}/\text{ml}$. The MBC and MFC values represent the lowest concentration of compound that produces in the range of 96–98% end point reduction of the used test bacterial and fungal species.

Conclusion: In general all synthesized derivatives exhibited good antimicrobial activity. Among them, compounds **2**, **5**, **10**, **11** and **12** have significant antimicrobial activity against used bacterial and fungal strains and also found to be more active than the standard drugs.

Keywords: Pyrimidine derivatives, Antibacterial activity, Antifungal activity

Background

Antimicrobial agents are one of the most important weapons in the resistance of infection caused by bacterial strains. In the past few years, increase the resistance of microorganisms toward antimicrobial agents become a serious health problem so there is a need of safe, potent and novel antimicrobial agents [1]. Pyrimidine aromatic heterocyclic moiety containing nitrogen atom at 1st and 3rd positions and play an important role to forms the central core for different necessity of biological active compounds [2]. Pyrimidine is the structural unit of DNA and RNA which play an imperative role in various existence progressions. Pyrimidines are present among the three isomeric diazines. Most abundant pyrimidine is uracil, cytosine and thymine [3]. These derivatives are

also known as *m*-diazine or 1,3-diazone can be regarded as cyclic amine and shows the various biological activities i.e. antiviral [4, 5]; anticancer [6]; antimicrobial [7]; anti-inflammatory [8]; analgesic [9]; antioxidant [10]; antimalarial [11].

Pyrimidine is used as parent substance for the synthesis of a wide variety of heterocyclic compounds and raw material for the synthesis of new molecule [12]. Pyrimidine ring complexes with different heterocyclic moiety found to be an essential part of natural products agrochemicals and veterinary products. A large measure of antimicrobial drugs such as ciprofloxacin, chloramphenicol, griseofulvin and nystatin are available for bacterial and fungal infections [13].

Recently, it was reported that *p*-methoxyphenyl group present on pyrimidine nucleus improved the antimicrobial activity of the pyrimidine derivative (**I**) [13], *p*-Chloro phenyl group present on pyrimidine nucleus

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[14] improved the anticancer activity of the pyrimidine derivatives (II), *p*-Methoxyphenyl group present on pyrimidine derivatives (III) improved the antioxidant [15], *p*-Methoxyphenyl group present on pyrimidine ring (IV) improved the antitubercular activity of the pyrimidine derivatives [16], *p*-Hydroxy group present on pyrimidine nucleus (V) improved the antimicrobial of the pyrimidine compound [10]. The electron releasing ($-OH$ and $-OCH_3$) and electron withdrawing ($-Cl$) groups are present on different position of pyrimidine nucleus (I, II, III, IV and V) enhanced the biological activity of the pyrimidine derivatives, from this facts we developed a design of reported biological active agents and proposed antimicrobial agent which is presented in Fig. 1. In light of abovementioned facts, we hereby report to design, synthesis and antimicrobial screening of 4-(substituted phenyl)-6-(4-nitrophenyl) pyrimidin-2-ol/thiol/amine derivatives (Scheme 1a, b).

Results and discussion

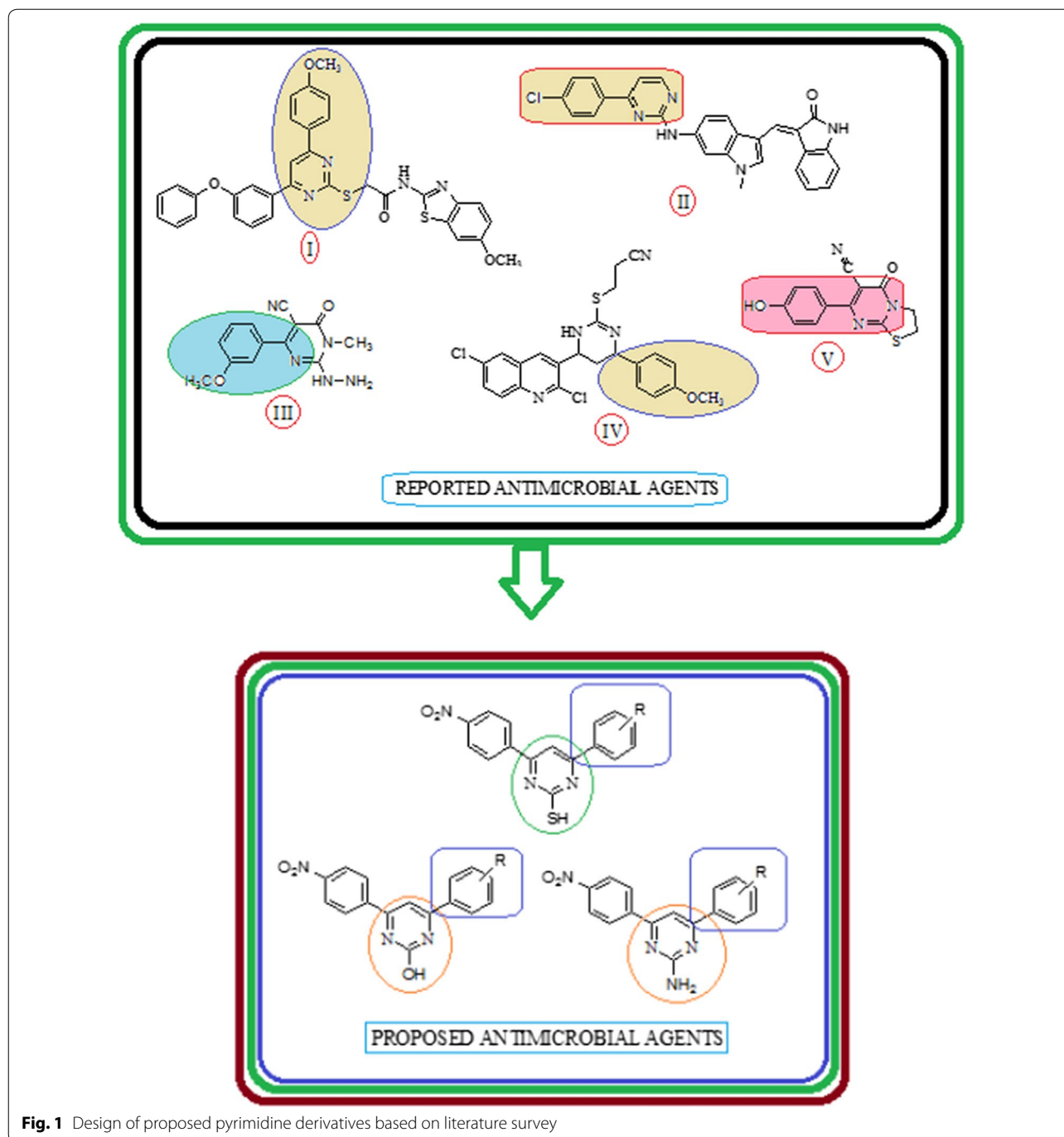
Chemistry

Synthesis of pyrimidine derivatives (1–13) followed the general procedure discussed in synthetic Scheme 1a, b. The reaction of substituted chalcone in the presence of guanidine hydrochloride/urea/thiourea in methanolic solvent resulted in the formation of the final compounds. The physicochemical properties of newly synthesized compounds are presented in Table 1. The molecular structures of the synthesized compounds (1–13) were confirmed by FT-IR (KBr pellets, cm^{-1}) and $^1H/^{13}C$ -NMR ($CDCl_3$, δ ppm) spectral and elemental studies. The appearance of IR absorption band at 1404 cm^{-1} in the spectral data of synthesized derivatives (1–13) displayed the presence of Ar-OH (C-O str. and O-H in plane bend. vib.) category on the aromatic ring. The IR absorption band in the scale of $645\text{--}623\text{ cm}^{-1}$ corresponds to the C-Br stretching of aromatic-bromo compounds (10 and 11). The existence of Ar- NO_2 group asymmetric Ar- NO_2 stretches in the scale of $1550\text{--}1510\text{ cm}^{-1}$. The existence of an arylalkyl ether category (Ar- OCH_3) in compounds 8 and 9 are established by the existence of an IR absorption band around $2842\text{--}2829\text{ cm}^{-1}$. Halogen group in compounds 1–7 and 12 is indicated by the existence of Ar-Cl stretching vibrations at $732\text{--}848\text{ cm}^{-1}$. The impression of IR stretching at $2602\text{--}2627$ and $623\text{--}709\text{ cm}^{-1}$ in the spectral data of synthesized compounds specified the existence of S-H and C-S group respectively. The appearance of IR stretching at $3379\text{--}3349\text{ cm}^{-1}$ spectral data of synthesized compounds specified the existence of $-NH_2$ group. The impression of IR stretching vibration at $3100\text{--}3000$ and $1580\text{--}1600\text{ cm}^{-1}$ in the spectral data of synthesized compounds specified the existence of C-H and C=C group respectively.

The appearance of IR stretching $1670\text{--}1709\text{ cm}^{-1}$ in the spectral data of all synthesized compounds specified the existence of C=N group. The multiplet signals between 6.33 and $8.34\text{ }\delta$ ppm in 1H -NMR spectra is indicative of aromatic proton of synthesized derivatives. The compounds 8 and 9 showed singlet at $3.01\text{--}3.34\text{ }\delta$ ppm due to the existence of OCH_3 of Ar- OCH_3 . All compounds showed singlet at $7.51\text{--}8.43$ and $6.85\text{--}841\text{ }\delta$ ppm due to the existence of N=CH and $-CH$ groups in pyrimidine ring respectively. Compound 13 showed singlet at $2.19\text{ }\delta$ ppm due to existence of $-N(CH_3)_2$ at the *para* position. Compounds, 1, 3, 5, 8, 11 and 12 showed singlet at $4.0\text{--}4.3\text{ }\delta$ ppm due to existence of $-NH_2$ at the *para* position and 2, 4, 6 and 10 showed singlet at $3.01\text{--}3.34\text{ }\delta$ ppm due to existence of $-SH$ group at the *para* position of the pyrimidine ring. The elemental screened studies of the 4-(substituted phenyl)-6-(4-nitrophenyl) pyrimidin-2-ol/thiol/amine were found within $\pm 0.39\%$ of the theoretical results.

In vitro antimicrobial activity

All the newly synthesized pyrimidine derivatives were examined for their in vitro antimicrobial activity against Gram positive *S. aureus* (MTCC 3160), *B. subtilis* (MTCC 441), Gram negative species: *E. coli* (MTCC 443), *P. aeruginosa* (MTCC 3542), *S. enteric* (MTCC 1165) and fungus species: *A. niger* (MTCC 281) and *C. albicans* (MTCC 227) strain using tube dilution method [17]. Dilutions of test and standard compounds were prepared in double strength nutrient broth for bacterial strains and sabouraud dextrose broth for fungal strains [18]. The minimum inhibitory concentration (MIC i.e. lowest concentration required of test substance to complete growth inhibition) values of standard drugs and synthesized compounds are presented in Table 2. From the results of antimicrobial evaluation it was observed that the entire synthesized compounds showed appreciable antimicrobial activity and different compounds were found to be active against different microorganisms. In case of Gram positive bacteria, compounds 12 ($MIC_{sa} = 0.87\text{ }\mu M/ml$) showed significant activity against *S. aureus* and 5 ($MIC_{bs} = 0.96\text{ }\mu M/ml$) exhibited most potent antibacterial activity against *B. subtilis*. In case of Gram negative bacteria, compounds 10 ($MIC_{se} = 1.55\text{ }\mu M/ml$) showed significant activity against *Salmonella enteric*, 2 ($MIC_{ec} = 0.91\text{ }\mu M/ml$) displayed more potent antibacterial activity against *E. coli* and 10 ($MIC_{pa} = 0.77\text{ }\mu M/ml$) exhibited most potent antibacterial activity against *P. aeruginosa*. Compound 12 ($MIC_{ca} = 1.73\text{ }\mu M/ml$) showed significant activity against *C. albicans* and 11 ($MIC_{an} = 1.68\text{ }\mu M/ml$) was found to be most potent antifungal agent against *A. niger*. All synthesized compounds having more antimicrobial potential than the standard



cefadroxil (antibacterial) and fluconazole (antifungal) drugs and these compounds may be used as lead for the further discovery of new antimicrobial agents.

Determination of MBC/MFC

After recorded the MIC results of the synthesized compounds in concentration of (50, 25, 12.5, 6.25, 3.125, 1.56) $\mu\text{M}/\text{ml}$ against microbial species i.e. Gram positive

bacteria (*S. aureus* and *B. subtilis*), Gram negative bacteria (*E. coli*, *P. aeruginosa* and *S. enterica*) and fungal strain (*C. albicans* and *A. niger*) then their minimum bactericidal concentration (MBC) and fungicidal concentration (MFC) were determined by petri dish method using nutrient agar media (antibacterial) and sabouraud dextrose agar media (antifungal) by subculturing 100 μl of culture from each test tube that remained clear in the

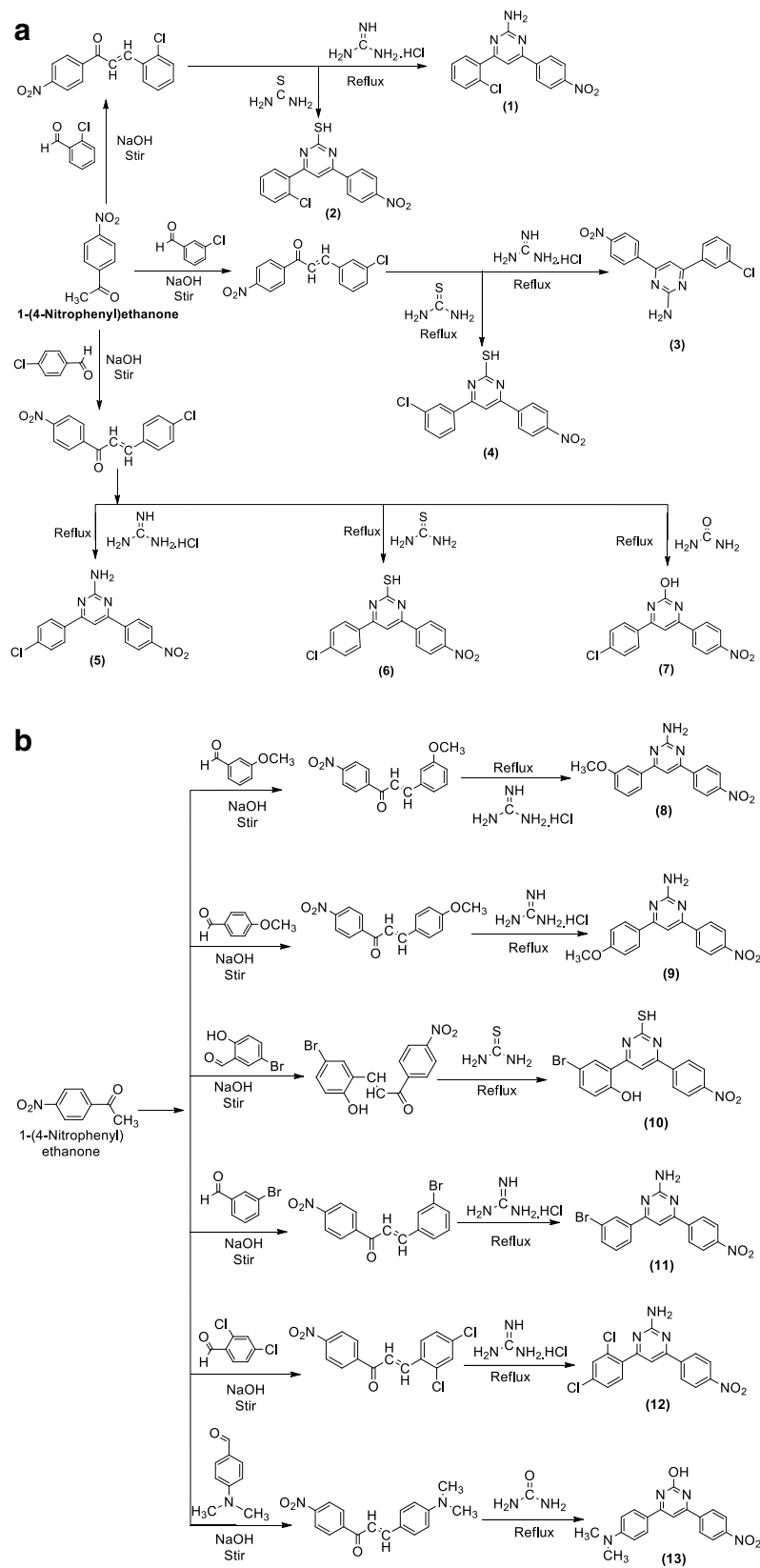


Table 1 The physicochemical properties of synthesized 4-(substituted phenyl)-6-(4-nitrophenyl) pyrimidin-2-ol/thiol/amine derivatives

Compounds	M. formula	M. weight	M.pt. (°C)	R _f value ^a	% Yield
Physicochemical properties					
1.	C ₁₆ H ₁₁ ClN ₄ O ₂	326	80–82	0.45	75.00
2.	C ₁₆ H ₁₀ ClN ₃ O ₂ S	343	61–63	0.57	84.72
3.	C ₁₆ H ₁₁ ClN ₄ O ₂	326	76–78	0.60	78.78
4.	C ₁₆ H ₁₀ ClN ₃ O ₂ S	343	90–92	0.62	72.54
5.	C ₁₆ H ₁₁ ClN ₄ O ₂	326	122–124	0.58	82.22
6.	C ₁₆ H ₁₀ ClN ₃ O ₂ S	343	63–65	0.56	75.00
7.	C ₁₆ H ₁₀ ClN ₃ O ₃	327	127–129	0.60	84.72
8.	C ₁₇ H ₁₄ N ₄ O ₃	322	66–68	0.51	73.43
9.	C ₁₇ H ₁₄ N ₄ O ₃	322	89–91	0.56	76.47
10.	C ₁₆ H ₁₀ BrN ₃ O ₃ S	404	59–61	0.61	81.81
11.	C ₁₆ H ₁₁ BrN ₄ O ₂	371	153–155	0.41	64.00
12.	C ₁₆ H ₁₀ ClN ₄ O ₂	361	87–89	0.42	87.61
13.	C ₁₈ H ₁₆ N ₄ O ₃	336	156–158	0.45	77.38

^a TLC mobile phase-benzene**Table 2 Antimicrobial activity (MIC = μM/ml) of synthesized 4-(substituted phenyl)-6-(4-nitrophenyl) pyrimidin-2-ol/thiol/amine derivatives**

Compounds no.	Minimum inhibitory concentration (MIC = μM/ml)						
	Bacterial strains					Fungal strains	
	Gram positive		Gram negative			<i>C. albicans</i> (MTCC 227)	<i>A. Niger</i> (MTCC 281)
	<i>S. aureus</i> (MTCC 3160)	<i>B. subtilis</i> (MTCC 441)	<i>E. coli</i> (MTCC 443)	<i>P. aeruginosae</i> (MTCC 3542)	<i>S. enteric</i> (MTCC 1165)		
1.	1.91	3.83	1.91	1.91	1.91	3.83	3.83
2.	1.82	3.64	0.91	1.82	1.82	1.82	3.64
3.	1.91	3.83	0.96	1.91	3.83	3.83	3.83
4.	3.64	3.64	1.82	0.91	3.64	3.64	3.64
5.	1.91	0.96	1.91	1.91	3.83	1.91	3.83
6.	1.82	3.64	1.82	1.82	3.64	1.82	3.64
7.	3.81	3.81	1.91	1.91	3.81	1.91	3.81
8.	3.88	3.88	1.94	3.88	3.88	1.94	3.88
9.	1.94	3.88	1.94	3.88	3.88	3.88	3.88
10.	3.09	1.55	1.55	0.77	1.55	3.09	3.09
11.	1.68	3.37	1.68	3.37	3.37	3.37	1.68
12.	0.87	1.73	1.73	1.73	1.73	1.73	3.46
13.	0.93	3.72	1.86	3.72	3.72	1.86	3.72
DMSO	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Cefadroxil	1.72	1.72	1.72	1.72	1.72	–	–
Fluconazole	–	–	–	–	–	2.04	2.04

MIC determination into fresh medium. The MBC and MFC values represent the lowest concentration of compound that produces in the range of 96–98% end point reduction of the used test bacterial and fungal species [19].

SAR (structure activity relationship) studies

From the antimicrobial testing results of synthesized 4-(substituted phenyl)-6-(4-nitrophenyl)pyrimidin-2-ol/thiol/amine derivatives, the subsequent structure activity relationship can be derived in Fig. 2.

- Presence of electron withdrawing group (–Cl, Compounds **2**, **5** and **12**) on benzylidene portion improved the antimicrobial activity of the synthesized compounds against *S. aureus*, *E. coli*, *B. subtilis* and *C. albicans*.
- Presence of electron withdrawing group (–Br, Compound **11**) improved the antifungal activity of the synthesized compounds against *A. niger*.
- Using 5-bromo-2-hydroxybenzaldehyde (Compound **10**) improved the antibacterial activity of the synthesized compounds against Gram negative *S. enterica* and *P. aeruginosa*.
- NO₂ group presence on benzylidene portion of acetophenone play an important role to enhanced the antimicrobial activity against bacterial and fungal microorganism.

Experimental section

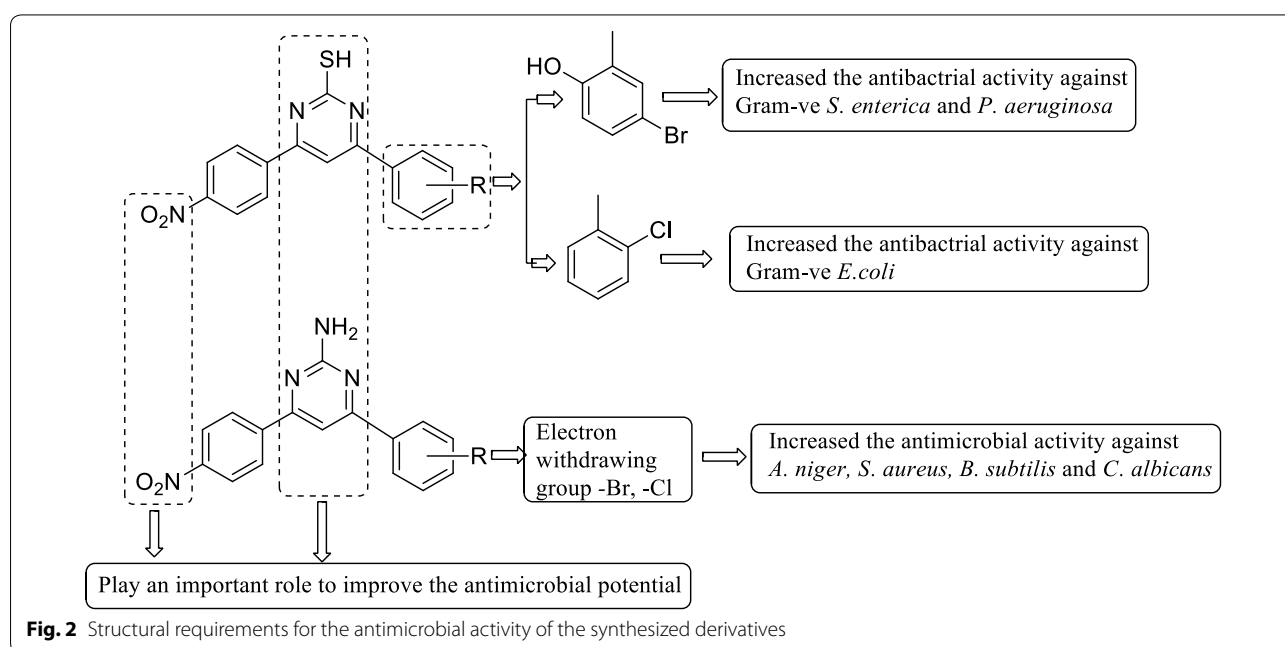
Starting materials were obtained from commercial sources and were used without any type of further purification. The completion of the chemical reaction was observed by thin layer chromatography (TLC) making use of silica gel G plates of 0.5 mm thickness as stationary phase and benzene as mobile phase for final compounds. Melting points of final compounds were determined by open capillary tubes method. The molecular structures of the compounds were characterized by ¹H/¹³C-NMR (CDCl₃, δ ppm), FT-IR and Mass spectral studies. The Mass spectral data were confirmed by Waters Micromass Q-ToF Micro instrument. ¹H nuclear magnetic resonance (¹H-NMR) spectra was recorded on Bruker Avance

400 MHz spectrometer in appropriate CDCl₃ solvents and are expressed in parts per million (δ, ppm) downfield from tetramethyl silane (internal standard). ¹H-NMR data are given as multiplicity (s, singlet; d, doublet; t, triplet; m, multiplet) and number of protons. Infrared (IR) spectra were recorded on Bruker 12060280, Software: OPUS 7.2.139.1294 spectrometer in the range of 400–4000 using KBr pellets and the value of λ max were reported in cm⁻¹.

General procedure for synthesized pyrimidine analogues

Step i: synthesis of substituted chalcone (intermediate-I) The reaction mixture of 1-(4-nitrophenyl)ethanone (0.01 mol) and corresponding aldehyde (0.01 mol) were stirred for 2–3 h in methanol (5–10 ml) followed by drop wise addition of sodium hydroxide solution (10 ml 40%) with constant stirring at room temperature. Then reaction mixture was taken overnight at room temperature and then was poured into ice cold water and acidified with hydrochloric acid and the precipitated substituted chalcone was filtered, dried and recrystallized from methanol [20].

Step ii: synthesis of 4-(substituted phenyl)-6-(4-nitrophenyl)pyrimidin-2-ol/thiol/amine derivatives The solution of substituted chalcone (0.01 mol) [synthesized in “Step i: synthesis of substituted chalcone (intermediate-I)”] in methanol (50 ml) was added with 0.01 mol of potassium hydroxide and 40 ml of 0.25 M solution of thiourea/urea/guanidine hydrochloride and refluxed for 3–4 h. The reaction mixture was then cooled and acidified with few drops



of hydrochloric acid (20 ml of 0.5 M solution) and the resultant precipitate 4-(substituted phenyl)-6-(4-nitrophenyl)pyrimidin-2-ol/thiol/amine was separated dried and recrystallized from methanol.

Spectral analysis determined by FT-IR (KBr pellets, cm^{-1}) and $^1\text{H}/^{13}\text{C}$ -NMR (CDCl_3 , δ ppm).

4-(2-Chlorophenyl)-6-(4-nitrophenyl)pyrimidin-2-amine (1) M. Formula: $\text{C}_{16}\text{H}_{11}\text{ClN}_4\text{O}_2$; Yield: 75.00%; MS ES + (ToF): m/z 326 [$\text{M}^+ + 1$]; IR (KBr pellets, cm^{-1}): 2931 (C–H str.), 1596 (C=C str.), 700 (C–C str.), 1688 (C=N str. or N=CH str., pyrimidine ring), 1344 (C–N str., pyrimidine), 754 (C–Cl str.), 1521 (NO_2 asym str.), 854 (C–N str., Ar– NO_2), 3379 (NH_2 asym str.); ^{13}C -NMR (CDCl_3 - d_6 , δ , ppm): 163.4, 163.6, 160.1, 148.3, 139.8, 132.4, 130.1, 129.2, 128.3, 127.4, 121.7, 95.2; ^1H -NMR (CDCl_3 , δ , ppm): 7.13–8.25 (m, 8H, Ar–H), 6.71 (s, 1H, CH of pyrimidine ring), 4.2 (s, 2H, NH_2).

4-(2-Chlorophenyl)-6-(4-nitrophenyl)pyrimidine-2-thiol (2) M. Formula: $\text{C}_{16}\text{H}_{10}\text{ClN}_3\text{O}_2\text{S}$; Yield: 84.72%; MS ES + (ToF): m/z 343 [$\text{M}^+ + 1$]; IR (KBr pellets, cm^{-1}): 2858 (C–H str.), 1596 (C=C str.), 703 (C–C str.), 1665 (C=N str.), 1342 (C–N str., pyrimidine), 753 (C–Cl str.), 1521 (NO_2 asym str.), 698 (C–N str., Ar– NO_2), 2627 (S–H str.), 621 (C–S str.); ^{13}C -NMR (CDCl_3 - d_6 , δ , ppm): 182.4, 163.5, 163.2, 160.1, 147.3, 139.6, 132.2, 130.1, 129.6, 128.3, 127.4, 121.7, 106.1; ^1H -NMR (CDCl_3 , δ , ppm): 7.35–8.34 (m, 8H, Ar–H), 8.40 (s, 1H, CH of pyrimidine ring), 3.01 (s, 1H, SH).

4-(3-Chlorophenyl)-6-(4-nitrophenyl)pyrimidin-2-amine (3) M. Formula: $\text{C}_{16}\text{H}_{11}\text{ClN}_4\text{O}_2$; Yield: 78.78%; MS ES + (ToF): m/z 326 [$\text{M}^+ + 1$]; IR (KBr pellets, cm^{-1}): 2923 (C–H str.), 1607 (C=C str.), 703 (C–C str.), 1670 (C=N str.), 1351 (C–N str., pyrimidine), 732 (C–Cl str.), 1525 (NO_2 asym str., phenyl ring), 674 (C–N str., Ar– NO_2), 3387 (NH_2 asym str.); ^{13}C -NMR (CDCl_3 - d_6 , δ , ppm): 163.2, 160.1, 147.2, 138.6, 132.0, 134.3, 130.1, 129.2, 128.1, 127.4, 125.3, 121.7, 95.3; ^1H -NMR (CDCl_3 , δ , ppm): 7.26–9.02 (m, 8H, Ar–H), 6.0 (s, 1H, CH of pyrimidine ring), 4.3 (s, 2H, NH_2).

4-(3-Chlorophenyl)-6-(4-nitrophenyl)pyrimidine-2-thiol (4) M. Formula: $\text{C}_{16}\text{H}_{10}\text{ClN}_3\text{O}_2\text{S}$; Yield: 72.54%; MS ES + (ToF): m/z 343 [$\text{M}^+ + 1$]; IR (KBr pellets, cm^{-1}): 2991 (C–H str.), 1570 (C=C str.), 709 (C–C str.), 1701 (C=N str. pyrimidine ring), 1303 (C–N str.), 748 (C–Cl str.), 1521 (NO_2 asym str.), 659 (C–N str., Ar– NO_2), 2597 (S–H str.), 709 (C–S str.); ^{13}C -NMR (CDCl_3 - d_6 , δ , ppm): 181.4, 163.5, 163.2, 160.1, 146.3, 139.6, 132.2, 130.1, 129.6, 128.3, 127.4, 125.3, 121.7, 103.1; ^1H -NMR (CDCl_3 , δ , ppm): 7.83–8.25 (m, 8H, Ar–H), 7.41 (s, 1H, CH of pyrimidine ring), 3.06 (s, 1H, SH).

4-(4-Chlorophenyl)-6-(4-nitrophenyl)pyrimidin-2-amine (5) M. Formula: $\text{C}_{16}\text{H}_{11}\text{ClN}_4\text{O}_2$; Yield: 82.22%; MS ES + (ToF): m/z 326 [$\text{M}^+ + 1$]; IR (KBr pellets, cm^{-1}): 2942 (C–H str.), 1598 (C=C str.), 703 (C–C str.), 1673 (C=N str.), 1346 (C–N str., pyrimidine), 755 (C–Cl str.), 1523 (NO_2 asym str.), 822 (C–N str., Ar– NO_2), 3349 (NH_2 asym str.); ^{13}C -NMR (CDCl_3 - d_6 , δ , ppm): 162.2, 160.1, 146.2, 138.6, 131.0, 134.3, 130.1, 129.2, 128.1, 127.4, 124.3, 121.7, 96.3; ^1H -NMR (CDCl_3 , δ , ppm): 7.33–8.34 (m, 8H, Ar–H), 7.85 (s, 1H, CH of pyrimidine ring), 4.14 (s, 2H, NH_2).

4-(4-Chlorophenyl)-6-(4-nitrophenyl)pyrimidine-2-thiol (6) M. Formula: $\text{C}_{16}\text{H}_{10}\text{ClN}_3\text{O}_2\text{S}$; Yield: 75.00%; MS ES + (ToF): m/z 343 [$\text{M}^+ + 1$]; IR (KBr pellets, cm^{-1}): 2927 (C–H str.), 1596 (C=C str.), 709 (C–C str.), 1345 (C–N str., pyrimidine), 824 (C–Cl str.), 1480 (NO_2 asym str.), 698 (C–N str., Ar– NO_2), 645 (C–S str.), 2602 (S–H str.); ^{13}C -NMR (CDCl_3 - d_6 , δ , ppm): 182.4, 163.2, 163.2, 161.1, 148.3, 139.6, 131.2, 130.1, 129.6, 128.3, 126.4, 121.7, 103.4; ^1H -NMR (CDCl_3 , δ , ppm): 7.83–8.25 (m, 8H, Ar–H), 7.45 (s, 1H, CH of pyrimidine ring), 3.34 (s, 1H, SH).

4-(4-Chlorophenyl)-6-(4-nitrophenyl)pyrimidin-2-ol (7) M. Formula: $\text{C}_{16}\text{H}_{10}\text{ClN}_3\text{O}_3$; Yield: 84.72%; MS ES + (ToF): m/z 327 [$\text{M}^+ + 1$]; IR (KBr pellets, cm^{-1}): 2941 (C–H str.), 1595 (C=C str.), 705 (C–C str.), 1672 (C=N str.), 1342 (C–N str., pyrimidine), 756 (C–Cl str.), 1523 (NO_2 asym str.), 3374 (O–H str.), 822 (C–N str., Ar– NO_2), 1404 (C–O str., and O–H in plane bending vib.); ^{13}C -NMR (CDCl_3 - d_6 , δ , ppm): 160.4, 160.4, 153.2, 148.2, 139.1, 134.1, 131.1, 129.2, 128.2, 121.2, 88.1; ^1H -NMR (CDCl_3 , δ , ppm): 7.43–8.56 (m, 8H, Ar–H), 6.61 (s, 1H, CH of pyrimidine ring), 5.04 (s, 1H, OH).

4-(3-Methoxyphenyl)-6-(4-nitrophenyl)pyrimidin-2-amine (8) M. Formula: $\text{C}_{17}\text{H}_{14}\text{N}_4\text{O}_3$; Yield: 73.43%; MS ES + (ToF): m/z 322 [$\text{M}^+ + 1$]; IR (KBr pellets, cm^{-1}): 2947 (C–H str.), 692 (C–C str.), 1709 (C=N str. pyrimidine ring), 1344 (C–N str., pyrimidine), 784 (C–N str., Ar– NO_2), 1041 (C–O–C str., $-\text{OCH}_3$), 2839 (C–H str., R– CH_3); ^{13}C -NMR (CDCl_3 - d_6 , δ , ppm): 163.2, 160.1, 146.2, 139.6, 131.0, 134.3, 130.1, 128.1, 121.7, 119.3, 114.3, 111.3, 96.3, 55.2; ^1H -NMR (CDCl_3 , δ , ppm): 6.33–8.44 (m, 8H, Ar–H), 6.85 (s, 1H, CH of pyrimidine ring), 4.2 (s, 2H, NH_2), 3.34 (s, 1H, OCH_3).

4-(4-Methoxyphenyl)-6-(4-nitrophenyl)pyrimidin-2-amine (9) M. Formula: $\text{C}_{17}\text{H}_{14}\text{N}_4\text{O}_3$; Yield: 76.47%; MS ES + (ToF): m/z 322 [$\text{M}^+ + 1$]; IR (KBr pellets, cm^{-1}): 2937 (C–H str.), 1604 (C=C str.), 694 (C–C str.), 1661 (C=N str.), 1349 (C–N str., pyrimidine), 1502 (NO_2 asym str., phenyl ring), 752 (C–N str., Ar– NO_2),

1108 (C–O–C str., –OCH₃), 2842 (C–H str., R–CH₃); ¹³C-NMR (CDCl₃-d₆, δ, ppm): 163.1, 160.1, 148.2, 139.6, 128.1, 125.3, 121.7, 114.3, 95.3; ¹H-NMR (CDCl₃, δ, ppm): 6.33–8.71 (m, 8H, Ar–H), 6.35 (s, 1H, CH of pyrimidine ring), 4.23 (s, 2H, NH₂), 3.01 (s, 1H, OCH₃).

4-Bromo-2-(2-mercapto-6-(4-nitrophenyl)pyrimidin-4-yl)phenol (10) M. Formula: C₁₆H₁₀BrN₃O₃S; Yield: 81.81%; MS ES + (ToF): *m/z* 404 [M⁺ + 1]; IR (KBr pellets, cm⁻¹): 2869 (C–H str.), 1592 (C=C str.), 691 (C–C str.), 1680 (C=N str.), 1349 (C–N str., pyrimidine), 623 (C–Br str.), 1521 (NO₂ asym str., phenyl ring), 844 (C–N str., Ar–NO₂), 2597 (S–H str.), 623 (C–S str.); ¹³C-NMR (CDCl₃-d₆, δ, ppm): 182.4, 163.2, 161.1, 154.3, 148.3, 139.6, 134.2, 133.1, 128.3, 121.2, 122.4, 115.2, 118.2, 103.4; ¹H-NMR (CDCl₃, δ, ppm): 7.93–8.35 (m, 7H, Ar–H), 8.41 (s, 1H, CH of pyrimidine ring), 3.05 (s, 1H, SH), 5.97 (s, 1H, OH).

4-(3-Bromophenyl)-6-(4-nitrophenyl)pyrimidin-2-amine (11) M. Formula: C₁₆H₁₁BrN₄O₂; Yield: 64.00%; MS ES + (ToF): *m/z* 371 [M⁺ + 1]; IR (KBr pellets, cm⁻¹): 3064 (C–H str.), 1596 (C=C str.), 692 (C–C str.), 1671 (C=N str.), 1342 (C–N str., pyrimidine), 1500 (NO₂ asym str., phenyl ring), 783 (C–N str., Ar–NO₂), 645 (C–Br str.); ¹³C-NMR (CDCl₃-d₆, δ, ppm): 163.2, 160.1, 148.2, 139.6, 131.0, 134.3, 130.1, 129.2, 128.1, 126.3, 121.7, 95.3; ¹H-NMR (CDCl₃, δ, ppm): 6.11–8.41 (m, 8H, Ar–H), 7.35 (s, 1H, CH of pyrimidine ring), 4.00 (s, 2H, NH₂).

4-(2,4-Dichlorophenyl)-6-(4-nitrophenyl)pyrimidin-2-amine (12) M. Formula: C₁₆H₁₀ClN₄O₂; Yield: 87.61%; MS ES + (ToF): *m/z* 361 [M⁺ + 1]; IR (KBr pellets, cm⁻¹): 1600 (C=C str.), 695 (C–C str.), 1669 (C=N str.), 1346 (C–N str., pyrimidine), 848 (C–Cl str.), 1415 (NO₂ asym str., phenyl ring), 735 (C–N str., Ar–NO₂); ¹H-NMR (CDCl₃, δ, ppm): 6.34–8.67 (m, 7H, Ar–H), 6.15 (s, 1H, CH of pyrimidine ring), 4.30 (s, 2H, NH₂); ¹³C-NMR (CDCl₃-d₆, δ, ppm): 163.6, 160.1, 147.2, 139.4, 133.3, 135.1, 129.2, 128.1, 127.3, 121.7, 95.6.

4-(4-(Dimethylamino)phenyl)-6-(4-nitrophenyl)pyrimidin-2-ol (13) M. Formula: C₁₈H₁₆N₄O₃; Yield: 77.38%; MS ES + (ToF): *m/z* 336 [M⁺ + 1]; IR (KBr pellets, cm⁻¹): 2923 (C–H str.), 1524 (C=C str.), 704 (C–C str.), 1670 (C=N str. or N=CH str., pyrimidine ring), 1348 (C–N str., phenyl ring), 733 (NO₂ asym str., phenyl ring), 806 (C–N str., Ar. nitro group), 2858 (C–H str., R–CH₃), 3393 (O–H str.); ¹³C-NMR (CDCl₃-d₆, δ, ppm): 160.5, 154.3, 149.2, 139.6, 128.1, 122.7, 121.3, 114.3, 87.2, 41.1; ¹H-NMR (CDCl₃, δ, ppm): 6.11–8.26 (m, 8H, Ar–H), 6.75 (s, 1H, CH of pyrimidine ring), 5.30 (s, 1H, OH), 2.19 (s, 6H, (CH₃)₂).

Conclusion

Summarizing, we may conclude that the synthesized compounds (**2**, **5**, **10**, **11** and **12**) displayed appreciable antibacterial and antifungal activities against Gram positive bacteria (*S. aureus* and *B. subtilis*), Gram negative bacteria (*E. coli*, *S. enterica* and *P. aeruginosa*) and fungal strains (*C. albicans* and *A. niger*). The electron withdrawing group's play an important role to enhanced the antimicrobial potential of compounds **2**, **5**, **11** and **12** and these compound more active than standard drugs cefadroxil and fluconazole. The MBC and MFC values represent the lowest concentration of compound that produces in the range of 96–98% end point reduction of the used test bacterial and fungal species.

Authors' contributions

PKV designed and finalized the scheme; SN performed research work and SK analyzed the spectral and biological data and wrote the paper. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

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