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Original article

Synthesis of novel 6-phenyl-2,4-disubstituted pyrimidine-5-carbonitriles as potential antimicrobial agents

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ABSTRACT

New series of 6-phenyl-2,4-disubstituted pyrimidine-5-carbonitriles namely, 2-substitued thio-6-phenyl-3,4-dihydro-4-oxopyrimidine-5-carbonitriles (**5a**–**d**, **6**, **7a**–**d**, **8**), 2-(4-chlorobenzylthio)-4-chloro-6-phenylpyrimidine-5-carbonitrile (**9**), 2-(4-chlorobenzylthio)-4-arylthio-6-phenylpyrimidine-5-carbonitriles (**10a**–**d**) and 2-(4-chlorobenzylthio)-4-arylamino-6-phenylpyrimidine-5-carbonitriles (**11a**–**d**) was synthesized and tested for *in vitro* activities against a panel of Gram-positive and Gram-negative bacteria and the yeast-like pathogenic fungus *Candida albicans*. Compounds **5b**, **5c**, **6**, **7a**, **7b**, **7c**, **9** and **11a** displayed marked antibacterial activity particularly against the tested Gram-positive bacteria, while compounds **6**, **7c**, **7d** and **9** were moderately or weakly active against *C. albicans*.

1. Introduction

Pyrimidines occupy a distinct and unique place in medicine. The chemotherapeutic efficacy of pyrimidine derivatives is related to their ability to inhibit vital enzymes responsible for DNA biosynthesis as dihydrofolate reductase (DHFR), thymidylate synthetase (TSase), thymidine phosphorylase (TPase) and reverse transcriptase (RTase). Large array of pyrimidine non-nucleoside derivatives possess a variety of pharmacological properties. These properties include anticancer [1–5], antiviral [6–14], antibacterial [15–18], antifungal [19–22], antiprotozoal [23–29], antihypertensive [30–32], antihistaminic [33,34], anti-inflammatory [35–37] and central nervous activities [38–41]. Moreover, several pyrimidine carbonitrile derivatives were reported to possess antiviral and antimicrobial activities [42–45].

In continuation to our interest in the chemical and pharmacological properties of pyrimidine derivatives [46–50], we report herein the synthesis of new series of 6-phenyl-2,4-disubstituted pyrimidine-5-carbonitriles and related derivatives as potential antimicrobial agents.

2. Results and discussion

2.1. Chemistry

6-Phenyl-2-thiouracil-5-carbonitrile **4** was prepared *via* prolonged heating of benzaldehyde **1**, ethyl cyanoacetate **2** and thiourea **3** in ethanol, in the presence of potassium carbonate [51]. Compound **4** was allowed to react with the appropriate benzyl- or 4-substituted benzyl chloride in the presence of potassium carbonate, in DMF at room temperature for 12 h to yield the target derivatives **5a**–**d** in 77–94% yields. The reaction of compound **4** with 2-bromomethyl-5-nitrofuran in acetone under the same conditions yielded the 2-(5-nitrofuran-2-ylmethylthio) analogue **6** in 52% yield. Similarly, the 2-(2-alkyloxy- or alkylthioethylthio) and the 2-(benzyloxymethylthio) derivatives **7a-d** and **8** were obtained in relatively lower yields *via* the reaction of compound **4** with the appropriate 2-chloroethyl alkyl ether, 2-chloroethyl alkyl sulphide or benzyl chloromethyl ether (Scheme 1, Table 1).

The reaction of compound **5c** with phosphorus oxychloride and *N,N*-dimethylaniline yielded the 4-chloropyrimidine derivative **9** in 62% yield. The pronounced reactivity of 4-halopyrimidines towards nucleophilic reagents is utilized for the synthesis of several 2-(4-chlorobenzylthio)-4-arylthio-6-alkylpyrimidine-5-carbonitriles. Thus, attempted reaction of the 4-chloropyrimidines **9** with thiophenol or thiocresols in ethanol, in the presence of potassium

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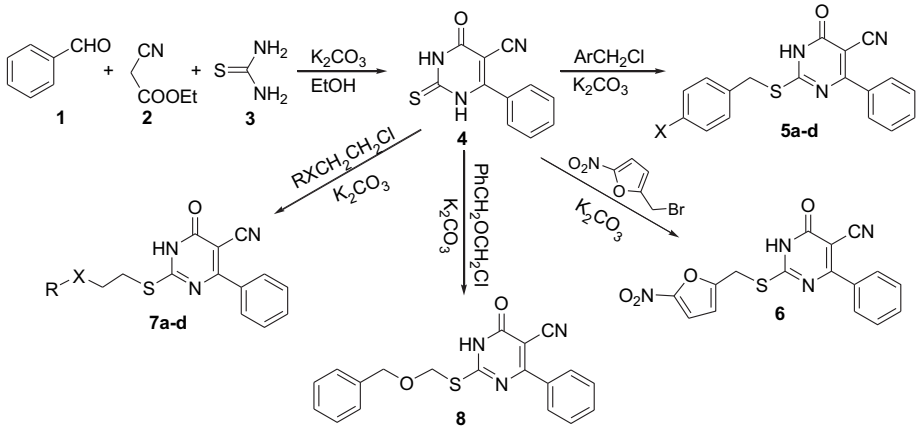


Scheme 1. Synthesis of compounds **5a**–**d**, **6**, **7a**,**b**, and **8**.

carbonate, *via* prolonged heating for up to 24 h to yield the corresponding 4-arylthio derivatives **10a**–**d** was unsuccessful, and the starting materials were recovered unchanged. On the other hand, carrying out the reaction in pyridine by heating for 4 h yielded the target compounds in good yields. On the other hand, the more nucleophilic arylamines were smoothly reacted with compound **9** in boiling ethanol, in the presence of potassium carbonate as hydrogen chloride acceptor to yield the corresponding 4-arylamino derivatives **11a**–**d** in 50–66% yields (Scheme 2, Table 1). The structures of all the newly synthesized compounds were confirmed by elemental analyses in addition to the IR, ^{1}H NMR, ^{13}C NMR, and ESI-MS spectral data, which were in full agreement with their structures.

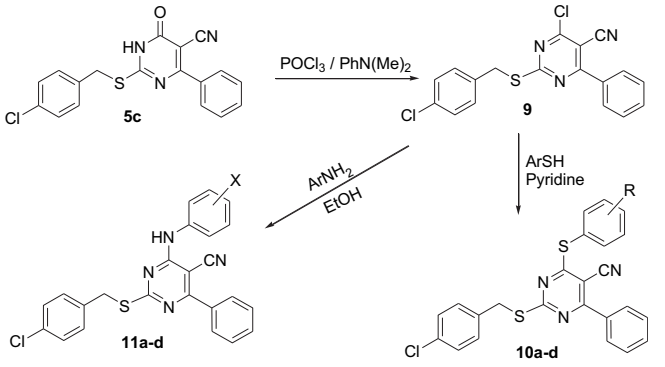
2.2. In vitro antimicrobial activity

The newly synthesized compounds **5a**–**d**, **6**, **7a**–**d**, **8**, **9**, **10a**–**d** and **11a**–**d** were tested for their *in vitro* growth inhibitory activity against the standard strains of the Institute of fermentation of Osaka (IFO) namely; *Staphylococcus aureus* IFO 3060, *Bacillus subtilis* IFO 3007, *Micrococcus luteus* IFO 3232 (Gram-positive bacteria), *Escherichia coli* IFO 3301, *Pseudomonas aeuroginosa* IFO 3448 (Gram-negative bacteria), and the yeast-like pathogenic fungus *Candida albicans* IFO 0583. The primary screening was carried out using the agar disc-diffusion method using Müller-Hinton agar medium [52].

The results of the preliminary antimicrobial testing of compounds **5a**–**d**, **6**, **7a**–**d**, **8**, **9**, **10a**–**d** and **11a**–**d** (200 μg/disc), the antibacterial antibiotics Ampicillin trihydrate, Gentamicin (100 μg/disc) and the antifungal drug Clotrimazole (100 μg/disc) are shown in Table 2. The results revealed that the compounds showed varying degrees of inhibition against the tested microorganisms. In general, strong activity was displayed by the compounds **5b**, **5c**, **6**, **7a**, **7b**, **7c**, **9**, **11a**, which produced growth inhibition zones ≥18 mm against one or more of the tested microorganisms. Meanwhile, compounds **5a**, **5d**, **7d**, **8**, **10a**, **10d**, **11c** and **11d** showed moderate activity (growth inhibition zones 14–17 mm), compound **11b** exhibited weak activity (growth inhibition zones 10–13 mm) and compounds **10b** and **10c** were practically inactive against the tested microorganisms. The Gram-positive bacteria *B. subtilis* and *S. aureus* and to a lesser extent *M. luteus* are considered the most sensitive among the tested microorganisms. The tested compounds were generally inactive against the Gram-negative bacteria, only three compounds (**5b**, **6** and **7c**) showed strong activity against *E. coli* and *P. aeuroginosa*. The inhibitory activity of the compounds against *C. albicans* was rather lower than their antibacterial activity, only compounds **6**, **7c**, **7d** and **9** displayed moderate or weak activity. The minimal inhibitory concentrations (MIC) [53] for the most active compounds **5b**, **5c**, **6**, **7a**, **7b**, **7c**, **9** and **11a**, which are shown in Table 3, were in accordance with the results obtained in the primary screening.

Table 1
Melting points, crystallization solvents, yield percentages, molecular formulae, molecular weights and of compounds **5a**–**d**, **6**, **7a**–**d**, **8**, **9**, **10a**–**d** and **11a**–**d**.

Comp. No.	R	X	Cryst. Solvent	M.p. (°C)	Yield (%)	Mol. Formula (Mol. Wt.)
5a	–	H	EtOH	205–7	81	$C_{18}H_{13}N_3OS$ (319.38)
5b	–	F	EtOH	225–7	77	$C_{18}H_{12}FN_3OS$ (337.37)
5c	–	Cl	EtOH	242–4	92	$C_{18}H_{12}ClN_3OS$ (353.83)
5d	–	NO_2	EtOH	266–8	94	$C_{18}H_{12}N_4O_3S$ (364.38)
6	–	–	EtOH/H_2O	201–3	52	$C_{16}H_{10}N_4O_4S$ (354.34)
7a	CH_3	O	EtOH/H_2O	183–5	45	$C_{14}H_{13}N_3O_2S$ (287.34)
7b	C_2H_5	O	EtOH	161–3	42	$C_{15}H_{15}N_3O_2S$ (301.36)
7c	CH_3	S	EtOH	193–5	38	$C_{14}H_{13}N_3OS_2$ (303.40)
7d	C_2H_5	S	EtOH	188–90	46	$C_{15}H_{15}N_3OS_2$ (317.43)
8	–	–	EtOH/H_2O	127–9	59	$C_{19}H_{15}N_3O_2S$ (349.41)
9	–	–	*n*-Hexane	66–8	62	$C_{18}H_{11}Cl_2N_3S$ (372.27)
10a	H	–	EtOH	152–4	69	$C_{24}H_{16}ClN_3S_2$ (445.99)
10b	2-CH_3	–	EtOH	134–6	62	$C_{25}H_{18}ClN_3S_2$ (460.01)
10c	3-CH_3	–	EtOH	150–2	65	$C_{25}H_{18}ClN_3S_2$ (460.01)
10d	4-CH_3	–	EtOH	174–6	77	$C_{25}H_{18}ClN_3S_2$ (460.01)
11a	–	4-F	EtOH/H_2O	188–90	58	$C_{24}H_{16}ClFN_4S$ (446.93)
11b	–	2-CF_3	EtOH/H_2O	162–4	50	$C_{25}H_{16}ClF_3N_4S$ (496.93)
11c	–	3-CF_3	EtOH	145–7	61	$C_{25}H_{16}ClF_3N_4S$ (496.93)
11d	–	4-CF_3	EtOH	195–7	66	$C_{25}H_{16}ClF_3N_4S$ (496.93)



Scheme 2. Synthesis of compounds **9**, **10a**–**d** and **11a**–**d**.

According to the results of the antimicrobial activity, it would be conclude that the 2-(substituted thio) substituents in the 6-phenyl-3,4-dihydro-4-oxopyrimidine-5-carbonitriles derivatives **5a**–**d**, **6**, **7a**–**d** and **8**, which are generally active, greatly influenced the antibacterial activity. In the benzyl series **5a**–**d**, the activity of the 4-fluoro- and chlorobenzyl derivatives **5b** and **5c** was superior to the benzyl and 4-nitrobenzyl analogues **5a** and **5d**. In addition, the activity of the 2-alkoxyethylthio derivatives **7a** and **7b** were higher than their alkythioethylthio analogues **7c** and **7d**, which possessed higher activity against the tested Gram-negative bacteria, beside moderate activity against *C. albicans*. The 2-(5-nitrofuran-2-ylmethylthio) substituent (compound **6**) was optimal for antimicrobial activity, while the 2-benzyloxymethylthio substituent (compound **8**) diminished the antimicrobial activity. The antimicrobial activity of the 2-(4-chlorobenzylthio)-4-substituted 6-phenylpyrimidine-5-carbonitriles derivatives **9**, **10a**–**d** and **11a**–**d** derivatives was greatly influenced by the nature of the 4-substituents, the optimal activity was attained in the 4-chloro analogue **9** which possessed potent and broad spectrum activity.

Table 2
Antimicrobial activity of compounds **5a**–**d**, **6**, **7a**–**d**, **8**, **10a**–**d** and **11a**–**d** (200 μg/8 mm disc), the broad spectrum antibacterial drugs Gentamicin (100 μg/8 mm disc), Ampicillin (100 μg/8 mm disc) and the antifungal drug Clotrimazole (100 μg/8 mm disc) against *Staphylococcus aureus* IFO 3060 (*SA*), *Bacillus subtilis* IFO 3007 (*BS*), *Micrococcus luteus* IFO 3232 (*ML*), *Escherichia coli* IFO 3301 (*EC*), *Pseudomonas aeuroginosa* IFO 3448 (*PA*), and *Candida albicans* IFO 0583 (*CA*).

Comp. No.	Diameter of Growth Inhibition Zone (mm)[a]					
	SA	*BS*	*ML*	*EC*	*PA*	*CA*
5a	16	17	16	–	–	–
5b	22	26	21	18	14	–
5c	20	19	19	14	12	–
5d	14	17	13	12	–	–
6	33	28	24	22	21	11
7a	18	22	17	12	10	–
7b	21	24	17	14	11	–
7c	16	18	15	21	18	14
7d	12	16	12	16	14	12
8	14	16	13	–	–	–
9	24	26	18	16	14	12
10a	12	14	12	–	–	–
10b	–	–	–	–	–	–
10c	–	–	–	–	–	–
10d	14	13	11	–	–	–
11a	16	18	19	–	–	–
11b	12	12	10	–	–	–
11c	14	14	11	–	–	–
11d	15	17	13	–	–	–
Gentamicin	26	25	18	20	19	NT
Ampicillin	23	21	19	17	17	NT
Clotrimazole	NT	NT	NT	NT	NT	21

[a] (-): Inactive (inhibition zone < 10 mm). (NT): Not tested.

Table 3
The minimal inhibitory concentrations (MIC, μg/ml) of compounds **5b**, **5c**, **6**, **7a**, **7b**, **7c**, **9**, **11a**, the broad spectrum antibacterial drugs Gentamicin and Ampicillin against *Staphylococcus aureus* IFO 3060 (*SA*), *Bacillus subtilis* IFO 3007 (*BS*), *Micrococcus luteus* IFO 3232 (*ML*), *Escherichia coli* IFO 3301 (*EC*) and *Pseudomonas aeuroginosa* IFO 3448 (*PA*).

Comp. No.	Minimal Inhibitory Concentration (MIC, μg/ml)[a]				
	SA	*BS*	*ML*	*EC*	*PA*
5b	4	2	4	8	ND
5c	8	8	8	ND	ND
6	0.5	1	1	2	4
7a	8	2	ND	ND	ND
7b	4	4	ND	ND	ND
7c	ND	8	ND	ND	ND
11a	2	1	4	ND	ND
Gentamicin	2	2	2	0.5	1
Ampicillin	2	0.5	2	2	2

[a] ND: Not determined.

The replacement of the 4-chloro substituent with an arylthio group dramatically diminished the antimicrobial activity (compounds **10a**–**d**). On the other hand, the activity is partially retained against the tested Gram-positive bacteria in the 4-arylamino derivatives **11a**–**d**.

3. Conclusion

In this study, new series of 6-phenyl-2,4-disubstituted pyrimidine-5-carbonitriles were synthesized and their antimicrobial activity against a panel of Gram-positive and Gram-negative bacteria and the yeast-like pathogenic fungus *C. albicans*. Several newly synthesized derivatives displayed promising antibacterial activity compared to known antibacterial drugs. Though, the mechanism of the antibacterial activity needs further investigations, which are in progress.

4. Experimental protocols

Melting points (°C) were measured in open glass capillaries using a Branstead 9001 electrothermal melting point apparatus and are uncorrected. NMR spectra were obtained on a Bruker AC 500 Ultra Shield NMR spectrometer (Fällanden, Switzerland) operating at 500.13 MHz for ^{1}H and 125.76 MHz for ^{13}C, the chemical shifts are expressed in δ (ppm) downfield from tetramethylsilane (TMS) as internal standard; coupling constants (J) are expressed in Hz. Electrospray ionization mass spectra (ESI-MS) were recorded an Agilent 6410 Triple Quad tandem mass spectrometer at 4.0 and 3.5 kV for positive and negative ions, respectively. Elemental analyses (C, H, N, S) were in full agreement with the proposed structures within ±0.4% of the theoretical values. Monitoring the reactions and checking the purity of the final products were carried out by thin layer chromatography (TLC) using silica gel precoated aluminium sheets (60 F_{254}, Merck) and visualization with ultraviolet light (UV) at 365 and 254 nm. The bacterial strains and *C. albicans* fungus were obtained from the Institute of Fermentation of Osaka (IFO), Osaka, Japan. The reference drugs Gentamicin sulphate (CAS 1405-41-0), Ampicillin trihydrate (CAS 7177-48-2) and Clotrimazole (CAS 23593-75-1) were obtained from Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany.

*4.1. 2-(Benzyl- or 4-substituted benzylthio)-6-phenyl-3,4-dihydro-4-oxopyrimidine-5-carbonitriles (**5a**–**d**)*

To a solution of compound **4** (2.13 g, 0.01 mol) in DMF (10 mL), the appropriate arylmethyl chloride (0.01 mol) and anhydrous potassium carbonate (1.38 g, 0.01 mol) were added and the mixture

was stirred at room temperature for 12 h. Water (15 mL) was added and the mixture was stirred for further 30 min. The separated solid was filtered, washed with cold water, dried and crystallized from ethanol. **5a**: ^{1}H NMR (DMSO-d$_6$): δ 4.55 (s, 2H, CH_2), 7.26–7.34 (m, 3H, Ar–H), 7.42 (d, 2H, Ar–H, J = 7.0 Hz), 7.57–7.64 (m, 3H, Ar–H), 7.94 (d, 2H, Ar–H, J = 7.0 Hz), 13.86 (s, 1H, NH). ^{13}C NMR: 34.73 (CH_2), 93.70 (C-5), 116.29 (CN), 127.99, 129.01, 129.06, 129.21, 129.52, 132.25, 132.70, 136.88 (Ar–C), 161.50 (C-2), 166.15 (C]O), 168.02 (C-6). ESI-MS, *m/z* (Rel. Int.): 317.3 (M^-, 100), 91.1 (23). **5b**: ^{1}H NMR (DMSO-d$_6$): δ 4.54 (s, 2H, CH_2), 7.13–7.17 (m, 2H, Ar–H), 7.45–7.48 (m, 2H, Ar-H), 7.57–7.65 (m, 3H, Ar–H), 7.93–7.95 (m, 2H, Ar-H), 13.80 (s, 1H, NH). ^{13}C NMR: 33.86 (CH_2), 93.79 (C-5), 116.31 (CN), 115.71, 129.08, 128.20, 131.59, 132.27, 133.27, 135.69, 162.92 (Ar–C), 160.98 (C-2), 166.06 (C]O), 167.78 (C-6). ESI-MS, *m/z* (Rel. Int.): 336.3 (M^-, 100), 109.1 (11). **5c**: ^{1}H NMR (DMSO-d$_6$): δ 4.54 (s, 2H, CH_2), 7.37 (d, 2H, Ar-H, J = 8.5 Hz), 7.44 (d, 2H, Ar-H, J = 8.5 Hz), 7.57–7.64 (m, 3H, Ar–H), 7.92–7.94 (m, 2H, Ar–H), 13.82 (s, 1H, NH). ^{13}C NMR: 33.86 (CH_2), 93.79 (C-5), 116.29 (CN), 128.93, 129.08, 129.20, 131.37, 132.27, 132.57, 135.64, 136.26 (Ar–C), 161.61 (C-2), 165.98 (C]O), 167.78 (C-6). ESI-MS, *m/z* (Rel. Int.): 354.1 (M^- +2, 29), 352.3 (M^-, 100), 126.1 (6), 124.0 (22). **5d**: ^{1}H NMR (DMSO-d$_6$): δ 4.44 (s, 2H, CH_2), 7.47–7.48 (m, 3H, Ar–H), 7.69 (d, 2H, Ar–H, J = 8.5 Hz), 7.68–7.76 (m, 2H, Ar–H), 8.15 (d, 2H, Ar–H, J = 8.5 Hz), 13.82 (s, 1H, NH). ^{13}C NMR: 33.53 (CH_2), 90.06 (C-5), 115.20 (CN), 120.15, 123.88, 128.57, 130.31, 130.48, 137.94, 146.78, 148.27 (Ar–C), 160.01 (C-2), 167.63 (C]O), 170.87 (C-6). ESI-MS, *m/z* (Rel. Int.): 363.2 (M^-, 100), 136.1 (8).

4.2. 2-(5-Nitrofuran-2-ylmethylthio)-6-phenyl-3,4-dihydro-4-oxopyrimidine-5-carbonitrile (**6**)

To a solution of compound **4** (2.13 g, 0.01 mol), in acetone (15 mL), 2-bromomethyl-5-nitrofuran (2.06 g, 0.01 mol) and anhydrous potassium carbonate (1.38 g, 0.01 mol) were added and the mixture was stirred at room temperature for 12 h. The solvent was then distilled *in vacuo* at room temperature. Water (15 mL) was added to the residue the mixture was stirred for further 30 min. The obtained solid was filtered, washed with cold water, dried and crystallized from aqueous-ethanol. ^{1}H NMR (DMSO-d$_6$): δ 4.71 (s, 2H, CH_2), 6.72 (d, 1H, Furan-H, J = 3.5 Hz), 7.55–7.59 (m, 2H, Ar–H), 7.62–7.65 (m, 2H, Ar–H), 7.96–7.98 (m, 2H, Furan-H & Ar–H), 13.88 (s, 1H, NH). ^{13}C NMR: 27.18 (CH_2), 93.85 (C-5), 116.17 (CN), 113.16, 129.08, 129.39, 132.38, 135.48 (Ar–C), 112.92, 114.76, 151.39, 155.79 (Furan-C), 161.85 (C-2), 165.06 (C]O), 167.74 (C-6). ESI-MS, *m/z* (Rel. Int.): 353.2 (M^-, 100).

4.3. 2-(2-Alkoxyethylthio or 2-alkylthioethylthio)-6-phenyl-3,4-dihydro-4-oxopyrimidine-5-carbonitriles (7a-d) and 2-benzylyloxymethylthio-6-phenyl-3,4-dihydro-4-oxopyrimidine-5-carbonitrile (**8**)

To a solution of compound **4** (2.13 g, 0.01 mol) in DMF (10 mL), the appropriate halide (2-chloroethyl alkyl ether, 2-chloroethyl alkyl sulphide or benzyl chloromethyl ether) (0.01 mol) and anhydrous potassium carbonate (1.38 g, 0.01 mol) were added and the mixture was stirred at room temperature for 12 h. Water (15 mL) was added and the mixture was stirred for further 30 min. The separated solid was filtered, washed with cold water, dried and crystallized. **7a**: ^{1}H NMR (DMSO-d$_6$): δ 3.27 (s, 3H, CH_3), 3.44–3.46 (m, 2H, CH_2S), 3.62–3.64 (m, 2H, OCH_2), 7.57–7.64 (m, 3H, Ar–H), 7.94 (d, 2H, Ar–H, J = 7.0 Hz), 13.89 (s, 1H, NH). ^{13}C NMR: 30.45 (CH_2S), 58.41 (CH_3), 70.21 (OCH_2), 93.59 (C-5), 116.35 (CN), 129.08, 129.15, 132.25, 135.74 (Ar–C), 161.26 (C-2), 165.89 (C]O), 167.70 (C-6). ESI-MS, *m/z* (Rel. Int.): 286.3 (M^-, 100), 76.0 (4). **7b**: ^{1}H NMR (DMSO-d$_6$): δ 1.08 (t, 3H, CH_3, J = 7.0 Hz), 3.43–3.48 (m, 4H, CH_2S & CH_3CH_2), 3.65–3.67 (m, 2H, OCH_2), 7.56–7.64 (m, 3H, Ar–H), 7.94–7.95 (m, 2H, Ar–H), 13.85 (s, 1H, NH). ^{13}C NMR: 15.51 (CH_3), 30.69 (CH_2S), 65.90 ($CH_3\mathbf{C}H_2$), 68.23 (OCH_2), 93.59 (C-5), 116.43 (CN), 129.06, 129.15, 132.25, 135.75 (Ar–C), 161.46 (C-2), 166.39 (C]O), 167.77 (C-6). ESI-MS, *m/z* (Rel. Int.): 300.3 (M^-, 100), 76.0 (3). **7c**: ^{1}H NMR (DMSO-d$_6$): δ 2.07 (s, 3H, CH_3), 2.81–2.84 (m, 2H, SCH_2), 3.45–3.48 (m, 2H, CH_2S), 7.56–7.62 (m, 3H, Ar–H), 7.93–7.95 (m, 2H, Ar–H), 13.62 (s, 1H, NH). ^{13}C NMR: 14.38 (CH_3), 30.47 (CH_2S), 32.96 (SCH_2), 93.68 (C-5), 116.34 (CN), 129.01, 129.17, 132.24, 135.74 (Ar–C), 161.45 (C-2), 166.24 (C]O), 167.90 (C-6). ESI-MS, *m/z* (Rel. Int.): 302.3 (M^-, 100). **7d**: ^{1}H NMR (DMSO-d$_6$): δ 1.12 (t, 3H, CH_3, J = 7.2 Hz), 2.77–2.80 (m, 2H, SCH_2), 3.38–3.41 (m, 4H, CH_2S & CH_3CH_2), 7.55–7.63 (m, 3H, Ar–H), 7.65–7.88 (m, 2H, Ar–H), 13.75 (s, 1H, NH). ^{13}C NMR: 13.25 (CH_3), 28.55 (CH_2S), 31.85 ($CH_3\mathbf{C}H_2$), 33.65 (SCH_2), 94.05 (C-5), 115.99 (CN), 128.20, 129.65, 132.80, 136.25 (Ar–C), 160.85 (C-2), 165.45 (C]O), 169.05 (C-6). ESI-MS, *m/z* (Rel. Int.): 316.4 (M^-, 100). **8**: ^{1}H NMR (DMSO-d$_6$): δ 4.75 (s, 2H, PhCH_2O), 5.58 (s, 2H, OCH_2S), 7.21–7.65 (m, 10H, Ar–H), 13.08 (s, 1H, NH). ^{13}C NMR: 69.65 (O$\mathbf{C}H_2S$), 77.89 (Ph$\mathbf{C}H_2$O), 95.65 (C-5), 114.95 (CN), 127.22, 127.56, 128.0, 128.12, 128.26, 128.70, 135.74, 138.24 (Ar–C), 160.92 (C-6), 166.48 (C]O), 177.35 (C-2). ESI-MS, *m/z* (Rel. Int.): 348.3 (M^-, 100), 91.1 (34).

4.4. 2-(4-Chlorobenzylthio)-4-chloro-6-phenylpyrimidine-5-carbonitrile (**9**)

Compound **5c** (17.7 g, 0.05 mol) was added portionwise to a mixture of phosphorus oxychloride (19.2 mL) and *N,N*-dimethylaniline (10.3 mL) over a period of 10 min with stirring. The mixture was then heated under reflux for 1 h. On cooling, the mixture was poured onto crushed ice (200 g), stirred for 30 min and extracted with diethyl ether (2 × 200 mL). The ethereal extract was dried over anhydrous sodium sulphate and evaporated under vacuum at room temperature to yield the crude product as oily liquid. The crude product was purified by flash silica gel column chromatography using chloroform as an eluent. ^{1}H NMR (DMSO-d$_6$): δ 4.35 (s, 2H, CH_2), 7.24 (d, 2H, Ar–H, J = 7.5), 7.20 (d, 2H, Ar–H, J = 7.5), 7.23–7.46 (m, 5H, Ar–H). ^{13}C NMR: 42.95 (CH_2), 106.10 (C-5), 116.25 (CN), 127.31, 128.52, 128.90, 130.75, 132.01, 133.99, 134.80, 137.05 (Ar–C), 160.66 (C-4), 171.50 (C-6), 174.20 (C-2). ESI-MS, *m/z* (Rel. Int.): 376.4 (M^+ +4, 11), 374.4 (M^+ +2, 70), 372.4 (M^+, 100).

4.5. 2-(4-Chlorobenzylthio)-4-arylthio-6-phenylpyrimidine-5-carbonitriles (**10a–d**)

To a solution of compound **9** (1.86 g, 5 mmol) in dry pyridine (5 mL), the appropriate arylthiol (5 mmol) was added and the mixture was heated under reflux for 4 h. On cooling, the solvent was then distilled *in vacuo*, and water (10 mL) was added to the residue. The separated precipitate was filtered, washed with cold water, dried and crystallized from ethanol. **10a**: ^{1}H NMR (DMSO-d$_6$): δ 4.09 (s, 2H, CH_2), 6.80–7.22 (m, 5H, Ar–H), 7.26 (d, 2H, Ar–H, J = 8.5 Hz), 7.50–7.64 (m, 7H, Ar–H). ^{13}C NMR: 44.90 (CH_2), 101.0 (C-5), 116.80 (CN), 124.66, 127.05, 128.24, 128.66, 129.0, 129.34, 130.08, 130.99, 132.60, 133.98, 136.90, 138.22 (Ar–C), 170.52, 172.98, 176.26 (C-2, C-4 & C-6). ESI-MS, *m/z* (Rel. Int.): 448.3 (M^+ +2, 38), 446.3 (M^+, 100). **10b**: ^{1}H NMR (DMSO-d$_6$): δ 2.44 (s, 3H, CH_3), 4.12 (s, 2H, CH_2), 6.88–7.20 (m, 4H, Ar–H), 7.24 (d, 2H, Ar–H, J = 8.5 Hz), 7.45–7.68 (m, 7H, Ar–H). ^{13}C NMR: 20.05 (CH_3), 44.91 (CH_2), 100.95 (C-5), 116.80 (CN), 124.66, 125.28, 126.40, 128.04, 128.80, 129.45, 129.30, 130.06, 131.98, 132.65, 134.06, 136.95, 138.25, 143.50 (Ar–C), 169.88, 173.08, 176.20 (C-2, C-4 & C-6). ESI-MS, *m/z* (Rel. Int.): 462.3 (M^+ +2, 40), 460.3 (M^+, 100). **10c**: ^{1}H NMR (DMSO-d$_6$): δ 2.39 (s, 3H, CH_3), 4.10 (s, 2H, CH_2), 6.82–7.18 (m, 6H, Ar–H), 7.22 (d, 2H, Ar–H, J = 8.5 Hz), 7.36–7.60 (m, 5H, Ar–H). ^{13}C NMR: 21.26 (CH_3), 44.90

(CH_2), 98.85 (C-5), 117.06 (CN), 125.25, 125.45, 127.44, 128.26, 129.06, 129.26, 129.35, 129.99, 131.25, 132.05, 136.25, 136.95, 138.25, 139.50 (Ar–C), 169.85, 173.10, 176.55 (C-2, C-4 & C-6). ESI-MS, *m/z* (Rel. Int.): 462.3 (M^+ +2, 41), 460.3 (M^+, 100). **10d**: ^{1}H NMR (DMSO-d_6): δ 2.36 (s, 3H, CH_3), 4.15 (s, 2H, CH_2), 6.85 (d, 2H, Ar–H, J = 8.5 Hz), 7.01–7.18 (m, 4H, Ar–H), 7.23 (d, 2H, Ar–H, J = 8.5 Hz), 7.38–7.49 (m, 5H, Ar–H). ^{13}C NMR: 23.05 (CH_3), 42.95 (CH_2), 97.85 (C-5), 116.25 (CN), 125.35, 126.38, 126.90, 127.22, 127.98, 128.24, 128.98, 129.36, 130.05, 132.0, 136.16, 138.06 (Ar–C), 168.95, 173.15, 176.80 (C-2, C-4 & C-6). EI-MS, *m/z* (Rel. Int.): 462.3 (M^+ +2, 44), 460.3 (M^+, 100).

4.6. 2-(4-Chlorobenzylthio)-4-arylamino-6-phenylpyrimidine-5-carbonitriles (**11a–d**)

To a solution of compound **9** (1.86 g, 5 mmol) in ethanol (8 mL), the appropriate aromatic amine (5 mmol) and anhydrous potassium carbonate (0.7 g, 5 mmol) were added and the mixture was heated under reflux for 6 h. On cooling, the solvent was then distilled *in vacuo*, and water (10 mL) was added to the residue. The separated precipitate was filtered, washed with cold water, dried and crystallized. **11a**: ^{1}H NMR (DMSO-d_6): δ 4.20 (s, 2H, CH_2), 6.82–7.01 (m, 4H, Ar–H), 7.15–7.25 (m, 3H, Ar–H), 7.34 (d, 2H, Ar–H, J = 8.2 Hz), 7.38–7.52 (m, 4H, Ar–H), 10.24 (s, 1H, NH). ^{13}C NMR: 36.24 (CH_2), 85.50 (C-5), 115.18 (CN), 116.89, 117.24, 126.65, 128.0, 129.14, 130.52, 132.75, 133.88, 136.93, 137.99, 138.22, 156.52 (Ar–C), 162.88, 171.08, 174.02 (C-2, C-4 & C-6). ESI-MS, *m/z* (Rel. Int.): 449.4 (M^+ +2, 32), 447.4 (M^+, 100). **11b**: ^{1}H NMR (DMSO-d_6): δ 4.22 (s, 2H, CH_2), 6.75–6.90 (m, 2H, Ar–H), 7.04–7.18 (m, 4H, Ar–H), 7.34 (d, 2H, Ar–H, J = 8.2 Hz), 7.42–7.50 (m, 5H, Ar–H), 10.20 (s, 1H, NH). ^{13}C NMR: 37.66 (CH_2), 86.51 (C-5), 115.22 (CN), 115.44, 117.24, 124.55, 126.65, 127.82, 128.0, 128.55, 129.14, 130.52, 131.24, 132.75, 133.88, 137.22, 137.99, 138.22 (Ar–C & CF_3), 163.12, 170.88, 174.06 (C-2, C-4 & C-6). ESI-MS, *m/z* (Rel. Int.): 501.3 (M^+ +2, 29), 498.3 (M^+, 100). **11c**: ^{1}H NMR (DMSO-d_6): δ 4.21 (s, 2H, CH_2), 6.85–7.22 (m, 6H, Ar–H), 7.33 (d, 2H, Ar–H, J = 8.2 Hz), 7.40–7.44 (m, 3H, Ar–H), 7.55–7.62 (m, 2H, Ar–H), 10.04 (s, 1H, NH). ^{13}C NMR: 37.06 (CH_2), 87.16 (C-5), 115.22 (CN), 115.44, 117.24, 126.65, 127.82, 128.0, 128.55, 129.14, 130.52, 131.24, 132.75, 133.88, 137.22, 137.99, 138.22, 143.15 (Ar–C & CF_3), 162.14, 169.90, 173.84 (C-2, C-4 & C-6). ESI-MS, *m/z* (Rel. Int.): 501.3 (M^+ +2, 34), 498.3 (M^+, 100). **11d**: ^{1}H NMR (DMSO-d_6): δ 4.21 (s, 2H, CH_2), 6.82 (d, 2H, Ar–H, J = 7.5 Hz), 6.99–7.33 (m, 7H, Ar–H), 7.32 (d, 2H, Ar–H, J = 8.2 Hz), 7.50–7.59 (m, 2H, Ar–H), 10.10 (s, 1H, NH). ^{13}C NMR: 37.25 (CH_2), 87.18 (C-5), 115.29 (CN), 116.25, 120.25, 123.89, 126.45, 127.12, 127.35, 128.36, 128.55, 129.14, 130.52, 131.24, 137.24, 144.68 (Ar–C & CF_3), 162.14, 169.90, 173.84 (C-2, C-4 & C-6). ESI-MS, *m/z* (Rel. Int.): 501.3 (M^+ +2, 35), 498.3 (M^+, 100).

4.7. Determination of antimicrobial activity (agar disc-diffusion method)

Sterile filter paper discs (8 mm diameter) were moistened with the compound solution in dimethylsulphoxide of specific concentration (200 μg/disc), the antibacterial antibiotics Gentamicin and Ampicillin trihydrate (100 μg/disc) and the antifungal drug Clotrimazole (100 μg/disc) were carefully placed on the agar culture plates that had been previously inoculated separately with the microorganisms. The plates were incubated at 37 °C, and the diameter of the growth inhibition zones were measured after 24 h in case of bacteria and 48 h in case of *C. albicans*.

4.8. Determination of the minimal inhibitory concentration (MIC)

Compounds **5b**, **5c**, **6**, **7a**, **7b**, **7c**, **9**, **11a**, Gentamicin and Ampicillin trihydrate were dissolved in dimethylsulphoxide at concentration of 128 μg/mL. The twofold dilutions of the solution were prepared (128, 64, 32, …, 0.5 μg/mL). The microorganism suspensions at 106 CFU/mL (colony forming unit/mL) concentrations were inoculated to the corresponding wells. The plates were incubated at 36 °C for 24 and 48 h for the bacteria and *C. albicans*, respectively. The MIC values were determined as the lowest concentration that completely inhibited visible growth of the microorganism as detected by unaided eye.

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