Design, synthesis, antimicrobial and cytotoxicity study on human colorectal carcinoma cell line of new 4,4′-(1,4-phenylene) bis(pyrimidin-2-amine) derivatives

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Abstract
Background: Pyrimidine molecules attracted organic chemists very much due to their biological and chemotherapeutic importance. Their related fused heterocycles are of interest as potential bioactive molecules so, we have designed and prepared a new class of 4,4′-(1,4-phenylene)bis(pyrimidin-2-amine) molecules and screened for their in vitro antibacterial, antifungal and cytotoxicity studies.

Results: The structures of synthesized bis-pyrimidine molecules were confirmed by physicochemical and spectral means. The synthesized compounds were further evaluated for their in vitro biological potentials i.e. antimicrobial activity using tube dilution method and anticancer activity against human colorectal carcinoma (HCT116) cancer cell line by Sulforhodamine B assay.

Conclusions: The biological study demonstrated that compounds s7, s8, s11, s14, s16, s17 and s18 have shown more promising antimicrobial activity with best MIC values than the cefadroxil (antibacterial) and fluconazole (antifungal) and compound s3 found to have better anticancer activity against human colorectal carcinoma (HCT116) cancer cell line.

Keywords: Pyrimidine molecules, Design, Synthesis, Antimicrobial, Cytotoxicity, HCT116

Background
Among a wide variety of heterocyclic that have been explored for developing medicinally important molecules [1]. Pyrimidine derivatives attracted organic chemists very much due to their biological and chemotherapeutic importance especially the fused heterocycles are of interest as potential bioactive molecules. Pyrimidine derivatives are known to exhibit biological activities i.e. anticancer [2, 3], antiviral [4], anti-inflammatory [5], antimalarial [6], antibacterial [1, 7] and antifungal [8] etc. As pathogenic bacteria continuously evolve mechanisms of resistance to currently used antibacterial, so the discovery of novel and potent antibacterial drugs is the best way to overcome bacterial resistance and develop effective therapies [9].

Cancer is one of the most serious health problems all over the world and one of the leading causes of death. Thus, in the past for several decades, researchers have been struggling to find effective clinical approaches for the treatment of cancer and search for novel anticancer agents. Recently, accumulating evidences have illustrated that heterocyclic derivatives are considered to be the most promising molecules as leads for the discovery of novel synthetic drugs. In particular, substituted pyrimidines, present in the cores of many physiologically active molecules, display interesting therapeutic properties, especially antitumor activities with different bio targets.

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and mechanisms by means of inhibiting several enzymes as well as modulating the activity of many receptors [10]. Pyrimidine is found as a core structure in a large variety of compounds that exhibit important biological activity, specifically pyrimidines known to inhibit Pneumocystis carinii (pc), Toxoplasma gondii (tg) of tumour cell lines in culture and the activity is attributed to inhibition of dihydrofolate reductase (DHFR) [11]. 2,4-Disubstituted and 2,4,6-trisubstituted pyrimidines have shown potent anticancer activity as CDK inhibitors, TNF-α inhibitors, Ab1 tyrosine protein kinase inhibitors, PI-3 kinase inhibitors, Akt kinase inhibitors and cytokines inhibitors [12]. Design of pyrimidine molecules for antimicrobial and anticancer potentials based on literature is presented in Fig. 1. Selected marketed drug contains pyrimidine ring presented in Fig. 2 [13].

On the basis of these observations, we here in report the synthesis, in vitro antimicrobial and cytotoxicity activities of 4,4′-(1,4-phenylene)bis(pyrimidin-2-amine) derivatives.

Results and discussion

Chemistry

Synthesis of the intermediate and target molecules was performed according to the reactions outlined in Scheme 1 (based on Claisen-Schmidt condensation). Initially, the bis-chalcone was prepared by the reaction of 1-(2,4-dichlorophenyl)ethanone and terephthalaldehyde. The cyclization of bis-chalcone (int-I) to yield bis-pyrimidine (int-II) was effected with guanidine bis-pyrimidine (int-II) to yield hydrochloride. The reaction of bis-pyrimidine (int-I) was displayed by the existence of an IR absorption band around 3363.97 and 1692.49 cm⁻¹ indicated the presence of –NH₂ and N=CH str. The molecular structure of the intermediate-I and its cyclized products were further confirmed by proton–NMR spectral data. The ¹H-NMR spectrum of intermediate-I showed two doublets at 7.59 ppm (J = 15.1 Hz) and 8.06 ppm (J = 15.1 Hz) indicating that the CH=CH group in the enone linkage is in a trans-conformation. The ¹H-NMR spectrum of (II) showed a multiplet signals between 7.42 and 8.01 δ ppm confirming the cyclisation of the 3,3′-(1,4-phenylene)bis(1-(2,4-dichlorophenyl)prop-2-en-1-one) (I) to give 6,6′-(1,4-phenylene)bis(4-(2,4-dichlorophenyl)pyrimidin-2-amine) (II). The ¹H-NMR spectrum of intermediate-II showed a sharp singlet at 7.09 δ ppm due to the NH₃ protons and it also showed a sharp singlet at 7.85 δ ppm due to HC=C group, which confirmed the cyclization of the bis-chalcone into a bis-pyrimidine ring. The IR stretching vibrations at 733.88–750.53 cm⁻¹ in the spectral data of synthesized derivatives (s1–s18) displayed the presence of halogen group (Ar–Cl) on the aromatic nucleus substituted at the ortho, meta and para-position. The existence of Ar=NO₂ functional group in compounds s3, s6 and s7 was displayed by the existence of symmetric Ar=NO₂ stretches in the scale of 1372.55–1373.82 cm⁻¹. The existence of an arylalkyl ether group (Ar–OCH₃) in compounds, s5, s9, s10, s12 and s13 are established by the existence of an IR absorption band around 1038.33–3089.60 cm⁻¹. The impression of IR stretching vibration at 3088.93–2972.97 cm⁻¹ and 1599.67–1595.05 cm⁻¹ in the spectral data of synthesized derivatives (s1–s18) specified the existence of C–H and C=C group, respectively. The appearance of IR stretching 1698.99–1663.17 cm⁻¹ in the spectral data of synthesized derivatives (s1–s18) specified the existence of N=CH group. The impression of IR absorption band at 3461.41–3345.04 cm⁻¹ in the spectral data s2, s4, s13 and s15 displayed the presence of Ar=OH group on the aromatic ring at ortho and para position. The multiplet signals between 6.77 and 8.34 δ ppm in proton-NMR spectra is indicative of aromatic proton of synthesized derivatives. The compounds, s5, s9, s10, s12 and s13 showed singlet at 3.84–3.85 δ ppm due to the existence of OCH₃ of Ar–OCH₃. The synthesized compounds showed singlet at 9.01–10.05 δ ppm due to the existence of N=CH in pyrimidine ring. Compounds showed singlet at 10.00–10.15 δ ppm due to the existence of –CH in pyrimidine ring. Compound s8 showed singlet at 3.04 δ ppm due to existence of –N(CH₃)₃ at the para position. The compound s16 showed quadrate at 3.38–3.49 δ ppm and triplet at 1.07–1.15 δ ppm due to presence of –N(C₃H₇)₂ at para position. The
13C-NMR spectral data and elemental analysis studies of the synthesized pyrimidine derivatives were found within ± 0.4% of the theoretical results of synthesized compounds are given in the “Experimental section”.

**In vitro antimicrobial activity**

Antimicrobial screening of synthesized 4,4′-(1,4-phenylene)bis(pyrimidin-2-amine) molecules against Gram positive and Gram negative bacterial and fungal strains was done by tube dilution technique. Antimicrobial activity results indicated (Table 2) particularly; compounds, s7, s8, s11, s14, s16, s17 and s18 have shown more promising antimicrobial activity as compared to standard drugs cefadroxil (antibacterial) and fluconazole (antifungal) while other derivatives are moderately active. In the case of Gram positive bacterial study, compound s11 (MIC<sub>sa</sub> = 0.14 µmol/mL, MIC<sub>bc</sub> = 0.07 µmol/mL) was found to be most potent one against *S. aureus* and *B. cereus*. In the case of Gram negative bacterial study, compound s7 and s18 displayed appreciable antibacterial activity against *Providencia rettgeri* with MIC value of 0.08 µmol/mL. Compound s8 (MIC<sub>pa</sub> = 0.15 µmol/mL) exhibited excellent activity against *Pseudomonas aeruginosa* and compound s11 showed good antibacterial activity against *Salmonella typhi* and *Escherichia coli* with the MIC values of 0.29 and 0.23 µmol/mL, respectively. The antifungal screening results demonstrated that compounds s11 displayed appreciable antifungal activity against *Aspergillus niger* and *Aspergillus flavus* with the MIC values of 0.58 and 0.14 µmol/mL, respectively. Compounds, s14 and s17 (MIC<sub>af</sub> = 0.31 µmol/mL) were found to be most potent ones against *Aspergillus fumigatus* and compound s16 (MIC<sub>af</sub> = 0.14 µmol/mL) was found to be most effective one against *Aspergillus flavus*.

The antimicrobial screening results of synthesized molecules (s7, s8, s11, s14, s16, s17 and s18) have more than standard drugs and may be used as a lead compound to discover novel antimicrobial scaffolds.
In vitro anticancer activity

The in vitro anticancer activity of synthesized 4,4′-(1,4-phenylene)bis(pyrimidin-2-amine) molecules was carried out against human colorectal cancer cell line [HCT-116 (ATCC CCL-247)] and compared with 5-fluorouracil (reference drug) and the results of anticancer studies have been presented in Table 3, Fig. 3. Anticancer screening results revealed that in general pyrimidin exhibited good anticancer potential against human colorectal cancer cell line, especially, compound s3 (IC$_{50}$ = 1.16 µmol/mL) displayed anticancer activity comparable to the reference drug 5-fluorouracil (IC$_{50}$ = 0.83 µmol/mL).

Structure–activity relationship

From the in vitro antibacterial, antifungal and anticancer results, the structure–activity relationship of synthesized 4,4′-(1,4-phenylene)bis(pyrimidin-2-amine) molecules (SAR, are presented in Fig. 4).

From structure activity relationship study, we may conclude that different structural requirements are required for a compound to be effective against different targets. The aforementioned facts are supported by the earlier research findings [3, 14, 16, 17].

Experimental section

Preliminary materials (glasswares, chemicals etc.) for the research work were obtained from commercial sources [Loba Chemie, Pvt Ltd. Mumbai, India; Central Drug House (CDH) Pvt. Ltd., New Delhi, India and HiMedia Laboratory Pvt. Ltd., Delhi, India] used without further purification. All reactions were monitored by thin-layer chromatography on 0.25 mm silica gel (Merck) plates, using benzene, chloroform: methanol as mobile phase and spots were observed by exposure to iodine vapours. Melting points of synthesized 4,4′-(1,4-phenylene) bis(pyrimidin-2-amine) molecules was determined in open capillary tube technique. Mass spectra of the...
The synthesized compounds were recorded on Waters Micro-mass Q-ToF Micro instrument. An infrared spectrum (IR) was recorded (KBr-pellets) in Bruker 12060280, Software: OPUS 7.2.139.1294 spectrometer. $^{1}$$H$-NMR and $^{13}$$C$-NMR were recorded at 600 and 150 MHz respectively on Bruker Avance III 600 NMR spectrometer by appropriate deuterated solvents. The results are conveyed in parts per million ($\delta$, ppm) downfield from tetramethyl silane (internal standard). $^{1}$$H$-NMR spectral details of the synthesized derivatives are represented with multiplicity like singlet (s); doublet (d); triplet (t); quartet (q); multiplet (m) and the number hydrogen ion. Elemental analysis of the synthesized 4,4$'$(1,4-phenylene) bis(pyrimidin-2-amine) molecules was obtained by Perkin–Elmer 2400 C, H and N analyzer. All the compounds

![Scheme 1](image-url)
Table 1  Physicochemical properties of the synthesized bis-pyrimidine molecules

| Comp. no | Structure | Molecular formula | Color       | M.pt. (°C) | R<sub>f</sub> value | % Yield |
|----------|-----------|-------------------|-------------|------------|---------------------|---------|
| s1       | ![](image1) | C<sub>40</sub>H<sub>22</sub>Cl<sub>6</sub>N<sub>6</sub> | Dark yellow | 133–135    | 0.46                | 85.45   |
| s2       | ![](image2) | C<sub>44</sub>H<sub>32</sub>Cl<sub>4</sub>N<sub>6</sub>O<sub>4</sub> | Light yellow | 113–115    | 0.25                | 75.56   |
| s3       | ![](image3) | C<sub>40</sub>H<sub>22</sub>Cl<sub>4</sub>N<sub>8</sub>O<sub>4</sub> | Cream yellow | 140–142    | 0.31                | 69.03   |
| s4       | ![](image4) | C<sub>40</sub>H<sub>24</sub>Cl<sub>4</sub>N<sub>6</sub>O<sub>2</sub> | Pure yellow | 133–135    | 0.26                | 82.56   |
| s5       | ![](image5) | C<sub>44</sub>H<sub>32</sub>Cl<sub>4</sub>N<sub>6</sub>O<sub>4</sub> | Dark yellow | 145–147    | 0.20                | 69.23   |
| s6       | ![](image6) | C<sub>40</sub>H<sub>22</sub>Cl<sub>4</sub>N<sub>8</sub>O<sub>4</sub> | Medallion yellow | 146–148    | 0.35                | 70.00   |
| s7       | ![](image7) | C<sub>40</sub>H<sub>22</sub>Cl<sub>4</sub>N<sub>8</sub>O<sub>4</sub> | Light yellow | 142–144    | 0.32                | 75.65   |
| s8       | ![](image8) | C<sub>44</sub>H<sub>34</sub>Cl<sub>4</sub>N<sub>8</sub>O<sub>4</sub> | Light yellow | 123–125    | 0.39                | 78.12   |
| s9       | ![](image9) | C<sub>42</sub>H<sub>28</sub>Cl<sub>4</sub>N<sub>6</sub>O<sub>2</sub> | Honey yellow | 129–131    | 0.25                | 65.01   |
| Comp. no | Structure | Molecular formula | Colour  | M.pt. (°C) | Rf value | % Yield |
|----------|-----------|-------------------|---------|------------|----------|---------|
| s10.     | ![Structure Image](image1) | C_{42}H_{28}Cl_{4}N_{6}O_{2} | Pure yellow | 124–126 | 0.23 | 80.45 |
| s11.     | ![Structure Image](image2) | C_{40}H_{20}Cl_{8}N_{6} | Lemon yellow | 80–82 | 0.21 | 79.34 |
| s12.     | ![Structure Image](image3) | C_{42}H_{28}Cl_{4}N_{6}O_{2} | Light yellow | 134–135 | 0.58 | 82.23 |
| s13.     | ![Structure Image](image4) | C_{42}H_{28}Cl_{4}N_{6}O_{4} | Pure yellow | 129–131 | 0.41 | 89.45 |
| s14.     | ![Structure Image](image5) | C_{40}H_{22}Cl_{6}N_{6} | Medallion yellow | 56–58 | 0.43 | 85.56 |
| s15.     | ![Structure Image](image6) | C_{40}H_{24}Cl_{4}N_{6}O_{2} | Dark yellow | 79–81 | 0.50 | 87.23 |
| s16.     | ![Structure Image](image7) | C_{48}H_{42}Cl_{4}N_{8} | Cream yellow | 75–77 | 0.37 | 66.33 |
| s17.     | ![Structure Image](image8) | C_{40}H_{22}Cl_{6}N_{6} | Dark yellow | 56–58 | 0.57 | 68.12 |
| s18.     | ![Structure Image](image9) | C_{44}H_{28}Cl_{4}N_{6} | Light yellow | 63–65 | 0.50 | 62.23 |
gave C, H and N analysis within ± 0.4% of the theoretical results.

General procedure for the synthesis of 4,4′-(1,4-phenylene)bis(pyrimidin-2-amine) derivatives (s1–s18)

Step i: Synthesis of 3,3′-(1,4-phenylene)bis(1-(2,4-dichlorophenyl)prop-2-en-1-one) (Int-I)
A mixture of 1-(2,4-dichlorophenyl)ethanone (0.02 mol) and terephthalaldehyde (0.01 mol) were stirred in ethanol (10–20 mL) for 2–3 h and 10 mL 40% sodium hydroxide solution was added drop wise with constant stirring at room temp till a light brown mass was obtained. Then the mixture was kept overnight at room temperature and the contents were poured on crushed ice and acidified with dilute hydrochloric acid, which resulted in the precipitation of chalcone. The crude 3,3′-(1,4-phenylene) bis(1-(2,4-dichlorophenyl)prop-2-en-1-one) was filtered, dried and recrystallized from methanol [18, 19].

Step ii: Synthesis of 6,6′-(1,4-phenylene)bis(4-(2,4-dichlorophenyl)pyrimidin-2-amine) (Int-II)
To a mixture of 3,3′-(1,4-phenylene)bis(1-(2,4-dichlorophenyl)prop-2-en-1-one) (0.01 mol) (synthesized in previous step-i) and potassium hydroxide (0.01 mol) in 80 mL absolute ethanol, 40 mL 0.50 M solution of guanidine hydrochloride in ethanol was added. After addition, the mixture was refluxed for 4–5 h (50 °C). The progress of reaction was monitored by TLC and the reaction mixture was cooled at room temperature and quenched with 20 mL of 0.5 M solution of hydrochloric acid in water. The reaction mixture was shaken to ensure mixing and concentrated to obtain solid which was recrystallized from ethanol [18].

Step iii: Synthesis of final compounds (s1–s18)
A mixture of 6,6′-(1,4-phenylene)bis(4-(2,4-dichlorophenyl)pyrimidin-2-amine) (0.01 mol) (synthesized in previous step-ii) and substituted aldehyde (0.02 mol) were

Table 2 Antimicrobial activity results of synthesized bis-pyrimidine molecules

| Compound no. | Antimicrobial activity (MIC = µmol/mL) | Fungal species |
|--------------|----------------------------------------|---------------|
|              | Bacterial species                      |               |
|              | Gram positive                          | Gram negative |
|              | S.A. B.C.                              | S.T. P.A. E.C. P.R. | A.N. A.F. A.F. |
| s1.          | 0.16 0.08 – – 0.25 0.16                | 0.63 1.26 –    |
| s2.          | 2.36 1.18 – 2.36 – 2.36                | 2.36 1.18 0.59 |
| s3.          | 0.61 0.61 – – 0.15                     | 0.61 1.22 0.61 |
| s4.          | 0.16 0.08 0.33 0.16 0.26 0.16          | 2.63 0.66 0.16 |
| s5.          | 0.59 0.59 – – 0.15                     | – 1.18 1.18    |
| s6.          | – 0.15 2.44 1.22 0.61 –               | 2.44 0.61 0.15 |
| s7.          | 0.61 – 1.22 0.61 2.44 0.08             | – 1.22 –       |
| s8.          | – 1.23 0.31 0.15 0.61 –               | 2.46 0.61 0.15 |
| s9.          | 0.16 0.08 0.32 0.16 0.25 0.16          | – – 0.32       |
| s10.         | 1.27 0.63 1.27 2.54 – 0.63             | 0.63 1.27 0.32 |
| s11.         | 0.14 0.07 0.29 1.16 0.23 0.14          | 0.58 – 0.14    |
| s12.         | 1.27 0.63 – 2.54 – 0.63                | – 1.27 0.32    |
| s13.         | 1.22 0.61 – – 1.22 0.61                | 1.22 1.22 –    |
| s14.         | 2.51 1.26 – 1.26 – 2.51                | – 0.31 0.63    |
| s15.         | 0.66 0.66 – – 0.66 0.16                | 0.66 0.33 –    |
| s16.         | 0.57 0.57 – – 0.57 0.14                | 2.30 0.57 0.14 |
| s17.         | – 0.16 2.51 1.26 0.63 1.26             | 0.63 0.31 1.26 |
| s18.         | 0.64 – 1.28 0.64 2.56 0.08             | 1.28 – 0.32    |
| Acetone      | NA NA NA NA NA NA                    | NA NA NA      |
| Broth control| NG NG NG NG NG NG                    | NG NG NG     |
| Std.         | 0.34c 0.34c 0.68c 0.68c 0.34d 0.68d   | 0.82d 0.82d 0.82d |

S.A.: Staphylococcus aureus; B.C.: Bacillus cereus; S.T.: Salmonella typhi; P.A.: Pseudomonas aeruginosa; E.C.: Escherichia coli; P.R.: Providencia rettgeri; A.N.: Aspergillus niger
A.F.: Aspergillus fumigatus; A.F.: Aspergillus flavus; Resist –; NA no activity, NG No growth
Std.: Cefadroxil; Fluconazole

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refluxed in minimum amount of ethanol in presence of small amount of glacial acetic acid as catalyst for 2–3 h (40 °C). The progress of reaction was monitored by TLC plates. The mixture was cooled and poured in ice cold water. The precipitated solid (different yellow color) was filtered and recrystallized with methanol.

In vitro antimicrobial assay
The in vitro antimicrobial study of the synthesized 6,6′-(1,4-phenylene)bis(4-(2,4-dichlorophenyl)pyrimidin-2-amine) molecules was determined against human colorectal carcinoma [HCT-116 (ATCC (American Type Culture Collection) CCL-247)] cancer cell line using Sulforhodamine-B (SRB) assay. In this study, the cells were fixed with trichloroacetic acid and then stained with 0.4% (w/v) Sulforhodamine B mixed with 1% acetic acid. Unbound dye was discarded by five washes of 1% acetic acid solution and protein-bound dye was solubilised with 10 mM Tris base for confirmation of optical density in a computer-interfaced, 96-well microtiter plate reader. The anticancer activity results recorded in IC50 value [22].

### Table 3 Anticancer activity results of the synthesized bis-pyrimidine molecules

| Compound no. | Cancer cell line (HCT-116) | Cancer cell line (HCT-116) |
|--------------|---------------------------|---------------------------|
| s1           | 12.56                     | s10.                      |
| s2           | 5.16                      | s11.                      |
| s3           | 1.16                      | s12.                      |
| s4           | 13.16                     | s13.                      |
| s5           | 11.79                     | s14.                      |
| s6           | 12.22                     | s15.                      |
| s7           | 6.72                      | s16.                      |
| s8           | 4.91                      | s17.                      |
| s9           | 3.81                      | s18.                      |
| 5-Fluorouracil| 0.83                      | 5-Fluorouracil            |

HCT-116 human colorectal carcinoma

**In vitro anticancer assay**

The in vitro cytotoxicity screening of synthesized molecules was performed against human colorectal carcinoma [HCT-116 (ATCC (American Type Culture Collection) CCL-247)] cancer cell line using 5-Fluorouracil (ATCC 23564), *Salmonella typhi* (ATCC 15499) and *Providencia rettgeri* (MTCC-8099) and fungal strains- *Aspergillus niger* (MTCC-281); *Aspergillus fumigatus* (2483); *Aspergillus flavus* (1783) by tube dilution method [20]. The stock solution was prepared for the test compounds (s1–s18) and reference drugs (cefadroxil and fluconazole) in acetone to get a concentration of 100 µg/mL and this stock solution was used for further tube dilution with six concentration of 20, 10, 5.0, 2.5, 1.25, 0.625 µg/mL for the antimicrobial study [21]. Dilution of test and standard compounds were prepared double strength nutrient broth—I.P (antibacterial) and sabouraud dextrose broth—I.P (antifungal). The samples were incubated at 37 ± 1 °C for 24 h (bacteria), 25 ± 1 °C for 7 days (A. niger), 30 ± 1 °C for 15 days (A. flavus), 35 ± 1 °C for 72 h (A. fumigates) respectively and results were recorded in terms of MIC (The lowest concentration of test substances which inhibited microbial growth) are presented in the Table 2.
Spectral characteristics of the synthesized pyrimidine compounds (s1–s18)

6,6′-(1,4-Phenylene)

bis(N-(4-chlorobenzylidene)-4-(2,4-dichlorophenyl) pyrimidin-2-amine) (s1)

IR (KBr, cm⁻¹): 3088.93 (C–H str.), 1596.06 (C=C str.), 1665.82 (N=CH str.), 1332.11 (C–N str.), 735.72 (Ar–Cl);

MS ES⁺ (ToF): m/z 797 [M++1]; 1H-NMR (δ, DMSO-d₆): 7.36–7.86 (m, 18H, Ar–H), 10.05 (s, 2H, N=CH), 10.22 (s, 2H, (CH)₂ of pyrimidine ring);

13C-NMR (δ, DMSO-d₆): 160.2, 145.7, 138.9, 136.2, 131.2, 130.2, 129.98, 129.94, 128.17, 128.13, 127.6; CHN: Calc. C₄₀H₂₂Cl₆N₆: C, 60.10; H, 2.77; N, 10.51; Found: C, 60.18; H, 2.71; N, 10.56.

4,4′-(((6,6′-(1,4-Phenylene)bis(4-(2,4-dichlorophenyl)pyrimidine-6,2-diyl))bis(azanylylidene))bis(methanylylidene))bis(2-ethoxyphenol) (s2)

IR (KBr, cm⁻¹): 2978.51 (C–H str.), 1595.22 (C=C str.), 1665.80 (N=CH str.), 1331.51 (C–N str.), 736.16 (Ar–Cl), 1104.41 (C–O–C₂H₅, aralkyl ether), 3345.04 (O–H str.);

MS ES⁺ (ToF): m/z 849 [M++1]; 1H-NMR (δ, DMSO-d₆): 6.78–8.27 (m, 16H, Ar–H), 9.70 (s, 2H, N=CH), 10.03 (s, 2H, (CH)₂ of pyrimidine ring), 3.98 (t, 4H, (CH₃)₂), 1.32 (d, 6H, (CH₃)₂); 13C-NMR (δ, DMSO-d₆): 165.67, 164.08, 145.7, 138.9, 136.2, 131.2, 130.2, 129.98, 128.17, 128.13, 127.6; CHN: Calc. C₄₄H₃₂Cl₄N₆O₄: C, 62.13; H, 3.79; N, 9.88; Found: C, 62.10; H, 3.68; N, 9.81.

6,6′-(1,4-Phenylene)bis(4-(2,4-dichlorophenyl)-N-(3-nitrobenzylidene)pyrimidin-2-amine) (s3)

IR (KBr, cm⁻¹): 3088.09 (C–H str.), 1595.51 (C=C str.), 1665.33 (N=CH str.), 1337.08 (C–N str.), 734.25 (Ar–Cl); 1373.82 (C=NO₂, str., NO₂);

MS ES⁺ (ToF): m/z 819 [M⁺1]; 1H-NMR (δ, DMSO-d₆): 7.42–8.34 (m, 18H, Ar–H), 9.85 (s, 2H, N=CH), 10.10 (s, 2H, (CH)₂ of pyrimidine ring); 13C-NMR (δ, DMSO-d₆): 165.56, 164.35, 145.90, 138.92, 137.86, 137.49, 136.22, 135.37, 131.90, 130.49, 129.49, 129.88, 128.21, 127.61, 124.45; CHN: Calc. C₄₀H₂₂Cl₄N₈O₄: C, 58.56; H, 2.70; N, 13.66; Found: C, 58.50; H, 2.73; N, 13.70.

4,4′-(((6,6′-(1,4-Phenylene)bis(4-(2,4-dichlorophenyl)pyrimidine-6,2-diyl))bis(azanylylidene))bis(methanylylidene))diphenol (s4)

IR (KBr, cm⁻¹): 3028.00 (C–H str.), 1595.49 (C=C str.), 1664.04 (N=CH str.), 1332.80 (C–N str.), 735.82 (Ar–Cl), 3461.39 (O–H str.);

MS ES⁺ (ToF): m/z 761 [M++1]; 1H-NMR (δ, DMSO-d₆): 7.33–8.34 (m, 16H, Ar–H), 9.85 (s, 2H, N=CH), 10.10 (s, 2H, (CH)₂ of pyrimidine ring), 6.92 (s, 2H, Ar–OH); 13C-NMR (δ, DMSO-d₆): 165.65, 164.36, 137.87, 136.45, 134.93, 130.24, 130.18, 129.94, 129.05, 128.03, 127.62, 116.30, 107.26; CHN: Calc. C₄₀H₂₄Cl₄N₆O₂: C, 63.01; H, 3.17; N, 11.02; Found: C, 63.05; H, 3.19; N, 11.08.

6,6′-(1,4-Phenylene)bis(4-(2,4-dichlorophenyl)-N-(3,4-dimethoxybenzylidene)pyrimidin-2-amine) (s5)

IR (KBr, cm⁻¹): 3027.69 (C–H str.), 1596.45 (C=C str.), 1663.75 (N=CH str.), 1332.00 (C–N str.), 735.35 (Ar–Cl), 3088.33 (C–O–CH₃, aralkyl ether); MS ES⁺ (ToF): m/z 849 [M⁺1]; 1H-NMR (δ, DMSO-d₆): 7.01–8.34 (m, 16H, Ar–H), 9.85 (s, 2H, N=CH), 10.10 (s, 2H, (CH)₂ of pyrimidine ring), 3.84 (s, 12H, (OCH₃)₄); 13C-NMR (δ, DMSO-d₆): 165.65, 164.36, 137.87, 136.45, 134.93, 131.83, 130.43, 129.95, 128.22, 128.17, 127.62, 111.82, 107.25.
6,6′-(1,4-Phenylenedioxy)bis(4-(2,4-dichlorophenyl)-N-(2-nitrobenzylidene)pyrimidin-2-amine) (s6)

IR (KBr, cm⁻¹): 3028.75 (C–H str.), 1595.16 (C=C str.), 1664.50 (N=CH str.), 1333.19 (C–N str.), 736.40 (Ar–Cl); 1372.97 (C=N O2 sym. str., NO2); MS ES + (ToF): m/z 819 [M⁺⁺]; 2H-NMR (δ, DMSO-d₆): 7.02–8.34 (m, 18H, Ar–H), 10.00 (s, 2H, N=CH), 10.10 (s, 2H, (CH₂)₂ of pyrimidine ring); 13C-NMR (δ, DMSO-d₆): 165.55, 164.36, 142.36, 137.87, 134.93, 132.66, 131.37, 130.27, 130.22, 129.99, 129.98, 128.16, 128.13, 127.61, 127.10; CHN: Calc. C₄₀H₂₂Cl₄N₈O₄: C, 58.56; H, 2.70; N, 13.66; Found: C, 58.60; H, 2.74; N, 13.69.

6,6′-(1,4-Phenylenedioxy)bis(4-(2,4-dichlorophenyl)-N-(4-nitrobenzylidene)pyrimidin-2-amine) (s7)

IR (KBr, cm⁻¹): 3088.00 (C=O str.), 1595.42 (C=C str.), 1664.00 (N=CH str.), 1334.07 (C–N str.), 735.84 (Ar–Cl), 1372.55 (C=N O2 sym. str., NO2); MS ES + (ToF): m/z 819 [M⁺⁺]; 2H-NMR (δ, DMSO-d₆): 7.02–8.34 (m, 18H, Ar–H), 10.00 (s, 2H, N=CH), 10.10 (s, 2H, (CH₂)₂ of pyrimidine ring); 13C-NMR (δ, DMSO-d₆): 165.55, 164.37, 163.17, 145.88, 140.07, 137.86, 136.94, 134.93, 132.79, 131.80, 129.84, 128.16, 128.10, 124.71, 107.1; CHN: Calc. C₄₀H₂₂Cl₄N₈O₄: C, 58.56; H, 2.70; N, 13.66; Found: C, 58.61; H, 2.76; N, 13.70.

6,6′-(1,4-Phenylenedioxy)bis(4-(2,4-dichlorophenyl)-N-(4-dimethylamino)benzylidene)pyrimidin-2-amine) (s8)

IR (KBr, cm⁻¹): 3028.00 (C=O str.), 1590.10 (C=C str.), 1662.86 (N=CH str.), 1331.98 (C–N str., 733.88 (Ar–Cl), 2826.15 (C–H str., –CH₃); MS ES + (ToF): m/z 815 [M⁺⁺]; 1H-NMR (δ, DMSO-d₆): 6.77–8.34 (m, 18H, Ar–H, 9.67 (s, 2H, N=CH)), 10.04 (s, 2H, (CH₂)₂ of pyrimidine ring), 3.04 (s, 12H, (CH₃)₂); 13C-NMR (δ, DMSO-d₆): 165.54, 164.36, 145.70, 142.74, 137.87, 136.40, 133.40, 131.37, 130.27, 130.18, 129.93, 128.16, 127.60, 111.54, 107.26; CHN: Calc. C₄₄H₃₄Cl₄N₈: C, 64.72; H, 4.20; N, 13.72; Found: C, 64.76; H, 4.26; N, 13.74.

6,6′-(1,4-Phenylenedioxy)bis(4-(2,4-dichlorophenyl)-N-(3-methoxysobenzylidene)pyrimidin-2-amine) (s9)

IR (KBr, cm⁻¹): 3028.79 (C=O str.), 1595.05 (C=C str.), 1664.77 (N=CH str.), 1332.13 (C–N str.), 736.46 (Ar–Cl), 3089.60 (C=O–CH₃, aralkyl ether); MS ES + (ToF): m/z 789 [M⁺⁺]; 1H-NMR (δ, DMSO-d₆): 7.03–8.34 (m, 18H, Ar–H), 10.04 (s, 2H, N=CH), 10.10 (s, 2H, (CH₂)₂ of pyrimidine ring), 3.84 (s, 6H, (OCH₃)₂); 13C-NMR (δ, DMSO-d₆): 165.55, 164.36, 145.86, 137.86, 136.94, 134.93, 139.79, 130.94, 129.99, 128.19, 128.12, 127.61, 116.6, 109.26, 56.8; CHN: Calc. C₃₂H₂₈Cl₂N₆O₂: C, 63.81; H, 3.57; N, 10.63; Found: C, 63.85; H, 3.60; N, 10.68.

4,4′-(1,1,1-Tris(5-Methyl-2-(4,4-dichlorophenyl)pyrimidine-6,2-diyl))bis(azanylylidene)bis(methanylylidene)bis(2-methoxyphenol) (s13)

IR (KBr, cm⁻¹): 3027.22 (C=H str.), 1596.30 (C=C str.), 1663.95 (N=CH str.), 1331.37 (C–N str.), 3461.41 (O=H str.), 3088.44 (C=O–CH₃, aralkyl ether), 736.07 (Ar–Cl); MS ES + (ToF): m/z 821 [M⁺⁺]; 1H-NMR (δ, DMSO-d₆): 7.03–8.34 (m, 16H, Ar–H), 10.04 (s, 2H, N=CH), 10.10 (s, 2H, (CH₂)₂ of pyrimidine ring), 3.85s (6H, (OCH₃)₂); 13C-NMR (δ, DMSO-d₆): 165.55, 164.36, 151.01, 149.01, 137.71, 136.94, 134.93, 132.78, 131.84, 130.94, 130.34, 129.88, 128.16, 128.05, 107.61, 61.35;
CHN: Calc. C_{63}H_{32}Cl_{2}N_{6}O_{3}: C, 61.33; H, 3.43; N, 10.22; Found: C, 61.38; H, 3.48; N, 10.27.

6,6′-(1,4-Phenylene)bis(N(2-chlorobenzylidene)-4-(2,4-di chlorophenyl)pyrimidin-2-amine) (s14)
IR (KBr, cm⁻¹): 2973.44 (C=H str.), 1599.67 (C=C str.), 1666.78 (N=CH str.), 1329.19 (C–N str.), 750.40 (Ar–Cl); MS ES⁺ (ToF): m/z 797 [M⁺+1]; ¹H-NMR (δ, DMSO-d₆): 7.24–8.00 (m, 18H, Ar–H), 9.01 (s, 2H, N=CH), 10.10 (s, 2H, (CH)₂ of pyrimidine ring); ¹³C-NMR (δ, DMSO-d₆): 166.55, 164.35, 162.50, 146.75, 136.24, 134.37, 131.85, 130.31, 130.22, 130.18, 129.90, 129.20, 128.03, 120.02, 128.00, 127.88, 100.90; CHN: Calc. C_{40}H_{22}Cl_{6}N_{6}: C, 60.10; H, 2.77; N, 10.51; Found: C, 60.17; H, 2.80; N, 10.55.

2,2′-(((6,6′-(1,4-Phenylene)bis(4-(2,4-dichlorophenyl)pyrimidine-6,2-diyi))bis(azanylylidene))diphenol (s15)
IR (KBr, cm⁻¹): 2972.97 (C=H str.), 1598.70 (C=C str.), 1698.99 (N=CH str.), 1330.19 (C–N str.), 750.53 (Ar–Cl); 3360.91 (O=H str.); MS ES⁺ (ToF): m/z 761 [M⁺+1]; ¹H-NMR (δ, DMSO-d₆): 7.27–7.99 (m, 18H, Ar–H), 9.99 (s, 2H, N=CH), 10.07 (s, 2H, (CH)₂ of pyrimidine ring); ¹³C-NMR (δ, DMSO-d₆): 165.55, 164.36, 137.78, 136.46, 130.31, 129.90, 128.12, 117.08, 110.04; CHN: Calc. C_{40}H_{22}Cl_{6}N_{6}O_{2}: C, 63.01; H, 3.17; N, 11.02; Found: C, 63.05; H, 3.19; N, 11.07.

6,6′-(1,4-Phenylene)bis(4-(2,4-dichlorophenyl)-N-(4-(diethy lamino)benzylidene)pyrimidin-2-amine) (s16)
IR (KBr, cm⁻¹): 2974.01 (C=H str.), 1590.98 (C=C str.), 1695.19 (N=CH str.), 1352.37 (C–N str.), 750.16 (Ar–Cl), 2826.51 (C=H str., –C₂H₅); MS ES⁺ (ToF): m/z 871 [M⁺+1]; ¹H-NMR (δ, DMSO-d₆): 7.26–8.00 (m, 18H, Ar–H), 9.63 (s, 2H, N=CH), 10.00 (s, 2H, (CH)₂ of pyrimidine ring), 3.38–3.49 [q, 8H, (CH₂)₄], 1.07–1.15 [t, 12H, (CH₃)₃]; ¹³C-NMR (δ, DMSO-d₆): 167.55, 164.36, 159.36, 136.42, 131.86, 130.30, 129.91, 128.94, 127.49, 126.62, 124.49, 111.0, 44.43, 12.74; CHN: Calc. C_{68}H_{42}Cl_{14}N_{6}: C, 66.06; H, 4.85; N, 12.84; Found: C, 66.10; H, 4.90; N, 12.88.

6,6′-(1,4-Phenylene)bis(N(3-chlorobenzylidene)-4-(2,4-di chlorophenyl)pyrimidin-2-amine) (s17)
IR (KBr, cm⁻¹): 2974.44 (C=H str.), 1579.02 (C=C str.), 1693.29 (N=CH str.), 1328.36 (C–N str.), 750.11 (Ar–Cl); MS ES⁺ (ToF): m/z 797 [M⁺+1]; ¹H-NMR (δ, DMSO-d₆): 7.25–8.03 (m, 18H, Ar–H), 10.00 (s, 2H, N=CH), 10.04 (s, 2H, (CH)₂ of pyrimidine ring); ¹³C-NMR (δ, DMSO-d₆): 165.55, 164.36, 136.91, 131.85, 131.77, 130.31, 130.23, 129.92, 129.21, 128.98, 128.10, 127.49, 126.65, 100.90; CHN: Calc. C_{48}H_{28}Cl_{14}N_{6}: C, 60.10; H, 2.77; N, 10.51; Found: C, 60.15; H, 2.80; N, 10.48.

4-(2,4-Dichlorophenyl)-6-(4-((E)-3-phenylallylidene)aminopyrimidin-4-yl phenyl)-N-(((E)-3-phenylallylidene)pyrimidin-2-amine) (s18)
IR (KBr, cm⁻¹): 2973.44 (C=H str.), 1597.13 (C=C str.), 1669.71 (N=CH str.), 1329.59 (C–N str.), 749.84 (Ar–Cl), 2829.12 (C–H str. aliphatic); ¹H-NMR (δ, DMSO-d₆): 7.45–8.04 (m, 20H, Ar–H), 7.55 [d, 2H, (CH)₂ of N=CH], 9.00 (s, 2H, (CH)₂ of pyrimidine ring); 6.86 [t, 2H, (CH)₂], 7.34 [d, 2H, (CH)₂]; ¹³C-NMR (δ, DMSO-d₆): 167.89, 164.23, 163.9, 135.6, 134.69, 133.56, 130.20, 130.87, 129.88, 128.00, 128.34, 127.02, 129.70, 120.12, 110.1; CHN: Calc. C_{44}H_{26}Cl_{14}N_{6}: C, 67.53; H, 3.61; N, 10.74; Found: C, 67.51; H, 3.68; N, 10.77.

Conclusion
In conclusion, we have developed a simple and efficient protocol for the synthesis of new bis-pyrimidine molecules (s1–s18) with appreciable yields. The in vitro antibacterial, antifungal and anticancer potential of all the synthesized compounds were investigated. It is evident that synthesized compounds, s7, s8, s11, s14, s16, s17 and s18 have excellent antimicrobial activity and compound s3 exhibited good anticancer activity. For the above compounds a significant improvement in their antibacterial and antifungal activities has been examined over the earlier reported compounds. The 4,4’-((1,4-phenylene)bis(pyrimidin-2-amine) molecules reported have a probability to emerge as a valuable lead series with great potential to be used as antibacterial, antifungal and anticancer agents and as promising candidates for further efficacy evaluation.

Authors’ contributions
BN and SK have designed, synthesized and carried out the antimicrobial activity and SML, KR, MV and SAAS have carried out the spectral analysis, interpretation and cytotoxicity study of synthesized compounds. All authors read and approved the final manuscript.

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