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**RESEARCH ARTICLE**

# Synthesis and biological evaluation of novel series of aminopyrimidine derivatives as urease inhibitors and antimicrobial agents

Laxman K. Adsul[1], Babasaheb P. Bandgar[1], Hemant V. Chavan[1], Shivkumar S. Jalde[1], Valmik D. Dhakane[1], and Amol L. Shirfule[2]

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**Abstract**

A novel series of carbazole substituted aminopyrimidines (**5a-p**) were synthesized and screened for their *in vitro* urease inhibition and antimicrobial activity. Among the compounds, 4-(2,4-dichlorophenyl)-6-(9-methyl-9H-carbazol-3-yl)-pyrimidin-2-amine **(5i)** was found to be the most potent showing urease inhibitory activity with an $IC_{50}$ value 19.4 ± 0.43 μM. Compounds **5c**, **5g, 5j** and **5o** showed good activity against all selected bacterial strains and compounds **5b**, **5c, 5m** and **5o** showed good activity against selected fungal strains. All the compounds were subjected for ADME predictions by computational method.

**Keywords:** Carbazole, aminopyrimidines, urease inhibition, antimicrobial activity



## Introduction

Urease inhibitors are considered as new targets for antiulcer drugs[1]. The activity of bacterial urease has been shown to be important virulence factor in the development of many harmful clinical conditions for human and animal health as well as agriculture[2]. Bacterial ureases have been reported to be involved in the formation of infectious stones and development of peptic ulcers and stomach cancer[3]. Ureases also contribute to the development of urolithiasis, pyelonephritis, hepatic encephalopathy, hepatic coma, and urinary catheter encrustation[4]. In the near past, a number of compounds have been proposed as urease inhibitors to reduce environmental problems and enhance the uptake of urea nitrogen by plants[5–8]. The treatment of infections caused by urease producing bacteria may also be possible by urease inhibition. In recent years, a variety of urease inhibitors have been studied, including acetohydroxamic acid, flurofamide, omeprazole, lansoprazole, rabaprazole, 1,4-benzoquinone and its derivatives, and inorganic metal salts, etc[9–11]. Various heterocyclic compounds were reported as urease inhibitors by several research groups. Khan et al. have synthesized biscoumarins which were shown as urease inhibitors[8]. Akhtar et al. have synthesized 3-substituted-4-amino-5-thioxo-1*H*-4*H*-1,2,4-triazoles derivatives showing their urease inhibition activity[12]. Rauf et al. have reported the synthesis and urease inhibition activity of barbituric and thiobarbituric acid derivatives[13]. More recently, Khan et al. also reported arylidene barbiturates as urease inhibitors[14]. Amines derived from deoxybenzoins are also reported as urease inhibitors[15].

The pyrimidine entity is one of the most prominent structures found in nucleic acid chemistry. Pyrimidine derivatives including uracil, thymine, cytosine, adenine, and guanine are fundamental building blocks for deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). Vitamin B1 (thiamine) is a well-known example of a naturally occurring pyrimidine that is encountered in our

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daily lives[16,17]. Pyrimidine derivatives form a component in a number of useful drugs and are associated with many biological, pharmaceutical and therapeutical activities[18]. Condensed pyrimidine derivatives have been reported as antimicrobial[19], analgesic[20], antiviral, anti-inflammatory[21], anti-HIV[22], antitubercular[23], antitumour[24], antineoplastic[25], antimalarial[26], diuretic[27] and antiplatelet agents[28].

Like pyrimidines, carbazole compounds also exhibits diverse biological properties. Carbazole and its derivatives are the important class of heterocyclic compounds endowed with various pharmacological activities such as anticancer, antimicrobial, antiviral, anti-inflammatory and antioxidant activity[29]. Carbazole scaffold is present in many drugs such as carvedilol and carprofen (Figure 1). Carvedilol, an antihypertensive drug, acts as a nonspecific β-adrengic antagonist[30]. Carprofen, a nonsteroidal anti-inflammatory drug (NSAID), is a selective COX-2 inhibitor[31]. More recently, a series of phenylcarbazole (Figure 1) molecules have been reported as antitumour agent[32], carbazole sulphonamides (Figure 1) are a novel class of antimitotic agents against solid tumours[33]. Syutkin et al.[34] have reported carbazole containing chalcones and pyrimidines as light emitting diodes.

It was envisaged that these two active pharmacophores, if linked together would generate novel molecular templates which are likely to exhibit interesting biological properties. The above-cited applications pyrimidines and barbiturates prompted us to synthesize a series of carbazole containing pyrimidine derivatives. Owing to this importance and in continuation of our work on synthesis of biologically active compounds[35–37], now we wish to describe the synthesis of carbazole containing aminopyrimidine derivatives (Scheme 1). The newly synthesized compounds were screened for their i*n-vitro* urease inhibition and antimicrobial activity. We also subjected all synthesized compounds for ADME predictions by computational method.

## Results and discussion

### Chemistry

The target compounds were synthesized as shown in Scheme 1. Carbazole (**1**) on methylation with methyl iodide gave 9-methyl carbazole (**2**), which on Vilsmeier-Haack formylation gave 3-formyl-9-methylcarbazole (**3**). The 3-formyl-9-methylcarbazole (**3**) on Claisen-Schimdt condensation with various aromatic acetophenones and heteroaromatic acetophenones in aqueous sodium hydroxide afforded compounds **4a-p**[37]. This on further treatment with guanidine hydrochloride in presence of NaH in DMF gave the target compounds **5a-p**. Compounds have been characterized by IR, $^1$H NMR and mass spectroscopy. In the $^1$H NMR spectra of these compounds, the pyrimidine ring proton was observed downfield as a singlet at δ ~7.80 and $NH_2$ proton appears at δ ~6.65 as a singlet.

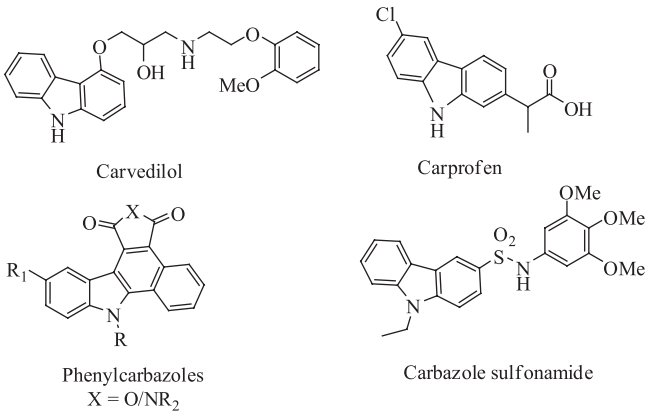
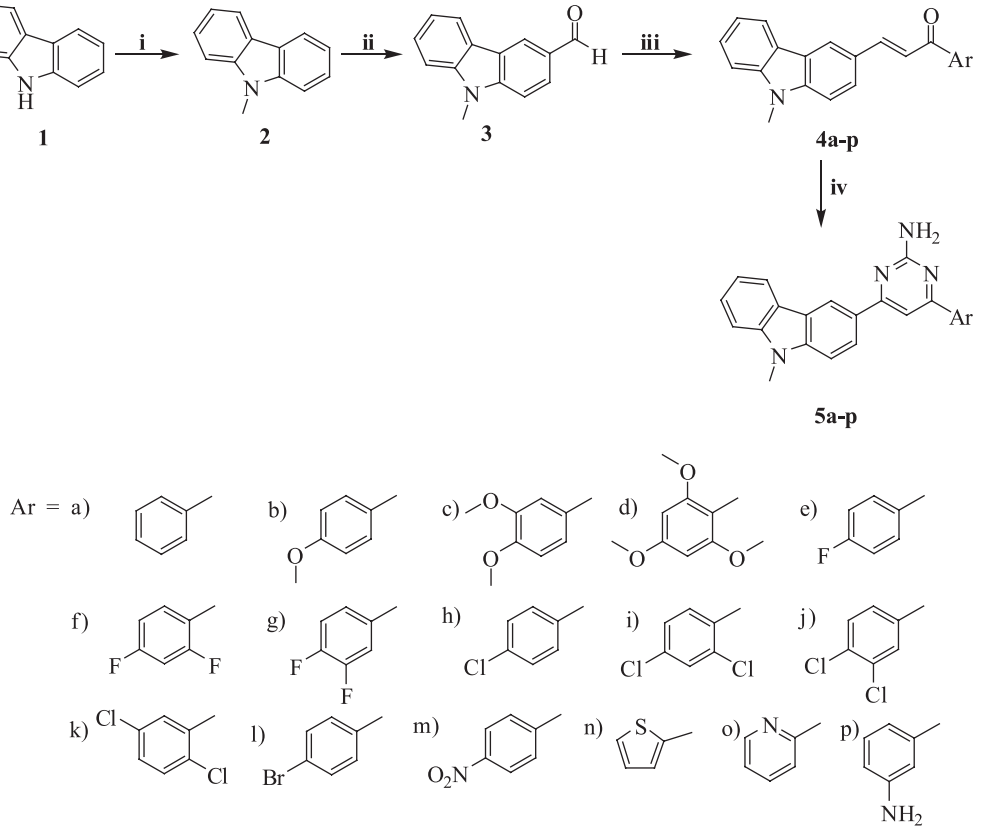



Figure 1. Pharmacologically active carbazole derivatives.

### Biological evaluation

The urease inhibition activity was carried out according to the literature protocol[38] using thiourea as the standard inhibitor having an $IC_{50}$ value of 21.0 ± 0.14 μM and the results are presented in Table 1. Out of 16 screened compounds, compounds **5c**, **5f**, **5g**, **5i**, **5j**, and **5k** were found to be active against urease. Compound **5i** proved to be the most potent showing urease inhibitory activity with an $IC_{50}$ value 19.4 ± 0.23 μM as compared to the standard thiourea. The compounds **5j** ($IC_{50}$ = 23.2 ± 0.58) and **5k** ($IC_{50}$ = 28.1 ± 0.59) exhibited good activity with an $IC_{50}$ value as compared to that of standard thiourea. Compound **5c** with 3,4-dimethoxy substituent was found to be good inhibitor with an $IC_{50}$ value of 30.7 ± 0.94. Compounds **5f** and **5g** were displayed moderate urease inhibitory activities with an $IC_{50}$ value 40.2 ± 0.31 and 45.2 ± 0.63, respectively. From these results of urease inhibitory activities by pyrimidine derivatives, following tentative results can be drawn; it appears that disubstituted halogen compounds were found to inhibit urease enzyme. Dichloro substituted compounds were showing promising urease inhibitory activity than difluoro and dimethoxy substituted compounds. Whereas, monosubstituted compound with electron donating and withdrawing substituent are weak inhibitors of urease. Heteroaryl substituted pyrimidine derivatives (compounds **5n** and **5o**) are also weak inhibitors of urease.

The synthesized pyrimidine derivatives were tested for their *in vitro* antimicrobial against five bacterial and two fungal species using disk diffusion method. Tetracycline and nystatin were used as standards against bacteria and fungi, respectively. The antibacterial activity against two Gram positive bacteria i.e. *Bacillus subtilis* (BS) and *Staphylococcus aureus* (SA), and three Gram negative bacteria, i.e. *Escherichia coli* (EC), *Shigella flexenari* (SF) and *Salmonella typhi* (ST) was tested at a sample concentration of 1 mg/mL in DMSO. The antibacterial activity data is tabulated in Table 2. The result indicated that among the tested compounds, compounds **5c**, **5g, 5j** and **5o** showed good activity against all selected bacterial strains at concentration of 1 mg/mL as compared to standard drug tetracycline. Compound **5o** showed comparable activity as that of standard, against *B. subtilis*




Scheme 1. Reagents and conditions: (i) $CH_3I$, NaH, DMF, rt, 3 h; (ii) DMF, $POCl_3$, 80°C, 3 h; (iii) Substituted acetophenones, NaOH, Ethanol, rt, 24 h; (iv) Guanidine.HCl, DMF, NaH, 110°C, 10 h.

Table 1. Urease inhibition activity.

| Compound | $IC_{50}$ ± SEM (μM)* |
|---|---|
| **5a** | >100 |
| **5b** | 95.4 ± 0.52 |
| **5c** | 30.7 ± 0.94 |
| **5d** | 73.7 ± 0.45 |
| **5e** | >100 |
| **5f** | 40.2 ± 0.31 |
| **5g** | 45.2 ± 0.63 |
| **5h** | >100 |
| **5i** | 19.4 ± 0.43 |
| **5j** | 23.2 ± 0.58 |
| **5k** | 28.1 ± 0.59 |
| **5l** | 95.1 ± 0.43 |
| **5m** | 87.2 ± 0.60 |
| **5n** | >100 |
| **5o** | >100 |
| **5p** | 83.7 ± 0.94 |
| **Thiourea** | 21.0 ± 0.14 |

*$IC_{50}$ values represent mean ± SD from three different experiments.

(BS), *S. aureus* (SA) and *S. flexenari* (SF). The remaining compounds showed moderate activity against all of the tested bacterial strains except *S. typhi* (ST).

The antifungal activity of pyrimidine derivatives against two fungal species, i.e. *Candida albicans* (CA) and *Aspergillus niger* (AN) was tested at a sample concentration of 1 mg/mL in DMSO using nystatin as standard. The antifungal activity data are tabulated in Table 2. The result indicated that among the tested compounds, compounds **5b**, **5c, 5m** and **5o** showed good activity against selected fungal strains at concentration of 1 mg/mL as compared to standard drug nystatin. Compounds **5m** and **5o** showed comparable activity as that of standard, against *C. albicans* (CA) and *A. niger* (AN). The remaining compounds showed moderate activity against *C. albicans* (CA). While, all remaining compounds showed less activity against *A. niger* (AN).

The bioavailability of the target compounds was assessed using ADME (absorption, distribution, metabolism, and excretion) prediction methods. In particular, we calculated the compliance of compounds to the Lipinski's rule of five[39,40]. Predictions of ADME properties for the studied compounds are shown in Table 3. The results showed that all the tested compounds comply with the rules. Compounds **5h, 5i, 5j, 5k** and **5l** have c Log *P* value more than 5 however, these compounds have molecular weight, hydrogen bond donor, hydrogen bond acceptor within the range of Lipinski rule of five and these compounds have low polar surface area (PSA) and molar volume (MV). Molecular polar surface area (PSA) is a very useful parameter for the prediction of drug transport properties. All the tested compounds have maximum absorption as they are having low PSA and MV. Drug-likeness score (a combined effect of physico-chemical properties, pharmacokinetics and pharmacodynamics of a compound and is represented by a numerical value) was computed by MolSoft (MolSoft 2007) software for

the 16 molecules under study. Maximum drug-likeness score was found to be 0.85 for the compound **5b**. Hence, these compounds do not pose absorption and permeation difficulty.

## Conclusion

A new series of carbazole substituted pyrimidine derivatives were synthesized and screened for their *in-vitro* urease inhibition and antimicrobial activity. Compounds **5c**, **5f**, **5g**, **5i**, **5j**, and **5k** were found to be active against urease. Compound, 4-(2,4-dichlorophenyl)-6-(9-methyl-9H-carbazol-3-yl)-pyrimidin-2-amine **5i** was found to be the most potent showing urease inhibitory activity with an $IC_{50}$ value 19.4 ± 0.43 μM. Compounds **5c**, **5g, 5j** and **5o** showed good activity against all selected bacterial strains and compounds **5b**, **5c, 5m** and **5o** showed good activity against selected fungal strains. ADME prediction revealed that the compounds could be considered as good candidates for drug development.

## Experimental section

### General

All the reagents and solvents used were of analytical grade and were used as supplied unless otherwise stated.

Table 2. Antimicrobial activity of pyrimidine derivatives.

| | Antibacterial activity | | | | | Antifungal activity | |
|---|---|---|---|---|---|---|---|
| Compound | EC | BS | SA | SF | ST | CA | AN |
| **5a** | – | – | 14 | – | – | 9 | 8 |
| **5b** | – | 11 | 11 | 13 | – | 14 | 12 |
| **5c** | 9 | 13 | 14 | 12 | 9 | 12 | 12 |
| **5d** | – | 10 | 10 | 8 | – | 9 | – |
| **5e** | – | 12 | 12 | 11 | – | 12 | 9 |
| **5f** | – | 16 | 11 | 10 | – | 13 | 10 |
| **5g** | 8 | 12 | 16 | 13 | – | 14 | 8 |
| **5h** | – | 9 | 8 | 9 | – | 8 | 8 |
| **5i** | – | 12 | 13 | 14 | 8 | 12 | 10 |
| **5j** | 9 | 13 | 14 | 12 | – | 9 | 9 |
| **5k** | – | 11 | 12 | 11 | 9 | 10 | 8 |
| **5l** | – | 10 | 13 | 10 | – | – | – |
| **5m** | – | 11 | 12 | 11 | – | 14 | 10 |
| **5n** | 8 | 9 | 10 | 12 | – | 9 | – |
| **5o** | 10 | 18 | 18 | 18 | – | 15 | 12 |
| **5p** | 9 | 12 | 15 | 14 | – | 10 | 8 |
| **Tetracycline** | 16 | 20 | 20 | 16 | 16 | – | – |
| **Nystatin** | – | – | – | – | – | 18 | 16 |

Concentration: 1 mg/mL in DMSO; Size of well 6 mm in diameter.
(–): Compounds showing no inhibition.
EC, *E. coli*; BS, *B. subtilis*; SA, *S. aureus*; SF, *S. flexenari*; ST, *S. typhi*; CA, *C. albicans*; AN, *A. niger*.

Table 3. In silico pharmacological parameters for bioavailability.

| Compounds | MW | c log p | HBA | HBD | PSA | MV | Drug likeness score |
|---|---|---|---|---|---|---|---|
| **5a** | 350 | 4.69 | 2 | 2 | 42.54 | 332.76 | –0.17 |
| **5b** | 380 | 4.75 | 3 | 2 | 50.92 | 364.61 | 0.85 |
| **5c** | 410 | 4.31 | 4 | 2 | 58.63 | 396.21 | 0.47 |
| **5d** | 440 | 4.15 | 5 | 2 | 66.85 | 425.79 | –0.36 |
| **5e** | 368 | 4.81 | 2 | 2 | 42.54 | 338.68 | –0.41 |
| **5f** | 386 | 4.45 | 2 | 2 | 42.54 | 343.60 | –0.26 |
| **5g** | 386 | 4.96 | 2 | 2 | 42.54 | 343.99 | –0.71 |
| **5h** | 384 | 5.38 | 2 | 2 | 42.54 | 349.96 | –0.21 |
| **5i** | 418 | 5.85 | 2 | 2 | 42.54 | 365.33 | 0.03 |
| **5j** | 418 | 5.99 | 2 | 2 | 42.54 | 365.28 | –0.43 |
| **5k** | 418 | 5.70 | 2 | 2 | 42.54 | 365.33 | –0.35 |
| **5l** | 428 | 5.59 | 2 | 2 | 42.54 | 354.62 | –0.72 |
| **5m** | 395 | 4.59 | 3 | 2 | 85.18 | 356.53 | –0.51 |
| **5n** | 356 | 4.40 | 3 | 2 | 43.56 | 327.64 | 0.52 |
| **5o** | 351 | 3.43 | 3 | 2 | 51.86 | 327.04 | –0.26 |
| **5p** | 365 | 3.86 | 2 | 4 | 63.62 | 340.34 | –0.85 |

HBA, hydrogen bond acceptors; HBD, hydrogen bond donors; MV, molecular volume; PSA, polar surface area.

Melting points were recorded in open capillaries with electrical melting point apparatus and were uncorrected. IR spectra (KBr disks) were recorded using a Perkin–Elmer 237 spectrophotometer. 1H NMR (200 MHz) spectra were recorded on a Bruker Avance spectrometer using DMSO-*d*6 as solvent. Mass spectra were recorded on a Shimadzu LCMS-QP 1000 EX. Thin layer chromatography (TLC) was performed on silica gel-coated plates for monitoring the reactions.

#### *General procedure for the synthesis of 4-(9-methyl-9H-carbazol-3-yl)-6-phenylpyrimidin-2-amine (5a-p)*

Chalcones **4a-p** (1.0 mmol) was dissolved in DMF (15 mL) and guanidine hydrochloride (2 mmol) was added to it. To this reaction mixture, NaH (4.0 mmol) was added and the reaction mixture was heated up to 110°C for 10 h. After completion of reaction (TLC), the reaction mixture was cooled to room temperature and water (15 mL) was added to it, solid product started to separate out. The solid was filtered and purified by column chromatography using chloroform as eluent to afford target compounds **5a-p**.

## Spectral data for synthesized compounds

#### *Synthesis of 4-(9-methyl-9H-carbazol-3-yl)-6-phenylpyrimidin-2-amine (5a)*

Yield: 90%; MP: 234–236°C; IR (KBr, $cm^{-1}$): 3368, 3325, 3214, 3020, 1641, 1559, 1372, 1216, 1149; $^1$H NMR (200 MHz, DMSO-$d_6$, δ in ppm): 3.82 (s, 3H, $NCH_3$), 6.66 (s, 2H, $NH_2$), 7.23 (t, 1H, *J* = 8 Hz, ArH), 7.48–7.58 (m, 4H, ArH), 7.61–7.73 (m, 2H, ArH), 7.80 (s, 1H, ArH), 8.23–8.28 (m, 3H, ArH), 8.40 (d, 1H, *J* = 8 Hz, ArH), 9.07 (s, 1H, ArH); MS: m/e 351 (M + 1).

#### *4-(4-methoxyphenyl)-6-(9-methyl-9H-carbazol-3-yl)-pyrimidin-2-amine (5b)*

Yield: 87%; MP: 210–212°C; IR (KBr, $cm^{-1}$): 3368, 3320, 3214, 3019, 1641, 1559, 1372, 1220, 1149, 930; $^1$H NMR (200 MHz, DMSO-$d_6$, δ in ppm): 3.80 (s, 3H, $OCH_3$), 3.87 (s, 3H, $NCH_3$), 6.66 (s, 2H, $NH_2$), 6.90 (d, 2H, *J* = 8 Hz, ArH), 7.23 (t, 1H, *J* = 8 Hz, ArH), 7.48–7.58 (m, 2H, ArH), 7.61–7.73 (m, 2H, ArH), 7.80 (s, 1H, ArH), 8.23–8.28 (m, 2H, ArH), 8.40 (d, 1H, *J* = 8 Hz, ArH), 9.07 (s, 1H, ArH); MS: m/e 381 (M + 1).

#### *4-(3,4-dimethoxyphenyl)-6-(9-methyl-9H-carbazol-3-yl)-pyrimidin-2-amine (5c)*

Yield: 86%; MP: 218–221°C; IR (KBr, $cm^{-1}$): 3368, 3320, 3214, 3019, 1641, 1559, 1372, 1220, 1149, 930; $^1$H NMR (200 MHz, DMSO-$d_6$, δ in ppm): 3.82 (s, 3H, $OCH_3$), 3.87 (s, 3H, $OCH_3$), 4.00 (s, 3H, $NCH_3$), 7.26 (d, 1H, *J* = 8 Hz, ArH), 7.38 (t, 1H, *J* = 8 Hz, ArH), 7.58 (t, 1H, *J* = 8 Hz, ArH), 7.70 (s, 1H, ArH), 7.81 (d, 1H, *J* = 8 Hz, ArH), 7.90 (s, 1H, ArH), 8.00–8.08 (m, 2H, ArH), 8.31 (d, 1H, *J* = 8 Hz, ArH), 8.45–8.50 (m, 1H, ArH), 9.24 (s, 1H, ArH). MS: m/e 411 (M+1).

#### *4-(9-methyl-9H-carbazol-3-yl)-6-(2,4,6-trimethoxyphenyl)-pyrimidin-2-amine (5d)*

Yield: 86%; MP: 208–211°C; IR (KBr, $cm^{-1}$): 3368, 3320, 3214, 3019, 1641, 1559, 1372, 1220, 1149, 930; $^1$H NMR (200 MHz, DMSO-$d_6$, δ in ppm): 3.80 (s, 3H, $OCH_3$), 3.85 (s, 6H, $OCH_3$), 4.01 (s, 3H, $NCH_3$), 6.35 (s, 2H, ArH), 6.66 (s, 2H, $NH_2$), 7.38 (t, 1H, *J* = 8 Hz, ArH), 7.58 (t, 1H, *J* = 8 Hz, ArH), 7.61–7.70 (m, 1H, ArH), 7.90 (s, 1H, ArH), 8.00–8.08 (m, 2H, ArH), 8.31 (d, 1H, *J* = 8 Hz, ArH), 9.24 (s, 1H, ArH); MS: m/e 441 (M + 1).

#### *4-(4-fluorophenyl)-6-(9-methyl-9H-carbazol-3-yl)-pyrimidin-2-amine (5e)*

Yield: 92%; MP: 230–232°C; IR (KBr, $cm^{-1}$): 3417, 3330, 3296, 3020, 1596, 1566, 1443, 1369, 1212, 1155, 1045, 932, 663; $^1$H NMR (200 MHz, DMSO-$d_6$, δ in ppm): 3.85 (s, 3H, $NCH_3$), 6.66 (s, 2H, $NH_2$), 7.23–7.58 (m, 6H, ArH), 7.80 (s, 1H, ArH), 8.23–8.28 (m, 4H, ArH), 9.12 (s, 1H, ArH); MS: m/e 369 (M + 1).

#### *4-(2,4-difluorophenyl)-6-(9-methyl-9H-carbazol-3-yl)-pyrimidin-2-amine (5f)*

Yield: 87%; MP: 112–116°C; IR (KBr, $cm^{-1}$): 3417, 3330, 3296, 3020, 1596, 1566, 1443, 1369, 1212, 1155, 1140, 930; $^1$H NMR (200 MHz, DMSO-$d_6$, δ in ppm): 3.85 (s, 3H, $NCH_3$), 6.66 (s, 2H, $NH_2$), 7.23 (t, 1H, *J* = 8 Hz, ArH), 7.48–7.58 (m, 3H, ArH), 7.61–7.73 (m, 2H, ArH), 7.80 (s, 1H, ArH), 8.23–8.28 (m, 2H, ArH), 8.40 (d, 1H, *J* = 8 Hz, ArH), 9.08 (s, 1H, ArH); MS: m/e 387 (M + 1).

#### *4-(3,4-difluorophenyl)-6-(9-methyl-9H-carbazol-3-yl)-pyrimidin-2-amine (5g)*

Yield: 83%. MP: 190–192°C; IR (KBr, $cm^{-1}$): 3417, 3330, 3296, 3020, 1596, 1566, 1443, 1369, 1212, 1155, 1140, 930; $^1$H NMR (200 MHz, DMSO-$d_6$, δ in ppm): 3.85 (s, 3H, $NCH_3$), 6.66 (s, 2H, $NH_2$), 7.23 (t, 1H, *J* = 8 Hz, ArH), 7.48–7.58 (m, 3H, ArH), 7.61–7.73 (m, 2H, ArH), 7.80 (s, 1H, ArH), 8.23–8.28 (m, 2H, ArH), 8.41 (d, 1H, *J* = 8 Hz, ArH), 9.07 (s, 1H, ArH); MS: m/e 387 (M + 1).

#### *4-(4-chlorophenyl)-6-(9-methyl-9H-carbazol-3-yl)-pyrimidin-2-amine (5h)*

Yield: 93%. MP: 220–222°C; IR (KBr, $cm^{-1}$): 3350, 3019, 2926, 2400, 1597, 1535, 1425, 1123, 1015, 928; $^1$H NMR (200 MHz, DMSO-$d_6$, δ in ppm): 3.87 (s, 3H, $NCH_3$), 6.77 (s, 2H, $NH_2$), 7.31 (t, 1H, *J* = 8 Hz, ArH), 7.48–7.58 (m, 5H, ArH), 7.83 (s, 1H, ArH), 8.22–8.38 (m, 3H, ArH), 8.45 (d, 1H, *J* = 8 Hz, ArH), 9.12 (s, 1H, ArH); MS: m/e 385 (M + 1).

#### *4-(2,4-dichlorophenyl)-6-(9-methyl-9H-carbazol-3-yl)-pyrimidin-2-amine (5i)*

Yield: 82%; MP: 210–212°C; IR (KBr, $cm^{-1}$): 3360, 3325, 3019, 2926, 2400, 1597, 1535, 1425, 1123, 1015, 930; $^1$H NMR (200 MHz, DMSO-$d_6$, δ in ppm): 3.87 (s, 3H, $NCH_3$), 6.80 (s, 2H, $NH_2$), 7.28 (t, 1H, *J* = 8 Hz, ArH), 7.48–7.58 (m, 5H, ArH), 7.83 (s, 1H, ArH), 8.25–8.38 (m, 3H, ArH), 9.02 (s, 1H, ArH); MS: m/e 419 ($M^+$).

***4-(3,4-dichlorophenyl)-6-(9-methyl-9H-carbazol-3-yl)-pyrimidin-2-amine (5j)***

Yield: 80%; MP: 184–187°C; IR (KBr, $cm^{-1}$): 3360, 3325, 3019, 2926, 2400, 1597, 1535, 1425, 1123, 1015, 930; $^1H$ NMR (200 MHz, DMSO-$d_6$, δ in ppm): 3.87 (s, 3H, $NCH_3$), 6.80 (s, 2H, $NH_2$), 7.32 (t, 1H, *J* = 8 Hz, ArH), 7.48–7.77 (m, 5H, ArH), 7.87 (s, 1H, ArH), 8.30 (d. 1H, *J* = 7.2 Hz, ArH), 8.37 (s, 1H, ArH), 8.48 (d, 1H, *J* = 8 Hz, ArH), 9.14 (s, 1H, ArH); MS: m/e 419 ($M^+$).

***4-(2,5-dichlorophenyl)-6-(9-methyl-9H-carbazol-3-yl)-pyrimidin-2-amine (5k)***

Yield: 86%; MP: 210–212°C; IR (KBr, $cm^{-1}$): 3360, 3325, 3019, 2926, 2400, 1597, 1535, 1425, 1123, 1015, 930; $^1H$ NMR (200 MHz, DMSO-$d_6$, δ in ppm): 3.87 (s, 3H, $NCH_3$), 6.80 (s, 2H, $NH_2$), 7.28 (t, 1H, *J* = 8 Hz, ArH), 7.48–7.58 (m, 5H, ArH), 7.83 (s, 1H, ArH), 8.20–8.40 (m, 3H, ArH), 9.02 (s, 1H, ArH); MS: m/e 419 ($M^+$).

***4-(4-bromophenyl)-6-(9-methyl-9H-carbazol-3-yl)-pyrimidin-2-amine (5l)***

Yield: 82%; MP: 260–262°C; IR (KBr, $cm^{-1}$): 3365, 3330, 3296, 3020, 1596, 1566, 1443, 1369, 1212, 1155, 1045, 932; $^1H$ NMR (200 MHz, DMSO-$d_6$, δ in ppm): 3.87 (s, 3H, $NCH_3$), 6.77 (s, 2H, $NH_2$), 7.31 (t, 1H, *J* = 8 Hz, ArH), 7.48–7.58 (m, 5H, ArH), 7.81 (s, 1H, ArH), 8.25–8.37 (m, 3H, ArH), 8.40 (d, 1H, *J* = 8 Hz, ArH), 9.10 (s, 1H, ArH); MS: m/e 431 (M + 2).

***4-(9-methyl-9H-carbazol-3-yl)-6-(4-nitrophenyl)-pyrimidin-2-amine (5m)***

Yield: 94%; MP: 144–146°C; IR (KBr, $cm^{-1}$): 3360, 3320, 3020, 1596, 1566, 1443, 1369, 1212, 1155, 1045, 932; $^1H$ NMR (200 MHz, DMSO-$d_6$, δ in ppm): 3.85 (s, 3H, $NCH_3$), 6.88 (s, 2H, $NH_2$), 7.28 (t, 1H, *J* = 8 Hz, ArH), 7.48–7.74 (m, 4H, ArH), 8.02 (s, 1H, ArH), 8.23–8.65 (m, 5H, ArH), 9.11 (s, 1H, ArH); MS: m/e 396 (M + 1).

***4-(9-methyl-9H-carbazol-3-yl)-6-(thiophen-2-yl)-pyrimidin-2-amine (5n)***

Yield: 80%; MP: 144–146°C; IR (KBr, $cm^{-1}$): 3360, 3325, 3019, 1595, 1560, 1445, 1369, 1212, 1155, 1045, 928; $^1H$ NMR (200 MHz, DMSO-$d_6$, δ in ppm): 3.87 (s, 3H, $NCH_3$), 6.77 (s, 2H, $NH_2$), 7.23 (t, 1H, *J* = 8 Hz, ArH), 7.35 (t, 1H, *J* = 3.9 and 4.8 Hz, ArH), 7.48–7.60 (m, 3H, ArH), 7.80 (s, 1H, ArH), 7.93 (d, 1H, *J* = 2.1 Hz, ArH), 8.23–8.28 (m, 2H, ArH), 8.40 (d, 1H, *J* = 8 Hz, ArH), 9.12 (s, 1H, ArH); MS: m/e 357 (M + 1).

***4-(9-methyl-9H-carbazol-3-yl)-6-(pyridin-2-yl)-pyrimidin-2-amine (5o)***

Yield: 65%; MP: 161–164°C; IR (KBr, $cm^{-1}$): 3368, 3325, 3019, 1595, 1560, 1445, 1369, 1212, 1155, 1045, 932; $^1H$ NMR (200 MHz, DMSO-$d_6$, δ in ppm): 3.87 (s, 3H, $NCH_3$), 6.80 (s, 2H, $NH_2$), 7.29 (t, 1H, *J* = 8 Hz, ArH), 7.48–7.58 (m, 4H, ArH), 7.61–7.73 (m, 2H, ArH), 7.80 (s, 1H, ArH), 8.25–8.38 (m, 2H, ArH), 8.40 (d, 1H, *J* = 8 Hz, ArH), 9.12 (s, 1H, ArH); MS: m/e 352 (M + 1).

***4-(3-aminophenyl)-6-(9-methyl-9H-carbazol-3-yl)-pyrimidin-2-amine (5p)***

Yield: 70%; MP: 178–180°C; IR (KBr, $cm^{-1}$): 3360, 3320, 3020, 2925, 1595, 1560, 1445, 1369, 1212, 1155, 1045, 930; $^1H$ NMR (200 MHz, DMSO-$d_6$, δ in ppm): 3.87 (s, 3H, $NCH_3$), 6.40 (s, 2H, $NH_2$), 6.79 (s, 2H, $NH_2$), 7.23 (t, 1H, *J* = 8 Hz, ArH), 7.48–7.58 (m, 4H, ArH), 7.61–7.73 (m, 2H, ArH), 7.80 (s, 1H, ArH), 8.23–8.28 (m, 2H, ArH), 8.40 (d, 1H, *J* = 8 Hz, ArH), 9.09 (s, 1H, ArH); MS: m/e 366 (M + 1).

## Urease inhibition assay

Mixture of 25 µL of urease enzyme (Jack bean) solution containing 1 unit of enzyme (1 unit = 0.02 mg of enzyme) was dissolved in phosphate buffer of pH 6.8, 55 µL of buffer containing 100 mM urea, and 5 µL of test compounds (0.5 mM concentration) was incubated at 30°C for 15 min in 96-well plates. Urease activity was determined by measuring ammonia production using the iodophenol method[38]. Briefly, 45 µL each of phenol reagent and 70 µL of alkali reagent were added to each well. The increase in absorbance at 630 nm was measured after 50 min, using a microplate reader (Molecular Device, CA, USA). All reactions were performed in triplicate in a final volume of 200 µL. The results (change in absorbance per min) were processed using Soft-Max Pro software (Molecular Device, CA, USA). Thiourea was used as the standard inhibitor and percentage inhibitions were calculated as follow: $100 - (OD_{testwell}/OD_{control}) \times 100$.

## Antimicrobial activity

Antimicrobial activities of all the synthesized compounds were determined by disc diffusion method[41]. All the bacterial and fungal species used were procured from Institute of Microbial Type Culture Collection (IMTCC), Chandigarh, India, and National Collection of Industrial Microorganisms (NCIM), Pune, India, namely, *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, *Shigella flexenari*, *Salmonella typhi*, *Candida albicans*, and *Aspergillus niger*. The test compounds were dissolved in dimethyl sulfoxide (DMSO) at a concentration of 1 mg/mL. 20 mL of sterilized agar media was poured into each presterilized Petri dish. Excess of suspension was decanted and plates were dried by placing in an incubator at 37°C for an hour. About 60 µL of 24-h-old culture suspension were poured and neatly swabbed with the presterilized cotton swabs. 6-mm diameter well was then punched carefully using a sterile cork borer and 30 µL of test solutions were added into each labelled well. Inoculated plates in duplicate were then incubated at 37 ± 0.5°C for antibacterial activity for 24 h and at 25 ± 0.5°C for antifungal activity for 48 h. After incubation, the antimicrobial activity was measured in terms of the zone of inhibition in mm.

## Calculation of drug-likeness properties

The physicochemical properties such as molecular weight, c Log *P*, HBA, HBD, polar surface area, molecular volume and drug likeness of the synthesized compounds

are studied from online Osiris property explorer for drug bioavailability of chemical compounds[42].

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## Declaration of interest

The authors report no conflicts of interest.

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