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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

# Synthesis and biological studies of a novel series of 4-(4-(1*H*-imidazol-1-yl)phenyl)-6-arylpyrimidin-2-amines

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**Abstract** A novel series of eleven 4-(4-(1*H*-imidazol-1-yl)phenyl)-6-arylpyrimidin-2-amines has been prepared from synthesized 3-[4-(1*H*-imidazol-1-yl) phenyl]prop-2-en-1-ones and evaluated for phosphodiesterase (PDE) inhibition and antimicrobial activities. *N*-arylation of imidazole with 4-fluorobenzaldehyde using hexadecyltrimethylammonium bromide as catalyst gave 4-(1*H*-imidazol-1-yl) benzaldehyde which on treatment with substituted acetophenones yielded corresponding chalcones (**1a**–**1k**). Each chalcone on further reaction with guanidine hydrochloride resulted in title compounds (**2a**–**2k**). Pyrimidines thus synthesized were subjected to biological studies. Some compounds showed marked activities in PDE inhibition and anti-bacterial and anti-fungal bioassays.

**Keywords** Arylpyrimidin-6-amine · Phosphodiesterases · N-arylation · Anti-fungal

## Introduction

Phosphodiesterases (PDEs) form a unique class of enzymes that hydrolyse cyclic nucleotides and thus play a vital role in cell function by regulating intracellular levels of cyclic adenine monophosphate (cAMP) and cyclic guanine monophosphate (cGMP). Theophylline and Papaverine have historically been used as drugs and are known to be weak inhibitors of PDE. The discovery of several isoenzyme families provides a pace for the development of isoenzyme selective inhibitors for the treatment of various ailments. The role of PDE3 inhibitors for congestive heart failure (Barnes *et al*., 1988; Lugnier, 2006; Nicholson *et al*., 1991), PDE4 inhibitors for inflammatory airways (Muller *et al*., 1996; Torphy and Undem, 1991) and most successfully, PDE5 inhibitors for erectile dysfunction (Murray, 1993) is widely recognised. The structure of one of the famous PDE5 inhibitors, Sildinafil and its analogues is based on pyrimidine scaffold. (Toque *et al*., 2008)

Pyrimidine ring system is present in pyrimidine and purine bases of DNA and RNA. In purines, both pyrimidine as well as imidazole rings are fused together. Pyrimidine derivatives are reported as highly potentially biologically active compounds including antimicrobial (Ballell *et al*., 2007; Rao *et al*., 2003), anticancer (Miyazaki *et al*., 2005), anti-inflammatory and analgesic (Breault and Pease, 2000; Venu *et al*., 2008; Zienab *et al*., 2011), anti-HCV (Chamakura *et al*., 2007), anti-HIV (Malik *et al*., 2006), anti-oxidant (Biagi *et al*., 1996), anti-aging (Bbizhayev, 2006) and several others. A variety of synthetic imidazole derivatives themselves are extensively used as amoebicidal (Metronidazole or Flagyl®, Tinidazole, Timorazole), anti-fungal (Emami *et al*., 2008) (Miconazole, Ketoconazole), anti-thyroid (Carbizole®), anti-ulcer (Omeprazole, Cimetidine, e.g. Cimet®), anxiolytic (Loprazolam®) and several other drugs available in the market. Recent research shows that chalcones based on imidazole scaffold exhibit anti-oxidant, anti-fungal and anti-leishmanial activities (Hussain *et al*., 2009).

This work is dedicated to our beloved teacher, the Late **Professor Dr. Hamid Latif Siddiqui**, University of the Punjab, Lahore-Pakistan.

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As both the ring systems play a vital role in our life, we planned to study their synergic effect as PDE inhibitors and as anti-bacterial and anti-fungal agents.

## Results and discussion

### Chemistry

*Synthesis of 3-[4-(1H-imidazol-1-yl) phenyl]prop-2-en-1-ones (**1a–1k**)*

Imidazole was N-arylated with 4-fluorobenzaldehyde in the presence of hexadecyltrimethylammonium bromide as a catalyst. 4-(1*H*-Imidazol-1-yl) benzaldehyde thus synthesized was then treated with various substituted acetophenones using 10 % NaOH solution in MeOH to get the corresponding chalcones (**1a–1k**).

*Synthesis of 4-(4-(1H-imidazol-1-yl)phenyl)-6-arylpyrimidin-2-amines (**2a–2k**)*

Each chalcone was treated with guanidine hydrochloride and 50 % aqueous KOH solution in EtOH at reflux temperature followed by portion wise addition of 30 % $H_2O_2$ solution under the same conditions (Scheme 1) (Varga *et al*., 2003). The reaction was frequently monitored visualising TLC, and after completion of reaction, precipitates formed were filtered, dried and thoroughly washed with cold MeOH and then cold water. The semi-pure product was purified by column chromatography using $CHCl_3$/MeOH (4:1) solvent system. Each title compound was characterized by spectral studies and elemental analysis which was found in accordance to the calculated values (Scheme 1) (Hussain *et al*., 2009).

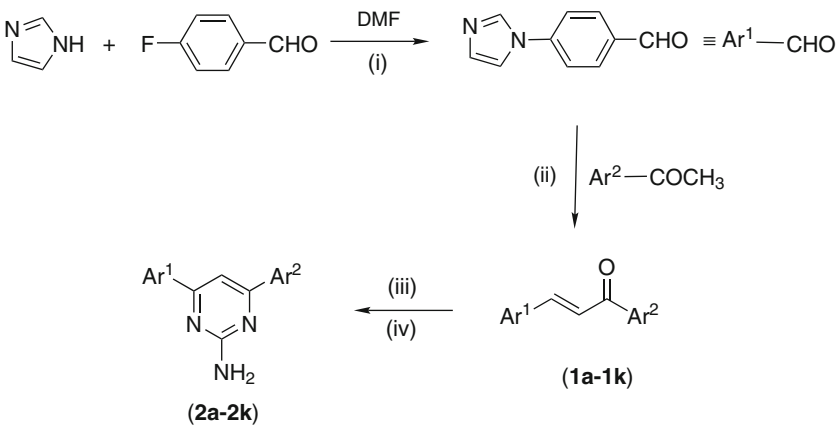
### Characterisation

All the pyrimidines were characterized with the help of NMR, IR and MS spectral studies and CHN analysis. The IR spectra of each compound synthesized showed characteristic absorptions for $NH_2$, N–C, N=C and aromatic functionalities present in each compound. Absorption at $\sim$1550 $cm^{-1}$ is characteristic for pyrimidine skeleton. A characteristic peak in the range of $\delta$ 6.67–7.08 ppm in $^1$H-NMR as a broad singlet integrated for two protons was assigned to $NH_2$ protons (Varga *et al*., 2003). A singlet was assigned to imidazole H-2 at near $\delta$ 7.15 ppm. Another singlet, in most cases was differentiable in the aromatic region which may be assigned to H-5 of pyrimidine ring. Table 1

### Biological activities of compounds 2a–2k

*Phosphodiesterase inhibition activity of compounds **2a–2k***

It has been reported that Imazodan CI-914 (I), CI-930 (II), and related compounds 4,5-dihydro-6-[4-(1*H*-imidazol-1-yl)phenyl]-3(2H)-pyridazinones, have positive inotropic activity(Bristol *et al*., 1984; Sircar *et al*., 1985). Series of heterocyclic systems have been investigated for their inotropic activity. These studies revealed the contribution of the (I*H*-imidazol-1-yl)phenyl moiety for superior inotropic activity in comparison with other more conventional aromatic substituents. Keeping in view this fact, we studied the phosphodiesterase inhibition activity of the compounds. The results are shown in Table 2.




**Scheme 1** Synthesis of 4-(4-(1*H*-imidazol-1-yl)phenyl)-6-arylpyrimidin-2-amines (**2a–2k**) from 3-[4-(1*H*-imidazol-1-yl) phenyl]prop-2-en-1-ones (**1a–1k**)

**Table 1** General description of 3-[4-(1H-imidazol-1-yl) phenyl]-prop-2-en-1-ones (**1a–1k**) and 4-(4-(1H-imidazol-1-yl)phenyl)-6-arylpyrimidin-2-amines (**2a–2k**)

| Compounds | $Ar^2$ | Compounds | $Ar^2$ |
|---|---|---|---|
| **1a** | 4-chlorophenyl | **2a** | 4-chlorophenyl |
| **1b** | 4-bromophenyl | **2b** | 4-bromophenyl |
| **1c** | 4-methoxyphenyl | **2c** | 4-methoxyphenyl |
| **1d** | 3-methoxyphenyl | **2d** | 3-methoxyphenyl |
| **1e** | 3,4-dimethoxyphenyl | **2e** | 3,4-dimethoxyphenyl |
| **1f** | 2,4-dichlorophenyl | **2f** | 2,4-dichlorophenyl |
| **1g** | 4-fluorophenyl | **2g** | 4-fluorophenyl |
| **1h** | 2,3,4-trichlorophenyl | **2h** | 2,3,4-trichlorophenyl |
| **1i** | 2,5-dichlorophenyl | **2i** | 2,5-dichlorophenyl |
| **1j** | 4-iodophenyl | **2j** | 4-iodophenyl |
| **1k** | Phenyl | **2k** | Phenyl |

**Table 2** Phosphodiesterase inhibition activity of compounds **(2a–2k)**

| Compound | Conc. (mM) | % Inhibition |
|---|---|---|
| **2a** | 0.2 | Negative |
| **2b** | 0.1 | 9.2 |
| **2c** | 0.2 | 12.0 |
| **2d** | 0.1 | 22.4 |
| **2e** | 0.1 | Negative |
| **2f** | 0.2 | Negative |
| **2g** | 0.1 | Negative |
| **2h** | 0.2 | 12.0 |
| **2i** | 0.1 | Negative |
| **2j** | 0.1 | 11.7 |
| **2k** | 0.2 | Negative |

*Anti-bacterial activities*

The compounds (**2a–2k**) were subjected to anti-bacterial studies. Some compounds exhibited moderate activity against *Bacillus subtillis, Escherichia coli* and *Staphylococcus aureus.* Particularly, compound **2a**, **2h** and **2j** showed significant activity against *E. coli.* Compound **2j** exhibited marked activity also against *S. aureus* and *B. subtillis.* This compound with *p*-iodophenyl moiety may be undertaken for further biological investigations. All the compounds showed insignificant activity against *Shigella flexenari, Pseudomonas aeruginosa* and *Salmonella typhi.* The results of anti-bacterial activity are summarized in Table 3.

*Antifungal activities*

The synthesized compounds were tested for anti-fungal activities against three fungal species viz. *Alternaria alternata, Aspergillus flavus* and *Aspergillus fumigatus* procured from Biofertilizers and Biopesticide Laboratory, Institute of Mycology & Plant Pathology, University of the Punjab Lahore, Pakistan. The results are summarized in Table 4.

## Conclusion

In short, we evaluated 4-(4-(1*H*-imidazol-1-yl)phenyl)-6-arylpyrimidin-2-amines (**2a–2k**) for their antimicrobial and Phosphodiesterase Inhibition Activity. These compounds are structural hybrids of two excellent bioactive heterocycles i.e. imidazole and pyrimidine. Thus, it is proposed that the title compounds may possess the biological activities of parent ring systems and could be used as template for further discoveries.

## Experimental

Conversion of imidazole to 4-(4-(1*H*-imidazol-1-yl)phenyl)-6-arylpyrimidin-2-amines

*Procedure for synthesis of 4-(1H-imidazol-1-yl)benzaldehyde*

A mixture of imidazole (3.40 g, 50 mmol), anhydrous potassium carbonate (6.90 g, 50 mmol), 4-fluorobenzaldehyde (6.2 g, 50 mmol), hexadecyltri-*n*-butylphosphonium bromide (10 mg) and dimethylformamide (30.0 mL) was stirred for a period of 15 h at 100 °C. The contents were poured onto ice cold water (100 mL) after cooling to room temperature. Pale yellow precipitates obtained were filtered, dried and crystallized from methanol. Yield: 78.3 %; mp 151–153 °C (Hussain *et al*., 2009).

*Procedure for synthesis of 3-(4-(1H-imidazol-1-yl) phenyl)prop-2-en-1-ones (**1a–1k**)*

All chalcones were synthesized according to the procedure which has already been reported by us (Hussain *et al*., 2009). A solution of NaOH (40 %; 10.0 mL) was added drop wise to a mixture of 4-(1*H*-imidazol-I-yl)benzaldehyde (10.0 mmol, 1.72 g), corresponding substituted acetophenone (10.0 mmol) and methanol (50 mL) over a period of 30–40 min with continuous stirring at ambient temperature till completion of the reaction monitored frequently by TLC. Precipitates thus obtained, were filtered, washed with cold MeOH followed by cold water. Finally, recrystallization from MeOH gave the title compound.

**Table 3** Anti-bacterial activity (zone of inhibition in mm) of compounds (**2a–2k**)

| Compound | *Bacillus subtillis* | *Escherichia coli* | *Staphylococcus aureus* | *Shigella flexneri* | *Pseudomonas aeruginosa* | *Salmonella typhi* |
|---|---|---|---|---|---|---|
| **2a** | 12 | 18 | _ | 11 | _ | _ |
| **2b** | 13 | 12 | 12 | 12 | _ | _ |
| **2c** | 14 | 12 | _ | 11 | 11 | _ |
| **2d** | _ | 13 | 14 | _ | 12 | _ |
| **2e** | _ | 15 | 13 | _ | 10 | _ |
| **2f** | _ | 14 | 11 | 10 | _ | _ |
| **2g** | _ | 16 | _ | 12 | _ | _ |
| **2h** | 15 | 18 | 15 | 11 | _ | _ |
| **2i** | 12 | 12 | _ | 12 | _ | _ |
| **2j** | 21 | 20 | 16 | _ | _ | _ |
| **2k** | 13 | 11 | _ | _ | _ | _ |
| Imipenem | 30 | 35 | 30 | 30 | 31 | 25 |

**Table 4** Anti-fungal activities of compounds (**2a–2k**)

| Compounds | MIC (mg/mL) | | |
|---|---|---|---|
| | *Alternaria alternata* | *Aspergillus fumigatus* | *Aspergillus flavus* |
| **2a** | 1.0 | 1.0 | 1.0 |
| **2b** | >1.0 | 1.0 | 1.0 |
| **2c** | >1.0 | 1.0 | >1.0 |
| **2d** | 1.0 | >1.0 | 1.0 |
| **2e** | >1.0 | >1.0 | 1.0 |
| **2f** | 1.0 | >1.0 | >1.0 |
| **2g** | 1.0 | 0.50 | 1.0 |
| **2h** | >1.0 | >1.0 | >1.0 |
| **2i** | >1.0 | >1.0 | >1.0 |
| **2j** | 1.0 | 1.0 | 1.0 |
| **2k** | >1.0 | 1.0 | 1.0 |
| Mancozeb | 0.25 | 0.25 | 0.25 |

A typical procedure for the synthesis of 4-(4-(1*H*-imidazol-1-yl)phenyl)-6-arylpyrimidin-2-amines (**2a–2k**)

*4-(4-(1H-imidazol-1-yl)phenyl)-6-(4-chlorophenyl)pyrimidin-2-amine (**2a**)*

3-(4-(1*H*-Imidazol-1-yl) phenyl)-1-(4-chlorophenyl)prop-2-en-1-one (2.8 g, 9.08 mmol), guanidine hydrochloride (1.3 g, 1.5 mmol), ethanol (20 mL) and 50 % aqueous KOH solution (4 mL,) were mixed together, then heated up and stirred at reflux temperature for 1 h. Under the same conditions, 30 % aqueous $H_2O_2$ (3.1 mL, 27.3 mmol) was added to the above mixture in small portions over a period of I hr. The ethanol was removed under reduced pressure in a rotary evaporator and distiled water ($\sim$20 mL) was added to the residue. The product was easily isolated as precipitates and was washed repeatedly with pure water. The still crude product was recrystallized from ethanol and was dried finally in a vacuum desiccator over $P_2O_5$/KOH.

Compound **2a**: (52 %, Pale yellow crystals), m. p. 252–254 °C. IR (KBr) $\nu_{max}$ $cm^{-1}$: 3328, 3205, 1690, 1546, 675; $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 6.81 (2H, br. s, $NH_2$), 7.14 (1H, s, H-2 imidazole), 7.56–7.60 (3H, m, ArH), 7.81–7.87 (4H, m, ArH), 8.27 (2H, d, $J = 8.4$ Hz, $^{Ar2}$H-3 + $^{Ar2}$H-5), 8.36–8.39 (2H, m, $^{Ar2}$H-2 + $^{Ar2}$H-6). MS *m/z*: 347.09 ($M^+$). $^{13}$C NMR: 100.8, 115.5, 115.7, 120.9, 126.0, 126.2 126.4, 126.6, 128.9 130.2, 130.4, 131.6, 133.5, 134.0, 135.1, 135.3, 136.8, 164.4, 167.2. Anal. Calc. for $C_{19}H_{14}ClN_5$; C, 65.61; H, 4.06; N, 20.14: Found: C, 65.64; H, 4.05; N, 20.15.

*4-(4-(1H-imidazol-1-yl)phenyl)-6-(4-bromophenyl)pyrimidin-2-amine (**2b**)*

The experimental procedure was similar to that described for compound **2a** starting from 3-(4-(1*H*-imidazol-1-yl) phenyl)-1-(4-bromophenyl)prop-2-en-1-one.

Compound **2b**: (46 %, Yellow powder), m. p. 225–227 °C, IR (KBr) $\nu_{max}$ $cm^{-1}$: 3310, 3195, 1680, 1532, 670. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 7.08 (2H, br. s, $NH_2$), 7.26 (3H, d, $J = 5.6$ Hz), 7.50–7.58 (6H, m, ArH), 7.71 (1H, s, H-5), 8.21–8.25 (2H, m, ArH). $^{13}$C NMR: 101.6, 115.6, 115.8, 120.7, 122.9, 125.1, 125.4, 125.6, 125.8, 130.4, 133.3, 133.6, 133.9 134.6, 135.4, 136.5, 162.4, 164.1, 167.1. MS *m/z*: 391.04 ($M^+$). Anal. calc. for $C_{19}H_{14}BrN_5$; C, 58.18; H, 3.60; N, 17.85; Found: C, 58.20; H, 3.61; N, 17.82.

*4-(4-(1H-imidazol-1-yl)phenyl)-6-(4-methoxyphenyl)-pyrimidin-2-amine (**2c**)*

The experimental procedure was similar to that described for compound **2a** starting from 3-(4-(1*H*-imidazol-1-yl) phenyl)-1-(4-methphxyenyl)prop-2-en-1-one.

Compound **2c**: (55 %, Whitish amorphous solid), m. p. 211–213 °C. IR (KBr) $\nu_{max}$ $cm^{-1}$: 3380, 3086, 1645, 1545, 688. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 3.82 (3H, s, $OCH_3$), 6.78 (2H, br. s, $NH_2$), 7.05 (1H, d, $J = 6.8$ Hz, ArH), 7.16 (1H, s, $^{Ar1}$H-2, imidazole), 7.77 (1H, s, H-5), 7.81–7.84 (4H, m, ArH), 7.89 (2H, d, $J = 8.4$ Hz, ArH), 8.03–8.07 (3H, m, ArH). $^{13}$C NMR: 23.5, 102.2, 115.5, 115.9, 118.5, 123.5, 124.0, 129.2 (2C), 130.1, 130.3, 131.2, 132.1, 133.1, 135.1, 135.6, 136.5, 161.2, 165.0, 168.5. MS *m/z*: 343.14 ($M^+$). Anal. calc. for $C_{20}H_{17}N_5O$; C, 69.96; H, 4.99; N, 20.40; Found: C, 69.95; H, 4.98; N, 20.38.

*4-(4-(1H-imidazol-1-yl)phenyl)-6-(3-methoxyphenyl)-pyrimidin-2-amine (**2d**)*

The experimental procedure was similar to that described for compound **2a** starting from 3-(4-(1*H*-imidazol-1-yl) phenyl)-1-(3-methphxyenyl)prop-2-en-1-one.

Compound **2d**: (44 %, Yellow amorphous solid), m. p. 232–234 °C. IR (KBr) $\nu_{max}$ $cm^{-1}$. 3390, 3080, 1635, 1533, 690. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 3.84 (3H, s, $OCH_3$), 6.82 (2H, br. s, $NH_2$), 7.15 (1H, d, $J = 8.4$ Hz, ArH), 7.19 (1H, s, $^{Ar1}$H-2, imidazole), 7.67 (1H, s, ArH), 7.76–7.80 (3H, m, ArH), 7.84 (1H, s, ArH), 7.93 (2H, d, $J = 8.4$ Hz, ArH), 8.01–8.03 (3H, m, ArH). $^{13}$C NMR: 21.6, 101.2, 114.5, 114.9, 118.5, 123.8, 124.0, 128.2, 128.5, 129.2, 130.1, 132.1, 133.1, 135.1, 135.6, 136.5, 138.1, 162.2, 164.0, 167.1. MS *m/z*: 343.14 ($M^+$). Anal. calc. for $C_{20}H_{17}N_5O$; C, 69.96; H, 4.99; N, 20.40; Found: C, 69.95; H, 4.98; N, 20.38.

*4-(4-(1H-imidazol-1-yl)phenyl)-6-(3,4-dimethoxyphenyl)-pyrimidin-2-amine (**2e**)*

The experimental procedure was similar to that described for compound **2a** starting from 3-(4-(1*H*-imidazol-1-yl) phenyl)-1-(3,4-dimethoxyphenyl)prop-2-en-1-one.

Compound **2e**: (55 %, Whitish amorphous solid), m. p. 318–320 °C. IR (KBr) $\nu_{max}$ $cm^{-1}$: 3410, 3090, 1640, 1545, 692. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 3.83 (3H, s, $OCH_3$), 3.87 (3H, s, $OCH_3$), 6.67 (2H, br. s, $NH_2$), 7.08 (1H, d, $^{Ar2}$H-5), 7.14 (1H, s, $^{Ar1}$H-2, imidazole), 7.72 (1H, s, H-5), 7.79–7.82 (3H, m, ArH), 7.85–7.88 (2H, m, ArH), 8.34–8.38 (3H, m, ArH). $^{13}$C NMR: 19.6, 21.5, 100.2, 115.5, 115.9, 118.5, 123.1, 123.3, 128.3, 128.5, 129.2, 129.9, 130.1, 131.1, 135.1, 135.6, 137.0, 139.1, 162.2, 164.1, 167.0. MS *m/z*: 373.15 ($M^+$). Anal. calc. for $C_{21}H_{19}N_5O_2$; C, 67.55; H, 5.13; N, 18.76; Found: C, 67.55; H, 5.13; N, 18.75.

*4-(4-(1H-imidazol-1-yl)phenyl)-6-(2,4-dichlorophenyl)-pyrimidin-2-amine (**2f**)*

The experimental procedure was similar to that described for compound **2a** starting from 3-(4-(1*H*-imidazol-1-yl) phenyl)-1-(2,4-dichlorophenyl)prop-2-en-1-one.

Compound **2f**: (54 %, Yellowish solid), m. p. 224–226 °C. IR (KBr) $\nu_{max}$ $cm^{-1}$: 3428, 3110, 1680, 1570, 655. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 6.89 (2H, br. s, $NH_2$), 7.13 (1H, s, H-2 imi), 7.41 (1H, s, $^{Ar2}$H-3), 7.56 (1H, d, $J = 7.6$ Hz, $^{Ar2}$H-5), 7.64 (1H, d, $J = 8.0$ Hz ArH), 7.77–7.85 (4H, m, ArH), 8.25 (2H, d, $J = 8.0$ Hz, $^{Ar1}$H-2 + $^{Ar1}$H-6), 8.37 (1H, s, H-5). $^{13}$C NMR: 102.9, 115.6, 115.9, 119.5, 124.4, 125.2, 129.3, 129.5, 130.8, 131.1, 131.9, 132.1, 135.1, 135.6, 136.5, 162.7, 164.2, 165.9, 168.1. MS *m/z*: 381.05 ($M^+$). Anal. calc. for $C_{19}H_{13}Cl_2N_5$; C, 59.70; H, 3.43; N, 18.32: Found: C, 59.72; H, 3.40; N, 18.35.

*4-(4-(1H-imidazol-1-yl)phenyl)-6-(4-fluorophenyl)-pyrimidin-2-amine (**2g**)*

The experimental procedure was similar to that described for compound **2a** starting from 3-(4-(1*H*-imidazol-1-yl) phenyl)-1-(4-fluorophenyl)prop-2-en-1-one.

Compound **2g**: (63 %, Yellow powder), m. p. 255–256 °C. IR (KBr) $\nu_{max}$ $cm^{-1}$: 3446, 3100, 1670, 1580, 685. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 6.77 (2H, br. s, $NH_2$), 7.14 (1H, s, H-2 imidazole), 7.35 (2H, m, $^{Ar2}$H-3 + $^{Ar2}$H-5), 7.77–7.83 (3H, m, ArH), 7.87 (1H, s, H-5), 8.29–8.32 (2H, m, ArH), 8.36–8.39 (3H, m, ArH). $^{13}$C NMR: 100.4, 115.6, 116.9, 118.5, 118.7, 120.4, 127.2, 127.4, 129.2, 130.1, 130.3, 131.2, 135.1, 135.6, 136.5, 163.2, 164.0, 165.6, 167.8. MS *m/z*: 331.12 ($M^+$). Anal. calc. for $C_{19}H_{14}FN_5$; C, 68.87; H, 4.26; N, 21.14: Found: C, 68.91; H, 4.27; N, 21.11.

*4-(4-(1H-imidazol-1-yl)phenyl)-6-(2,3,4-trichlorophenyl)-pyrimidin-2-amine (**2h**)*

The experimental procedure was similar to that described for compound **2a** starting from 3-(4-(1*H*-imidazol-1-yl) phenyl)-1-(2,3,4-trichlorophenyl)prop-2-en-1-one.

Compound **2h**: (46 %, Bright yellow powder, m. p. 306–308 °C. IR (KBr) $\nu_{max}$ $cm^{-1}$: 3428, 3110, 1680, 1570, 655. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 6.94 (2H, br. s, $NH_2$), 7.13 (1H, s, H-2 imidazole), 7.41 (1H, s, H-5), 7.56–7.65 (2H, m, ArH), 7.77–7.85 (3H, m, ArH), 8.27 (2H, d, $J = 8.0$ Hz, ArH), 8.37 (1H, d, $J = 6.8$ Hz, ArH). $^{13}$C NMR: 100.5, 115.5, 115.7, 119.4, 129.2, 129.3, 129.7, 130.5, 131.1, 132.1, 133.5, 134.6, 135.4, 135.6, 135.8, 138.6, 161.1, 162.2, 166.7. MS *m/z*: 415.02 ($M^+$). Anal.

calc. for $C_{19}H_{12}Cl_3N_5$; C, 54.77; H, 2.90; N, 16.81; Found: C, 54.80; H, 2.94; N, 16.80.

*4-(4-(1H-imidazol-1-yl)phenyl)-6-(2,5-dichlorophenyl)-pyrimidin-2-amine (**2i**)*

The experimental procedure was similar to that described for compound **2a** starting from 3-(4-(1*H*-imidazol-1-yl) phenyl)-1-(2,5-dichlorophenyl)prop-2-en-1-one.

Compound **2i**: (46 %, Whitish solid), m. p. >340 °C. IR (KBr) $\nu_{max}$ $cm^{-1}$: 3300, 3195, 1670, 1565, 680. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 6.88 (2H, br. s, $NH_2$), 7.14 (1H, s, H-2 imidazole), 7.48 (1H, s, H-5), 7.54–7.58 (3H, m, ArH), 7.77–7.85 (3H, m, ArH), 8.23 (2H, d, $J$ = 8.4 Hz, ArH), 8.31 (1H, s, $^{Ar2}$H-6). $^{13}$C NMR: 101.2, 116.3, 116.4, 121.3, 128.5, 128.6, 128.7, 129.9, 130.1, 130.4, 132.0, 132.6, 135.6, 135.8, 136.2, 138.1, 163.1, 164.0, 166.2. MS *m/z*: 381.05 ($M^+$). Anal. calc. for $C_{19}H_{13}Cl_2N_5$; C, 59.70; H, 3.43; N, 18.32: Found: C, 59.72; H, 3.40; N, 18.35.

*4-(4-(1H-imidazol-1-yl)phenyl)-6-(4-iodophenyl)-pyrimidin-2-amine (**2j**)*

The experimental procedure was similar to that described for compound **2a** starting from 3-(4-(1*H*-imidazol-1-yl) phenyl)-1-(4-iodophenyl)prop-2-en-1-one.

Compound **2j**: (68 %, Off white powder) m. p. 189–191 °C. IR (KBr) $\nu_{max}$ $cm^{-1}$: 3310, 3180, 1680, 1584, 685. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 6.79 (2H, br. s, $NH_2$), 7.14 (1H, s, H-2 imidazole), 7.27 (1H, d, $J$ = 8.5 Hz, ArH), 7.36 (1H, d, $J$ = 8.0 Hz, ArH), 7.50 (2H, d, $J$ = 8.0 Hz, $^{Ar1}$H-3 + $^{Ar1}$H-5), 7.70–7.74 (2H, m, $^{Ar1}$H-2 + $^{Ar1}$H-6), 7.79–7.91 (2H, m, $^{Ar2}$H-3 + $^{Ar2}$H-5), 8.22 (1H, s, H-5), 8.31–8.39 (2H, m, $^{Ar2}$H-2 + $^{Ar2}$H-6). $^{13}$C NMR: 98.2, 102.1, 115.6, 115.8, 119.0, 128.2, 128.3, 129.3, 129.5, 130.3, 134.5, 135.3, 135.5, 137.8, 138.1, 138.3, 163.5, 165.1, 168.0. MS *m/z*: 439.03 ($M^+$). Anal. calc. for $C_{19}H_{14}IN_5$; C, 51.95; H, 3.21; N, 15.94; Found: C, 51.97; H, 3.21; N, 15.92.

*4-(4-(1H-imidazol-1-yl)phenyl)-6-phenylpyrimidin-2-amine (**2k**)*

The experimental procedure was similar to that described for compound **2a** starting from 3-(4-(1*H*-imidazol-1-yl) phenyl)-1phenylprop-2-en-1-one.

Compound **2k**: (36 %, Cream coloured powder), m.p. 210–212 °C. IR (KBr) $\nu_{max}$ $cm^{-1}$: 3305, 3165, 1655, 1556, 674. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 7.08 (2H, br. s, $NH_2$), 7.27 (2H, m, ArH), 7.36 (2H, m, ArH),7.49–7.56 (4H, m, ArH), 7.70 (1H, s, H-5), 8.21 (2H, m, $^{Ar2}$H-3 + $^{Ar2}$H-5), 8.29 (2H, m, $^{Ar2}$H-2 + $^{Ar2}$H-6). $^{13}$C NMR: 100.7, 115.3, 115.5, 120.5, 129.4, 129.6, 130.2(2C), 131.9, 132.0, 132.2, 132.4, 136.5, 136.7, 140.1, 140.4, 163.5, 164.1, 166.9. MS *m/z*: 313.13 ($M^+$). Anal. calc. for $C_{19}H_{15}N_5$; C, 72.83; H, 4.82; N, 22.35: Found: C, 72.83; H, 4.82; N, 22.38.

## Phosphodiesterase inhibition assay

Activity against snake venom was determined by taking 0.66 mM bis-(*p*-nitrophenyl) phosphate (Sigma N-3002) as substrate and 66 mM Tris–HCl buffer of pH 8.8, 60 mM Mg-acetate with final concentration of 0.0001484 U/well of enzyme using a microtitre plate assay. EDTA and Cystein (Merck) were used as positive controls ($IC_{50}$ = 274 ± 0.007 µM, 748 ± 0.015 µM, respectively). Enzyme activity was monitored by spectrophotometer at 410 nm after 30-min pre-incubation of the enzyme with test samples, at 37 °C on a microtitre plate reader (SpectraMax, Molecular Devices) by following change in O.D/min (rate) of release of *p*-nitrophenol from *p*-nitrophenyl phosphate. All assays were conducted in triplicate (Goding *et al*., 1998; Johnson *et al*., 2001).

## Anti-bacterial activity

All the synthesized pyrimidines were tested against six bacterial species viz. *S. aureus, E. coli, B. subtilis, S. flexenari, P. aeruginosa* and *S. typhi.* Each compound (dissolved in DMSO) was subjected to anti-bacterial screening for determining the zone of inhibition by well diffusion method. The Petri plates were inoculated in cultures of bacteria on potato dextrose agar medium. Plates were incubated at 37 °C for 24 h for bacteria. After inoculation, the diameter of clear zone of inhibition surrounding the sample was taken as a measure of the inhibitory power of the sample against the particular test organism. (Gaulejac *et al*., 1999)

## Antifungal activity

Three fungal species viz. *A. alternata*, *A. flavus* and *A. fumigatus* were procured from Biofertilizers and Biopesticide Laboratory, Institute of Mycology & Plant Pathology, University of the Punjab, Lahore, Pakistan.

Pure cultures of *A. alternata, A. flavus* and *A. fumigatus* were prepared in Petri plates using agar medium of malt extract which was autoclaved at 121 °C for 60 min. Then it was poured to plates and after its setting, media was inoculated with all fungal strains and incubated for 5 days at 37 °C to obtain pure cultures without any contamination.

2 % malt extract (ME) broth was autoclaved at 121 °C for 30 min and cooled at room temperature. 6 mg of each of the synthetic compounds was dissolved in 0.7 mL dimethyl sulfoxide (DMSO). Appropriate quantity of ME broth was added to make the volume 6 mL. Lower concentrations of these stock solutions (1 mg/mL) viz. 0.500, 0.250 and 0.125 mg/mL were prepared by double dilution.

Control treatments were similarly prepared without the addition of compounds. A commercial fungicide mancozeb was used as a reference compound.

One-milliliter solution of each of the concentration of the synthetic compounds, mancozeb and control was poured into a sterilized 5 mL culture tube. Two drops (0.01 mL) of suspension of spores/conidia of each of the three test fungal species was added to each culture tube. Culture tubes were completely closed with cotton plugs and incubated at room temperature for 48 h. After 48 h, tubes were observed for appearance of fungal mycelia. The effectiveness of a compound was assessed in terms of 100 % inhibition of germination of spores. The minimum inhibitory concentration (MIC) of each compound was recorded.

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