Journal of Enzyme Inhibition and Medicinal Chemistry, 2010; 25(3): 347–353

informa
healthcare

RESEARCH ARTICLE

4-(4-Morpholinophenyl)-6-arylpyrimidin-2-amines: synthesis, spectral analysis, and in vitro microbiological evaluation

J. Thanusu, V. Kanagarajan, and M. Gopalakrishnan

Synthetic Organic Chemistry Laboratory, Department of Chemistry, Annamalai University, Annamalainagar, Tamil Nadu, India

Abstract

Compounds 4-(4-morpholinophenyl)-6-phenylpyrimidin-2-amine **20**, 4-(4-methoxyphenyl)-6-(4-morpholinophenyl)pyrimidin-2-amine **23**, 4-(4-bromophenyl)-6-(4-morpholinophenyl)pyrimidin-2-amine **25**, 4-(3-chlorophenyl)-6-(4-morpholinophenyl) pyrimidin-2-amine **27**, and 4-(3-fluorophenyl)-6-(4-morpholinophenyl) pyrimidin-2-amine **28** exerted excellent antibacterial activity against *V. cholerae*. Compounds 4-(4-chlorophenyl)-6-(4-morpholinophenyl)pyrimidin-2-amine **22**, **25**, 4-(4-morpholinophenyl)-6-(3-nitrophenyl)pyrimidin-2-amine **26**, and **28**, which contained electron-withdrawing chloro, bromo, nitro, and fluoro functional groups, respectively, at the *para*/*meta* position of the phenyl ring attached to the pyrimidine ring promoted much activity against *S. aureus*. Compounds 4-(4-fluorophenyl)-6-(4-morpholinophenyl)pyrimidin-2-amine **24** and **25** (against β-hemolytic *Streptococcus*) and compound **28** (against *S. felxneri*) showed pronounced activity. Compounds **26** and **28** (against *K. pneumoniae*) and compounds **24**, **25**, and **28** (against *P. aeruginosa*) exerted strong antibacterial activity. Compounds **22** and **25** promoted much antifungal activity against *A. flavus*, while compounds **24** and **25** registered maximum activity against *Mucor*. Compounds **23** (against *C. albicans*) and **27** and **28** (against *Rhizopus*) promoted good activity.

Keywords: *4-(4-morpholinophenyl)-6-arylpyrimidin-2-amines; guanidine nitrate; synthesis; antibacterial activity; antifungal activity*

Introduction

Pyrimidines consititute the basic nucleus in nucleic acids and have been associated with a number of biological activities. Some notable biological activities of pyrimidine derivatives include as adenosine receptor antagonists[1], as kinase inhibitors[2], analgesic[3], anti-inflammatory[3], as inhibitors of cyclin-dependent kinases 1 and 2[4], as calcium channel antagonists[5], antihistaminic[6], and antitubercular[7] activities. Substituted aminopyrimidine nuclei are common in marketed drugs such as anti-atherosclerotic aronixil, anti-histaminic thonzylamine, anti-anxiolytic buspirone, anti-psoriatic enazadrem, and other medicinally relevant compounds.

Morpholine is a simple heterocyclic compound with great industrial importance. It is used as an anticorrosive agent and as a chemical intermediate: catalyst, solvent, and antioxidant, in the production of various pharmaceuticals and pesticides. 4-Phenyl morpholine derivatives[8] are reported to possess antimicrobial, anti-inflammatory, and central nervous system activities. Linezolide (commercially available antimicrobial) also possesses a 4-phenylmorpholine substituent. These derivatives are reported to exert a number of important physiological activities such as antidiabetic[9], antiemetic[10], platelet aggregation inhibition, antihyperlipoproteinemic[9], bronchodilatory, growth stimulatory[11], and antidepressant[12]. They have also been used in the treatment of inflammatory diseases, pain, migraine, and asthma[13].

Recently, we exploited the synthesis of 6-aryl-1,2,4,5-tetrazinane-3-thiones[14], fused indazoles[15], pyrimidinyl thiazolidin-4-ones[16], and 2,6-diarylpiperidin-4-one derivatives[17–19] with a view to incorporating various other

Address for Correspondence: M. Gopalakrishnan, Synthetic Organic Chemistry Laboratory, Department of Chemistry, Annamalai University, Annamalainagar-608 002, Tamil Nadu, India. Tel: +91 4144 228 233. E-mail: profmgk@yahoo.co.in

(Received 29 March 2009; revised 31 May 2009; accepted 01 June 2009)

ISSN 1475-6366 print/ISSN 1475-6374 online © 2010 Informa UK Ltd
DOI: 10.3109/14756360903179468

bioactive heterocyclic nuclei such as 1,2,3-selenadiazoles, 1,2,3-thiadiazoles, and diazepans, intact, for the evaluation of associated antibacterial and antifungal activities. In view of the above and as part of the ongoing research on antimicrobials, we planned to synthesize a system that comprises both *N*-functionalized morpholine and 2-amino-4,6-diarylpyrimidine components together to give a compact structure as the title 4-(4-morpholinophenyl)-6-arylpyrimidin-2-amines.

Experimental

Chemistry

Thin layer chromatography (TLC) was performed to assess the reactions and the purity of the products. All the reported melting points were taken in open capillaries and are uncorrected. Infrared (IR) spectra were recorded in KBr (pellet form) on a Nicolet Avatar-330 Fourier transform (FT)-IR spectrophotometer, and noteworthy absorption values (cm^{-1}) alone are listed. ^1H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded at 400 MHz and 100 MHz, respectively, on a Bruker AMX 400 NMR spectrometer using dimethylsulfoxide (DMSO)-*d* as solvent. Electrospray ionization (ESI)-+ve mass spectra (MS) were recorded on a Bruker Daltonics LC-MS spectrometer. Satisfactory microanalysis was obtained on a Carlo Erba 1106 CHN analyzer.

General procedure[20] for the synthesis of (E)-1-(4-morpholinophenyl)-3-aryl-prop-2-en-1-ones (11–19)

To an ethanolic solution of 1-(4-morpholinophenyl) ethanone (0.001 mol) and substituted benzaldehyde (0.001 mol), aqueous sodium hydroxide (0.005 mol) was added dropwise with stirring on a mechanical stirrer for 10 min, and stirring was continued for 4–6 h. After completion of reaction, the crude product isolated by suction was washed with water, dried, and recrystallized from ethanol.

(E)-1-(4-Morpholinophenyl)-3-phenyl-prop-2-en-1-one ***(11)*** Reaction time: 4 h; m.p.: 148–150°C; Yield: 95%; IR (KBr) ν (cm^{-1}): 3007, 2962, 2924, 2852, 1646, 1606, 1190, 769; MS: *m/z* 294 $(M + 1)^+$; Molecular formula: $C_{19}H_{19}NO_2$; ^1H NMR (δ ppm): 3.33–3.36 (t, 4H, $N(CH_2)_2$), 3.87–3.89 (t, 4H, $O(CH_2)_2$), 6.93–6.95 (d, 1H, H_2, *J* = 8.9 Hz); 7.38–7.82 (m, 10H, H_{arom}); 8.01–8.03 (d, 1H, H_3, *J* = 8.9 Hz); ^{13}C NMR (δ ppm): 47.7 $N(CH_2)$, 66.5 $O(CH_2)$, 122.3 C-2, 143.2 C-3, 113.6, 128.8–130.3 $-C_{arom}$, 128.2, 135.4, 154.1 *ipso*-C, 188.1 C-1.

(E)-1-(4-Morpholinophenyl)-3-p-tolyl-prop-2-en-1-one ***(12)*** Reaction time: 5 h; m.p.: 178–180°C; Yield: 92%; IR (KBr) ν (cm^{-1}): 3012, 2923, 2924, 2851, 1645, 1600, 1194, 810; MS: *m/z* 308 $(M + 1)^+$; Molecular formula: $C_{20}H_{21}NO_2$; ^1H NMR (δ ppm): 1.57 (*s*, 3H, CH_3 at phenyl ring), 3.32–3.35 (t, 4H, $N(CH_2)_2$), 3.86–3.89 (t, 4H, $O(CH_2)_2$), 6.92–6.94 (d, 1H, H_2, *J* = 8.8 Hz); 7.21–7.80 (m, 9H, H_{arom}); 8.00–8.02 (d, 1H, H_3, J = 8.8 Hz); ^{13}C NMR (δ ppm): 21.0 CH_3 at phenyl ring, 47.7 $N(CH_2)$, 66.5 $O(CH_2)$, 121.2 C-2, 143.3 C-3, 113.5, 129.3–130.5 $-C_{arom}$, 128.2, 132.7, 140.5, 154.1 *ipso*-C, 188.2 C-1.

(E)-3-(4-Chlorophenyl)-1-(4-morpholinophenyl)prop-2-en-1-one ***(13)*** Reaction time: 5 h; m.p.: 142–144°C; Yield: 90%; IR (KBr) ν (cm^{-1}): 3087, 2967, 2920, 2859, 1597, 1654, 1202, 817; MS: *m/z* 328 $(M + 1)^+$; Molecular formula: $C_{19}H_{18}NO_2Cl$; ^1H NMR (δ ppm): 3.34–3.37 (t, 4H, $N(CH_2)_2$), 3.89–3.91 (t, 4H, $O(CH_2)_2$), 6.97–6.99 (d, 1H, H_2, *J* = 8.8 Hz); 7.35–7.76 (m, 9H, H_{arom}); 8.00–8.02 (d, 1H, H_3, *J* = 8.9 Hz); ^{13}C NMR (δ ppm): 47.6 $N(CH_2)$, 66.5 $O(CH_2)$, 121.2 C-2, 141.8 C-3, 113.5, 129.4–130.6 $-C_{arom}$, 129.1, 133.8, 135.0, 154.0 *ipso*-C, 192.2 C-1.

(E)-3-(4-Methoxyphenyl)-1-(4-morpholinophenyl)prop-2-en-1-one ***(14)*** Reaction time: 4 h; m.p.: 110–112°C; Yield: 90%; IR (KBr) ν (cm^{-1}): 3010, 2961, 2918, 2841, 1645, 1601, 1225; MS: *m/z* 324 $(M + 1)^+$; Molecular formula: $C_{20}H_{21}NO_3$; ^1H NMR (δ ppm): 3.32–3.35 (t, 4H, $N(CH_2)_2$), 3.87–3.90 (t, 4H, $O(CH_2)_2$), 3.86 (*s*, 3H, OCH_3 at phenyl ring), 7.59–7.61 (d, 1H, H_2, *J* = 8.6 Hz); 6.92–7.46 and 7.75–7.79 (m, 9H, H_{arom}); 8.00–8.02 (d, 1H, H_3, *J* = 8.7 Hz); ^{13}C NMR (δ ppm): 47.6 $N(CH_2)$, 55.3 OCH_3 at phenyl ring, 66.5 $O(CH_2)$, 119.6 C-2, 143.1 C-3, 113.5, 129.2–130.4 $-C_{arom}$, 127.9, 129.9, 153.9, 161.3 *ipso*-C, 187.8 C-1.

(E)-3-(4-Fluorophenyl)-1-(4-morpholinophenyl)prop-2-en-1--one ***(15)*** Reaction time: 4 h; m.p.: 159–161°C; Yield: 95%; IR (KBr) ν (cm^{-1}): 3009, 2969, 2919, 2849, 1650, 1602, 1227; MS: *m/z* 312 $(M + 1)^+$; Molecular formula: $C_{19}H_{18}NO_2F$; ^1H NMR (δ ppm): 3.33–3.36 (t, 4H, $N(CH_2)_2$), 3.87–3.89 (t, 4H, $O(CH_2)_2$), 6.93–6.95 (d, 1H, H_2, *J* = 8.9 Hz); 7.08–7.78 (m, 9H, H_{arom}); 8.00–8.02 (d, 1H, H_3, *J* = 8.9 Hz); ^{13}C NMR (δ ppm): 47.5 $N(CH_2)$, 66.5 $O(CH_2)$, 121.6 C-2, 141.9 C-3, 113.4, 115.8, 130.1, 131.5 $-C_{arom}$, 128.8, 130.6, 154.1, 162.5 *ipso*-C, 187.8 C-1.

(E)-3-(4-Bromophenyl)-1-(4-morpholinophenyl)prop-2-en-1--one ***(16)*** Reaction time: 2 h; m.p.: 138–139°C; Yield: 94%; IR (KBr) ν (cm^{-1}): 3001, 2960, 2923, 2845, 1657, 1612, 1227; MS: *m/z* 372 $(M + 1)^+$; Molecular formula: $C_{19}H_{18}NO_2Br$; ^1H NMR (δ ppm): 3.32–3.35 (t, 4H, $N(CH_2)_2$), 3.86–3.87 (t, 4H, $O(CH_2)_2$), 6.94–6.96 (d, 1H, H_2, *J* = 8.8 Hz); 7.18–7.82 (m, 9H, H_{arom}); 8.01–8.03 (d, 1H, H_3, *J* = 8.6 Hz); ^{13}C NMR (δ ppm): 47.9 $N(CH_2)$, 65.6 $O(CH_2)$, 121.8 C-2, 142.3 C-3, 113.8, 115.1, 130.7, 131.7 $-C_{arom}$, 128.5, 131.2, 154.7, 162.7 *ipso*-C, 188.8 C-1.

(E)-1-(4-Morpholinophenyl)-3-(3-nitrophenyl)prop-2-en-1-one ***(17)*** Reaction time: 6 h; m.p.: 134–316°C; Yield: 87%; IR (KBr) ν (cm^{-1}): 3087, 2966, 2923, 2862, 1651, 1608, 1224; MS: *m/z* 339 $(M + 1)^+$; Molecular formula: $C_{19}H_{18}N_2O_4$; ^1H NMR (δ ppm): 3.36–3.38 (t, 4H, $N(CH_2)_2$), 3.88–3.90 (t, 4H, $O(CH_2)_2$), 6.95–6.97 (d, 1H, H_2, *J* = 8.9 Hz); 7.27–7.91 and 8.23–8.25 (m, 9H, H_{arom}); 8.03–8.05 (d, 1H, H_3, *J* = 8.9 Hz); ^{13}C NMR (δ ppm): 47.3 $N(CH_2)$, 66.9 $O(CH_2)$, 122.0 C-2, 140.1 C-3, 113.3, 124.2–134.2 $-C_{arom}$, 128.9, 137.1, 148.7, 154.3 *ipso*-C, 187.8 C-1.

(E)-1-(4-Morpholinophenyl)-3-(3-chlorophenyl)prop-2-en-1--one ***(18)*** Reaction time: 6 h; m.p.: 138–140°C; Yield: 90%; IR (KBr) ν (cm^{-1}): 3093, 2969, 2928, 2857, 1593, 1652, 1212, 830; MS: *m/z* 328 $(M + 1)^+$; Molecular formula: $C_{19}H_{18}NO_2Cl$; ^1H NMR (δ ppm): 3.33–3.36 (t, 4H, $N(CH_2)_2$), 3.89–3.91 (t, 4H, $O(CH_2)_2$), 6.96–6.98 (d, 1H, H_2, *J* = 8.9 Hz); 7.33–7.81 (m, 9H, H_{arom}); 7.98–8.00 (d, 1H, H_3, *J* = 8.7 Hz); ^{13}C NMR (δ ppm): 47.8 $N(CH_2)$, 66.4 $O(CH_2)$, 121.2 C-2, 141.6 C-3, 113.3, 128.8–130.1 $-C_{arom}$, 129.3, 133.7, 145.2, 154.1 *ipso*-C, 192.3 C-1.

(E)-1-(4-Morpholinophenyl)-3-(3-fluorophenyl)prop-2-en-1-one ***(19)*** Reaction time: 5 h; m.p.: 152–157°C; Yield: 91%; IR (KBr) ν (cm^{-1}): 3018, 2974, 2924, 2843, 1649, 1605,

1226; MS: *m/z* 312 $(M + 1)^+$; Molecular formula: $C_{19}H_{18}NO_2F$; ^{1}H NMR (δ ppm): 3.33–3.36 (t, 4H, $N(CH_2)_2$), 3.86–3.88 (t, 4H, $O(CH_2)_2$), 6.92–6.94 (d, 1H, H_2, J = 8.8 Hz); 7.18–7.68 (m, 9H, H_{arom}); 7.92–7.94 (d, 1H, H_3, J = 8.7 Hz); ^{13}C NMR (δ ppm): 47.5 $N(CH_2)$, 66.6 $O(CH_2)$, 121.3 C-2, 141.8 C-3, 113.4, 125.3–130.1 -C_{arom}, 128.6, 130.5, 154.3, 162.7 *ipso*-C, 187.5 C-1.

General method for the synthesis of 4-(4-morpholinophenyl)-6-arylpyrimidin-2-amines (20–28)

A mixture of (*E*)-1-(4-morpholinophenyl)-3-aryl-prop-2-en-1-ones **(11–19)** (0.001 mol) and guanidine nitrate (0.001 mol) in ethanol (50 mL) was refluxed, while a solution of sodium hydroxide (0.005 mol) in water (10 mL) was added portion-wise for 2 h. Refluxing was continued for a further 10 h and the mixture was poured into ice-cold water. The formed solid was separated by filtration, and purified by column chromatography using silica gel (100–200 mesh), with ethyl acetate–petroleum ether (b.p. 40–60°C) in the ratio 2:8 as eluent.

*4-(4-Morpholinophenyl)-6-phenylpyrimidin-2-amine (**20**)* IR (KBr) (cm^{-1}): 3355, 3459, 3060, 2961, 2920, 1661, 1599, 1229, 928, 824, 776, 697, 634; ^{1}H NMR (δ ppm): 3.33–3.38 (t, 4H, $N(CH_2)_2$), 3.88–3.89 (t, 4H, $O(CH_2)_2$), 5.23 (s, 2H, NH_2), 7.38–7.85 (m, 10H, H_{arom}), the signal for H-5 proton may be merged with the aromatic protons; ^{13}C NMR (δ ppm): 46.3 $N(CH_2)$, 67.3 $O(CH_2)$, 103.4 C-5, 163.8 C-2, 164.7 C-6, 165.0 C-4, 127.0–131.5 -C_{arom}, 142.1, 153.9 *ipso*-C.

*4-(4-Morpholinophenyl)-6-p-tolylpyrimidin-2-amine (**21**)* IR (KBr) (cm^{-1}): 3432, 3200, 2967, 2923, 1625, 1599, 1229, 928, 815, 645; ^{1}H NMR (δ ppm): 2.31 (s, 3H, CH_3), 3.34–3.38 (t, 4H, $N(CH_2)_2$), 3.86–3.89 (t, 4H, $O(CH_2)_2$), 5.25 (s, 2H, NH_2), 7.20–8.12 (m, 9H, H_{arom}), the signal for H-5 proton may be merged with the aromatic protons; ^{13}C NMR (δ ppm): 25.4 CH_3, 46.7 $N(CH_2)$, 67.5 $O(CH_2)$, 104.1 C-5, 163.8 C-2, 164.1 C-6, 164.3 C-4, 126.0–131.4 -C_{arom}, 143.8, 152.4 *ipso*-C.

*4-(4-Chlorophenyl)-6-(4-morpholinophenyl)pyrimidin-2-amine (**22**)* IR (KBr) (cm^{-1}): 3396, 3217, 3027, 2962, 2920, 1656, 1229, 930, 819, 684; ^{1}H NMR (δ ppm): 3.35–3.39 (t, 4H, $N(CH_2)_2$), 3.89–3.91 (t, 4H, $O(CH_2)_2$), 5.28 (s, 2H, NH_2), 7.26–7.85 (m, 9H, H_{arom}), the signal for H-5 proton may be merged with the aromatic protons; ^{13}C NMR (δ ppm): 47.6 $N(CH_2)$, 66.5 $O(CH_2)$, 104.5 C-5, 163.8 C-2, 164.1 C-6, 164.7 C-4, 127.0–139.1 -C_{arom}, 141.5, 152.5 *ipso*-C.

*4-(4-Methoxyphenyl)-6-(4-morpholinophenyl)pyrimidin-2-amine (**23**)* IR (KBr) (cm^{-1}): 3447, 3200, 2972, 2922, 1659, 1600, 1243, 929, 821, 607; ^{1}H NMR (δ ppm): 3.32–3.35 (t, 4H, $N(CH_2)_2$), 3.86 (s, 3H, OCH_3), 3.87–3.90 (t, 4H, $O(CH_2)_2$), 5.26 (s, 2H, NH_2), 7.18–7.82 (m, 9H, H_{arom}), the signal for H-5 proton may be merged with the aromatic protons; ^{13}C NMR (δ ppm): 46.7 $N(CH_2)$, 55.0 OCH_3, 67.3 $O(CH_2)$, 103.9 C-5, 163.7 C-2, 164.7 C-6, 165.0 C-4, 127.5–142.1 -C_{arom}, 153.8, 154.2 *ipso*-C.

*4-(4-Fluorophenyl)-6-(4-morpholinophenyl)pyrimidin-2-amine (**24**)* IR (KBr) (cm^{-1}): 3434, 3200, 2967, 2923, 1624, 1599, 1226, 928, 815, 645; ^{1}H NMR (δ ppm): 3.34–3.36 (t, 4H, $N(CH_2)_2$), 3.87–3.88 (t, 4H, $O(CH_2)_2$), 5.29 (s, 2H, NH_2), 7.30–8.03 (m, 9H, H_{arom}), the signal for H-5 proton may be merged with the aromatic protons; ^{13}C NMR (δ ppm): 46.6 $N(CH_2)$, 67.3 $O(CH_2)$, 103.5 C-5, 163.8 C-2, 164.2 C-6, 164.4 C-4, 126.5–140.0 C_{arom}, 140.6, 154.0 *ipso*-C.

*4-(4-Bromophenyl)-6-(4-morpholinophenyl)pyrimidin-2-amine (**25**)* IR (KBr) (cm^{-1}): 3398, 3219, 3029, 2965, 2922, 1659, 1231, 934, 821, 687; ^{1}H NMR (δ ppm): 3.36–3.39 (t, 4H, $N(CH_2)_2$), 3.87–3.91 (t, 4H, $O(CH_2)_2$), 5.29 (s, 2H, NH_2), 7.29–7.87 (m, 9H, H_{arom}), the signal for H-5 proton may be merged with the aromatic protons; ^{13}C NMR (δ ppm): 47.8 $N(CH_2)$, 66.6 $O(CH_2)$, 104.8 C-5, 163.9 C-2, 164.3 C-6, 164.8 C-4, 127.5–139.7 -C_{arom}, 152.6, 141.6 *ipso*-C.

*4-(4-Morpholinophenyl)-6-(3-nitrophenyl)pyrimidin-2-amine (**26**)* IR (KBr) (cm^{-1}): 3400, 3200, 3060, 2961, 2920, 1661, 1566, 1229, 928, 824, 776, 697; ^{1}H NMR (δ ppm): 3.36–3.39 (t, 4H, $N(CH_2)_2$), 3.88–3.92 (t, 4H, $O(CH_2)_2$), 5.28 (s, 2H, NH_2), 7.27–8.28 (m, 9H, H_{arom}), the signal for H-5 proton may be merged with the aromatic protons; ^{13}C NMR (δ ppm): 47.3 $N(CH_2)$, 67.6 $O(CH_2)$, 104.4 C-5, 163.8 C-2, 164.2 C-6, 164.4 C-4, 125.2–131.5 -C_{arom}, 146.7, 153.9 *ipso*-C.

*4-(3-Chlorophenyl)-6-(4-morpholinophenyl)pyrimidin-2-amine (**27**)* IR (KBr) (cm^{-1}): 3398, 3219, 3028, 2963, 2923, 1655, 1228, 929, 817, 686; ^{1}H NMR (δ ppm): 3.36–3.38 (t, 4H, $N(CH_2)_2$), 3.88–3.90 (t, 4H, $O(CH_2)_2$), 5.29 (s, 2H, NH_2), 7.16–7.74 (m, 9H, H_{arom}), the signal for H-5 proton may be merged with the aromatic protons; ^{13}C NMR (δ ppm): 47.7 $N(CH_2)$, 66.6 $O(CH_2)$, 104.4 C-5, 163.6 C-2, 164.3 C-6, 164.8 C-4, 126.2–138.8 -C_{arom}, 146.5, 152.3 *ipso*-C.

*4-(3-Fluorophenyl)-6-(4-morpholinophenyl)pyrimidin-2-amine (**28**)* IR (KBr) (cm^{-1}): 3437, 3204, 2965, 2928, 1623, 1597, 1225, 921, 811, 649; ^{1}H NMR (δ ppm): 3.33–3.35 (t, 4H, $N(CH_2)_2$), 3.87–3.88 (t, 4H, $O(CH_2)_2$), 5.27 (s, 2H, NH_2), 7.28–8.05 (m, 9H, H_{arom}), the signal for H-5 proton may be merged with the aromatic protons; ^{13}C NMR (δ ppm): 46.8 $N(CH_2)$, 67.4 $O(CH_2)$, 103.7 C-5, 163.9 C-2, 164.4 C-6, 164.5 C-4, 125.9–140.3 -C_{arom}, 146.6, 154.2 *ipso*-C.

Microbiology

Materials

All the clinically isolated bacterial strains, namely *Staphylococcus aureus*, β-hemolytic *Streptococcus*, *Vibreo cholerae*, *Shigella felxneri*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* and fungal strains, namely *Aspergillus flavus*, *Candida albicans*, *Mucor*, and *Rhizopus* were obtained from the Faculty of Medicine, Annamalai University, Annamalainagar, Tamil Nadu, India.

In vitro antibacterial and antifungal activity

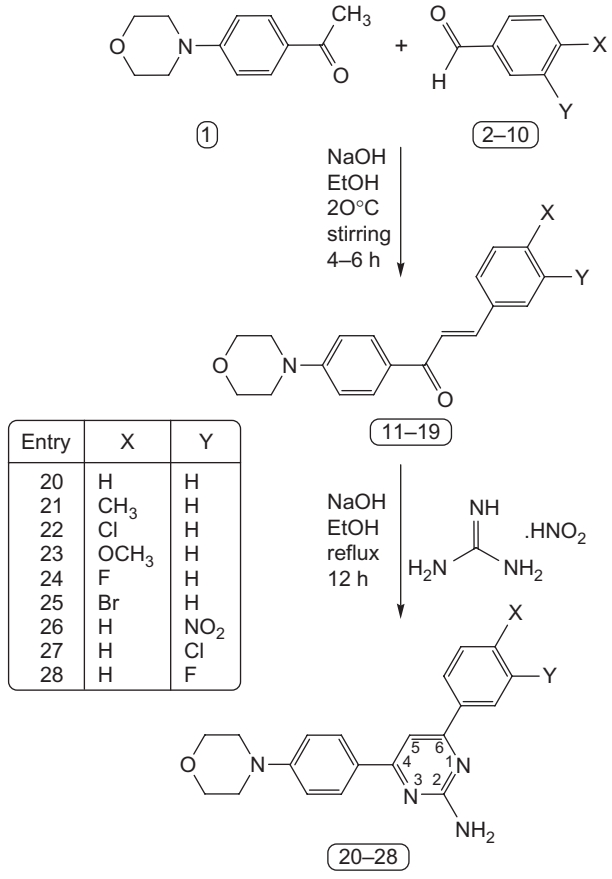
Minimum inhibitory concentration (MIC) values in μg/mL were determined by the two-fold serial dilution method[21]. The respective test compounds **(20–28)** were dissolved in DMSO to obtain 1 mg mL^{-1} stock solution. Seeded broth (broth containing microbial spores) was prepared in nutrient broth (NB) from 24-h-old bacterial cultures on nutrient agar (Hi-media, Mumbai) at 37 ± 1°C, while fungal spores from 1- to 7-day-old Sabouraud agar (Hi-media) slant cultures were suspended in Sabouraud dextrose broth (SDB). The colony forming units (cfu) of the seeded broth were determined by

the plating technique and adjusted in the range of 10^4–10^5 cfu/mL. The final inoculum size was 10^5 cfu/mL for the antibacterial assay and $1.1–1.5 \times 10^2$ cfu/mL for the antifungal assay. Testing was performed at pH 7.4 ± 0.2 for bacteria (NB) and at pH 5.6 for fungi (SDB). Exactly 0.4 mL of test compound solution was added to 1.6 mL of seeded broth to form the first dilution. One milliliter of this was diluted with a further 1 mL of seeded broth to give the second dilution, and so on till six such dilutions were obtained. A set of assay tubes containing only seeded broth were kept as control. The tubes were incubated in biochemical oxygen demand (BOD) incubators at 37 ± 1°C for bacteria and 28 ± 1°C for fungi. The minimum inhibitory concentrations (MICs) were recorded by visual observation after 24 h (for bacteria) and 72–96 h (for fungi) of incubation. Ciprofloxacin was used as the standard for bacterial studies and fluconazole was used as the standard for fungal studies.

Results and discussion

Chemistry

(*E*)-1-(4-Morpholinophenyl)-3-aryl-prop-2-en-1-ones **(11–19)** were synthesized by stirring commercially available 1-(4-morpholinophenyl) ethanone and substituted benzaldehyde in the presence of sodium hydroxide in ethanol at 20°C for 1 h. Treatment of compounds **11–19** with guanidine nitrate[22] in the presence of sodium hydroxide in refluxing ethanol for 8 h yielded the respective 4-(4-morpholinophenyl)-6-aryl-pyrimidin-2-amines **(20–28)**. A schematic representation and analytical data of compounds **20–28** are shown in Scheme 1 and Table 1, respectively. The structure of the newly synthesized compounds **20–28** was confirmed by the melting points, elemental analysis, and MS, FT-IR, and one-dimensional NMR (^{1}H and ^{13}C) spectroscopic data.



Entry	X	Y
20	H	H
21	CH_3	H
22	Cl	H
23	OCH_3	H
24	F	H
25	Br	H
26	H	NO_2
27	H	Cl
28	H	F

Scheme 1. Synthesis reaction pathway for formation of 4-(4-morpholinophenyl)-6-aryl-pyrimidin-2-amines.

Antibacterial activity

Novel 4-(4-morpholinophenyl)-6-aryl-pyrimidin-2-amines **(20–28)** were tested for their antibacterial activity *in vitro* against *S. aureus*, β-hemolytic *Streptococcus*, *V. cholerae*, *S. felxneri*, *K. pneumoniae*, and *P. aeruginosa*. Ciprofloxacin was used as the standard drug. Minimum inhibitory concentration (MIC) values are reproduced in Table 2. Compound **20**, which had no substitution at the *para* position of the phenyl ring, only exerted moderate activities against all the used bacterial strains except against *V. cholerae*, with pronounced activity at 12.5 μg/mL, besides compounds **23**, **25**, **27**, and **28**, which were also potently active. Compounds **21** (against *S. felxneri*) and **23** (against β-hemolytic *Streptococcus*) did not exhibit antibacterial activity even at higher concentrations, i.e. 200 μg/mL. Compounds **22**, **25**, **26**, and **28**, which contained electron-withdrawing chloro, bromo, nitro, and fluoro functional groups, respectively, at the *para*/*meta* position of the phenyl ring attached to the pyrimidine ring promoted much activity against *S. aureus*. Compounds **24** and **25** (against β-hemolytic *Streptococcus*) and compound **28** (against *S. felxneri*) showed pronounced activity. Compounds **26** and **28** (against *K. pneumoniae*) and compounds **24**, **25**, and **28** (against *P. aeruginosa*) exerted strong antibacterial activity.

Antifungal activity

The *in vitro* antifungal activity of 4-(4-morpholinophenyl)-6-aryl-pyrimidin-2-amines **(20–28)** was studied against the fungal strains *A. flavus*, *C. albicans*, *Mucor*, and *Rhizopus*. Fluconazole was used as the standard drug. The MIC values are reproduced in Table 3. Compound **20**, which had no substitution at the *para* position of the phenyl ring, compound **21**, which had an electron-donating CH_3 group, and compound **26**, which had an electron-withdrawing NO_2 group attached at the *meta* position of the phenyl ring attached to the pyrimidine ring, were less active against all tested fungal strains, whereas compound **27** did not exhibit antifungal activity against *A. flavus* and *C. albicans*. Further introduction of an electron-withdrawing chloro/bromo functional group at the *para* position of the phenyl ring attached to the pyrimidine ring in compounds **22** and **25** promoted much activity against *A. flavus*, while against *Mucor* compounds **24** and **25** registered maximum activity. Compound **23**, which had an electron-donating methoxy group at the *para* position of the phenyl ring attached to the pyrimidine ring, was more potent against *C. albicans*. Instead, where the chloro/fluoro group was attached at the *meta* position of the phenyl

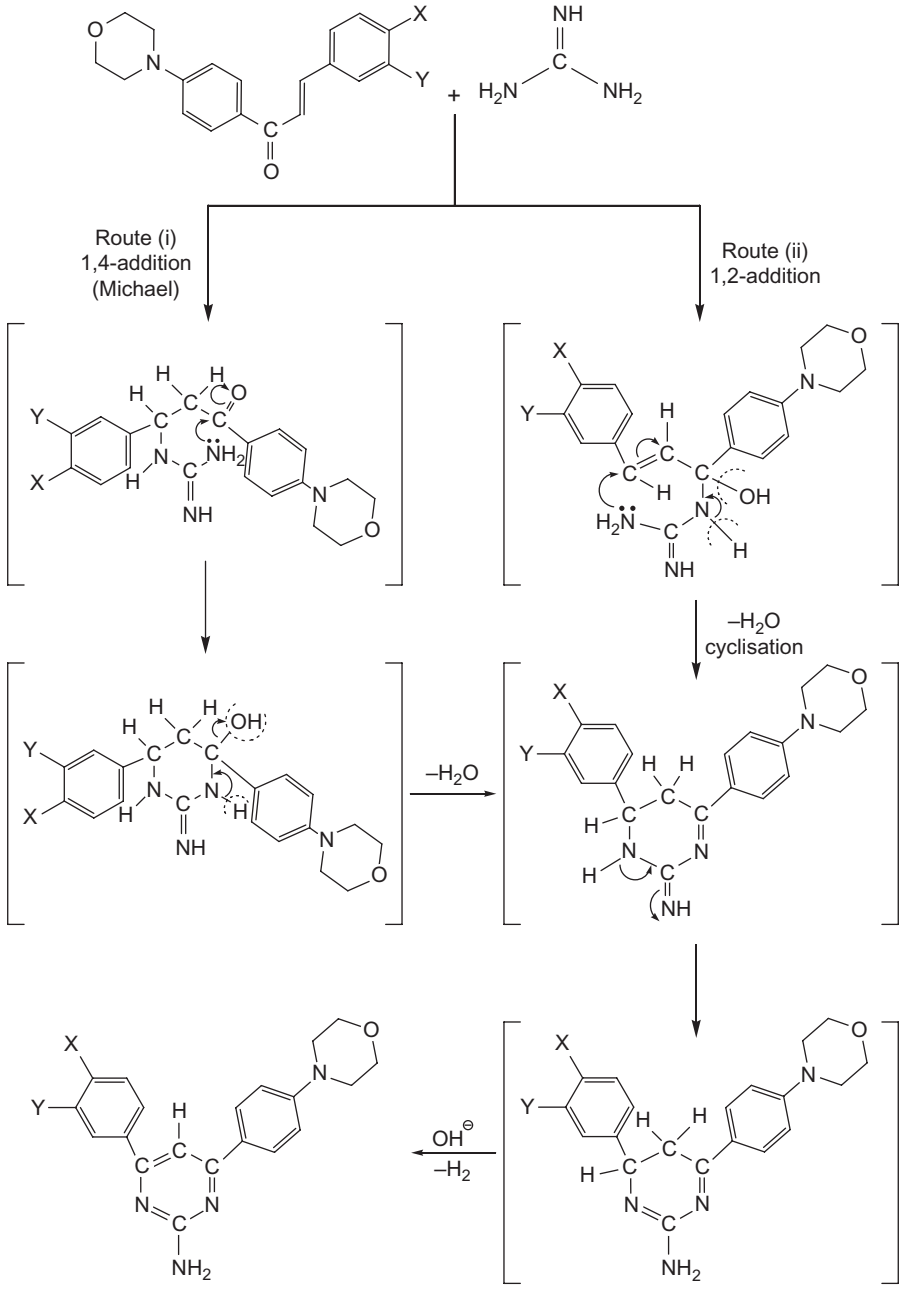
Table 1. Physical and analytical data of compounds **11–28**.

Entry	X	Y	Yield (%)	m.p. (°C)	Elemental analysis (%) C, Calc. (Found)	H, Calc. (Found)	N, Calc. (Found)	m/z $(M+1)^+$, molecular formula
11	H	H	95	149	77.81	6.48	4.77	294
					(77.78)	(6.46)	(4.76)	$C_{19}H_{19}NO_2$
12	CH_3	H	92	179	78.17	6.84	4.56	308
					(78.15)	(6.81)	(4.54)	$C_{20}H_{21}NO_2$
13	Cl	H	90	143	69.72	5.50	4.28	328
					(69.70)	(5.47)	(4.26)	$C_{19}H_{18}NO_2Cl$
14	OCH_3	H	90	111	74.30	6.50	4.33	324
					(74.29)	(6.48)	(4.31)	$C_{20}H_{21}NO_3$
15	F	H	95	160	73.31	5.78	4.50	312
					(73.29)	(5.76)	(4.48)	$C_{19}H_{18}NO_2F$
16	Br	H	85	145	61.30	4.87	3.76	372
					(61.27)	(4.83)	(3.74)	$C_{19}H_{18}BrNO_2$
17	H	NO_2	87	135	67.45	5.32	8.28	339
					(67.43)	(5.29)	(8.26)	$C_{19}H_{18}N_2O_4$
18	H	Cl	90	138	69.72	5.50	4.28	328
					(69.69)	(5.48)	(4.25)	$C_{19}H_{18}NO_2Cl$
19	H	F	90	154	73.31	5.78	4.50	312
					(73.28)	(5.74)	(4.47)	$C_{19}H_{18}NO_2F$
20	H	H	80	118	72.09	6.30	16.80	333
					(72.05)	(6.28)	(16.77)	$C_{20}H_{21}N_4O$
21	CH_3	H	85	73	72.64	6.62	16.12	347
					(72.61)	(6.59)	(16.10)	$C_{21}H_{23}N_4O$
22	Cl	H	78	123	65.33	5.44	15.23	367
					(65.31)	(5.41)	(15.20)	$C_{20}H_{20}N_4OCl$
23	OCH_3	H	80	91	69.44	6.33	15.41	363
					(69.41)	(6.31)	(15.38)	$C_{21}H_{23}N_4O_2$
24	F	H	90	87	68.40	5.69	15.94	351
					(68.38)	(5.66)	(15.91)	$C_{20}H_{20}N_4OF$
25	Br	H	90	93	58.40	4.66	13.62	411
					(58.36)	(4.64)	(13.59)	$C_{20}H_{19}BrN_4O$
26	H	NO_2	85	176	65.96	5.49	15.38	364
					(65.92)	(5.47)	(15.35)	$C_{20}H_{20}N_5O_3$
27	H	Cl	80	135	65.33	5.44	15.23	367
					(65.30)	(5.42)	(15.20)	$C_{20}H_{20}N_4OCl$
28	H	F	78	96	68.40	5.69	15.94	351
					(68.37)	(5.68)	(15.92)	$C_{20}H_{20}N_4OF$

ring attached to the pyrimidine ring, **27** and **28** promoted good activity against *Rhizopus*.

Conclusion

Microbiological screening studies carried out to evaluate the antibacterial and antifungal potencies of the newly synthesized 4-(4-morpholinophenyl)-6-aryl-pyrimidin-2-amines **(20–28)** are clearly illustrated by Tables 2 and 3. Close inspection of the *in vitro* antibacterial and antifungal activity profiles in different electron-donating (CH_3 and OCH_3) and electron-withdrawing (-F, -Cl, -Br, and $-NO_2$) functional group-substituted phenyl rings of the novel 4-(4-morpholinophenyl)-6-aryl-pyrimidin-2-amines **(20–28)** showed that they exerted strong antibacterial activity against all tested bacterial strains. Compounds **20**, **23**, **25**, **27**, and **28** exerted excellent antibacterial activity against *V. cholerae*, and compounds **21** (against *S. felxneri*) and **23** (against β-hemolytic *Streptococcus*) did not exhibit antibacterial activity even at higher concentrations, i.e. 200 μg/mL. Compounds **22**, **25**, **26**, and **28**, which contained electron-withdrawing chloro, bromo, nitro, and fluoro functional groups, respectively, at the *para*/*meta* position of the phenyl ring attached to the pyrimidine ring promoted much activity against *S. aureus*. Compounds **24** and **25** (against β-hemolytic *Streptococcus*) and compound **28** (against *S. felxneri*) showed pronounced activity. Compounds **26** and **28** (against *K. pneumoniae*) and compounds **24**, **25**, and **28** (against *P. aeruginosa*) exerted strong antibacterial activity. Results of the antifungal activity study showed that the nature of the substituent on the phenyl ring, namely chloro, fluoro, and bromo, and methoxy functions at the *para*/*meta* positions of the aryl moieties, were determinants of the nature and extent of the antifungal activity of



Scheme 2. Plausible reaction mechanism for formation of target molecules.

Table 2. *In vitro* antibacterial activity (MIC) values for compounds **20–28**.

			Minimum inhibitory concentration (MIC) (μg/mL)					
Compound	X	Y	*S. aureus*	β-Hemolytic *Streptococcus*	*V. cholerae*	*S. felxneri*	*K. pneumoniae*	*P. aeruginosa*
20	**H**	**H**	100	100	12.5	100	200	25
21	**CH_3**	**H**	50	100	100	—[a]	100	100
22	**Cl**	**H**	6.25	50	100	100	50	50
23	**OCH_3**	**H**	100	—[a]	6.25	100	50	50
24	**F**	**H**	100	12.5	200	100	100	6.25
25	**Br**	**H**	6.25	25	12.5	25	25	12.5
26	**H**	**NO_2**	12.5	50	200	100	6.25	50
27	**H**	**Cl**	25	100	50	50	100	12.5
28	**H**	**F**	12.5	25	12.5	6.25	6.25	25
Ciprofloxacin	—	—	25	50	25	50	50	25

[a]No inhibition even at higher concentration, i.e. 200 μg/mL.

Table 3. *In vitro* antifungal activity (MIC) values for compounds **20–28**.

			Minimum inhibitory concentration (MIC) (μg/mL)			
Compound	X	Y	*A. flavus*	*C. albicans*	*Mucor*	*Rhizopus*
20	**H**	**H**	50	100	25	200
21	**CH_3**	**H**	50	200	100	25
22	**Cl**	**H**	6.25	100	200	100
23	**OCH_3**	**H**	100	6.25	25	50
24	**F**	**H**	200	100	6.25	100
25	**Br**	**H**	6.25	25	12.5	12.5
26	**H**	**NO_2**	100	100	100	100
27	**H**	**Cl**	—[a]	—[a]	12.5	6.25
28	**H**	**F**	50	100	25	12.5
Fluconazole	—	—	25	50	50	50

[a]No inhibition even at higher concentration, i.e. 200 μg/mL.

all synthesized compounds **20–28** on the fungal strains *A. flavus*, *C. albicans*, *Mucor*, and *Rhizopus*. The introduction of an electron-withdrawing chloro/bromo functional group at the *para* position of the phenyl ring attached to the pyrimidine ring in compounds **22** and **25** promoted much activity against *A. flavus*, while compounds **24** and **25** registered maximum activity against *Mucor*. Compound **23**, which had an electron-donating methoxy group at the *para* position of the phenyl ring attached to the pyrimidine ring, was more potent against *C. albicans*. Instead, when the chloro/fluoro group was attached at the *meta* position of the phenyl ring attached to the pyrimidine ring, compounds **27** and **28** promoted pronounced activity against *Rhizopus*. The method of action of these compounds is unknown. These observations may promote progress of our research in this field. Further development of this group of 4-(4-morpholinophenyl)-6-aryl-pyrimidin-2-amines **(20–28)** may lead to compounds with better pharmacological profiles than those of standard antibacterial and antifungal drugs.

Acknowledgements

The authors are thankful to the NMR Research Center, Indian Institute of Science, Bangalore for recording spectra.

***Declaration of interest*:** One of the authors (V.K.) is grateful to the Council of Scientific and Industrial Research (CSIR), New Delhi, Republic of India for providing financial support in the form of a CSIR-Senior Research Fellowship (SRF) in Organic Chemistry. Another author (J.T.) wishes to thank the Annamalai University authorities for providing financial support in the form of a Research Fellowship.

References

1. Van Veldhoven JP, Chang LC, von Frijtag Drabbe Künzel JK, Mulder-Krieger T, Struensee-Link R, Beukers MW, et al. A new generation of adenosine receptor antagonists: from di- to trisubstituted aminopyrimidines. Bioorg Med Chem 2008;16:2741–52.
2. Hughes TV, Emanuel SL, Beck AK, Wetter SK, Connolly PJ, Karnachi P, et al. 4-Aryl-5-cyano-2-aminopyrimidines as VEGF-R2 inhibitors: synthesis and biological evaluation. Bioorg Med Chem Lett 2007;17:3266–70.
3. Chhabria MT, Bhatt HG, Raval HG, Oza PM. Synthesis and biological evaluation of some 5-ethoxycarbonyl-6-isopropylamino-4-(substitutedphenyl)amino pyrimidines as potent analgesic and anti-inflammatory agents. Bioorg Med Chem Lett 2007;17:1022–4.
4. Sayle KL, Bentley JF, Boyle T, Calvert AH, Cheng Y, Curtin NJ, et al. Structure-based design of 2-arylamino-4-cyclohexylmethyl-5-nitroso-6-aminopyrimidine inhibitors of cyclin-dependent kinases 1 and 2. Bioorg Med Chem Lett 2003;13:3079–82.
5. Pastor A, Alajarin R, Vaquero JJ, Alvarez-Builla J, Fau de Casa-Juana M, Sunkel C, et al. Synthesis and structure of new pyrido[2,3-d]pyrimidine derivatives with calcium channel antagonist activity. Tetrahedron 1994;50:8085–98.
6. Youssouf MS, Kaiser P, Singh GD, Singh S, Bani S, Gupta VK, et al. Anti-histaminic, anti-inflammatory and bronchorelaxant activities of 2, 7-dimethyl-3-nitro-4H pyrido [1,2-a] pyrimidine-4-one. Int Immunopharmacol 2008;8:1049–55.
7. Gasse C, Douguet D, Huteau V, Marchal G, Munier-Lehmann H, Pochet S. Substituted benzyl-pyrimidines targeting thymidine monophosphate kinase of Mycobacterium tuberculosis: synthesis and in vitro anti-mycobacterial activity. Bioorg Med Chem 2008;16:6075–85.
8. Panneerselvam P, Nair RR, Vijayalakshmi G, Subramanian EH, Sridhar SK. Synthesis of Schiff bases of 4-(4-aminophenyl)-morpholine as potential antimicrobial agents. Eur J Med Chem 2005;40:225–9.
9. Manfred R, Michael M, Robert S, Wolfgang G. ^{1}H and ^{13}C NMR spectra of N-substituted morpholines. Eur Pat Appl EP 334146:28. Chem Abstr 1989;112:178999.
10. Hale JJ, Mills SG, MacCross M, Dorn CP, Finke PE, Budhu RJ, et al. Phosphorylated morpholine acetal human neurokinin-1 receptor antagonists as water-soluble prodrugs. J Med Chem 2000;43:1234–7.
11. Fisher MH, Wyvratt MJ. Morpholine derivatives: compositions and uses. US Patent 5077290:10. Chem Abstr 1991;116:214513.
12. Avramova P, Danchev N, Buyukliev R, Bogoslovova T. Synthesis, toxicological, and pharmacological assessment of derivatives of 2-aryl-4-(3-arylpropyl)morpholines. Arch Pharm 1998;331:342–6.
13. Dorn CP, Hale JJ, MacCross M, Mills SG. Morpholine compounds are prodrugs useful as tachykinin receptor antagonists. US Patent 5691336:82. Chem Abstr 1997;128:48231.
14. Gopalakrishnan M, Sureshkumar P, Kanagarajan V, Thanusu J. Design, "one-pot" synthesis, characterization, antibacterial and antifungal activities of novel 6-aryl-1,2,4,5-tetrazinan-3-thiones in dry media. J Sulf Chem 2007;28:383–92.
15. Gopalakrishnan M, Sureshkumar P, Thanusu J, Kanagarajan V. Synthesis, spectral analysis, antibacterial and antifungal activities of some 4,6-diaryl-4,5-dihydro-3-hydroxy-2[H]-indazole—a novel fused indazole derivative. J Enzyme Inhib Med Chem 2008;23:974–9.
16. Gopalakrishnan M, Sureshkumar P, Thanusu J, Kanagarajan V. Design, synthesis, spectral analysis and in vitro microbiological evaluation of biolabile 2-phenyl-3-(4,6-diarylpyrimidin-2-yl)thiazolidin-4-ones. J Enzyme Inhib Med Chem 2009;in press.
17. Gopalakrishnan M, Sureshkumar P, Thanusu J, Kanagarajan V. Design, synthesis, characterization, antibacterial and antifungal activities of novel class of 5,7-diaryl-4,4-dimethyl-4,5,6,7-tetrahydropyridino[3,4-d]-1,2,3-selenadiazoles. J Enzyme Inhib Med Chem 2008;23:347–51.
18. Gopalakrishnan M, Thanusu J, Kanagarajan V. Synthesis and biological evaluation of 5,7-diaryl-4,4-dimethyl-4,5,6,7-tetrahydropyridino[3,4-d]-1,2,3-thiadiazoles. Med Chem Res 2007;16:392–401.
19. Gopalakrishnan M, Sureshkumar P, Thanusu J, Kanagarajan V, Govindaraju R, Jayasri G. A convenient "one-pot" synthesis and *in vitro* microbiological evaluation of novel 2,7-diaryl-[1,4]-diazepan-5-ones. J Enzyme Inhib Med Chem 2007;22:709–15.
20. Guthrie W, Wang XP. The aldol condensation of acetophenone with acetone. Can J Chem 1991;69:339–40.
21. Dhar MH, Dhar MM, Dhawan BN, Mehrotra BN, Ray C. Screening of Indian plants biological activity. Part I. Indian J Exp Biol 1968;6:232–47.
22. Kothari S, Singhal M, Vijayvergia D, Vyas R, Verma BL. Synthesis and biocidal activity of some new 2-amino-4,6-diaryl-substituted-pyrimidines. J Indian Chem Soc 2000;77:329–31.