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ORIGINAL ARTICLE

Synthesis, spectral characterization, and *in vitro* antibacterial and antifungal activities of novel 1,3-thiazine-2-amines comprising morpholine nucleus

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

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

Abstract

A collection of 4-(4-morpholinophenyl)-6-aryl-6*H*-1,3-thiazin-2-amines (**20–28**) were synthesized and their *in vitro* antimicrobial activity was investigated. Compound **21** against *P. aeruginosa*, **23** against *B. subtilis*, **24** against *V. cholerae* and *P. aeruginosa*, **26** against *S. aureus* and *B. subtilis*, **27** against *B. subtilis* and *E. coli*, and **28** against all tested bacterial strains exerted excellent antibacterial activity. Compound **20** against *A. flavus* and *Rhizopus*, **21**, **26** against *Rhizopus*, **22**, **27** against *Mucor*, **23** against *A. flavus*, **24** against both *A. flavus* and *Mucor*, **25** against all tested strains, and **28** against *Rhizopus* and *M. gypseum* exerted excellent antifungal activity.

Keywords: *Morpholino thiazines; thiourea; synthesis; antibacterial activity; antifungal activity*

Introduction

2-Amino-1,3-thiazines and their derivatives are chemically and pharmaceutically interesting entities, and have been used as antimicrobial agents[1], as cannabinoid receptor agonists[2], in the discovery of CB2 receptors[3], in the phagocytic activity of human neutrophils[4], as vasopressin receptor antagonists[5], and have central nervous system (CNS) and antioxidant properties[6], analgesic and anti-inflammatory activities[7], antifilarial properties[8], metalloprotease inhibition activity[9], and antihypertensive activity[10]. The presence of the N–C–S linkage is believed to account for the extensive biological activities of the thiazine nucleus.

Promising diverse pharmacological activities are shown by various *N*-fuctionalized morpholines. They are reported to exert a number of important physiological activities such as antidiabetic[11], antiemetic[12], platelet aggregation inhibition, antihyperlipoproteinemic[13], bronchodilatation, growth stimulant[14], and antidepressant[15]. They are also used in the treatment of inflammatory diseases, pain, migraine, and asthma[16]. 4-Phenyl morpholine derivatives are reported to possess antimicrobial[17], anti-inflammatory[18], and CNS[19] activities.

It is known (Scheme 1) that the antibiotic activities of cephalosporins (**A**) are due to the presence of the 1,3-thiazine part[19]. Inhibition studies with 2-amino-5,6-dihydro-4*H*-1, 3-thiazine (**B**) have indicated its use in the expression and immunoaffinity purification of human inducible nitric-oxide synthase[20]. Xylazine (**C**) is used for sedation, anesthesia, muscle relaxation, and analgesia in animals[21]. Naphtho[2,3-b][1,4]-thiazine-5,10-diones and 3-substituted-1,4-dioxo-1,4-dihydronaphthalen-2-yl-thioalkanoate derivatives (**D**) have been screened for their biological evaluation as potential antibacterial and antifungal agents[22]. Tridemorph, a morpholine derivative (**E**), is used as an antifungal agent[23]. Drugs derived from morpholine-incorporated compounds include dextromoramide (**F**), a narcotic analgesic, and doxapram·HCl (**G**), a respiratory stimulant; Dopram® is used in the treatment of respiratory depression following anesthesia.

The synthesis of molecules that are novel but still resemble known biologically active molecules by virtue of the presence of some critical structural features is an essential component of the search for new leads in drug design programs. As part of our research program aimed at the synthesis of

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

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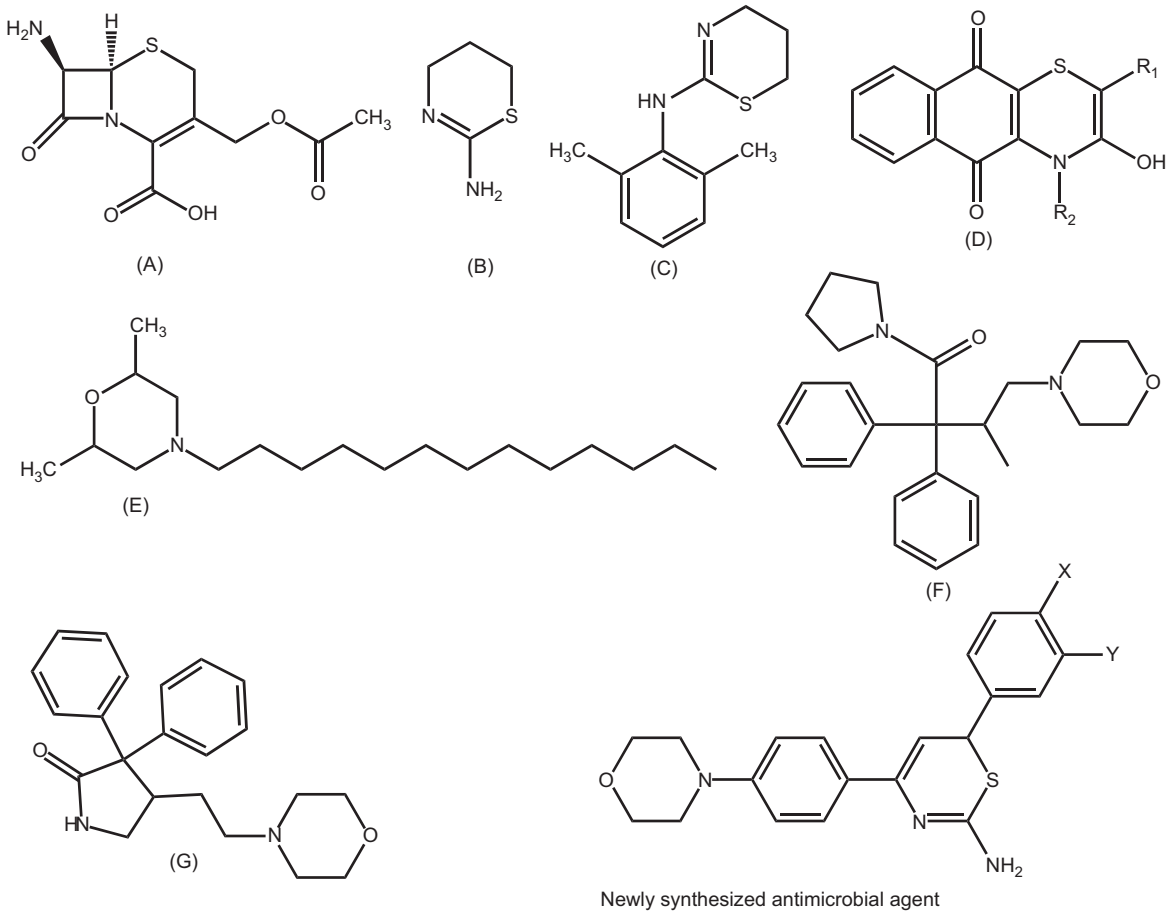


Scheme 1. Novel synthesized compounds having core thiazine and morpholine nuclei of therapeutic importance.

structurally diverse nitrogen/sulfur and selenium containing heterocycles[24–29] comprising piperidino 1,2,3-selenadiazoles, 1,2,3-thiadiazoles, 1,2,4-triazolidine-3-thiones, diazepans, 1,2,4,5-tetrazinan-3-thiones, and indazoles, we performed the synthesis of 4-(4-morpholinophenyl)-6-aryl-2*H*-1,3-thiazine-2-amines (**20–28**) from (*E*)-1-(4-morpholinophenyl)-3-aryl-prop-2-en-1-ones (**11–19**) and evaluated their *in vitro* antibacterial and antifungal activities.

Experimental

Chemistry

The progress of the reaction was monitored by thin layer chromatography (TLC) analysis. 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_6 as solvent. Electrospray ionization mass spectrometry (ESI +ve MS) spectra were recorded on a Bruker Daltonics LC-MS spectrometer. Satisfactory microanalyses were obtained on a Carlo Erba 1106 CHN analyzer.

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

To 50 mL ethanolic solution of 1-(4-morpholinophenyl) ethanone **1** (0.001 mol) and substituted benzaldehyde **2–10** (0.001 mol), aqueous sodium hydroxide (0.005 mol) was added drop-wise with stirring on a mechanical stirrer for 10 min, and stirring was continued for 1 h. After completion of the 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*** IR (KBr) ν (cm^{-1}): 3007, 2962, 2924, 2852, 1646, 1606, 1190, 769; ^1H NMR (δ ppm): 3.33–3.36 (t, 4H, $N(CH_2)_2$, J = 4.7 Hz), 3.87–3.89 (t, 4H, $O(CH_2)_2$, J = 4.7 Hz), 6.93–6.95 (d, 1H, H_2, J = 8.9 Hz), 7.38–7.82 (m, 9H, H_{arom}), 8.01–8.03 (d, 1H, H_3, J = 8.9 Hz); ^{13}C NMR (δ ppm): 47.7 $N(CH_2)_2$, 66.5 $O(CH_2)_2$, 122.3 C-2, 143.2 C-3, 113.6, 128.8–130.3 -C_{arom}, 128.2 *ipso*-C to C=O, 135.4 Ar-ring *ipso*-C, 154.1 morpholine *ipso*-C, 188.1 C-1.

(E)-3-(4-Methylphenyl)-1-(4-morpholinophenyl)prop-2-en-1-one ***12*** IR (KBr) ν (cm^{-1}): 3012, 2923, 2924, 2851, 1645, 1600, 1194, 810; ^1H NMR (δ ppm): 1.57 (s, 3H, CH_3 at phenyl ring), 3.32–3.35 (t, 4H, $N(CH_2)_2$, J = 4.8 Hz), 3.86–3.89 (t, 4H, $O(CH_2)_2$, J = 4.8 Hz), 6.92–6.94 (d, 1H, H_2, J = 8.8 Hz), 7.21–7.80 (m, 8H, 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 $N(CH_2)_2$, 66.5

$O(CH_2)_2$, 121.2 C-2, 143.3 C-3, 113.5, 129.3–130.5 -C_{arom}, 128.2 *ipso*-C to C=O, 132.7 Ar-ring *ipso*-C, 140.5 Ar-substitutent *ipso*-C, 154.1 morpholine *ipso*-C, 188.2 C-1.

(E)-3-(4-Chlorophenyl)-1-(4-morpholinophenyl)prop-2-en-1-one ***13*** IR (KBr) ν (cm^{-1}): 3087, 2967, 2920, 2859, 1597, 1654, 1202, 817; ^{1}H NMR (δ ppm): 3.34–3.37 (t, 4H, $N(CH_2)_2$, J = 4.7 Hz), 3.89–3.91 (t, 4H, $O(CH_2)_2$, J = 4.8 Hz), 6.97–6.99 (d, 1H, H_2, J = 8.8 Hz), 7.35–7.76 (m, 8H, H_{arom}), 8.00–8.02 (d, 1H, H_3, J = 8.9 Hz); ^{13}C NMR (δ ppm): 47.6 $N(CH_2)_2$, 66.5 $O(CH_2)_2$, 121.2 C-2, 141.8 C-3, 113.5, 129.4–130.6 -C_{arom}, 129.1 *ipso*-C to C=O, 133.8 Ar-ring *ipso*-C, 135.0 Ar-substitutent *ipso*-C, 154.0 morpholine *ipso*-C, 192.2 C-1.

(E)-3-(4-Methoxyphenyl)-1-(4-morpholinophenyl)prop-2-en-1-one ***14*** IR (KBr) ν (cm^{-1}): 3010, 2961, 2918, 2841, 1645, 1601, 1225; ^{1}H NMR (δ ppm): 3.32–3.35 (t, 4H, $N(CH_2)_2$, J = 4.8 Hz), 3.87–3.90 (t, 4H, $O(CH_2)_2$, J = 4.8 Hz), 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 & 7.75–7.79 (m, 8H, H_{arom}), 8.00–8.02 (d, 1H, H_3, J = 8.7 Hz); ^{13}C NMR (δ ppm): 47.6 $N(CH_2)_2$, 55.3 OCH_3 at phenyl ring, 66.5 $O(CH_2)_2$, 119.6 C-2, 143.1 C-3, 113.5, 129.2–130.4 -C_{arom}, 127.9 *ipso*-C to C=O, 129.9 Ar-ring *ipso*-C, 153.9 Ar-substitutent *ipso*-C, 161.3 morpholine *ipso*-C, 187.8 C-1.

(E)-3-(4-Fluorophenyl)-1-(4-morpholinophenyl)prop-2-en-1-one ***15*** IR (KBr) ν (cm^{-1}): 3009, 2969, 2919, 2849, 1650, 1602, 1227; ^{1}H NMR (δ ppm): 3.33–3.36 (t, 4H, $N(CH_2)_2$, J = 4.7 Hz), 3.87–3.89 (t, 4H, $O(CH_2)_2$, J = 4.8 Hz), 6.93–6.95 (d, 1H, H_2, J = 8.9 Hz), 7.08–7.78 (m, 8H, H_{arom}), 8.00–8.02 (d, 1H, H_3, J = 8.9 Hz); ^{13}C NMR (δ ppm): 47.5 $N(CH_2)_2$, 66.5 $O(CH_2)_2$, 121.6 C-2, 141.9 C-3, 113.4, 115.8, 130.1, 131.5 -C_{arom}, 128.8 *ipso*-C to C=O, 130.6 Ar-ring *ipso*-C, 154.1 Ar-substitutent *ipso*-C, 162.5 morpholine *ipso*-C, 187.8 C-1.

(E)-3-(4-Bromophenyl)-1-(4-morpholinophenyl)prop-2-en-1-one ***16*** IR (KBr) ν (cm^{-1}): 3001, 2960, 2923, 2845, 1657, 1612, 1227; ^{1}H NMR (δ ppm): 3.32–3.35 (t, 4H, $N(CH_2)_2$, J = 4.5 Hz), 3.86–3.87 (t, 4H, $O(CH_2)_2$, J = 4.6 Hz), 6.94–6.96 (d, 1H, H_2, J = 8.8 Hz); 7.18–7.82 (m, 8H, H_{arom}), 8.01–8.03 (d, 1H, H_3, J = 8.6 Hz); ^{13}C NMR (δ ppm): 47.9 $N(CH_2)_2$, 65.6 $O(CH_2)_2$, 121.8 C-2, 142.3 C-3, 113.8, 115.1, 130.7, 131.7 -C_{arom}, 128.5 *ipso*-C to C=O, 131.2 Ar-ring *ipso*-C, 144.7 Ar-substitutent *ipso*-C, 162.7 morpholine *ipso*-C, 188.8 C-1.

(E)-1-(4-Morpholinophenyl)-3-(3-nitrophenyl)prop-2-en-1-one ***17*** IR (KBr) ν (cm^{-1}): 3087, 2966, 2923, 2862, 1651, 1608, 1224; ^{1}H NMR (δ ppm): 3.36–3.38 (t, 4H, $N(CH_2)_2$, J = 4.5 Hz), 3.88–3.90 (t, 4H, $O(CH_2)_2$, J = 4.6 Hz), 6.95–6.97 (d, 1H, H_2, J = 8.9 Hz), 7.27–7.91 & 8.23–8.25 (m, 8H, H_{arom}), 8.03–8.05 (d, 1H, H_3, J = 8.9 Hz); ^{13}C NMR (δ ppm): 47.3 $N(CH_2)_2$, 66.9 $O(CH_2)_2$, 122.0 C-2, 140.1 C-3, 113.3, 124.2–134.2 -C_{arom}, 128.9 *ipso*-C to C=O, 137.1 Ar-ring *ipso*-C, 148.7 Ar-substitutent *ipso*-C, 154.3 morpholine *ipso*-C, 187.8 C-1.

(E)-1-(4-Morpholinophenyl)-3-(3-chlorophenyl)prop-2-en-1-one ***18*** IR (KBr) ν (cm^{-1}): 3093, 2969, 2928, 2857, 1593, 1652, 1212, 830; ^{1}H NMR (δ ppm): 3.33–3.36 (t, 4H, $N(CH_2)_2$, J = 4.6 Hz), 3.89–3.91 (t, 4H, $O(CH_2)_2$, J = 4.6 Hz), 6.96–6.98 (d, 1H, H_2, J = 8.9 Hz), 7.33–7.81 (m, 8H, H_{arom}), 7.98–8.00 (d, 1H, H_3, J = 8.7 Hz); ^{13}C NMR (δ ppm): 47.8 $N(CH_2)_2$, 66.4 $O(CH_2)_2$, 121.2 C-2, 141.6 C-3, 113.3, 128.8–130.1 -C_{arom}, 129.3 *ipso*-C to C=O, 133.7 Ar-ring *ipso*-C, 145.2 Ar-substitutent *ipso*-C, 154.1 morpholine *ipso*-C, 192.3 C-1.

(E)-1-(4-Morpholinophenyl)-3-(3-fluorophenyl)prop-2-en-1-one ***19*** IR (KBr) ν (cm^{-1}): 3018, 2974, 2924, 2843, 1649, 1605, 1226; ^{1}H NMR (δ ppm): 3.33–3.36 (t, 4H, $N(CH_2)_2$, J = 4.8 Hz), 3.86–3.88 (t, 4H, $O(CH_2)_2$, J = 4.7 Hz), 6.92–6.94 (d, 1H, H_2, J = 8.8 Hz), 7.18–7.68 (m, 8H, H_{arom}), 7.92–7.94 (d, 1H, H_3, J = 8.7 Hz); ^{13}C NMR (δ ppm): 47.5 $N(CH_2)_2$, 66.6 $O(CH_2)_2$, 121.3 C-2, 141.8 C-3, 113.4, 125.3–130.1 -C_{arom}, 128.6 *ipso*-C to C=O, 130.5 Ar-ring *ipso*-C, 154.3 morpholine *ipso*-C, 162.7 Ar-substitutent *ipso*-C, 187.5 C-1.

General method for the synthesis of 4-(4-morpholinophenyl)-6-aryl-6H-1,3-thiazin-2-amines 20–28

A mixture of (*E*)-1-(4-morpholinophenyl)-3-aryl-prop-2-en-1-ones **11–19** (0.001 mol) and thiourea (0.001 mol) in ethanol (50 mL) was refluxed, while a solution of potassium hydroxide (0.005 mol) in water (10 mL) was added portion-wise for 2 h. Refluxing was continued for a further 4 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 1:9 as eluent.

4-(4-Morpholinophenyl)-6-phenyl-6H-1,3-thiazin-2-amine ***20*** IR (KBr) (cm^{-1}): 3399, 3289, 3000, 2965, 2921, 2852, 1660, 1600, 1240, 929, 821, 766, 700, 605; ^{1}H NMR (δ ppm): 3.33–3.36 (t, 4H, $N(CH_2)_2$, J = 4.6 Hz), 3.87–3.89 (t, 4H, $O(CH_2)_2$, J = 4.9 Hz), 5.23–5.25 (d, 1H, H_6, J = 12.2 Hz), 5.54–5.57 (d, 1H, H_5, J = 11.9 Hz), 7.11–7.82 (m, 9H, H_{arom}), the signal for NH_2 protons was exchanged with H_2O in the solvent (d_6-DMSO); ^{13}C NMR (δ ppm): 46.7 $N(CH_2)_2$, 55.0 C-6, 65.6 $O(CH_2)_2$, 112.8 C-5, 140.6 C-4, 126.8–134.3 -C_{arom}, 142.1 153.8 *ipso*-C, 170.6 C-2.

6-(4-Methylphenyl)-4-(4-morpholinophenyl)-6H-1,3-thiazin-2-amine ***21*** IR (KBr) (cm^{-1}): 3429, 3300, 2960, 2922, 2852, 1648, 1604, 1227, 928, 820, 768, 662; ^{1}H NMR (δ ppm): 2.33 (s, 3H, CH_3), 3.33–3.37 (t, 4H, $N(CH_2)_2$, J = 4.5 Hz), 3.86–3.89 (t, 4H, $O(CH_2)_2$, J = 4.8 Hz), 5.22–5.24 (d, 1H, H_6, J = 12.4 Hz), 5.55–5.56 (d, 1H, H_5, J = 11.1 Hz), 7.26–7.89 (m, 8H, H_{arom}), the signal for NH_2 protons was exchanged with H_2O in the solvent (d_6-DMSO); ^{13}C NMR (δ ppm): 24.4 CH_3, 46.7 $N(CH_2)_2$, 55.0 C-6, 65.7 $O(CH_2)_2$, 113.0 C-5, 141.3 C-4, 125.9–134.3 -C_{arom}, 154.0, 161.0 *ipso*-C, 171.6 C-2.

6-(4-Chlorophenyl)-4-(4-morpholinophenyl)-6H-1,3-thiazin-2-amine ***22*** IR (KBr) (cm^{-1}): 3428, 3202, 2965, 2922, 2852, 1648, 1603, 1226, 928, 814, 744, 634; ^{1}H NMR (δ ppm): 3.35–3.38 (t, 4H, $N(CH_2)_2$, J = 4.6 Hz), 3.88–3.91 (t, 4H, $O(CH_2)_2$, J = 4.7 Hz), 5.24–5.27 (d, 1H, H_6, J = 12.7 Hz), 5.57–5.60 (d, 1H, H_5, J = 11.8 Hz), 7.30–7.93 (m, 8H, H_{arom}), the signal for NH_2 protons was exchanged with H_2O in the solvent (d_6-DMSO); ^{13}C NMR (δ ppm): 46.8 $N(CH_2)_2$, 55.6 C-6, 66.5 $O(CH_2)_2$, 113.5 C-5, 140.5 C-4, 126.6–134.9 -C_{arom}, 143.9, 154.1 *ipso*-C, 172.8 C-2.

6-(4-Methoxyphenyl)-4-(4-morpholinophenyl)-6H-1,3-thiazin-2-amine ***23*** IR (KBr) (cm^{-1}): 3425, 3300, 2967, 2922, 2853, 1644, 1601, 1226, 819, 669; ^{1}H NMR (δ ppm): 3.33–3.38

(t, 4H, $N(CH_2)_2$, J = 4.8 Hz), 3.86 (s, 3H, OCH_3), 3.87–3.92 (t, 4H, $O(CH_2)_2$, J = 4.6 Hz), 5.23–5.26 (d, 1H, H_6, J = 11.9 Hz), 5.57–5.60 (d, 1H, H_5, J = 11.3 Hz), 7.20–8.03 (m, 8H, H_{arom}), the signal for NH_2 protons was exchanged with H_2O in the solvent (d_6-DMSO); ^{13}C NMR (δ ppm): 46.7 $N(CH_2)_2$, 55.1 OCH_3, 55.9 C-6, 65.8 $O(CH_2)_2$, 112.9 C-5, 141.3 C-4, 127.9–134.6 -C_{arom}, 152.5, 153.9 *ipso*-C, 170.8 C-2.

6-(4-Fluorophenyl)-4-(4-morpholinophenyl)-6H-1,3-thiazin-2-amine ***24*** IR (KBr) (cm^{-1}): 3429, 3200, 2972, 2922, 2853, 1649, 1602, 1227, 929, 817, 669; ^{1}H NMR (δ ppm): 3.33–3.36 (t, 4H, $N(CH_2)_2$, J = 4.8 Hz), 3.87–3.90 (t, 4H, $O(CH_2)_2$, J = 5.1 Hz), 5.23–5.25 (d, 1H, H_6, J = 12.9 Hz), 5.58–5.61 (d, 1H, H_5, J = 11.1 Hz), 7.27–8.09 (m, 8H, H_{arom}), the signal for NH_2 protons was exchanged with H_2O in the solvent (d_6-DMSO); ^{13}C NMR (δ ppm): 46.8 $N(CH_2)_2$, 55.2 C-6, 65.9 $O(CH_2)_2$, 113.8 C-5, 140.5 C-4, 126.6–132.1 -C_{arom}, 153.9, 152.1 *ipso*-C, 171.3 C-2.

6-(4-Bromophenyl)-4-(4-morpholinophenyl)-6H-1,3-thiazin-2-amine ***25*** IR (KBr) (cm^{-1}): 3429, 3200, 2967, 2922, 2855, 1646, 1602, 1226, 928, 817, 743, 631; ^{1}H NMR (δ ppm): 3.34–3.39 (t, 4H, $N(CH_2)_2$, J = 4.7 Hz), 3.86–3.89 (t, 4H, $O(CH_2)_2$, J = 4.4 Hz), 5.24–5.26(d, 1H, H_6, J = 12.5 Hz), 5.57–5.61 (d, 1H, H_5, J = 11.6 Hz), 7.32–7.95 (m, 8H, H_{arom}), the signal for NH_2 protons was exchanged with H_2O in the solvent (d_6-DMSO); ^{13}C NMR (δ ppm): 46.9 $N(CH_2)_2$, 55.8 C-6, 66.7 $O(CH_2)_2$, 112.9 C-5, 140.7 C-4, 126.7–133.8 -C_{arom}, 153.4, 154.5 *ipso*-C, 172.3 C-2.

4-(4-Morpholinophenyl)-6-(3-nitrophenyl)-6H-1,3-thiazin-2-amine ***26*** IR (KBr) (cm^{-1}): 3419, 3200, 2965, 2920, 2848, 1660, 1599, 1241, 929, 820, 737, 606; ^{1}H NMR (δ ppm): 3.36–3.39 (t, 4H, $N(CH_2)_2$, J = 4.9 Hz), 3.88–3.90 (t, 4H, $O(CH_2)_2$, J = 4.8 Hz), 5.23–5.25 (d, 1H, H_6, J = 12.8 Hz), 5.57–5.60 (d, 1H, H_5, J = 10.9 Hz), 7.67–8.33 (m, 8H, H_{arom}), the signal for NH_2 protons was exchanged with H_2O in the solvent (d_6-DMSO); ^{13}C NMR (δ ppm): 46.7 $N(CH_2)_2$, 54.9 C-6, 65.8 $O(CH_2)_2$, 113.6 C-5, 142.1 C-4, 126.8–136.6 -C_{arom}, 147.2, 154.5 *ipso*-C, 170.1 C-2.

6-(3-Chlorophenyl)-4-(4-morpholinophenyl)-6H-1,3-thiazin-2-amine ***27*** IR (KBr) (cm^{-1}): 3427, 3201, 2967, 2926, 2850, 1647, 1602, 1229, 921, 817, 744, 637; ^{1}H NMR (δ ppm): 3.36–3.39 (t, 4H, $N(CH_2)_2$, J = 4.7 Hz), 3.86–3.88 (t, 4H, $O(CH_2)_2$, J = 4.6 Hz), 5.23–5.26 (d, 1H, H_6, J = 12.4 Hz), 5.57–5.60 (d, 1H, H_5, J = 11.4 Hz), 7.33–7.89 (m, 8H, H_{arom}), the signal for NH_2 protons was exchanged with H_2O in the solvent (d_6-DMSO); ^{13}C NMR (δ ppm): 46.6 $N(CH_2)_2$, 55.2 C-6, 66.9 $O(CH_2)_2$, 113.1 C-5, 140.8 C-4, 126.1–134.2 -C_{arom}, 148.2, 154.5 *ipso*-C, 171.8 C-2.

6-(3-Fluorophenyl)-4-(4-morpholinophenyl)-6H-1,3-thiazin-2-amine ***28*** IR (KBr) (cm^{-1}): 3434, 3209, 2969, 2927, 28513, 1648, 1604, 1225, 927, 814; ^{1}H NMR (δ ppm): 3.32–3.35 (t, 4H, $N(CH_2)_2$, J = 4.8 Hz), 3.86–3.89 (t, 4H, $O(CH_2)_2$, J = 5.1 Hz), 5.23–5.25 (d, 1H, H_6, J = 12.7 Hz), 5.57–5.60 (d, 1H, H_5, J = 11.3 Hz), 7.24–8.03 (m, 8H, H_{arom}), the signal for NH_2 protons was exchanged with H_2O in the solvent (d_6-DMSO); ^{13}C NMR (δ ppm): 46.7 $N(CH_2)_2$, 55.4 C-6, 66.1 $O(CH_2)_2$, 113.5 C-5, 140.7 C-4, 126.1–132.4 -C_{arom}, 148.3, 152.8 *ipso*-C, 171.8 C-2.

Microbiology

Materials

All the clinically isolated bacterial strains, namely *Staphylococcus aureus*, *Bacillus subtilis*, *Vibreo cholerae*, *Escherichia coli*, and *Pseudomonas aeruginosa* and fungal strains, namely *Aspergillus flavus*, *Mucor*, *Rhizopus*, and *Microsporum gypseum* were obtained from the Faculty of Medicine, Annamalai University, Annamalainagar, Tamil Nadu, India.

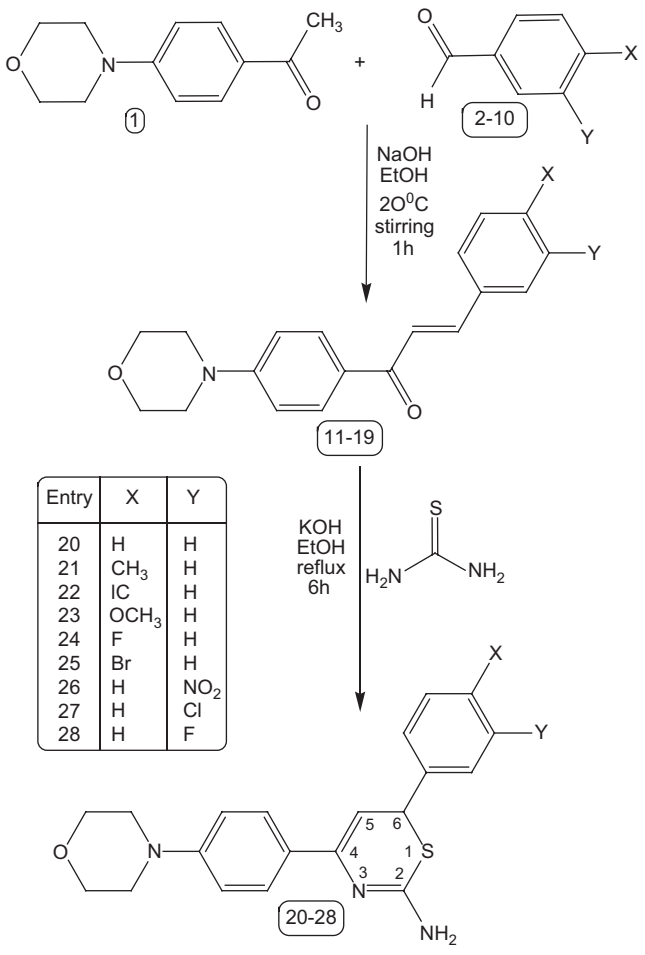
In vitro antibacterial and antifungal activity

The minimum inhibitory concentration (MIC) in µg/mL was determined by the two-fold serial dilution method[30]. The respective test compounds (**20–28**) were dissolved in DMSO to obtain 1 mg/mL stock solution. Seeded broth (broth containing microbial spores) was prepared in nutrient broth (NB) from 24-h-old bacterial cultures on nutrient agar (HiMedia, Mumbai) at 37 ± 1°C, while fungal spores from 1- to 7-day-old Sabouraud agar (HiMedia, Mumbai) slant cultures were suspended in Sabouraud dextrose broth (SDB). The number of 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 × 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 the solution of the test compound 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 BOD (biochemical oxygen demand) incubators at 37 ± 1°C for bacteria and 28 ± 1°C for fungi. The 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 as the standard for fungal studies.

Results and discussion

Chemistry

The classical approach for the synthesis of 4-(4-morpholinophenyl)-6-aryl-2*H*-1,3-thiazine-2-amines (**20–28**) was used as follows. (*E*)-1-(4-Morpholinophenyl)-3-aryl-prop-2-en-1-ones (**11–19**) were synthesized by the Claisen–Schmidt condensation of 1-(4-morpholinophenyl) ethanone and substituted benzaldehydes in the presence of alcoholic sodium hydroxide. Treatment of (*E*)-1-(4-morpholinophenyl)-3-aryl-prop-2-en-1-ones (**11–19**) with thiourea in the presence of potassium hydroxide in refluxing ethanol (Scheme 2, Table 1) afforded the respective target molecules (**20–28**). The structures of these compounds were confirmed by melting points, FT-IR, MS, ^{1}H NMR, and ^{13}C NMR spectral studies, and elemental analysis. The mechanism involves the formation of a Michael adduct and its subsequent heterocyclization (Scheme 3) with a tautomeric change to afford the title compounds.



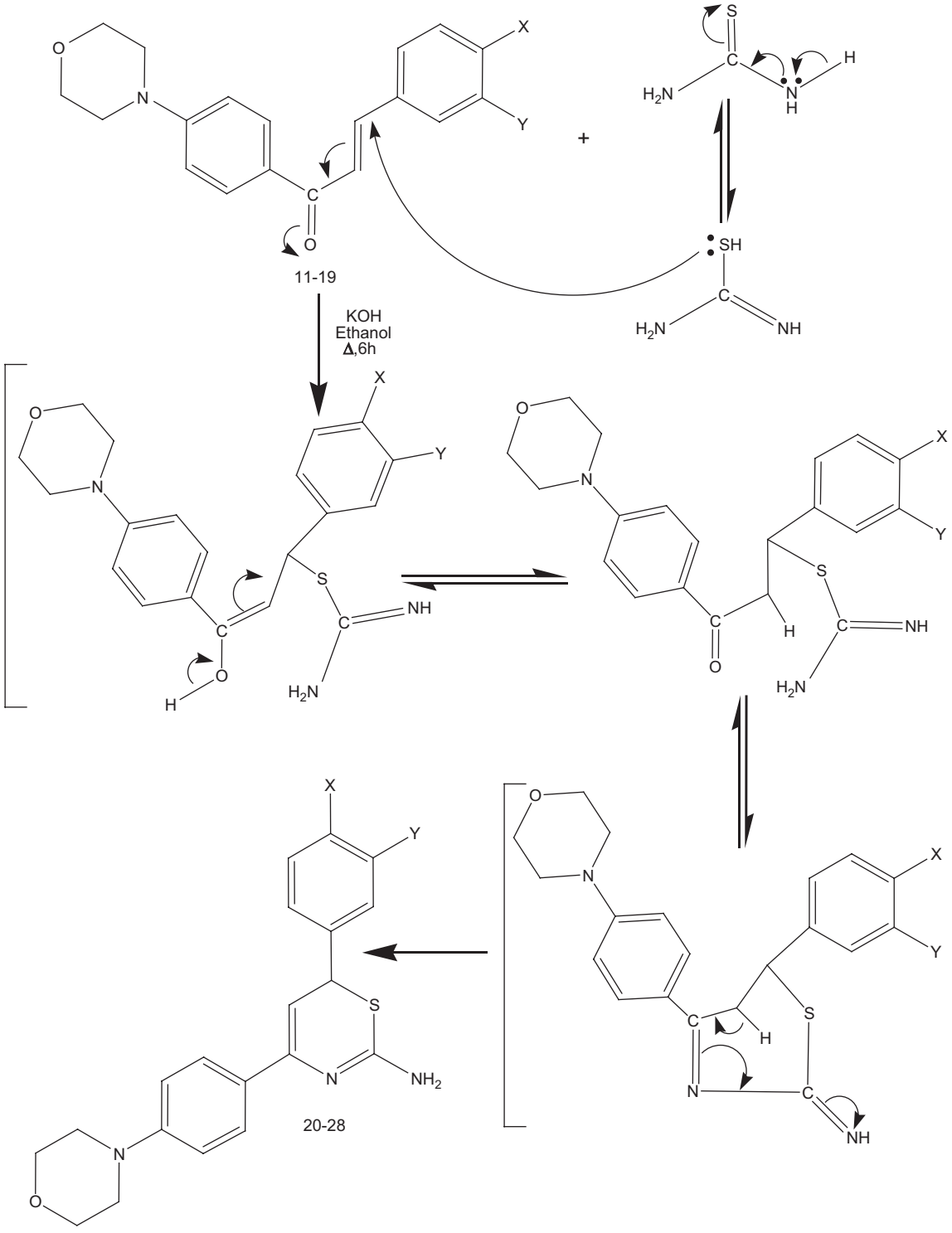
Entry	X	Y
20	H	H
21	CH_3	H
22	IC	H
23	OCH_3	H
24	F	H
25	Br	H
26	H	NO_2
27	H	Cl
28	H	F

Scheme 2. Synthesis reaction pathway for formation of 4-(4-morphlinophenyl)-6-aryl-6*H*-1,3-thiazin-2-amines.

The FT-IR spectrum of 4-(4-morpholinophenyl)-6-phenyl-6*H*-1,3-thiazin-2-amine **20** showed characteristic absorptions at 3342–3446 cm^{-1} due to N-H asymmetric and symmetric stretching vibrations of the primary amino group. The band at 1661 cm^{-1} is due to the presence of the C=N stretching frequency. The absorption frequency at 2961–3002 cm^{-1} is assigned to the aromatic C-H stretching vibration. The absorption band at 1229 cm^{-1} is consistent with the C-N stretching vibration. The observed NH_2, C=N, C-H, and C-N stretching vibrational bands are supporting evidence for the formation of synthesized compound **20**. Two doublets were obtained in the ^{1}H NMR spectrum of 4-(4-morpholinophenyl)-6-phenyl-6*H*-1,3-thiazin-2-amine **20**, due to H-5 and H-6 protons. The doublet at 5.14 ppm is assigned to the H-6 proton. The doublet observed in the downfield region at 5.55 ppm is due to the H-5 proton. The amino protons signal was exchanged with water in the solvent. Two triplets were observed, due to the methylene protons of the morpholine ring. The triplet observed in the region of 3.33–3.36 ppm corresponds to two protons and this signal is due to methylene protons $N(CH_2)_2$ of the morpholine ring. There was a another triplet in the region of 3.87–3.89 ppm, corresponding to two protons, which can be assigned to methylene protons $O(CH_2)_2$ of the morpholine ring. The aromatic protons appeared as a multiplet in the range 7.11–7.82 ppm. The ^{13}C resonance at 170.6 ppm is assigned to the amino group bearing carbon C-2 for 4-(4-morpholinophenyl)-6-phenyl-6*H*-1,3-thiazin-2-amine **20**. The ^{13}C resonances at 140.6 and 112.8 ppm are due to the C-4 and C-5 carbons, respectively. The ^{13}C resonance observed at 55.0 ppm is assigned to the C-6 carbon. Two signals were observed at 46.7 and 65.6 ppm. Of the two signals, one ^{13}C resonance at 46.7 ppm is due to the methylene carbon $N(CH_2)_2$ of the morpholine ring, and the ^{13}C resonance at 65.6 ppm is unambiguously assigned to the methylene

Table 1. Physical and analytical data of (*E*)-1-4-morpholinophenyl)-3-aryl-prop-2-en-1-ones (**11–19**) and 4-(4-morpholinophenyl)-6-aryl-6*H*-1,3-thiazin-2-amines (**20–28**).

					Elemental analysis			
Entry	X	Y	Yield (%)	m.p. (°C)	C found (Calculated)	H found (Calculated)	N found (Calculated)	*m/z* (M + 1)$^+$ Molecular formula
11	H	H	98	149	77.78 (77.81)	6.46 (6.48)	4.76 (4.77)	294 $C_{19}H_{19}NO_2$
12	CH_3	H	92	179	78.15 (78.17)	6.81 (6.84)	4.54 (4.56)	308 $C_{20}H_{21}NO_2$
13	Cl	H	90	143	69.70 (69.72)	5.47 (5.50)	4.26 (4.28)	328, 330 $C_{19}H_{18}NO_2Cl$
14	OCH_3	H	95	111	74.29 (74.30)	6.48 (6.50)	4.31 (4.33)	324 $C_{20}H_{21}NO_3$
15	F	H	95	160	73.29 (73.31)	5.76 (5.78)	4.48 (4.50)	312 $C_{19}H_{18}NO_2F$
16	Br	H	95	145	61.27 (61.30)	4.83 (4.87)	3.74 (3.76)	372, 374 $C_{19}H_{18}BrNO_2$
17	H	NO_2	90	135	67.43 (67.45)	5.29 (5.32)	8.26 (8.28)	339 $C_{19}H_{18}N_2O_4$
18	H	Cl	90	138	69.69 (69.72)	5.48 (5.50)	4.25 (4.28)	328, 330 $C_{19}H_{18}NO_2Cl$
19	H	F	95	154	73.28 (73.31)	5.74 (5.78)	4.47 (4.50)	312 $C_{19}H_{18}NO_2F$
20	H	H	75	61	68.31 (68.35)	6.05 (6.07)	11.94 (11.96)	352 $C_{20}H_{21}N_3OS$
21	CH_3	H	70	185	69.00 (69.01)	6.30 (6.34)	11.46 (11.50)	366 $C_{21}H_{23}N_3OS$
22	Cl	H	75	180	62.23 (62.25)	5.19 (5.22)	10.85 (10.89)	386, 388 $C_{20}H_{20}N_3OSCl$
23	OCH_3	H	70	124	66.08 (66.12)	6.04 (6.08)	10.98 (11.01)	382 $C_{21}H_{23}N_3O_2S$
24	F	H	80	182	64.97 (65.02)	5.42 (5.46)	11.33 (11.37)	370 $C_{20}H_{20}N_3OSF$
25	Br	H	80	161	55.77 (55.82)	4.65 (4.68)	9.71 (9.76)	431, 433 $C_{20}H_{20}N_3OSBr$
26	H	NO_2	85	157	60.55 (60.59)	4.65 (4.68)	9.73 (9.76)	397 $C_{20}H_{20}N_4O_3S$
27	H	Cl	85	184	62.20 (62.25)	5.18 (5.22)	10.86 (10.89)	386, 388 $C_{20}H_{20}N_3OSCl$
28	H	F	80	188	64.99 (65.02)	5.44 (5.46)	11.32 (11.37)	370 $C_{20}H_{20}N_3OSF$



Scheme 3. Proposed reaction mechanism for formation of target molecules.

carbon $O(CH_2)_2$ of the morpholine ring. The remaining ^{13}C signal at 153.8ppm is due to *ipso* carbon. Aromatic carbons were observed in the range of 126.8–134.3 ppm.

Antibacterial activity

Novel 4-(4-morpholinophenyl)-6-aryl-2*H*-1,3-thiazine-2-amines (**20–28**) were tested for their antibacterial activity *in vitro* against *S. aureus*, *B. subtilis*, *V. cholerae*, *E. coli*, and *P. aeruginosa*. Ciprofloxacin was used as the standard drug. The minimum inhibitory concentration (MIC) values in μg/mL are reproduced in Table 2. A close survey of the MIC values indicates that all the compounds (**20–28**) exhibited a varied range (6.25–200 μg/mL) of antibacterial activity against all the tested bacterial strains, except compound **23** against *P. aeruginosa* and compound **26** against *V. cholerae*, which did not have activity at a maximum concentration of 200 μg/mL.

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

	Minimum inhibitory concentration (MIC) (μg/mL)				
Compound	*S. aureus*	*B. subtilis*	*V. cholerae*	*E. coli*	*P. aeruginosa*
20	100	50	200	12.5	25
21	100	100	25	25	6.25
22	50	100	25	50	25
23	100	12.5	25	25	—[a]
24	100	50	12.5	25	6.25
25	50	25	50	50	50
26	6.25	12.5	—[a]	200	25
27	25	6.25	25	12.5	25
28	12.5	50	12.5	6.25	6.25
Ciprofloxacin	25	25	50	25	25

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

Compound **20**, which has no substitution at the *para* position of the phenyl rings attached to C-4 and C-6 carbons of the pyrimidine moiety, exerted two-fold increased activity at a MIC value of 12.5 μg/mL against *E. coli*, and showed moderate activity against all other tested bacterial strains. Compound **21**, with electron-donating methyl substitution at the *para* position of the phenyl rings attached to C-4 and C-6 carbons of the pyrimidine moiety, showed four-fold increased activity against *P. aeruginosa* at a MIC value of 6.25 μg/mL, and showed moderate activity against all other tested bacterial strains with a varied range of 100–25 μg/mL. Compound **22**, which has an electron-withdrawing chloro substitution at the *para* position of the phenyl rings attached to C-4 and C-6 carbons of the pyrimidine moiety, showed potent activity against all the tested bacterial strains. Compound **23**, with electron-donating methoxy substitution at the *para* position of the phenyl rings attached to C-4 and C-6 carbons of the pyrimidine moiety, showed two-fold increased activity against *B. subtilis* and *V. cholerae*. Compound **24**, with electron-withdrawing fluoro substitution at the *para* position of the phenyl rings attached to C-4 and C-6 carbons of the pyrimidine moiety, showed excellent activity against all the tested bacterial strains except *S. aureus*, with a MIC value of 100 μg/mL. Compound **24** showed four-fold increased activity against *V. cholerae* and *P. aeruginosa*. Bromo-substituted compound **25** exerted moderate activity against all the tested strains, whereas *meta*-substituted nitro compound **26** possessed excellent antibacterial activity against all the tested strains except *V. cholerae* and *E. coli*. Against *S. aureus*, compound **26** exerted four-fold increased activity, and against *B. subtilis*, it exerted two-fold increased activity. Compound **27**, which has electron-withdrawing chloro substitution at the *meta* position of the phenyl rings attached to C-4 and C-6 carbons of the pyrimidine moiety, showed four-fold increased activity against *S. aureus* and two-fold increased activity against *B. subtilis*. Compound **28**, which has electron-withdrawing fluoro substitution at the *meta* position of the phenyl rings attached to C-4 and C-6 carbons of the pyrimidine moiety, showed four-fold increased activity against *V. cholerae*, *E. coli*, and *P. aeruginosa*, and exerted two-fold increased activity against *S. aureus*.

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

	Minimum inhibitory concentration (MIC) (μg/mL)			
Compound	*A. flavus*	*Mucor*	*Rhizopus*	*M. gypseum*
20	12.5	100	12.5	100
21	25	100	12.5	50
22	—[a]	6.25	100	50
23	6.25	100	50	50
24	12.5	6.25	100	—[a]
25	25	25	12.5	6.25
26	50	100	6.25	25
27	100	12.5	50	25
28	50	—[a]	6.25	6.25
Fluconazole	50	25	25	25

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

Antifungal activity

The *in vitro* antifungal activity of 4-(4-morpholinophenyl)-6-aryl-2*H*-1,3-thiazine-2-amines (**20–28**) was studied against the fungal strains, viz. *Aspergillus flavus*, *Mucor*, *Rhizopus*, and *Microsporum gypseum*. Fluconazole was used as the standard drug. The minimum inhibitory concentration (MIC) values in μg/mL are reproduced in Table 3. A close survey of the MIC values indicates that all the compounds (**20–28**) exhibited a varied range (6.25–200 μg/mL) of antifungal activity against all the tested bacterial strains except compounds **22**, **24**, and **28**, which had no antifungal activity against *A. flavus*, *M. gypseum*, and *Mucor*, respectively, even at a high concentration of 200 μg/mL. Compound **20**, having no substitution at the phenyl rings attached to C-4 and C-6 carbons of the pyrimidine moiety, exerted four-fold increased activity against *A. flavus* and two-fold increased activity against *Rhizopus*, and showed moderate activity against *Mucor* and *M. gypseum*. Two-fold increased activity was noted against *A. flavus* and *Rhizopus* for compound **21**, which had electron-donating methyl substitution at the *para* position of the phenyl rings attached to C-4 and C-6 carbons of the pyrimidine moiety. Four-fold increased activity was noted against *Mucor* for compound **22**, with electron-withdrawing chloro substitution at the *para* position of the phenyl rings attached to C-4 and C-6 carbons of the pyrimidine moiety. Methoxy-substituted compound **23** showed four-fold increased activity against *A. flavus*, whereas fluoro-substituted compound **24** exerted four-fold increased activity against *A. flavus* and *Mucor*. Compound **25**, with electron-withdrawing bromo substitution at the *para* position of the phenyl rings attached to C-4 and C-6 carbons of the pyrimidine moiety, showed excellent activity against all the tested fungal strains. Against *Rhizopus*, compound **26**, which had an electron-withdrawing nitro functional group, exerted four-fold increased activity. Two-fold increased activity was noted against *Mucor* for compound **27**, with electron-withdrawing chloro substitution at the *meta* position of the phenyl rings attached to C-4 and C-6 carbons of the pyrimidine moiety. Compound **28**, with electron-withdrawing fluoro substitution at the *meta* position of the phenyl rings attached to C-4 and C-6 carbons of the pyrimidine moiety, showed four-fold increased activity against *Rhizopus* and *M. gypseum*.

Conclusion

Results of microbiological screening studies carried out to evaluate the antibacterial and antifungal potencies of the newly synthesized 4-(4-morpholinophenyl)-6-aryl-6*H*-1,3-thiazin-2-amines (**20–28**) are clearly known from Tables 2 and 3. Close inspection of the *in vitro* antibacterial and antifungal activity profiles in the differently 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-2*H*-1,3-thiazine-2-amines (**20–28**) shows that they exerted strong antibacterial activity against all the tested bacterial strains. Electron-donating methyl-substituted compound **21** against *P. aeruginosa*, electron-donating methoxy-substituted compound **23** against *B. subtilis*, and strong, small-size electron-withdrawing fluoro-substituted compound **24** against *V. cholerae* and *P. aeruginosa* exerted excellent antibacterial activity. Compound **26**, with an electron-withdrawing nitro group, exerted good activity against *S. aureus* and *B. subtilis*. Electron-withdrawing *m*-chloro-substituted compound **27** exerted excellent antibacterial activity against *B. subtilis* and *E. coli*, whereas electron-withdrawing fluoro-substituted compound **28** possessed good activity against all the tested bacterial strains. Results of the antifungal activity study show that the nature of the substituents on the phenyl ring, viz. chloro, fluoro, bromo, methyl, and methoxy functions at the *para*/*meta* positions of the aryl moieties, are determinants of the nature and extent of the antifungal activity of all the synthesized compounds **20–28** on the fungal strains, namely *A. flavus*, *Mucor*, *Rhizopus*, and *M. gypseum*. Compound **20** against *A. flavus* and *Rhizopus*, compound **21** against *Rhizopus*, compound **22** against *Mucor*, **23** against *A. flavus*, **24** against both *A. flavus* and *Mucor*, possessed excellent antifungal activity. Bulky bromo-substituted compound **25** exerted good activities against all the tested strains, whereas electron-withdrawing nitro-substituted compound **26** exerted good activity against *Rhizopus*. Electron-withdrawing chloro-substituted compound **27** against *Mucor* and strong electron-withdrawing fluoro-substituted compound **28** against *Rhizopus* and *M. gypseum* exerted excellent antifungal activity. The method of action of these compounds is unknown. These observations may promote further development of our research in this field. Further development of this group of morpholino 1,3-thiazin-2-amines may lead to compounds with better pharmacological profiles than standard antibacterial and antifungal drugs.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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