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Laboratory note

Synthesis of Schiff bases of 4-(4-aminophenyl)-morpholine as potential antimicrobial agents

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Abstract

In the present study, a novel series of Schiff bases of 4-(4-aminophenyl)-morpholine were synthesised and characterised by IR, ^{1}H-NMR, ^{13}C-NMR, Mass spectral and elemental analysis. The compounds were screened for antibacterial (*Staphylococcus aureus* (ATCC 9144), *Staphylococcus epidermidis* (ATCC 155), *Bacillus cereus* (ATCC 11778), *Micrococcus luteus* (ATCC 4678), and *Escherichia coli* (ATCC 25922)) and antifungal (*Candida albicans* (ATCC 2091) and *Aspergillus niger* (ATCC 9029)) activities. The minimum inhibitory concentrations of the compounds were also ascertained by agar streak dilution method. 4-(4-(4-Hydroxy-benzylidene-imino)phenyl)-morpholine (7) was found to be the most potent antimicrobial activity with MIC of 25, 19, 21, 16, 29, 20 and 40 μg/ml against *S. aureus*, *S. epidermidis*, *B. cereus*, *M. luteus*, *E. coli*, *C. albicans* and *A. niger*, respectively. All the other compounds exhibited moderate activity against the bacterial and fungal organisms tested.

Keywords: Morpholine; Antibacterial; Antifungal

1. Introduction

4-Phenyl-morpholine derivatives were reported to possess antimicrobial [1,2], anti-inflammatory [3–5] and central nervous system activities [5–7]. Schiff bases has been reported to possess antimicrobial properties [8–11] apart from other biological activities. In our previous work [12], it was observed that 4-phenyl-morpholine was a potential substituent to impart significant antimicrobial property to quinazoline moiety. Linezolide (commercially available antimicrobial) also possess a 4-phenyl-morpholine substituent. These observations led to the conception that Schiff bases of 4-(4-aminophenyl)-morpholine would possess potential antimicrobial properties. In the present study, a novel series of Schiff bases of 4-(4-aminophenyl)-morpholine were synthesised and characterised by IR, ^{1}H-NMR, ^{13}C-NMR, Mass spectral and elemental analysis. The compounds were screened for antibacterial and antifungal activities. The minimum inhibitory concentrations of the compounds were also ascertained by agar streak dilution method.

2. Chemistry

In the present study, 4-(4-nitrophenyl)-morpholine was synthesised starting from morpholine and 4-chloronitrobenzene. 4-(4-Nitrophenyl)-morpholine was reduced to 4-aminophenyl-morpholine. Schiff bases of 4-(4-aminophenyl)-morpholine was synthesised by condensing 4-(4-aminophenyl)-morpholine and appropriate aldehyde.

3. Biological investigation

The antibacterial (*Staphylococcus aureus* ATCC 9144, *Staphylococcus epidermidis* ATCC 155, *Bacillus cereus* ATCC 11778, *Micrococcus luteus* ATCC 4698 and *Escherichia coli* ATCC 25922) and antifungal (*Candida albicans* ATCC 2091 and *Aspergillus niger* ATCC 9029) activities of the com-

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pounds were evaluated by paper disc diffusion method. The minimum inhibitory concentrations of the compounds were also determined by agar streak dilution method.

4. Results and discussion

All the synthesised compounds exhibited significant antibacterial activity. All the compounds were active against all tested microorganism with a range of MIC values for *S. aureus* (20–47 μg/ml), *S. epidermidis* (17–38 μg/ml), *B. cereus* (21–42 μg/ml), *M. luteus* (29–46 μg/ml), *E. coli* (16–41 μg/ml), *C. albicans* (20–36 μg/ml) and *A. niger* (30–44 μg/ml). Compounds **2**, **6** and **9** exhibited significant activity against *S. aureus* (MIC: 20 μg/ml), *S. epidermidis* (17 μg/ml) and *A. niger* (30 μg/ml). 4-(4-(4-Hydroxy-benzylidene-imino)phenyl)-morpholine **(7)** exhibited significant activity against *B. cereus* (MIC: 21 μg/ml), *M. luteus* (29 μg/ml), *E. coli* (16 μg/ml) and *C. albicans* (20 μg/ml). Compounds **1, 3, 4, 5, 8, 10** and **11** exhibited moderate activity against all the bacteria and fungi tested. The results revealed that most of the synthesised compounds exhibited significant antibacterial activity but they showed moderate antifungal activity.

5. Experimental protocols

5.1. Chemistry

The melting points were taken in open capillary tube and are uncorrected. The IR spectra of the compounds were recorded on ABB Bomem FTIR spectrometer MB104 with KBr pellets. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on 300 MHz-Bruker DPX 200. The chemical shifts are reported as parts per million downfield from tetra methyl silane. Mass spectra were recorded on Finnigan MAT 8230. Microanalyses for C, H, N were performed in Heraeus CHN Rapid Analyzer. All the compounds gave satisfactory chemical analyses (±0.4%). The purity of the compounds was checked by TLC on precoated SiO_2 gel (HF_{254}, 200 mesh) aluminum plates (E Merck).

*5.1.1. General method of synthesis **1-5***

The starting material (4-(4-aminophenyl)-morpholine) for the synthesis of the title compounds has been previously published [13]. Equimolar quantities (0.01 mol) of 4-(4-aminophenyl)-morpholine and the appropriate aldehyde in absolute alcohol (100 ml) containing 2–3 drops of glacial acetic acid were refluxed for 4 h. The excess solvent was removed under reduced pressure. The resulting precipitate was filtered, vacuum dried and recrystallized using absolute alcohol.

*5.1.1.1. 4-(4-(Benzylidene-imino)-phenyl)-morpholine **1***. Yield = 86%, mp 135–137 °C. IR (KBr) cm^{-1}: 2963(Ar–H), 2852(N=CH), 1508(C=N), 1448(C=C), 1261(C–N), 1120(C–O–C), 822(Ar–H). ^{1}H-NMR ($CDCl_3$) Δ: 8.52(s, 1H; N=CH), 7.89–7.71(m, 2H; 2″,6″-H), 7.49–7.47(m, 3H; 3″,4″,5″-H) 7.28(d, J = 8.9 Hz, 2H; 3′,5′-H), 6.98(d, J = 9 Hz, 2H; 2′,6′-H), 3.91(t, J = 7.8 Hz, 4H; 2,6-CH_2), 3.22(t, J = 7.7 Hz, 4H; 3,5-CH_2). ^{13}C-NMR ($CDCl_3$) Δ: 157.78(N=CH), 147.27(C-1′), 142.66(C-4′), 133.71(C-1″), 130.83(C-4″), 128.61(C-2″ & C-6″), 128.41(C-3″ & C-5″), 121.97(C-3′ & C-5′), 116.03(C-2′ & C-6′), 116.03(C-2 & C-6), 66.78(C-3 & C-5). EI-MS m/z (M^+): 266(Calcd for $C_{17}H_{18}N_2O$: 266.3). Anal. $C_{17}H_{18}N_2O$.

*5.1.1.2. 4-(4-(2-Chlorobenzylidene-imino)-phenyl)-morpholine **2***. Yield = 87%, mp 163–165 °C. IR (KBr) cm^{-1}: 2970(Ar–H), 2854(N=CH), 1507(C=N), 1446(C=C), 1332(C–N), 1123(C–O–C), 826, 767(Ar–H). ^{1}H-NMR ($CDCl_3$) Δ: 8.23(s, 1H; N=CH), 7.43–7.26(m, 6H; 3′,5′,3″,4″,5″,6″-H), 6.95(d, J = 9 Hz, 2H; 2′,6′-H), 3.88(t, J = 7.8 Hz, 4H; 2,6-CH_2), 3.20(t, J = 7.7 Hz, 4H; 3,5–CH_2). ^{13}C-NMR ($CDCl_3$) Δ: 154.14(N=CH), 150.27(C-1′), 143.73(C-4′), 135.72(C-2″), 133.54(C-4″), 131.67(C-1″), 129.89(C-6″), 128.29(C-3″), 127.08(C-5″), 122.43(C-3′ & C-5′), 116.03(C-2′ & C-6′), 66.86(C-2 & C-6), 49.29(C-3 & C-5). EI-MS m/z (M^+): 300(Calcd for $C_{17}H_{17}ClN_2O$: 300.8). Anal. $C_{17}H_{17}ClN_2O$.

*5.1.1.3. 4-(4-(3-Nitrobenzylidene-imino)-phenyl)-morpholine **3***. Yield = 65%, mp 111–113 °C. IR (KBr) cm^{-1}: 2961(Ar–H), 2826(N=CH), 1530(C=N), 1441(C=C), 1354(NO_2), 1304(C–N), 1120(C–O–C), 831,738(Ar–H). ^{1}H-NMR ($CDCl_3$) Δ: 8.72(s, 1H; 2″-H), 8.58(s, 1H; N=CH), 8.30–8.22(m, 2H; 4″,6″-H), 7.67–7.61(m, 1H; 5″-H), 7.30(d, J = 8.9 Hz, 2H; 3′,5′-H), 6.96(d, J = 8.9 Hz, 2H; 2′,6′-H), 3.88(t, J = 7.8 Hz, 4H; 2,6-CH_2), 3.21(t, J = 7.7 Hz, 4H; 3,5-CH_2). ^{13}C-NMR ($CDCl_3$) Δ: 153.94(N=CH), 150.61(C-3″), 148.66(C-1′), 142.46(C-4′), 138.32(C-6″), 133.73(C-1″), 129.70(C-5″), 125.02(C-4″), 123.16(C-2″), 122.42(C-3′ & C-5′), 115.89(C-2′ & C-6′), 67.07(C-6), 66.82(C-2), 49.08(C-3 & C-5). EI-MS m/z (M^+): 311(Calcd for $C_{17}H_{17}N_3O_3$: 311.3). Anal. $C_{17}H_{17}N_3O_3$.

*5.1.1.4. 4-(4-(1-Phenyl-propenyl-imino)-phenyl)-morpholine **4***. Yield = 83%, mp 150–152 °C. IR (KBr) cm^{-1}: 2959(Ar–H), 2853(N=CH), 1506(C=N), 1446(C=C), 1262(C–N), 1118(C–O–C), 824(Ar–H). ^{1}H-NMR ($CDCl_3$) Δ: 8.34(t, J = 8 Hz, 1H; N=CH), 7.55(d, J = 2.2 Hz, 1H; N=CH–C<u>H</u>), 7.43–7.36(m, 4H; 2′,3,5′,6′-H), 7.28–7.13(m, 5H; 2″,3″,4″,5″,6″-H), 6.95(d, J = 6.5 Hz, 1H; CH=C<u>H</u>–Ph), 3.89(t, J = 7.8 Hz, 4H; 2,6-CH_2), 3.20(t, J = 7.7 Hz, 4H; 3,5-CH_2). ^{13}C-NMR ($CDCl_3$) Δ: 158.79(N=CH), 149.89(C-1′), 143.48(C-4′), 142.63 (N=CH–<u>C</u>H), 135.7(CH=C<u>H</u>–Ph), 131.24(C-1″), 129.2(C-3″ & C-5″), 128.78(C-4″), 127.2(C-2″ & C-6″), 122.01(C-3′ & C-5′), 115.93(C-2′ & C-6′), 66.76(C-2 & C-6), 49.17(C-3 & C-5). EI-MS m/z (M^+): 292(Calcd for $C_{19}H_{20}N_2O$: 292.4). Anal. $C_{19}H_{20}N_2O$.

*5.1.1.5. 4-(4-(Furfuryl-imino)-phenyl)-morpholine **5***. Yield = 59%, mp 144–146 °C. IR (KBr) cm^{-1}: 2958(Ar–H),

2833(N=CH), 1508(C=N), 1447(C=C), 1333(C–N), 1121(C–O–C), 829(Ar–H). ^{1}H-NMR ($CDCl_3$) Δ: 8.32(s, 1H; N=CH), 7.59(d, J = 7.2 Hz, 1H; 5″-H), 7.28(d, J = 8.7 Hz, 2H; 3′,5′-H), 6.95–6.51(m, 4H; 2′,6′,3″,4″-H), 3.87(t, J = 7.8 Hz, 4H; 2,6-CH_2), 3.19(t, J = 7.7 Hz, 4H; 3,5-CH_2). ^{13}C-NMR ($CDCl_3$) Δ: 152.36(N=CH), 150.02(C-2″), 145.32(C-5″), 145.03(C-1′), 143.22(C-4′), 122.18(C-3′ & C-5′), 116.97(C-2′ & C-6′), 115.41(C-4″), 112.07(C-3″), 66.84(C-2 & C-6), 49.23(C-3 & C-5). EI-MS *m/z* (M^+): 256(Calcd for $C_{15}H_{16}N_2O_2$: 256.3). Anal. $C_{15}H_{16}N_2O_2$.

5.1.2. General method of synthesis ***6-11***

Equimolar quantities (0.01 mol) of 4-(4-aminophenyl)-morpholine and the appropriate aldehyde in absolute alcohol (30 ml) containing 3–4 drops of piperidine were refluxed for 2 h. The reaction mixture was cooled, the precipitate formed was filtered, vacuum dried and recrystallized using absolute alcohol.

5.1.2.1. 4-(4-(2-Hydroxybenzylidene-imino)-phenyl)-morpholine ***6***. Yield = 85%, mp 162–164 °C. IR (KBr) cm^{-1}: 3221(OH), 2957(Ar–H), 2865(N=CH), 1618(C=N), 1445(C=C), 1282(C–N), 1119(C–O–C), 821, 756(Ar–H). ^{1}H-NMR ($CDCl_3$) Δ: 8.61(s, 1H; N=CH), 7.37–7.26(m, 4H; 3″,4″,5″,6″-H), 7.02–6.89(m, 4H; 2′,3′,5′,6′-H), 3.86(t, J = 7.8 Hz, 4H; 2,6-CH_2), 3.18(t, J = 7.7 Hz, 4H; 3,5-CH_2). ^{13}C-NMR ($CDCl_3$) Δ: 160.92(N=CH), 159.53(C-2″), 150.40(C-1′), 140.16(C-4′), 132.48(C-4″), 131.82(C-6″), 122.5(C-3′ & C-5′), 122.10(C-5″), 118.92(C-1″), 117.06(C-3″), 115.97(C-2′ & C-6′), 66.76(C-6), 66.51(C-2), 49(C-3 & C-5). EI-MS *m/z* (M^+): 282(Calcd for $C_{17}H_{18}N_2O_2$: 282.3). Anal. $C_{17}H_{18}N_2O_2$.

5.1.2.2. 4-(4-(4-Hydroxybenzylidene-imino)-phenyl)-morpholine ***7***. Yield = 76%, mp 200–202 °C. IR (KBr) cm^{-1}: 3241(OH), 2951(Ar–H), 2834(N=CH), 1604(C=N), 1449(C=C), 1285(C–N), 1117(C–O–C), 831, 819(Ar–H). ^{1}H-NMR ($CDCl_3$) Δ: 8.40(s, 1H; N=CH), 7.77(d, J = 8.4 Hz, 2H; 2″,6″-H), 7.23(d, J = 8.4 Hz, 2H; 3′,5′-H), 6.95–6.90(m, 4H; 2′,6′,3″,5″-H), 3.88(t, J = 7.8 Hz, 4H; 2,6-CH_2), 3.17(t, J = 7.7 Hz, 4H; 3,5-CH_2). ^{13}C-NMR ($CDCl_3$) Δ: 160.92(N=CH), 159.52(C-4″), 150.39(C-1′), 140.15(C-4′), 132.47(C-2″), 131.81(C-6″), 122.5(C-3′ & C-5′), 122.09(C-5″), 118.91(C-1″), 117.07(C-3″), 115.97(C-2′ & C-6′), 66.76(C-6), 66.50(C-2), 49.01(C-3 & C-5). EI-MS *m/z* (M^+): 282(Calcd for $C_{17}H_{18}N_2O_2$: 282.3). Anal. $C_{17}H_{18}N_2O_2$.

5.1.2.3. 4-(4-(4-Methoxybenzylidene-imino)-phenyl)-morpholine ***8***. Yield = 58%, mp 160–162 °C. IR (KBr) cm^{-1}: 2959(Ar–H), 2855(N=CH), 2835(OCH_3), 1511(C=N), 1445(C=C), 1309(C–N), 1252, 1122(C–O–C), 820(Ar–H). ^{1}H-NMR ($CDCl_3$) Δ: 8.42(s, 1H; N=CH), 7.83(d, J = 9 Hz, 2H; 2″,6″-H), 7.24(d, J = 9 Hz, 2H; 3″,5″-H), 6.99-6.93(m, 4H; 2′,3′5′,6′-H), 4.17(s, 3H; 4″-OCH_3), 3.88(t, J = 7.8 Hz, 4H; 2,6-CH_2), 3.17(t, J = 7.7 Hz, 4H; 3,5-CH_2). ^{13}C-NMR ($CDCl_3$) Δ: 153.94(N=CH), 150.26(C-1′), 143.72(C-4′), 135.72(C-2″), 133.53(C-4″), 131.66(C-1″), 129.89(C-6″), 128.28(C-3″ & C-5″), 122.42(C-3′ & C-5′), 116.03(C-2′ & C-6′), 66.86(C-2 & C-6), 61.48(OCH_3), 42.29(C-3 & C-5). EI-MS *m/z* (M^+): 296(Calcd for $C_{18}H_{20}N_2O_2$: 296.4). Anal. $C_{18}H_{20}N_2O_2$.

5.1.2.4. 4-(4-(3-Methoxy-4-hydroxybenzylidene-imino)-phenyl)-morpholine ***9***. Yield = 75%, mp 184–185 °C. IR (KBr) cm^{-1}: 3312(OH), 2962(Ar–H), 2863(N=CH), 2863(OCH_3), 1510(C=N), 1458(C=C), 1282(C–N), 1243, 1115, 1033(C–O–C), 826, 805, 751(Ar–H). ^{1}H-NMR ($CDCl_3$) Δ: 8.38(s, 1H; N=CH), 7.61(s, 1H; 2″-H), 7.26–7.21(m, 4H; 2′,3′,5′,6′-H), 6.99–6.93(m, 2H; 5″,6″-H), 3.99(s, 3H; 3″-OCH_3), 3.87(t, J = 7.8 Hz, 4H; 2,6-CH_2), 3.18(t, J = 7.7 Hz, 4H; 3,5-CH_2). ^{13}C-NMR ($CDCl_3$) Δ: 157.81(N=CH), 149.60(C-3″), 148.63(C-4″), 147.06(C-1′), 129.44(C-1″), 124.86(C-3′ & C-5′), 121.93(C-6″), 116.24(C-5″), 114.41(C-2″), (C-4′), 108.22(C-2′ & C-6′), 66.91(C-2 & C-6), 56.09(3″-OCH_3), 49.53(C-3 & C-5). EI-MS *m/z* (M^+): 312(Calcd for $C_{18}H_{20}N_2O_3$: 312.4). Anal. $C_{18}H_{20}N_2O_3$.

5.1.2.5. 4-(4-(3,4-Dimethoxybenzylidene-imino)-phenyl)-morpholine ***10***. Yield = 56%, mp 140–142 °C. IR (KBr) cm^{-1}: 2954(Ar–H), 2858(N=CH), 2824(OCH_3), 1509(C=N), 1445(C=C), 1330(C–N), 1241, 1124, 1019(C–O–C), 824, 750(Ar–H). ^{1}H-NMR ($CDCl_3$) Δ: 8.41(s, 1H; N=CH), 7.61(s, 1H; 2″-H), 7.30–7.22(m, 3H; 3′,5′,6″-H), 6.96-6.91(m, 3H; 2′,6′,5″-H), 3.97(s, 6H; 3″,4″-OCH_3), 3.88(t, J = 7.8 Hz, 4H; 2,6-CH_2), 3.18(t, J = 7.7 Hz, 4H; 3,5-CH_2). ^{13}C-NMR ($CDCl_3$) Δ: 153.94(N=CH), 150.26(C-1′), 143.72(C-4′), 135.72(C-2″), 133.53(C-4″), 131.66(C-1″), 129.89(C-6″), 128.28(C-3″), 127.08(C-5″), 122.42(C-3′ & C-5′), 116.03(C-2′ & C-6′), 66.86(C-2 & C-6), 58.03(3″,4″-O$\underline{C}H_3$), 49.29(C-3 & C-5). EI-MS *m/z* (M^+): 326(Calcd for $C_{19}H_{22}N_2O_3$: 326.4). Anal. $C_{19}H_{22}N_2O_3$.

5.1.2.6. 4-(4-(3,4,5-Trimethoxybenzylidene-imino)-phenyl)-morpholine ***11***. Yield = 72%, mp 125–127 °C. IR (KBr) cm^{-1}: 2699(Ar–H), 2852(N=CH), 2825(OCH_3), 1509(C=N), 1448(C=C), 1328(C–N), 1237, 1123, 1068(C–O–C), 824, 784(Ar–H). ^{1}H-NMR ($CDCl_3$) Δ: 8.39(s, 1H; N=CH), 7.27–7.14(m, 4H; 2′,3′,5′,6′-H), 6.94(s, 2H; 2″,6″-H), 3.95(s, 9H; 3″,4″,5″-OCH_3), 3.86(t, J = 7.8 Hz, 4H; 2,6-CH_2), 3.18(t, J = 7.7 Hz, 4H; 3,5-CH_2). ^{13}C-NMR ($CDCl_3$) Δ: 155.94(N=CH), 15.26(C-1′), 143.72(C-4′), 135.72(C-2″), 133.53(C-4″), 131.66(C-1″), 129.89(C-6″), 128.28(C-3″ & C-5″), 122.42(C-3′ & C-5′), 116.03(C-2′ & C-6′), 66.86(C-2 & C-6), 59.25(3″,4″,5″-O$\underline{C}H_3$), 49.29(C-3 & C-5). EI-MS *m/z* (M^+): 356(Calcd for $C_{20}H_{24}N_2O_4$: 356.4). Anal. $C_{20}H_{24}N_2O_4$.

5.2. Antimicrobial activity

The antibacterial activity of the synthesised compounds was tested against *S. aureus*, *S. epidermidis*, *B. cereus*, *M. luteus* and *E. coli* using nutrient agar medium (Hi-Media Laboratories, India). The antifungal activity of the com-

Table 1
Antimicrobial activity of the synthesised compounds

Compound	In vitro activity—zone of inhibition in mm (MIC in μg/ml)						
	Staphylococcus aureus ATCC 9144	*Staphylococcus epidermidis* ATCC 155	*Bacillus cereus* ATCC 11778	*Micrococcus luteus* ATCC 4698	*Escherichia coli* ATCC 25922	*Candida albicans* ATCC 2091	*Aspergillus niger* ATCC 9029
1	14(30)	16(24)	12(39)	11(39)	15(24)	13(31)	11(43)
2	16(20)	17(21)	16(25)	13(32)	17(18)	15(23)	12(39)
3	11(43)	13(36)	14(32)	11(42)	11(41)	12(36)	10(44)
4	11(47)	13(38)	13(36)	10(45)	13(37)	14(28)	11(42)
5	14(30)	15(25)	13(34)	10(43)	14(29)	15(24)	11(40)
6	16(23)	17(17)	15(26)	12(38)	16(22)	15(24)	11(39)
7	16(25)	17(19)	16(21)	15(29)	17(16)	16(20)	12(40)
8	13(36)	15(26)	14(33)	11(41)	15(25)	13(31)	10(44)
9	15(28)	17(24)	15(26)	12(36)	17(19)	15(23)	14(30)
10	14(31)	16(22)	14(29)	10(44)	15(26)	14(28)	10(43)
11	14(33)	16(19)	11(42)	8(46)	15(27)	14(25)	13(33)
Ciprofloxacin (100 μg/disc)	25(0.2)	22(0.4)	24(0.3)	25(0.2)	20(0.1)	–	–
Norfloxacin (100 μg/disc)	24(1.6)	22(3)	23(1.2)	23(0.9)	21(1.4)	–	–
Ketoconazole (100 μg/disc)	–	–	–	–	–	22(1)	20(6.1)
Di methyl formamide	–	–	–	–	–	–	–

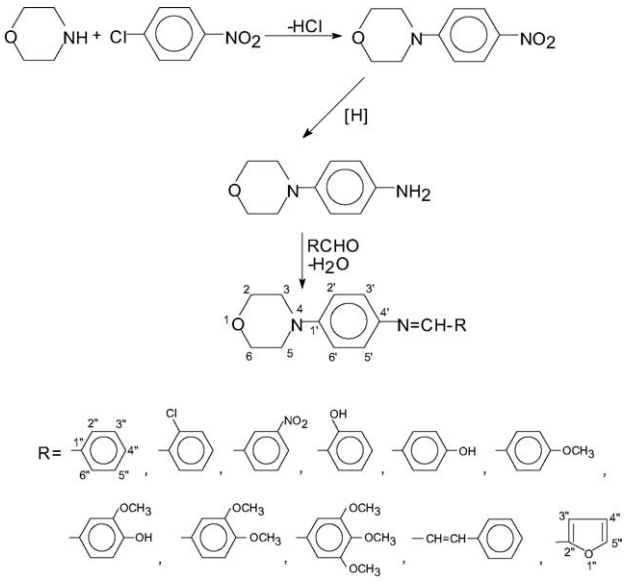
pounds was tested against *C. albicans* and *A. niger* using sabouraud dextrose agar medium (Hi-Media Laboratories, India).

5.2.1. Paper disc diffusion method

The sterilized [14] (autoclaved at 120 °C for 30 min) medium (40–50 °C) was inoculated (1 ml/100 ml of medium) with the suspension (10^5 cfu/ml) of the microorganism (matched to McFarland barium sulphate standard) and poured into a petridish to give a depth of 3–4 mm. The paper impregnated with the test compounds (50 μg/ml in dimethyl formamide) was placed on the solidified medium. The plates were preincubated for 1 h at room temperature and incubated at 37 °C for 24 and 48 h for antibacterial and antifungal activity respectively. Ciprofloxacin (Dr. Reddy's Laboratories, Batch No. IC 666E04, India), Norfloxacin (Petrosolv India Ltd., Batch No. 20112009) and ketoconazole (Wuhan Shengmao Corporation ,Batch No. SBML/kch/403, China) were used as standard for antibacterial and antifungal activity respectively. The observed zone of inhibition is presented in Table 1.

5.2.2. Minimum inhibitory concentration

Minimum inhibitory concentration (MIC) [15] Scheme 1of the test compounds was determined by agar streak dilution method. A stock solution of the synthesised compound (50 μg/ml) in dimethyl formamide was prepared and graded quantities of the test compounds were incorporated in specified quantity of molten sterile agar (nutrient agar for antibacterial activity and sabouraud dextrose agar medium for antifungal activity). A specified quantity of the medium (40–50 °C) containing the compound was poured into a petridish to give a depth of 3–4 mm and allowed to solidify. Suspension of the microorganism was prepared to contain approximately 10^5 cfu/ml and applied to plates with serially diluted



Scheme 1. Synthesis of Schiff bases of 4-(4-aminophenyl) morpholine

compounds in dimethyl formamide to be tested and incubated at 37 °C for 24 and 48 h for bacteria and fungi, respectively. The MIC was considered to be the lowest concentration of the test substance exhibiting no visible growth of bacteria or fungi on the plate. The observed MIC is presented in Table 1.

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