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RESEARCH ARTICLE

Synthesis, Characterization and Biological Evaluation of Novel Morpholine Derivatives

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ABSTRACT

Considering the biological and chemical relevance of Morpholine derivatives, we have devised novel derivatives of morpholine. The series of morpholine derivatives were synthesized in high yield and quality by reaction of 4-(4-aminophenyl)morpholin-3-one with various aromatic o-hydroxy aldehyde giving schiff base, which on reduction and further reaction with chloroacetyl chloride gives chloro derivatives. The obtained chloro derivative is cyclized using strong base to give novel morpholine derivatives. The synthesized compounds were confirmed by NMR (1H, ^{13}C), IR, Mass spectroscopy, elemental analysis. All the compounds were screened vitro antibacterial activities. The Compound **6a** exhibited good inhibition towards antimicrobial activity compared to the other compounds.

KEYWORDS

4-(4-Aminophenyl) Morpholin-3-one, Morpholine Derivatives, Aromatic o-Hydroxy Aldehyde Derivatives, Chloroacetyl Chloride, Base

INTRODUCTION

The development of drug resistance towards the clinically used antibacterial agents has increased the demand for the design and synthesis of new chemical entity that possess antimicrobial activity[1-2]. Moreover, in some cases, especially in patients with impaired liver or kidney functions, the use of antimicrobial drugs to treat infections causes several problems[3-4]. Thus, these trends have required the urgent need for new, more effective antibacterial agents with lack of side effect. The novel morpholine derivatives[5-8] are obtained staring from 4-(4-aminophenyl)morpholin-3-one **(1)**, converted into corresponding schiff base **(3)** by reaction with various aromatic o-hydroxy aldehyde **(2)**.

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The obtained schiff base was reduced to give free base **(4)**, which on further reaction with chloroacetyl chloride gives corresponding chloro derivatives **(5)**. These chloro compounds are cyclized using NaOH to produced novel morpholine derivatives (6a-e) (Figure 1)[9-13].

EXPERIMENTAL

All reagents were of analytical reagent grade and were used without further purification. The moiety 4-(4-aminophenyl)mopholin-3-one is commercially available and is also available in Sigma-Aldrich. This can be also synthesized as per reported literature[9]. Substituents, aromatic O-hydroxy aldehyde derivatives are purchase from Aldrich. Melting points were determined in open capillaries on a Veego (model VMP-D) electronic apparatus and are uncorrected. Thin-layer chromatography was performed on microscope slides (2 cm: 97.5 cm) coated with silica get G for monitoring of the reactions as

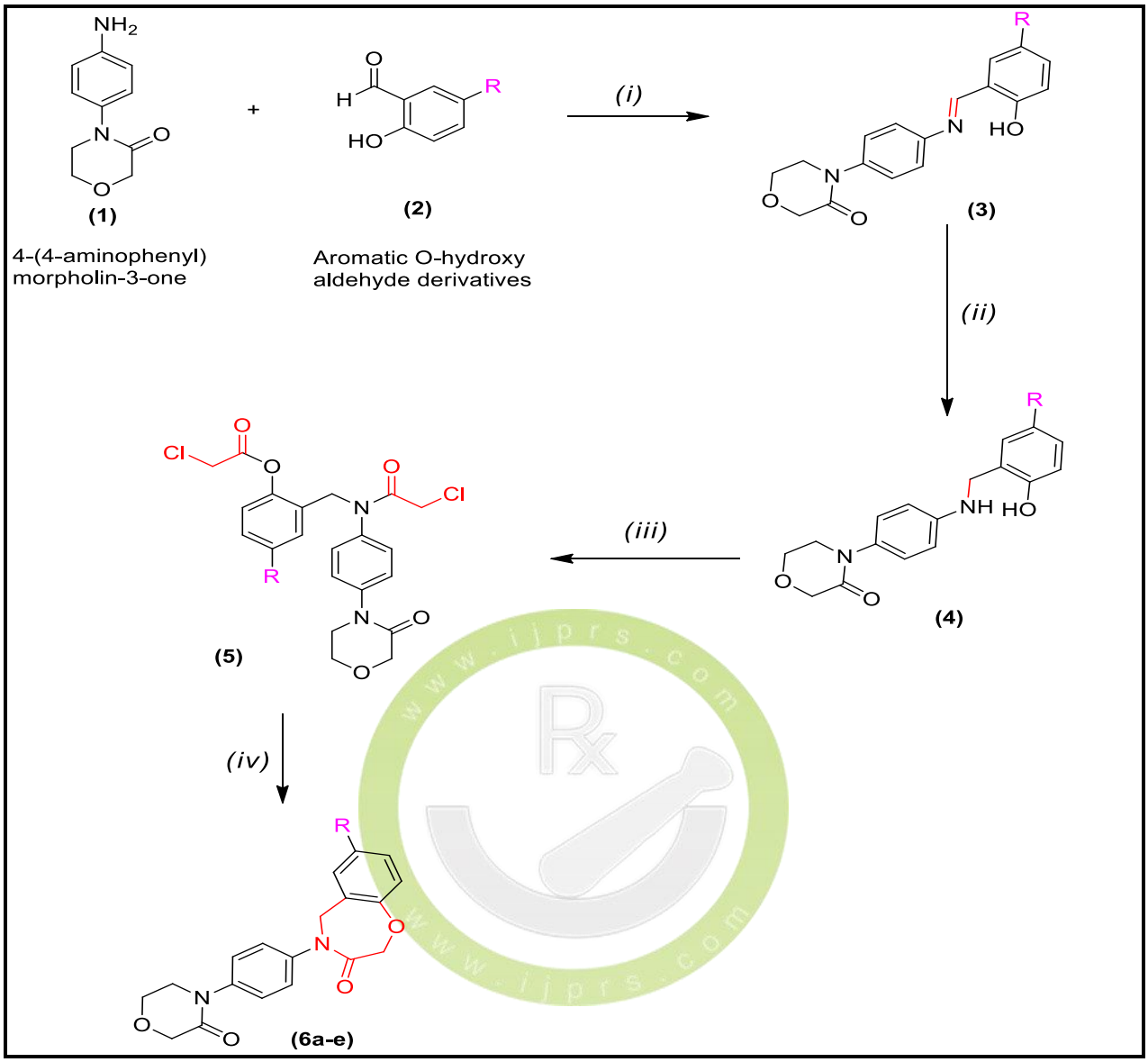

Figure 1: General synthesis of novel morpholine derivatives, (i) Methanol, (ii) Methanol, sodium borohydride, (iii) Chloroacetyl chloride, Toluene, (iv) Sodium hydroxide, water

Table 1: Over all yield of compounds 6a–e

Entry	Substituent (R)	Over all yield of last stage (%)
6a	-H	85.0
6b	-F	81.0
6c	$-NO_2$	77.0
6d	-Cl	83.0
6e	-Br	75.0

well as to establish the identity and purity of the compounds. Ethylacetate: cyclohexane was used as mobile phase and spots were visualized under UV irradiation. Elemental analysis (C, H, and N) was performed by Perkin-Elmer, USA, 2400-II CHN analyzer. Mass spectra were obtained with a Agilent LC-MS-6120 mass spectrometer. FTIR spectra (4,000-400 cm^{-1}) were recorded on Shimadzu 8400-S spectrophotometer using KBr disks. Nuclear magnetic resonance spectra were recorded on a Varian 400 MHz spectrometer using DMSO-d6 as a solvent and TMS as an internal reference (chemical shifts in ppm).

General Procedure for Preparation of Compounds (6a-e).

A mixture of 4-(4-aminophenyl) morpholin-3-one (1 mol. Eq.), aromatic O-hydroxy aldehyde derivatives **(3)** (1 mol. Eq.) (Table 1) in methanol (10 volume) were taken in round bottom flask. The reaction mixture was stirred for 3-5 hours at 25-35°C, reaction was monitored by TLC (ethylacetate:cyclohexane = 1:1), after completion of reaction, solid material was filtered and washed with methanol to get pure compound **(3)**.

To a stir suspension of compound **(3)** (1 mol. Eq.) in methanol (10 volume), sodium borohydride (0.5 mol. Eq.) were added lot wise at 25-35°C. The reaction mixture was stirred for 3-4 hours at 25-35°C, reaction was monitored by TLC (ethylacetate:cyclohexane = 6:4), after completion of reaction, solid material was filtered and washed with methanol to get pure compound **(4)**.

To a stir suspension of compound **(4)** (1 mol. Eq.) in toluene (10 volume), chloroacetyl chloride (1 mol. Eq.) was added in single lot. The reaction mixture was heated to 90-100°C and stir for 2-3 hours at this temperature. The reaction was monitored by TLC (ethylacetate:cyclohexane = 6:4), after completion of reaction, the reaction mixture was cool to 25-35°C. The reaction mixture was wash with water and solid material was obtained after distillation of toluene under reduced pressure below 60°C to get compound **(5)**.

The compound **(5)** (1 mol. Eq.) was taken in water (10 volume) and sodium hydroxide (2 mol. Eq.) were added in single lot. The reaction mixture was heated to 80-90°C and stir for 4-5 hours at this temperature. The reaction was monitored by TLC (ethylacetate:cyclohexane = 9:1), after completion of reaction, the reaction mixture was cool to 25-35°C and solid material was filtered off and wash with water to get pure cyclized product **(6a-e)**.

4-(4-(3-Oxomorpholino)phenyl)-4,5-dihydrobenzo[f][1,4]oxazepin-3(2H)-one (6a).

Creamish color solid, Yield 95 %; m.p. 185-187°C; 1 H NMR (400 MHz, DMSO-d6), 7.17-7.19(dd, 1H), 6.97-7.06(m, 3H), 6.81-6.83(dd, 1H), 6.71-6.75(t, 1H), 6.54-6.56(d, 2H), 4.48(s, 2H), 4.23(s, 2H), 4.13(s, 2H), 3.90-3.92(t, 2H), 3.57-3.59(t, 2H); 13C NMR (100 MHz, DMSO-d6), C= 166.7, 164.5, 156.5, 137.7, 135.0, 127.3, CH= 131.6, 127.9, 127.7, 120.9, 112.2, CH_2= 73.5, 72.3, 66.1, 55.0, 52.0; FTIR (KBr): 1785 cm^{-1} (C=O Stretching), 1724.2 cm^{-1} (Aromatic C-H Stretching), 1590.8 cm^{-1} (Aromatic C-C Stretching), 1475 cm^{-1} (Aliphatic -CH_2 Stretching), 1150 cm^{-1} (R-O-R, C-O Stretching), 840 cm^{-1} (Para disubstituted Aromatic C-H out of plane), 770 cm^{-1} (Ortho disubstituted Aromatic C-H out of plane); ESI/MS *m/z* 339.2(M+1); Anal. Calc. for $C_{19}H_{18}N_2O_4$ (338.36): C, 67.44; H, 5.36; N, 8.28; Found: C, 67.20; H, 5.26; N, 8.12.

7-Fluoro-4-(4-(3-oxomorpholino)phenyl)-4,5-dihydrobenzo[f][1,4]oxazepin-3(2H)-one (6b).

A light green color solid, Yield 92 %; m.p. 221-223°C; 1 H NMR (400 MHz, DMSO-d6), 7.00-7.02(d, 1H), 6.80-6.83(d, 1H), 6.75-6.78(t, 2H), 6.71-6.73(d, 2H), 6.61(s, 1H),4.56(s, 2H), 4.31(s, 2H), 4.25(s, 2H), 3.78-3.81(t, 2H), 3.50-3.55(t, 2H); 13C NMR (100 MHz, DMSO-d6), C= 166.2, 164.2, 155.0, 152.7, 137.5, 135.3, 128.6, CH= 131.2, 119.2, 114.5, 114.2, CH_2= 73.2, 72.1, 66.0, 55.2, 52.2; FTIR (KBr): 1780 cm^{-1} (C=O Stretching), 1720.2 cm^{-1} (Aromatic C-H Stretching), 1589.3 cm^{-1} (Aromatic C-C Stretching), 1474.5 cm^{-1} (Aliphatic -CH_2 Stretching), 1348.5 cm^{-1} (Aromatic -C-F Stretching), 1149 cm^{-1} (R-O-R, C-O Stretching),

841 cm^{-1} (Para disubstituted Aromatic C-H out of plane), 769 cm^{-1} (Ortho disubstituted Aromatic C-H out of plane); ESI/MS *m/z* 357.5(M+1); Anal. Calc. for $C_{19}H_{17}FN_2O_4$ (356.35): C, 64.04; H, 4.81; N, 7.86; Found: C, 64.13; H, 4.92; N, 7.90.

7-Nitro-4-(4-(3-oxomorpholino)phenyl)-4,5-dihydrobenzo[f][1,4]oxazepin-3(2H)-one (6c).

A yellow color solid, Yield 95 %; m.p. 210-212°C; 1H NMR (400 MHz, DMSO-d6), 7.98(s, 1H),7.83-7.85(d, 1H), 7.11-7.13(d, 1H), 6.75-6.78(t, 2H), 6.60-6.62(d, 2H), 4.79(s, 2H), 4.41(s, 2H), 4.19(s, 2H), 3.92-3.95(t, 2H), 3.62-3.65(t, 2H); 13C NMR (100 MHz, DMSO-d6), C= 166.9, 164.7, 160.4, 140.9, 137.8, 132.3, 129.6, CH= 131.9, 124.8, 123.2, 115.2, CH_2= 73.8, 72.7, 66.5, 55.4, 52.4; FTIR (KBr): 1786.2 cm^{-1} (C=O Stretching), 1725.2 cm^{-1} (Aromatic C-H Stretching), 1595.2 cm^{-1} (Aromatic C-C Stretching), 1525 cm^{-1} (Aromatic N-O Stretching), 1477.1 cm^{-1} (Aliphatic -CH_2 Stretching), 1151.1 cm^{-1} (R-O-R, C-O Stretching), 771.2 cm^{-1} (Ortho disubstituted Aromatic C-H out of plane), 842.5 cm^{-1} (Para disubstituted Aromatic C-H out of plane); ESI/MS *m/z* 384.6(M+1); Anal. Calc. for $C_{19}H_{17}N_3O_6$ (383.35): C, 59.53; H, 4.47; N, 10.96; Found: C, 59.2; H, 4.25; N, 10.75.

7-Chloro-4-(4-(3-oxomorpholino)phenyl)-4,5-dihydrobenzo[f][1,4]oxazepin-3(2H)-one (6d).

An off-white color solid, Yield 96 %; m.p. 214-216°C; 1 H NMR (400 MHz, DMSO-d6), 7.98(s, 1H),7.83-7.85(d, 1H), 7.11-7.13(d, 1H), 6.75-6.78(t, 2H), 6.60-6.62(d, 2H), 4.79(s, 2H), 4.41(s, 2H), 4.19(s, 2H), 3.92-3.95(t, 2H), 3.62-3.65(t, 2H); 13C NMR (100 MHz, DMSO-d6), C= 166.9, 164.7, 160.4, 140.9, 137.8, 132.3, 129.6, CH= 131.9, 124.8, 123.2, 115.2, CH_2= 73.8, 72.7, 66.5, 55.4, 52.4; FTIR (KBr): 1787.6 cm^{-1} (C=O Stretching), 1725.5 cm^{-1} (Aromatic C-H Stretching), 1587.8 cm^{-1} (Aromatic C-C Stretching), 1479 cm^{-1} (Aliphatic -CH_2 Stretching), 1229.2 cm^{-1} (Aromatic -C-Cl Stretching), 1147.2 cm^{-1} (R-O-R, C-O Stretching), 775 cm^{-1} (Ortho disubstituted Aromatic C-H out of plane), 835.9 cm^{-1} (Para disubstituted Aromatic C-H out of plane); ESI/MS *m/z* 373.6(M+1); Anal. Calc. for $C_{19}H_{17}ClN_2O_4$ (372.35); C, 61.21; H, 4.60; N, 7.51; Found: C, 61.09; H, 4.51; N, 7.40.

7-Bromo-4-(4-(3-oxomorpholino)phenyl)-4,5-dihydrobenzo[f][1,4]oxazepin-3(2H)-one (6e).

A light brownish color solid, Yield 91 %; m.p. 215-218°C; 1 H NMR (400 MHz, DMSO-d6), 7.61(s, 1H),7.23-7.25(d, 1H), 6.84-6.86(d, 1H), 6.71-6.73(t, 2H), 6.57-6.59(d, 2H), 4.79(s, 2H), 4.41(s, 2H), 4.19(s, 2H), 3.92-3.95(t, 2H), 3.62-3.65(t, 2H); 13C NMR (100 MHz, DMSO-d6), C= 166.8, 164.3, 156.0, 137.9, 135.3, 129.3, 112.6, CH= 131.9, 130.2, 130.0, 113.2, CH_2= 73.8, 72.7, 66.5, 55.4, 52.4; FTIR (KBr): 1760.9 cm^{-1} (C=O Stretching), 1735.6 cm^{-1} (Aromatic C-H Stretching), 1598.5 cm^{-1} (Aromatic C-C Stretching), 1480.1 cm^{-1} (Aliphatic -CH_2 Stretching), 1249.8 cm^{-1} (Aromatic -C-Br Stretching), 1142.7 cm^{-1} (R-O-R, C-O Stretching), 765.8 cm^{-1} (Ortho disubstituted Aromatic C-H out of plane), 843.2 cm^{-1} (Para disubstituted Aromatic C-H out of plane); ESI/MS *m/z* 418.41(M+1); Anal. Calc. for $C_{19}H_{17}BrN_2O_4$ (417.25); C, 54.69; H, 4.11; N, 6.71; Found: C, 54.52; H, 4.09; N, 6.65.

RESULT AND DISCUSSION

The elemental analysis data showed good agreement between the experimentally determined values and the theoretically calculated values within the limits of permissible error. Yield and substitution of the synthesized compounds are listed Table 1.

Biological Evaluation

Antibacterial Activity

Antibacterial activity of the synthesized compounds was determined against Gram-positive bacteria (*Bacillus subtilis and Staphylococcus aureus*) and Gram-negative bacteria (*Escherichia coli and Xanthomonas malvacearum*) in DMF by disc diffusion method on nutrient agar medium[14].

The sterile medium (nutrient agar medium, 15mL) in each petri plates was uniformly smeared with cultures of Gram-positive and Gram-negative bacteria. Sterile discs of 10 mm

diameter (Hi-Media) were made in each of the petri plates to which 50 µL (1 mg/mL, that is, 50µg/disc) of the different synthesized compounds was added. The treatments also included 50 µL of DMF as negative control and streptomycin (1 mg/mL; 10µg/disc) as positive control for comparison. For each treatment, three replicates were maintained. The plates were incubated at 37 ± 2°C for 24 h, and the size of the resulting zone of inhibition, if any was determined.

The investigation of antibacterial screening data revealed that synthesized compounds showed comparable activity against *Bacillus subtilis*, *Staphylococcus aureus*, and *Escherichia coli*. Compound **6a** exhibited good activity with the zone of inhibition in the range of 16 mm against pathogenic bacteria strain.

Antifungal Activity

The synthesized compounds were screened for their antifungal activity against *Fusarium oxysporum* in DMF by poisoned food technique[15].

Potato dextrose agar (PDA) media were prepared, and about 15 mL of PDA was poured into each petri plate and allowed to solidify 5 mm disc of seven-day-old culture of the test fungi was placed at the center of the petri plates and incubated at 26°C for 7 days.

After incubation, the percentage inhibition was measured and three replicates were maintained for each treatment. Nystatin was used as standard.

All the synthesized compounds and nystatin were tested (at the dosage of 500 µL of the compounds/petri plate, where concentration was 0.1 mg/mL) by poisoned food technique. The antifungal activity of synthesized compounds was evaluated and compared with standard drug nystatin. All the synthesized compounds showed moderate inhibitory activity and compound **6a** showed good antifungal activity with the 56.1% inhibition against *F. oxyspo- rum*, compared to other compound. Among the synthesized compounds, inhibitory activity is in the order of **6a** > **6b–d** > **6e** against tested fungi. Antimicrobial screening results of the tested compounds are shown in Table 2.

CONCLUSION

In this study, the synthesis of some morpholine derivatives (6a-e) was performed and their structures were confirmed by IR, ^{1}H NMR, ^{13}C NMR, Mass spectroscopy and elemental analysis techniques. In addition, the newly synthesized compounds were screened for their antimicrobial and antifungal activities. Some of them were found to possess good antifungal activity.

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Table 2: *In Vitro* antibacterial and antifungal activities of synthesized compounds
Diameter of inhibition zone (mm) % inhibition

	B. subtilis	S. aureus	X. malvacearum	E. coli	F. oxysporum
6a	13	15	16	14	56.1
6b	11	17	11	13	52.7
6c	14	11	11	12	47.2
6d	13	15	14	14	44.6
6e	11	14	12	13	41.9
Streptomycin	18	20	18	19	—
Nystatin	—	—	—	—	85.2

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