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# Synthesis and Antimicrobial Evaluation of Novel 1, 3-Thiazolan-4-one Derivatives.

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Research Article

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## ABSTRACT

A series of new 3-(2-morpholinophenyl)-2-aryl/heteryl-1,3-thiazolan-4-one 6a-j were synthesized by the reaction of N-(2-morpholinophenyl)-N-[1-aryl/heterylmethylidene]amine 5a-j and thioglycolic acid. The chemical structures of newly synthesized compounds were elucidated by IR, 1H NMR, MS and elemental analyses. The compounds 6a-j were evaluated for their anticaterial activity against Gram-positive bacteria viz. Bacillus subtilis (MTCC 441), Bacillus sphaericus (MTCC 11) and Staphylococcus aureus (MTCC 96), and Gram-negative bacteria viz. Pseudomonas aeruginosa (MTCC 741), Klobsinella aerogenes (MTCC 39) and Chromobacterium violaceum (MTCC 2656). Compounds 6e, 6f, 6i, and 6j exhibited potent inhibitory activity compared to standard drug at the tested concentrations. The results also reveal that the presence 4-methoxyphenyl (6e) or 2-hydroxyphenyl (6f) or 2-furyl (6i) or 2-thienyl (6j) on thiazolan-4-one ring might be the reason for the significant inhibitory activity.

## INTRODUCTION

Morpholine derivative plays an important role in the treatment of several diseases. Hetero cyclic ring systems having morpholine nucleus have aroused great interest in recent years due to their variety of biological activities [1]. Morpholine derivatives were reported to possess anti-inflammatory[2], analgesic [3], local anesthetic[4], anti-HIV[5], anticancer[6], appetite suppressant[7], antidepressant[8], antiplatelet[9], selective inhibitor of protein kinase C[10], neuroprotective[11], antitumor[12], antituberculosis[13], antimalarial[14], antiparasitic[15], hypocholesterolemic and hypolipidemic activities[16].

Further, there has been considerable interest in the chemistry of thiazolidine-4-one ring system, which is a core structure in various synthetic pharmaceuticals displaying a broad spectrum of biological activities[17]. Thiazolidin-4-one ring also occurs in nature; thus actithiazic acid isolated from *streptomyces* strains exhibits highly specific *in vitro* activity against *mycobacterium tuberculosis*[18]. Thiazolidin-4-one derivatives are known to exhibit diverse bioactivities such as anti-convulsant[19], antidiarrheal[20], anti-platelet activating factor[21], anti-histaminic[22], anti-diabetic[23], cyclooxygenase (COX) inhibitory[24], $Ca^{2+}$-channel blocker[25], platelet activating factor (PAF) antagonist[26], cardioprotective[27], anti-cancer[28] and tumor necrosis factor-α antagonist[29] activities. Following the successful introduction, inspired by the biological profile of morpholine and thiazolan-4-one derivatives and their increasing importance in pharmaceutical and biological fields in continuation of our research on biologically active heterocycles[30-34], and also to know the biological effects of the compounds incorporating the two active pharmacophores in a single molecular frame work, it is considered worthwhile to synthesize certain new chemical entities such as 3-(2-morpholinophenyl)-2-aryl/heteryl-1,3-thiazolan-4-one derivatives and to evaluate their *in vitro* antibacterial activity.

## MATERIALS AND METHODS

### Experimental Chemistry

Compounds 4-(2-nitrophenyl)morpholine (3), 2-morpholinoaniline (4), *N*-(2-morpholinophenyl)-*N*-[1-aryl/heterylmethylidene]amine (5a-j), 3-(2-morpholinophenyl)-2-aryl/heteryl-1,3-thiazolan-4-one (6a-j) are synthesized.

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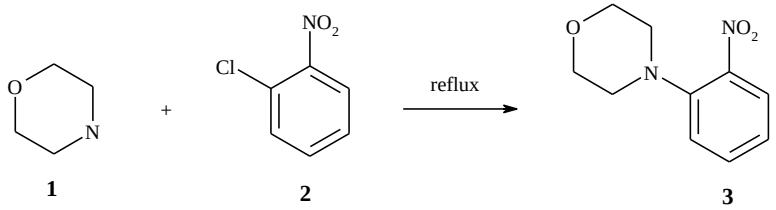
The identification of compounds 3, 4, 5a-j and 6a-j is made by using melting point, infrared, $^1$H NMR, $^{13}$C NMR, MS spectra and elemental analyses. Melting points was determinate on Electro thermal apparatus (Fischer Scientific 2555) in an open capillary tube and are uncorrected. Infrared spectra were recorded in cm-1 using KBr pellets on a Perkin–Elmer FTIR spectrometer. The $^1$H NMR, $^{13}$C NMR spectra were recorded on a Varian Gemini spectrometer (300 MHz for $^1$H and 75 MHz for $^{13}$C). Chemical shifts are reported in δ ppm units with respect to TMS as internal standard and coupling constants (*J*) are reported in Hz units. Mass spectra were recorded on a VG micro mass 7070H spectrometer.

Elemental analyses (C, H, N) determined by means of a Perkin–Elmer 240 CHN elemental analyzer, were within ± 0.4% of theory. Reactions were monitored by thin-layer chromatography (TLC) on pre-coated silica gel $F_{254}$ plates from Merck, and compounds visualized either by exposure to UV light. Chromatographic columns 70–230 mesh silica gel for separations were used.

### Synthesis of 4-(2-nitrophenyl)morpholine (3)

A mixture of 1-chloro-2-nitrobenzene **2** (0.01 mol) and morpholine **1** (0.2 mol) was heated on a water bath at reflux temperature with occasional stirring for 24 h. The obtained solid was cooled and collected on a filter paper and recrystallized from methanol to gave a orange crystals of 96% yield, melting point 148-150$^o$C (**Scheme 1**). IR (KBr): 3071, 1540, 1410, 1370 cm$^{-1}$; $^1$H NMR (DMSO-$d_6$): *δ* 7.86 (m, 1H, ArH), 6.90-6.80 (m, 3H, ArH), 3.67-3.50 (m, 4H, $CH_2$-N), 3.40-3.30 (m, 4H, $CH_2$-O).

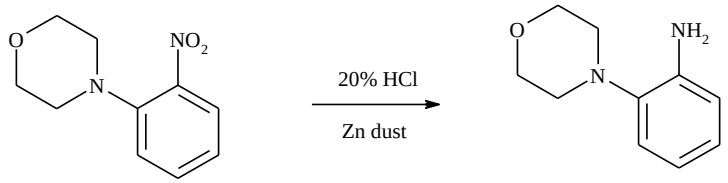



**1** **2** **3**

Scheme 1: Synthesis of 4-(2-nitrophenyl)morpholine (3)

### Synthesis of 2-morpholinoaniline (4)

A solution of compound **3** (0.01 mol) in 20% hydrochloric acid (75 mL) was treated with small portion of zinc dust with stirring and gentle warming until all the orange colour of nitro compound had disappeared. The mixture was filtered to remove the excess zinc and the filtrate was neutralized with NaOH. The crude product obtained was filtered, dried and crystallized from methanol to give pure compound of 84% yield, melting point 128-130$^o$C (**Scheme 2**). IR (KBr): 3370-3310, 3056, 1410, 1074 cm$^{-1}$; $^1$H NMR (DMSO-$d_6$): *δ* 6.30-6.20 (m, 2H, ArH), 6.75-6.80 (m, 2H, ArH), 3.90-4.00 (bs, 2H, $NH_2$), 3.60-3.50 (m, 4H, $CH_2O$), 2.90-2.80 (m, 4H, $CH_2$-N).




**3** **4**

Scheme 2: Synthesis of 2-morpholinoaniline (4)

### Synthesis of *N*-(2-morpholinophenyl)-*N*-[1-aryl/heterylmethylidene]amine (5a-j)

A mixture of compound **4** (0.01 mol), corresponding aryl/heteryl aldehyde (0.01 mol) and acetic acid (0.5 mL) was refluxed in toluene for 3 h using a Dean–Stark apparatus and the water formed was removed azeiotropically. The progress of the reaction was checked by TLC using toluene: ethyl acetate (4:1) as an eluent. After completion of the reaction, solvent was removed by distillation to give the solid, which was filtered, and recrystallized from methyl alcohol to get the pure compounds **5a-j** (**Scheme 3**).

**5a:** Yellow solid, yield 71%, m.p. 149-151 $^o$C; IR (KBr): 3054, 2988, 1617, 1610, 1067 cm$^{-1}$; $^1$H NMR (DMSO-$d_6$): *δ* 8.10 (s, 1H, CH=N), 7.20-7.30 (m, 6H, ArH), 6.50-6.40 (m, 3H, ArH), 3.15-3.20 (m, 4H, $CH_2$-N), 3.65-3.55 (m, 4H, $CH_2$-O).

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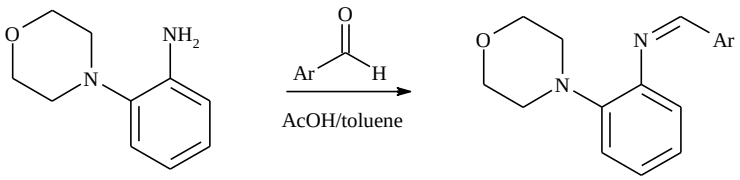



**5**: **a**) phenyl; **b**) 4-chlorophenyl; **c**) 4-methylphenyl; **d**) 4-nitrophenyl; **e**) 4-methoxyphenyl; f) 2-hydroxyphenyl; **g**) 4-dimethylaminophenyl; **h**) 2-pyridyl; **i**) 2-furyl; **j**) 2-thienyl

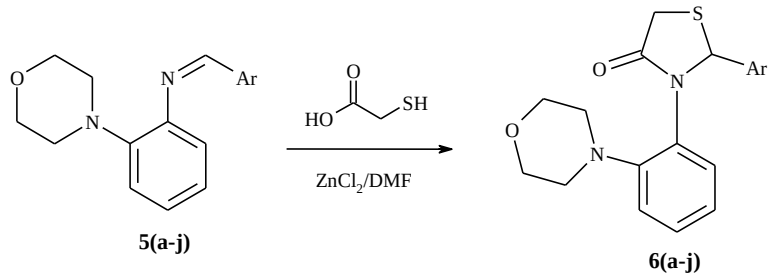
Scheme 3: Synthesis of *N*-(2-morpholinophenyl)-*N*-[1-aryl/heterylmethylidene]amine (5a-j)

## Synthesis of compounds (6a-j)

A mixture of compound **5a-j** (0.01 mol), thioglycolic acid (0.02 mol) in *N*, *N*-dimethylformamide (20 mL) with a pinch of anhydrous $ZnCl_2$, was refluxed for 6 h. The progress of the reaction was checked by TLC using toluene: ether (3:1) as an eluent. The reaction mixture was cooled to room temperature and then poured into crushed ice. It was set-aside for overnight at room temperature. The solid thus separated was filtered, washed several times with water, and purified by column chromatography on silica-gel with hexane-ethyl acetate as eluent to get the pure compound **6a-j** (**Scheme 4**).

**6a:** Brown solid, yield 71%, m.p. 152-154 $^oC$; IR (KBr): 3062, 1698, 1612, 1604, 1475, 1066, 712 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$): $\delta$ 7.40-7.30 (m, 5H ArH), 6.80-6.90 (m, 3H, ArH), 6.32 (m, 1H, ArH), 5.94 (s, 1H, N-CH-S), 3.70-3.60 (m, 6H, $CH_2$-S + $CH_2$-O), 2.75-2.80 (m, 4H, $CH_2$-N); $^{13}C$ NMR (DMSO-$d_6$): $\delta$ 41.2, 53.1, 64.7, 68.4, 114.2, 121.6, 124.6, 125.3, 125.9, 128.5, 129.3, 132.3, 135.2, 141.4, 169.4; MS: *m/z* 341 ($M^+$ + 1). *Anal*. Calcd for $C_{19}H_{20}N_2O_2S$: C, 67.03; H, 5.92; N, 8.23. Found: C, 67.00; H, 5.86; N, 8.18.

**6e:** Brown solid, yield 70%, m.p. 147-149 $^oC$; IR (KBr): 3067, 1696, 1610, 1604, 1437, 1060, 712 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$): $\delta$ 7.33 (d, 2H, *J* = 8.3 Hz, ArH), 7.29 (d, 2H, *J* = 8.3 Hz, ArH), 6.90-6.80 (m, 4H, ArH), 5.82 (s, 1H, N-CH-S), 3.70-3.60 (m, 6H, $CH_2$-S + $CH_2$-O), 3.52 (s, 3H, $OCH_3$), 2.75-2.80 (m, 4H, $CH_2$-N); $^{13}C$ NMR (DMSO-$d_6$): $\delta$ 41.2, 52.6, 53.2, 61.6, 70.2, 112.8, 114.2, 124.5, 125.1, 127.1, 127.8, 129.2, 132.3, 145.8, 157.8, 170.7; MS: *m/z* 370 ($M^+$). *Anal*. Calcd for $C_{20}H_{22}N_2O_3S$: C, 64.84; H, 5.99; N, 7.56. Found: C, 64.78; H, 5.92; N, 7.52.




**6**: **a**) phenyl; **b**) 4-chlorophenyl; **c**) 4-methylphenyl; **d**) 4-nitrophenyl; **e**) 4-methoxyphenyl; f) 2-hydroxyphenyl; **g**) 4-dimethylaminophenyl; **h**) 2-pyridyl; **i**) 2-furyl; **j**) 2-thienyl

Scheme 4: Synthesis of compounds (6a-j)

## Antibacterial Assay

All the newly synthesized compounds **6a-j** were screened for their antibacterial activity against Gram-positive bacteria *viz*. *Bacillus subtilis* (MTCC 441), *Bacillus sphaericus* (MTCC 11) and *Staphylococcus aureus* (MTCC 96), and Gram-negative bacteria *viz*. *Pseudomonas aeruginosa* (MTCC 741), *Klobsinella aerogenes* (MTCC 39) and *Chromobacterium violaceum* (MTCC 2656) by disc diffusion method[35]. For the antibacterial assay standard inoculums (1-2 × $10^7$ c.f.u/mL 0.5 Mc Farland standards) were introduced on to the surface of sterile agar plates, and a sterile glass spreader was used for even distribution of the inoculums. The discs measuring 6.26 mm in diameter were prepared from Whatman no.1 filter paper and sterilized by dry heat at 140 $^oC$ for 1 h. The sterile discs previously soaked in a known concentration of the test compounds were placed in nutrient agar medium. The plates were inverted and incubated for 24 h at 37$^oC$. The inhibition zones were measured and compared with the standard drug streptomycin and zone of inhibition are presented in **Table 1**.

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Table 1: Antibacterial activity of compounds 6a-j

| Compound | Zone of inhibition at 50 μg/mL (mm) | | | | | |
|---|---|---|---|---|---|---|
| | *B. subtilis* | *B. sphaericus* | *S. aureus* | *P. aeruginosa* | *K. aerogenes* | *C. violaceum* |
| **6a** | 12 | 14 | 12 | 15 | 10 | 12 |
| **6b** | 10 | 12 | 10 | 12 | 11 | 18 |
| **6c** | 14 | 10 | 14 | 10 | 14 | 16 |
| **6d** | 15 | 14 | 16 | 10 | 12 | 14 |
| **6e** | 26 | 28 | 28 | 29 | 25 | 30 |
| **6f** | 25 | 24 | 24 | 26 | 24 | 28 |
| **6g** | 10 | 10 | 13 | 10 | 10 | 18 |
| **6h** | 10 | 12 | 12 | 12 | 10 | 14 |
| **6i** | 22 | 26 | 28 | 30 | 24 | 29 |
| **6j** | 25 | 28 | 28 | 28 | 25 | 26 |
| streptomycin | 25 | 30 | 30 | 30 | 25 | 30 |

The antibacterial screening data reveal that all the tested compounds **6a-j** showed moderate to good inhibition towards all the tested strains. Compounds **6e**, **6f**, **6i**, and **6j** exhibited potent inhibitory activity compared to standard drug at the tested concentrations. The results also reveal that the presence of The results also reveal that the presence 4-methoxyphenyl (**6e**) or 2-hydroxyphenyl (**6f**) or 2-furyl (**6i**) or 2-thienyl (**6j**) on thiazolan-4-one ring might be the reason for the significant inhibitory activity. The presence of 4-methoxyphenyl moiety in the molecules would enhance the inhibitory activity as shown by **6e**. However, presence of 4-methylphenyl (**6c**) and 2-pyridyl (**6h**) did not show significant inhibition. Further, the comparison of zone of inhibition values (in mm) of the selected compounds **6** and standard drug against different bacteria is presented in **Figure 1**.

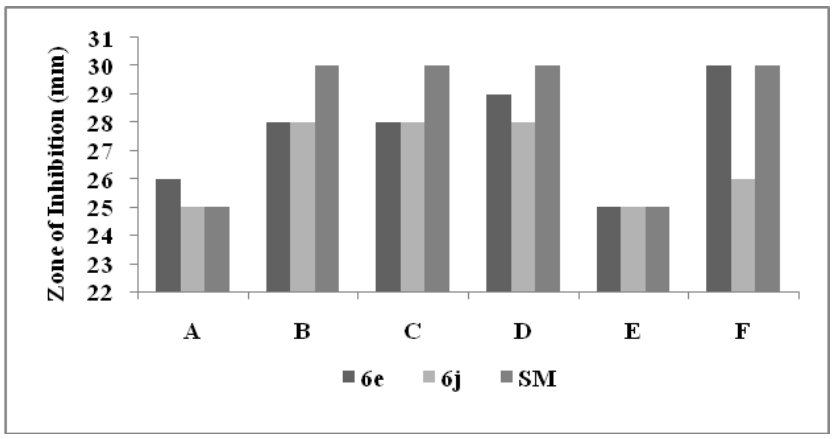


**Figure 1. Comparison of zone of inhibition values (mm) of selected compounds and standard drugs at 50 μg/mL against different bacteria. A) *B. subtilis*; B) *B. sphaericus*; C) *S. aureus*; D) *P. aeruginosa*; E) *K. aerogenes*; F) *C. Violaceum***

## CONCLUSION

In conclusion, a series of new 3-(2-morpholinophenyl)-2-aryl/heteryl-1,3-thiazolan-4-one **6a-j** has been synthesized. The antibacterial activity of these compounds was evaluated against various bacteria. Among the synthesized compounds **6e**, **6f**, **6i** and **6j** were found to be most active against all the microorganisms employed. Further, the presence of 4-methoxyphenyl moiety in the molecule enhanced the inhibitory activity and emerged as potential molecules for further development.

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