Full Papers

Synthesis of Mannich Bases of Some 2,5-Disubstituted 4-Thiazolidinones and Evaluation of Their Antimicrobial Activities

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Summary

5-Phenyl/methyl-5-morpholinomethyl/pyrrolidinomethyl-2-(5-aryl-1,3,4-oxadiazol-2-yl) imino]-4-thiazolidinones (**5a–m**) were synthesized by the reaction of 5-phenyl/methyl-2-[(5-aryl-1,3,4-oxadiazol-2-yl)imino]-4-thiazolidinones (**4a–j**) with formaldehyde and morpholine or pyrrolidine. The structures of the compounds were determined by analytical and spectral (IR, ^{1}H-NMR, EIMS) methods. The antibacterial activities of the novel compounds against *Staphylococcus aureus* ATCC 6538, *Staphylococcus epidermidis* ATCC 12228, *Escherichia coli* ATCC 8739, *Klebsiella pneumoniae* ATCC 4352, *Pseudomonas aeruginosa* ATCC 1539, *Salmonella typhi*, *Shigella flexneri* and *Proteus mirabilis* and antifungal activity against *Candida albicans* ATCC 10231 were tested using the disk diffusion method. Compounds **5a**, **5b**, **5c**, **5e**, **5g**, and **5h** were found to be active against *S. aureus* ATCC 6538 (MIC: 312.5; 39; 19.5; 39; 156; and 78 µg/mL respectively) and compounds **5c** and **5h** against *S. flexneri* (MIC: both 312.5 µg/mL). The minimal inhibitory concentrations of these compounds were determined using the micro dilution method.

Introduction

In our literature search we found that Mannich bases have antimicrobial activities [1–6] besides various other activities. In our previous work we had synthesized some thiazolidinone derivatives which were shown to have high antibacterial activity [7]. The present study was carried out with the purpose of finding out the effects of aminomethylation of 4-thiazolidinones on antibacterial activity. In the literature it was shown that when thiazolidinones or theophylline were reacted with formaldehyde and secondary amines at room temperature, new derivatives in which the aminomethyl group was bonded to 3- or 7-position, respectively were obtained [8–10] otherwise when the reaction was carried out under reflux the aminomethyl group was bonded to the 5- or the 8-position, respectively [10, 11]. In this work, in line with literature findings, refluxing 5-phenyl/methyl-2-[(5-aryl-1,3,4-oxadiazol-2-yl)imino]-4-thiazolidinones with formaldehyde and morpholine or pyrrolidine yielded 5-phenyl/methyl-5-morpholinomethyl/pyrrolidinomethyl-2-[(5-aryl-1,3,4-oxadiazol-2-yl)imino]-4-thiazolidinones. The antibacterial activity against *S. aureus* ATCC 6538, *S. epidermidis* ATCC 12228, *E. coli* ATCC 8739, *K. pneumoniae* ATCC 4352, *P. aeruginosa* ATCC 1539, *S. typhi*, *S. flexneri*, and *P. mirabilis* and antifungal activity against *C. albicans* ATCC 10231 were investigated.

Results and Discussion

Aromatic aldehyde semicarbazones (**1a–f**) [12] suspended in glacial acetic acid were stirred with bromine and anhydrous sodium acetate to give 5-aryl-2-amino-1,3,4-oxadiazoles (**2a–f**) [13]. Reaction of these with α-chloro-α-phenylacetyl chloride or α-bromopropionyl bromide yielded 5-aryl-2-[(α-chloro-α-phenylacetyl/α-bromopropionyl)amino-1,3,4-oxadiazoles (**3a–j**) [7]. Compounds **3a–j** were heated with ammonium thiocyanate to give 5-phenyl/methyl-2-[(5-aryl-1,3,4-oxadiazol-2-yl)imino]-4-thiazolidinones (**4a–j**) [7, 14].

Compounds **4a–j** were heated under reflux after the addition of 37% formaldehyde and morpholine or pyrrolidine subsequently to give 5-phenyl/methyl-5-morpholinomethyl/-pyrrolidinomethyl-2-[(5-aryl-1,3,4-oxadiazol-2-yl)imino]-4-thiazolidinones (**5a–m**) (Scheme 1, Table 1). The formulas of the compounds **5a–m** were confirmed by the elemental analyses and their structures were determined by IR, ^{1}H-NMR, and EI mass spectral data. The IR spectra of the compounds displayed the characteristic N-H stretching vibration at 3374–3489 cm^{-1} which showed that the substitution took place at the 5-position rather than 3-position (Table 2). Also the absence of C_5-H of **4a** in the ^{1}H-NMR spectrum of **5a** and the singlet assigned to the CH_3 group at 1.56 ppm which was a doublet in **4a** have proven that the proton at 5-position of **4a** was replaced by the aminomethyl group. The singlet at 12.46 ppm in the spectrum of **5a** showed that the nitrogen still had a proton, which further supported the substitution at the 5-position.

In the ^{1}H-NMR spectrum of **5c**, the CH_2 protons absorbed as an AB system at δ 2.90 and 3.02 ppm with coupling constants of 13.7 and 13.8 Hz characteristic for geminal protons in rigid environments.

EIMS of two compounds chosen as prototypes were taken. MS of compound **5d** showed the molecular ion peak (M^+) with low intensity, while MS of compound **5a** did not show any molecular ion peak but showed the peaks due to fragments that supported the expected structures and which were in accordance with the fragmentation routes (Scheme 2) [13–15].

Experiments were performed to evaluate the antibacterial activity against *S. aureus* ATCC 6538, *S. epidermidis* ATCC 12228, *E. coli* ATCC 8739, *K. pneumoniae* ATCC 4352, *P. aeruginosa* ATCC 1539, *S. typhi*, *S. flexneri*, and

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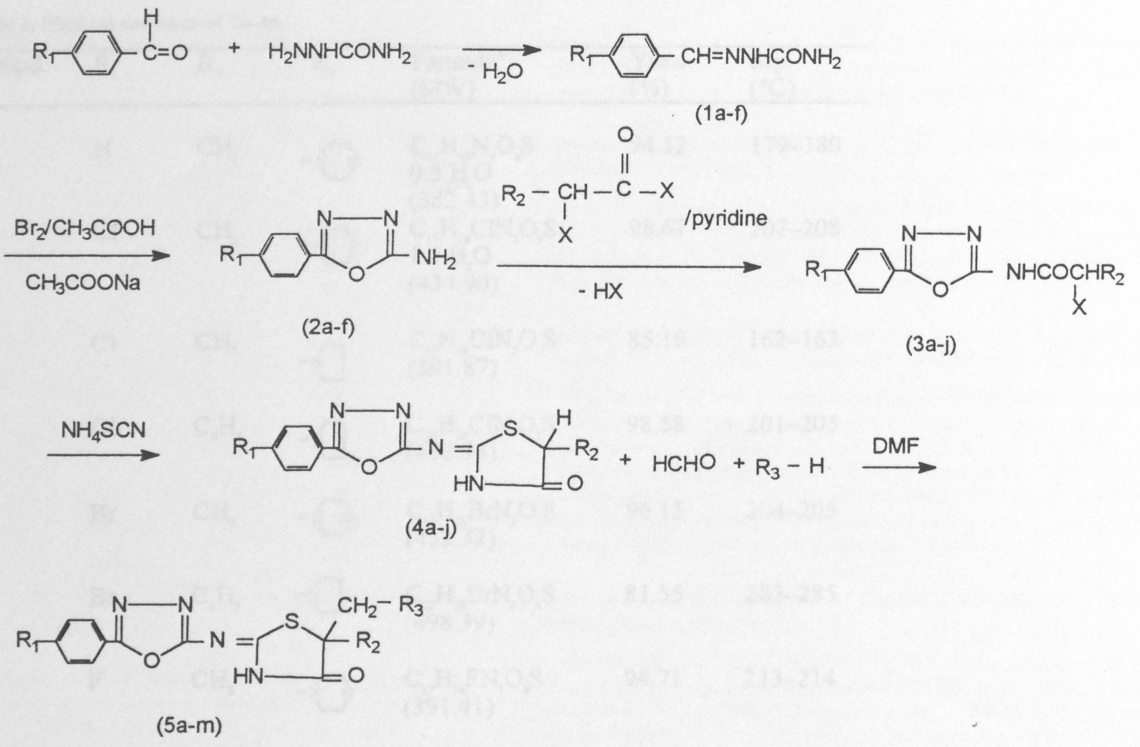
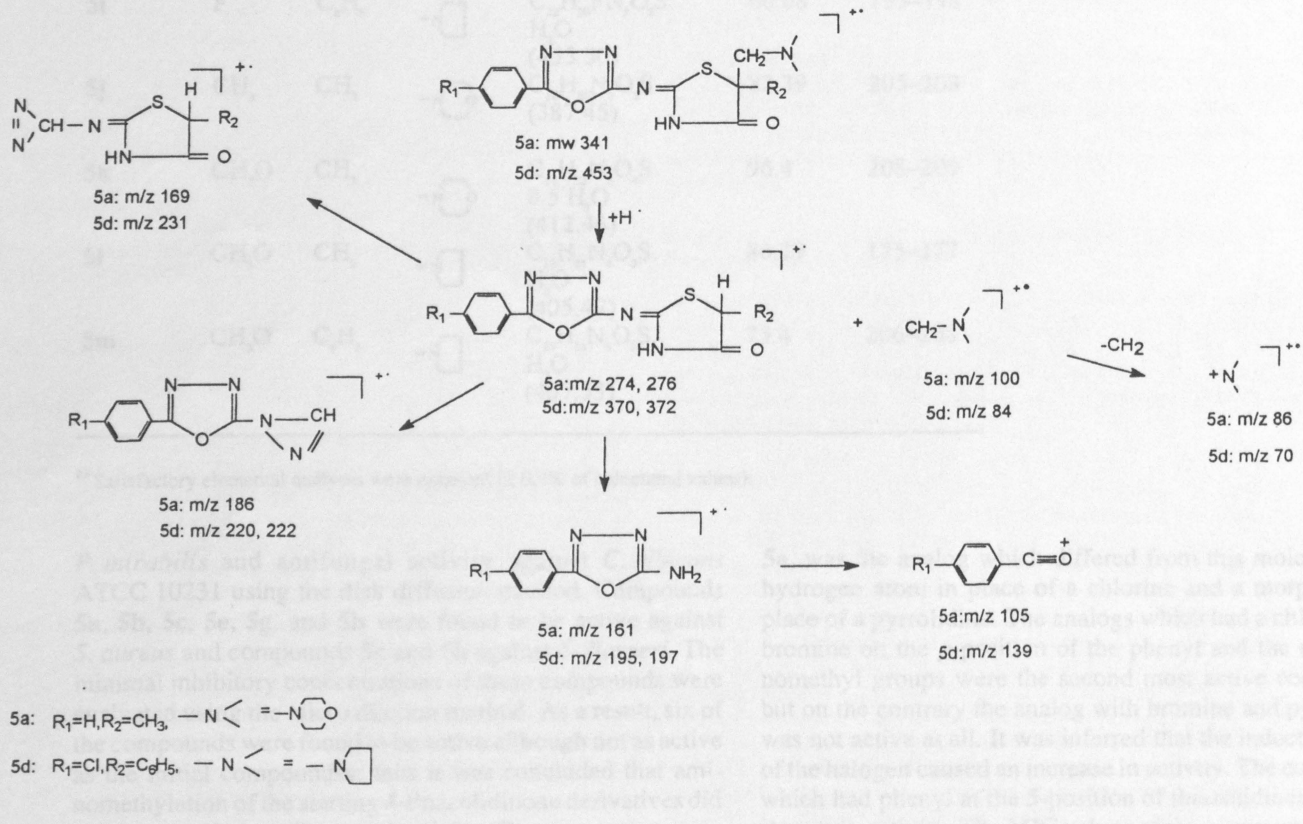


Scheme 1



Scheme 2

Table 1. Physical constants of **5a–m**.

Compd.	R_1	R_2	R_3	Formula[a] (MW)	Yield (%)	Mp (°C)
5a	H	CH_3		$C_{17}H_{19}N_5O_3S.$ $0.5\ H_2O$ (382.43)	94.12	179–180
5b	Cl	CH_3		$C_{17}H_{18}ClN_5O_3S.$ $1.5\ H_2O$ (434.90)	98.67	207–208
5c	Cl	CH_3		$C_{17}H_{18}ClN_5O_2S$ (391.87)	85.19	162–163
5d	Cl	C_6H_5		$C_{22}H_{20}ClN_5O_2S$ (453.94)	98.58	201–205
5e	Br	CH_3		$C_{17}H_{18}BrN_5O_3S$ (452.32)	96.15	204–205
5f	Br	C_6H_5		$C_{22}H_{20}BrN_5O_2S$ (498.39)	81.55	283–285
5g	F	CH_3		$C_{17}H_{18}FN_5O_3S$ (391.41)	94.71	213–214
5h	F	CH_3		$C_{17}H_{18}FN_5O_2S.$ $2H_2O$ (411.44)	88.5	200–201
5i	F	C_6H_5		$C_{22}H_{20}FN_5O_2S.$ H_2O (455.50)	60.08	195–198
5j	CH_3	CH_3		$C_{18}H_{21}N_5O_3S$ (387.45)	82.39	205–208
5k	CH_3O	CH_3		$C_{18}H_{21}N_5O_4S.$ $0.5\ H_2O$ (412.46)	96.4	208–209
5l	CH_3O	CH_3		$C_{18}H_{21}N_5O_3S.$ H_2O (405.47)	86.29	175–177
5m	CH_3O	C_6H_5		$C_{23}H_{23}N_5O_3S.$ H_2O (467.53)	75.4	206–208

[a] Satisfactory elemental analyses were obtained (± 0.4% of calculated values).

P. mirabilis and antifungal activity against *C. albicans* ATCC 10231 using the disk diffusion method. Compounds **5a**, **5b**, **5c**, **5e**, **5g**, and **5h** were found to be active against *S. aureus* and compounds **5c** and **5h** against *S. flexneri*. The minimal inhibitory concentrations of these compounds were evaluated using the micro dilution method. As a result, six of the compounds were found to be active although not as active as the initial compoundss; thus it was concluded that aminomethylation of the starting 4-thiazolidinone derivatives did not enhance their antibacterial activity. The most active compound was compound **5c** which had a *p*-chlorophenyl group on the oxadiazole, a methyl and a pyrrolidinomethyl at the 5-position of the thiazolidinone, while the least active one, **5a**, was the analog which differed from this molecule by a hydrogen atom in place of a chlorine and a morpholine in place of a pyrrolidine. The analogs which had a chlorine or a bromine on the *p*-position of the phenyl and the morpholinomethyl groups were the second most active compounds, but on the contrary the analog with bromine and pyrrolidine was not active at all. It was inferred that the inductive effect of the halogen caused an increase in activity. The compounds which had phenyl at the 5-position of thiazolidinone did not show any activity. The MIC values of the compounds **5a**, **5b**, **5c**, **5e**, **5g**, and **5h** against *S. aureus* were 312.5, 39, 19.5, 39, 156, and 78 µg/mL, respectively and of the compounds **5c** and **5h** against *S. flexneri* were both 312.5 µg/mL (Table 3).

Table 2. Characteristic bands of compounds **5a–m** in IR spectra.

Compound	N-H	C=O
5a	3447	1733
5b	3447	1742
5c	3420	1734
5d	3420	1727
5e	3462	1744
5f	3374	1694
5g	3448	1734
5h	3464	1650
5i	3419	1728
5j	3453	1741
5k	3448	1735
5l	3422	1734
5m	3489	1693

Table 3. MIC values (μg/ml) of compounds **5a**, **5b**, **5c**, **5e**, **5g**, and **5h**.

Compound	*S. aureus* ATCC 6538	*S. flexneri*
5a	312.5	–
5b	39	–
5c	19.5	312.5
5e	39	–
5g	156	–
5h	78	312.5
Cefuroxim Na	1.2	2.4

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Experimental Part

Chemistry

Melting points were estimated with a Büchi 530 apparatus in open capillaries and are uncorrected. Elemental analyses were performed on a Carlo Erba 1106 elemental analyzer. IR spectra were recorded on KBr discs, using a Perkin-Elmer 1600 spectrophotometer. ^{1}H-NMR spectra were taken on a Bruker ARX 200 (^{1}H: 200 MHz, [d_6] DMSO) spectrophotometer. EIMS were determined on a VG Zab Spec (70 eV) mass spectrometer.

*Synthesis of 5-Aryl-2-amino-1,3,4-oxadiazoles (**2a–f**)* [13]

A solution of bromine (5.7 mL) in glacial acetic acid (20 mL) was added dropwise with stirring to a suspension of aromatic aldehyde semicarbazone (0.1 mol) and of sodium acetate (32.8 g) in glacial acetic acid (120 mL). Then the mixture was stirred for an additional hour at room temperature and poured into ice water to precipitate. The residue was filtered off and washed with water and recrystallized from ethanol.

*Synthesis of 5-Aryl-2-[(α-chloro-α-phenylacetyl/α-bromopropionyl)-amino]-1,3,4-oxadiazoles (**3a–j**)* [7]

5-(*p*-Substituted phenyl)-2-amino-1,3,4-oxadiazole (0.01 mol) in a mixture of benzene (4 mL) and dry pyridine (1 mL) was reacted with α-chloro-α-phenylacetyl chloride (0.01 mol) (or α-bromopropionyl bromide (0.01 mol)) in benzene (3 mL) for 1 h at room temperature. The crude product was washed with water and recrystallized from ethanol.

*Synthesis of 5-phenyl/methyl-2-[(5-aryl-1,3,4-oxadiazol-2-yl)imino]-4-thiazolidinones (**4a–j**)* [7]

5-(*p*-Substituted phenyl)-2-[(α-chloro-α-phenylacetyl/α-bromopropionyl)amino-1,3,4-oxadiazole (0.05 mol) and ammonium thiocyanate (0.1 mol) in 96% ethanol (50 mL) was refluxed on a water bath for 1 h, left overnight, filtered, and the precipitate recrystallized from ethanol.

*Synthesis of 5-Phenyl/Methyl-5-morpholinomethyl/pyrrolidinomethyl-2-[(5-aryl-1,3,4-oxadiazol-2-yl)imino]-4-thiazolidinones (**5a–m**)*

A solution of 37% formaldehyde (0.5 mL) and morpholine or pyrrolidine (0.002 mol) was added dropwise with vigorous stirring to a suspension of 5-phenyl/methyl-2-[(5-aryl-1,3,4-oxadiazol-2-yl)imino]-4-thiazolidinone (0.002 mol) in absolute ethanol. After addition of DMF (20 mL) to the medium the mixture was refluxed for 4 h. Then the solution was evaporated under vacuum to half its volume and poured into ice water to precipitate. The residue was filtered off and recrystallized from ethanol.

*5-Methyl-5-morpholinomethyl-2-[(5-phenyl-1,3,4-oxadiazol-2-yl)imino]-4-thiazolidinone (**5a**)*

IR: ν = 3447 cm^{-1} (N-H), 1733 cm^{-1} (C=O).– ^{1}H-NMR: δ = 1.56 (s, 3H, CH_3), 2.46–2.64 (m, 4H, morpholine N-CH_2), 2.85 (s, 2H, CH_2), 3.48 (t, 4H, morpholine O-CH_2), 7.58–7.60 (m, 3H, phenyl $C_{3,4,5}$-H), 7.93–7.98 (m, 2H, phenyl $C_{2,6}$-H), 12.46 (s, 1H, N-H).– EIMS: *m/z* (%) = 274 (0.7), 187 (5), 186 (0.7), 169 (0.5), 161 (0.5), 145 (4), 129 (0.5), 105 (3), 100 (100) (base peak), 86 (0.7), 70 (23), 69 (1.5), 56 (9).

*5-Methyl-5-pyrrolidinomethyl-2-[(5-(p-chlorophenyl)-1,3,4-oxadiazol-2-yl)imino]-4-thiazolidinone (**5c**)*

IR: ν = 3420 cm^{-1} (N-H), 1734 cm^{-1} (C=O).– ^{1}H-NMR: δ = 1.55 (s, 3H, CH_3), 1.63 (bs, 4H, pyrrolidine $C_{3,4}$-H), 2.61–2.64 (m, 4H, pyrrolidine $C_{2,5}$-H), 2.90 and 3.02 (dd, *J* = 13.8 Hz and *J* = 13.7 Hz, 2H, CH_2), 7.47 (d, *J* = 8.6 Hz, 2H, phenyl $C_{3,5}$-H), 7.88 (d, *J* = 8.4 Hz, 2H, phenyl $C_{2,6}$-H), N-H peak disappeared since the proton on nitrogen was exchanged with deuterium of DMSO-d_6.

*5-Phenyl-5-pyrrolidinomethyl-2-[(5-(p-chlorophenyl)-1,3,4-oxadiazol-2-yl)imino]-4-thiazolidinone (**5d**)*

IR: ν = 3420 cm^{-1} (N-H), 1727 cm^{-1} (C=O).– EIMS: *m/z* (%) = 453 (M^+) (0.35), 372 (2.5), 370 (7), 340 (2.5), 338 (7), 231 (6), 222 (4.5), 220 (7.5), 218 (3), 197 (6), 195 (8), 181 (5.5), 179 (16), 176 (4.5), 154 (10.5), 152 (14), 141 (1.5), 139 (46.5), 137 (51), 127 (11), 126 (13), 125 (14.5), 124 (12), 121 (15), 113 (16.5), 111 (35), 90 (5), 85 (46), 84 (18), 78 (96), 77 (22), 71 (68), 70 (31.5), 69 (48), 57 (100) (base peak).

Microbiology

Derivatives **5a–m** were tested *in vitro* for antimicrobial activity against *S. aureus* ATCC 6538, *S. epidermidis* ATCC 12228, *E. coli* ATCC 8739, *K. pneumoniae* ATCC 4352, *P. aeruginosa* ATCC 1539, *S. typhi*, *S. flexneri*, and *P. mirabilis* and antifungal activity against *C. albicans* ATCC 10231 using disk diffusion method where each disc contained 200 μg of the tested compound. For this method, Mueller-Hinton agar (Difco) was melted at 100 °C and after cooling to 56 °C, was poured into Petri plates of 9 cm diameter in quantities of 20 mL, and left on a flat surface to solidify and the surface of the medium was dried at 37 °C. Then, the cultures of each bacteria and yeast strain, after being kept in Mueller-Hinton broth (Difco) at 37 °C for 18–24 h and diluted with Mueller-Hinton broth to 10^5 cfu/mL, were pipetted into the Mueller-Hinton agar plate prepared as described above. The surface of the medium was allowed to dry. The 10 000 μg/mL (in DMSO) compound impregnated discs were applied to the surface of inoculated plates. The Petri plates were placed in an incubator at 37 °C. After 18–24 h of incubation, the Petri plates were examined and it was found that compounds **5a**, **5b**, **5c**, **5e**, **5g**, and **5h** were active against *S. aureus* ATCC 6538 and compounds **5c** and **5h** against *S. flexneri*.

The minimum inhibitory concentrations (MIC) of these compounds were determined by the microbroth dilution technique using Mueller-Hinton broth. Serial two-fold dilutions ranged from 2500 to 2.4 mg/L for compounds.

The inoculum was prepared in broth which had been kept overnight at 37 °C and which had been diluted with Mueller-Hinton broth to give a final concentration of 10^5 cfu/mL in the test tray. The trays were covered and placed in plastic bags to prevent drying. After incubation at 37 °C for 18–24 h, the MIC was defined as the lowest concentration of compound giving complete inhibition of visible growth . MIC values of the compounds are given in Table 3.

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