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Synthesis and evaluation of antibacterial activity of some 2-[[α-(4-substituted benzoyloxy)-α-phenylacetyl or methylacetyl]amino]-5-(4-methoxyphenyl)-1,3,4-oxadiazoles

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Abstract

In this study, a new series of 2-[[α-(4-substitutedbenzoyloxy)-α-phenylacetyl or methylacetyl]amino]-5-(4-methoxyphenyl)-1,3,4-oxadiazoles were obtained by condensation of 2-[(α-chloro-α-phenylacetyl or α-bromopropionyl)amino]-5-(4-methoxyphenyl)-1,3,4-oxadiazoles with sodium salts of 4-substituted benzoic acids. Structures of the compounds were assigned on the basis of spectral data (UV, IR, ^{1}H NMR, EI MS) and elemental analyses. 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 disk diffusion method. Compounds **4a**, **4d** and **4g** were found to be active against *S. aureus* ATCC 6538 (MIC, 78, 39 and 78 μg ml^{-1}, respectively) and compound **4e** against *S. epidermidis* ATCC 12228 (MIC, 156 μg ml^{-1}).

Keywords: Antibacterial drugs; 5-Aryl-2-[[α-(4-substituted benzoyloxy)-α-phenylacetyl or methylacetyl]amino]-1,3,4-oxadiazoles; Synthesis; Microbiology

1. Introduction

In one of our previous works, we synthesized some 5-aryl-2-(α-haloacyl)amino-1,3,4-oxadiazole derivatives, which were shown to have high antibacterial activity [1]. In our effort to modify these compounds and enhance their potency first their dithiocarbamic acid esters were synthesized [2], then the present study was carried out with the purpose of finding out the effects of esterification of these compounds on antibacterial activity. In the first study mentioned above [1] 12 analogs were obtained, but in this study we chose, in order to prepare the esterified 1,3,4-oxadiazole derivatives (**4a**–**g**) (Table 1), 2-[(α-chloro-α-phenylacetyl or α-bromopropionyl)amino]-5-(4-methoxyphenyl)-1,3,4-oxadiazoles (**3a**, **b**) which seemed interesting to us because 2-[(α-chloro-α-phenylacetyl)amino]-5-(4-methoxyphenyl)-1,3,4-oxadiazole (**3a**) was found to be the second most active compound of all while 2-[(α-bromopropionyl)amino]-5-(4-methoxyphenyl)-1,3,4-oxadiazole (**3b**) was the least active one. The antimicrobial activity of these novel compounds was evaluated and the correlation between the chemical structures of the studied compounds and their respective biological activities was discussed.

2. Chemistry

4-Methoxybenzaldehyde semicarbazone (**1**) [3] suspended in glacial acetic acid was stirred with bromine and anhydrous sodium acetate to give 5-(4-methoxyphenyl)-2-amino-1,3,4-oxadiazole (**2**) [4]. Reaction of these with α-chloro-α-phenylacetyl chloride or α-bromopropionyl bromide yielded 5-(4-methoxyphenyl)-2-[(α-chloro-α-phenylacetyl or α-bromopropionyl)-

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amino]-1,3,4-oxadiazoles, respectively (**3a**,**b**) [1]. Compounds **3a**,**b** were reacted with an appropriate sodium benzoate [5,6] in DMF and 2-[[α-(4-substituted benzoyloxy)-α-phenylacetyl or methylacetyl]amino]-5-(4-methoxyphenyl)-1,3,4-oxadiazoles (**4a**–**g**) were obtained.

3. Experimental

3.1. Chemistry

Melting points were determined on a Büchi 530 apparatus in open capillaries and are uncorrected. UV spectra were determined on Shimadzu UV 2100 S spectrophotometer. IR spectra were recorded on KBr discs, using a Perkin–Elmer 1600 spectrophotometer. ^{1}H NMR spectra were obtained on a Bruker AC 200 (200 MHz) spectrometer using TMS as the internal standard. EI MS were recorded on a VG Zab Spec (70 eV) instrument. Elemental analyses were performed on a Carlo Erba 1106 apparatus. All the compounds gave satisfactory C, H, N analyses.

3.1.1. 2-[[α-(4-Substituted benzoyloxy)-α-phenylacetyl or methylacetyl]amino]-5-(4-methoxyphenyl)-1,3,4-oxadiazoles (**4a**–**g**)

To a solution of 0.005 mol of 2-[(α-chloro-α-phenylacetyl or α-bromopropionyl)amino]-5-(4-methoxyphenyl)-1,3,4-oxadiazole in 15 ml of DMF (*N*,*N*-dimethylformamide) were added 0.005 mol of an appropriate sodium benzoate [5,6] and the mixture was refluxed for 6–10 h. After cooling, the mixture was poured into ice-water. The precipitate formed was filtered, dried and recrystalyzed from ethanol (Table 1 and Scheme 1).

3.1.2. Spectral data of **4d**; UV [λ_{max}, nm (ε), EtOH]: 284 (24190), 243 (20690)

IR [ν, cm^{-1}, KBr]: 3322 (NH), 1722, 1657 (C=O ester, amide).

^{1}H NMR [200 MHz, δ, ppm, DMSO-d_6]: 1.61 (d, $J = 6.82$ Hz, 3H, CH_3), 3.84 (s, 3H, CH_3), 5.38 (q, $J = 6.92$ Hz, 1H, CH), 7.13 (d, $J = 8.81$ Hz, 2H, $C_{3,5}$–H of the phenyl adjacent to the oxadiazole), 7.64 (d, $J = 8.50$ Hz, 2H, $C_{3,5}$–H of the phenyl adjacent to the ester group), 7.86 (d, $J = 8.76$ Hz, 2H, $C_{2,6}$–H of the phenyl adjacent to the oxadiazole), 8.03 (d, $J = 8.41$ Hz, 2H, $C_{2,6}$–H of the phenyl adjacent to the ester group), 12.17 (s, 1H, NH).

EI MS (70 eV) [m/z (rel. int. %)]: 386 (3), 368 (2), 341 (5), 328 (1), 264 (5), 256 (34), 218 (2), 213 (14), 191 (4), 175 (3), 157 (9), 149 (19), 139 (6.5), 135 (10), 133 (4), 73 (64), 69 (100) (base peak), 57 (68), 43 (67.5).

3.1.3. Spectral data of **4e**; UV [λ_{max}, nm (ε), EtOH]: 286 (16762), 243 (14057)

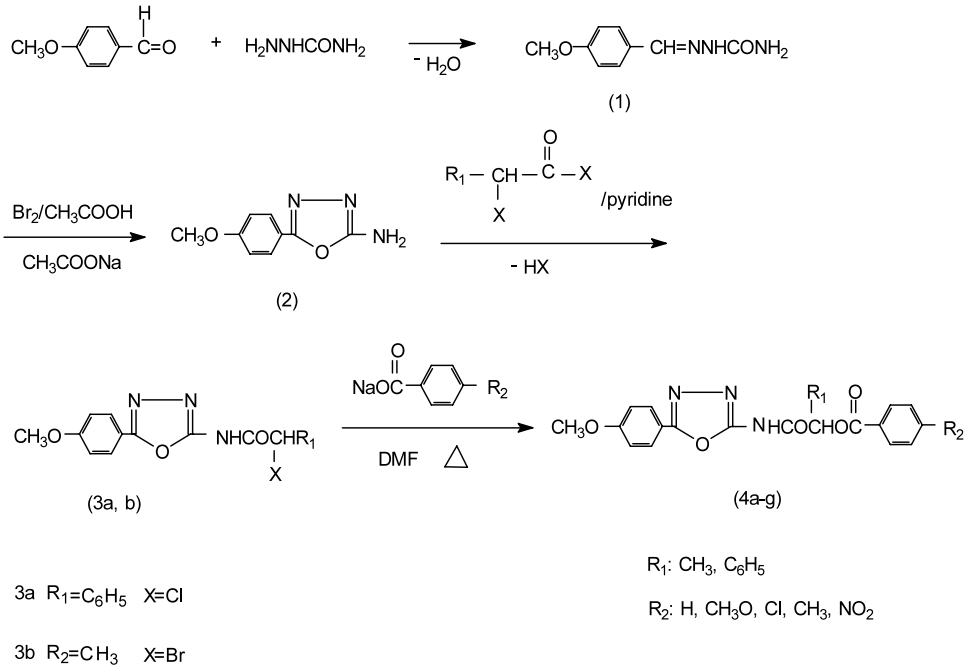
IR [ν, cm^{-1}, KBr]: 3205 (NH), 1708, 1654 (C=O ester, amide).

^{1}H NMR [200 MHz, δ, ppm, DMSO-d_6]: 2.41 (s, 3H, CH_3), 3.84 (s, 3H, CH_3O), 6.32 (s, 1H, CH), 7.12 (d, $J = 8.80$ Hz, 2H, $C_{3,5}$–H of the phenyl adjacent to the oxadiazole), 7.38 (d, $J = 8.07$ Hz, 2H, $C_{3,5}$–H of the phenyl adjacent to the ester group), 7.46–7.71 (m, 5H, phenyl–H), 7.84 (d, $J = 8.86$ Hz, 2H, $C_{2,6}$–H of the phenyl adjacent to the oxadiazole), 7.96 (d, $J = 8.08$ Hz, 2H, $C_{2,6}$–H of the phenyl adjacent to the ester group), 12.38 (s, 1H, NH).

EI MS (70 eV) [m/z (rel. int. %)]: 444 (2) ($M + 1$), 324 (1), 253 (11.5), 218 (36), 191 (3.5), 175 (7), 148 (1.5), 135 (42), 133 (16), 120 (7), 119 (100) (base peak), 107 (4.5), 105 (10), 91 (45), 90 (17.5), 77 (20).

Table 1
Some characteristics of compounds **4a**–**g**

Compound	R_1	R_2	Yield (%)	M.p. (°C)	Molecular formula (mol. wt.)	Elemental analyses (calc./found)		
						C	H	N
4a	C_6H_5	H	67.13	235–237	$C_{24}H_{19}N_3O_5$ (429.41)	67.12 66.92	4.46 4.11	9.79 9.79
4b	C_6H_5	CH_3O	56.64	208–209	$C_{25}H_{21}N_3O_6$ (459.44)	65.35 65.44	4.61 4.32	9.15 9.18
4c	CH_3	CH_3O	69.02	206–208	$C_{20}H_{19}N_3O_6$ (397.37)	60.45 60.04	4.82 4.46	10.57 10.60
4d	CH_3	Cl	48.67	206	$C_{19}H_{16}ClN_3O_5$ (401.793)	56.79 56.84	4.01 3.41	10.46 10.47
4e	C_6H_5	CH_3	72.23	208–210	$C_{25}H_{21}N_3O_5$ (443.44)	67.71 67.64	4.77 4.60	9.48 9.13
4f	C_6H_5	NO_2	43.88	131–134	$C_{24}H_{18}N_4O_7 \cdot 1/2H_2O$ (483.42)	59.62 59.56	3.96 4.54	11.58 12.21
4g	CH_3	NO_2	53.40	205–208	$C_{19}H_{16}N_4O_7$ (412.35)	55.34 54.80	3.91 3.67	13.59 13.31



Scheme 1.

*3.1.4. Spectral data of **4g**; UV [λ_{max}, nm (ε), EtOH]: 279 (30679)*

IR [v, cm^{-1}, KBr]: 3322 (NH), 1725, 1656 (C=O ester, amide).

^{1}H NMR [200 MHz, δ, ppm, DMSO-d_6]: 1.64 (d, $J = 6.94$ Hz, 3H, CH_3), 3.84 (s, 3H, CH_3O), 5.45 (q, $J = 6.94$ Hz, 1H, CH), 7.13 (d, $J = 8.81$ Hz, 2H, $C_{3,5}$–H of the phenyl adjacent to the oxadiazole), 7.86 (d, $J = 8.80$ Hz, 2H, $C_{2,6}$–H of the phenyl adjacent to the oxadiazole), 8.26 (d, $J = 8.89$ Hz, 2H, $C_{2,6}$–H of the phenyl adjacent to the ester group), 8.39 (d, $J = 8.79$ Hz, 2H, $C_{3,5}$–H of the phenyl adjacent to the ester group), 12.21 (s, 1H, NH).

EI MS (70 eV) [m/z (rel. int. %)]: 412 (19) (M^+), 262 (2), 222 (13), 218 (100) (base peak), 192 (15), 175 (28), 150 (66), 148 (20), 147 (1), 135 (40), 133 (18), 120 (36), 107 (10), 92 (15), 77 (9).

3.2. Microbiology

Derivatives **4a**–**g** were tested for in vitro antimicrobial activity 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 using disk diffusion method where each disk 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, 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^{-1}, were pipetted into the Mueller–Hinton agar plate prepared as described above. The surface of the medium was allowed to dry. The 10 mg ml^{-1} (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 **4a**, **4d** and **4g** were active against *S. aureus* ATCC 6538 and compound **4e** against *S. epidermidis*.

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^{-1} 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 μg ml^{-1} 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 2.

Table 2
MIC values (μg ml^{-1}) of compounds **4a**, **4b**, **4e** and **4g**

Compound	*S. aureus* ATCC 6538	*S. epidermidis* ATCC 12228
4a	78	–
4d	39	–
4e	–	156
4g	78	–
Cefuroxim Na	1.2	4.9

4. Results and discussion

Treatment of equimolar amounts of 2-[(α-chloro-α-phenylacetyl or α-bromopropionyl)amino]-1,3,4-oxadiazole and an appropriate sodium benzoate in DMF resulted in the formation of 2-[[α-(4-substituted benzoyloxy)-α-phenylacetyl or methylacetyl]amino]-5-(4-methoxyphenyl)-1,3,4-oxadiazoles (**4a**–**g**).

The structures of the compounds were established by elemental analysis and spectrometric data (UV, IR, ^{1}H NMR and EI MS).

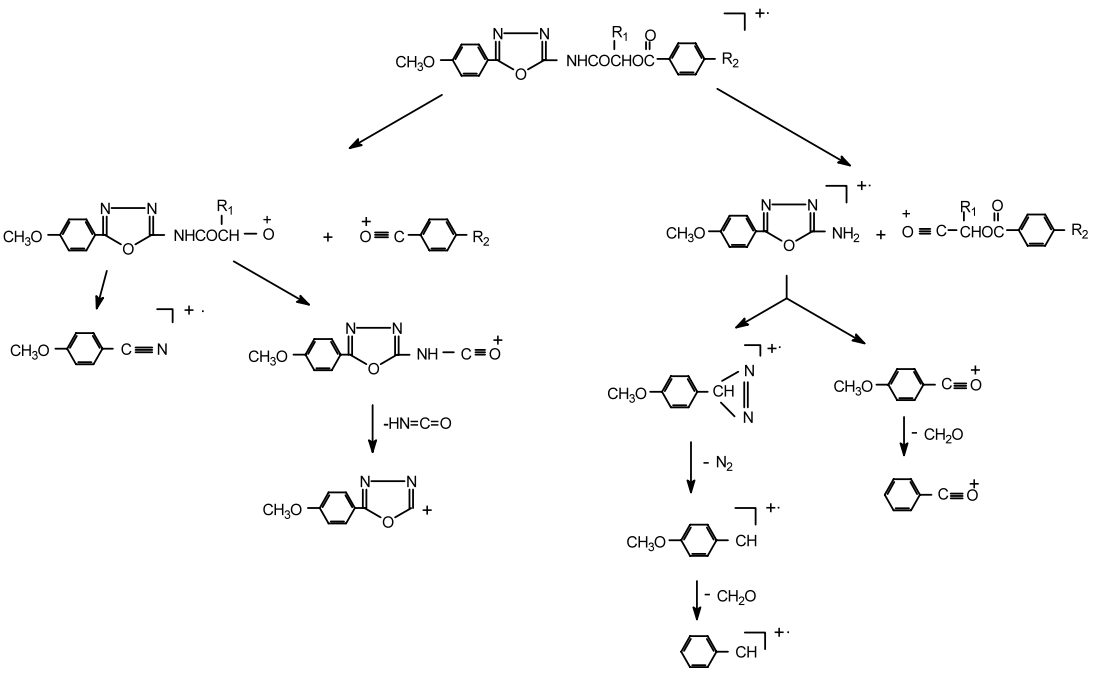
The UV spectra of compounds **4d** and **4e** showed two absorption bands in the 284–286 and 243 nm regions, which are characteristic for oxadiazoles [7,8], while compound **4g** showed just one band at 279 nm but with higher molar absorptivity.

The IR spectra of **4a**–**g** exhibited N–H and C=O bands in 3322–3203 and 1728–1607 cm^{-1}, respectively. The absorption bands associated with other functional groups appeared in the expected regions.

In the ^{1}H NMR spectra of compounds **4d**, **4e** and **4g**, the protons of the phenyl adjacent to the oxadiazole and the phenyl adjacent to the ester group were all observed as doublets. In the ^{1}H NMR of compound **4g**, both of the doublets of the protons of the phenyl adjacent to the oxadiazole were observed first, while the doublets of the protons of the phenyl adjacent to the ester group were observed later.

Molecular ion and $(M+1)$ ion observed in the EI MS of **4g** and **4e**, respectively, confirmed their molecular weights. Compound **4d** did not show the molecular ion peak but showed the peaks due to fragments that supported the expected structures and which were in accordance with the fragmentation routes given in literature [9–11]. In general, the major fragmentation route of all the compounds mentioned above involved the cleavage of the NH–CO bond of the amide function and the O–CO bond of ester function which was followed by further fragmentations (Scheme 2).

The title compounds were evaluated for 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. Compounds **4a**, **4d** and **4g** were found to be active against *S. aureus* ATCC 6538 (MIC, 39, 78 and 78 μg ml^{-1}, respectively) and compound **4e** against *S. epidermidis* ATCC 12228 (MIC, 156 μg ml^{-1}). As can be seen, compound **4d** which had a chlorine on the *p*-position of the benzoate residue and a methyl substitution on the acyl side-chain was more active than compounds **4a** and **4g**.



Scheme 2.

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