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Synthesis, spectroscopic characterization and pharmacological evaluation of oxazolone derivatives

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Abstract: A series of six 4-(arylmethylidene)-2-phenyl/methyl-5(4*H*)-oxazolone derivatives were synthesized using a reported method by condensation of aldehydes with *N*-benzoyl/*N*-acetyl glycine in the presence of zinc oxide as a catalyst and acetic anhydride at room temperature in ethanol. Five of the compounds are new derivatives. The structures of the compounds were evaluated based on ^{1}H-NMR, ^{13}C-NMR, EI-MS and FT-IR spectroscopy and elemental analysis. All the compounds were screened for their antibacterial and urease inhibition activity. The antibacterial activity was tested by the agar well diffusion method using Mueller–Hinton agar medium. Compound **2** showed excellent activity against *Staphylococcus aureus* exhibiting 16 mm (80 %) inhibition and above 24 mm (70 %) against *Salmonella typhi*. Compound **6** was the most active compound against *Escherichia coli* having 20 mm (80 %) inhibition followed by compound **5** having above 18 mm (70 %) inhibition. Urease inhibition activity of all the compounds was determined by the indophenol method. Compounds **3**, **6** and **7** showed significant inhibition against Jack bean urease.

Keywords: aldehyde; antibacterial; urease inhibition activities; oxazolones; synthesis; zinc oxide.

INTRODUCTION

For many decades, increasing resistance against human pathogens that cause serious infections is one of the main topics of interest for medicinal chemists. During the last century, many medicines were developed against bacterial infections. However, the available antibacterial drugs could cause undesirable effects

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and might be toxic. In view of these facts, it is important to explore additional drugs that have no adverse effects due to their different mode of action. In recent years, there has also been an increased interest in urease inhibiting drugs. Urease is a major cause of peptic and gastric ulcer. Compounds having urease inhibitory potential play an important role in pharmaceutical companies.[1] Activities of urease (E. C. 3.5.1.5) has been shown to be important virulence determinant in the pathogenesis of many clinical conditions, which are detrimental for human and animal health. Urease is directly involved in the formation of infection stones and contributes to the pathogenesis of urolithiasis, pyelonephritis, ammonia and heptic encephalopathy, hepatic coma and urinary catheter encrustation.[2,3] For the above reasons, a wide range of resistant drugs are important and required to treat patients. Mostly nitrogen-, sulfur- and oxygen-containing five- and six-membered heterocyclic compounds have enormous significance in the field of medicinal chemistry.[4] Oxazolones are five-membered heterocyclic compounds containing nitrogen and oxygen as hetero atoms. The C-2 and C-4 positions of oxazolone are responsible for their various biological activities. Oxazolones perform an important role in the synthesis of several organic molecules, including amino acids,[5] amino alcohols, thiamine[6] and peptides.[7] Certain natural and synthetic oxazolones also possess important biological activities, such as anti-inflammatory,[8] antimicrobial,[9] anticancer,[10] anti-HIV,[11] anti-angiogenic,[12] anticonvulsant,[13] antitumor, antagonistic, sedative[14–16] and cardiotonic activity.[17]. Oxazolone plays a very vital role in the manufacture of various biologically active drugs, such as analgesic, anti-inflammatory, antidepressant, anticancer, antimicrobial, antidiabetic and anti-obesity compounds.[18,19]

In the literature, the syntheses of oxazolone derivatives were achieved by condensation of aldehydes with *N*-benzoyl/*N*-acetyl glycine in the presence of various reagents and catalysts, such as, $ZnCl_2$,[20] KF/NaOAc,[21] Al_2O_3–H_3BO_3,[22] Bi(III) salts[23] and $Pb(OAc)_2$.[24] Herein, the synthesis of a series of 4-(arylmethylidene)-2-phenyl/methyl-5(4*H*)-oxazolone derivatives (**2**–**7**) using a literature method[25] by the condensation of an aldehyde with *N*-benzoyl/*N*-acetyl glycine in the presence of zinc oxide as a catalyst at room temperature in ethanol is reported. Compounds **2**–**6** are new. All the synthesized compounds were tested for their urease inhibition activity and antibacterial activity against *Escherichia coli*, *Bacillus subtilis*, *B. cereus*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Proteus mirabilis.*

EXPERIMENTAL

All chemicals were of AR grade. The melting points were measured using Gallen Kemp MF-370 apparatus. The IR spectra were recorded on Nicolet Avatar 300 DTGS instrument. The ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker AV-300 instrument operating at 300 and 75 MHz, respectively, in dimethyl sulfoxide (DMSO) with trimethylsilane (TMS) as an internal standard. The EI-MS were recorded on a Finnigan MAT-311 A. CHN analysis was

performed on a Carlo Erba Strumentazione-Mod-1106, Italy. Purity of the product was determined by using silica gel $60F_{254}$ TLC, pre-coated cards (0.2 mm thickness) and visualized under UV light (254 and 366 nm) and by iodine vapors.

Antibacterial assay

Antibacterial activity was tested by the agar well diffusion method using Mueller Hinton Agar medium for the assay. The microorganisms were activated by inoculating a loopful of the strains in the nutrient broth (25 mL) and incubating at room temperature on a rotary shaker. Then 0.2 mL of inoculum (inoculum concentration was 10^8 cells mL^{-1} as per the McFarland standard) was inoculated into the molten Mueller–Hinton agar media and after proper homogenization, it was poured into 20 mm×100 mm Petri dishes. For the agar well diffusion, a well was made in the seeded plates with the help of a cup-borer (8.5 mm). The reference standard and test sample (1 %) 0.1 mL was introduced into the well and all the plates were incubated at 37 °C for 24 h. The microbial growth was determined by measuring the zone of inhibition (mm) and the mean values are presented. Percentage inhibitions of the compounds were determined by comparing the zone of inhibition to that of standard drug against each organism.

Urease inhibition assay

Reaction mixtures comprising 25 μL of enzyme (jack bean urease) solution and 55 μL of buffers containing 100 mM urea were incubated with 5 μL of the test compounds (1 mM concentration) at 30 °C for 15 min in 96-well plates. The urease activity was determined by measuring ammonia production using the indophenol method as described by Weatherburn.[26] Briefly, 45 μL each of phenol reagent (1 % w/v phenol and 0.005 % w/v sodium nitroprusside) and 70 μL of alkali reagent (0.5 % w/v NaOH and 0.1 % (w/v) active chlorine NaOCl) were added to each well. The increasing absorbance at 630 nm was measured after 50 min, using a microplate reader (Molecular Device, USA). All reactions were performed in triplicate in a final volume of 200 μL. The results (change in absorbance per min) were processed using Soft Max Pro software (Molecular Device, USA). All the assays were performed at pH 8.2 (0.01 M $K_2HPO_4 \cdot 3H_2O$, 1 mM EDTA and 0.01 M LiCl). The percentage inhibitions were calculated from the formula $100–(OD_{testwell}/OD_{control})\times100$. Thiourea was used as the standard inhibitor of urease.

Synthesis of N*-benzoylglycine (**1a**)*

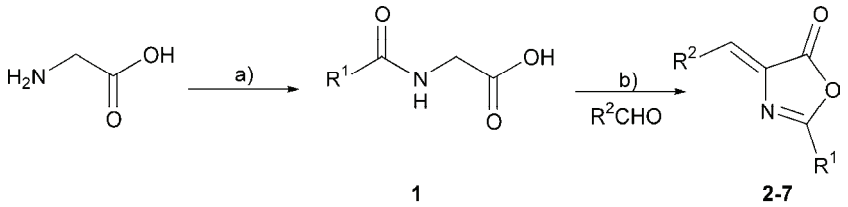
Glycine (10 g, 133 mmol) was dissolved in 10 % NaOH solution (100 mL) and benzoyl chloride (21.6 ml, 186 mmol) was added in portions to this solution in 500mL conical flask. After each addition, the flask was stoppered and shaken vigorously until all the acid chloride had reacted. The solution was cooled by adding few grams of crushed ice and concentrated hydrochloric acid (pH 2–3) was added. The so-formed precipitates were filtered and washed several times with cold distilled water, dried, and recrystallized from hot water. The required compound was obtained as a crystalline white solid.

*Synthesis of N-acetylglycine (**1b**)*

Glycine (10 g, 133 mmol) and water (30 mL) in a 500 mL conical flask was vigorously stirred until the solid glycine had completely dissolved. Acetic anhydride (25 mL, 266 mmol) was added in portions and stirred vigorously for 20 min. The exothermic reaction produced solid *N*-acetylglycine, which showed the end-point of the reaction. The reaction mixture was then placed in refrigerator overnight for the complete formation of the product. The solid was filtered, washed with ice-cooled water and recrystallized from hot water.

*Synthesis of the 4-(arylmethylidene)-2-phenyl/methyl-5-(4H)-oxazolones derivatives (**2**–**7**)*

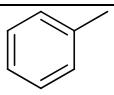
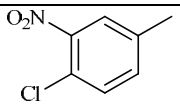
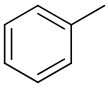
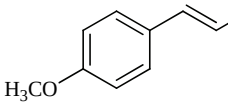
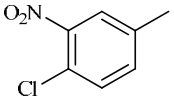
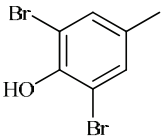
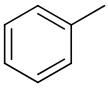
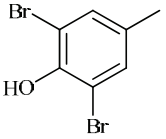
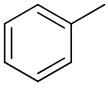
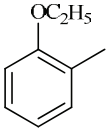
A mixture of the required aldehyde (1 mmol), *N*-benzoylglycine/*N*-acetylglycine (1 mmol), acetic anhydride (3 mmol), ZnO as a catalyst (0.05 mmol) and 15 mL ethanol was stirred at room temperature, 25 °C (Scheme 1). After a certain period, the syrupy reaction mixture was solidified and the reaction was completed (Table I). After the final product had been obtained, 20 mL more of cold ethanol was added. The solid was filtered off, dried and washed with hot water before recrystallization of the product.



1 **2-7**

Scheme 1. Synthesis of oxazolones **2**–**7**; a) acetic anhydride or benzoyl chloride, 10 % NaOH, H_2O; b) ZnO, acetic anhydride, C_2H_5OH, reflux under stirring at r.t.

TABLE I. Synthesis of oxazolone derivatives (**2**–**7**) with their physical parameters

Compound	R^1	R^2	Yield %	Molecular weight g mol^{-1}	Reaction time min	M.p. °C
2			89	328	10	227
3			92	305	15	118
4	—CH_3		80	266	12	160
5	—CH_3		70	360	19	166
6			82	423	15	149
7			70	293	10	197

RESULTS AND DISCUSSION

Chemistry

Oxazolone derivatives were synthesized by condensation of substituted aromatic aldehydes with *N*-benzoyl/*N*-acetyl glycine using zinc oxide as a catalyst in ethanol at room temperature. All the synthesized compounds were characterized using different spectroscopic techniques. The IR spectrum of compound **3,** as typical, showed characteristic band of carbonyl group at 1781 and C=N at 1652 cm^{-1}. Also, typical ^{1}H-NMR spectrum showed characteristic pattern of peaks. The methyl protons appeared in the region of 3.84 ppm, whereas the aromatic protons appeared at 6.89–8.12 ppm. The electron ionization mass spectrometric fragmentation patterns of the compounds were the same.

The complete analytic and spectral data of the obtained products are given in the Supplementary material to this paper.

Antibacterial activity

All the compounds (**2**–**7**) were tested for their antibacterial activity against *Escherichia coli*, *Bacillus subtilis*, *B. cereus*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Proteus mirabilis.* The results were compared with those of the standard 0.3 % gentamycin. The antibacterial activity of compounds **2–7** are shown in Fig 1. The bacterial zones of inhibition values are summarized in Table II. Compounds **5** and **6** showed excellent activities against *E. coli*, which is very difficult to treat with traditionally used antibiotics. The most active compound against *E. coli* was compound **6** having 20 mm inhibition zone (80 % inhibition), followed by compound **5** having above 18 mm inhibition zone (70 %). Thus, compounds **5** and **6** would be the better choice for the treatment of infections caused by *E. coli* than the derivatives **1**–**4** or **7**. All the screened compounds showed low to moderate activities against *B. cereus*, except

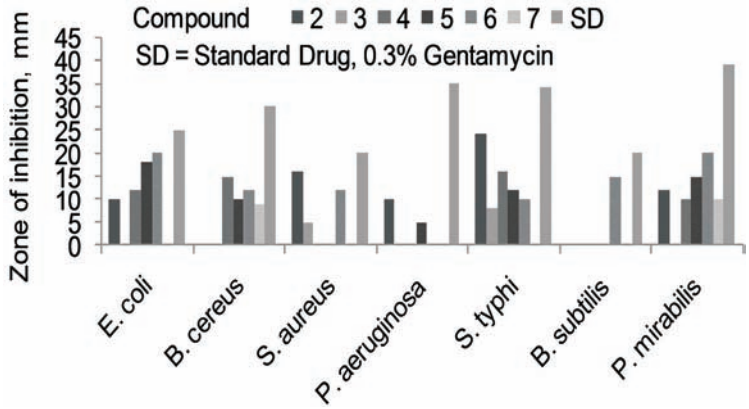


Fig. 1. Antibacterial activity of compounds **2**–**7**.

compound **4** that showed 50 % inhibition. *S. aureus* is responsible for various throat infections and cholic diseases. Therefore, compound **2** would be the best choice to treat infections caused by *S. aureus* as it had a 16 mm inhibition zone (80 %). None of the compounds showed inhibition against *P. aeruginosa* except compound **2** that had less than 30 % inhibition. *S. typhi*, responsible for typhoid, could be inhibited by compound **2**, showing an inhibition zone of 24 mm (70 %). Compound **4** also showed 50 % inhibition followed by compounds **5**–**7**. *B. subtilis* was only inhibited by compound **6**, which showed an inhibition zone of 15 mm (75 %). Compound **6** also showed ≈20 mm inhibition zone (50 %) against *P. mirabilis*, followed by compounds **4**, **5** and **7**.

TABLE II. Antibacterial activity (concentration used: 1 % of each compound); zone of inhibition was measured in mm; SD – standard drug, 0.3 % gentamycin

Bacterium	Compound						SD
	2	**3**	**4**	**5**	**6**	**7**	
E. coli	10	00	12	18	20	00	25
B. cereus	00	00	15	10	12	09	30
S. aureus	16	05	00	00	12	00	20
P. aeruginosa	10	00	00	05	00	00	35
S typhi	24	08	16	12	10	00	34
B. subtilis	00	00	00	00	15	00	20
P. mirabilis	12	00	10	15	20	10	39

Compounds **5** and **6** proved themselves to be very effective antibacterial agents. Compound **5** showed inhibition against four and compound **6** showed inhibitions against six different bacterial strains. The C-2 and C-4 positions of the oxazolones are responsible for their crucial biological activities (Fig. 2). A phenyl group instead of a methyl group at C-2 plays a vital role in the enhancement of the activities, as shown by compounds **5** and **6**. Compared to compound **5**, compound **6** showed a greater tendency of inhibition against different bacterial strains due to the phenyl group at C-2. Electron withdrawing substituents at C-4 position are also a good choice for the enhancement of the activities.

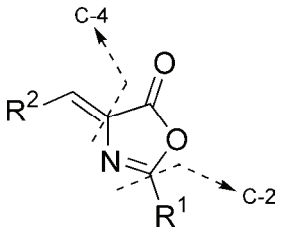


Fig. 2. Structure of oxazolones.

Urease inhibition activity

All the synthesized compounds were screened for their urease inhibition activity. Compounds **3**, **6** and **7** showed strong inhibition against jack bean urease, having IC_{50} values near to the standard inhibitor thiourea as determined by the indophenol method. The results are listed in Table III.

TABLE III. Urease inhibition activity of compounds **2**–**7**; Standard IC_{50} = 21.4 μM

Compound	Urease inhibition, %	IC_{50} / μM
2	21	Not determined
3	85.2	62
4	44	NA
5	49	NA
6	82.5	65.3
7	73.2	58

CONCLUSION

Based on the biological activities of the synthesized oxazolone derivatives, it could be concluded that they are therapeutic antibacterial agents and urease inhibitors.

SUPPLEMENTARY MATERIAL

Analytic and spectral data for the synthesized compounds are available electronically from http://www.shd.org.rs/JSCS/, or from the corresponding author on request.

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ИЗВОД

СИНТЕЗА, СПЕКТРАЛНА КАРАКТЕРИЗАЦИЈА И ФАРМАКОЛОШКА ИСПИТИВАЊА ДЕРИВАТА ОКСАЗОЛОНА

GHULAM FAREED[1,2], NIGHAT AFZA[1], MUHAMMAD ALI VERSIANI[2], NAZIA FAREED[2], UZMA RASHEED MUGHAL[3], MAHBOOB ALI KALHORO[1], LUBNA IQBAL[1] и MEHREEN LATEEF[1]

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Синтетисана је серија деривата 4-(арилметилиден)-2-фенил/метил-5(4*H*)-оксазолона (**2**–**7**) од којих су деривати **2**–**6** синтетисани први пут. Једињења су синтетисана кондензацијом алдехида и *N*-бензоил/*N*-ацетил-глицина у присуству цинк-оксида као катализатора и анхидрида сирћетне киселине, на собној температури у етанолу као растварачу. Структура деривата одређена је спектралном (^{1}H-NMR, ^{13}C-NMR, EI-MS и FT-IR) и елементалном анализом. Свим једињењима одређена је антибактеријска активност и инхибиторна активност према уреази. Антибактеријска активност је тестирана према методи дифузије у агару помоћу Mueller–Hinton агар медијума. Једињење **2** показује одличну активност према *Staphylococcus aureus* ≈16 mm (80 %) инхибиције и преко

24 mm (70 %) према *Salmonella typhi*. Према *Escherichia coli* најактивније је једињење **6** са 20 mm (80 %) инхибиције, а затим једињење **5** са преко 18 mm (70 %) инхибиције. Инхибиторна активност према уреази за сва једињења одређена је индофенолном методом. Једињења **3**, **6** и **7** показују значајну активност према уреази пасуља сорте Jack.

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