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Synthesis & Biological evaluation of Oxazolone derivatives

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ABSTRACT

Some new [4-[3-chloro-benzylidine]-2-phenyl oxazol-5-one & 4-[2-nitro-benzylidine]-2-phenyl oxazol-4-5-one were synthesized in microwave and there structures were confirmed by I.R. and 1H NMR and their antimicrobial screening against E. Coli , Staphylococcus aureus and Candida albicans were established by zone of inhibition.

Key words: oxazolone derrivatives, synthesis, characterization, pharmacological activity

1. INTRODUCTION

In recent years much attention has been focused on the synthesis of oxazolone and their derivatives because of their wide spectrum biological properties. A large number of Nitrogen containing heterocycles display antifungal, antibacterial, anticonvulsant, insecticidal, anti-inflammatory, tuberculotherapeutic and anti-HIV activity [1-6]. It was thought of interest to synthesize some substituted [4-[3-chloro-benzylidine]-2-phenyl oxazol-5-one & 4-[2-nitro-benzylidine]-2-phenyl oxazol-4-5-one for their antibacterial and antifungal activity [7-14].

2. MATERIALS AND METHODS

All the chemicals and solvents were obtained from commercial sources like S. D. Fine Chem. Research Lab. and purified using standard procedure whenever required. Melting points were measured in open capillary tube on VEEGO (VMP -D) melting point apparatus and are uncorrected. IR spectra were recorded in KBr pellets on a SHIMADZU FTIR 8400S infrared spectrophotometer. The 1H-NMR spectra were determined in DMSO-d6 at 300 MHz on a JEOL FT NMR spectrophotometer using TMS as an internal standard. The progress of the reaction and the purity of co mpounds were monitored by TLC using silica gel plates.

Synthesis of Oxazolone (Figure 1)

Oxazolone derivatives were prepared by refluxing benzoyl glycine with substituted aromatic aldehyde in presence of sodium acetate and acetic anhydride.

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Preparation of Benzoyl glycine

Glycine was dissolved in 10 percent sodium hydroxide solution contained in a conical flask. Benzoyl chloride was added in five portions to the solution. Stopper the vessel and shake vigorously after each addition until all the chloride has reacted. The solution was transferred into a beaker and rinsed the conical flask with a little water. A few gram of crushed ice was placed in the solution and add concentrated hydrochloric acid slowly and with stirring until the mixture was acidifying to Congo red paper. The resulting crystalline precipitate of benzoyl glycine was collected, which was contaminated with a little benzoic acid, upon a Buchner funnel, washed with cold water and drain well. The obtained solid product was placed in a beaker with carbon tetrachloride, the beaker was covered with a watch glass and boil gently for 10 minutes (fume cupboard); this extracts any benzoic acid which may be present. The resulting mixture was allowed to cool slightly and filter with carbon tetra chloride.

The dried product was recrystallized from boiling water with the addition of a little decolorizing charcoal, and then filters through a hot water funnel and allow crystallizing. The benzoyl glycine was collected from Buchner funnel and it was dried in an oven.

Preparation of Oxazolone Derivatives

A mixture of benzaldehyde, benzoyl glycine, acetic anhydride, and anhydrous sodium acetate was taken in a 500ml conical flask and heated on an electric hot plate with constant shaking. As soon as the mixture has liquefied completely, then the flask was transferred to a water bath and heated for 2 hours.

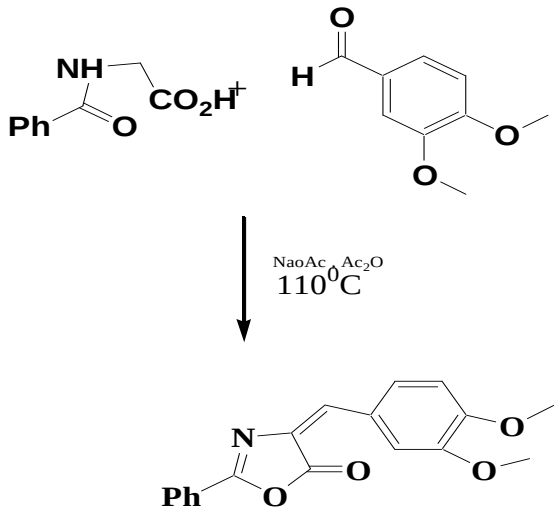


Fig 1: Synthesis of Oxazolone

Then ethanol was added slowly to the contents of the flask and allows the mixture to stand overnight. The crystalline product was filtered with suction, and washed with two portions of ice cold alcohol and then washed with two portions of boiling water, dried at 100°C. Then crude oxazolone was obtained and recrystallized by benzene.

4-[furfurylidine]-2-phenyl-oxazol-5-one; $C_{14}H_9NO_3$ was prepared by refluxing benzoyl glycine with heterocyclic aromatic aldehyde (furfuraldehyde) in presence of sodium acetate and acetic anhydride.

Fig 2. Scheme of Synthesis

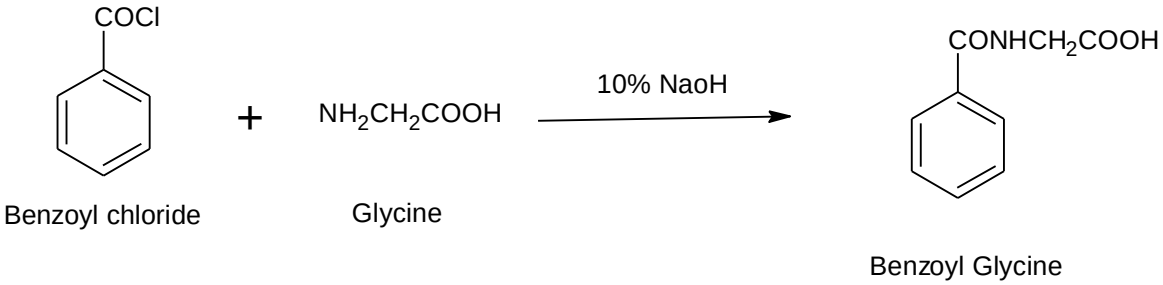
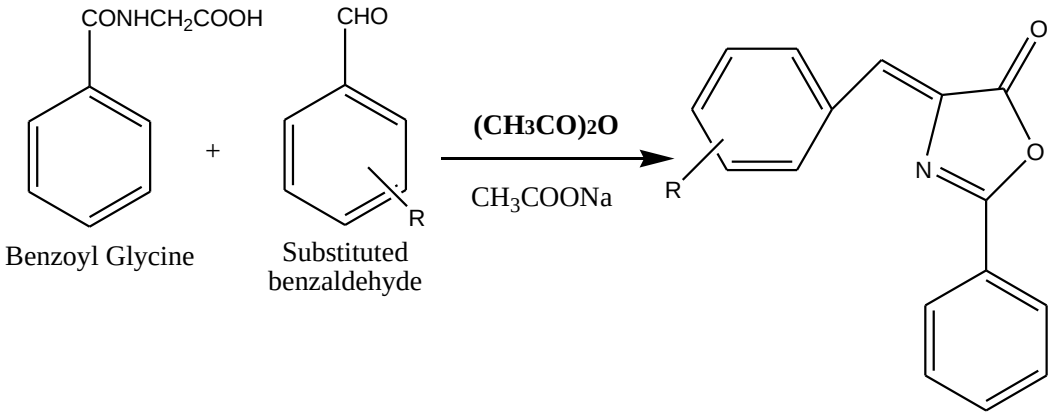


Fig 3. Scheme of Synthesis



Biological evaluation (Antibacterial)

Acute toxicity study

10 mice were taken and fasted overnight before the start of the test. The synthesized compound was administered orally, by making a suspension with 0.5 % CMC. In ascending and widely spaced doses, say, 10, 30, 300, and 1500 mg/kg body weight. All the mice were observed continuously for two hours, recoded and occasionally further four hours and finally over night. The LD50 was found at 1000mg/kg body weight which was also verified from the literature.

Zone of Inhibition of different synthesized compounds against different bacterial strains

Antimicrobial activity of the synthesized compounds was carried out against different bacterial strains by determining the Zone of Inhibition and test was done by agar dilution method.

3. RESULTS AND DISCUSSION

Oxazolones were synthesised by the Erlenmeyer reaction. The melting points of the synthesized compounds were determined by open end capillary tube method in melting point apparatus (*Thiele tube*) and were uncorrected. The purity of the compounds was checked by thin layer chromatography using silica gel as stationary phase, benzene: methanol (8:2) as mobile phase and spot was visualized by iodine vapour. The *Lambda* max (λ_{max}) or maximum absorption has been measured in UV- Pharma Spec 1700 (SHIMADZU) UV-visible spectrophotometer using the concentration of 0.01% of the synthesized oxazolone compounds in benzene solvent. The structures of the compounds were characterized by FTIR- 8400S, Fourier Transform (SHIMADZU) Infrared spectrophotometer and ^{1}H NMR (Proton Nuclear Magnetic Resonance) by using Chloroform ($CHCl_3$) as a solvent. The molecular weight of the compounds was determined by Mass spectrophotometer.

The analgesic activity of oxazolone derivatives in Hot-Plate method, 30 minutes after drug administration reaction time was increased significantly for the test and standard drugs when compared to the pre-drug reaction time. In this case, all the compounds were more effective. Out of all compounds OXZ1 and OXZ2 having more activity. The results were found to be significant in comparison to the control.

In the carrageen an-induced paw edema method in rats 100 mg/kg per oral produce inhibition of paw edema. The test and standard drugs produced significant inhibition of paw edema as compared to the control. After 4 hours it was observed that all the compounds given equivalent activity.

The synthesized compounds were screened for their antibacterial activity against S. aureus, S. paratyphi, E. coli, V. cholera, S. dysenteriae by Cup-Plate method [15-17].

From the activity data it was concluded that all the compounds shows antibacterial activity against gram +ve and gram –ve bacteria as compared to reference standard streptomycin. All

compounds shows equivalent antibacterial activity against gram +ve and gram –ve bacteria.

Statistical analysis of the differences observed between control and treated groups were carried out using ANOVA. P value <0.05 was considered significant. Dennett's Post ANOVA test has done.

Table 1: Physical data of compound 1

1	Molecular formula	$C_{16}H_{10}NO_2Cl$
2	Molecular weight	283
3	Chemical name	4-[3-chloro-benzylidine]-2-phenyl oxazol-5-one
4	Colour & Nature	Pale Yellow , Powder
5	Percentage of yield	49.20%
6	Melting Point (^{0}C)	172-177
7	R_f value	0.66
8	UV spectra	(λ –MAX 390 nm) Peak- 410.5 0.460, 390.5 0.544 Valley- 459 -0.37, 404 0.443, 315 -0.010
9	IR spectra	1745(C=O), 1656,1650(C=N), 1455(H-C=C), 3039(Aro-C-H), 680(C-Cl), 1548,1540,1450,1427(Ar,C=C)
10	NMR spectra	7.13 (^{1}H, H-4) J=1.00 , 7.39 (^{1}H, H-3) J=2.02 , 7.40 (^{1}H, H-5) , 7.51 (^{1}H, H-2) , 7.60 (^{1}H, H-6), 7.98 (s,^{1}H, Alkenyl) J=1.05 , 8.16,8.18 (d,^{2}H, H-2`,6`) J=1.89 , 8.27 (^{1}H, H-4`) J=1.08
11	Mass spectra	m/e = 286

Table 2: Physical data for compound 2

1	Molecular formula	$C_{16}H_{10}N_2O_4$
2	Molecular weight	294
3	Chemical name	4-[2-nitro-benzylidine]-2-phenyl oxazol-5-one
4	Colour & Nature	Light Brown , Powder
5	Percentage of yield	47.10%
6	Melting Point (^{0}C)	162-167
7	R_f value	0.62
8	UV spectra	(λ –MAX 365 nm) Peak- 386 0.716 , 365 1.005 Valley- 379 0.637
9	IR spectra	1745(C=O), 1651,1649(C=N), 1475(H-C=C), 3050(Ar-C-H), 1594,1538,1519(Ar-C=C), 1350(C-NO2)
10	NMR spectra	7.43 (1H, H-4) , 7.53 (1H, H-3) , 7.55 (1H, H-5) , 7.58 (1H, H-2) , 7.64 (1H, H-6) , 7.75 (1H, Alkenyl) , 8.03, 8.05 (2H, H-2`,6`) J=1.17 , 8.13, 8.16 (d, 1H, H-5`) J=2.18 , 8.63 (1H, H-4`) J=1.00
11	Mass spectra	m/e= 286

Analysis of variance (one way)

The data were analyzed by one way ANOVA followed by Dunnet′s test using Graph pad Instat software. n= 6 animals in each group; F= 31.451, df (6, 14), **p< 0.0001, *p< 0.05

Table 3: Evaluation of Antibacterial Activity

Entry	Microorganism	Zone of inhibition (in mm) µg/ml			
		100	250	500	750
1.	Staphylococcus aureus MTCC-6571	12	15	17	18
2.	Shigella paratyphi $A_2(SL_2)$				
3.	Escherichia coli $(TG_1)4$				
4.	Vibrio choleri 71	5	10	15	19
5.	Shigella dysentriae 6				
		9	14	17	21
		10	11	15	17
		12	16	18	20

Table 4: Analysis of variance (one way)

Source of variation	Degree of freedom	Sum of square	Mean square
Treatments (between columns)	6	4.381	0.7301
Residuals (within columns)	14	0.3250	0.2321
Total	20	4.706	

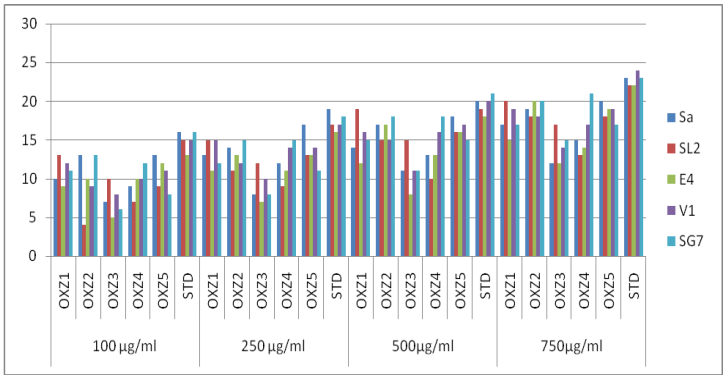


Fig 4: Zone of Inhibition (in mm) of Standard against bacteria

S_1^+--- *Staphylococcus aureus* MCTC 6571 SL_2^- — *Shigella paratyphi* A_2

E_4^- --- *Escherichia coli* HD 10 SG_7^- --- *Shigella dysenteriae* 6

V_1^- --- *Vibrio choleri* 71

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