Research Article

Comparison of Yields of Synthesis, of 2, 4 di-substituted oxazolone Derivatives taking Aldehydes vs Ketones as Starting Reagent and Evaluation of Biological Activities

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ABSTRACT

Conventionally, a series of five derivatives of oxazolone were synthesized by two schemes using different carbonyl compounds with benzoyl glycine in presence of acetic anhydride as dehydrating agent, zinc oxide as catalyst and ethanol as solvent. The yield values and rate of reaction in term of time was compared which concluded the aldehydes are effective and convenient for the synthesizing the oxazolone derivatives in terms of higher yield and less reaction time. The synthesized compounds were assayed for the inhibition for the protein denaturation through In-vitro testing and found the Oxz-4 as a potent anti-arthritic activity among all others derivatives. Further Oxz-1and Oxz-2 were screened for antihelminthic activity and both of them gave satisfactory result, Out of five synthesized compounds, novel compounds Oxz-1,2 and 3 were characterized by spectral data and other two were already synthesized earlier

Keywords: 4-Arylidene-2-phenyl-5(4H)-oxazolone, Synthons, Anti-arthritic, Protein denaturation.

INTRODUCTION

In conventional method of chemical synthesis, the thermal energy produce by furnace or oil bath makes reactive molecules encounter one another through random thermal motion in a liquid or vapor phase. The supplied energy is transmitted through the system by convection or conduction process. However, due to many disadvantages of conventional method of synthesis over microwave irradiation method, low investment cost and easy handling process makes the conventional method of synthesis still in use of small and practical case of study.[1, 2]

Heterocyclic nucleus possesses heteroatoms such as Nitrogen, and Sulphur and sometimes also contains oxygen, phosphorus and selenium and are significantly used in organic and Medicinal chemistry so, heterocyclic compounds acquired importance in these decades. Oxazolone are one of those five membered heterocyclic compounds which contain Nitrogen and Oxygen as hetero atoms[3]. Oxazolone are used in diversity oriented synthesis, it is important synthons for synthesizing various biologically active compounds such as anti-microbial, anti-diabetic[4], Tyrosinase inhibitor[5], anti-viral, anti-fungal, anti-cancer, cardio protective, contact allergen, anti-inflammatory, anti-obesity, anti-depressant, anti-HIV, anti-angiogenic, anti-convulsant, sedative, fungicide and herbicide[6]. One of the derivatives of oxazolone, 4-Arylidene-2-phenyl-5(4H)-oxazoloneare important synthons used for synthesizing several organic molecules including amino acids, peptides, antimicrobials, and antitumor[7] and are utilized in Synthesis of various heterocyclic nucleus such as 1,2,4-Triazinone, Oxadiazole and Imidazole Derivatives.[8]

As in literatures, a number of methods using conventional and/or microwave irradiation methods has been reported for synthesizing oxazolone derivatives involving use of aldehyde or ketone, with glycine derivative in presence or absence of solvent and catalysts[9,10].

In continuation of our research work we compare the role of aldehydes and ketones in yield, time of product formation, and determine the anti-arthritic activity through in-vitro inhibition of protein denaturation assay of synthesized compounds. Both aldehydes and ketones contain carbonyl group and are referred as carbonyl compound. They resemble with each other in most of their physical and chemical properties but due to difference in their structure, aldehydes are quite easily oxidized and are more reactive towards nucleophilic addition reaction than its tautomer ketones.[11]

In view of all these, the present study was carried out conventionally by using aldehydes or ketones with benzoyl glycine in presence of acetic anhydride and zinc oxide with a hope to obtain the desired compounds which shows potent anti-arthritic and antihelminthic activity. Generally, compound those possess piperazine and benzimidazole moiety(as per marketed drugs) are found to possess antihelminthic activities so here an attempt for trial has been made to find out whether oxazolone moiety can also possess antihelminthic activity.

MATERIALS AND METHODS

All chemicals and solvents were obtained from commercial sources and used without further purification. Melting points were recorded by open capillary tube method and are uncorrected.

Synthesis

Methods of synthesis

The 2,4disubstitutedoxazolones were prepared either from ketone or from aldehyde. The first scheme leads to synthesis of oxazolone form ketones and second scheme involves use of aldehyde to form final product.

Scheme 1

Step 1

General method of synthesis of hippuric acid

The entire synthetic procedures were performed in accordance to (*Furniss S.B. et al, A text book of practical organic chemistry, Vogels fifth edition, 1989, 1155)and (Pasha M.A. et al 2007)* with slight modifications.

25g (0.33mol) of glycine in 250 ml of 10 percent sodium hydroxide solution was taken in a conical flask. Then, 54 g (45 ml, 0.385mol) of benzoyl chloride on five portions was added to the solution. The vessel was stoppered and shaken vigorously after each addition until all the chloride has reacted. The solution was transferred to the beaker and the conical flask and was rinsed with little water. Then few grams of crushed ice were poured in the solution and concentrated hydrochloric acid was added slowly with stirring until the mixture was acid to congo red paper. The resulting crystalline precipitate of benzoylglycine, which was contaminated with little benzoic acid, was filtered at Buchner funnel, was washed with cold water and drained well.100 ml of carbon tetrachloride was added and the beaker was covered with a watch glass and boil gently for 10 minutes, which extract any benzoic acid present. The mixture was cooled slightly and was filtered under gentle suction and washed the product at the pump with 10-20 ml of carbon tetrachloride. Recrystallization was carried out by boiling water (about 500 ml) with the addition of a little decolorizing charcoal. Thus, pure benzoyl glycine was obtained. The pure benzoylglycine in the Buchner funnel was collected, and dried in oven. The yield was found to be 74.31 %

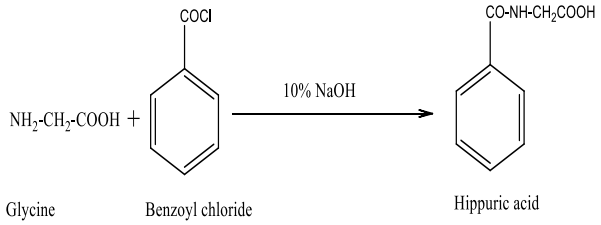
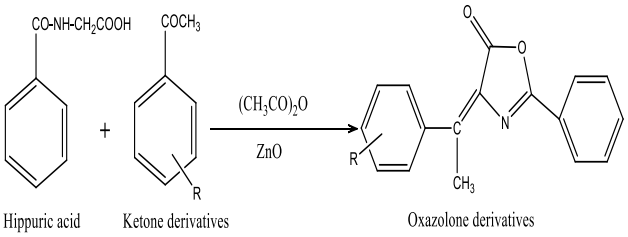


Fig: Synthesis of Hippuric acid

Step 2

Procedure of synthesis of 2,4 di substituted Oxazaolone

A mixture of aromatic ketone derivative (10 mmol), hippuric acid (10 mmol, 1.79 gm), acetic anhydride (15 mmol), catalytic amount of Zinc oxide (ZnO) (6 mmol, 0.49 gm) and ethyl alcohol 5ml was taken in 100 ml conical flask and the mixture was melted on heating mantle. After melting, the mixture was stirred by magnetic stirrer at room temperature for a period of time until the syrupy mixture was obtained, which was washed with little amount of ethanol, then it was transferred to the aluminium petri-plate and the ethanol was made to evaporated by gentle heating on hot plate. The crude product was obtained after complete evaporation of solvent. The product was completely dried and then it was re-crystallized from ethanol to obtain pure crystals of final product.



R= 4-Cl, H, 4-OH

Scheme: Synthesis of oxazolone derivatives Oxz-1, Oxz-2 and Oxz-3

Scheme 2

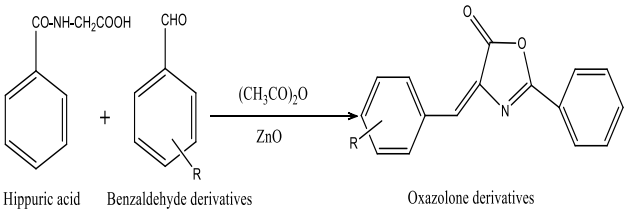
Step 1

Hippuric acidhas been prepared as it was mentioned in step 1 of Scheme 1.

Step 2

Synthesis of 2,4 di substituted Oxazolone

A mixture of Benzaldehyde derivative (10 mmol), hippuric acid (10 mmol, 1.79 gm), acetic anhydride (15 mmol), catalytic amount of Zinc oxide (ZnO) (6 mmol, 0.49 gm) and ethyl alcohol 5 ml was taken in 100 ml conical flask and the mixture was melted on heating mantle. After melting, the mixture was stirred by magnetic stirrer at room temperature for a period of time until a syrupy mixture was formed in the conical flask which was washed with ethanol, and the content was transferred to an aluminium petri-plate and the ethanol was made to evaporate by gentle heating on hot plate. The crude product was obtained after complete evaporation of solvent. The product was completely dried and then it was re-crystallized from ethanol to obtain pure crystals of final product.



Where R= 4-Cl, 4-N(CH3)2

Scheme: Synthesis of oxazolone derivatives Oxz-4 and Oxz-5

Biological activity

Anti-arthritic activity/ Anti-inflammatory

The anti-arthritic activity was carried out in-vitro by the determination of inhibition of albumin denaturation by

the method described in protocol[14]. The different concentration 750, 500, 250, and 125 µg/ml of test solution was prepared in DMSO. Then, the reaction mixture (5ml) consisted of 0.2 ml of egg albumin (from fresh hen's egg), 2.8 ml of phosphate buffer saline (pH 6.4) and 2ml of varying concentration of test solution was prepared. Similar volume of distilled water was served as control. Then the mixture were incubated at 37+2°C for 15 mins and then heated at 70°C for 5 mins. After cooling, their absorbance was measured at 660 nm by using DMSO as blank. Diclofenac sodium at final concentration of 750, 500, 250, and 125µg/ml was used as reference drug and treated similarly for the determination of absorbance. The % inhibition of protein denaturation was calculated by using formula:

% of Inhibition = [Vt/Vc-1] X 100

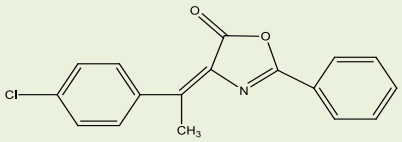
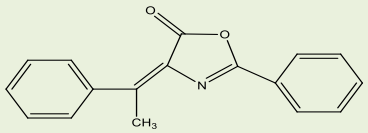
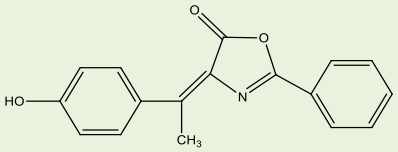
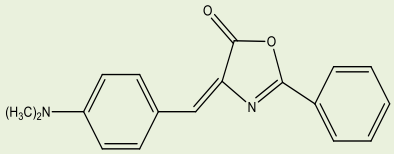
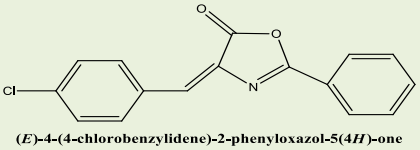
Where, Vt= absorbance of test sample and Vc= absorbance of control and the data was analyzed by statistical method.

Antihelmenthic activity

The synthesized compounds (Oxz-1 and Oxz-2) were evaluated in vitro for their antihelminthic activities on adult Indian earthworms [15]. For preliminary evaluation of antihelminthic activity test samples of synthesized compounds was prepared in the concentration of (1000, 800, 600, 400) µg/ml in DMSO (6%) solution and diluted with normal saline and 6 worms *Pheretimaposthuma* of each 8-10cm were placed in Petridish containing 25 ml of above test solutions of synthesized compounds. Mebendazole (4mg/ml) was used as positive control and normal saline with DMSO (6%) is used as negative control. All the test solutions and standard solutions were prepared freshly before starting the experiment. Observations are made for the time taken for paralysis when movement was lost or no movement. Worms should not relieve even in normal saline. Time for death of worms were recorded after ascertaining that worms neither moved when shaken vigorously nor when dipped in warm water and fading of color of worms

RESULTS

Table 1: Physiochemical parameter of synthesized compounds along with their compound code.

Compound Code	Structure	Yield %	Time	Melting point
Oxz-1	 (*E*)-4-(1-(4-chlorophenyl)ethylidene)-2-phenyl oxazol-5(4*H*)-one	56.36%	45 min	(110-115) ^{0}C
Oxz-2	 (*E*)-2-phenyl-4-(1-phenylethylidene)oxazol-5(4*H*)-one	32.88%	45 min	(200-205) ^{0}C
Oxz-3	 (*E*)-4-(1-(4-hydroxyphenyl)ethylidene)-2-phenyl oxazol-5(4*H*)-one	44.44%	50 min	(210-215) ^{0}C
Oxz-4	 (*E*)-4-(4-(dimethylamino)benzylidene)-2-phenyloxazol-5(4*H*)-one	80.61%	30 min	(215-220) ^{0}C
Oxz-5	 (*E*)-4-(4-chlorobenzylidene)-2-phenyloxazol-5(4*H*)-one	86.26%	20 min	(200-205) ^{0}C

(E)-4-(1-(4-chlorophenyl)ethylidene)-2-phenyl oxazol-5(4H)-one[Oxz-1]

Cream colored crystalline powder; IR (KBr)1617.02 C=N (vibration), 1488.78 C=C (Stretching aromatic), 1617.02 C-C (Stretching), 678.498 C-Cl (stretching), 1299.79 C-O (Stretching), 1397.17 C-H (Aliphatic) [Ar-H, prob. Of Para disubstituted benzene], Mass m/z (%): 299 (32), 298 (18) $[M+1]^+$, 297 (M^+ 100) 105 (45), 89 (15), 77 (39), 63 (9), 51 (28).1H NMR (400 MHz, DMSO): (δ, ppm), 6.5-7.8(m, 9H,Ar-H &=CH-),(C=C-CH_3);(δ, ppm),(2.2-2.5).

(E)-2-phenyl-4-(1-phenylethylidene) oxazol-5(4H)-one [Oxz-2]

Light grey colored Powdered; IR (KBr) 1616.06 C=O (stretching), 1298.82 C-O (Stretching) 1578.45 C=C (Aromatic) 1396.21 C-H (Aliphatic),1616.06 C=N (Stretching) [Ar-H, prob. of mono substituted benzene] Mass;m/z (%): 264 (18) $[M+1]^+$, 263 (M^+100), 265 (2), 105 (45), 89 (15), 77 (39), 63 (9), 51 (28). 1H NMR (400 MHz, DMSO): d6.5-7.8(m, 9H,Ar-H &=CH-),(C=C-CH_3);d(2.2-2.5)1H-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.

(E)-4-(1-(4-hydroxyphenyl) ethylidene)-2-phenyl oxazol-5(4H)-one:[Oxz-3]

Greenish colored powdered1643.05 C=O (Stretching) 1643.05 C=N (Stretching) 3289.96-OH (Hydrogen bonded alcohol and phenol) 1488.78 C=C (Stretching) 923.736 C-O(Stretching) 1396.21 C-H (Aliphatic) [Ar-H, prob. of Para disubstituted benzene] Mass: 281 (1), 280 (18), 279 (100), 105 (45), 89 (15), 77 (39), 63 (9), 51 (28).d6.5-7.8(m, 9H,Ar-H &=CH-),(C=C-CH_3);d(2.2-2.5)1H-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.

Table 2: Inhibition of protein denaturation activity shown by synthesized compounds as compared with standard at varying concentration.

Test Sample	Concentration	% Inhibition
Control	-	-
Standard	750	233.39±2.47
	500	212.14±2.55
	250	178.07±2.32
	125	126.09±2.51
Oxz-1	750	168.51±3.59
	500	61.44±4.26
	250	18.94±3.88
	125	8.93±3.09
Oxz-2	750	158.15±4.79
	500	144.33±3.59
	250	120.77±3.44
	125	73.99±2.84
Oxz-3	750	173.37±3.33
	500	121.70±3.42
	250	106.31±3.19
	125	62.54±3.51
Oxz-4	750	190.52±3.59
	500	135.45±3.16
	250	106.39±3.26
	125	82.51±2.88
Oxz-5	750	161.03±3.88
	500	122.84±3.02
	250	94.22±3.00
	125	61.66±3.46

Values are expressed in term of mean±SEM; (n=5) P<

Table 3: Anti-helminthic activity of synthesized compounds

Compound code	Antihelminthic activity		
	Concentration(ug/ml)	Paralyzing time(min)	Death time(min)
Oxz-1	1000ug/ml	27	72
	800ug/ml	36	80
	600ug/ml	52	95
	400ug/ml	63	119
Oxz-2	1000 µg/ml	32	64
	800 µg/ml	45	80
	600 µg/ml	54	105
	400 µg/ml	65	120
Standard	4mg/ml	15	25
Control (6% DMSO)	-	-	-

DISCUSSION

The present study was carried out for synthesizing Novel as well as reported derivatives of Oxazolone. Ultimately, 2, 4 di-substituted oxazolone was obtained through different scheme, viz: scheme 1, scheme 2 as described in procedures. The condensation of aldehyde with hippuric acid in presence of acetic anhydride, zinc oxide as catalyst and ethanol as solvent to give oxazolone[13]. In first scheme, hippuric acid was prepared then it was further reacted with different derivatives of Benzaldehydes and with Aromatic ketones in second scheme, to obtained final product. Here comparisons in yield values were done of those final oxazolone derivatives prepared by aldehydes and ketones as a starting material for preparations of oxazolone derivatives from two different schemes. The synthesized compounds were analyzed form their physiochemical parameters.

In those entire schemes, sodium acetate was not used because strong base like sodium acetate causes gum formation probably due to rapid self-condensation of hippuricacid[17].The catalyst zinc oxide was taken because *it* has been proved *as an economical and efficient* catalyst, other catalyst such as potassium paramagnet was also used in one attempt, but use of that catalyst affects the color of final product. Presence of spectral dates confirmed the compounds to be the actual one. Hence it can be concluded that, the desired compound of this study were achieved successfully.

The synthesized compounds were tested for the anti-arthritic activity through in-vitro protein denaturation assay, according to the literature protocols. During activity, it was observed that, the increments in absorbance of reference standard and test samples with respect to control which indicated stabilization of protein as reported in (Bhattacharya S. et.al.). Those derivatives of oxazolone showed mild to moderate inhibition of protein denaturation at different concentration as compared with standard. In this study, compound Oxz-1 and Oxz-2 shows mild activity as compared to the derivatives Oxz-3, 4 and 5. While comparing the structure of the tested compounds with the biological activity, we found that the inhibition of protein denaturation activity depends upon the presence of substituents at C-4 position of oxazolone ring. Among all the synthesized compounds, Oxz-4 shows good activity than other synthesized derivatives but shows lower activity when compared with the standard drugs Diclofenac in all the four ranging concentration viz; at 750, 500, 250 and 125 µg/ml concentration, the percentage inhibition of protein denaturation of Oxz-4 derivatives are 190.52±3.59, 135.45±3.16, 106.31±3.19, 82.51±2.88 respectively; the range of % inhibition of protein denaturation of other four compounds at different concentration are 158.15±4.79 to 173.37±3.33, 61.44±4.26 to 144.33±3.59, 18.94±3.88 to 120.77±3.44 and 8.93±3.09 to 73.99±2.84 at concentrations 750, 500, 250 and 125 µg/ml and the relation of concentration with % inhibition of protein denaturation of standard are 233.39±2.47, 212.14±2.55, 178.07±2.32, 126.09±2.51 respectively at 750, 500,250 and 125 µg/ml concentration. Among all synthesized derivatives of oxazolone, compound Oxz-1 and Oxz-2 were tested for anti-helminthic activity. Both of the compounds showed dose-dependent paralysis activity ranging from loss of motility to loss of response to external stimuli, which eventually progressed to death.

CONCLUSION

Present studies suggested that, while synthesizing oxazolone derivatives use of aldehyde derivatives results in high yield, and less time to obtain the final and desired product comparative than use of ketones. The inhibition of protein denaturation activity shown by the derivative synthesized from the 4-dimethyl aminobenzaldehyde derivatives proved comparatively more active than other synthesized derivatives. So, it can be concluded that, aldehydes derivatives are important substituent for synthesizing oxazolone derivatives which is again beneficial in terms of inhibition of protein denaturation as shown by compound Oxz-4. Thus, use of aldehyde for

synthesizing oxazolone derivatives can be used in increasing in yield, decreasing the time of reaction and showing potent inhibition of protein denaturatuion which can further be used in synthesizing potent Anti-arthritic or Anti-inflammatory compounds. The anti-arthritics activity for those derivatives concluded that all the derivatives showed the activity but in mild to moderate as compare with standard. The comparison between structure and biological activity(anti-arthritic activity) of compound shows that, may be due incorporation of electron donating species such as $-N(CH_3)_2$i.e: Oxz-4and –OHi.e: Oxz-3 group in benzyl ring may shows comparative good anti-arthritic activity than substitution by electron withdrawing species such as –Cl in benzyl ringi.e: Oxz 1 and Oxz-5.When screening for antihelminthic activity, it can be concluded that, Oxz-1 and Oxz-2 showed mild to moderate antihelminthic activity. The anti-helminthic activity is slightly better may be due incorporation of chlorine group as in Oxz-1

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