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Synthesis and Study of Oxazolone Derivatives Showing Biological Activity

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Abstract

Synthesis of the oxazolone ring was performed by the condensation of 4-n –alkoxy benzoyl glycine with appropriate 4-n-alkoxy benzaldehyde in presence of acetic anhydride and anhydrous sodium acetate. Eight compounds were prepared using two different types of 4-n-alkoxy benzaldehyde molecules and compare their percentage yields as well as their activities. Also in both the respective series, the oxazolone rings containing a terminal nitro group were prepared to observe the effect of the electron withdrawing nitro group as compared to that of the electron releasing alkoxy groups. The antibacterial activity was checked against Micrococcus luteus and Escherichia coli for all the compounds. The lower members of each series showed better antibacterial activity than the higher members. Also, the cytotoxicity was checked against dicots (moong seeds; Phaseolus aureus). The presence of alkoxy groups showed no profound effect on the seed germination while the presence of nitro group showed growth inhibitory action. The structures of the synthesized compounds have been characterized by elemental analysis, TLC and spectral data.

Keywords: Liquid crystals, nematic, oxazolone, antibacterial, cytotoxicity, antifungal etc.

Introduction

A novel class of compounds, which contain oxazolone rings in its structure, known as peptide nucleic acids[1], bind complementary DNA and RNA strands more strongly than the corresponding natural DNA or RNA strands, and exhibit increased sequence specificity and solubility. This peptidomimetic synthesis can lead to enhanced properties of the required proteins, which are required to do characteristic functions. Functionalized oxazole derivatives[2-4] are key precursors to *N*-acyliminium ions, critical intermediates for the syntheses of natural products such as conhydrine, *o*-methyl pallidinine, and morphine. The improved method of the synthesis of tetracycline involves an intermediate oxazolone moiety, with a controlled introduction of stereo center at C4a has been presented by Stork *et al*.[5.]

Material and Methods

The synthetic route adopted for the synthesis of oxazolone derivatives is as shown in figure-1. The microanalysis of compounds were recorded on Coleman instrument. The I.R. spectra were recorded on Perkin - Elmer spectrophotometer. NMR spectrum was recorded on BRUKER-300 instrument. Melting points were investigated on Leitz- Labourlux 12 Pol (Germany) polarizing microscope, provided with Kofler heating stage.

Preparation of 4-n-alkoxybenzoic acid[6]: 4-hydroxy benzoic acid (0.1 mole), appropriate alkyl halide (0.12 mole) and potassium hydroxide (0.25mole) were dissolved in 100 ml ethanol and refluxed for 7 to 8 hours. Ten percent aqueous potassium hydroxide solution (25 ml) was added and reflux was continued for two hours to hydrolyze any ester formed. The solution was cooled and acidified with 1:1 cold hydrochloric acid to precipitate the acid. The n-alkoxy acids were crystallized several times from ethanol until sharp melting point / transition temperatures were obtained. The percentage yield is about 60-65%.

Preparation of 4-n-alkoxybenzoyl chloride[6]: 4-n-Alkoxy benzoyl chlorides were prepared by reacting the corresponding 4-n-alkoxy benzoic acid with excess of thionyl chloride and heating on a water-bath till evolution of hydrogen chloride gas ceased. Excess of thionyl chloride was distilled off under reduced pressure using water pump and the acid chloride left behind as a residue was used in next reaction without further purification.

Preparation of 4-n-alkoxybenzoyl glycines[6]: Dissolve 0.11 mole of glycine in 25 ml of 10% NaOH solution in a conical flask. 0.10 mole of 4-n alkoxy benzoyl chloride is added in two portions to the solution. The flask is stoppard and shaken vigorously after each addition till all 4-n-alkoxy benzoyl chloride is reacted. The solution is transferred to a 100 ml beaker and the conical flask is rinsed with a little water. A few grams of crushed ice is added to the solution and conc. HCl is added slowly with stirring till the mixture is acidic to Congo red paper. The resulting crystalline 4-n-alkoxy benzoyl glycines are filtered upon a Buchner funnel, washed with cold water and drained well. The product is crystallized from aqueous alcohol until sharp melting points are obtained. The compounds are confirmed by elemental analysis as well as the

purity of the compound is observed by TLC (20:80; ethyl acetate: petroleum ether). IR data are recorded in table-3.

Preparation of 4-Benzylidene-2-phenyloxazol-5-one derivatives[7-8]: Place a mixture of 0.25 mole of 4-n-alkoxy benzaldehyde, 0.25 mole n-alkoxy benzoyl glycine, 0.75 moles of acetic anhydride and 0.25 mole of anhydrous sodium acetate in a round bottom flask and heat on a steam bath with constant shaking , using a water condenser and guard tube. As soon as solid dissolve, reflux was continue for 2 hours. Cool and add 100 ml ethanol slowly to the contents of the flask and allow the mixture to stand overnight. Filter the crystallized product with suction and wash with hot water and then with a little volume of ice cold 1:1 methanol. Recrystallize from methanol. The purity of compounds was checked using TLC. The compounds are characterized by elemental analysis, I.R. spectra and NMR spectrum (figure-2) and spectral data are recorded in table-4.

Table-3
I.R. Spectra of Compound 4-n-heptyloxybenzoyl glycine

Sr.No.	Frequency (cm^{-1})	Probable Functional Group
1.	2980 , 2846	$-CH_3$, $-CH_2-$ stretching
2.	1683	-C=O stretching of amide carbonyl
3.	2554-3426	-O-H stretching of carboxylic acid
4.	3426	-N-H stretching of 2^0 amide (1 band)
5.	1603 , 1515	-C-C- (skeletal) stretching of benzene ring
6.	1030	-C-O stretching of ether
7.	845 , 824	Ar-H bending in p-substituted benzene ring

Table-4
I.R. Spectra of Compound Pn1

Sr.No.	Frequency (cm^{-1})	Probable Functional Group
1.	2950 , 2873	$-CH_3$, $-CH_2-$ stretching
2.	1784	-C=O stretching of lactone
3.	1644	-C=N- stretching (cyclic)
4.	1602 , 1504	-C-C- (skeletal) stretching of benzene ring
5.	1035	-C-O stretching of ether
6.	851 , 832 , 808	Ar-H bending in p-substituted benzene ring

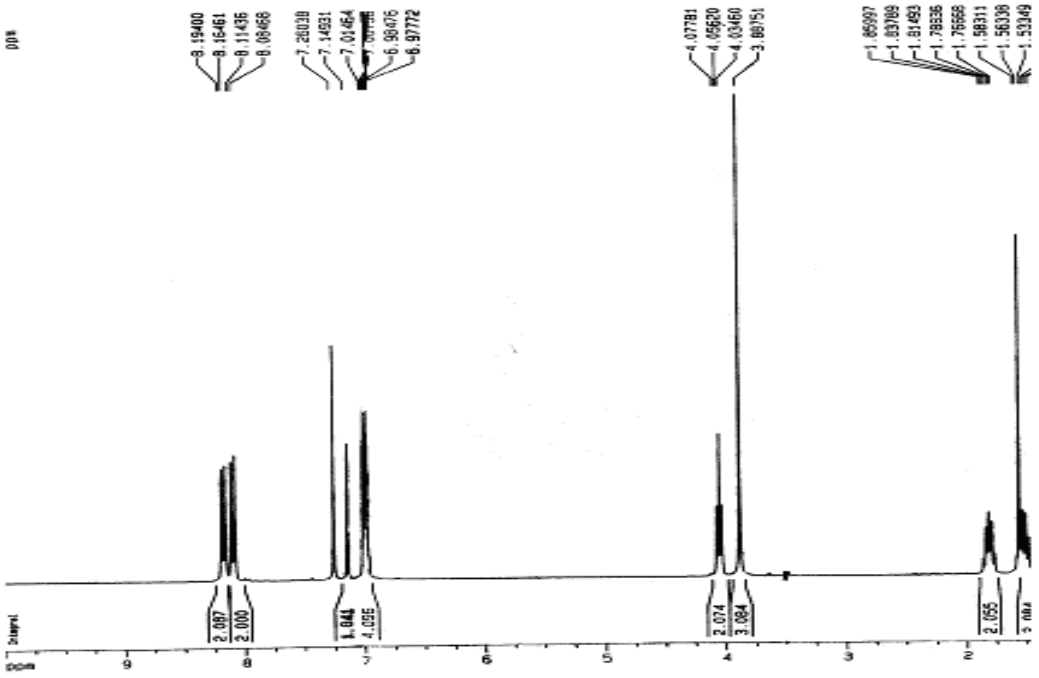

Figure-2
^{1}H NMR (300 MHz, $CDCl_3$) spectrum of Pn1

The n-pentyloxy, n-hexyloxy, n-heptyloxy and m-nitro derivatives formed using anisaldehyde (series I) with substituted glycines are respectively coded as Pn1, He1, Hp1 and Nt1. Similarly those formed using 4-Ethoxy-3-Methoxybenzaldehyde and substituted glycines are coded as Pn2, He2, Hp2 and Nt2 (series II).

NMR Spectrum for Compound Pn1: ^{1}H NMR (300 MHz, $CDCl_3$) 0.99 (t, 3H, $-CH_3$), 1.56 (m, 2H, $-CH_2-$), 1.81 (m, 4H, 2-CH_2-), 3.88 (s , 3H , $-OCH_3$), 4.05 (t , 2H , $-CH_2-$), 6.97-7.00 (d , 2H , J=8.9 Hz , Ar-H), 6.98-7.01 (d , 2H , J=8.9 Hz , Ar-H), 7.14 (s , 1H , -C=C-H), 8.08-8.11 (d, 2H, J=8.9 Hz, Ar-H), 8.16-8.19 (d, 2H, J=8.8 Hz, Ar-H).

Results and Discussion

A new class of compounds was synthesized, containing oxazolone ring, by using 4-n-alkoxybenzoic acids as the starting compounds. 4-n-alkoxybenzoyl glycine derivatives were synthesized by condensing acid chloride of 4-n-alkoxy benzoic acids with glycine in presence of aqueous NaOH (figure-1). These derivatives do not show liquid crystalline properties, possibly due to hydrogen bonding of amide linkage. The final compounds were synthesized by condensing 4-n-alkoxy benzoyl glycines with appropriate 4-n-alkoxy aldehydes. The percentage yields of the final products are about 23-38% in case of anisaldehyde derivatives and about 20-33% in case of the 4-ethoxy-3-methoxybenzaldehyde derivatives (table-1). An increase in carbon chain length of the alkoxy group usually gives lesser yield due to such attractive interactions with the carbonyl oxygen[8].

Liquid Crystal Property: None of the compounds are observed to exhibit liquid crystal phases except 4-n alkoxy benzoic acids exhibit but melt softly at their respective melting temperatures.

Antibacterial Activity: Experimental: The bacteria are grown in the sterile nutrient broth under submerged conditions a few hours prior to the experiment. About 0.1 ml of this culture medium is inoculated on the nutrient agar plate uniformly. Once set, wells are bored using a sterile cork borer. Solution containing 10 mg /0.1 ml is prepared in dimethyl sulfoxide and 0.1 ml of this solution is added to the wells using a micropipette. The plates are incubated at 25 degree Celsius for 15-16 hours. The zone of inhibition around the wells is checked and measured (table-5).

Effect on Seed Germination: (Cytotoxicity[9-10]) Seed infusion technique: Experimental: For Seed infusion, dry seeds are immersed for 3-4 hours in acetone, containing the synthesized compounds. Seeds are then transferred to a petridish and incubated at 25-27 degree celsius temperature and the extent of germination is observed after 4-5 days. The concentration of solutions used for these experiments was 15 mg / ml. Six petridishes each were used in case of both monocot for dicot (moong seeds–figure-3) (table-6)

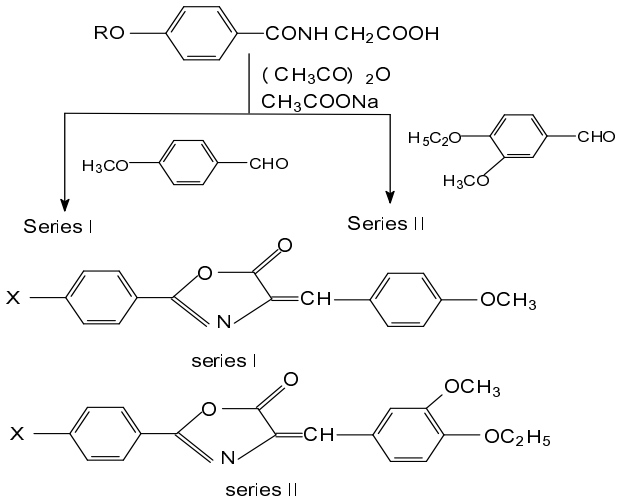

Figure-1
Synthetic route

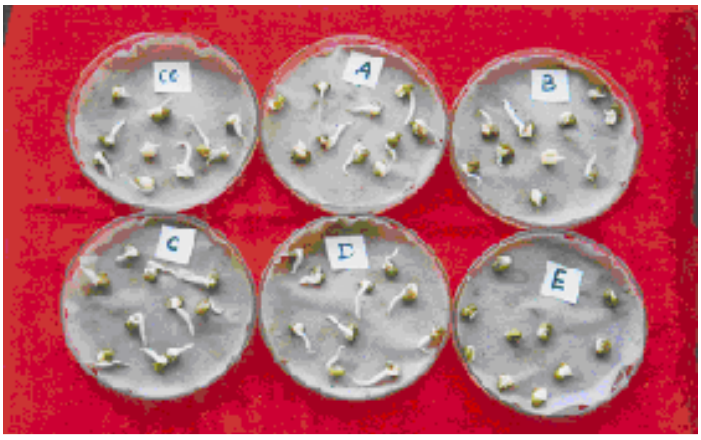

Figure-3
Showing the effect of the compounds on Moong seed germination

Table-1
Percentage Yields of Compounds

Series-I	Percentage yield (%)	Series-II	Percentage yield (%)
Pn1	38	Pn2	33
He1	30	He2	28
Hp1	28	Hp2	23
Nt1	23	Nt2	20

Table-2
Melting Points / Transition Temperatures of Compounds at Various Stages of the Synthetic Route

Code Of Compd.	n-alkoxy benzoic acid (degree Celsius)	Condensed with Glycine (degree Celsius)	Series-I (degree Celsius) With code no.	Series-II (degree Celsius) With code no.
1.	124 N 151 Iso*	130	150 (Pn1)	230 (Pn2)
2.	105 N 153 Iso*	126	205 (He1)	170 (He2)
3.	92Sm 98 N145Iso*	135	120 (Hp1)	196 (Hp2)
4.	220	198	170 (Nt1)	210 (Nt2)

*Indicates transition temperatures(Liquid crystals) observed under polarizing microscope

Table0-5
Antibacterial activity

Code of Compound	Gram negative bacteria	Zone of Inhibition (mm)	Gram positive bacteria	Zone of inhibition (mm)
Pn1	positive	10	(-)	(-)
He1	positive	08	(-)	(-)
Hp1	(-)	(-)	(-)	(-)
Nt1	(-)	(-)	(-)	(-)
Pn2	(-)	(-)	positive	12
He2	(-)	(-)	positive	10
Hp2	(-)	(-)	positive	07
Nt2	(-)	(-)	(-)	(-)

(-) indicates negative test
The gram positive bacteria used was *Micrococcus Luteus* while the gram negative bacteria used was *Escherichia Coli (photograph A).*

Table-6
Seed Germination for Moong Seeds

	In ethyl acetate		In acetone	
Solution used for seed infusion	**Percentage Germination (%)**	**Average radicle length (cm)**	**Percentage Germination (%)**	**Average radicle length (cm)**
Pn1	98.33	1.30	94.30	1.47
He1	85.66	0.95	82.42	2.10
Hp1	87.13	1.26	73.80	1.03
Nt1	-	-	-	-
Water	84.62	1.48	64.33	2.56
Acetone	-	-	93.54	0.85

The seed infusion technique for dicots, when carried out using both ethyl acetate and acetone as solvents, shows that the presence of p-substituted alkoxy groups does not inhibit germination and the subsequent growth of the radicle and plumule, whereas the presence of p-substituted -NO_2 group completely inhibits the germination of seeds and thus hinders its propagation and proliferation.

Antifungal Activity[11]: None of the compounds showed any inhibitory action towards the growth of the fungus *Alternaria alternata* whose growth covered the entire plate over a period of 5 days. To study the effect of the compounds the experiment was repeated using a more slow growing fungus *Phoma multirostata* which exhibited similar results as the previous experiment. Here the fungus took 5-6 days to grow over the whole plate entirely, without any inhibitory action from the compounds.

Conclusion: Structure-activity relationship of the compounds revealed that first three members exhibited antibacterial activity. None of the compounds showed any inhibitory action towards the growth of the fungus. The most striking observation is that compounds with nitro group causes total inhibition of seed germination even after five days of incubation. Thus the presence of nitro group makes the compound cytotoxic in case of barley and moong.

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References

1. Buchardt D., Michael E., Peter N. and Henrik R., *U. S. Patent No*. 5736336 (**1998**)

2. David C. P., *Oxazoles: Synthesis, Reactions and Spectroscopy*, Part John Wiley and Sons. London (**2004**)

3. Nicolaou K.C., Claiborner C.F., Nantermet P.G., Liu, J.J., Ueno H., Renaud J. and Guy R.K., *Nature*, **367** ,630-633 (**1994**)

4. Li Q. and Sham H., *Jr . Expert Opinion on Therapeutic Patents,* **12**, 1663-1669 (**2002**)

5. Stork G., La Clair, J.J., Spargo P., Nargund R.P. and Totah N., *J. Am. Chem. Soc*., **118**, 5304-5309 (**1996**)

6. Dave J.S. and Vora R.A., *Liquid crystals and ordered fluids*, Plenum press, London, 477-480 (**1970**)

7. *Vogel's textbook of Practical Organic Chemistry*, fifth edition, Pearson Education Limited, 1073 (**2005**)

8. Mc Donald M.B. and Copeland L.O., *Seed Science and Technology Laboratory Manual*, Scientific Publishers, Jodhpur, 232 (**1999**)

9. Nazaritarhan H., Hosseinzadeh A., Alibadi A and Gholamine B., Cytotoxic and apoptogenic properties of 2-phenylthiazole-4-carboxamide derivatives in human carcinoma cell lines, *Res. J. Recent Sci.*, **7(5) (2012)**

10. Vosooghi M., Rajabalian S., Sorkhi M., Badinloo M., Negahbani A. S., Asadipour A., Mahdavi M., Shafiee A. and Foroumadi A., Synthsis and cytotoxic activity of some 2-amino-4-aryl-3-cyano-7-(dimethylaamino) -4H-chromenes, *Res. J. Recent Sci.,* 9-14 **(2010)**

11. Dalili D., Samadi N. and Sabeti B., The effect of antifungal compounds on cell surface hydrophobicity and adhesion Candida albicans to buccal epithelial cells, Res. *J. Recent Sci.,* **7(5) (2012)**