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## Note

# Synthesis and study of some compounds containing oxazolone ring, showing biological activity

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Synthesis of the oxazolone ring has been performed by the condensation of 4-substituted alkoxy benzoyl glycine with appropriate 4-substituted alkoxy benzaldehyde, in the presence of acetic anhydride and anhydrous sodium acetate. The antibacterial activity has been checked against *Micrococcus luteus* and *Escherichia coli* and antifungal activity against *Alternaria alternate* and *Phoma multirostata* for all the compounds. The cytotoxicity has been checked against the monocots *barley seeds; Hordeum vulgare L* and dicots *moong seeds; Phaseolus aureus*. The compounds having electron releasing group exhibit antibacterial activity. Compounds with nitro group cause total inhibition of seed germination, exhibiting cytotoxic behaviour. The structures of the synthesized compounds have been characterized by elemental analysis and spectral data and the purity of the compounds has been checked by TLC method.

**Keywords**: Phenyloxazol-5-one, benzoyl glycine, antibacterial activity, antifungal activity, seed germination, liquid crystalline property

Recently amino acids incorporating furan and pyrrole rings have been synthesized[1,2]. They have been found to exhibit biological activity. The common structural units like amino groups and carboxylic acid groups can be incorporated in new compounds having other functional groups. A novel class of compounds, which contain oxazolone rings in its structure, known as peptide nucleic acids[3], bind complementary DNA and RNA strands more strongly than the corresponding natural DNA or RNA strands, and exhibit increased sequence specificity and solubility. Functionalized oxazole derivatives[4] are key precursors to *N*-acyliminium ions, critical intermediates for the syntheses of natural products such as conhydrine, *o*-methylpallidinine and morphine[5,6].

The improved method for the synthesis of tetracycline involves an intermediate oxazolone moiety, with a controlled introduction of stereo center has been presented by Stork *et al.*[7]. Deflazacort[8] is an oxazolone derived from prednisone, and has anti-inflammatory and immunosuppressive effects. It was thought proper to synthesize compounds having oxazole as a central ring and to study the biological activity.

Starting compounds 4-substituted benzoic acids were synthesized from 4-hydroxy benzoic acid and the respective alkyl halides by known procedure[9]. The melting points are found to be in good accordance with the literature. 4-Substituted alkoxy benzoyl glycine were synthesized from 4-substituted alkoxy benzoyl chloride and glycine in the presence of aq. NaOH solution. None of the acids exhibit liquid crystalline properties. All members have high melting points.

4-Benzylidene-2-phenyloxazol-5-one derivatives were synthesized by condensing 4-substituted benzoyl glycine and appropriate aldehydes in the presence of acetic anhydride and sodium acetate (**Figure 1**). The purity of compounds was confirmed by spectral and elemental analysis. The characterization data of the newly synthesized compounds are tabulated in **Table I**.

## Results and Discussion

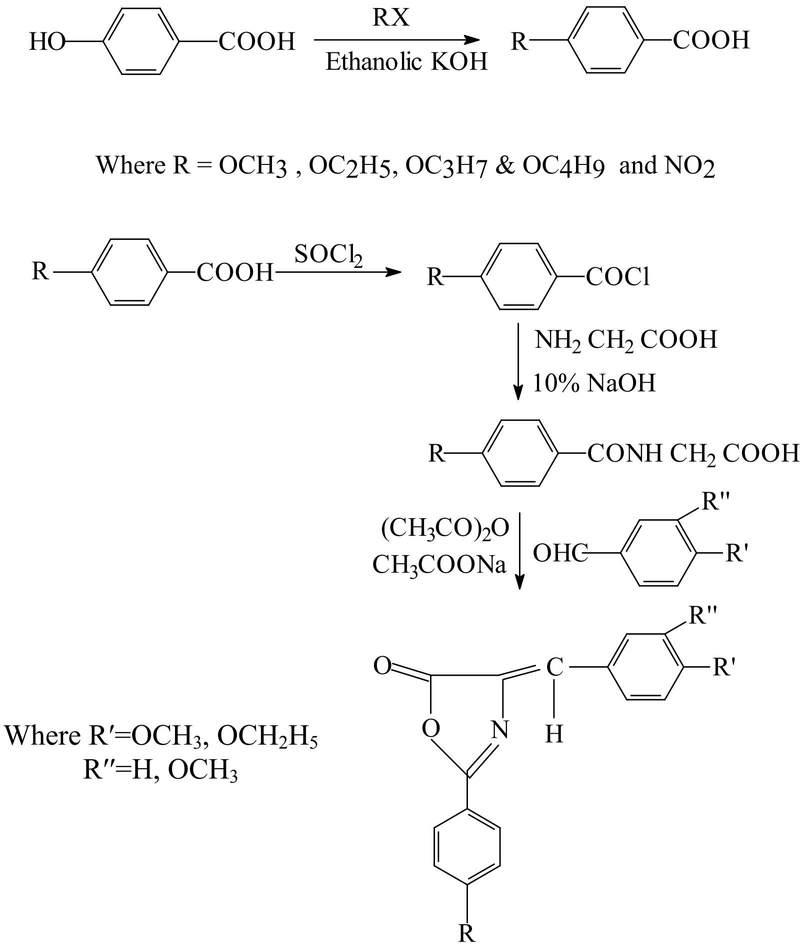
### Antibacterial activity

The bacteria were grown in the sterile nutrient broth under submerged conditions a few hours prior to the experiment. About 0.1 mL of this culture medium was inoculated on the nutrient agar plate uniformly. Once set, wells were bored using a sterile cork borer. Solution containing 10 mg /mL of the compound to be tested was prepared in dimethyl sulfoxide and 0.1 mL of this solution was added to the wells using a micropipette. The plates were incubated at 30°C for 12-18 hr. The zone of inhibition around the wells was checked and measured (**Table II**).

### Effect on seed Germination: (Cytotoxicity)

### Seed infusion technique

For seed infusion[10], dry seeds were immersed for one to four hours in acetone, containing the synthesized compounds. Seeds were then transferred to a petridish and incubated at 25-30°C temperature and the extent of germination was observed after 3-5 days. The concentration of solutions used for these experiments was 15 mg/mL. Six petridishes each



Where R = $OCH_3$ , $OC_2H_5$, $OC_3H_7$ & $OC_4H_9$ and $NO_2$

Where R′=$OCH_3$, $OCH_2H_5$
R″=H, $OCH_3$

**Figure 1** — Synthetic Route

were used in case of both monocot (barley seeds) as well as for dicot (moong seeds). In case of barley seeds the compound **2a** fails to germinate when the solvent used is ethyl acetate, but germinates well in case of acetone as solvent.

Similarly the compound **2b** germinates well when ethyl acetate is used as solvent but fails when the solvent used is acetone (**Table III**).

These observations can thus be attributed to the effect of the conditions to which the seeds are subjected and not due to cytotoxicity.

The most striking observation is that of compound **2e** where the presence of -$NO_2$ group causes total inhibition of seed germination even after 5 days of incubation. The presence of -$NO_2$ group makes the compound cytotoxic in case of both barley and moong (**Table III**).

The seed infusion technique for both monocots and dicots, when carried out using both ethyl acetate and acetone as solvents, shows that the presence of 4-substituted alkoxy groups does not inhibit germination and the subsequent growth of the radicle and plumule, whereas the presence of 4-substituted $NO_2$ group completely inhibits the germination of seeds and thus hinders its propagation and proliferation.

### Antifungal activity

The antifungal screening of the compounds, **2a-e** and **3a-e** was carried out *in vitro* by paper disc method against *Alternaria alternate* and *Phoma multirostata* at 10 mg/mL concentration.

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 7-8 days to grow over the whole plate entirely, without any inhibitory action from the compounds.

**Table I** — Characterization data of synthesized compounds

| Compd | R | R′ | R″ | Mol. Formula | m.p. °C | Yield (%) | Found % (Calculated) | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | C | H | N |
| **1a** | $OCH_3$ | - | - | $C_{10}H_{11}NO_4$ | 150 | 41 | 57.23 | 5.13 | 6.60 |
| | | | | | | | (57.42 | 5.26 | 6.70) |
| **1b** | $OC_2H_5$ | - | - | $C_{11}H_{13}NO_4$ | 120 | 39 | 58.87 | 5.74 | 6.17 |
| | | | | | | | (59.19 | 5.83 | 6.28) |
| **1c** | $OC_3H_7$ | - | - | $C_{12}H_{15}NO_4$ | 153 | 37 | 60.56 | 6.26 | 5.83 |
| | | | | | | | (60.76 | 6.33 | 5.91) |
| **1d** | $OC_4H_9$ | - | - | $C_{13}H_{17}NO_4$ | 149 | 36 | 62.01 | 6.59 | 5.61 |
| | | | | | | | (62.15 | 6.77 | 5.58) |
| **1e** | $NO_2$ | - | - | $C_9H_8N_2O_5$ | 220 | 33 | 48.08 | 3.47 | 12.41 |
| | | | | | | | (48.06 | 3.47 | 12.41) |
| **2a** | $OCH_3$ | $OCH_3$ | H | $C_{18}H_{15}NO_4$ | 190 | 45 | 69.79 | 4.69 | 4.45 |
| | | | | | | | (69.90 | 4.85 | 4.53) |
| **2b** | $OC_2H_5$ | $OCH_3$ | H | $C_{19}H_{17}NO_4$ | 265 | 43 | 70.52 | 5.20 | 4.20 |
| | | | | | | | (70.59 | 5.26 | 4.33) |
| **2c** | $OC_3H_7$ | $OCH_3$ | H | $C_{20}H_{19}NO_4$ | 130 | 38 | 71.04 | 5.60 | 4.10 |
| | | | | | | | (71.22 | 5.64 | 4.15) |
| **2d** | $OC_4H_9$ | $OCH_3$ | H | $C_{21}H_{21}NO_4$ | 129 | 35 | 71.77 | 5.84 | 3.90 |
| | | | | | | | (71.79 | 5.98 | 3.99) |
| **2e** | $NO_2$ | $OCH_3$ | H | $C_{17}H_{12}N_2O_5$ | 175 | 30 | 62.86 | 3.80 | 8.60 |
| | | | | | | | (62.96 | 3.70 | 8.64) |
| **3a** | $OCH_3$ | $OC_2H_5$ | $OCH_3$ | $C_{20}H_{19}NO_5$ | 250 | 44 | 67.84 | 5.40 | 3.89 |
| | | | | | | | (67.99 | 5.38 | 3.97) |
| **3b** | $OC_2H_5$ | $OC_2H_5$ | $OCH_3$ | $C_{21}H_{21}NO_5$ | 180 | 42 | 68.55 | 5.63 | 3.80 |
| | | | | | | | (68.67 | 5.72 | 3.82) |
| **3c** | $OC_3H_7$ | $OC_2H_5$ | $OCH_3$ | $C_{22}H_{23}NO_5$ | 205 | 36 | 69.14 | 6.10 | 3.58 |
| | | | | | | | (69.29 | 6.04 | 3.68) |
| **3d** | $OC_4H_9$ | $OC_2H_5$ | $OCH_3$ | $C_{23}H_{25}NO_5$ | 124 | 30 | 69.82 | 6.15 | 3.47 |
| | | | | | | | (69.87 | 6.33 | 3.54) |
| **3e** | $NO_2$ | $OC_2H_5$ | $OCH_3$ | $C_{19}H_{16}N_2O_6$ | 230 | 30 | 61.89 | 4.31 | 7.70 |
| | | | | | | | (61.96 | 4.35 | 7.61) |

## Experimental Section

The microanalysis of compounds was recorded on Coleman instrument. Satisfactory C, H, N analysis were obtained for all the compounds. The IR spectra were recorded on Perkin-Elmer spectrophotometer. $^1H$ NMR spectra was recorded on Bruker-300 instrument. ESI-MS were recorded on LC-MS Shimadzu-2010 Instrument and mass values are recorded. Melting points were determined on a Leitz-Labourlux 12 Pol (Germany) polarizing microscope, provided with Kofler heating stage.

### Preparation of 4-*n*-alkoxybenzoic acids[9]

4-Hydroxy benzoic acid (0.1 mole), appropriate alkyl halide (0.12 mole) and potassium hydroxide (0.25 mole) were dissolved in 100 mL ethanol and refluxed for 7 to 8 hr. Ten percent aqueous potassium hydroxide solution (25 mL) was added and refluxed was continued for two hr to hydrolyse any ester formed. The solution was cooled and acidified with 1:1 cold hydrochloric acid to precipitate the acid. The 4-alkoxy benzoic acids were crystallized from ethanol until sharp melting points were obtained. The melting points are in good accordance with the literature. The percentage yield is about 80%.

### Preparation of 4-substituted benzoyl chloride[9]

4-Substituted benzoyl chlorides were prepared by reacting the corresponding 4-substituted 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.

**Table II** — Antibacterial activity

| Compd | Gram negative bacteria | Zone of Inhibition (mm) | Gram positive bacteria | Zone of inhibition (mm) |
|---|---|---|---|---|
| **2a** | positive | 12 | (-) | (-) |
| **2b** | positive | 11 | (-) | (-) |
| **2c** | positive | 10 | (-) | (-) |
| **2d** | (-) | (-) | (-) | (-) |
| **2e** | (-) | (-) | (-) | (-) |
| **3a** | (-) | (-) | positive | 11 |
| **3b** | (-) | (-) | positive | 7 |
| **3c** | (-) | (-) | (-) | (-) |
| **3d** | (-) | (-) | (-) | (-) |
| **3e** | (-) | | | |

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

**Preparation of 4-substituted benzoyl glycines[11] (Compounds 1a to 1e)**:

Dissolve glycine (0.11 mole) in 25 mL of 10% NaOH solution in a conical flask. 4-Substituted benzoyl chloride (0.10 mole) was added in two portions to the solution. The flask was stoppered and shaken vigorously after each addition till all 4-substituted benzoyl chloride was reacted. The solution was transferred to a 100 mL beaker and the conical flask was rinsed with a little water. A few grams of crushed ice was added to the solution and conc. hydrochloric acid was added slowly with stirring till the mixture was acidic to congo red paper. The resulting crystalline 4-substituted benzoyl glycine was filtered upon a Buchner funnel, washed with cold water and drained well. The solid was placed in a conical flask containing 10 mL of $CCl_4$ and boiled gently for about 2 min. The mixture was allowed to cool and was filtered by suction. The product was

**Table III** — Effect of compounds on seed germination

**A) For Barley seeds:**

| Solution used for seed infusion | In ethyl acetate | | | In acetone | | |
|---|---|---|---|---|---|---|
| | Percentage Germination (%) | Average Radicle Length (cm) | Average plumule Length (cm) | Percentage Germination (%) | Average Radicle Length (cm) | Average plumule Length (cm) |
| **2a** | - | - | - | 92.00 | 4.65 | 1.90 |
| **2b** | 100 | 2.89 | 0.56 | - | - | - |
| **2c** | 100 | 2.71 | 0.40 | 77.77 | 4.72 | 2.88 |
| **2d** | 100 | 2.16 | 0.60 | 80.00 | 4.04 | 1.93 |
| **2e** | - | - | - | - | - | - |
| Water | 100 | 2.86 | 0.61 | 92.85 | 6.01 | 2.69 |
| Acetone | - | - | - | 88.88 | 4.42 | 2.30 |

**B) For Moong seeds**

| Solution used for seed infusion | In ethyl acetate | | In acetone | |
|---|---|---|---|---|
| | Percentage Germination (%) | Average radicle length (cm) | Percentage Germination (%) | Average radicle length (cm) |
| **2a** | 90.44 | 1.20 | 92.30 | 1.53 |
| **2b** | 86.63 | 0.77 | 88.46 | 1.97 |
| **2c** | 90.34 | 1.28 | 75.86 | 1.12 |
| **2d** | 90.12 | 1.66 | 80.76 | 1.02 |
| **2e** | - | - | - | - |
| Water | 88.62 | 1.38 | 68.96 | 2.86 |
| Acetone | - | - | 96.87 | 0.78 |

washed with a little volume of $CCl_4$. The product was recrystallized from methanol until sharp melting points are obtained (**Table I**). The compounds were confirmed by elemental analysis. The purity of the compound was observed by TLC (30:70; ethyl acetate: petroleum ether).

**Compound 1d**: IR(KBr) 3423 –N-H stretching of $2^{o}$ amide (1 band), 2983, 2844 -$CH_3$,-$CH_2$- stretching, 1686 -C=O stretching of amide carbonyl, 2557, 3423 (br) -O-H stretching of carboxylic acid, 1603, 1515 -C-C- (skeletal) stretching of benzene ring, 1026 -C-O- stretching of ether, 845, 824 cm$^{-1}$Ar-H bending in 4-substituted benzene ring.

### Preparation of 4-benzylidene-2-phenyloxazol-5-one derivatives[12] (Compounds 2a to 3d)

Place a mixture of 0.25 mole of 4-substituted benzaldehyde, 0.25 mole 4-substituted 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 solids dissolve, reflux was continued for 2 hr. The mixture was cooled and 100 mL ethanol was added slowly to the contents of the flask and the mixture was allowed to stand overnight. The crystallized product was filtered with suction and washed with hot water and then with a little volume of ice cold 1:1 methanol. Compounds were recrystallized from ethanol. The purity of compounds was checked using TLC. The compounds were characterized by elemental analysis (**Table I**), IR, mass and $^{1}$H NMR spectrum.

The methoxy, ethoxy, *n*-propoxy, *n*-butoxy and nitro derivatives formed using anisaldehyde and substituted glycines are respectively coded as **2a**, **2b, 2c, 2d** and **2e**. Similarly those formed using 4-ethoxy-3-methoxybenzaldehyde and substituted glycines are coded as **3a, 3b, 3c, 3d** and **3e**.

**Compound 2d**: IR(KBr) 2956, 2870 (-$CH_3$, -$CH_2$- stretching), 1786 (-C=O stretching of lactone), 1649 (-C=N- stretching) (cyclic), 1604, 1508 (-C-C- (skeletal) stretching of benzene ring), 1033(-C-O stretching of ether), 851, 832, 808 cm$^{-1}$ (Ar-H bending in 4-substituted benzene ring); MS: *m/z* 352 [M + H].$^{+\cdot}$

**Compound 3d**: IR(KBr) 2957, 2874 (-$CH_3$,-$CH_2$- stretching), 1791 (-C=O stretching of lactone), 1648 (-C=N- stretching) (cyclic), 1604, 1509 (-C-C- (skeletal) stretching of benzene ring), 1038 (-C-O stretching of ether), 842, 832, 806 cm$^{-1}$ (Ar-H bending in 4-substituted benzene ring); $^{1}$H NMR (300 MHz, $CHCl_3$): δ 0.99 (t, 3H, -CH3), 1.56 (m, 2H, -CH2-), 1.81 (m, 2H, -CH2-), 3.88 (s, 3H, -OCH3), 4.05 (t, 2H, -CH2-), 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); MS: *m/z* 395 [M]$^{+}$.

The spectral data matches with similar compounds[13,14].

## 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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