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Research Article

# IN-VITRO STUDIES OF SOME CHALCONES ON ALKALINE PHOSPHATASE

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**ABSTRACT**

Chalcones, a class of naturally occurring metabolites, precursors of flavanoids and isoflavanoids abundant in edible plants are known to possess diverse pharmacological, activities e.g. anti-inflammatory, analgesic, antimicrobial, antioxidant, anticancer, antimalarial, antiviral, and antitubercular, etc. The pharmacological potential of the chalcones can be an outcome of their potency to inhibit several important enzymes in cellular system such as fumarate reductase, mitochondrial dehydrogenases. In the present work we have evaluated the effect of chalcones on the activity of alkaline phosphatase of two different sources.

**Keywords**: Alkaline Phosphatase, Chalcones, Moong bean, Liver

## INTRODUCTION

Chalcones (1,3-diphenyl-2-propen-1-ones) have been a subject of great interest for chemists and biochemists all over due to their ease of synthesis, vast and interesting pharmacological activities. These are one of the major classes of natural products with widespread distribution in spices, tea, beer, fruits and vegetables. Chalcones also act as intermediate compounds for various heterocyclic compounds. Chalcones serve as a precursor unit in flavonoid[1] biosynthesis in plants. Chemically, they are open-chain flavonoids in which the two aromatic rings are joined by a three-carbon $\alpha$, $\beta$-unsaturated carbonyl system. Various compounds with the backbone of chalcones have been reported to possess various biological activities such as antimicrobial[2], anti-inflammatory[3], analgesic, antiplatelet[4], antiulcerative[5,] antimalarial[6], anticancer[7], antiviral[8] antileishmanial[9] antioxidant[10], antitubercular[11], antitumour[12], antihyperglycemic[13], immunomodulatory[14], antiangeogenic[15], antiparasitic[16], inhibition of chemical mediators release[17], inhibition of leukotriene $B_4$[18], inhibition of tyrosinase[19] and inhibition of aldose reductase[20]activities. The molecules which interfere with the metabolic system of the host will lead to alteration in metabolic processes and will certainly be having some side effects. Alkaline phosphatase [EC 3.1.3.1] are important class of enzyme. Alkaline phosphatase (ALP) is an important enzyme mainly derived from the liver, bones and in lesser amounts from intestines, placenta, kidneys and leukocytes which hydrolyse phosphate group from a variety of substrate at alkaline pH. Alkaline phosphatases perform diverse functions[21] to remove phosphate groups from a variety of substrates such as nucleotides, proteins and alkaloids etc. Low concentration of this enzyme results in hypophosphatasia that is characterized by hypocalcaemia and include skeletal defects[22].

An increase in ALP levels in the serum is frequently associated with a variety of diseases. This clearly indicates the physiological importance of this enzyme. Use of molecules as anti-infective or anti parasitic agent that affects the host enzyme system can cause enzyme related side effects. In the present study we report the effect of differently substituted chalcones as these are gaining attention for their use in the treatment of various parasitic diseases on the activity of alkaline phosphatase, a physiologically important enzyme isolated from two different sources, moong bean (a plant source) and liver (an animal source).

## MATERIALS AND METHODS

### General method for the synthesis of chalcones

Substituted chalcones were synthesized by claisen-Schmidt reaction by the established routes as in our previous work[23] in alkaline medium taking equimolar ratio of substituted acetophenone were stirred in methanol in ice bath for 30 minutes and then substituted benzaldehyde (equimolar ratio) was added and stirred again for 3 hours in ice bath then at room temperature for overnight. The reaction was worked up in ice cold water. It was then filtered, washed with ice cold water, dried and recrystallised from ethyl alcohol. Their melting points are reported in table I.

The reactions were monitored by thin layer chromatography. Thin layer chromatography was performed on glass plates coated with silica-gel G (suspended in $CHCl_3$-EtOH) and plates were viewed under Iodine vapours. Melting points were determined by electrochemical capillary Melting point apparatus and are thus uncorrected. The Spectrofuge was used for centrifugation purpose. Elisa plate reader was used for measuring absorbance in the visible range.

### Isolation of alkaline phosphatase activity

Goat liver purchased from local slaughter house was washed with cold isotonic saline solution. It was then disintegrated in a mixer-cum-blender and 10% homogenate was prepared in 0.1 M Glycine-NaOH buffer pH 10.5 containing 0.2 M NaCl. The homogenate was centrifuged at 4$^o$C to obtain a clear solution which was further used as enzyme source. Similarly 10% homogenate was prepared with freshly sprouted moong beans.

### Assay of alkaline phosphatase activity

The enzyme was estimated using p-nitrophenylphosphate as substrate at pH 10.5[24]

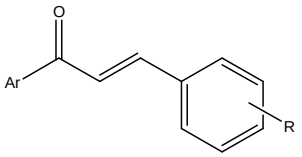
### Assay of alkaline phosphatase activities in presence compounds 1a-1j, 2a-2j, 3a-3j and 4a-4j

Enzyme homogenate (50 µl) was incubated with 0.1 M Glycine-NaOH buffer pH 10.5 containing 0.2 M NaCl containing 1mM concentrations of compounds 1a-1j, 2a-2j, 3a-3j and 4a-4j, separately. After half an hour the residual enzyme activities were measured using p-nitrophenyl phosphate as substrate. Control experiments were also run along with. The results are presented in Table 1 as % residual activity left in solution with respect to control after the interaction of alkaline phosphatase with the individual compound for 30 minutes.

## RESULTS AND DISCUSSION

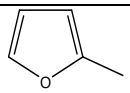
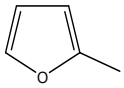
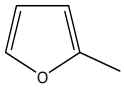
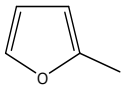
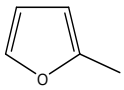
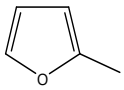
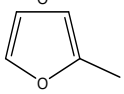
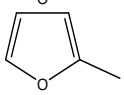
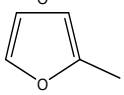
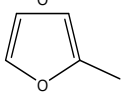
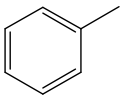
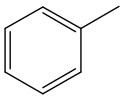
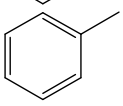
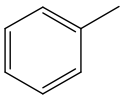
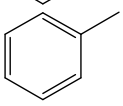
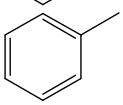
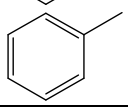
Firstly the chalcones of differently substituted benzaldehydes were synthesized by the established routes. The progress of reaction and purity of the compounds was checked by TLC. The synthesis of chalcones was confirmed with the help of their IR and $^1$H NMR spectra. The synthesized chalcones shows >C=O and the C=C stretching vibrations at their respective positions. Thereafter the effect of these compounds was evaluated on the activity of alkaline phosphatases isolated from goat liver and moong bean. The results are presented in the following Table.

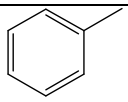
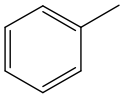
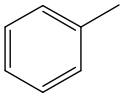
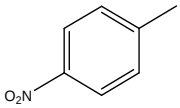
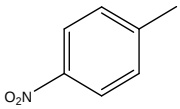
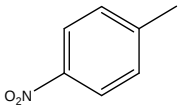
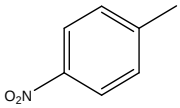
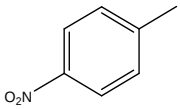
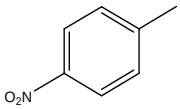
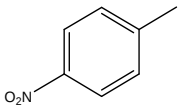
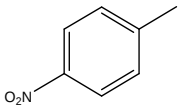
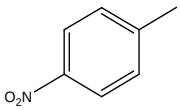
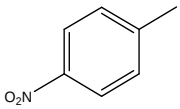
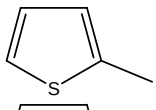
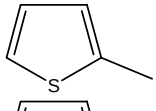
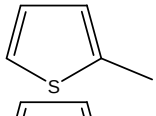
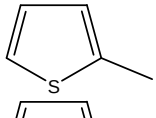
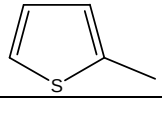
It can be observed from the results that these compounds do not have so much effect on the activity of alkaline phosphatase at 1mM concentration. And if these compounds are used in drugs then there will be no change in the physiological value of the alkaline phosphatase enzyme.

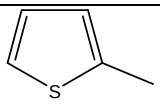
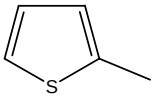
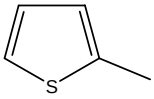
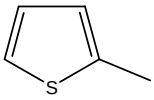
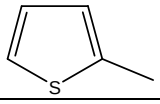



**General structure of chalcone**

**Table: Effect of various chalcones on the activity of alkaline phosphatase**

| S.No | Compound no. | Ar | R | Melting point $^0$C | % Residual activity of alkaline phosphatase in presence of compound at 1mM conc. | |
|---|---|---|---|---|---|---|
| | | | | | Moong bean | Goat liver |
| 1. | 1a |  | H | 90-93 | 101.80±3.02 | 100.23±1.45 |
| 2. | 1b |  | o-Cl | 62-65 | 80.32±3.31 | 92.96±2.63 |
| 3. | 1c |  | m-Cl | 60-62 | 84.89±2.82 | 87.43±4.65 |
| 4. | 1d |  | p-Cl | 120-123 | 80.02±1.84 | 91.45±4.62 |
| 5. | 1e |  | o-$OCH_3$ | 80-82 | 78.73±4.02 | 87.75±3.92 |
| 6. | 1f |  | m-$OCH_3$ | 60-63 | 82.43±1.23 | 87.49±2.03 |
| 7. | 1g |  | p-$OCH_3$ | 70-74 | 75.42±5.84 | 108.34±4.62 |
| 8. | 1h |  | o-$NO_2$ | 110-113 | 74.67±3.72 | 83.49±4.72 |
| 9. | 1i |  | m-$NO_2$ | 175-178 | 87.47±3.12 | 93.72±3.65 |
| 10. | 1j |  | p-$NO_2$ | 225-229 | 83.95±0.56 | 92.79±2.94 |
| 11. | 2a |  | H | 57-59 | 87.29±1.32 | 102.84±2.64 |
| 12. | 2b |  | o-Cl | 50-52 | 85.67±1.08 | 92.27±4.62 |
| 13. | 2c |  | m-Cl | 68-70 | 78.54±4.65 | 90.49±3.25 |
| 14. | 2d |  | p-Cl | 112-114 | 102.27±2.84 | 95.78±2.67 |
| 15. | 2e |  | o-$OCH_3$ | 54-56 | 98.44±5.63 | 94.89±2.95 |
| 16. | 2f |  | m-$OCH_3$ | 56-58 | 100.76±1.35 | 94.67±2.85 |
| 17. | 2g |  | p-$OCH_3$ | 75-77 | 79.49±3.45 | 81.65±3.75 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 18. | 2h |  | o-$NO_2$ | 127-129 | 77.31±7.56 | 95.61±2.54 |
| 19. | 2i |  | m-$NO_2$ | 144-146 | 80.60±4.75 | 84.05±4.56 |
| 20. | 2j |  | p-$NO_2$ | 157-159 | 90.93±2.98 | 99.65±2.46 |
| 21. | 3a |  | H | 100-104 | 83.41±6.43 | 84.47±5.76 |
| 22. | 3b |  | o-Cl | 140-143 | 98.88±3.74 | 94.44±3.65 |
| 23. | 3c |  | m-Cl | 95-98 | 96.53±2.85 | 89.93±5.34 |
| 24. | 3d |  | p-Cl | 142-145 | 96.32±0.18 | 82.02±5.64 |
| 25. | 3e |  | o-$OCH_3$ | 144-148 | 87.74±3.54 | 94.82±4.12 |
| 26. | 3f |  | m-$OCH_3$ | 95-98 | 102.34±2.65 | 91.99±5.73 |
| 27. | 3g |  | p-$OCH_3$ | 160-164 | 90.22±4.53 | 100.43±2.72 |
| 28. | 3h |  | o-$NO_2$ | 150-152 | 92.76±2.43 | 82.48±4.73 |
| 29. | 3i |  | m-$NO_2$ | 205-208 | 90.93±3.45 | 101.32±2.56 |
| 30. | 3j |  | p-$NO_2$ | 120-123 | 102.43±3.14 | 93.52±3.54 |
| 31. | 4a |  | H | 90-92 | 89.94±4.56 | 99.76±4.86 |
| 32. | 4b |  | o-Cl | 130-131 | 101.34±1.23 | 90.02±6.74 |
| 33. | 4c |  | m-Cl | 62-65 | 87.45±1.19 | 94.31±2.64 |
| 34. | 4d |  | p-Cl | 118-120 | 105.80±3.65 | 97.08±5.86 |
| 35. | 4e |  | o-$OCH_3$ | 80-82 | 79.40±5.87 | 89.06±5.72 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 36. | 4f |  | m-$OCH_3$ | 50-52 | 90.03±4.98 | 87.13±4.63 |
| 37. | 4g |  | p-$OCH_3$ | 144-146 | 78.43±5.76 | 84.88±3.72 |
| 38. | 4h |  | o-$NO_2$ | 120-122 | 80.29±3.48 | 79.65±6.43 |
| 39. | 4i |  | m-$NO_2$ | 141-144 | 96.26±2.57 | 100.56±1.34 |
| 40. | 4j |  | p-$NO_2$ | 200-203 | 98.10±3.52 | 98.54±3.65 |

The results are mean ± S.D. of a typical experiment conducted in triplicate. The values are calculated as % residual activities w.r.t. control having an equivalent amount of solvent as in experimental.

In an effort to discover various targets for biologically active chalcones, the present work is focussed on the effect of chalcones on the activity of alkaline phosphatases. It can be observed from the results that these compounds do not alter the activity of alkaline phosphatase at 1mM concentration to a significant extent. Therefore the chalcones can't be used as inhibitors to alkaline phosphatases and such moieties are of limited use in the treatment of diseases where elevated alkaline phosphatase level such as Cholestasis, Cholecystitis, Cholangitis, Cirrhosis, Hepatitis, Fatty liver, Liver tumour, Liver metastasis, Pagets disease, Osteosarcoma, Bone metastasis, Multiple myeloma (only when associated with fractures), Osteomalacia. At the same time it is also suggested that if these compounds are used in the treatment of some diseases, there will be no effect on the physiological role of the enzyme alkaline phosphatase, therefore the side effects due to low activities of alkaline phosphatases are negligible. In addition the present study suggests that the alkaline phosphatases from an animal and a plant source behave similarly towards chalcones. Similar type of results has been reported earlier with semicarbazones, hydrazones and phenylhydrazones[25] of various carbonyl compounds, few of these have also been reported to be biologically active[26]. These derivatives were found to be inhibitory to protease activity[27]. In addition chalcones were also found to be ineffective on acid phosphatase[28].

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