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IN-VITRO STUDIES OF SOME CHALCONES ON ACID PHOSPHATASE

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ABSTRACT: Chemicals/compounds interact with biologically significant molecules such as enzymes, proteins, receptors, nucleic acids etc. due to the presence of various reactive groups. These interactions may result in physiological changes and are also responsible for a compound to be pharmacologically/ therapeutically active. Chalcones are known to possess significant therapeutic activities such as antiinflammatory, analgesic, antimicrobial, antioxidant, anticancer, antimalarial, antiviral, antitubercular, etc. In the present work we have evaluated the effect of chalcones on the activity of acid phosphatase of two different sources.

Key Words: Acid Phosphatase, chalcones, moong bean, goat liver

INTRODUCTION

Chalcones (1, 3-diphenyl-2-propen-1-ones) are one of the major classes of natural products with widespread distribution in spices, tea, beer, fruits and vegetables. These have been a subject of great interest for chemists and biochemists all over due to their ease of synthesis, vast and interesting pharmacological activities. Chalcones also act as synthons for various heterocyclic compounds. Chalcones serve as a precursor unit in flavonoid (Dicarlo, et. al., 1999) biosynthesis in plants. Chemically, they are open-chain flavonoids in which the two aromatic rings are joined by a three-carbon α,β-unsaturated carbonyl system.

Various compounds with the backbone of chalcones have been reported to possess various biological activities such as antimicrobial (Mokle, et.al., 2004), anti-inflammatory (Hsieh, et. al., 2000, Nowakowska, et.al., 2007, Viana, et. al., 2003), analgesic, antiplatelet (Zhao, et.al., 2005), antiulcerative (Mukarami, et.al., 1991), anticancer (Go, et.al. 2005, Katsori et. al., 2009, Miranda et. al., 2000, Shah et.al., 2008), antiviral (Onyilagna, et. al., 1997), antioxidant, antitumour (Echeverria et. al., 2009), antihyperglycemic, immunomodulatory, antiangeogenic (Boumendjel, et.al., 2009), antiparasitic (Nielsen, et.al., 1995), antimalarial (Liu, et.al. 2001), antileishmanial (Dimmock, et. al., 1999), antitubercular. These molecules also cause inhibition of chemical mediators release, inhibition of leukotriene B_4, inhibition of tyrosinase and inhibition of aldose reductase activities. The molecules which interfere with the metabolic system of the host will lead to alteration in metabolic process and will certainly be having some side effects. Acid phosphatases [EC 3.1.3.2] constitute an important class of enzyme which hydrolyse phosphate group from a variety of substrate at acidic pH. These perform diverse physiological functions (Lin, et.al., 1983, Loor, et.al., 1981, Yam, et.al., 1974). For example, lysosomal acid phosphatase and tartarate resistant acid phosphatase are essential for the processing of non collagenous proteins such as osteopontin (Suter, et.al., 2001). It has also been reported that acid phosphatase activity was deficient in patients with a new familial metabolic disorder (characterised by intermittent vomiting, hypotonia, lethargy, opisthotonos, terminal bleeding And death in early infancy) in the lysosomal fraction of homogenates of cultivated fibroblast, brain, liver, spleen, and kidney (Nadler and Egan., 1970). It is also reported that intracellular level and activity of human prostate acid phosphatase are greatly diminished in prostate cancer cells (Veeramani, et.al., 2005). This clearly indicates the physiological importance of this enzyme. Use of molecules as antiinfective 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 acid phosphatase, a physiologically important enzyme isolated from two different sources, moong bean (a plant source) and liver (an animal source).

EXPERIMENTAL

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

General method for the synthesis of chalcones

Substituted chalcones were synthesized by taking KOH(1 mol) in methanol and stirred for 30 min. in ice bath after that substituted acetophenone (1 mol) and substituted benzaldehyde (1 mol) was added and stirred for 3 hrs 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 ethanol. Their melting points are reported in table.

Isolation of acid phosphatase activity

Fresh sprouted moong beans or goat liver was crushed and 10% homogenate was prepared in 0.1M acetate buffer pH 5.3 cotaining 0.2 M NaCl. The homogenate was centrifuged at 4^{o}C to obtain a clear solution which was further used as enzyme source.

Assay of acid phosphatase activity

The enzyme was estimated using p-nitrophenylphosphate as substrate at pH 5.3 (Plummer, 1987)

Assay of acid phosphatase activities in presence compounds 1a-1j, 2a-2j, 3a-3j & 4a-4j.

Enzyme homogenate (50 µl) was incubated with 0.1 M acetate buffer pH 5.3 containing 1mM concentration of compouds 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. The results were compared with the controls run alongwith the experiments. Table represent the % residual activity left in solution after the interaction of acid phosphatase with the individual compound for 30 minutes.

RESULTS AND DISCUSSION

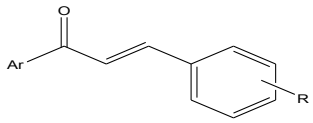
In the first phase of the present work, chalcones of differently substituted benzaldehydes were synthesized by the established routes. The purity of the compounds was checked by TLC. Their preparation was confirmed by comparing the melting points/boiling points from literature. Their IR spectra were also studied and in the synthesized compounds >C=O and the C=C stretching vibrations were observed at their respective positions. Thereafter the effect of these compounds was evaluated on the activity of acid phosphatases isolated from goat liver and moong beans. The results are presented in the Table.1

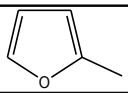
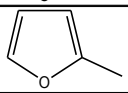
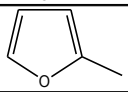
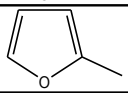
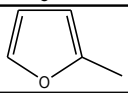
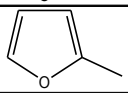
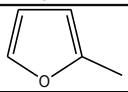
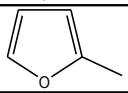
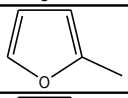
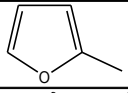
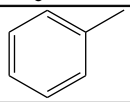
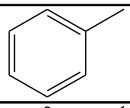
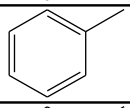
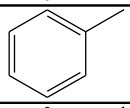
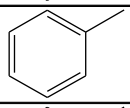
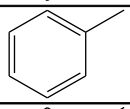
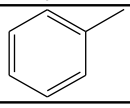
The results presented here are mean % residual activity ± SD of a typical experiment conducted in triplicate. The % residual activity was calculated w.r.t. control where no chalcone was added but an equivalent amount of solvent was present.

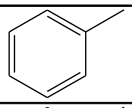
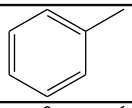
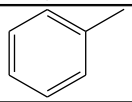
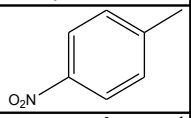
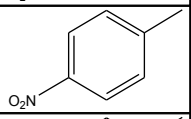
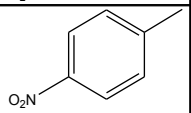
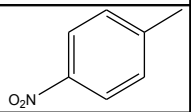
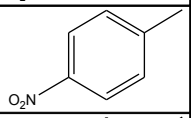
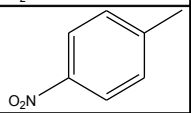
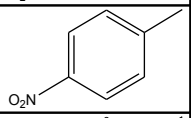
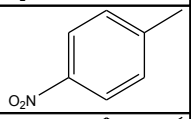
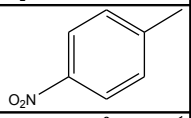
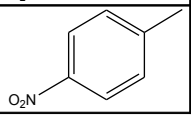
It can be observed from the results that these compounds do not have so much effect on the activity of acid phosphatase at 1mM concentration. In an effort to discover various targets for biologically active chalcones, the present work is focussed on the effect of chalcones on the activity of acid phosphatases. We found that the chalcones can't be used as inhibitors for acid phosphatases and therefore such moieties are of limited use in the treatment of diseases where elevated acid phosphatase level such as Gaucher's disease, prostate cancer, Paget's disease are used as markers. At the same time we also suggest that if these compounds are used in the treatment of some diseases, there will be no effect on the physiological role of the enzyme acid phosphatase and therefore will not be causing any side effects as observed in cases of low acid phosphatases level. In addition the present study suggests that the acid phosphatases from an animal and a plant source behave similarly towards chalcones. Similar type of results have been reported earlier with semicarbazones, thiosemicarbazones, hydrazones and phenylhydrazones (N. Raghav, et. al., 2010).

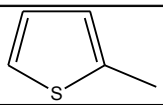
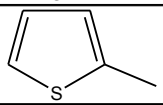
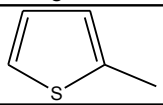
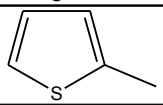
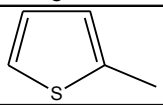
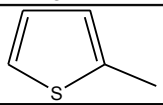
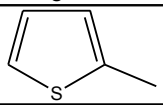
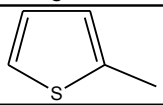
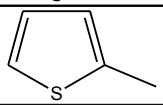
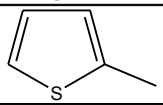
Acknowledgements

Among the authors Suman Jangra and Mamta Singh are thankful to CSIR for providing JRF and Ravinder Kaur acknowledges K.U. Kurukshetra for providing URS.



S.No	Compound no.	Ar	R	Meltingpoint ^{0}C	% Residual activity of acid phosphatase in presence of compound at 1mM conc.	
					Moong bean	Goat liver
1.	1a		H	90-93	76.07±4.07	93.34±3.54
2.	1b		o-Cl	62-65	84.27 ± 5.27	85.63±2.65
3.	1c		m-Cl	60-62	105.01±6.12	90.78±7.23
4.	1d		p-Cl	120-123	89.00±2.02	89.12±1.02
5.	1e		o-OCH_3	80-82	73.56±4.50	90.34±0.44
6.	1f		m-OCH_3	60-63	86.13±1.05	79.58±2.45
7.	1g		p- OCH_3	70-74	78.92±3.23	84.67±1.82
8.	1h		o-NO_2	110-113	92.02±6.55	77.74±3.78
9.	1i		m-NO_2	175-178	99.2±5.58	95.64±2.51
10.	1j		p-NO_2	225-229	81.15±0.35	82.56±2.24
11.	2a		H	57-59	81.52±1.43	76.87±5.34
12.	2b		o-Cl	50-52	99.72±2.86	100.45±1.85
13.	2c		m-Cl	68-70	90.37±6.34	89.34±1.98
14.	2d		p-Cl	112-114	96.10±3.90	80.23±5.78
15.	2e		o-OCH_3	54-56	106.22±11.22	86.98±4.54
16.	2f		m-OCH_3	56-58	96.20±7.02	97.06±2.08
17.	2g		p- OCH_3	75-77	80.07±2.68	79.34±1.86

18.	2h		o-NO_2	127-129	85.42±1.64	75.67±3.84
19.	2i		m-NO_2	144-146	85.60±2.86	95.75±5.74
20.	2j		p-NO_2	157-159	91.92±7.89	86.23±0.85
21.	3a		H	100-104	82.90±2.42	82.04±0.67
22.	3b		o-Cl	140-143	109.45±4.54	87.32±2.78
23.	3c		m-Cl	95-98	92.37±1.47	87.56±4.63
24.	3d		p-Cl	142-145	94.90±0.70	86.45±2.87
25.	3e		o-OCH_3	144-148	102.6±14.70	90.78±2.58
26.	3f		m-OCH_3	95-98	84.65±2.59	82.87±2.84
27.	3g		p- OCH_3	160-164	105.07±11.78	95.83±8.95
28.	3h		o-NO_2	150-152	102.30±1.20	98.38±3.96
29.	3i		m-NO2	205-208	89.12±6.92	95.56±5.36
30.	3j		p-NO2	120-123	86.57±12.72	84.86±2.75

31.	4a		H	90-92	101.82±6.42	95.45±6.24
32.	4b		o-Cl	130-131	96.47±1.97	89.47±3.74
33.	4c		m-Cl	62-65	84.82±13.22	92.78±3.74
34.	4d		p-Cl	118-120	93.12±0.23	93.92±2.64
35.	4e		o-OCH_3	80-82	94.05±3.85	92.73±4.82
36.	4f		m-OCH_3	50-52	94.55±7.25	91.78±5.62
37.	4g		p- OCH_3	144-146	76.40±4.00	75.93±5.35
38.	4h		o-NO_2	120-122	83.13±5.50	86.93±4.82
39.	4i		m-NO_2	141-144	115.15±5.67	100.43±6.83
40.	4j		p-NO_2	200-203	92.62±5.90	94.26±4.85

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