IN-VITRO STUDIES OF SOME CARBONYL DERIVATIVES ON LIVER ACID PHOSPHATASE

N. Raghav*, Mamta, Suman, Anita, Ravinder and Priyanka

*Department of Chemistry, Kurukshetra University Kurukshetra-132119, Haryana (INDIA)

ABSTRACT: For a compound to be pharmacologically/ therapeutically active interaction of the compound with biologically significant molecules exists. It may be with a protein molecule, receptor site, enzyme, nucleic acid etc. In the present work we have evaluated the effect of semicarbazones, thiosemicarbazones and hydrazones of simple aryl aldehydes on the activity of liver acid phosphatase.

Key Words: Acid Phosphatase, liver, semicarbazones, thiosemicarbazone, hydrazones and phenyl hydrazones

INTRODUCTION

Recent studies on semicarbazones (Cerecetto, et.al., 1998, 2000, 2002), thiosemicarbazones (Klayman, et.al., 1979, Wilson, et.al., 1974, Du, et.al., 2002, Chiyanzu, et.al., 2003, Lambros, et.al., 1982, Chipeleme, et.al., 2007, Mallari, et.al., 2008) and hydrazones (Gemma, et. al., 2006) have proposed their use in the treatment of leishmaniasis, trypanosomiasis and malaria due to their potency to inhibit the cysteine proteases of the causal organism i.e. *Leishmania major*, *Trypanosoma cruzi* and *Plasmodium falciparum*. These compounds are also reported to possess antiviral (Mishra, et. al., 2002, Condit, et. al., 1991,), antimicrobial (Pandeya, et. al., 2006, Savini, et. al., 2001, Gurkok, et. al., 2009, Kucukauzel, et. al., 1999) and antifungal (Sengupta, et. al.,2002, Arya and Ahmad 2007, Rai, et. al., 2007) activities. In addition to study the effect of therapeutically effective compounds on the activities of the enzymes of infecting parasite or infecting agents, these should also be screened for their inhibitory/activating effect on physiologically important enzymes of the host. The molecules which interfere with the metabolic systems 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] are omnipresent and are important class of enzymes which hydrolyze phosphate group from a variety of substrates at acidic pH. These perform diverse physiological functions (Loor, et.al., 1981, Yam, et.al., 1974, Lin, et.al., 1983). For example, lysosomal acid phosphatase and tartrate 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 (characterized by intermittent vomiting, hypotonia, lethargy, opisthotonos, terminal bleeding and death in early infancy) in the lysosomal fraction of homogenates of cultivated fibroblasts, brain, liver, spleen and kidney (Nadler and Egan., 1970). It is also reported that intracellular level and activity of human prostrate 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 antiparasitic agent that affects the host enzyme systems can cause enzyme related side effects. In the present study we report the effect of different types of carbonyl derivatives 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 liver.

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 points 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 semicarbazones, thiosemicarbazone, hydrazones and phenyl hydrazone

The semicarbazones, thiosemicarbazone, hydrazones and phenyl hydrazones were synthesized by the established routes (Raghav, et. al., 2010a, 2010b, 2011) . Ethanolic solution of aldehyde was mixed with aqueous/alcoholic solution of corresponding reagent in equimolar ratio, heated/refluxed for 2-24 h, cooled and obtained the products. It was then filtered, washed with cold water, dried and recrystallised from ethanol. Their melting points /boiling points are reported in table I.

Isolation of acid phosphatase activity

Fresh goat liver purchased from local slaughter house was washed with cold isotonic saline solution and was disintegrated in a mixer-cum-blender 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.

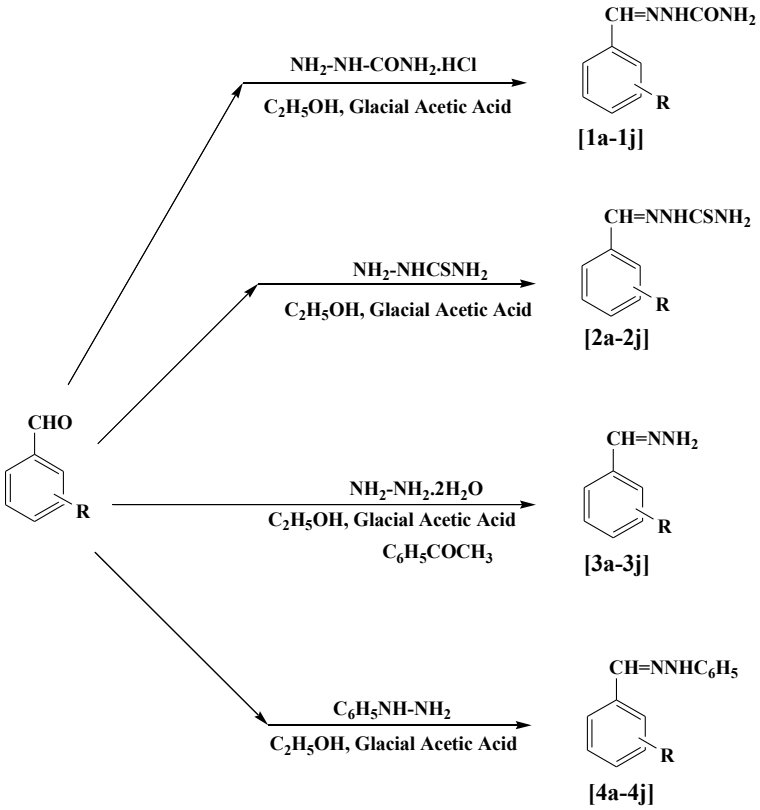
Effect of compounds, 1a-1j, 2a-2j, 3a-3j and 4a-4j on the activity of liver acid phosphatase

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 (Plummer, 1987). The results were compared with the controls run alongwith the experiments. Table I represent the % residual activity left in solution after the interaction of acid phosphatase with the individual compound for 30'.

RESULTS AND DISCUSSION

In the first phase of the present work the semicarbazone, thiosemicarbazone, hydrazone and phenylhydrazone derivatives 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 the synthesized compounds possessed the >C=N stretch at 1603-1630 cm^{-1} and the N-H stretching vibrations were observed at their respective positions. Thereafter the effect of these compounds was evaluated on the activity of liver acid phosphatase. The results are presented in Table I.

It can be observed from the figure. I that there is little or no effect on the activity of acid phosphatase isolated from of goat liver at 1mM final concentration. The results suggest that use of these structural moieties for the treatment of parasitic diseases or antimicrobial activities will not alter the physiological roles of acid phosphatase, resposible for the hydrolysis of phosphate group from a variety of substrates, and are involved in metabolism of osteopontin (Suter, et.al., 2001). Prostatic acid phosphatase is reported as an ectonucleotidase which suppresses pain by generating adenosine (Zylka, et. al, 2008).



a, R = -H
b, R = *o*-Cl
c, R= *m*- Cl
d, R= *p*- Cl
e, R= *o*-OMe
f, R= *m*-OMe
g, R = *p*–OMe
h, R = *o*–NO2
i, R= *m*-NO2
j, R= *p*-NO2

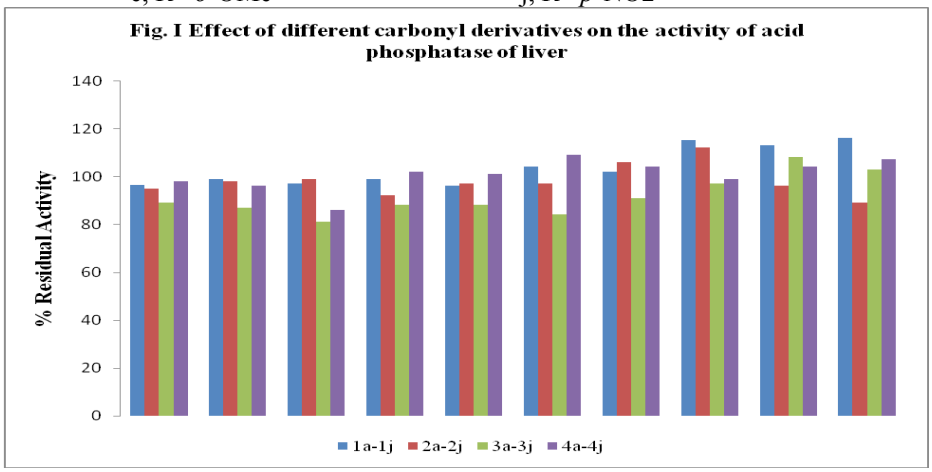


Figure. I-represents the % residual activities of acid phosphatase in presence of 1mM final concentration of semicarbazones, thiosemicarbazones, hydrazones and phenyl hydrazones. The results are mean of two different experiments conducted in duplicate sets.

Table I- Effect of different carbonyl derivatives on the activity of liver acid phosphatase

S. No.	Compound no.	Name of compound	Melting point/Boiling point* ° C	% Residual activity in presence of compound at 1mM concentration
1	1a	Benzaldehyde semicarbazone	222-224 (222)	96.51 ± 4.57
2	1b	o-chlorobenzaldehyde semicarbazone	222-225 (226)	99.05 ± 0.24
3	1c	m- chlorobenzaldehyde semicarbazone	230-232 (228)	97.16 ± 2.47
4	1d	p- chlorobenzaldehyde semicarbazone	234 -236 (230)	99.58 ± 1.87
5	1e	o-methoxybenzaldehyde semicarbazone	206-208 (215)	96.53 ± 4.62
6	1f	m- methoxybenzaldehyde semicarbazone	224-226 (233)	104.32 ± 5.86
7	1g	p- methoxybenzaldehyde semicarbazone	206-208 (210)	102.54 ± 3.23
8	1h	o-nitrobenzaldehyde semicarbazone	250-252 (256)	115.31 ± 10.86
9	1i	m-nitrobenzaldehyde semicarbazone	238-240 (246)	113.54 ± 7.45
10	1j	p- nitrobenzaldehyde semicarbazone	210-212 (221)	116.12 ± 12.65
11	2a	benzaldehyde thiosemicarbazone	160-164 (167-169)	95.23 ± 4.13
12	2b	o-chlorobenzaldehyde thiosemicarbazone	206-210 (207-210)	98.87 ± 2.56
13	2c	m- chlorobenzaldehyde thiosemicarbazone	182-185	99.37 ± 0.76
14	2d	p- chlorobenzaldehyde thiosemicarbazone	210-212(209)	92.63 ± 5.76
15	2e	o-methoxybenzaldehyde thiosemicarbazone	180-182	97.13 ± 3.87
16	2f	m- methoxybenzaldehyde thiosemicarbazone	208° -210°	97.83 ± 2.30
17	2g	p- methoxybenzaldehyde thiosemicarbazone	217° -220°	106.48 ± 3.49
18	2h	o-nitrobenzaldehyde thiosemicarbazone	210-212(214-215)	112.61 ± 7.52
19	2i	m- nitrobenzaldehyde thiosemicarbazone	240	96.82 ± 6.74
20	2j	p- nitrobenzaldehyde thiosemicarbazone	240-242	89.37 ± 9.51
21	3a	Benzaldehyde hydrazone	88	89.67 ± 10.43
22	3b	o-chlorobenzaldehyde hydrazone	140*	87.84 ± 6.83
23	3c	m- chlorobenzaldehyde hydrazone	80*	81.37 ± 10.83
24	3d	p-chlorobenzaldehyde hydrazone	40-42 (46)	88.68 ± 7.96
25	3e	o-methoxybenzaldehyde hydrazone	98*	88.73 ± 4.51
26	3f	m-methoxybenzaldehyde hydrazone	103*	84.33 ± 9.36
27	3g	p-methoxybenzaldehyde hydrazone	168	91.86 ± 7.49
28	3h	o- nitrobenzaldehyde hydrazone	150	97.25 ± 0.73
29	3i	m-nitrobenzaldehyde hydrazone	190	108.37 ± 6.49
30	3j	p-nitrobenzaldehyde hydrazone	133-135 (135 -137)	103.84 ± 1.84
31	4a	Benzaldehyde phenylhydrazone	158-160	98.49 ± 2.01
32	4b	o-chlorobenzaldehyde phenylhydrazone	56-58	96.84 ± 2.74
33	4c	m- chlorobenzaldehyde phenylhydrazone	126-128	86.61 ± 6.72
34	4d	p-chlorobenzaldehyde phenylhydrazone	122-124 (127)	102.02 ± 2.05
35	4e	o-methoxybenzaldehyde phenylhydrazone	110*	101.47 ± 1.42
36	4f	m-methoxybenzaldehyde phenylhydrazone	55-60	109.95 ± 3.86
37	4g	p-methoxybenzaldehyde phenylhydrazone	120 (122)	104.47 ± 2.38
38	4h	o- nitrobenzaldehyde phenylhydrazone	154 (156)	99.64 ± 0.62
39	4i	m-nitrobenzaldehyde phenylhydrazone	122 (121)	104.83 ± 1.46
40	4j	p-nitrobenzaldehyde phenylhydrazone	156 (159)	107.15 ± 2.73

The results presented are mean of two different experiments conducted in duplicate sets. The values are % residual activity ± S.D. with respect to control containing an equivalent amount of solvent

Acknowledgements:
Among the authors, M. Singh and Suman are thankful to CSIR for providing JRF, R. Kaur acknowledges K. U. Kurukshetra for providing URS and Priyanka acknowledges DST for providing JRF.

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