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Journal of Chemical and Pharmaceutical Research

J. Chem. Pharm. Res., 2010, 2(4):801-807

ISSN No: 0975-7384
CODEN(USA): JCPRC5

In-vitro studies of various carbonyl derivatives on liver alkaline phosphatase

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ABSTRACT

The present work describes the effect of semicarbazones, thiosemicarbazones, hydrazones and phenyl hydrazones of simple aryl aldehydes on the activity of liver alkaline phosphatase. The structural moieties under investigation have gained considerable attention in the field of parasitic and infective diseases. These compounds are reported as potent inhibitors of cruzain, falcipain and rhodesain of infecting parasites. We propose that the molecules which possess potential therapeutic activities should also be screened for their effect on the activities of physiologically important enzymes of the hosts. In the present work we have evaluated the effect of semicarbazones, thiosemicarbazones and hydrazones of simple aryl aldehydes on the activity of liver alkaline phosphatase.

Key Words: Alkaline phosphatase, liver, thiosemicarbazones, semicarbazones, hydrazones and phenyl hydrazones

INTRODUCTION

In the recent years, thiosemicarbazones [1, 2], semicarbazones [3] and hydrazones [4] have gained significant attention because of their proposed use in the treatment of leishmaniasis, trypanosomiasis and malaria. These compounds are found to be potential inhibitors to the cysteine proteases [1, 5] of the causal organisms i.e. *Leishmania major*, *Trypanosoma cruzi* and *Plasmodium falciparum*. In addition, these compounds are also reported to possess antiviral [6], antimicrobial [7] and antifungal [8] activities. Alkaline phosphatases [EC 3.1.3.1] are a group of enzymes primarily found in liver and bone, also present in intestine, placenta and kidney, perform diverse functions [9] 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 [10]. We propose that the compounds or structural moieties which are potential drugs should also be screened for their effects on the activities of clinically significant enzymes. In the present work we have reported the effect of semicarbazones, thiosemicarbazones, hydrazones and phenyl hydrazones of simple aryl aldehydes on the activity of liver alkaline phosphatase. These types of compounds are proposed to treat chaga's disease, malaria, leishmaniasis [1-5] etc.

EXPERIMENTAL SECTION

The reactions were monitored by thin layer chromatography. Thin layer chromatography was performed with silica-gel G (suspended in $CHCI_3$-EtOH) and plates were viewed under Iodine vapors. Melting points were determined by electrochemical capillary Melting points apparatus and are uncorrected. The Spectrofuge was used for centrifugation purpose. Elisa plate reader was used for measuring absorbance in the visible range.

Synthesis of semicarbazones, thiosemicarbazones, hydrazones and phenyl hydrazones

The mentioned carbonyl derivatives were synthesized by the established routes [11]. Alcoholic solution of aldehydes was mixed with aqueous/alcoholic solution of corresponding reagent in equimolar ratio. The reaction mixture was heated/refluxed for 2-24 h. The product was isolated after cooling, which was then filtered, washed with cold water, dried and recrystallised from ethanol. Their melting points /boiling points are reported in table 1.

Isolation of alkaline phosphatase activity

Fresh 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^oC 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 alkaline phosphatase

Enzyme homogenate (50 µl) was incubated with 0.1 M Glycine-NaOH buffer pH 10.5 containing 1mM concentration 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 [12]. The results were compared with the controls run along with the experiments. Table 1 represents the % residual activity left in solution after the interaction of alkaline phosphatase with the individual compound for 30'.

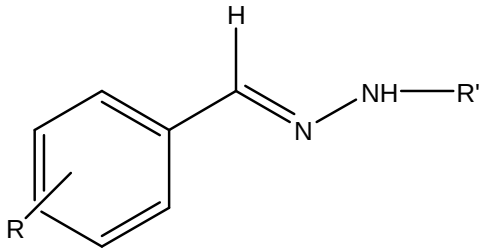


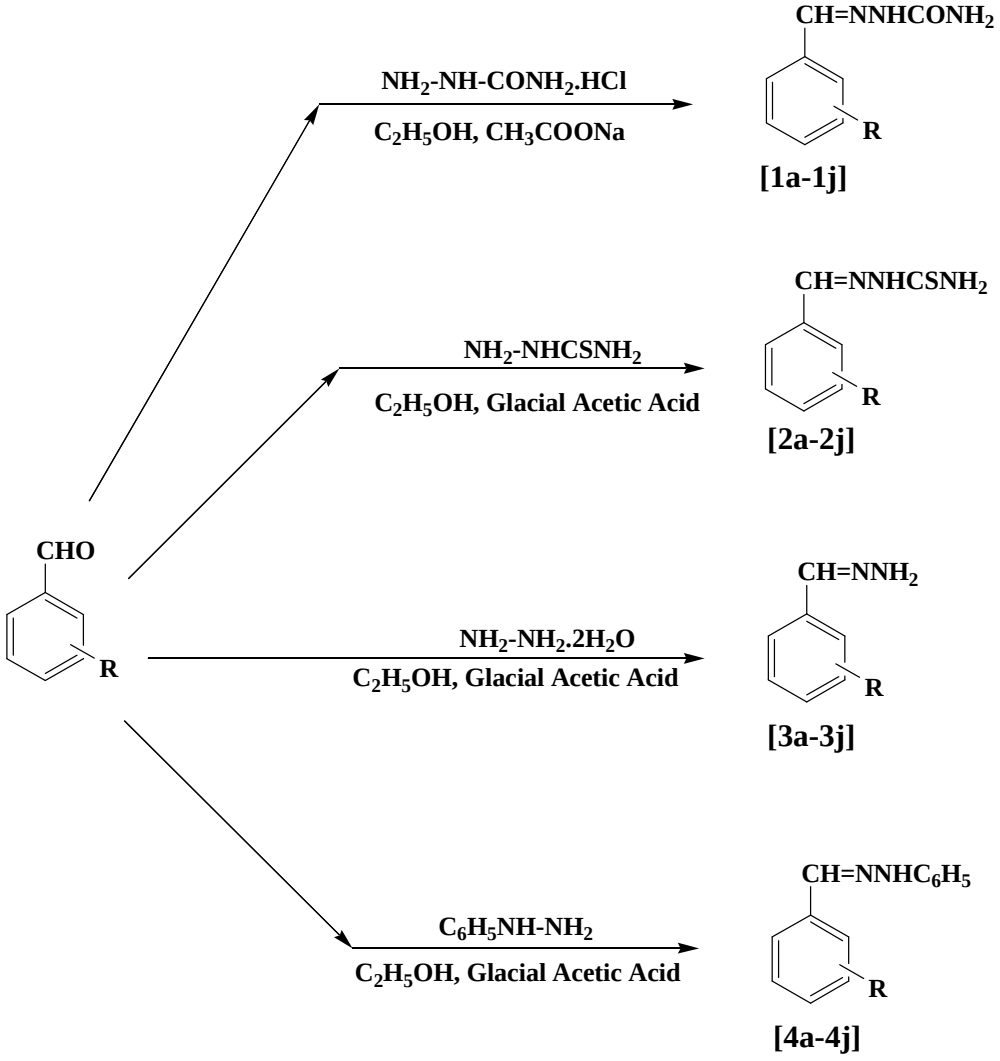
Table 1- Effect of different carbonyl derivatives on the activity of liver alkaline phosphatase

Compound no.	R-	R'-	Name of compound	Melting point/Boiling point* ° C	% Residual activity in presence of compound at 1mM concentration
1a	H	$-CONH_2$	Benzaldehyde semicarbazone	222-224 (222)	83.45 ± 3.59
1b	2-Cl	$-CONH_2$	o-chlorobenzaldehyde semicarbazone	222-225 (226)	94.03 ± 4.63
1c	3-Cl	$-CONH_2$	m- chlorobenzaldehyde semicarbazone	230-232 (228)	88.73 ± 2.86
1d	4-Cl	$-CONH_2$	p- chlorobenzaldehyde semicarbazone	234 -236 (230)	98.56 ± 6.34
1e	2-OCH_3	$-CONH_2$	o-methoxybenzaldehyde semicarbazone	206-208 (215)	94.95 ± 0.64
1f	3-OCH_3	$-CONH_2$	m- methoxybenzaldehyde semicarbazone	224-226 (233)	91.34 ± 3.75
1g	4-OCH_3	$-CONH_2$	p- methoxybenzaldehyde semicarbazone	206-208 (210)	91.62 ± 2.63
1h	2-NO_2	$-CONH_2$	o-nitrobenzaldehyde semicarbazone	250-252 (256)	103.34 ± 4.28
1i	3-NO_2	$-CONH_2$	m-nitrobenzaldehyde semicarbazone	238-240 (246)	91.14 ± 2.59
1j	4-NO_2	$-CONH_2$	p- nitrobenzaldehyde semicarbazone	210-212 (221)	83.66 ± 4.82
2a	H	$-CSNH_2$	benzaldehyde thiosemicarbazone	160-164 (167-169)	93.04 ± 5.28
2b	2-Cl	$-CSNH_2$	o-chlorobenzaldehyde thiosemicarbazone	206-210(207-210)	92.85 ± 4.71
2c	3-Cl	$-CSNH_2$	m- chlorobenzaldehyde thiosemicarbazone	182-185	90.65 ± 1.53
2d	4-Cl	$-CSNH_2$	p- chlorobenzaldehyde thiosemicarbazone	210-212(209)	83.36 ± 2.47
2e	2-OCH_3	$-CSNH_2$	o-methoxybenzaldehyde thiosemicarbazone	180-182	95.11 ± 3.61
2f	3-OCH_3	$-CSNH_2$	m- methoxybenzaldehyde thiosemicarbazone	208° -210°	91.07 ± 4.78
2g	4-OCH_3	$-CSNH_2$	p- methoxybenzaldehyde thiosemicarbazone	217° -220°	82.57 ± 3.64
2h	2-NO_2	$-CSNH_2$	o-nitrobenzaldehyde thiosemicarbazone	210-212(214-215)	84.45 ± 7.39
2i	3-NO_2	$-CSNH_2$	m- nitrobenzaldehyde thiosemicarbazone	240	89.22 ± 3.61
2j	4-NO_2	$-CSNH_2$	p- nitrobenzaldehyde thiosemicarbazone	240-242	84.39 ± 4.63
3a	H	-H	Benzaldehyde hydrazone	88	81.40 ± 0.52
3b	2-Cl	-H	o-chlorobenzaldehyde hydrazone	140*	89.24 ± 4.60
3c	3-Cl	-H	m- chlorobenzaldehyde hydrazone	80*	85.38 ± 1.38
3d	4-Cl	-H	p-chlorobenzaldehyde hydrazone	40-42 (46)	88.43 ± 3.41
3e	2-OCH_3	-H	o-methoxybenzaldehyde hydrazone	98*	83.63 ± 4.86
3f	3-OCH_3	-H	m-methoxybenzaldehyde hydrazone	103*	80.15 ± 4.27
3g	4-OCH_3	-H	p-methoxybenzaldehyde hydrazone	168	90.52 ± 4.28
3h	2-NO_2	-H	o- nitrobenzaldehyde hydrazone	150	89.45 ± 6.20
3i	3-NO_2	-H	m-nitrobenzaldehyde hydrazone	190	82.27 ± 3.72
3j	4-NO_2	-H	p-nitrobenzaldehyde hydrazone	133-135 (135 -137)	90.18 ± 1.96
4a	H	$-C_6H_5$	Benzaldehyde phenylhydrazone	158-160	83.76 ± 4.62
4b	2-Cl	$-C_6H_5$	o-chlorobenzaldehyde phenylhydrazone	56-58	90.64 ± 6.72
4c	3-Cl	$-C_6H_5$	m- chlorobenzaldehyde phenylhydrazone	126-128	84.93 ± 1.26
4d	4-Cl	$-C_6H_5$	p-chlorobenzaldehyde phenylhydrazone	122-124 (127)	91.67 ± 3.59
4e	2-OCH_3	$-C_6H_5$	o-methoxybenzaldehyde phenylhydrazone	110*	92.27 ± 3.23
4f	3-OCH_3	$-C_6H_5$	m-methoxybenzaldehyde phenylhydrazone	55-60	94.34 ± 4.31
4g	4-OCH_3	$-C_6H_5$	p-methoxybenzaldehyde phenylhydrazone	120 (122)	103.61 ± 6.71
4h	2-NO_2	$-C_6H_5$	o- nitrobenzaldehyde phenylhydrazone	154 (156)	96.83 ± 5.21
4i	3-NO_2	$-C_6H_5$	m-nitrobenzaldehyde phenylhydrazone	122 (121)	97.57 ± 4.76
4j	4-NO_2	$-C_6H_5$	p-nitrobenzaldehyde phenylhydrazone	156 (159)	83.03 ± 2.37

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

RESULTS AND DISCUSSION

In the present work first of all semicarbazones, thiosemicarbazones, hydrazones and phenylhydrazones of differently substituted benzaldehydes were synthesized by the established routes [11]. The progress of reaction and purity of the compounds was checked by TLC. Their preparation was confirmed by comparing the melting points/boiling points from literature. Their



a, R = -H	**f, R=3-OMe**
b, R = 2-Cl	**g, R = 4-OMe**
c, R= 3-Cl	**h, R = 2-NO2**
d, R= 4-Cl	**i, R= 3-NO2**
e, R= 2-OMe	**j, R= 4-NO2**

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. The NMR studies also confirmed the synthesis of respective derivatives, where the usual Ar-H resonance was observed at δ 6.5-8.2 as m or dd, depending upon the position and reactivity of substituent; -CH=N- resonated at δ 8.2-8.8 as singlet; -NH and $–NH_2$ protons in different

derivatives were revealed at respective positions as reported in literature [13]. Thereafter the effect of these compounds was evaluated on the activity of alkaline phosphatase isolated from liver. The results are presented in Table 1 and Fig. 1.

It can be observed from the fig. 1 that there is little effect on the activity of alkaline phosphatase isolated from of goat liver. There is no inhibition in few compounds at 1mM final concentration and in some maximum inhibition achieved is up to 20%.

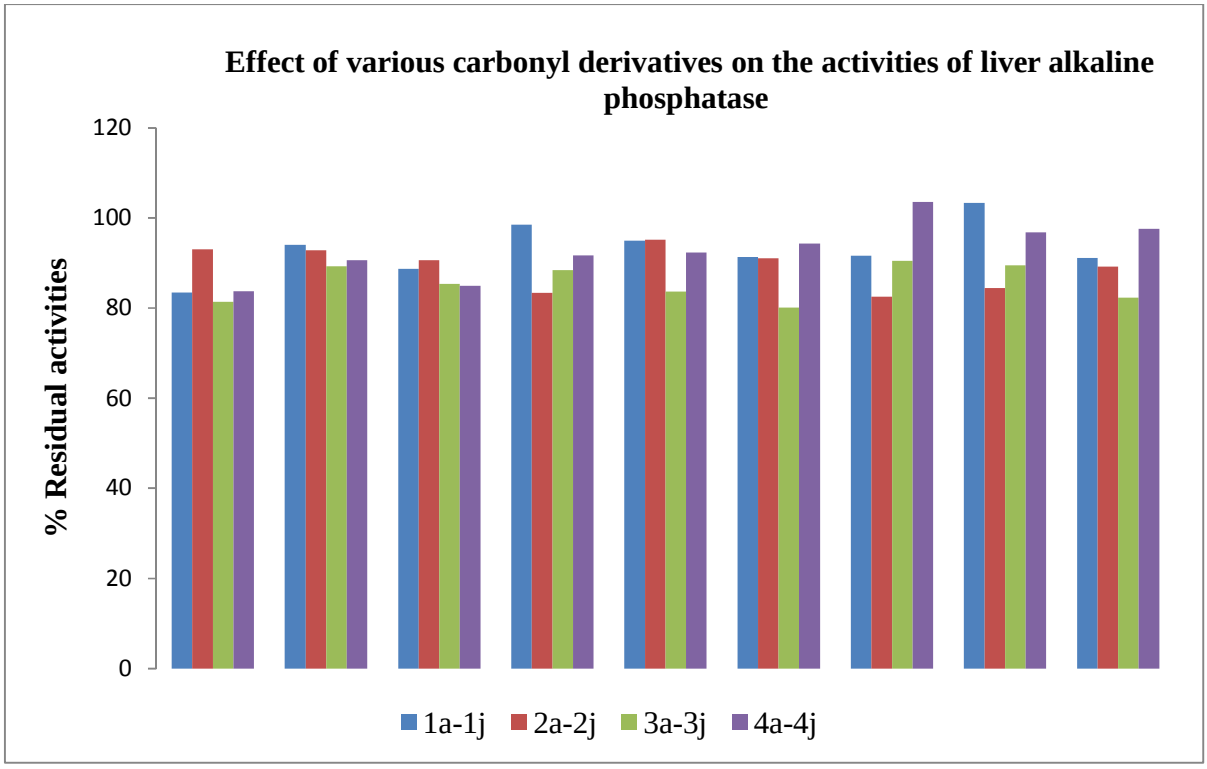


Fig. 1-represents the % residual activities of acid phosphatase in presence of 1mM final concentration of semicarbazones, thiosemicarbazones, hydrazones and phenyl hydrazones.
The results are mean of two different experiments conducted in duplicate sets.

The present study suggests that the compounds 1a-1j, 2a-2j, 3a-3j and 4a-4j, do not effectively inhibit liver alkaline phosphatase at 1mM concentration, whereas the inhibition of cysteine proteases of infecting organisms is achieved at very low concentration of compounds having similar structural moieties. The IC_{50} levels for cruzain, falcipain and rhodesain for these classes of compounds lies in the range of µM and nM range [1, 5]. Therefore the compounds under investigation will not alter the physiological roles of this phosphomonoesterase of liver. Moreover, use of these structural moieties for the treatment of parasitic diseases or antimicrobial activities will also not affect the diagnostic value of alkaline phosphatase, responsible for the hydrolysis of phosphate group from a variety of substrates at alkaline pHs [14]. In addition the results also suggest that these derivatives cannot be used as specific or nonspecific inhibitors for alkaline phosphatase at a concentration where these act as inhibitors to parasitic enzyme, although this enzyme is reported to be inhibited by a diverse group of inhibitors [15].

Acknowledgements
Among the authors, M. Singh and S. Jangra are thankful to CSIR; P. Malik acknowledges DST for providing JRF and R. Kaur acknowledges K. U. Kurukshetra for providing URS.

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