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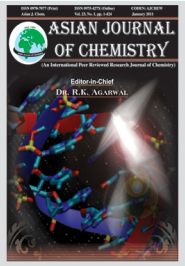

NOTE

Proteolytic Studies in Liver Homogenate in Presence of Substituted Aryl Hydrazones

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Hydrazones of different arylaldehydes were synthesized and their effect on endogenous proteolysis in liver was studied. It was observed that different functional groups on the benzene moiety altered the enzymatic activity and *para*-nitrobenzaldehyde hydrazone exhibited maximum inhibitory effect.

Key Words: Hydrazones, Endogenous proteolysis, Liver homogenate.

Proteases, also known as proteinases or proteolytic enzymes, belong to the class of enzyme, hydrolase which catalyzes the hydrolysis of peptide bonds in proteins. Proteases are currently classified into different categories according to the nature of active site group[1]. Proteolytic enzymes are very important in digestion of proteins. These have also been used as therapeutic agents. Their use is gaining more and more attention because of their involvement in oncology, inflammatory conditions, blood rheology control and immune regulation *etc*. A detailed study of the inhibitory mechanisms may deliver prospects for the treatment of diverse disorders that result from defective control of proteolytic processes[2]. At present, an estimated 5-10 % of all pharmaceutical targets are proteases. Several small molecules as drugs, targeting proteases are already in the market and many more are in development[3]. In the present study we report the effect of various hydrazones on endogenous protein hydrolysis in liver.

Hydrazones have been demonstrated to possess antimicrobial[4], analgesic[5], anticonvulsant[6], antitumoral[7], antiinflammatory[8], antimalarial[9], antitubercular[10] and antiproliferative[11] activities. For example, 2,4-dinitrophenylhydrazone derivatives exhibited cytotoxicity in the range of 50-70 % against MCF-7 and ZR-75-1 human malignant breast cell lines[7]. Noval anticonvulsant hydrazones, semicarbazones and thiosemicarbazones derived from pyridyl ketones are useful as excitatory amino acid inhibitors and potent orally active antiepileptic pharmaceutical compositions with no neurotoxicity[12].

Thin layer chromatography was performed with silicagel G (suspended in $CHCl_3$-MeOH) and plates were viewed under iodine vapours. Melting points were determined by electro thermal capillary melting point apparatus and are uncorrected. Elisa plate reader was used for measuring absorbance in the visible range. Goat liver was purchased freshly from the local slaughter house. Spectrofuge-16M was used for centrifugation purposes.

General method for the synthesis of hydrazones: Hydrazones were prepared readily by the reactions of substituted benzaldehydes with hydrazine hydrate[13,14]. Arylaldehyde was refluxed with hydrazine hydrate in presence of glacial acetic acid for 3-4 h in ethanol. The reaction was monitored with the help of TLC. The reaction mixture was then poured in ice and the resulting solid was collected on filter and washed with water. It was then recrystallized from alcohol.

Preparation of liver homogenate: The fresh goat liver was first washed with cold isotonic saline solution. The tissue was then homogenized in 0.1 M sodium acetate buffer pH 5.3 containing 0.2 M NaCl, 1 mM EDTA and 0.1 % Triton X-100 in a mixer-cum-blender to obtain 10 % (w/v) homogenate. It was then stored at 4 ºC.

Assay for proteolytic activity: The proteolysis was carried out at pHs 3.5 and 5.0 at 37 ºC using 0.08 M citrate buffer. The homogenate was mixed with the buffer at these pHs separately and was incubated at 37 ºC for 3 h. The reaction was stopped by the addition of trichloroacetic acid and the resulting solution was centrifuged to precipitate proteins. Trichloroacetic acid soluble proteins were estimated in supernatant using Bradford method[15]. The experiment was conducted in triplicate and the results are presented in the Table-1.

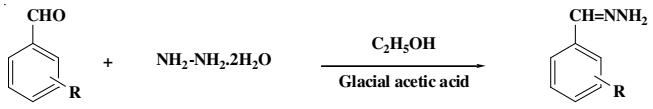
The hydrazones were synthesized by reacting hydrazine hydrate with substituted benzaldehydes in presence of ethanol and glacial acetic acid by usual method[13]. The synthesized

TABLE-1
EFFECT ON HYDRAZONES ON ENDOGENOUS PROTEIN HYDROLYSIS IN LIVER HOMOGENATE

Hydrazones	b.p. (ºC)	m.p. ºC (Lit.)	Effective conc. (mM)	Absorbance at 630 nm	
				pH 3.5	pH 5.0
–	–	–	–	0.943 ± 0.034	1.083 ± 0.051
Benzaldehyde	–	88	0.50	0.798 ± 0.014	0.075 ± 0.018
			0.05	–	0.973 ± 0.141
o-Chlorobenzaldehyde	140	–	0.50	0.929 ± 0.027	0.266 ± 0.049
m-Chlorobenzaldehyde	80	–	0.50	0.761 ± 0.020	0.789 ± 0.028
p-Chlorobenzaldehyde	–	40-42	0.50	0.541 ± 0.010	0.051 ± 0.026
		(46)	0.05	–	0.413 ± 0.081
o-Methoxybenzaldehyde	98	–	0.50	0.677 ± 0.044	1.078 ± 0.021
m-Methoxybenzaldehyde	103	–	0.50	0.863 ± 0.019	0.719 ± 0.046
p-Methoxybenzaldehyde	–	168	0.50	0.247 ± 0.038	0.117 ± 0.057
			0.05	–	0.863 ± 0.083
o-Nitrobenzaldehyde	–	150	0.50	0.565 ± 0.007	0.387 ± 0.018
m-Nitrobenzaldehyde	–	190	0.50	0.616 ± 0.026	1.036 ± 0.019
p-Nitrobenzaldehyde	–	133-135	0.50	0.737 ± 0.021	0.023 ± 0.011
		(135 -137)	0.05	–	0.220 ± 0.041

Proteolytic activity of the liver homogenate at pH 3.5 and pH 5.0; after proteolysis for 3 h at 37ºC in presence of 0.5 and 0.05 mM conc. of substituted benzaldehyde hydrazones. The TCA soluble peptides were estimated by Bradford method at 630 nm and the results are the mean and ± SD of one of the experiment conducted in triplicate and are calculated as protease activity/h/mL in 1% liver homogenate.

products were confirmed by comparing the melting points with literature[14].



R=o-Cl, m-Cl, p-Cl , o-OCH_3, m-OCH_3, p-OCH_3, o-NO_2, m-NO_2, p-NO_2, -H

Inhibitory activity of protease on endogenous protein substrate at pH 3.5 and 5.0 were measured. The liver homogenate was mixed with buffers at pH 3.5 and 5.0, separately in presence of substituted benzaldehyde at 0.5 mM concentration. After 3 h of incubation at 37 ºC, the reaction was stopped by adding trichloroacetic acid solution and the acid soluble proteins were estimated by Bradford method[15].

It was observed that endogenous protein hydrolysis was maximally inhibited in presence of *p*-substituted benzaldehyde hydrazones in comparison to *o*- and *m*-substituted hydrazones. The results are similar to those observed earlier[16] in our laboratory while working with similarly substituted aryl semicarbazones involved in proteolysis. The effect has been found to be more pronounced on *p*-substituted hydrazones whereas, *o*- and *m*- substituted hydrazones affected to a lesser extent. Since, *in vivo* activity depends highly on complex physiological interactions; therefore at this moment it is not possible to rationalize all the enzymatic activities. Another important observation was that inhibition levels in each case were different at pH 3.5 and 5.0, suggesting that these hydrazone derivatives effect differently on enzymes active at these pHs. Inhibitory effect is more pronounced at pH 5.0 than 3.5 suggesting that the effect is more on proteases which are active at pH 5.0 rather than those active at pH 3.5. The results are similar to those reported earlier with substituted aryl semicarbazones[17-19].

Further the effect of *p*-substituted hydrazones at 0.05 mM concentration indicate that the effect is more in nitro substituted hydrazone followed by chloro and methoxy substituted hydrazone in that order. Such studies on inhibition of endogenous proteolytic hydrolysis may contribute to explore newer possibilities for the identification of functional groups as potent inhibitors and detailed understanding of the inhibitory mechanisms may provide prospects for treatment of the diverse disorders which results from defective control of proteolytic processes[2].

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