ISSN: 0975-766X

Available Online through
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Research Article

PROTEOLYTIC STUDIES IN LIVER HOMOGENATE IN PRESENCE OF PHENYLHYDRAZONES

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Received on 24-07-2010 ***Accepted on 08-08-2010***

Abstract:

Phenylhydrazones of benzaldehyde and differently positioned chlorobenzaldehydes, methoxybenzaldehydes and nitrobenzaldehydes were synthesized and their effect on endogenous protein hydrolysis in liver was studied. It was observed that introduction of different functional groups on the benzene moiety altered the enzymatic activity appreciably and *p*-nitrobenzaldehyde phenylhydrazone exhibited the maximum inhibitory effect.

Key words: Phenylhydrazones, Endogenous proteolysis, Proteolytic studies, Liver homogenate.

Introduction:

Proteases are one of the largest and best-characterized families of enzymes in the human proteome[1]. Proteases are naturally expressed in all organisms and constitute 1-2% of the human genome. Cysteine proteases have been identified as promising targets for the development of antiparasitic chemotherapy. An attractive aspect of these enzymes is their widespread importance in both protozoan and helminth parasites of domestic animals and humans. Concerns about the ability to selectively inhibit parasite proteases without affecting host homologues have been addressed in recent studies of *Trypanosoma cruzi* and *Plasmodium falciparum*[2]. Increased understanding of the structure and mechanism of this class of enzymes has brought on a new fervor in the design of small molecule inhibitors with the hope of producing specific, therapeutic drugs for diseases such as arthritis, allergy, multiple sclerosis, atherosclerosis, Alzheimer's disease and cancer[3].

At present, an estimated 5-10 % of all pharmaceutical targets are proteases[4]. Protease signaling pathways are strictly regulated, and the dysregulation of protease activity can lead to pathologies such as cardiovascular and inflammatory diseases, cancer, osteoporosis and neurological disorders[5]. The precise control of proteolytic processes is essential for appropriate functioning of cells and whole organism. This is achieved at many levels from regulation of protease expression, secretion and maturation through specific degradation of mature enzymes, by inhibition of proteolytic activity [6].

Few dipeptidylbenzoylhydrazones have been found to be selective inhibitors of the cysteine proteases[7]. A series of 4-quinolylhydrazone were synthesized by reaction of 4-quinolylhydrazine and aryl or heteroaryl carboxaldehydes and tested against *M. tuberculosis*. Most of the derivatives had antitubercular properties, two compounds were identified with the highest activity and they were also tested against *M. aviam*[8]. A series of Indole–3–aldehyde and 5-Bromoindole–3-aldehyde hydrazide and hydrazone was evaluated for their *in vitro* antimicrobial activities using 2-fold serial dilution technique against *S. aureus, E. Coli, B. subtilis and C. albicans*[9].

Experimental:

Thin layer chromatography was performed with silica-gel G (suspended in $CHCl_3$-NaOH) and plates were viewed under Iodine vapors. Melting points were determined by Electrothermal 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 was used for centrifugation purposes.

Preparation of liver homogenate:

The fresh goat liver was first washed with cold isotonic saline solution. The tissue was then homogenized in 0.1M sodium acetate buffer pH 5.3 containing 0.2M NaCl, 1mM 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.

General method for the synthesis of hydrazones:

Phenylhydrazones were prepared readily by the reactions of substituted benzaldehydes with phenylhydrazine. For the synthesis of phenylhydrazones, substituted benzaldehydes in ethanol was added to phenylhydrazine. A few drops of glacial acetic acid were added. The reaction mixture was heated at 80^{o}C for 24 hours. After heating, the reaction mixture was cooled in an ice bath for 2 hour. Resulting solid was collected on filter paper and washed with alcohol. It was recrystallised twice from alcohol.

Assay for proteolytic activity:

The proteolysis was carried out at pHs 3.5 and 5.0 at 37^{o}C using 0.1 M citrate buffer as the incubation medium. The homogenate was mixed with the buffer at these pHs separately and was incubated at 37^{o}C for 3h. The reaction was stopped by the addition of TCA and the resulting solution was centrifuged to precipitate proteins. The acid soluble proteins were quantitated in the supernatant using Bradford method[10]. The experiment was conducted in triplicate and the results are presented in table.

TABLE: E**ffect of phenylhydrazones on endogenous protein hydrolysis in liver homogenate.**

Phenylhydrazones	**M.P./ B.P.* ^{0}C (Lit.)**	**Effective Conc.**	**Absorbance at 630 nm**	
			pH 3.5	**pH 5.0**
-	-	0.5mM	0.905±0.050	0.891±0.038
Benzaldehyde	158-160	0.5mM	0.714±0.054	0.075±0.037
		0.05mM	-	0.679±0.062
o-chlorobenzaldehyde	56-58	0.5Mm	0.608±0.014	0.952±0.035
m- chlorobenzaldehyde	126-128	0.5mM	0.191±0.048	0.131±0.032
p-chlorobenzaldehyde	122-124 (127)	0.5mM	0.061±0.014	0.111±0.054
		0.05mM	-	0.566±0.060

o-methoxybenzaldehyde	110*	0.5mM	0.779±0.017	0.667±0.032
m-methoxybenzaldehyde	55-60	0.5mM	0.537±0.009	0.383±0.022
p-methoxybenzaldehyde	120 (122)	0.5mM	0.485±0.033	0.117±0.057
		0.05mM	-	0.905±0.130
o- nitrobenzaldehyde	154 (156)	0.5mM	0.896±0.019	0.770±0.021
m-nitrobenzaldehyde	122 (121)	0.5mM	0.877±0.021	0.294±0.037
p-nitrobenzaldehyde	156 (159)	0.5mM	0.336±0.084	0.009±0.005
		0.05mM	-	0.045±0.010
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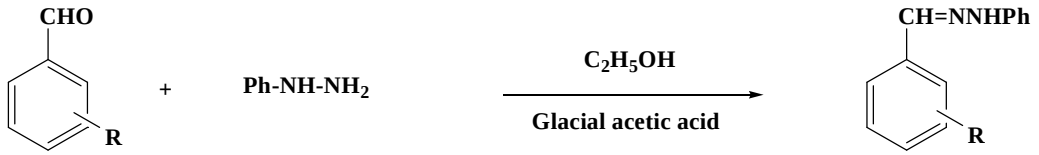
Proteolytic activity of the liver homogenate at pH 3.5 and pH 5.0; after proteolysis for 3h at 37^{o}C in presence of 0.5 and 0.05mM concentration of substituted benzaldehyde phenylhydrazones. First value denotes the protease activity/h/ml in respective controls which contained equal amount of solvent instead of the compound. The TCA soluble peptides were estimated at 630 nm[10] and the results are the mean and S.D. of the experiment conducted in triplicate and is calculated as protease activity/h/ml in 0.1% liver homogenate.

Result and discussion:

The phenylhydrazones were synthesized by reacting phenylhydrazine with substituted benzaldehydes in presence of ethanol and glacial acetic acid by usual method. The synthesized products were confirmed by comparing the melting points with literature[11]. The products were also confirmed by IR spectra where a C=N stretch was observed in the range 1635-1680 cm-^{1}.



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 activities of different phenylhydrazones on endogenous protein substrate at pH 3.5 and 5.0 were measured. The liver homogenate was mixed with buffers of pH 3.5 and 5.0 in presence of various phenylhydrazones at 0.5mM concentration. After three hours of incubation at 37^{o}C, the reaction was stopped by adding TCA solution and the acid soluble proteins were estimated by Bradford method[10].

It was observed that endogenous protein hydrolysis was inhibited maximum by p-substituted benzaldehyde phenylhydrazones in comparison to o- and m- substituted phenylhydrazones, indicating thereby

involvement of some steric factor at the interacting site. Therefore, it may be possible that the change in these groups alters the enzyme-substrate interactions. Another important observation was that inhibition levels in each case were different at pH 3.5 and 5.0, suggesting that different types of enzymes are 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 this pH[12]. After evaluating the inhibitory effect at 0.5 mM concentrations, the p-substituted benzaldehyde phenylhydrazones were tested at 0.05 mM final concentration. It was found that p-nitro benzaldehyde phenylhydrazones inhibited the protease activity maximally (~95%) followed by p-chloro benzaldehyde phenylhydrazones (~35%), benzaldehyde phenylhydrazones (~25%) and p-methoxy benzaldehyde phenylhydrazones (nearly no inhibition) in that order. The results are similar to those reported earlier for semicarbazones[13].

Conclusion:

From the results it can be concluded that phenylhyrazones of substituted benzaldehydes are inhibitory to *in vitro* protein hydrolysis in liver. Also the substituents at *para* position have inhibitory effects on endogenous protein hydrolysis and electron withdrawing nitro group exerts maximum effect.

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.

References:

1. Daisuke Kato, Kelly M Boatright, Alicia B Berger, Tamim Nazif, Galia Blum, Ciara Ryan, Kareem A H Chehade , Guy S Salvesen and Matthew Bogyo, Nature Chemical Biology 2005 , Vol *1*, pp33 - 38.
2. James H. McKerrow, Int. J. Parasitology 1999, Vol *29(6)*, pp833-837.
3. Rebecca Roberts, Drug news &perspectives 2005, Vol *18(10)*, pp 605.

4. Sreten Bogdanovic and Beata Langlands, Proteases as Drug Targets: Technologies and Opportunities for Drug Discovery**,** D&MD Publications Inc, 2004.

5. Turk Boris ,Nature Reviews Drug Discovery 2006, Vol *5*, pp785-799.

6. Rzychon M., Chmiel D. and Stec-Niemczyk J., Acta Biochimica Polonica 2006, Vol *51(4)*, pp861-873.

7. Charles L. Cywin, R.A. Firestone, D.W. McNeil, C.A. Grygon, J.D.Peterson, E.L. Raymond, M.L.Brown and D.M. Spero, Bioorganic & Medicinal Chemistry 2003, Vol *11(5*), pp 733-740.

8. Savini L., Chasserini L., Gaeta A. and Pellerano C., Bioorg. Med. Chem. 2001, Vol *10*, pp2193-2198.

9. Gurkok G., Altanlar N. and Suzen S., Int. J. of Exp. and Clinical Chemo.2009, Vol *55(1*), pp15-19.

10. Bradford M.M., Analytical Biochem.1976, Vol *72*, pp248.; Stoschek C.M., Methods in enzymology 1990, Vol *182*, pp50.

11. Mokhtari J., Naimi-Jamal M.R., Hamzehali H. and Dekamin M.G., 11th Int. Elec. Conf. on Syn. Org. Chem.2007.

12. Kamboj R.C., Pal S., Raghav N. and Singh H., J. Indian Chem. Soc. 1992, Vol *69*, pp270.

13. Raghav N., Kaur R., Singh M., Suman and Priyanka, *Asian J. Chem.* 2010, Vol *22(9).* (In Press).

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