Asian Journal of Chemistry Vol. 22, No. 6 (2010), 4567-4572

Antioxidant and Hepatoprotective Activities of β-N-Cyanoethyl Acyl Hydrazides Derivatives

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In the present study, β-N-cyanoethyl acyl hydrazides derivatives **1-9** were investigated for antioxidant and hepatoprotective properties. Compounds **2**, **3**, **5**-**8** exhibited significant free radical scavenging activities against 1,1-diphenyl-2-picrylhydrazyl radicals, while rest of the compounds **1**, **4** and **9** have shown moderate activities. Hepatoprotective activity of these compounds was investigated in carbon tetrachloride induced liver toxicity model. Animals were pretreated at a dose level of 10 mg/Kg body weight before the injection of carbon tetrachloride and serium was analyzed after 48 h of carbon tetrachloride induced hepatotoxicity model in Wistar rats. Results showed that the levels of serum biochemical parameters sAST, sALT and bilirubins were largely maintained ($p < 0.05$) by most of the compounds (**3**-**6** and **8**) as compared to pathological control. In conclusion these β-N-cyanoethyl acyl hydrazides derivatives showed both antioxidant and hepatoprotective potential.

Key Words: β-N-Cyanoethyl acyl hydrazides, Antioxidant, DPPH radical, Hepatoprotective.

INTRODUCTION

The liver is the largest glandular organ of the body that filters and detoxicify harmful substances from the blood. Despite of its intensive regeneration ability, liver cells may undergo oxidative stress because of the over production of free radicals, leading to liver diseases. Free radicals such as reactive oxygen species (ROS) have been shown to initiate lipid peroxidation and cause various degenerative diseases like cardiovascular disease, diabetes, cancer, liver failure and aging. If free radicals are involved in the development of various ailments, then antioxidants should be important and play a role in their prevention.

Carbon tetrachloride is a pharmacological tool used to produce liver damage in animal models. In liver CCl_4 is metabolized to trichloromethyl free radical ($^{\bullet}CCl_3$) by NADPH-cytochrome P450 system, transferring an electron from NADPH to CCl_4. This free radical and related reactive species may cause cellular damage by initiating lipidoxidation, covalent binding to protein, depleting GHS (glutathione), or releasing iron, ultimately leading to cell death[1].

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Antioxidants play an important role in prevention of different pathological conditions including heart diseases[2], cancers[3] and inflammation[4]. There are two basic mechanisms followed by the compounds possessing antioxidant property. Compounds may scavenge free radicals by donating electrons and by inhibiting enzymes responsible for the production of free radicals like oxidases, lipoxigenase, cyclooxygenase, *etc.*, or by chelation of transition metal ions and absorption of UV radiations[5]. We therefore studied the antioxidant properties of various β-N-cyanoethyl acyl hydrazides derivatives.

Compounds with *in vitro* free radical scavenging activity were further tested for their effect on liver injury induced by carbon tetrachloride (CCl_4) in Wistar rats. Hepatotoxicity by CCl_4 in animals is a well known experimental model to evaluate the therapeutic potential of hepatoprotective drugs and antioxidants[6]. The synthesis and β-glucuronidase inhibition studies of these derivatives have already been published by our group[7]. The present study describes the antioxidant properties of β-N-cyanoethyl acyl hydrazides derivatives both *in vitro* and *in vivo*.

EXPERIMENTAL

Carbon tetrachloride, DPPH (1,1-diphenyl-2-picrylhydrazyl), propylgallat, methanol were purchased from MERK Germany, Sigma Chemical Co. and Fluka. Water used for buffer preparation was deionized by simplicity water purification system (Millipore). All other solvents were of analytical grade. Biochemical analysis of serum was performed by standard Kit method (Boehringer Meannheim) for enzyme estimation.

Animal studies were carried out using adult Wistar rats of either sex weighing (150-200 g) breed in animal house of H.E.J. Research Institute of Chemistry, International Center for Chemical Sciences, Karachi University. Animals were grouped and housed in polyacrylic cages with not more than 6 animals/cage and maintained under standard laboratory conditions *i.e.*, 12 h light-12 h dark cycle and fed a standard laboratory diet and tap water *ad libitum*.

DPPH radicals scavenging studies: The reaction mixture contains 5 μL of test samples and 95 μL of DPPH in ethanol. Concentration of DPPH was 300 μM in the reaction mixture. These reaction mixtures were taken in 96-well plates (Molecular Devices. USA) and incubated for 0.5 h at 37 °C. The absorbance was measured at 515 nm[8]. Per cent radical scavenging activity by sample treatment was determined by comparison with a DMSO treated control group. Propylgallate were used as positive controls.

Hepatoprotecitve studies by CCl_4-induced toxicity: Hepatoprotective studies were performed by the method of Kapil *et al.*[9] with a slight modification. Healthy Wistar rats were divided into three different groups each containing 6 rats. Group 1 served as normal control was kept on normal diet and water. Rats other than normal control received test compound (**1**-**9**) 10 mg/Kg body weight p.o. Pathological control animals received saline in same quantity. After 0.5 h all the rats got the i.p dose of

20 % CCl_4 diluted with dietary cooking oil (1 mL/100 g body weight). All the animals were sacrificed after 48 h of treatment. Blood samples were allowed to clot for 30-40 min. Serum was separated after centrifugation at 3000 rpm for 15 min. Propyl gallate was used to compare the activities of the test compounds. The enzymes, serum glutamyl pyruvate transaminase (SGPT), serum glutamyl oxaloacetic acid transaminase (SGOT), total bilirubin and direct bilirubin were analyzed by biochemical analysis of the serum using standard Kit method, Mannheim[10-13].

RESULTS AND DISCUSSION

DPPH radical scavenging activity: Free radical scavenging activity of β-N-cyanoethyl acyl hydrazides derivatives (**1**-**9**) was performed spectrophotometrically by measuring the decrease in absorption of 1,1-diphenyl-2-picrylhydrazyl radicals (DPPH) at 517 nm[8]. The results of free radical scavenging potential are presented in Table-1.

TABLE-1
In vitro DPPH RADICAL SCAVENGING ACTIVITY
OF β-N-CYANOETHYL ACYL HYDRAZIDES

Comp. No.	Compound Name	DPPH IC_{50} ± SEM* (μM)
1	Benzohydrazide	106.00 ± 2.72
2	4-(Tosyloxy) benzohydrazide	49.00 ± 2.00
3	Benzoyl (2-cyanoethylhydrazine)	71.00 ± 1.41
4	N-(2-Cyanoethyl)-4-hydroxybenzo-hydrazide	133.00 ± 2.72
5	N-(2-Cyanoethyl)-3-hydroxybenzo-hydrazide	87.00 ± 0.72
6	4-(Benzyloxy)-N-(2-cyanoethyl) benzo-hydrazide	72.00 ± 0.27
7	3-(Benzyloxy)-N-(2-cyanoethyl) benzo-hydrazide	69.00 ± 0.72
8	4-{[2-(2-Cyanoethyl) hydrazine] carbonylphenyl 4-methylbenzene sulfonate	35.00 ± 0.27
9	N-(2-Cyanoethyl)-2-(1*H*-indol-3-yl)acetohydrazide	550.00 ± 18.8
St	Propyl gallate	30.00 ± 0.25

[a]IC_{50} values are the mean ± standard mean (SEM) error of three assays. The IC_{50} values were calculated using with the EZ-fit enzyme kinetics program (Perrella Scientific Inc., Amherst, USA).

Almost all the compounds tested showed radical scavenging potential. Most of the compounds have IC_{50} value near to the standard (propyl gallate, IC_{50} = 30 μM). Among all these compounds, compound **8** was found to be the most active (35.0 ± 0.27 μM). It is apparent from the comparison of activities of all these compounds with their structure, that the cyanoethyl moiety is important for the anti radical activity. For example, the IC_{50} value of compound **1** is 160 μM (do not contain cyanoethyl moiety) which is almost double that of compound **3** (IC_{50} = 71.0 μM) having cyanoethyl group. A similar trend was found with other compound.

Hepatoprotective studies: β-N-Cyanoethyl acyl hydrazides derivatives **1**-**9** were also tested for hepatoprotective activity in CCl_4-induced hepatotoxicity in Wistar rats. The effect of CCl_4 on sAST (serum aspartate aminotransferase) and

sALT (alanine aminotransferase) and bilirubins was monitored. It is well known fact that CCl_4 induces hepatocellular damage that results in an increase in the enzyme levels in the serum1[4,15]. Elevated levels of serum enzymes, especially transaminases (ALT and AST), are indicative of cellular leakage and loss of functional integrity of the cell membrane in the liver[16]. Total and direct bilirubin also serves as markers for the assessment of the proper functioning and integrity of the liver. The effect of β-N-cyanoethyl acyl hydrazides derivatives on CCl_4-induced hepatotoxicity was studied by pretreatment of the animals at a dose of 10 mg/kg body weight. The normal control group received only a saline solution, whereas the pathological control was given an i.p. dose of CCl_4 (20 % in edible oil, 1 mL/100 g body weight). The effect of saline solution and CCl_4 was monitored by the estimation of sAST and sALT and bilirubins. There was a significant difference of hepatic transaminases and bilirubins between the pathological and normal control groups. The significant increase in the levels of serum bilirubins and sAST and sALT was observed in case of pathological control. The hepatoprotective effect of β-N-cyanoethyl acyl hydrazides derivatives was compared with the pathological control group and the results are presented in Table-2.

TABLE-2
HEPATOPROTECTIVE ACTIVITY OF COMPOUND **1-9** BY CCl_4-INDUCED HEPATOTOXICITY IN WISTAR RATS

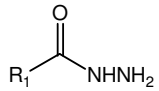
Compound	sAST (IU/L)	sALT (IU/L)	Bilirubin (mg %)		
			Total	Direct	Indirect
Pathological Control	1715.5±144.5	1557±48.71	0.824±0.0810	0.254±0.0310	0.576±0.0890
Normal Control	157.50±13.20	71.80±3.043	0.126±0.0600	0.023±0.0150	0.103±0.0660
1	1484.0±61.02	813.75*±27.77	0.1675*±0.0073	0.075*±0.0103	0.0925*±0.00739
2	746.25*±43.64	564.5*±8.189	0.1875*±0.0316	0.1025*±0.0316	0.085*±0.0075
3	132.0*±14.00	160.75*±23.06	0.203*±0.036	0.045*±0.00645	0.1975*±0.015
4	292.2*±47.77	131.2*± 14.35	0.142*±0.016	0.0625*±0.017	0.082*±0.023
5	391.2*±10.49	208.2*±10.47	0.2275*±0.012	0.164*±0.05	0.074*±0.039
6	135.75*±11.38	317.0*±104.6	0.1925*±0.04	0.11*±0.052	0.165*±0.0429
7	441.4*±55.04	210.6*±49.32	0.176*±0.038	0.065*±0.01	0.11*±0.053
8	173.75*±10.26	516.0*±63.20	0.155*±0.0064	0.0325*±0.006	0.1225*±0.0094
9	313.5*±17.76	170.75*±6.702	0.115*±0.0043	0.0675*±0.0089	0.0475*±0.0073
[a]Propyl gallate	559.00*±84.69	607.5*±127.9	0.318*±0.0560	0.145*±0.0120	0.173*±0.0300

*Statistically significant differences were defined as values less than $p \leq 0.05$. The results were evaluated by performing analysis of variance (ANOVA). [a] Standard antioxidant; Values of sAST, sALT, total bilirubin, direct bilirubin and indirect bilirubin are the ± SEM of 6 Wistar rats.

Among the β-N-cyanoethyl acyl hydrazides derivatives, compound **9** was found to be most active and showed highest hepatoprotective action as indicated by the lower values of serum biochemical parameters, sAST, sALT and total, direct and

indirect bilirubins. On the other hand, compound **1** showed least protection among all the tested compounds as indicated by the sAST level which is almost equal to the pathological control group. Whereas, standard antioxidant, propyl gallate showed moderate protection as indicated by the levels of sALT, sAST and bilirubins (Table-2).

STRUCTURES OF COMPOUNDS



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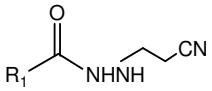
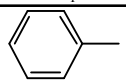
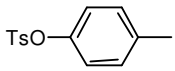
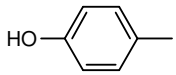
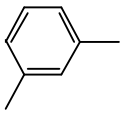
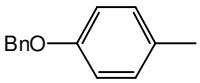
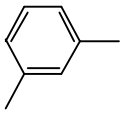
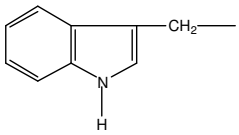


9-13, 15, 16

Compound No.	R_1
1,3	
2,8	
4	
5	
6	
7	
8	

Conclusion

The results of this study showed that β-N-cyanoethyl acyl hydrazides derivatives **1**-**9** possess antioxidant potential. The *in vivo* study indicated that the levels of sAST, sALT and total, direct and indirect bilirubins were significantly maintained ($p < 0.05$) by the pretreatment of most of the β-N-cyanoethyl acyl hydrazides derivatives (compound **2**-**9**). The contraction in the elevation of cytosolic enzymes is probably due to the interaction of CCl_4-generated free radicals with hepatic cells. Moreover, these compounds exhibited significant free radical scavenging activity in *in vitro* method, therefore it could be deduced that these compounds may provide hepatoprotection by acting as free radical scavengers. However, further investigations are needed to understand the exact mechanism of action of hepatoprotective activity of these compounds.

ACKNOWLEDGEMENTS

One of the authors (Shujaat) is thankful to Khalid, H.E.J. Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi and his student Shagufta Rahat for compounds provided in carrying out this study.

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(*Received*: 11 September 2009; *Accepted*: 15 February 2010) AJC-8444