β-*N*-Cyanoethyl Acyl Hydrazide Derivatives: A New Class of β-Glucuronidase Inhibitors

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Eight new β-*N*-substituted acyl hydrazides along with their corresponding acyl derivatives were synthesized and screened for *in vitro* β-glucuronidase inhibition and found to be active against the enzyme. All of these compounds were found to be noncompetitive inhibitors except for *N′*-(2-cyanoethyl)-4-hydroxy benzohydrazide (10), which was found to be an uncompetitive inhibitor. Structure–activity relationship studies indicated that the benzyloxy group present in compounds 12 and 13 is responsible for the β-glucuronidase inhibition activity.

Key words β-*N*-cyanoethylacyl hydrazide; β-glucuronidase; enzyme inhibition

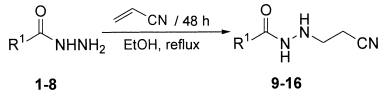
β-Glucuronidase (EC 3.2.1.31) is an exoglycosidase enzyme that catalyzes the cleavage of glucuronosyl-*O*-bonds.[1] The enzyme is present in many organs, body fluids, blood cells, liver, spleen, kidney, gastric juice, lung, muscle, bile, urine, and serum.[2,3] It has been reported that in certain diseases such as cancer, inflammatory joint disease, some hepatic diseases, and AIDS the activity of β-glucuronidase increases.[1] Human β-glucuronidase also has a role in the deconjugation of glycosaminoglycans. Endogenous biliary β-glucuronidase deconjugates the glucuronides of bilirubin and causes the development of cholelithiasis in human bile.[4] Many β-glucuronidase inhibitors such as 8-hydroxytricetin-7-glucuronide and isovitexin,[5] trihydroxy pipecolic acid,[6] and scoparic acid A and C[7] have already been isolated from different plants and some are used clinically.

During random screening of synthetic compounds, we found that hydrazide derivatives can inhibit β-glucuronidase enzyme. Although these hydrazides are not stronger than other inhibitors but have comparable inhibitory activity, to the best of our knowledge, this is the first example of synthetic inhibitors of this enzyme. The present report describes the synthesis of a series of hydrazide derivatives and their β-glucuronidase inhibitory activity with respect to structure–activity relationships.[7]

Results and Discussion

The synthesis of β-*N*-substituted acyl hydrazide derivatives from their corresponding acyl hydrazides were carried out as illustrated in Chart 1. The parent acyl hydrazides **1**—**8** were synthesized by treating their corresponding ethyl or methyl esters with hydrazine hydrate in ethanol. The Michael reaction between resulting acyl hydrazides and acrylonitrile gave exclusively β-*N*-substituted cyanoethyl derivatives **9**—**16** in high yields.[8] The formation of double Michael products was not observed. This may be due to a decline in nucleophilicity of the nitrogen atom after the first substitution.[9] The structures of synthesized compounds were identified and determined through their various physical and spectroscopic properties.

All the synthesized compounds **1**—**16** were screened for their β-glucuronidase inhibition activity.[10] On the basis of the results obtained from the preliminary screening of this series of compounds (Table 1), we selected compounds **4**, and **9**—**16** for IC_{50} and kinetic studies. The IC_{50} and K_i values and mode of inhibitions are given in Table 2. The K_i values (inhibitory constants) were determined directly from the Dixon plots.[11] These values were also confirmed, first by plotting the $1/V_{maxapp}$ against different concentrations of the respective inhibitor. $1/V_{maxapp}$ was calculated at each intersection point of lines of every inhibitory concentration on the *Y*-axis of the Lineweaver–Burk plot.[12] Second, the slope of each line of inhibitory concentration on the Lineweaver–Burk plot was plotted against inhibitor concentrations. For the uncompetitive type of inhibition, K_{mapp} was determined from the intersection of the lines for the inhibitory concentra-



1-8 **9-16**

Compound No.	R^1
1,9	
2,10	HO
3,11	HO
4,12	BnO
5,13	BnO
6,14	BzO
7,15	TsO
8,16	CH_2

Bn = Benzyl, Bz = Benzoyl, Ts = Tosyl

Chart 1

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Table 1. Results of Preliminary Screening of Compounds **1**—**16** against β-Glucuronidase at 0.5 mM/ml Concentration

Sr. no.	Compound no.	% inhibition
1	**1**	48.9
2	**2**	19.0
3	**3**	20.0
4	**4**	81.8
5	**5**	44.2
6	**6**	55.3
7	**7**	42.0
8	**8**	33.3
9	**9**	94.1
10	**10**	86.9
11	**11**	93.0
12	**12**	88.2
13	**13**	98.4
14	**14**	82.0
15	**15**	70.8
16	**16**	63.9

tions on the *X*-axis of the Lineweaver–Burk plot and plotted against inhibitor concentrations. The majority of the compounds exhibited a pure noncompetitive type of inhibition as the V_{max} values decreased without affecting the K_m values. Substitution of a benzyloxy group greatly enhanced the activity against β-glucuronidase, as in compounds **12** and **13**. It was also clear that substitution at the C-3 position is more effective than that at C-4. Similar results were obtained when a hydroxyl group (compounds **11**, **10**) was substituted at the C-3 and C-4 positions. The effect of a benzenesulfonyl group (compound **15**) on enzyme activity was same as in compound **10**, but the mode of action of compound **10** was found to be different from that of compound **15**, *i.e.*, uncompetitive. The graphic analysis of steady-state inhibition data of compound **13** for noncompetitive inhibition and of compound **10** for uncompetitive inhibition are shown in Figs. 1 and 2, respectively, providing an example of each type of inhibition.

Table 2. IC_{50} and K_i Values of Compounds **4** and **9**—**16** against β-Glucuronidase

Sr. no	Compound	IC_{50} (μM) Mean±S.E.M.[b)]	K_i[a)] (μM) Mean±S.E.M.	Type of inhibition
1	**4**	420.0±0.250	580.0±0.15	Noncompetitive
2	**9**	55.4±0.002	64.0±0.1	Noncompetitive
3	**10**	12.0±0.033	8.0±0.001	Uncompetitive
4	**11**	7.8±0.100	8.7±0.02	Noncompetitive
5	**12**	2.2±0.05	5.0±0.2	Noncompetitive
6	**13**	1.6±0.12	3.25±0.01	Noncompetitive
7	**14**	6.0±0.066	11.0±0.33	Noncompetitive
8	**15**	11.6±0.02	21.0±0.023	Noncompetitive
9	**16**	277.0±0.012	120.0±0.031	Noncompetitive

a) K_i is the mean of three values calculated using the Dixon plot and Lineweaver–Burk secondary plots. *b*) S.E.M. is the mean of five values in case of IC_{50}, and three values in case of K_i. IC_{50} of D-saccharic acid 1,4-lactone (standard)=0.8 μM±0.002.

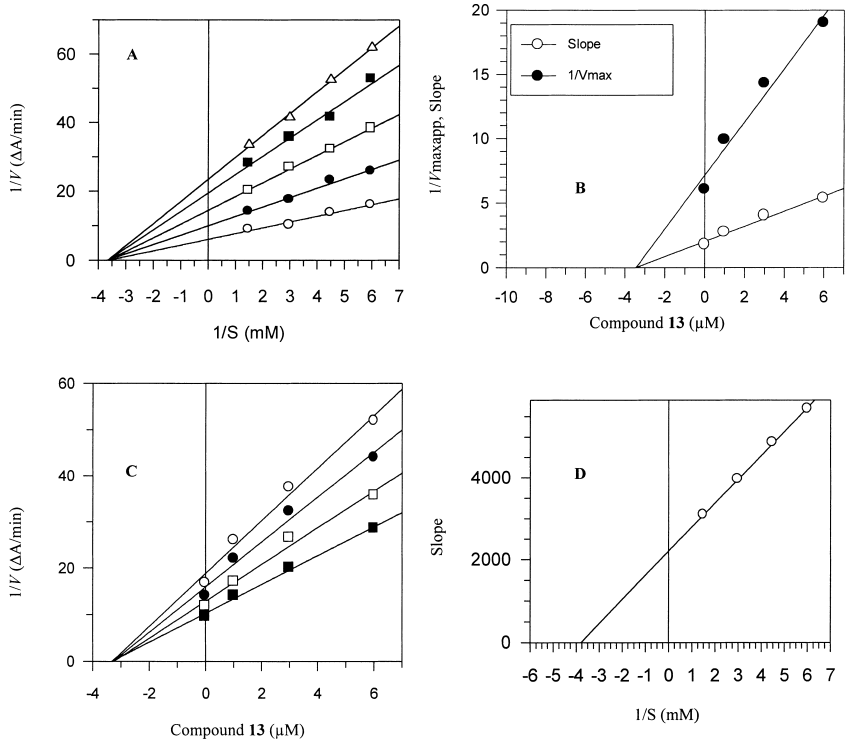

Fig. 1. Steady-State Inhibition of β-Glucuronidase by Compound **13** (3-(Benzyloxy)-*N*′-(2-cyanoethyl)benzohydrazide)

(A) Lineweaver–Burk plot of reciprocal of the initial velocities *versus* various substrate (*p*-nitrophenyl-β-D-glucuronide) concentrations. (B) Respective secondary replots of the Lineweaver–Burk plots, *i.e.*, $1/V_{maxapp}$ and slope *versus* various compound concentrations. (C) Dixon plot of reciprocal of the initial velocities *versus* various compound concentrations. (D) Secondary replot of the Dixon plot: slope *versus* reciprocal of substrate concentrations. Each point in the graphs represents the mean of three experiments.

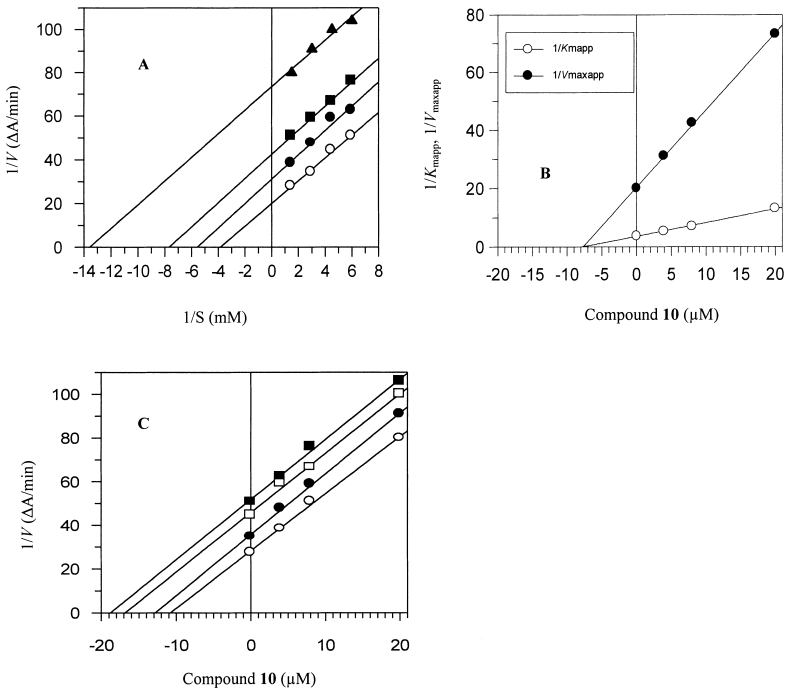

Fig. 2. Steady-State Inhibition of β-Glucuronidase by Compound **10** (*N*′-(2-cyanoethyl)-4-hydroxybenzohydrazide)

(A) Lineweaver–Burk plot of reciprocal of the initial velocities *versus* various substrate (*p*-nitrophenyl-β-D-glucuronide) concentrations. (B) Respective secondary replots of the Lineweaver–Burk plots, *i.e.*, $1/K_{maxapp}$ and $1/V_{maxapp}$ *versus* various compound concentrations. (C) Dixon plot of reciprocal of the initial velocities *versus* various compound concentrations. Each point in the graphs represents the mean of three experiments.

The present study provides a basis for further research on acyl hydrazides as β-glucuronidase inhibitors.

Experimental

Melting points were determined in open capillaries on a Büchi 535 apparatus and are uncorrected. Column chromatographic separations were carried out on silica gel (70—230 mesh, E. Merck). Infrared spectra (KBr) were recorded on a Shimadzu-IR-460 and IR ($CHCl_3$) were recorded on Jasco-A-302 spectrophotometer. The ^{1}H-NMR spectra were recorded on Bruker AM 300 and 400 spectrometers using the UNIX data system at 300 and 400 MHz, respectively. Electron impact (EI)-MS were recorded on MAT 311 A (Finnigan).

Material and Methods β-Glucuronidase (E.C. 3.2.1.31, from bovine liver, G-0251) and *p*-nitrophenyl-β-D-glucuronide (N-1627) were purchased from Sigma Chemical Co. (U.S.A.). Sodium carbonate anhydrous from Fluka and all other reagents were obtained from E. Merck and were of analytical grade. Anhydrous $CHCl_3$ and EtOH were dried using the standard methods. All other solvents and reagents were of reagent grade and used directly without purification, except for benzoyl chloride, which was distilled before use.

β-Glucuronidase Assay Protocol β-Glucuronidase activity was determined by measuring the absorbance at 405 nm of *p*-nitrophenol formed from the substrate by the spectrophotometric method developed by Collins *et al.*[10] with the following modifications. The total reaction volume was reduced to 250 μl with 190 μl of 0.1 M acetate buffer (13.608 g/l of sodium acetate, pH adjusted to 5.0 with 0.1 M acetic acid), and 5 μl of enzyme (30 U), 50 μl of 0.4 mM *p*-nitrophenyl-β-D-glucuronide were incubated at 37 °C for 30 min and 50 μl of 0.2 M Na_2CO_3 was used to quench the reaction. The plates were read on a multiplate reader (spectra MAX-340) at 405 nm. The inhibitory activities were determined as described previously,[7] except that 5 μl each of test compound was dissolved in DMSO, mixed with enzyme, and incubated at 37 °C for 15 min before the addition of substrate to start the reaction. The percentage inhibitory activity was calculated as follows: $(E-S)/E\times100$, where E is the activity of enzyme without test material and S is the activity of enzyme with test material. All assays were run in triplicate.

Determination of IC_{50} Values and Kinetic Parameters The concentrations of test compounds that inhibit the hydrolysis of *p*-nitrophenyl-β-D-glucuronide by 50% (IC_{50} value) were determined by monitoring the effect of increasing concentrations of these compounds in the assay. The IC_{50} values were then calculated using the EZ-Fit Enzyme Kinetic program (Perrella Scientific Inc., Amherst, MA, U.S.A. Dissociation constants (K_i values) were determined by the interpretation of Dixon plots.[11] Lineweaver–Burk plots[11] and their secondary plots using initial velocities were drawn over a substrate concentrations between 0.166 and 0.666 mM. Triplicate assays were conducted at each concentration, and graphs were plotted using the above method.

General Procedure for the Preparation of Acyl Hydrazides (1—8) The corresponding ethyl or methyl esters (0.06 mol) in small portions were added to hydrazine hydrate 99% (0.01 mol) in ethanol (3.7 ml) and then refluxed for 28 h. The solvents were removed *in vacuo* and the residues were crystallized overnight in a cold room.

Benzohydrazide (**1**): Colorless compound (1.24 g, 80%). mp 111 °C. UV λ_{max} MeOH: 219 (log ε=4.21) nm. IR (KBr): 3306, 3011, 1662, 1235, 1078, 748 cm^{-1}. ^{1}H-NMR (400 MHz, DMSO-d_6): δ 9.63 (1H, s, CONH), 7.61—7.40 (5H, m, Ar-H), 4.50 (2H, s, NH_2). FD-MS: *m*/*z* 136. *Anal.* Calcd for $C_7H_8N_2O$ (136): C, 61.75; H, 5.92; N, 20.57. Found: C, 61.73; H, 5.90; N, 20.56.

4-Hydroxybenzohydrazide (**2**): Colorless compound (1.32 g, 80%). mp 268 °C. UV λ_{max} MeOH: 245 (log ε=3.84) nm. IR (KBr): 3351, 3013, 1622, 1185, 1034, 832 cm^{-1}. ^{1}H-NMR (300 MHz, DMSO-d_6): δ 9.5 (1H, s, CONH), 7.68 (2H, d, *J*=8.8 Hz, H-2/H-6), 6.78 (2H, d, *J*=8.8 Hz, H-3/H-5), 4.35 (2H, s, NH_2). FD-MS: *m*/*z* 152. *Anal.* Calcd for $C_7H_8N_2O_2$ (152): C, 55.26; H, 5.3; N, 18.41. Found: C, 55.22; H, 5.32; N, 18.37.

3-Hydroxybenzohydrazide (**3**): Colorless compound (0.43 g, 75%). mp 153 °C. UV λ_{max} MeOH: 251 (log ε=4.46) nm. IR (KBr): 3315, 3114, 1623, 1191, 1064, 751 cm^{-1}. ^{1}H-NMR (400 MHz, DMSO-d_6): δ 9.65 (1H, s, CONH), 7.48 (1H, dt, *J*=8.3 Hz, *J*=1.7 Hz, H-6), 7.24 (1H, t, *J*=8.3 Hz, H-5), 7.18 (1H, t, *J*=1.7 Hz, H-2), 6.90 (1H, dt, *J*=8.3 Hz, *J*=1.7 Hz, H-4), 4.44 (2H, s, NH_2). FD-MS: *m*/*z* 152. *Anal.* Calcd for $C_7H_8N_2O_2$ (152): C, 55.26; H, 5.3; N, 18.4. Found: C, 55.22; H, 5.32; N, 18.37.

4-Benzyloxybenzohydrazide (**4**): Colorless compound (1.64 g, 73%). mp 140 °C. UV λ_{max} MeOH: 272 (log ε=4.69) nm. IR (KBr): 3293; 1687; 1611, 1213, 1053, 837 cm^{-1}. ^{1}H-NMR (300 MHz, DMSO-d_6): δ 9.58 (1H, s, CONH), 7.78 (2H, d, *J*=8.8 Hz, H-2/H-6), 7.45—7.30 (5H, m, Ar-H), 7.04 (2H, d, *J*=8.8 Hz, H-3/H-5), 5.14 (2H, s, Ph$\underline{CH_2}$O), 4.47 (2H, s, NH_2). FD-MS: *m*/*z* 242. *Anal.* Calcd for $C_{14}H_{14}N_2O_2$ (242): C, 69.40; H, 5.82; N, 11.56. Found: C, 69.37; H, 5.84; N, 11.47.

3-Benzyloxybenzohydrazide (**5**): Colorless compound (1.81 g, 72%). mp 155 °C. UV λ_{max} MeOH: 265 (log ε=4.73) nm. IR (KBr): 3312, 1663, 1613,

1375, 1043, 763 cm^{-1}. ^{1}H-NMR (300 MHz, DMSO-d_6): δ 9.63 (1H, s, CONH), 7.70—7.30 (5H, m, Ar-H), 7.51 (1H, dt, J=8.3 Hz, J=1.5 Hz, H-6), 7.24 (1H, t, J=8.3 Hz, H-5), 7.15 (1H, t, J=1.5 Hz, H-2), 6.95 (1H, dt, J=8.3 Hz, J=1.5 Hz, H-4), 5.21 (2H, s, Ph$\underline{CH_2}$O), 4.49 (2H, s, NH_2). FD-MS: m/z 242. *Anal.* Calcd for $C_{14}H_{14}N_2O_2$ (242): C, 54.90; H, 4.60; N, 9.14. Found: C, 54.87; H, 4.52; N, 9.17.

4-Benzoyloxybenzohydrazide (**6**): Colorless compound (1.74 g, 69%). mp 255 °C. UV λ_{max} MeOH: 283 (log ε=5.28) nm. IR (KBr): 3350, 1700, 1690, 1640 cm^{-1}. ^{1}H-NMR (300 MHz, DMSO-d_6): δ 9.53 (1H, s, CONH), 7.91—7.42 (5H, m, Ar-H), 7.71 (2H, d, J=8.1 Hz, H-2/H-6), 6.72 (1H, d, J=8.1 Hz, H-3/H-5), 4.42 (2H, s, NH_2). FD-MS: m/z 256. *Anal.* Calcd for $C_{14}H_{12}N_2O_3$ (256): C, 65.61; H, 4.72; N, 10.93. Found: C, 65.60; H, 4.68; N, 10.90.

4-(4′-Methylbenzenesulfonyl)benzohydrazide (**7**): Colorless compound (2.83 g, 63%). mp 105 °C. UV λ_{max} MeOH: 280 (log ε=5.11) nm. IR (KBr): 3354, 1693, 1645, 1382, 1053, 773 cm^{-1}. ^{1}H-NMR (300 MHz, DMSO-d_6): δ 9.51 (1H, s, CONH), 7.95 (2H, d, J=8.7 Hz, H-2/H-6), 7.76 (2H, d, J=8.9 Hz, H-3′/H-5′), 7.48 (2H, d, J=8.9 Hz, H-2′/H-6′), 6.77 (2H, d, J=8.7 Hz, H-3/H-5), 4.49 (2H, s, NH_2), 2.40 (3H, s, CH_3). FD-MS: m/z 306. *Anal.* Calcd for $C_{14}H_{14}N_2O_4S$ (306): C, 54.90; H, 4.60; N, 9.14. Found: C, 54.87; H, 4.51; N, 9.11.

2-(1*H*-Indol-3-yl)acetohydrazide (**8**): Colorless compound (1.66 g, 68%). mp 137 °C. UV λ_{max} MeOH: 261 (log ε=4.32) nm. IR (KBr): 3317, 1649, 1623, 1235, 1063, 812 cm^{-1}. ^{1}H-NMR (300 MHz, DMSO-d_6): δ 9.65 (1H, s, CONH), 7.59 (1H, dd, J=7.8 Hz, J=1.6 Hz, H-7), 7.30 (1H, dt, J=7.8 Hz, J=1.6 Hz, H-6), 7.09 (1H, dt, J=7.8 Hz, J=1.6 Hz, H-5), 6.98 (1H, d, J=7.8 Hz, H-4), 4.45 (2H, s, NH_2), 4.22 (2H, s, H_2-8), 3.40 (H, s, H-2). FD-MS: m/z 189. *Anal.* Calcd for $C_{10}H_{11}N_3O$ (189): C, 63.48; H, 5.86; N, 22.21. Found: C, 63.41; H, 5.80; N, 22.17.

General Procedure for the Preparation of Substituted Benzoyl[2-(2-cyanoethyl)hydrazines] (9—16) Acrylonitrile (1.5 mol) was added to a mixture of the corresponding acyl hydrazides **1**—**8** (0.5 mol) in ethanol (172 ml) and the mixture was heated under reflux conditions for 48 h. The solvent was removed *in vacuo* and the resulting residue was chromatographed over silica gel eluted with hexane–ethyl acetate (7 : 3) to obtain the crystalline substituted hydrazines **9**—**16**.

N′-(2-Cyanoethyl)benzohydrazide (**9**): Colorless compound (1.08 g, 80%). mp 105 °C. UV λ_{max} MeOH: 213 (log ε=4.11) nm. IR (KBr): 3314, 3011, 2315, 1663, 1624, 1135, 1062, 748 cm^{-1}. ^{1}H-NMR (400 MHz, DMSO-d_6): δ 10.01 (1H, s, N–H), 9.75 (1H, s, CONH), 7.81—7.41 (5H, m, Ar-H), 3.04 (2H, dd, J=11.3 Hz, J=6.4 Hz, H_2-10), 2.61 (2H, t, J=6.4 Hz, H_2-11). FD-MS: m/z 189. *Anal.* Calcd for $C_{10}H_{11}N_3O$ (189): C, 63.48; H, 5.9; N, 22.0. Found: C, 63.46; H, 5.92; N, 22.06.

N′-(2-Cyanoethyl)-4-hydroxybenzohydrazide (**10**): Colorless compound (1.53 g, 75%). mp 135 °C. UV λ_{max} MeOH: 236 (log ε=3.76) nm. IR (KBr): 3514, 3353, 2351, 1663, 1610, 1145, 1047, 765 cm^{-1}. ^{1}H-NMR (300 MHz, DMSO-d_6): δ 9.95 (1H, s, N–H), 9.72 (1H, s, CONH), 7.69 (2H, d, J=8.5 Hz, H-2/H-6), 6.79 (2H, d, J=8.5 Hz, H-3/H-5), 3.01 (2H, dd, J=11.1 Hz, J=6.4 Hz, H_2-10), 2.51 (2H, t, J=6.4 Hz, H_2-11). FD-MS: m/z 205. *Anal.* Calcd for $C_{10}H_{11}N_3O_2$ (205): C, 58.53; H, 5.40; N, 20.48. Found: C, 58.46; H, 5.28; N, 20.56.

N′-(2-Cyanoethyl)-3-hydroxybenzohydrazide (**11**): Colorless compound (1.46 g, 70%). mp 115 °C. UV λ_{max} MeOH: 246 (log ε=4.15) nm. IR (KBr): 3511, 3017, 2306, 1625, 1143, 1034, 762 cm^{-1}. ^{1}H-NMR (400 MHz, DMSO-d_6): δ 9.82 (1H, s, N–H), 9.54 (1H, s, CONH), 7.54 (1H, dt, J=8.4 Hz, J=1.9 Hz, H-6), 7.28 (1H, t, J=8.4 Hz, H-5), 7.14 (1H, t, J=1.9 Hz, H-2), 6.97 (1H, dt, J=8.4 Hz, J=1.9 Hz, H-4), 3.01 (2H, dd, J=11.2 Hz, J=6.1 Hz, H_2-10), 2.28 (2H, t, J=6.1 Hz, H_2-11). FD-MS: m/z 205. *Anal.* Calcd for $C_{10}H_{11}N_3O_2$ (205): C, 58.53; H, 5.40; N, 20.48. Found: C, 58.46; H, 5.28; N, 20.56.

4-(Benzyloxy)-*N*′-(2-cyanoethyl)benzohydrazide (**12**): Colorless compound (1.77 g, 66%). mp 139 °C. UV λ_{max} MeOH: 263 (log ε=4.58) nm. IR (KBr): 3312, 1682, 1633, 1135, 1053, 821 cm^{-1}. ^{1}H-NMR (300 MHz, DMSO-d_6): δ 9.90 (1H, s, N–H), 9.61 (1H, s, CONH), 7.80 (2H, d, J=8.7 Hz, H-2/H-6), 7.42—7.30 (5H, m, Ar-H), 7.05 (2H, d, J=8.7 Hz, H-3/H-5), 5.25 (2H, s, Ph$\underline{CH_2}$O), 3.04 (2H, dd, J=10.8 Hz, J=6.3 Hz, H_2-10), 2.62 (2H, t, J=6.3 Hz, H_2-11). FD-MS: m/z 295. *Anal.* Calcd for $C_{17}H_{17}N_3O_2$ (295): C, 69.14; H, 5.80; N, 14.23. Found: C, 69.10; H, 5.86; N, 14.17.

3-(Benzyloxy)-*N*′-(2-cyanoethyl)benzohydrazide (**13**): Colorless compound (2.58 g, 72%). mp 68 °C. UV λ_{max} MeOH: 263 (log ε=4.58) nm. IR (KBr): 3307, 2241, 1682, 1625, 1134, 1058, 779 cm^{-1}. ^{1}H-NMR (300 MHz, DMSO-d_6): δ 9.79 (1H, s, N–H), 9.59 (1H, s, CONH), 7.73—7.48 (5H, m, Ar-H), 7.33 (1H, dt, J=8.2 Hz, J=1.8 Hz, H-6), 7.22 (1H, t, J=8.2 Hz, H-5), 7.19 (1H, t, J=1.8 Hz, H-2), 6.85 (1H, dt, J=8.2 Hz, J=1.8 Hz, H-4), 5.18 (2H, s, Ph$\underline{CH_2}$O), 3.05 (2H, dd, J=10.8 Hz, J=6.1 Hz, H_2-10), 2.31 (2H, t, J=6.1 Hz, H_2-11). FD-MS: m/z 295. *Anal.* Calcd for $C_{17}H_{17}N_3O_2$ (295): C, 69.14; H, 5.80; N, 14.23. Found: C, 69.12; H, 5.75; N, 14.19.

4-(Benzoyloxy)-*N*′-(2-cyanoethyl)benzohydrazide (**14**): Colorless compound (1.85 g, 50%). mp 128 °C. UV λ_{max} MeOH: 274 (log ε=4.87) nm. IR (KBr): 3315, 2234, 1725, 1672, 1625, 1142, 1063, 747 cm^{-1}. ^{1}H-NMR (300 MHz, DMSO-d_6): δ 9.78 (1H, s, N–H), 9.58 (1H, s, CONH), 7.90—7.43 (5H, m, Ar-H), 7.68 (2H, d, J=7.9 Hz, H-2/H-6), 6.79 (1H, d, J=7.9 Hz, H-3/H-5), 3.02 (2H, dd, J=11.2 Hz, J=6.4 Hz, H_2-10), 2.62 (2H, t, J=6.4 Hz, H_2-11). FD-MS: m/z 309. *Anal.* Calcd for $C_{17}H_{15}N_3O_3$ (309): C, 66.01; H, 4.89; N, 13.59. Found: C, 66.03; H, 4.81; N, 13.61.

4-(4′-Methylbenzenesulfonyl)-*N*′-(2-cyanoethyl)benzohydrazide (**15**): Colorless compound (1.25 g, 61%). mp 103 °C. UV λ_{max} MeOH: 278 (log ε=4.94) nm. IR (KBr): 3309, 2243, 1694, 1625, 1136, 1063, 749 cm^{-1}. ^{1}H-NMR (300 MHz, DMSO-d_6): δ 9.79 (1H, s, N–H), 9.54 (1H, s, CONH), 7.89 (2H, d, J=8.5 Hz, H-2/H-6), 7.65 (2H, d, J=8.8 Hz, H-3′/H-5′), 7.45 (2H, d, J=8.8 Hz, H-2′/H-6′), 6.79 (2H, d, J=8.5 Hz, H-3/H-5), 3.07 (2H, dd, J=10.5 Hz, J=6.2 Hz, H_2-10), 2.63 (2H, t, J=6.2 Hz, H_2-11), 2.38 (3H, s, CH_3). FD-MS: m/z 359. *Anal.* Calcd for $C_{17}H_{17}N_3O_4S$ (359): C, 56.83; H, 4.77; N, 11.70. Found: C, 56.84; H, 4.69; N, 11.71.

N′-(2-Cyanoethyl)-2-(1*H*-indol-3-yl)acetohydrazide (**16**): Colorless compound (1.64 g, 75%). mp 123 °C. UV λ_{max} MeOH: 256 (log ε=3.98) nm. IR (KBr): 3308, 2253, 1662, 1621, 1137, 1061, 823 cm^{-1}. ^{1}H-NMR (300 MHz, DMSO-d_6): δ 9.83 (1H, s, N–H), 9.57 (1H, s, CONH), 7.61 (1H, dd, J=8.3 Hz, J=1.9 Hz, H-7), 7.34 (1H, dt, J=8.3 Hz, J=1.9 Hz, H-6), 7.13 (1H, dt, J=8.3 Hz, J=1.9 Hz, H-5), 7.01 (1H, d, J=8.3 Hz, H-4), 5.12 (2H, s, H_2-8), 3.45 (H, s, H-2), 2.98 (2H, dd, J=11.4 Hz, J=6.3 Hz, H_2-12), 2.52 (2H, t, J=6.3 Hz, H_2-13). FD-MS: m/z 242. *Anal.* Calcd for $C_{13}H_{14}N_4O$ (242): C, 64.45; H, 5.82; N, 23.13. Found: C, 64.41; H, 5.75; N, 23.09.

Acknowledgments This work was financially supported by the Pakistan Science Foundation (Grant no. SKU/CHEM/372).

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