ISSN Print: 2394-7500
ISSN Online: 2394-5869
Impact Factor: 5.2
IJAR 2018; 4(7): 69-73
www.allresearchjournal.com
Received: 01-05-2018
Accepted: 07-06-2018

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Synthesis, characterization and *In vitro* antidiabetic activity of some substituted cyanoacetyl hydrazone derivatives

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Abstract
The present study describe about the synthesis, characterization and invitro antidiabetic activity of novel cyanoacetyl hydrazone derivatives. However their derivatives have been used in the fields of medicinal and pharmaceutical chemistry and reported to exhibit a variety of biological activities. The structures of all the synthesized compounds were elucidated by using spectral data. This in vitro study explores the antidiabetic properties of S1, S2 and S3 and it can be considered as a potential candidate for the management of type-II diabetes mellitus. The present findings exhibited a concentration dependent inhibition of α-amylase activity by S1, S2 and S3. The results of the study revealed that the antidiabetic activity of the S1 is much higher than that of S2 and S3 and near to the standard.

Keywords: cyanoacetyl hydrazone, diabetes mellitus, S1, S2, S3, Acarbose, α-amylase

Introduction
Heterocyclic systems having piperidine are found to possess better biological activity. They aroused great interest in the past and recent years due to their wide variety of biological properties and their presence in biologically active pharmaceutical ingredients. The emphasis on the synthesis of the above said heterocycles can be recognized owing to their presence in the molecular structure of numerous alkaloids and drugs.

Hydrazones and their derivatives constitute an important class of compounds that has found wide utility in organic synthesis. The chemistry of carbon-nitrogen double bond of hydrazone is becoming the backbone of condensation reaction in benzo-fused N-heterocycles, also it constitutes an important class of compounds for new drug development. NMR spectroscopy is an important tool for the study of heterocyclic compounds owing to its frequent use for the conformational analysis and in understanding the influence of electronic and conformational effects on chemical shifts and coupling constant values. Many reports are available on the conformation of variously substituted 2, 6-diarylpiperidin-4-ones. Pandiarajan *et al.* have elaborately discussed the conformation of 2, 6-diarylpiperidin-4-ones with or without alkyl substituent at C-3 and C-3/C-5 positions [1-3]. Certain small molecules act as highly functionalized scaffolds and are known pharmacophores of a number of biologically active and medicinally potent molecules. Recently, acetic hydrazones have attracted great attention due to their diverse biological and pharmacological properties.

Diabetes mellitus results from the defects in the insulin secretion and action, this may be characterized by chronic hyperglycemia, which is connected with the carbohydrates, protein and lipid metabolism [4]. Globally mortality rate 9% is recorded due to the diabetes. Diabetes mellitus a well-known endocrine disorder and it is most common in India now a day. The reason may be life style and genetic factors [5]. The treatment of diabetes need to spent vast amount of resources including medicines, diets, physical training and along with serious complications often resulting in high death rate. Therefore there is a need for searching of a new class of compounds to overcome diabetic problems [6]. Thus taken above into considerations synthesized compounds were screened for their in-vitro antidiabetic activity and to find out the comparative potential of the compounds. In the present study is to screen for *in vitro* inhibition of alpha-amylase enzyme activity of S1, S2 and S3 and compared with standard as Acarbose.

Materials and Methods

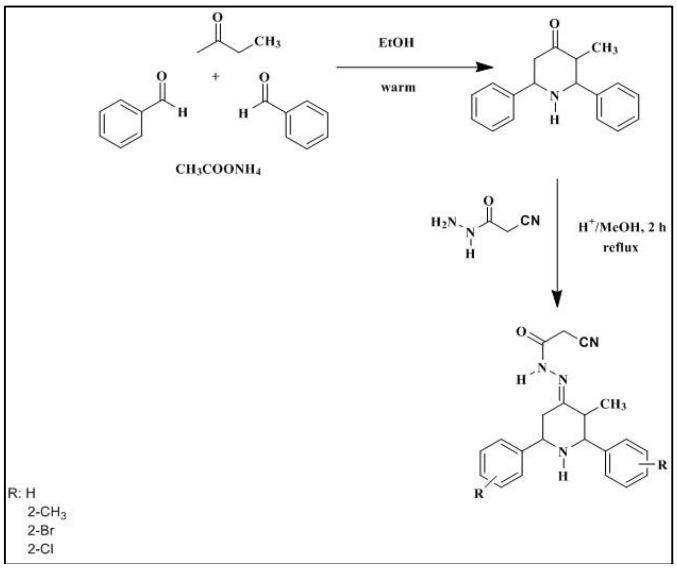
Chemicals were procured from E. Merck (India), S. D. Fine Chemicals (India) and reagent/solvents were used without distillation procedure. Melting points were taken in open capillary tubes and are uncorrected. IR (KBr) spectra were recorded on a Perkin-Elmer 157 infrared spectrometer (ν in cm–1) and NMR spectra were recorded on a Bruker spectrometer DPX-300MHz (Bruker, Germany) by using $CDCl_3$ as solvent with TMS as an internal standard. All the spectral data are consistent with the assigned structures of the desired product and the progress of the reactions was monitored on silica gel G plates using iodine vapour as visualizing agent.

Preparation of S1, S2 and S3

3-methyl-2,6-diphenylpiperidin-4-one was prepared by adopting the literature method. Condensation of 2-butanone, substituted aldehydes and ammonium acetate in warm ethanol in the ratio of 1:2:1 respectively afforded the formation of 3-methyl-2,6-diphenylpiperidin-4-ones.

Preparation of 3-methyl-2, 6-diphenylpiperidin-4-one cyanoacetyl hydrazone

A mixture of 3-methyl-2,6-diphenylpiperidin-4-one (0.1 mol), cyanoacetic hydrazide (0.1 mol) in the presence of few drops of concentrated acetic acid in methanol was refluxed for 2 hours. After the completion of reaction, the reaction mixture was cooled to room temperature. The solid product was separated by filtration and washed with warm water and recrystallized by methanol to afford 3-methyl-2,6-diphenylpiperidin-4-one cyanoacetyl hydrazone.



Scheme 1

In vitro antidiabetic activity

In vitro α-amylase inhibition study

In vitro α-amylase inhibition assay was carried out by the method of Apostolidis (2007) [7]

Reagents

- 20mm Phosphate buffer (pH 6.9).
- α-amylase (0.5mg/ml)
- 0.5% starch
- 96 % 3,5-dinitrosalicylic acid (DNS)

Procedure

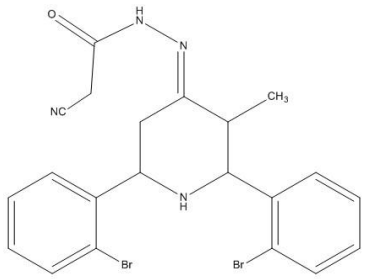
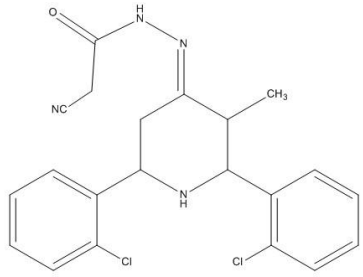
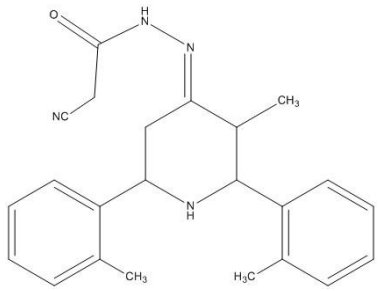
Various concentrations of the synthesized compounds were prepared i.e; 100 µg/ml, 200 µg/ml, 300 µg/ml, 400µg/ml & 500 µg/ml using phosphate buffer (pH 6.9). 500 µl of synthesized compounds to separate test tube and 500 µl of 20 % phosphate buffer pH 6.9, containing α-amylase at a concentration of 0.5 mg/ml were incubated at 25°C for 10 min. After pre incubation, 1000µl of 0.5% starch solution in 20 % phosphate buffer, pH 6.9 was added. The reaction mixtures were then incubated at 25°C for 10 min. The reaction was stopped with 500µl of 96 % 3, 5-dinitrosalicylic acid (DNS) color reagent. The test tubes were then incubated in a boiling water bath for 5 min and cooled to room temp. Absorbance (A) was measured at 540 nm. Acarbose was used as positive control and the inhibitory activity of α- amylase and percent of inhibition was calculated as follows:

% of inhibition = (Control O.D. – Test O.D.) / Control O.D.) × 100

Control incubations represent 100% enzyme activity and were conducted in a similar way by replacing sample. For blank incubation (to allow for absorbance produced by the extract), enzyme solution was replaced by buffer solution and absorbance recorded. Separate incubation carried out for reaction t = 0 was performed by adding samples to DNS solution immediately after addition of the enzyme. The experiment was done in triplicate. IC_{50} value was calculated by using regression analysis.

Result and Discussion

Table 1: The physical data of synthesized Cyanoacetyl hydrazone derivatives

Compound	Structure	Yield (%)	M. Formula	M. Weight	M. Point
S1		79.65	$C_{21}H_{18}Br_2N_4O$	502	174-178ºC
S2		80.69	$C_{21}H_{18}Cl_2N_4O$	412	180-182ºC
S3		82.6	$C_{23}H_{26}N_4O$	374	141-144 ºC.

3-methyl-2, 6 di(bis-*o*-bromo phenyl) piperidin-4-one cyanoacetyl hydrazone (S1): Yield. 79.65%. mp. 174-178 ºC. FT-IR (KBr) ν_{max} (cm^{-1}): 3099-2931 (C-H Aliphatic &Aromatic stretching), 1681 (C=O), 1567 (C=N), 2265 (C≡N), 3308-3179 (N-H). ^{13}C NMR(300 MHz, $CDCl_3$) δ ppm: 130.10 (C-2 ipso carbon), 130.62 (C-6 ipso carbon), 128.59-129.54 (Aromatic carbons), 166.34 (C=O), 159.54 (C=N), 125.18 (C≡N), 25.11 (CH_2 carbon of cyanoacetohydrazone moiety), 65.86 (C-2), 59.64 (C-6), 39.14 (C-3), 25.23 (C-5), 12.19 (3-CH_3). ^{1}H NMR(300 MHz, $CDCl_3$), δ ppm: 7.53- 7.31 (m,8H Aromatic protons) 10.79 (b s, 1H, N-H, Hydrazone Moiety), 2.50 (b s, 1H, N-H Piperidin moiety), 3.35 (q, 2H, CH_2 –Protons in hydrazone moiety), 0.91((d, J = 6.6Hz, 3H, 3-CH_3), 3.88 (dd, $J^3_{a,e}$ = 3Hz, $J^3_{a,a}$ = 10.2Hz, 1H, H-6a), 3.35(d, $J^3_{a,a}$ = 10.2Hz, 1H, H-2a), 2.50 (dd, $J^3_{a,e}$ = 11.4Hz, $J^3_{a,a}$ = 11.7 Hz, 1H, H-5a), 3.35 (dd, $J^3_{a,e}$ = 2.1 Hz, $J^2_{a,e}$ = 12Hz, 1H, H-5e), 2.51(m, 1H, H-3a Proton).

3-methyl-2,6 di(bis-*o*-chloro phenyl) piperidin-4-one cyanoacetyl hydrazone (S3): Yield. 80.69%. mp. 180-182ºC. FT-IR(KBr) ν_{max} (cm^{-1}): 3099-2933 ((C-H Aliphatic &Aromatic stretching), 1681 (C=O), 1570 (C=N), 2266 (C≡N), 3311-3183 (N-H). ^{13}C NMR(300 MHz, **$CDCl_3$) δ** ppm:132.27 (C-2 ipso carbon), 133.68 (C-6 ipso carbon), 127.51-129.27 (Aromatic carbons) 165.09 (C=O), 154.98 (C=N), 115.08 (C≡N), 24.17 (CH_2 carbon of cyanoacetohydrazone moiety), 63.31 (C-2), 56.55 (C-6), 34.00 (C-3), 28.21 (C-5), 11.14 (3-CH_3). ^{1}H NMR(300 MHz, $CDCl_3$), δ ppm: 7.42-7.32(m, 8H, Aromatic Protons), 10.09 (b s, 1H, N-H, Hydrazone Moiety), 2.24 (b s, 1H, N-H Piperidin moiety), 3.42 (q, 2H, CH_2 –Protons in hydrazone moiety), 0.971 (d, J = 6Hz, 3H, 3-CH_3) 3.73(dd, $J^3_{a,e}$ = 3Hz, $J^3_{a,a}$ = 10.2Hz, 1H, H-6a), 3.3(d, $J^3_{a,a}$ = 10.2Hz, 1H, H-2a), 2.98 (dd, $J^3_{a,e}$ = 11.4Hz, $J^3_{a,a}$ = 12 Hz, 1H, H-5a), 3.4(dd, $J^3_{a,e}$ = 2.1 Hz, $J^2_{a,e}$ = 12Hz, 1H, H-5e), 2.67(m, 1H, H-3a Proton).

3-methyl-2,6 di(bis-*o*-methyl phenyl) piperidin-4-one cyanoacetyl hydrazone (S3): Yield. 82.6%. mp. 141-144 ºC. FT-IR (KBr) ν_{max} (cm^{-1}): 3025-2852 (C-H Aliphatic & Aromatic stretching), 1674 (C=O), 1568 (C=N), 2267 (C≡N), 3440-3184 (N-H). ^{13}C NMR(300 MHz, $CDCl_3$) δ ppm:139.98 (C-2 ipso carbon), 140.49(C-6 ipso carbon), 126.49-129.77 (Aromatic carbons), 164.48 (C=O), 158.05 (C=N), 114.35 (C≡N), 24.16 (CH_2 carbon of cyanoacetohydrazone moiety), 76.57 (C-2), 56.12 (C-6), 44.89 (C-3), 34.56 (C-5), 11.15 (3-CH_3) 19.20 (*o*-CH_3). **^{1}H NMR(300 MHz, $CDCl_3$), δ ppm:** 7.32-7.13 (m, 8H,

Aromatic Protons), 10.09 (b s, 1H, N-H, Hydrazone Moiety), 2.09 (b s, 1H, N-H Piperidin moiety), 3.50 (q, 2H, CH_2 –Protons in hydrazone moiety), 0.92 (d, J = 6Hz, 3H, 3-CH_3), 3.89 (dd, $J^3_{a,e}$ = 3Hz, $J^3_{a,a}$ = 10.2Hz, 1H, H-6a), 3.11 (d, $J^3_{a,a}$ = 10.2Hz, 1H, H-2a), 2.39 (dd, $J^3_{a,e}$ = 11.4Hz, $J^3_{a,a}$ = 11.7 Hz, 1H, H-5a), 3.07(dd, $J^3_{a,e}$ = 2.1 Hz, $J^2_{a,e}$ = 12Hz, 1H, H-5e), 2.57 (m, 1H, H-3a Proton), 2.33 (s, 3H, *o*-CH_3 protons).

Antidiabetic activity of compounds S1, S2 and S3

α-Amylase inhibitory activity

The *in vitro* antidiabetic activity of the synthesized compounds were investigated through α-Amylase inhibitory activity. The inhibitory activities of compounds reported in Table 2. The compounds were comparable with standard antidiabetic drug viz. Acarbose. The compounds showed inhibitory effect on α-Amylase with varying degrees of inhibition. The maximum inhibition was seen with the standard drug Acarbose. Among the various doses (100 µg/ml, 200 µg/ml, 300 µg/ml, 400µg/ml & 500 µg/ml) of compounds.

The mean inhibition activity of compound S1 was 39.36±0.28% for 100 µg/ml, 49.20±0.34% for 200 µg/ml, 62.36±0.47% for 300 µg/ml, 73.39±0.52% for 400 µg/ml and 84.30±0.58% for 500µg/ml for α-Amylase.

The mean inhibition activity of compound S2 was 35.42±0.25% for 100 µg/ml, 48.08±0.42% for 200 µg/ml, 59.41±0.38% for 300 µg/ml, 65.74±0.44% for 400 µg/ml and 76.07±0.52% for 500µg/ml for α-Amylase.

The mean inhibition activity of compound S3 was 46.19±0.50% for 100 µg/ml, 47.25±0.40% for 200 µg/ml, 49.20±0.71% for 300 µg/ml, 50.61±0.09% for 400 µg/ml and 61.06±0.58% for 500µg/ml for α-Amylase.

The mean inhibition zone for standard is 19.63±0.57% for 100 µg/ml, 35.61±0.68% for 200 µg/ml, 56.84±0.68% for 300 µg/ml, 73.28±0.16% for 400 and 86.75±1.02% for 500 µg/ml for α-Amylase.

Table 2: In *vitro* α-amylase inhibition (S1, S2 and S3)

Concentrations	S1	S2	S3	Standard Acarbose
	% of inhibition			
100µg/ml	39.36±0.28	35.42±0.25	46.19±0.50	19.63±0.57
200µg/ml	49.20±0.34	48.08±0.42	47.25±0.40	35.61±0.68
300µg/ml	62.36±0.47	59.414±0.38	49.20±0.71	56.84±0.68
400µg/ml	73.39±0.52	65.74± 0.44	50.61±0.09	73.28±0.16
500 µg/ml	84.30±0.58	76.07±0.52	61.06±0.95	86.75±1.02
IC_{50} (mg/ml)	0.197	0.229	0.273	0.274

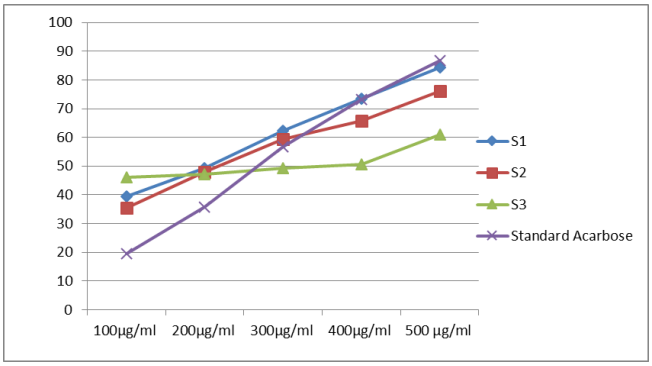


Fig 1: Effect of S1, S2 and S3 on inhibition of α Amylase activity

α-Amylase is one of the main enzymes in human body that is responsible for the breakdown of starch to more simple sugars. α-Amylases hydrolyze complex polysaccharides to produce oligosaccharides and disaccharides which are then hydrolyzed by α -glycosidase to monosaccharide which are absorbed through the small intestines into the hepatic portal vein and increase postprandial glucose levels [8]. The present findings exhibited a concentration dependent inhibition of α-amylase activity by the S1, S2 and S3. The lowest inhibition of α-amylase activity of S1, S2, S3 and Acarbose were 39.36±0.28, 35.42±0.25, 46.19±0.50 and 19.63±0.57 in the concentration of 100µg/ml respectively while the highest inhibition of α-amylase activity of S1, S2, S3 and Acarbose were 84.30±0.58, 76.07±0.52, 61.06±0.95and 86.75±1.02 % in the concentration of 500µg/ml respectively. The greatest effect of S1 (500 µg/ml) was found to be near to standard Acarbose. From the present study it can be concluded that S3 showed marked *in vitro* antidiabetic effect against the α-amylase activity (Table 2 and Figure 1). Present finding is in agreement with Gupta [9] study.

Amylase inhibitors are also known as starch blockers because they prevent dietary starch from being absorbed by the body and thereby lower postprandial glucose levels. Slowing the digestion and breakdown of starch may have beneficial effects on insulin resistance and glycemic index control in people with diabetes. In our investigation we found that compounds moderately inhibited α-amylase.

From the result it was observed that, compound S1 shows good anti diabetic activity than S2, and S3 due to the presence of electron with drawing group [10].

Conclusion

Synthesized a series of new substituted cyanoacetyl hydrazone derivatives obtained with good yield. All the compounds were characterized by using IR, ^{1}H-NMR and ^{13}C-NMR spectroscopy. The synthesized compounds possess potential antidiabetic activity compared to commercial drug Acarbose and hence clearly proved their pharmaceutical and medicinal importance of synthesized compounds. Among the three compounds, S1 has greated activity than S2 and S3. The antidiabetic activity in the following order: S1> S2> S3. Our findings support the reported therapeutic use of this compound as a antidiabetic agent in the Indian system of medicine.

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