

Original Research Article

Synthesis and *in vitro* antidiabetic activity of some alkyl carbazole compounds

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Abstract

Purpose: To synthesize some alkyl derivatives of carbazole and evaluate their *in vitro* inhibitory effect on alpha amylase and alpha glucosidase.

Methods: Synthesis of methylcarbazole, ethylcarbazole, propylcarbazole and butylcarbazole was carried out using acid-catalysed alkylation method while *in vitro* inhibitory assay on alpha amylase and alpha glucosidase enzymes on the synthesized compounds was evaluated using standard procedures. Acarbose was used as the reference compound.

Results: For carbazole, methylcarbazole, ethylcarbazole, propylcarbazole and butylcarbazole, the IC_{50} values of alpha amylase inhibitory assay were 87.47, 50.23, 47.20, 42.36 and 42.11 $\mu\text{g/mL}$ respectively. IC_{50} values of alpha glucosidase inhibitory assay for ethylcarbazole, propylcarbazole and butylcarbazole were 205.30, 153.93 and 152.90 $\mu\text{g/mL}$, respectively. Carbazole and methylcarbazole had no inhibitory effect on this enzyme but the reference drug (acarbose) had a better inhibitory effect towards the two enzymes than the synthesized products.

Conclusion: Some alkyl-carbazoles with anti-diabetic effect have been successfully synthesised. Alkylation of carbazole increased the alpha amylase inhibitory effect of carbazole. The inhibitory effect is directly proportional to the chain length of the alkyl group.

Keywords: Alkyl carbazole, alpha amylase, alpha glucosidase, synthesis

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INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder [1] which is the fourth main cause of death in most developed countries and one of the leading causes of kidney failure, heart attack, blindness and lower limb amputation. It has been projected

by International Diabetes Federation that about 330 million people will be diabetic by the year 2025, if adequate measures are not taken, and over 75 % of this will be in developing countries as compared with 62 % in 1995 [2]. One promising therapeutic approach is to decrease hyperglycaemia by decreasing the postprandial

rise in blood glucose concentration. This is achieved by retarding and reducing the digestion and absorption of ingested carbohydrates through the inhibition of carbohydrate hydrolyzing enzymes such as α -amylase and α -glucosidase [3]. Thus, the search for new leads for antidiabetic drugs with lower cost and better efficiency has therefore become a matter of major priority.

Carbazole and its derivatives are important type of nitrogen containing heterocyclic compounds that are widespread in nature [4]. The chemistry and biology of carbazole have attracted an increasing interest over the last 50 years. The presence of a desirable electronic and charge transport properties, and also large π -conjugated system in carbazole make the introduction of various functional groups into the structurally rigid carbazolyl ring easy, resulting in the extensive potential application of carbazole in the field of medicinal chemistry [5]. Some carbazole derivatives are known to possess anticancer, antimicrobial, anti HIV, antihypertensive, antiparkinsonian, antipsychotic, anti-emetic and antidiabetic activities [6]. Some antidiabetic compounds such as tetrahydroalstonine, lochnerinine, vindoline, harmine, reserpine, vincanidine and vinervine obtained from plants have carbazole-like nucleus which may be essential to their antidiabetic activity.

This work was therefore undertaken to synthesise and test some alkyl-carbazole compounds for their antidiabetic activity by evaluating their inhibitory effects on alpha amylase and alpha glucosidase activities. This could provide a 'lead' to the discovery of novel antidiabetic drugs.

EXPERIMENTAL

Carbazole and alkyl iodide were procured from Sigma-Aldrich, U.S.A. (CAS No: 86-74-8) and Kermel, India, respectively.

Synthesis of alkylcarbazole compounds

Alkylation of carbazole was carried out using acid catalysed synthesis method: carbazole (2.5g, 10 mmol) was dissolved in conc. Sulphuric acid (40 mL) and stirred vigorously in a round-bottomed flask for 2 h using a magnetic stirrer followed by addition of iodoalkane (0.1 mol). The reaction medium was suspended in ice and stirring continued for 5 h. This was rinsed into 100 mL iced water using 10 mL conc. sulphuric acid and stirred for 30 min in a cool water bath. 100 mL of conc. ammonia was further added to basify the

medium and was allowed to stand for 2 h. The cooled reaction medium was extracted using 50 mL acetone and was volatilized at room temperature to obtain crude alkylcarbazole.

Analysis of the compounds

The crude alkylcarbazole was purified by column chromatography using silica-gel (60-120 mesh). Elution was done with the following solvent systems: n-hexane (100%), n-hexane:chloroform (90:10v/v to 10:90v/v), chloroform (100%), chloroform:acetone (90:10v/v to 10:90v/v), and acetone (100%). Alkylcarbazole was obtained in the chloroform:acetone (30:70v/v) solvent system. TLC analysis of the alkyl carbazole fraction carried out on silica gel precoated plate (0.25mm) using toluene: ethyl acetate (7:2v/v) as mobile phase produced one spot.

Each of the synthesized compounds (10 mg) was dissolved in 25 mL of cyclohexane and pH of the solution was determined using Luton Electronic pH meter. The melting point was also determined using the Electrothermal Melting Point Apparatus (England. CAT. No. 1A6304).

The synthesized compounds were each dissolved in chloroform and the absorbance was measured at wavelength 200-400 nm using Spectro UV-VIS Dual Beam (8 Auto Cell UVS-270; Lambomed, Inc, USA). FTIR spectra of the synthesized compounds were obtained using FTIR-8400S Fourier Transform Infrared Spectrophotometer.

Ten milligram each of synthesized compound was analysed in GCMS equipment (GCMS-QP2010 PLUS) for molecular weight elucidation with the following acquisition parameters: Oven temperature programme (temperature and hold time, 70 °C and 280 °C, 0 and 5 minutes, respectively); Column flow (1.80 mL/min); Total flow (40.8 mL/min); purge flow (3 mL/min); washing volume (8 μ L); ion source temperature (200 °C); MS (start time, 3 mins, end time, 24 mins, event time, 0.50s). Identification of the compounds was done using the database of National Institute of Science and Technology.

Evaluation of alpha-amylase inhibitory activity

Assay was carried out according to the method reported by Sindhu *et al* [7]. The assay mixture containing 200 μ L of 0.02 M sodium phosphate buffer, 20 μ L of enzyme and the synthesized products in concentration range 20-100 μ g/mL were incubated for 10 minutes at room

temperature followed by addition of 200 μL of starch in all test tubes. The reaction was terminated with addition of 400 μL dinitrosalicylic acid reagent and placed in boiling water bath for 5 min, cooled and diluted with 15 mL of distilled water and absorbance was measured at 540 nm. The control sample was prepared without any synthesized product. The percentage Inhibition was calculated as:

$$\text{Inhibition (\%)} = (A_{\text{control}} - A_{\text{compound}}) \times 100/A_{\text{control}}$$

The concentration of alkylcarbazole required to inhibit 50 % of α -amylase activity was defined by the IC_{50} value determined from plot of percent inhibition versus log inhibitor concentration and was calculated by nonlinear regression analysis from the mean inhibitory values. Acarbose was used as the reference alpha-amylase inhibitor (positive control) and all tests were performed in triplicate.

Evaluation of alpha-glucosidase inhibitory activity

Alpha glucosidase inhibition assay was performed as reported by Adefegha and Oboh [8]. Alpha glucosidase was dissolved in 100 mM phosphate buffer (pH =6.8) and was used as the enzyme extract. p-Nitrophenyl- α -D-glucopyranoside was used as the substrate. Synthesized product were used in the concentration ranging from 20-100 $\mu\text{g/mL}$. Different concentrations of synthesized products were mixed with 320 μL of 100 mM phosphate buffer for 5 min. 3 mL of 50 mM sodium hydroxide was added to the mixture and the absorbance was read at 410 nm. The control samples were prepared without any synthesized products. The percentage inhibition was calculated as:

$$\text{Inhibition (\%)} = (A_{\text{control}} - A_{\text{compound}}) \times 100/A_{\text{control}}$$

The concentration of alkylcarbazole require to inhibit 50% of α -amylase activity was defined by the IC_{50} value determined from plot of percent inhibition versus log inhibitor concentration and was calculated by nonlinear regression analysis from the mean inhibitory values. Acarbose was used as the reference alpha-amylase inhibitor (positive control) and all tests were performed in triplicate.

RESULTS

Methylcarbazole: R_f , 0.68; (pH , 6.80); Melting points, 207-209 $^{\circ}\text{C}$; λ_{max} in chloroform and absorbance (309.00 nm, 1.979); IR absorption: 3423.76 (N-H), 1643.41 (C=C (Alkene)), 1452.45

(C=C (Aromatic)), 1449.55 (-CH₃ (bend)); Formula: C₁₃H₁₁N. Mol 182. GCMS Fragmentation: (m/e, Fragment) – 15 (CH₃ (methyl)), 167 (C₁₂H₉N (Carbazole)), 168 (C₁₂H₉N M+1), 181 (C₁₃H₁₁N (methylcarbazole)), 182 (C₁₃H₁₁N M+1).

Ethylcarbazole: R_f , 0.66; (pH, 6.33); Melting point, 142-145 $^{\circ}\text{C}$; λ_{max} in chloroform and absorbance (306.00 nm, 1.979); IR absorption: 3417.97 (N-H), 2892.04 (C-H Alkane (stretch)), 1627.01 (C=C (Aromatic)), 1465.17 (C=C (bend)), 1449.72 (-CH₃ (bend)); Formula: C₁₄H₁₃N. Mol Weight: 195. GCMS Fragmentation: (m/e, Fragment) - 15 (CH₃ (methyl)), 29 (CH₂CH₃ (ethyl)), 119 (C₈H₉N (indole)), 167 (C₁₂H₉N (Carbazole)), 195 (C₁₄H₁₃N (ethylcarbazole)), Ethylcarbazole.

Propylcarbazole: R_f , 0.65; (pH, 5.72); Melting points, 120-123 $^{\circ}\text{C}$; λ_{max} in chloroform and absorbance (346.00 nm, 1.915); IR absorption: 3427.62 (N-H), 2925.15 (C-H Alkane (stretch)), 1642.44 (C=C (Aromatic)), 1461.43 (-CH₂ (bend)), 1345.39 (-CH₃ (bend)); Formula: C₁₅H₁₅N. Mol Weight: 209. GCMS Fragmentation: (m/e, Fragment) - 15 (CH₃ (methyl)), 29 (CH₂CH₃ (ethyl)), 43 (CH₂CH₂CH₃ (propyl)), 119 (C₈H₉N (indole)), 167 (C₁₂H₉N (Carbazole)), 209 (C₁₅H₁₅N (propylcarbazole)).

Tert-butylcarbazole: R_f , 0.63; pH, 5.46; Melting points, 96-99 $^{\circ}\text{C}$; λ_{max} in chloroform and absorbance (350.00 nm, 1.928); IR absorption: 3417.01(N-H), 2878.77 (C-H Alkane (stretch)), 1634.73 (C=C (Aromatic)), 1464.66 (-CH₂ (bend)), 1449.55 (-CH₃ (bend)); Formula: C₁₆H₁₇N. Mol Weight: 223. GCMS Fragmentation: (m/e, Fragment) - 15 (CH₃ (methyl)), 27 (CCH₃), 42 (C (CH₃)₂), 57 (C (CH₃)₃), 119 (C₈H₉N (indole)), 167(C₁₂H₉N (Carbazole)), 223 (C₁₆H₁₇N (Tert-butylcarbazole)).

Result of *in vitro* alpha amylase and alpha glucosidase inhibitory assays are shown in Figure.

DISCUSSION

Methylcarbazole, ethylcarbazole, propylcarbazole and butylcarbazole have been successfully synthesized by alkylation of carbazole. *In vitro* inhibitory assay of synthesized products on alpha amylase showed a significant increase in inhibitory effect of the alkylcarbazole derivatives with increasing chain length of alkyl group.

The IC_{50} value of carbazole, methylcarbazole, ethylcarbazole, propylcarbazole, butylcarbazole

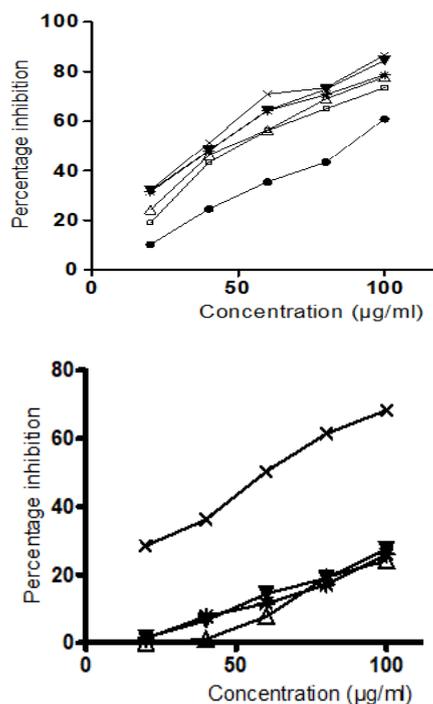


Figure 1: Percentage inhibition of (A) alpha amylase enzyme by carbazole, methylcarbazole, ethylcarbazole, propylcarbazole, butylcarbazole and acarbose, and (B) alpha glucosidase enzyme by ethylcarbazole, propylcarbazole, butylcarbazole and acarbose. Key: (□) methyl carbazole, (Δ) ethylcarbazole, (*) propylcarbazole, (▼) butylcarbazole, (●) carbazole, (X) acarbose

and acarbose (reference drug) decreased with increase in chain length of alkyl groups. This shows that inhibitory effect towards alpha amylase and hence antidiabetic activity increased with increase in alkyl group chain length (Figure 1). The alpha amylase inhibitory activity of propyl and butyl carbazole was comparable to that of Acarbose. For alphasglucosidase inhibitory activity, the IC_{50} values are 205.30, 153.93, 152.90 and 59.57 $\mu\text{g/mL}$ for ethylcarbazole, propylcarbazole, butylcarbazole and acarbose (reference drug) respectively. Carbazole, methylcarbazole and 20 $\mu\text{g/mL}$ ethylcarbazole did not inhibit the activity of alpha glucosidase (Figure 2). These results showed that the inhibitory effect of ethylcarbazole (40 – 100 $\mu\text{g/mL}$), propylcarbazole and butylcarbazole as compared with the reference drug (Acarbose) was not significant.

One way of reducing hyperglycaemia is to inhibit the activities of alpha amylase and alpha glucosidase. This is because pancreatic alpha amylase catalyses the breaking down of starch into disaccharides and oligosaccharides while intestinal alpha glucosidase catalyzes the breakdown of disaccharides to release glucose

which is later absorbed from small intestine into the blood circulation [9]. The alkyl carbazole compounds synthesized in this work inhibited alpha amylase activity and therefore may have the potential of reducing hyperglycaemia.

CONCLUSION

Four alkyl-carbozoles (methyl-, ethyl-, propyl- and butyl- carbazoles) with anti-diabetic effect have been successfully synthesised. These compounds have significant inhibitory activities against alpha amylase but alpha glucosidase. Alkylation of carbazole increases the alpha amylase inhibitory effect of carbazole. This inhibitory effect is directly proportional to the chain length of the alkyl group. Alkyl carbazole compounds could therefore serve as lead for novel alpha amylase inhibitory and hence antidiabetic compounds.

DECLARATIONS

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Conflict of interest

No conflict of interest associated with this work.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

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