Journal of Carcinogenesis
Covering Genesis to Therapeutics

Journal of Carcinogenesis, ISSN: 1477-3163
2025; Vol 24: Issue 2, https://carcinogenesis.com/

Synthesis, characterization and in vitro α-amylase inhibition potential of novel benzimidazole derivatives.

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ABSTRACT

Diabetes has become one of the foremost health concerns around the world, particularly in developing nations. By 2030, highest count of people encountering diabetes are anticipated to live in these areas. The amount of persons suffering from diabetes in India has grown dramatically. Estimates say that the number of cases would upsurge from 31.4 million in 2000 to 79.4 million by 2030, with urban populations having far higher rates than rural populations. This project aimed to synthesized and evaluate new benzimidazole derivatives as possible anti-diabetic drugs, focusing on their ability to block α-amylase. Using a simple two-step synthetic technique, benzimidazole derivatives were prepared and their properties were studied using spectral methods. The synthesized derivatives blocked α-amylase to different degrees, and a few of them worked as well as the conventional inhibitor acarbose. The results show that changing the structure at the 1-position of the benzimidazole nucleus affects the biological potency, with some substitutions making the inhibitory effects stronger. This study adds to the existing knowledge about benzimidazole derivatives as possible treatments for diabetes and lays the groundwork for more research and development in this area.

Keywords: *Benzimidazole, α-amylase, anti-diabetic, naphthyl, acarbose*

How to Cite: Megha Mishra, Gaurav Jain, Phool Singh, Kavita R Loksh, (2025) Synthesis, characterization and in vitro α-amylase inhibition potential of novel benzimidazole derivatives., *Journal of Carcinogenesis*, *Vol.24*, *No.2*, 134-143

1. INTRODUCTION

Diabetes constitutes a range of metabolic disorders that has emerged as a substantial universal health challenge[1]. The extraordinary economic growth and rapid expansion in Asian region, especially Indian subcontinent, have transitioned health problems from communicable towards non-communicable diseases. By 2030, more than 85 percent of the global diabetes population would reside in developing nations. The dominance of diabetic patients in India is anticipated to escalate to 79.4 million by 2030[2]. The frequency of Type 2 diabetes (T2D) is many a times greater in metropolitan regions than in rural regions[3]. The frequency of impaired glucose tolerance (IGT) among the village residents is notably high (7-8%), suggesting hereditary predisposition for T2D within the folkloric Indian demographic[4]. The Diabetes Control agencies have established that effective metabolic regulation, achieved through rigorous insulin treatment, diminished the threat of development of retinopathy, nephropathy, and neuropathy[5].

Some therapeutic agents are used to cure diabetes mellitus may be classified as α-glycosidase inhibitors, thiazolidinediones, biguanides, sulfonylureas, and gliptins.[6155] Various heterocyclic core containing structures like pyrrole, pyrazole, isatin, benzimidazole, triazole, oxadiazole, thiazolidinone, thiadiazole, benzoxazole, coumarin, pyrimidine, etc. have been reported to possess potent anti-diabetic actions by mechanisms involving α-amylase/α-glucosidase/dipeptidyl peptidase (DPP)-IV inhibition and/or peroxisome proliferator activated receptor (PPAR)-γ agonism.7,8 Benzimidazole-based compounds represent a constantly advancing and progressively dynamic area owing to their extensive prospective uses as pharmaceuticals, agricultural chemicals, synthetic compounds, artificial acceptors, supramolecular ligands, and biomimetic catalysts, among others9. Derivatives of benzimidazole have shown many pharmacological actions like antihypertensive, anticancer, antiviral, antidiabetic, antimicrobial etc10. Benzimidazole is intensively researched as a heterocyclic scaffold due to its structural similarity to purine-based nucleic acids, as they are isosteres of one another. Benzimidazoles are widely studied for their anti-diabetic action and reported to be potent comparable to standard drugs11. The substitution on 2-position or the nitrogen at position 1 of benzimidazole has been utilized primarily for imparting most of its pharmacological actions. Herein we report an efficient and simple synthesis of 1-

heterocycle/naphthyl linked to benzimidazole nitrogen with a carbon bridge as probable anti-diabetic compounds by α-amylase inhibition assay.

2. MATERIAL AND METHODS

General

The chemicals have been acquired from Loba Chemie, Sigma Aldrich, and CDH, and utilized unprocessed. Open capillary procedure was utilized to ascertain the melting temperatures of produced substances. Conventional literature methods were employed for solvent drying. Thin-layer chromatography (TLC) was steered using precoated Merck silica sheets using ethyl acetate/hexane solvent solution, followed by the observation of spots at 254 nm. The ^{1}H NMR (400 MHz) spectra were acquired in chloroform (CDCl3) using a Bruker Avance III spectrometer. The infrared spectra of synthesized compounds were obtained using an Agilent spectrophotometer using KBr powder as a reference in the range of 4,000–400 cm^{-1}. HRMS were obtained using a Jeol mass spectrometer.

Synthesis of 3a-h

2-phenylene diamine (1mmol) along with ammonium chloride (4mmol) were dissolved in 5 mL chloroform and stirred for 5 minutes. To it was added appropriate aldehyde (1mmol) and stirred till 4 hours. The reaction was observed by TLC (hexane-ethylacetate, 3.5-1.5). The mixture was kept aside to remove the solvent and then moved to a separating funnel with ethylacetate. It was washed using water (2x 10 mL) and the layers were separated. The product was collected in the organic layer and recrystallized using ethanol[12].

General method for synthesis of 4a-h

In a clean dry round bottom flask, introduce 10 mL ethanol and 0.01 mol sodium hydroxide and stir for 10 min. To this solution add 0.01 mol of step 1 product and continue stirring for 10 min. To it was mixed 0.01 mol of 1-(2-chloroethyl)piperidine and refluxed for 6 hour. Upon cooling, a solid was isolated, collected via filtering, dried, and recrystallized from ethanol[13].

General method to synthesize 5a-h

In a clean dry round bottom flask, introduce 10 mL ethanol and 0.01 mol sodium hydroxide and stir for 10 min. To this solution add 0.01 mol of step 1 product and continue stirring for 10 min. To the it was mixed 0.01 mol of 1-(chloromethyl) naphthyridine and refluxed for 6 hour. Upon cooling, a solid was isolated, collected via filtering, dried, and recrystallized from ethanol[13].

General method for synthesis of 6a-h

In a clean dry round bottom flask, introduce 10 mL ethanol and 0.01 mol sodium hydroxide and stir for 10 min. To this solution add 0.01 mol of step 1 product and continue stirring for 10 min. To it was mixed 0.01 mol of 2-(chloromethyl)pyridine and heated under reflux conditions for 6 hour. Upon cooling, a solid was isolated, collected via filtering, dried, and recrystallized from ethanol[13].

2-phenyl-1-(2-(piperidinyl)ethyl)-benzimidazole (4a)

Yield (%): 64; M.P (°C): 160; mass (m/z): 307; FTIR (cm^{-1}): 3100-3000 (s, C-H, Ar), 3000-2920 (m, C-H), 1570 (s, C=N), 1480 (m, CH_2 bend), 1440-1400 (w, C=C, Ar), 900-650 (s, C-H bend, oop); ^{1}HNMR (δ, ppm): 7.08-8.00 (C-H, Ar), 5.44-4.04 (CH_2, adj $N_{benzimidazole}$), 3.52 (CH_2, adj $N_{piperidine}$), 3.05-1.27 (CH_2 piperidine)

2-(2-nitrophenyl)- 1-(2-(piperidinyl)ethyl)-benzimidazole (4b)

Yield (%): 70; M.P (°C): 152; mass (m/z): 335; FTIR (cm^{-1}): 3100-3000 (s, C-H, Ar), 3000-2920 (m, C-H), 1570 (s, C=N), 1480 (m, CH_2 bend), 1440-1400 (w, C=C, Ar), 1380 (s, NO_2), 900-650 (s, C-H bend, oop); ^{1}HNMR (δ, ppm): 7.08-8.00 (C-H, Ar), 5.44-4.04 (CH_2, adj $N_{benzimidazole}$), 3.52 (CH_2, adj $N_{piperidine}$), 3.05-1.27 (CH_2 piperidine)

4-(1-(2-(piperidinyl)ethyl)-benzimidazole-2-yl)phenol (4c)

Yield (%): 68; M.P (°C): 159; mass (m/z): 286; FTIR (cm^{-1}): 3800-3500 (m, O-H), 3100-3000 (s, C-H, Ar), 3000-2920 (m, C-H), 1570 (s, C=N), 1480 (m, CH_2 bend), 1440-1400 (w, C=C, Ar), 900-650 (s, C-H bend, oop); ^{1}HNMR (δ, ppm): 7.08-8.00 (C-H, Ar), 5.44-4.04 (CH_2, adj $N_{benzimidazole}$), 3.52 (CH_2, adj $N_{piperidine}$), 3.05-1.27 (CH_2 piperidine)

2-(3,4-dimethylphenyl)- 1-(2-(piperidinyl)ethyl)-benzimidazole (4d)

Yield (%): 67; M.P (°C): 160; mass (m/z): 352; FTIR (cm^{-1}): 3100-3000 (s, C-H, Ar), 3000-2920 (m, C-H), 1570 (s, C=N), 1480 (m, CH_2 bend), 1440-1400 (w, C=C, Ar), 900-650 (s, C-H bend, oop); ^{1}HNMR (δ, ppm): 7.08-8.00 (C-H, Ar), 5.44-4.04 (CH_2, adj $N_{benzimidazole}$), 3.52 (CH_2, adj $N_{piperidine}$), 3.05-1.27 (CH_2 piperidine)

2-(4-chlorophenyl)- 1-(2-(piperidinyl)ethyl)-benzimidazole (4e)

Yield (%): 69; M.P (°C): 138; mass (m/z): 381; FTIR (cm^{-1}): 3100-3000 (s, C-H, Ar), 3000-2920 (m, C-H), 1570 (s, C=N),

1480 (m, CH_2 bend), 1440-1400 (w, C=C, Ar), 900-650 (s, C-H bend, oop), 630 (s, C-Cl); ^{1}HNMR (δ, ppm): 7.08-8.00 (C-H, Ar), 5.44-4.04 (CH_2, adj $N_{benzimidazole}$), 3.52 (CH_2, adj $N_{piperidine}$), 3.05-1.27 (CH_2 piperidine)

(E)-1-(2-(piperidinyl)ethyl)-2-styryl-benzimidazole (4f)

Yield (%): 69; M.P (°C): 183; mass (m/z): 331; FTIR (cm^{-1}): 3100-3000 (s, C-H, Ar), 3000-2920 (m, C-H), 1570 (s, C=N), 1480 (m, CH_2 bend), 1440-1400 (w, C=C, Ar), 900-650 (s, C-H bend, oop); ^{1}HNMR (δ, ppm): 7.08-8.00 (C-H, Ar), 5.44-4.04 (CH_2, adj $N_{benzimidazole}$), 3.52 (CH_2, adj $N_{piperidine}$), 3.05-1.27 (CH_2 piperidine)

2-(4-methoxyphenyl)- 1-(2-(piperidinyl)ethyl)-benzimidazole (4g)

Yield (%): 61; M.P (°C): 152; mass (m/z): 323; FTIR (cm^{-1}): 3100-3000 (s, C-H, Ar), 3000-2920 (m, C-H), 1570 (s, C=N), 1480 (m, CH_2 bend), 1440-1400 (w, C=C, Ar), 1350-1000 (s, C-O, ether), 900-650 (s, C-H bend, oop); ^{1}HNMR (δ, ppm): 7.08-8.00 (C-H, Ar), 5.44-4.04 (CH_2, adj $N_{benzimidazole}$), 4.54 (CH_3, methoxy), 3.52 (CH_2, adj $N_{piperidine}$), 3.05-1.27 (CH_2 piperidine)

2-methoxy-4-(1-(2-(piperidinyl)ethyl)-benzimidazole -2-yl)phenol (4h)

Yield (%): 62; M.P (°C): 151; mass (m/z): 351; FTIR (cm^{-1}): 3800-3500 (m, O-H), 3100-3000 (s, C-H, Ar), 3000-2920 (m, C-H), 1570 (s, C=N), 1480 (m, CH_2 bend), 1440-1400 (w, C=C, Ar), 1350-1000 (s, C-O, ether), 900-650 (s, C-H bend, oop); ^{1}HNMR (δ, ppm): 7.08-8.00 (C-H, Ar), 5.44-4.04 (CH_2, adj $N_{benzimidazole}$), 4.54 (CH_3, methoxy), 3.52 (CH_2, adj $N_{piperidine}$), 3.05-1.27 (CH_2 piperidine)

1-(naphthalen-2-ylmethyl)-2-phenyl-1H-indole (5a)

Yield (%): 65; M.P (°C): 240; mass (m/z): 302; FTIR (cm^{-1}): 3100-3000 (s, C-H, Ar), 3000-2920 (m, C-H), 1570 (s, C=N), 1480 (m, CH_2 bend), 1440-1400 (w, C=C, Ar), 900-650 (s, C-H bend, oop); ^{1}HNMR (δ, ppm): 7.28-8.15 (C-H, Ar), 5.04-5.14 (CH_2, adj $N_{benzimidazole}$)

1-(naphthalen-2-ylmethyl)-2-(2-nitrophenyl)-1H-indole (5b)

Yield (%): 66; M.P (°C): 215; mass (m/z): 335; FTIR (cm^{-1}): 3100-3000 (s, C-H, Ar), 3000-2920 (m, C-H), 1570 (s, C=N), 1480 (m, CH_2 bend), 1440-1400 (w, C=C, Ar), 1380 (s, NO_2), 900-650 (s, C-H bend, oop); ^{1}HNMR (δ, ppm): 7.28-8.15 (C-H, Ar), 5.04-5.14 (CH_2, adj $N_{benzimidazole}$)

4-(1-(naphthalenylmethyl)-indolyl)phenol (5c)

Yield (%): 65; M.P (°C): 226; mass (m/z): 363; FTIR (cm^{-1}): 3800-3500 (m, O-H), 3100-3000 (s, C-H, Ar), 3000-2920 (m, C-H), 1570 (s, C=N), 1480 (m, CH_2 bend), 1440-1400 (w, C=C, Ar), 900-650 (s, C-H bend, oop); ^{1}HNMR (δ, ppm): 7.28-8.15 (C-H, Ar), 5.04-5.14 (CH_2, adj $N_{benzimidazole}$)

2-(3,4-dimethylphenyl)-1-(naphthalen-2-ylmethyl)-1H-indole (5d)

Yield (%): 72; M.P (°C): 280; mass (m/z): 314; FTIR (cm^{-1}): 3100-3000 (s, C-H, Ar), 3000-2920 (m, C-H), 1570 (s, C=N), 1480 (m, CH_2 bend), 1440-1400 (w, C=C, Ar), 900-650 (s, C-H bend, oop); ^{1}HNMR (δ, ppm): 7.28-8.15 (C-H, Ar), 5.04-5.14 (CH_2, adj $N_{benzimidazole}$)

2-(4-chlorophenyl)-1-(naphthalen-2-ylmethyl)-1H-indole (5e)

Yield (%): 70; M.P (°C): 216; mass (m/z): 341; FTIR (cm^{-1}): 3100-3000 (s, C-H, Ar), 3000-2920 (m, C-H), 1570 (s, C=N), 1480 (m, CH_2 bend), 1440-1400 (w, C=C, Ar), 900-650 (s, C-H bend, oop), 630 (s, C-Cl); ^{1}HNMR (δ, ppm): 7.28-8.15 (C-H, Ar), 5.04-5.14 (CH_2, adj $N_{benzimidazole}$)

(E)-1-(naphthalen-2-ylmethyl)-2-styryl-1H-indole (5f)

Yield (%): 69; M.P (°C): 249; mass (m/z): 369; FTIR (cm^{-1}): 3100-3000 (s, C-H, Ar), 3000-2920 (m, C-H), 1570 (s, C=N), 1480 (m, CH_2 bend), 1440-1400 (w, C=C, Ar), 900-650 (s, C-H bend, oop); ^{1}HNMR (δ, ppm): 7.28-8.15 (C-H, Ar), 5.04-5.14 (CH_2, adj $N_{benzimidazole}$)

2-(4-methoxyphenyl)-1-(naphthalen-2-ylmethyl)-1H-indole (5g)

Yield (%): 61; M.P (°C): 219; mass (m/z): 320; FTIR (cm^{-1}): 3100-3000 (s, C-H, Ar), 3000-2920 (m, C-H), 1570 (s, C=N), 1480 (m, CH_2 bend), 1440-1400 (w, C=C, Ar), 1350-1000 (s, C-O, ether), 900-650 (s, C-H bend, oop); ^{1}HNMR (δ, ppm): 7.28-8.15 (C-H, Ar), 5.04-5.14 (CH_2, adj $N_{benzimidazole}$), 4.54 (CH_3, methoxy)

2-methoxy-4-(1-(naphthalenylmethyl)-indolyl)phenol (5h)

Yield (%): 62; M.P (°C): 244; mass (m/z): 333; FTIR (cm^{-1}): 3800-3500 (m, O-H), 3100-3000 (s, C-H, Ar), 3000-2920 (m, C-H), 1570 (s, C=N), 1480 (m, CH_2 bend), 1440-1400 (w, C=C, Ar), 1350-1000 (s, C-O, ether), 900-650 (s, C-H bend, oop); ^{1}HNMR (δ, ppm): 7.28-8.15 (C-H, Ar), 5.04-5.14 (CH_2, adj $N_{benzimidazole}$), 4.54 (CH_3, methoxy

2-phenyl-1-(pyridin-2-ylmethyl)-1H-indole (6a)

Yield (%): 62; M.P (°C): 167; mass (m/z): 361; FTIR (cm^{-1}): 3100-3000 (s, C-H, Ar), 3000-2920 (m, C-H), 1570 (s, C=N), 1480 (m, CH_2 bend), 1440-1400 (w, C=C, Ar), 900-650 (s, C-H bend, oop); ^{1}HNMR (δ, ppm): 7.07-8.59 (C-H, Ar), 5.55 (CH_2, adj $N_{benzimidazole}$)

2-(2-nitrophenyl)-1-(pyridin-2-ylmethyl)-1H-indole (6b)

Yield (%): 65; M.P (°C): 154; mass (m/z): 312; FTIR (cm^{-1}): 3100-3000 (s, C-H, Ar), 3000-2920 (m, C-H), 1570 (s, C=N), 1480 (m, CH_2 bend), 1440-1400 (w, C=C, Ar), 1380 (s, NO_2), 900-650 (s, C-H bend, oop); ^{1}HNMR (δ, ppm): 7.07-8.59 (C-H, Ar), 5.55 (CH_2, adj $N_{benzimidazole}$)

4-(1-(pyridinylmethyl)-indolyl)phenol (6c)

Yield (%): 67; M.P (°C): 164; mass (m/z): 337; FTIR (cm^{-1}): 3800-3500 (m, O-H), 3100-3000 (s, C-H, Ar), 3000-2920 (m, C-H), 1570 (s, C=N), 1480 (m, CH_2 bend), 1440-1400 (w, C=C, Ar), 900-650 (s, C-H bend, oop); ^{1}HNMR (δ, ppm): 7.07-8.59 (C-H, Ar), 5.55 (CH_2, adj $N_{benzimidazole}$)

2-(3,4-dimethylphenyl)-1-(pyridin-2-ylmethyl)-1H-indole (6d)

Yield (%): 63; M.P (°C): 164; mass (m/z): 365; FTIR (cm^{-1}): 3100-3000 (s, C-H, Ar), 3000-2920 (m, C-H), 1570 (s, C=N), 1480 (m, CH_2 bend), 1440-1400 (w, C=C, Ar), 900-650 (s, C-H bend, oop); ^{1}HNMR (δ, ppm): 7.07-8.59 (C-H, Ar), 5.55 (CH_2, adj $N_{benzimidazole}$)

2-(4-chlorophenyl)-1-(pyridin-2-ylmethyl)-1H-indole (6e)

Yield (%): 71; M.P (°C): 144; mass (m/z): 316; FTIR (cm^{-1}): 3100-3000 (s, C-H, Ar), 3000-2920 (m, C-H), 1570 (s, C=N), 1480 (m, CH_2 bend), 1440-1400 (w, C=C, Ar), 900-650 (s, C-H bend, oop), 630 (s, C-Cl); ^{1}HNMR (δ, ppm): 7.07-8.59 (C-H, Ar), 5.55 (CH_2, adj $N_{benzimidazole}$)

1-(pyridinylmethyl)-2-styrylindole (6f)

Yield (%): 62; M.P (°C): 201; mass (m/z): 353; FTIR (cm^{-1}): 3100-3000 (s, C-H, Ar), 3000-2920 (m, C-H), 1570 (s, C=N), 1480 (m, CH_2 bend), 1440-1400 (w, C=C, Ar), 900-650 (s, C-H bend, oop); ^{1}HNMR (δ, ppm): 7.07-8.59 (C-H, Ar), 5.55 (CH_2, adj $N_{benzimidazole}$)

2-(4-methoxyphenyl)-1-(pyridinylmethyl)indole (6g)

Yield (%): 69; M.P (°C): 157; mass (m/z): 381; FTIR (cm^{-1}): 3100-3000 (s, C-H, Ar), 3000-2920 (m, C-H), 1570 (s, C=N), 1480 (m, CH_2 bend), 1440-1400 (w, C=C, Ar), 1350-1000 (s, C-O, ether), 900-650 (s, C-H bend, oop); ^{1}HNMR (δ, ppm): 7.07-8.59 (C-H, Ar), 5.55 (CH_2, adj $N_{benzimidazole}$), 4.54 (CH_3, methoxy)

2-methoxy-4-(1-(pyridinylmethyl)indolyl)phenol (6h)

Yield (%): 64; M.P (°C): 151; mass (m/z): 332; FTIR (cm^{-1}): 3800-3500 (m, O-H), 3100-3000 (s, C-H, Ar), 3000-2920 (m, C-H), 1570 (s, C=N), 1480 (m, CH_2 bend), 1440-1400 (w, C=C, Ar), 1350-1000 (s, C-O, ether), 900-650 (s, C-H bend, oop); ^{1}HNMR (δ, ppm): 7.07-8.59 (C-H, Ar), 5.55 (CH_2, adj $N_{benzimidazole}$), 4.54 (CH_3, methoxy)

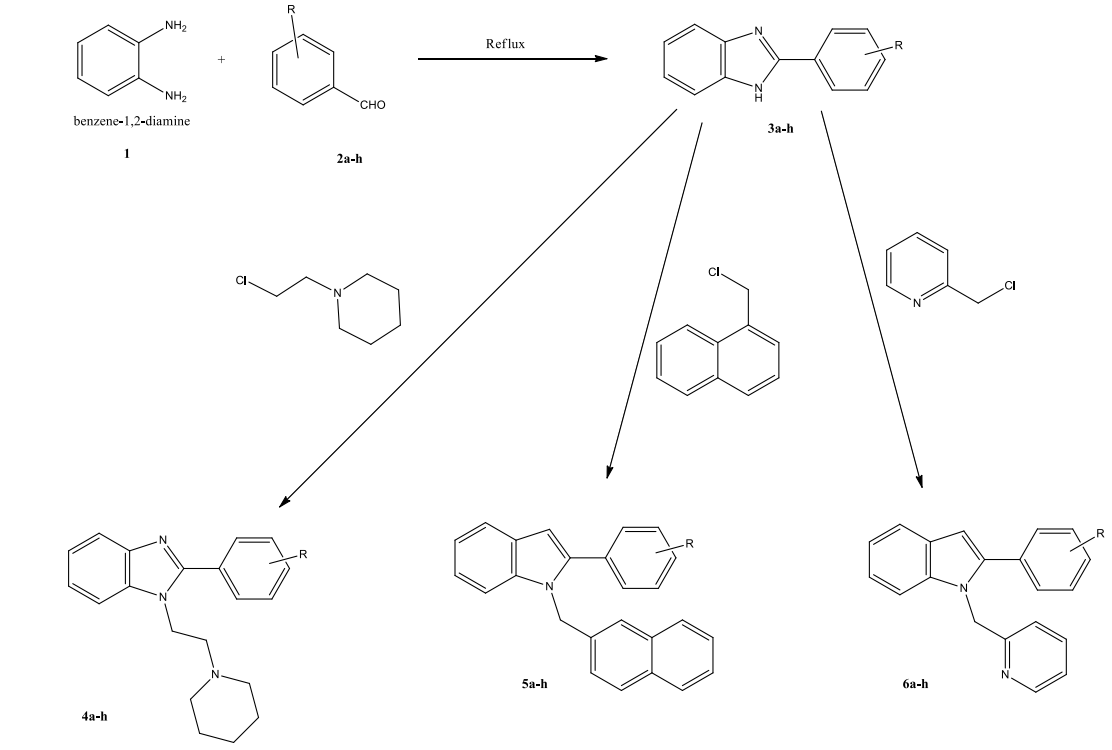
α-amylase inhibition action

The α-amylase blockage was assessed as per a previously published protocol. A 600 µL aliquot of the test sample (50 to 250 µg/mL) and 0.6 mL of solution of α-amylase (0.5 mg/mL) were kept for 10 min incubation at 25 °C. Following it, 0.5mL of 1% starch solution was mixed and 10 min incubated was done at 25 °C. The reaction was terminated using dinitrosalicylic acid colour reagent (1mL). The test samples were subsequently kept for incubation in water at 100 °C for 5 minutes and then cooled to ambient temperature. Dilution of these solutions to 10 mL by deionized water was done, and the absorbance measured at 540 nm[14] using acarbose for comparison. The calculation of percent inhibition was as follows:

$$\%\ \boldsymbol{amylase\ inhibtion} = \frac{\boldsymbol{Acontrol - Asample}}{\boldsymbol{Acontrol}} \times \mathbf{100}$$

where $A_{control}$ and A_{sample} represent the observed absorbance of the control and sample, respectively.

The IC_{50} value of each solution was calculated utilizing Graph Pad Prism.



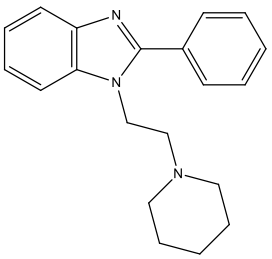
Substituted benzaldehydes used
Benzaldehyde, 2-nitrobenzaldehyde, 4-hydroxybenzaldehyde, 4-chlorobenzaldehyde, cinnamladehyde, 3,4-dimethyl benzaldehyde, vanillin, 4-methoxybenzaldehyde

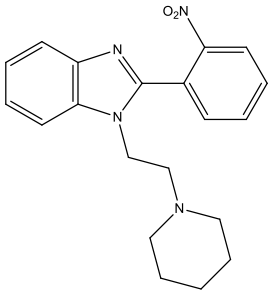
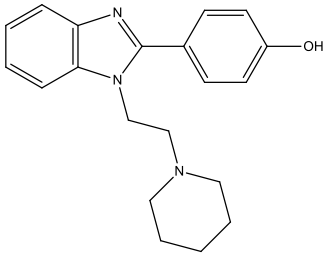
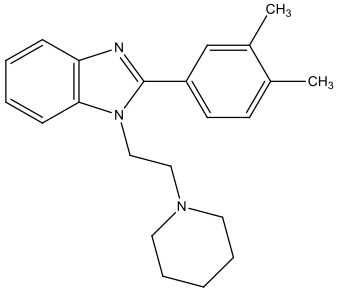
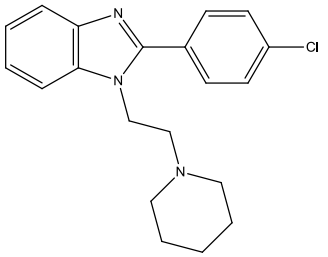
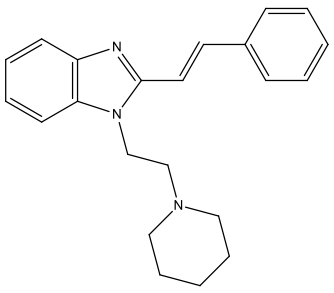
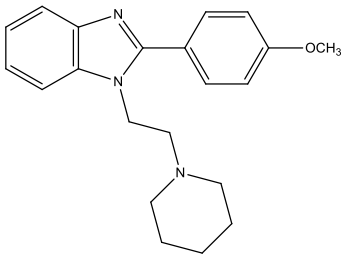
Scheme 1 Synthetic pathway

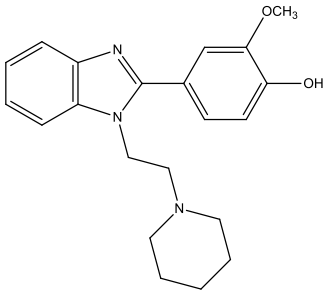
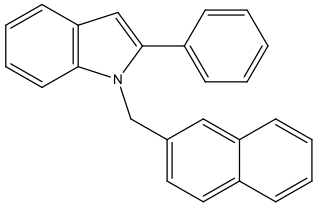
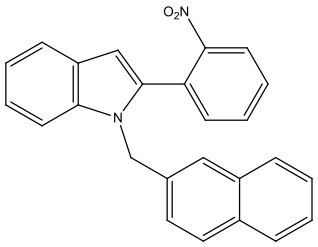
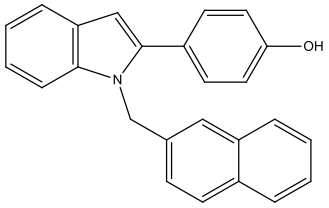
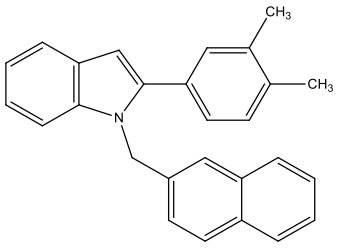
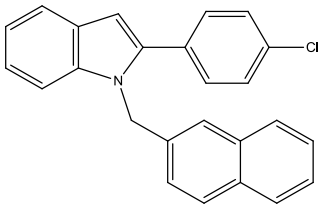
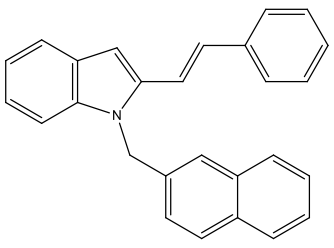
3. RESULTS AND DISCUSSION

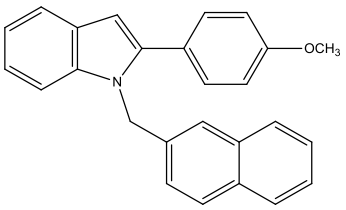
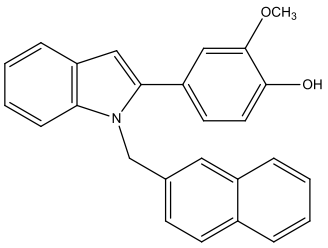
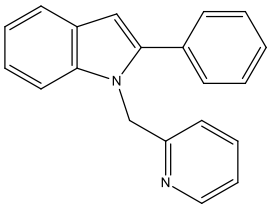
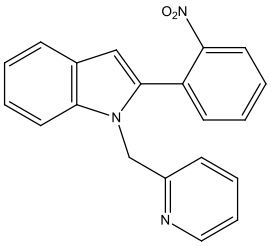
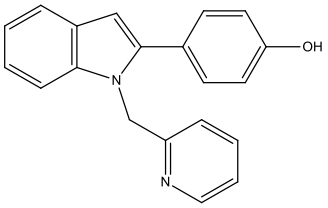
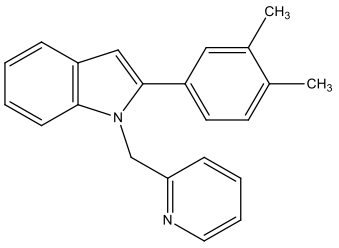
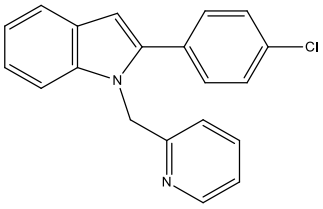
Several aromatic aldehydes and o-phenylene diamines on reaction afforded various 2-substituted benzimidazoles (3a-h). These benzimidazoles were reacted with 1-(2-chloroethyl)piperidine (4a-h), 1-(chloromethyl) naphthyridine (5a-h) and 2-(chloromethyl)pyridine (6a-h) to obtain the target compounds (Table 1).

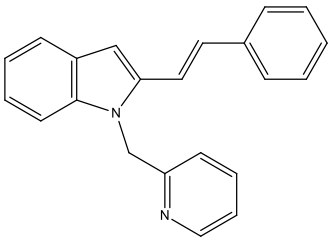
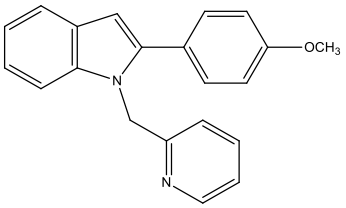
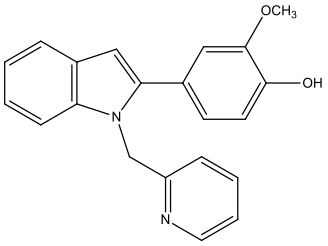
Table 1. *In vitro* α-amylase inhibition by benzimidazoles

Compound	Structure	α-Amylase inhibition IC_{50} (µg/mL)
4a		434.91

4b		34.28
4c		438.03
4d		596.03
4e		84.17
4f		632.77
4g		148.70

4h		185.97
5a		1285.72
5b		462.13
5c		764.22
5d		627.99
5e		464.78
5f		1027.25

5g		549.07
5h		589.22
6a		535.43
6b		77.99
6c		469.86
6d		570.68
6e		225.09

6f		650.22
6g		183.00
6h		219.66
Acarbose	-	7.87

The synthesis was achieved in two simple steps, the first involving condensation of ortho phenylene diamine with aromatic aldehydes to yield the benzimidazole (3a-h) followed by nucleophilic substitution on the nitrogen of the benzimidazole under alkaline conditions. The synthetic confirmation of the compounds was achieved through spectral study where the FTIR spectrum presented stretching and bending vibrations due to the presence of aromatic and aliphatic functional groups and imine in all the compounds and due to C-O, O-H in compounds having hydroxyl and methoxy substituents. The proton NMR spectrum of the compounds displayed chemical shifts of the aliphatic carbon bridge along with the aromatic protons in all the compounds. The protons adjacent to ring nitrogen resonated at higher frequencies and were visible in the spectrum.

Synthetic compounds 4a-h, 5a-h, 6a-h were subjected to study of *in vitro* α-amylase inhibition. All compounds revealed α-amylase inhibitory action in the range of IC_{50} = 34.28 to 1285.72 µg/mL in comparison to acarbose with IC_{50} = 7.87 µg/mL. Derivatives 4b, 4e, and 6b were found to be significantly active against the α-amylase, having IC_{50} of 34.28, 84.17, and 77.99 µg/mL, respectively. The remaining analogues exhibited moderate to poor α-amylase inhibition. In previous studies benzimidazole derivatives with 2-substitution have been found to be significant anti-diabetic compounds by their ability to lower serum glucose and oral glucose tolerance in animal, reducing glucose levels by 50-60% on the 9th day of treatment[15]. Another study on benzimidazole compound FK-614 significant PPAR-γ agonistic action for its anti-diabetic potential[16]. A recent structure activity relationship based study led to designing of new benzimidazole derivatives as α-amylase inhibitors with lower IC_{50} value compared to acarbose[17]. In yet another study, the benzimidazole derivatives with fluoro substitution were found to be having IC50 value comparable to acarbose for inhibition of α-amylase[14].

The presence of the substitution on 1-position of benzimidazole nucleus significantly affected the α-amylase inhibition potential. Substitution with a single ring heterocycle was found to be beneficial in comparison to naphthyl substitution at the 1-position. Also it was observed that the spacer length affected the biological potency. Compounds with the ring and benzimidazole nitrogen separated with two carbon (4a-h) were more potent α-amylase inhibitors in comparison to the compounds with single carbon bridge (6a-h).

4. CONCLUSION

The study effectively combined benzimidazole derivatives and tested how well they inhibited the action of α-amylase. Compounds 4b, 4e, and 6b were very good in inhibiting the action of α-amylase. Changing the 1-position of the benzimidazole nucleus had a big effect on how well it could inhibit α-amylase. It was better to use compounds with single ring heterocycles than those with naphthyl substitution. The biological activity was regulated by the length of the spacer between the ring and the benzimidazole nitrogen. Compounds with a two-carbon bridge were better at stopping things from happening.

5. ACKNOWLEDGEMENTS

Authors would like to thank RB Science Research Lab for providing antioxidant studies.

Conflict of Interests

None to declare

Sources of Funding

No funding from received for this project work

Author Contributions

Megha Mishra – Concept, implementation, manuscript preparation; Gaurav Jain – Concept, manuscript revision; Phool Singh – Interpretation of results and statistical analysis; Kavita R Loksh – Concept, manuscript corrections, interpretation of results

Data Availability

All data will be made available if required

Ethical Approval Statement

Not Applicable

Informed Consent

All authors declare that they are aware of the submission and provide their consent to submit the manuscript in its current form.

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