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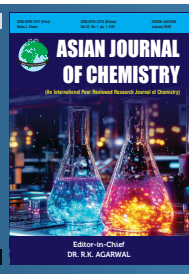

Exploring the Antidiabetic Potential of Novel Benzimidazole Analogs: Synthesis, Molecular Docking and DPP-4 Activity Evaluation

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Dipeptidyl peptidase-4 (DPP-4) inhibitors are frequently used as well-tolerated, safe antidiabetic medications. This work aimed to design and synthesize a series of benzimidazole compounds (**SS1A1**-**SS1A6** and **SS2A1**-**SS2A6**) as possible DPP-IV inhibitors. The compounds were characterized with FT-IR, ^{1}H NMR, ^{13}C NMR and mass spectrometry. Molecular docking, ADME analysis and Lipinski's drug-likeness rules were among the *in silico* experiments used to assess their binding affinity to DPP-4 and forecast their pharmacokinetic characteristics. *In vitro* DPP-4 inhibition tests were used to determine the biological activity of compounds and the findings indicated that none of them broke any of the main drug-likeness requirements. According to the molecular docking simulations, several derivatives showed stronger interactions and hydrogen bonds with ligands than the natural protein (PDB ID: 5Y7H). The most effective synthetic compound for inhibiting DPP-4 was compound **SS2A2**, which had an activity value of 1.68 µg/mL, much lower than the control 11.56 µg/mL. Based on both *in vitro* and *in silico* validation, these results imply that compound **SS2A2** is a good candidate for additional optimization as a DPP-4 inhibitor for antidiabetic treatment.

Keywords: DPP-4 inhibitors, Benzimidazoles, ADME, 5Y7H, Lipinski rule.

INTRODUCTION

Diabetes mellitus is a chronic metabolic disease that manifests as hyperglycemia and numerous other changes in the metabolism of proteins and carbohydrates [1]. Insulin resistance (type 1 diabetes) and insufficient insulin production and utilization by the body (type 2 diabetes) are the two primary causes of diabetes [2]. By 2025, there will be 380 million individuals with diabetes worldwide, up from 30 million in 1985, according to predictions from the International Diabetes Federation [3]. The DPP-4 (dipeptidyl peptidase-4) inhibitors are a well-established under classification of oral antidiabetic medications that help in treating type-2 diabetes [4]. The first medication to be introduced was sitagliptin in 2006 and other drugs followed soon after [5]. Anagliptin, teneligliptin, gemigliptin and other diabetic drugs are used in Asian nations [6]. Moreover, numerous national and international guidelines for treating non-insulin dependent diabetes mellitus (NIDDM) include DPP-IV inhibitors in their therapeutic algorithms. The majority of licensed DPP-IV inhibitors had ring systems with nitrogen atoms and an amide (-CONH) core, according to their structures.

Heterocyclic aromatic compounds like benzimidazole have shown great promise as building blocks for creating of a broad variety of physiologically active compounds [7] that includes antihistaminic, anticancer [8], cytotoxic [9], antitubercular [10], antimicrobial [11], antiviral [12] and anti-inflammatory [13], anticonvulsant, anti-depressive, antihypertensive, analgesic, enzyme inhibition those that treat diabetes [14]. In medicinal chemistry, benzimidazole has garnered a lot of interest due to its structural diversity and capacity to interact with important enzymes and receptors involved in glucose metabolism [15]. This scaffold is a desirable subject for medication research in diabetes treatment since it has demonstrated promise in blocking enzymes, regulating insulin secretion and enhancing overall glycemic control [16].

Due to this, benzimidazole was used as the foundation nucleus to form DPP-4 inhibitors. In benzimidazole, the aromatic ring and the imidazole ring's 4,5-positions combine to produce a fully planar ring structure [17]. The proton transfer from one N atom to other atoms NH and =N shows fast tautomerism [18]. Due to the NH group, benzimidazole has both acid and base properties and is amphoteric [19]. Benzimidazole derivatives have a complex mode of action when used to treat diabetes. The enzyme DPP-4 (dipeptidyl peptidase-4), which breaks down incretins like GLP-1 (glucagon-like peptide-1) and GIP (gastric inhibitory polypeptide), is one of the most prominent targets [20]. The present objective in this study was to synthesize 1-(1*H*-benzimidazol-2-yl)-2-methyl-propyl]-benzylidene-amine derivatives and 1*H*-benzimidazol-2-ylmethyl)benzylidene-amine derivatives to develop a strong DPP-4 inhibitor. For the proposed derivatives, this comprises simulations utilizing Lipinski criteria, ADME analysis and molecular docking. Wet-chemical synthesis and biological tests were applied to the substituents that successfully satisfied every criterion. Lastly, assessments of *in vivo* antidiabetic efficacy and *in vitro* enzymatic testing were also conducted.

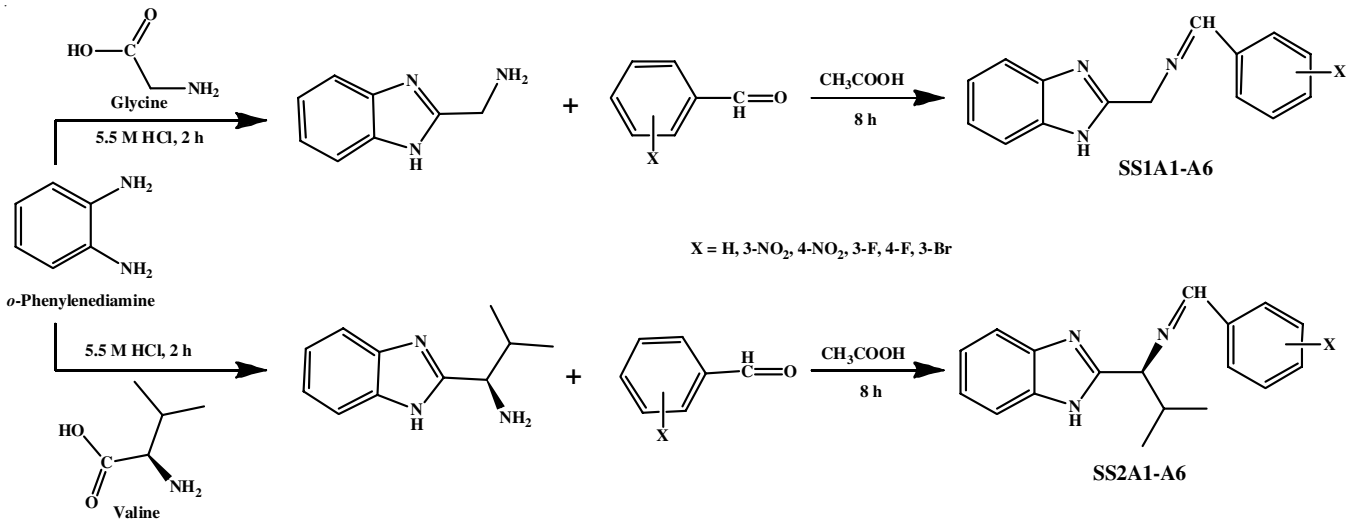
EXPERIMENTAL

The chemicals, reagents and solvents (LR-grade) were procured from different commercial suppliers. TLC separations were carried out with silica gel G (160-120 mesh) (Merck India Pvt. Ltd.) using the ethyl acetate:heptane solvent system. The melting points were measured using digital melting point equipment (Shimadzu DSC-50) and are unccorrected. On a Bruker 700 Hz apparatus, ^{1}H NMR and ^{13}C NMR spectra were scanned with solvents such as methanol (MeOD) and dimethyl sulfoxide (NMR grade d_6). Perkin-Elmer RX1 spectrometer was used to record in the 4000–400 cm^{-1} range. Mass spectra were noted with PE Sciex API300 mass spectrophotometer. The molecular docking investigations were conducted using Autodock 4.2 and docking result analysis was conducted using BIOVIA Discovery Studio Visualizer.

Synthesis of C-(1*H*-benzimidazol-2-yl)methylamine (SS1) and 1-(1*H*-benzimidazol-2-yl)-2-methyl-propylamine (SS2): Initially, C-(1*H*-benzimidazol-2-yl)methylamine (**SS1**) and 1-(1*H*-benzimidazol-2-yl)-2-methyl-propylamine (**SS2**) were synthesized by adopting the literature procedure with slight modification [21-23]. Accordingly, *o*-phenylenediamine (6 mmol) and glycine (12 mmol) in 12 mL HCl (5.5 mol) for **SS1** and *o*-phenylenediamine (12 mmol) and L-valine (24 mmol) in 24 mL HCl (5.5 mol) for **SS2**, The reactions were refluxed for 2 h, once reaction finishes as indicated by TLC, the reaction mixture was cooled to 0 ºC. Then this reaction mixture was quenched with 3 mol of NaOH solution till basified. The yellow (**SS1**) and brown precipitates (**SS2**) were obtained. The obtained products were washed with water and ethanol and dried under reduced pressure to obtain compounds (**SS1** and **SS2**) (**Scheme-I**).

Synthesis of 1*H*-benzimidazol-2-ylmethyl)benzylidene-amine derivatives (SS1A1-A6) and 1-(1*H*-benzimidazol-2-yl)-2-methyl-propyl]benzylidene-amine derivatives (SS2A1-A6): To a stirred solution of substituted benzaldehyde derivatives (benzaldehyde, 3-/4-nitro-benzaldehyde, 3-/4-floro-benzaldehyde and 3-bromo benzaldehyde (each of 0.05 mol) in glacial acetic acid (5 mL) was added dropwise a solution of C-(1*H*-benzimidazol-2-yl)-methylamine (**SS1**) and 1-(1*H*-benzimidazol-2-yl)-2-methyl-propylamine (**SS2**) separately in glacial acetic acid with continuously stirring [24-26] after addition of the reaction mixture was reflux for 8 h. After the completion of reaction, as indicated by TLC. The solvent was evaporated under reduced pressure to get a crude reaction mixture and was extracted using ethyl acetate and water. The organic layer was washed with brine, dried over anhydrous sodium sulphate and evaporated under reduced pressure. The residue was purified using column chromatography (100-200 mesh silica gel) using ethyl acetate/heptane as eluent.

(1*H*-Benzimidazol-2-ylmethyl)-benzylidene-amine (SS1A1): Yellowish brown colour, yield: 55%; m.p: 234-236 ºC; m.f.: $C_{15}H_{13}N_3$; R_f (ethyl acetate/hexane: 20/80): 0.76; IR (KBr, ν_{max}, cm^{-1}): 3321 (N-H *str.*, benzimidazole), 3057 (-Ar-



Scheme-I: Synthesis of noval (1*H*-benzimidazole-2-yl-methyl)benzylidene-amine derivatives (**SS1A1-A6**) and 1-(1*H*-benzimidazol-2-yl)-2-methyl-propyl]-benzylidene-amine derivatives (**SS2A1-A6**)

CH), 1631 (C=N imine *str.*), 1584 (C=C Ar); ^{1}H NMR (300 MHz, MeOD) δ ppm: 8.22 (s, 1H, CH), 7.74-7.77 (m, 4H, Ar-H), 7.55-7.59 (m, 3H, Ar-H), 7.31-7.37 (m, 2H, Ar-H), 5.11 (s, 1H, NH), 4.74 (s, 2H, CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 54.1 (CH_2-N), 116.3 (Ar, C), 123.5 (Ar, C), 129.1 (Ar, C), 131.1 (Ar, C), 137.1 (Ar, C), 138.2 (Ar, C), 142.1 (Ar, C), 164.1 (CH=N); HR-MS (ESI) *m/z*: $[M + H]^+$ Calcd. for $C_{15}H_{13}N_3^+$ 235.28; found 236.33.

(1*H*-Benzimidazol-2-ylmethyl)-(3-nitro-benzylidene)-amine (SS1A2): Light yellow colour, yield: 61%; m.p: 240-242 ºC; m.f.: $C_{15}H_{12}N_4O_2$; R_f (ethyl acetate/hexane: 50/50): 0.66; IR (KBr, ν_{max}, cm^{-1}): 3351 (N-H *str.*, benzimidazole), 3241 (C=N, benzimidazole), 3051 (Ar-CH), 1639 (C=N imine *str.*), 1591 (C=C Ar); ^{1}H NMR (300 MHz, MeOD) δ ppm: 8.80 (s, 1H, CH), 7.92-8.29 (m, 3H, Ar-H), 7.90-7.91 (m, 2H, Ar-H), 7.58-7.65 (m, 1H, Ar-H), 7.45-7.56 (m, 2H, Ar-H) 5.12 (s, 2H, CH_2), 4.11 (s, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 54.2 (CH_2-N), 116.5 (Ar, C), 123.1 (Ar, C), 124.5 (Ar, C), 126.1 (Ar, C), 128.9 (Ar, C), 134.9 (Ar, C), 137.9 (Ar, C), 138.1 (Ar, C), 142.3 (Ar, C), 148.9 (Ar, C-NO_2), 164.2 (CH=N); HR-MS (ESI) *m/z*: $[M + H]^+$ Calcd. for $C_{15}H_{12}N_4O_2$+ 280.28; found 281.10.

(1*H*-Benzimidazol-2-ylmethyl)-(4-nitro-benzylidene)-amine (SS1A3): Yellowish colour, yield: 52%; m.p: 180-182 ºC; m.f.: $C_{15}H_{12}N_4O_2$; R_f (ethyl acetate/hexane: 40/60): 0.72; IR (KBr, ν_{max}, cm^{-1}): 3345 (N-H *str.*, benzimidazole), 3210 (C=N, benzimidazole), 3045 (Ar-CH), 1625 (C=N imine *str.*), 1585 (C=C Ar); ^{1}H NMR (300 MHz, MeOD) δ ppm: 8.61 (s, 1H, CH), 8.25 (s, 2H, Ar-H), 7.90-7.92 (m, 2H, Ar-H), 7.57-7.64 (m, 2H, Ar-H), 7.30-7.56 (m, 2H, Ar-H), 5.10 (s, 2H, CH_2), 4.72 (s, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 54.4 (CH_2-N), 116.4 (Ar, C), 123.6 (Ar, C), 130.1 (Ar, C), 134.1 (Ar, C), 138.3 (Ar, C), 142.2 (Ar, C), 143.6 (Ar, C), 151.1 (Ar, C-NO_2), 164.3 (CH=N); HRMS (ESI) *m/z*: $[M + H]^+$ Calcd. for $C_{15}H_{12}N_4O_2^+$ 280.28; found 280.90.

1*H*-Benzimidazol-2-ylmethyl)-(3-fluoro-benzylidene)amine (SS1A4): Red brown colour; yield: 45%; m.p: 214-216 ºC; m.f.: $C_{15}H_{12}FN_3$; R_f (ethyl acetate/hexane: 30/70): 0.68; IR (KBr, ν_{max}, cm^{-1}): 3339 (N-H *str.*, benzimidazole), 3241 (C=N, benzimidazole), 3048 (Ar-CH), 1615 (C=N, imine *str.*), 1542 (C=C Ar) 1254 (C-F *str.*); ^{1}H NMR (300 MHz, MeOD) δ ppm: 8.18 (s, 1H, CH), 7.90-7.91 (m, 2H, Ar-H), 7.58-7.65 (m, 2H, Ar-H), 7.29-7.57 (m, 3H, Ar-H), 7.17-7.56 (m, 1H, Ar-H), 5.92 (s, 1H, NH), 4.92 (s, 2H, CH2); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 54.5 (CH_2-N), 116.5 (Ar, C), 118.1 (Ar, C), 123.2 (Ar, C), 125.2 (Ar, C), 130.4 (Ar, C), 138.4 (Ar, C), 139.1 (Ar, C), 141.9 (Ar, C), 162.2 (Ar, C-F), 164.4 (CH=N); HR-MS (ESI) *m/z*: $[M + H]^+$ Calcd. for $C_{15}H_{12}FN_3^+$ 253.10; found 254.10.

(1*H*-Benzimidazol-2-yl)-(5-fluoro-hexa-2,4-dienylidene)amine (SS1A5): Light orange colour; yield: 50%; m.p: 224-226 ºC; m.f.: $C_{14}H_{10}FN_3$; R_f (ethyl acetate/hexane: 35/65): 0.65; IR (KBr, ν_{max}, cm^{-1}): 3336 (N-H *str.*, benzimidazole), 3241 (C=N, benzimidazole), 3050 (-Ar-CH), 1621 (C=N imine *str.*), 1542 (C=C Ar) 1264 (C-F *str.*); ^{1}H NMR (300 MHz, MeOD) δ ppm: 8.17 (s, 1H, CH), 7.90-7.92 (m, 2H, Ar-H), 7.58-7.64 (m, 2H, Ar-H), 7.30-7.57 (m, 2H, Ar-H), 7.26-7.56 (m, 2H, Ar-H), 4.93 (s, 1H, NH), 4.81 (s, 2H, CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 50.1 (CH_2-N), 114.9 (Ar, C), 115.8 (Ar, C), 123.2 (Ar, C), 127.1 (Ar, C), 130.4 (Ar, C), 138.5 (Ar, C), 142.1 (Ar, C), 163.9 (Ar, C-F), 165.1 (CH=N); HR-MS (ESI) *m/z*: $[M + H]^+$ Calcd. for $C_{14}H_{10}FN_3^+$ 239.09; found 240.10.

(1*H*-Benzimidazol-2-yl)-(3-bromo-benzylidene)amine (SS1A6): Saffron colour; yield: 63%; m.p: 192-194 ºC; m.f.: $C_{14}H_{10}BrN_3$; R_f (ethyl acetate/hexane: 45/55): 0.45; IR (KBr, ν_{max}, cm^{-1}): 3330 (N-H *str.*, benzimidazole), 3196 (C=N, imidazole), 3045 (Ar-CH), 1634 (C=N imine *str.*): 0.45), 1597 (C=C, Ar) 1265 (C-Br *str.*); ^{1}H NMR (300 MHz, MeOD) δ ppm: 8.21 (s, 1H, CH), 7.90-7.91 (m, 3H, Ar-H), 7.57-7.65 (m, 2H, Ar-H), 7.20-7.54 (m, 3H, Ar-H), 4.91 (s, 1H, NH), 4.82 (s, 2H, CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 48.2 (CH_2-N), 114.9 (Ar, C), 123.2 (Ar, C), 123.7 (Ar, C), 127.9 (Ar, C), 130.4 (Ar, C), 131.9 (Ar, C), 133.1 (Ar, C), 134.6 (Ar, C), 138.3 (Ar, C), 142.9 (Ar, C), 164.4 (CH=N); HR-MS (ESI) *m/z*: $[M+H]^+$ Calcd. for $C_{14}H_{10}BrN_3^+$ 299.01; found 300.10.

[1-(1*H*-Benzimidazol-2-yl)-2-methyl-propyl]benzylidene-amine (SS2A1): Colourless solid; yield: 60%; m.p: 250-252 ºC; m.f.: $C_{15}H_{12}N_4O_2$; R_f (ethyl acetate/hexane:15/85): 0.60 IR (KBr, ν_{max}, cm^{-1}): 3320 (N-H *str.*, benzimidazole), 2972 (Ar-CH), 1612 (C=N imine *str.*), 1583 (C=C, Ar); ^{1}H NMR (300 MHz, MeOD) δ ppm: 8.11 (s, 1H, CH), 7.74-7.77 (m, 4H, Ar-H), 7.54-7.59 (m, 3H, Ar-H), 7.26-7.36 (m, 2H, Ar-H), 5.15 (s, 1H, NH) 4.31 (s, 1H, CH), 2.01-2.03 (m, 1H, CH), 1.03-1.05 (m, 6H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 17.5 (CH_3), 34.1 (CH_2), 70.2 (CH_2-N), 114.9 (Ar, C), 123.2 (Ar, C), 127.4 (Ar, C), 129.3 (Ar, C), 131.2 (Ar, C), 137.5 (Ar, C), 138.9 (Ar, C), 141.5 (Ar, C), 163.9 (CH=N), HR-MS (ESI) *m/z*: $[M + H]^+$ Calcd. for $C_{15}H_{12}N_4O_2^+$ 277.36; found 278.24.

[1-(1*H*-Benzimidazol-2-yl)-2-methyl-propyl]-(3-nitro-benzylidene)amine (SS2A2): Yellowish brown colour; yield: 64%; m.p: 264-266 ºC; m.f.: $C_{15}H_{12}N_4O_2$; R_f (ethyl acetate/hexane: 45/55): 0.56; IR (KBr, ν_{max}, cm^{-1}): 3310 (N-H *str.*, benzimidazole), 2962 (aliphatic-CH), 1625 (C=N imine *str.*), 1525 (NO_2) 1591 (C=C, Ar); ^{1}H NMR (300 MHz, MeOD) δ ppm: 8.81 (s, 1H, CH), 8.26 (s, 3H, Ar-H), 7.90-7.92 (m, 2H, Ar-H), 7.56-7.65 (m, 1H, Ar-H), 7.29-7.55 (m, 2H, Ar-H), 5.23 (s, 1H, NH), 4.71 (s, 1H, CH), 2.34-2.36 (m, 1H, CH), 1.28-1.30 (m, 6H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 17.6 (CH_3), 34.4 (CH_2), 70.3 (CH_2-N), 115.1 (Ar, C), 123.9 (Ar, C), 129.4 (Ar, C), 134.8 (Ar, C), 137.4 (Ar, C), 138.4 (Ar, C), 142.5 (Ar, C), 149.2 (Ar, C-NO_2), 164.5 (CH=N); HR-MS (ESI) *m/z*: $[M + H]^+$ Calcd. for : $C_{15}H_{12}N_4O_2^+$ 322.36; found 323.51.

[1-(1*H*-Benzimidazol-2-yl)-2-methyl-propyl]-(4-nitro-benzylidene)amine (SS2A3): Yellowish colour; yield: 47%; m.p: 268-270 ºC; m.f.: $C_{18}H_{18}N_4O_2$; R_f (ethyl acetate/hexane: 40/60): 0.79; IR (KBr, ν_{max}, cm^{-1}): 3305 (N-H *str.*, benzoimidazole), 2975 (aliphatic-CH) 1627 (C=N imine *str.*), 1520 (NO_2), 1584 (C=C, Ar); ^{1}H NMR (300 MHz, MeOD) δ ppm: 8.83 (s, 1H, CH), 8.13 (m, 3H, Ar-H), 7.90-7.91 (m, 2H, Ar-H), 7.57-7.64 (m, 1H, Ar-H), 7.40 (s, 2H, Ar-H), 5.21 (s, 1H, CH), 4.84 (s, 1H, NH), 2.11-2.12 (m, 1H, CH). 1.01-1.03 (m, 6H, CH3); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 17.5 (CH_3), 34.1 (CH_2), 70.2 (CH_2-N), 114.9 (Ar, C), 123.2 (Ar, C), 124.1 (Ar, C), 129.3 (Ar, C), 137.5 (Ar, C), 142.7 (Ar, C), 143.5 (Ar, C), 151.2

(Ar, C-NO_2), 164.6 (CH=N); HR-MS (ESI) *m/z*: $[M + H]^+$ Calcd. for $C_{18}H_{18}N_4O_2^+$ 322.36; found 323.51.

[1-(1*H*-Benzimidazol-2-yl)-2-methyl-propyl]-(3-fluoro-benzylidene)amine (SS2A4): Greenish yellow colour; yield: 48%; m.p: 228-230 ºC; m.f.: $C_{18}H_{18}FN_3$; R_f (ethyl acetate/hexane: 30/70): 0.58, IR (KBr, ν_{max}, cm^{-1}): 3310 (N-H *str.*, benzoimidazole), 2975 (aliphatic-CH), 1625 (C=N imine *str.*), 1584 (C=C, Ar), 1345 (NO_2); ^{1}H NMR (300 MHz, MeOD) δ ppm: 8.15 (s, 1H, CH), 7.92-7.95 (m, 2H, Ar-H), 7.58-7.65 (m, 2H, Ar-H), 7.38-7.64 (m, 2H, Ar-H), 7.17-7.34 (m, 2H, Ar-H), 5.04 (s, 1H, NH), 4.92 (s, 1H, CH), 2.01-2.03 (m, 1H, CH) 1.03-1.05 (m, 6H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm :17.7 (CH_3), 34.3 (CH_2), 70.3 (CH_2-N), 115.2 (Ar, C), 123.4 (Ar, C), 125.2 (Ar, C), 130.4 (Ar, C), 138.5 (Ar, C), 139.1 (Ar, C), 142.5 (Ar, C), 151.4 (Ar, C-F), 164.5 (CH=N), HR-MS (ESI) *m/z*: $[M + H]^+$ Calcd. for $C_{18}H_{18}FN_3^+$ 295.35; found 296.4.

[1-(1*H*-Benzimidazol-2-yl)-2-methyl-propyl]-(4-fluoro-benzylidene)amine (SS2A5): Yellow colour; yield: 47%; m.p: 202-204 ºC, m.f.: $C_{18}H_{18}FN_3$; R_f (ethyl acetate/hexane: 35/65): 0.55; IR (KBr, ν_{max}, cm^{-1}): 3322 (N-H *str.*, benzimidazole), 3022 (Ar-CH), 2895 (aliphatic-CH), 1631 (C=N imine *str.*), 1552 (C=C, Ar), 1261 (C-F *str.*); ^{1}H NMR (300 MHz, MeOD) δ ppm: 8.17 (s, 1H, CH), 7.65-7.91 (m, 2H, Ar-H), 7.58-7.64 (m, 2H, Ar-H), 7.56-7.57 (m, 2H, Ar-H) 7.58-7.38 (m, 2H, Ar-H), 5.12 (s, 1H, CH), 4.92 (s, 1H, NH), 2.01-2.04 (m, 1H, CH) 1.03-1.04 (m, 6H, CH3); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 17.5 (CH_3), 34.1 (CH_2), 70.2 (CH_2-N), 114.9 (Ar, C), 115.8 (Ar, C), 123.2 (Ar, C), 130.4 (Ar, C), 133.1 (Ar, C), 137.6 (Ar, C), 141.9 (Ar, C), 163.9 (Ar, C-F), 165.1 (CH=N); HR-MS (ESI) *m/z*:$[M +H]^+$ Calcd. for $C_{14}H_{10}FN_3^+$ 295.15; found 296.10

[1-(1*H*-Benzimidazol-2-yl)-2-methyl-propyl]-(3-bromo-benzylidene)amine (SS2A6): Brown colour: 0.48; yield: 43%; m.p: 218-220 ºC; m.f.: $C_{18}H_{18}BrN_3$; R_f (ethyl acetate/hexane: 40/60); IR (KBr, ν_{max}, cm^{-1}): 3334 (N-H *str.*, benzoimidazole), 3010 (Ar-CH), 2875 (aliphatic-CH), 1640 (C=N imine *str.*), 1593 (C=C, Ar), 1270 (C-Br *str.*); ^{1}H NMR (300 MHz, MeOD) δ ppm: 8.18 (s, 1H, CH), 7.90-7.92 (m, 3H, Ar-H), 7.57-7.65 (m, 2H, ArH), 7.30-7.56 (m, 3H, ArH), 5.16 (s, 1H, CH), 4.93 (s, 1H, NH), 2.01-2.03 (m, 1H, CH) 1.03-1.05 (m, 6H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 17.4 (CH_3), 34.6 (CH_2), 70.5 (CH_2-N), 115.3 (Ar, C), 123.4 (Ar, C), 123.9 (Ar, C), 127.9 (Ar, C), 130.5 (Ar, C), 131.9 (Ar, C), 134.6 (Ar, C), 137.8 (Ar, C), 140.1 (Ar, C), 141.5 (Ar, C), 163.8 (CH=N), HR-MS (ESI) *m/z*: $[M+H]^+$ Calcd. for $C_{18}H_{18}BrN_3^+$ 355.07; found 356.10.

Pharmacological activity

***In vitro* DPP-IV enzymatic assay:** *In vitro* assays were conducted to check whether the synthesized compounds could inhibit the DPP-IV enzyme. DPP-IV inhibition activity was performed using a 96-well plate and 10 µL samples of 1*H*-benzimidazol-2-ylmethyl)-benzylidene-amine derivatives (**SS1A1-A6**) and 1-(1*H*-benzimidazol-2-yl)-2-methyl-propyl]-benzylidene-amine derivatives (**SS2A1-A6**) were mixed with 200 µL. Gly-Pro-pNA (Sigma, USA) (0.5 mM Gly-Pro-pNA in 50 mM Tris buffer, pH 8.3, containing 0.1% Tween 20). DPP-IV activity was determined kinetically during 10 min at 37 ºC by measuring the velocities of pNA release (415 nm) from the chromogenic substrate with respect to control [27,28].

The calculation was performed to calculate DPP-IV activity:

$$\text{DPP-IV activity (U/mL)} = \frac{\Delta OD \times V}{\varepsilon * v * df}$$

where ΔOD = change in absorbance per minute at 405 nm; V = volume of the reaction mixture; v = volume of sample; ε = micromolar extinction coefficient at 405 nm (0.0102 $\mu M^{-1} cm^{-1}$); df = sample dilution factor.

ADME studies: The SwissADME online tools were used to determine the physico-chemical nature, lipophilic properties, solubility profiles, pharmacokinetic nature, drug likeness and molecular activity linkages of the synthesized compounds [29]. The lead likeness characteristic shows two exceptions to the shortening requirements. The log P value is less than 5 in each lipophilicity calculation. They predict the drug-likeness properties of novel 1*H*-benzimidazol-2-ylmethyl)benzylidene-amine derivatives (**SS1A1-A6**) and 1-(1*H*-benzimidazol-2-yl)-2-methyl-propyl]benzylidene-amine derivatives (**SS2A1-A6**) using the free online tool (http://www.swissadme.ch/).

Molecular docking: Molecular docking experiments were employed to elucidate the interactions between ligands and proteins in relation to common drugs. Auto Dock 4.2 software was used to molecularly dock ligands with distinct proteins (PDB ID: 5Y7H). Heteroatoms and water molecules were eliminated from the Swiss PDB viewer and Biovia drug discovery studios and the protein was obtained in PDB format from the RCSB protein data bank (https://www.rcsb.org/). The ligands were prepared in the Tripos.mol2 format using the Marvin Sketch structure drawing program. The Biovia drug discovery studio program was used to visualize the target protein and the ligand's two-dimensional interaction complex.

RESULTS AND DISCUSSION

The strategy of substituted 1*H*-benzimidazol-2-ylmethyl)-benzylidene-amine derivatives (**SS1A1-A6**) and 1-(1*H*-benzoimidazol-2-yl)-2-methyl-propyl]benzylideneamine derivatives (**SS2A1-A6**) was obtained in good yield. The chemical shift (δ) value of the NH group of benzimidazole moiety was observed in the range of 4.11-5.92 ppm, the δ value of a methyl group attached to the imine range 8.17-8.81, δ value of the aromatic region is 7.24-8.88 range. The δ value of the methyl group (isopropyl in **SS2** series) is 1.01-1.74 range. The δ value range of ^{13}C of all compounds between 17.5-165.1. The IR range of NH in the benzimidazole moiety showed between 3351-3305 cm^{-1} and the Schiff bases (C=N) range showed between 1640-1612 cm^{-1}. The mass range of synthesized compounds was between 235-356 g/mol.

***In vitro* DPP-IV enzyme assay:** *In vitro* enzyme assays were performed on each of the synthesized compounds. Based on the results obtained from the study, DPP-4 activity was measured in all samples and contrasted with a control. The outcome consistently depicts that DPP-4 activity was found to be decreased in all samples with respect to control and the lowest activity was found in **SS2A2** (1.68 µg/mL) (Table-1). The graph showing DPP-IV activity of all the synthesized compounds (**SS1A1-A6**

TABLE-1
In vitro ENZYME ASSAY OF SYNTHESIZED BENZIMIDAZOLE DERIVATIVES

	Enzyme activity (DPP-IV) (µg/mL)				
	Mean	SD		Mean	SD
Control	11.56	0.0397158	–	–	–
SS1A1	7.03	0.0241595	**SS2A1**	1.78	0.00613
SS1A2	3.71	0.0127279	**SS2A2**	1.68	0.00577
SS1A3	9.44	0.0324091	**SS2A3**	2.37	0.00813
SS1A4	3.64	0.0124922	**SS2A4**	6.62	0.02275
SS1A5	7.41	0.0254558	**SS2A5**	9.27	0.03184
SS1A6	2.68	0.0091924	**SS2A6**	3.4	0.01167

and **SS2A1-A6**) is mentioned in Fig. 1. It is clear that **SS2A2** ([1-(1*H*-benzimidazol-2-yl)-2-methyl-propyl]-(3-nitro-benzylidene)amine) is most effective in inhibiting the DPP-IV enzyme.

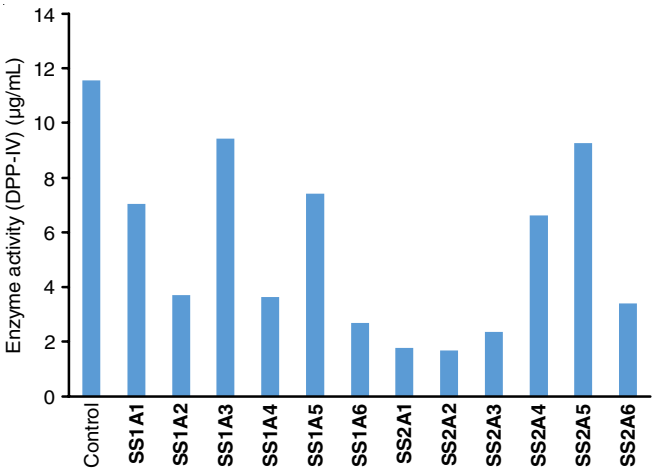


Fig. 1. DPP-IV activity of all the synthesized compounds

***In silico* ADMET analysis:** To ascertain whether the compounds were suitable for oral bioavailability, Lipinski's rule of five and Veber's criterion was applied (Table-2). To gain a better understanding of their pharmacokinetic profiles and drug-like characteristics, all synthesized compounds were examined for their ADME characteristics (Table-3). The physicochemical properties of all the synthesized benzimidazoles is tabulated in Table-4.

Every newly synthesized compound was subjected to the ADMET investigation. The log P values of all the compounds were between 2.23 and 4.31, indicating that the ideal level of lipophilicity was attained. The Log P value assesses a drug's capacity to permeate the body and reach its intended tissues [30]. Since the molecular weights of all the substances under investigation were less than 500 Da, it is likely that they can pass through biological membranes with ease [31,32]. Appreciatively, none of the substances had an adverse effect on the Lipinski rule of five's applicability. Veber's rule states that there should be fewer than 10 rotatable bonds. There was not a single synthetic chemical that violated Veber's law. It shows that these compounds are capable of being transformed into oral dosage forms. Many medications on the market are nevertheless approved by the FDA for oral use even if they violate rule 5.

The pharmacokinetics and drug-likeness characteristics of each molecule were examined and calculated in order to potentially enhance these substances further. For example, there was no proof that any of these substances could pass through the blood-brain barrier (BBB). The bioavailability and log Kp (skin penetration, cm/s) values fell well within the permissible ranges. All of the substances under investigation were shown to have a high degree of GI (gastrointestinal) absorption. It has been found that several substances inhibit cytochrome enzymes, which implies that these substances will probably affect how other drugs are metabolized. Since none of the synthesized compounds were P-gp substrate inhibitors, they do not hinder

TABLE-2
CALCULATIONS OF LIPINSKI'S RULE OF FIVE AND VEBER'S RULE FOR BENZIMIDAZOLE DERIVATIVES (**SS1A1-A6** AND **SS2A1-A6**)

Compound	Lipinski rule of five					Veber's rule
	Log P (<5)	m.w. (< 500 g/mol)	HBA (<10)	HBD (< 5)s	Violations	No. of rotatable bonds (< 10)
SS1A1	2.89	235.28	2	1	0	3
SS1A2	2.24	280.28	4	1	0	4
SS1A3	2.23	280.28	4	1	0	4
SS1A4	4.65	253.27	3	1	0	3
SS1A5	3.26	239.25	3	1	0	2
SS1A6	3.61	300.15	2	1	0	2
SS2A1	3.69	277.36	2	1	0	4
SS2A2	2.91	322.36	4	1	0	5
SS2A3	2.92	322.36	4	1	0	5
SS2A4	4.01	295.35	3	1	0	4
SS2A5	4.00	295.35	3	1	0	4
SS2A6	4.31	356.26	2	1	0	4

where: m.w., molecular weight; HBA, hydrogen bond acceptors; HBD, hydrogen bond donors.

TABLE-3
PHARMACOKINETICS AND DRUG-LIKE CHARACTERISTICS OF BENZIMIDAZOLE DERIVATIVES

Compound	Pharmacokinetics								Drug likeness	
	GI abs.	BBB pen.	P-gp sub.	Inhibitors					Log Kp (skin permeation, cm/s)	Bioavail-ability score
				CYP1A2	CYP2C19	CYP2C9	CYP2D6	CYP3A4		
SS1A1	High	Yes	No	Yes	Yes	No	Yes	No	-5.78	0.55
SS1A2	High	No	No	Yes	Yes	No	No	No	-6.17	0.55
SS1A3	High	No	No	Yes	Yes	No	No	No	-6.17	0.55
SS1A4	High	Yes	No	Yes	Yes	No	Yes	No	-5.81	0.55
SS1A5	High	Yes	No	Yes	Yes	No	No	No	-5.64	0.55
SS1A6	High	Yes	No	Yes	Yes	No	No	No	-5.43	0.55
SS2A1	High	Yes	No	Yes	Yes	Yes	Yes	Yes	-5.07	0.55
SS2A2	High	No	No	Yes	Yes	Yes	No	Yes	-5.46	0.55
SS2A3	High	No	No	Yes	Yes	Yes	No	Yes	-5.46	0.55
SS2A4	High	Yes	No	Yes	Yes	Yes	Yes	No	-5.11	0.55
SS2A5	High	Yes	No	Yes	Yes	Yes	Yes	No	-5.11	0.55
SS2A6	High	Yes	No	Yes	Yes	Yes	Yes	Yes	-5.05	0.55

where: GI abs., gastrointestinal absorption; BBB pen., blood-brain barrier penetration; P-gp sub., *p*-glycoprotein substrate

TABLE-4
PHYSICOCHEMICAL PROPERTIES OF BENZIMIDAZOLE DERIVATIVES

Compound	Formula	Num. heavy atoms	Num. arom. heavy atoms	Molar refractivity	Log S (ESOL)	Solubility (mg/mL)	Fraction Csp^3
SS1A1	$C_{15}H_{13}N_3$	18	15	74.04	-3.46	8.23×10^{-2}	0.07
SS1A2	$C_{15}H_{12}N_4O_2$	21	15	82.86	-3.47	9.41×10^{-2}	0.07
SS1A3	$C_{15}H_{12}N_4O_2$	21	15	82.86	-3.47	9.41×10^{-2}	0.07
SS1A4	$C_{15}H_{12}FN_3$	19	15	74.00	-3.6	6.39×10^{-2}	0.07
SS1A5	$C_{14}H_{10}FN_3$	18	15	69.75	-3.69	4.87×10^{-2}	0.00
SS1A6	$C_{14}H_{10}BrN_3$	18	15	77.49	-4.59	7.79×10^{-3}	0.00
SS2A1	$C_{18}H_{19}N_3$	21	15	88.46	-4.42	1.05×10^{-2}	0.22
SS2A2	$C_{18}H_{18}N_4O_2$	24	15	97.28	-4.46	1.12×10^{-2}	0.22
SS2A3	$C_{18}H_{18}N_4O_2$	24	15	97.28	-4.46	1.12×10^{-2}	0.22
SS2A4	$C_{18}H_{18}FN_3$	22	15	88.42	-4.57	7.94×10^{-3}	0.22
SS2A5	$C_{18}H_{18}FN_3$	22	15	88.42	-4.57	7.94×10^{-3}	0.22
SS2A6	$C_{18}H_{18}BrN_3$	22	15	96.16	-5.33	1.68×10^{-3}	0.22

the function of P-glycoprotein (P-gp), a protein that transports different substances out of cells. As a result, they do not stop P-gp substrates from being transported.

Molecular docking: The binding affinity, RMSD (root mean square deviation) lower bound and RMSD (root mean square upper) bound are mentioned in Table-5. The compound showed *in silico* activity at human DPP-4 receptor with binding affinity ranging from -6.4 to 7.4 kcal. RMSD lower bound ranges from 1.45 Å to 17.82 Å and RMSD upper bound ranges from 1.96 Å to 19.35 Å.

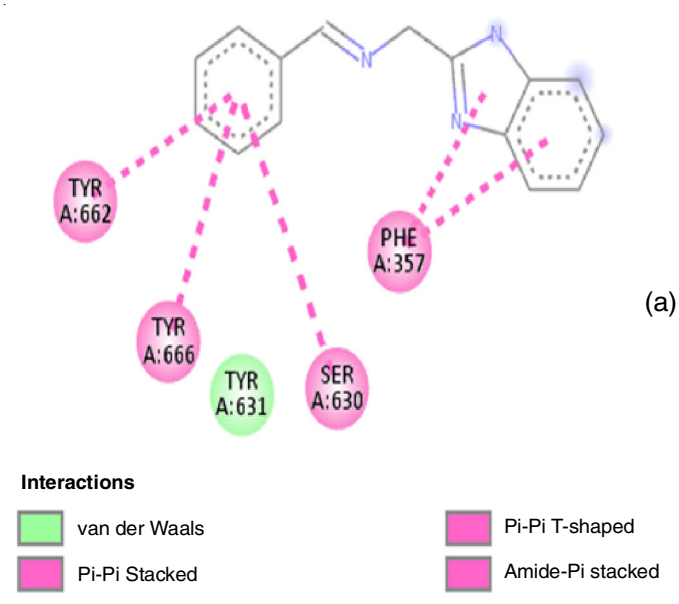
Fig. 2 represents the 2D and 3D interactions of **SS1A1**, **SS1A4**, **SS2A1** and **SS2A5** at the active site. The designed compounds interacted at the active site mainly through pi-pi stacking with amino acid TYR 54, PHE 3, TYR 662, TYR 666, SER 630, PHE 357, PHE 559 and pi-alkyl inter-action LEU 504, MET 509, PRO 475, LYS 512, ARG 560, ILE 63, ARG 61, LEU 69, ILE107 and TRP 157. The other inter-actions were Hydrogen bond interaction with amino acid GLN 553, PRO 510, carbon-hydrogen bond interaction with amino acid SER 552, van der Waals interaction with amino acid TYR 631, pi-sigma interactions with amino acid LEU477 and ILE 529 and halogen (fluorine) interaction with amino acid ASP 501.

TABLE-5
DOCKING SCORE OF SYNTHESIZED BENZIMIDAZOLE COMPOUNDS

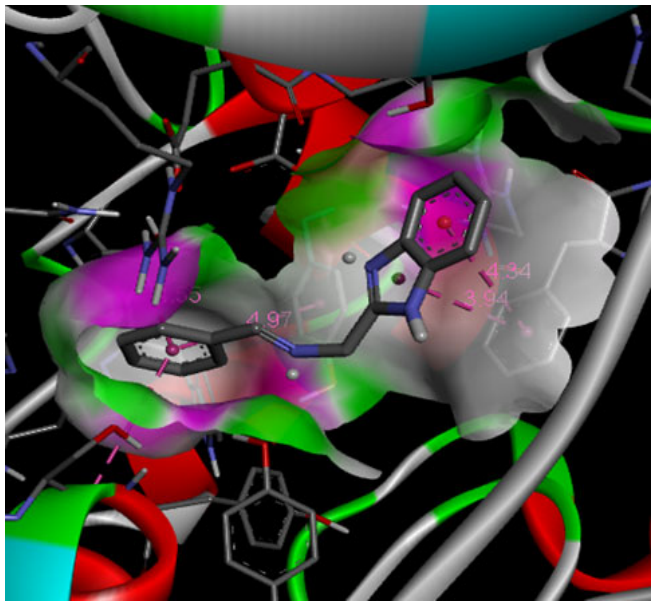
Compound	Binding affinity (kcal)	RMSD lower bound	RMSD upper bound
SS1A1	-7.36	1.45	1.96
SS1A2	-6.81	2.16	2.98
SS1A3	-7.13	2.45	3.45
SS1A4	-7.12	4.29	5.71
SS1A5	-6.60	5.34	6.24
SS1A6	-6.44	4.46	5.56
SS2A1	-7.50	17.82	19.35
SS2A2	-7.43	16.89	19.12
SS2A3	-7.24	16.14	18.42
SS2A4	-7.17	15.68	17.33
SS2A5	-7.33	16.13	18.28
SS2A6	-7.14	14.94	17.11

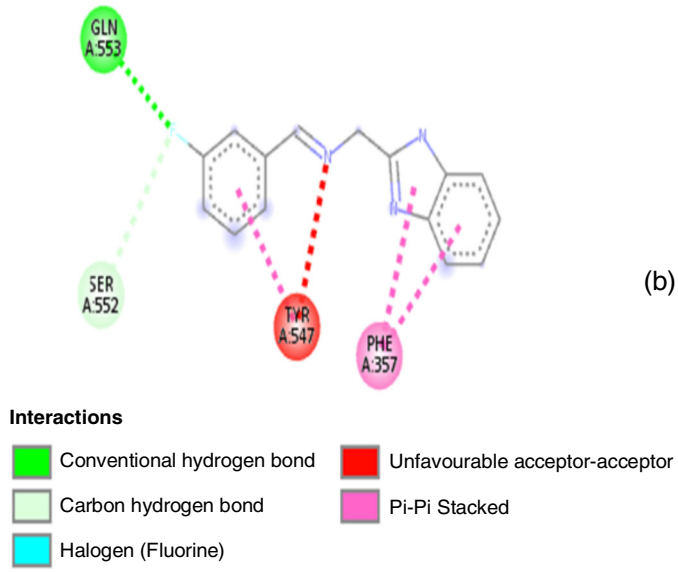
where, RMSD = root mean square deviation

Structure-activity relationship (SAR) of newly synthesized benzimidazole analogs: The biological action of the compounds depends on the benzimidazole core structure. Contributing to the binding affinity is the phenyl group in central core, which interacts hydrophobically with the target protein.

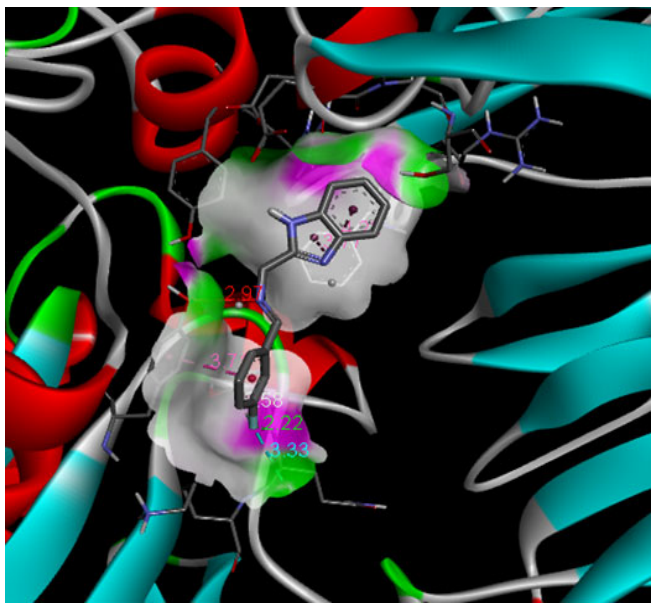

TYR A:662
PHE A:357
TYR A:666
TYR A:631
SER A:630
Interactions
van der Waals
Pi-Pi T-shaped
Pi-Pi Stacked
Amide-Pi stacked

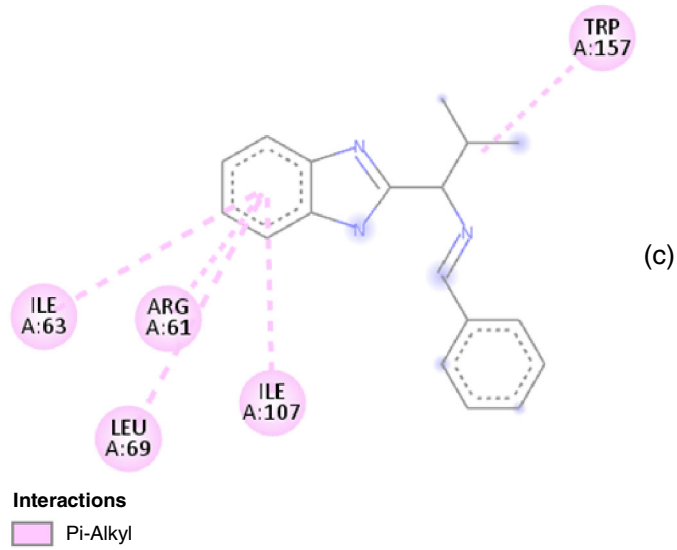
(a)



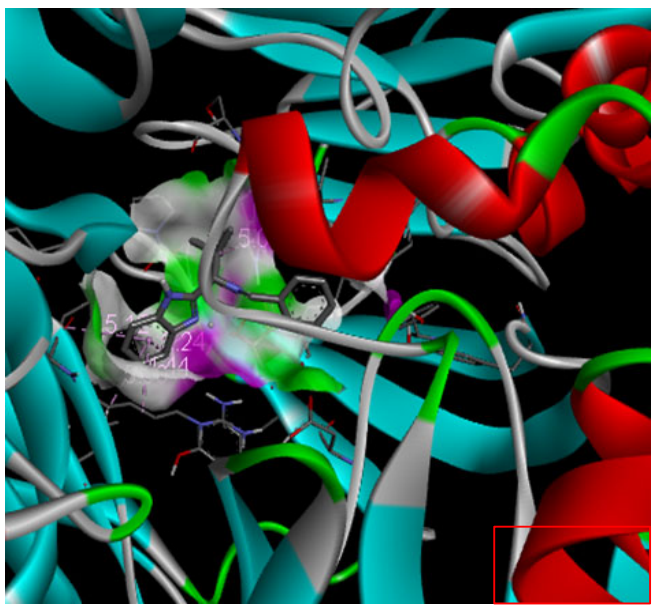

GLN A:553
SER A:552
TYR A:547
PHE A:357
Interactions
Conventional hydrogen bond
Unfavourable acceptor-acceptor
Carbon hydrogen bond
Pi-Pi Stacked
Halogen (Fluorine)

(b)




TRP A:157
ILE A:63
ARG A:61
ILE A:107
LEU A:69
Interactions
Pi-Alkyl

(c)



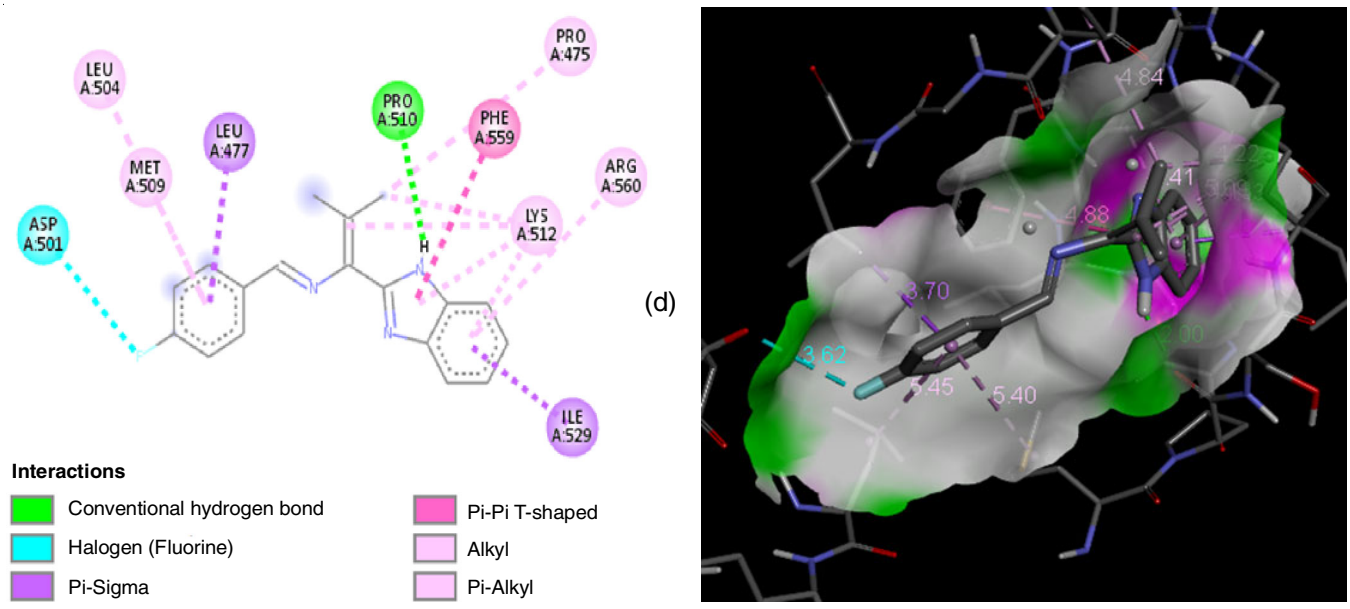


Fig. 2. 2D and 3D interaction images of compounds (a) **SS1A1**, (b) **SS1A4**, (c) **SS2A1** and (d) **SS2A5**

Furthermore, the benzimidazole ring's nitrogen atoms at positions one and two promote hydrogen bonding, which strengthens the link with the protein even more. It has been demonstrated that adding electron-donating groups at the third position to the Schiff base junction increases the compounds' biological activity. On the other hand, the newly synthesized series of compounds' antidiabetic activity is significantly enhanced by the addition of electron-withdrawing groups at the *meta* and *para* positions of the phenyl ring, as shown in Fig. 3. These structural changes seem to optimize the therapeutic potential by modifying the pharmacodynamic characteristics.

Conclusion

In summary, substituted 1*H*-benzimidazol-2-ylmethyl)-benzylidene-amine derivatives (**SS1A1-A6**) and 1-(1*H*-benzoimidazol-2-yl)-2-methyl-propyl]benzylidene-amine derivatives (**SS2A1-A6**) were synthesized, characterized and screened for the *in vitro* antidiabetic study. **SS2A2** in particular showed great promise in suppressing DPP-IV with an activity value of 1.68 µg/mL, significantly lower than the control (11.56 µg/mL), indicating promising results in the *in vivo* antidiabetic investigation. The processes of **SS2A2**'s interaction with the DPP-IV enzyme was further confirmed by molecular docking studies, which also highlighted the protein's advantageous binding stability and affinity. Furthermore, according to *in vivo* ADME research, **SS2A2** has a good pharmacokinetic profile, indicating that it could be a viable therapeutic candidate. According to these results, which have been validated both *in vitro* and *in silico*, **SS2A2** is a viable candidate for additional DPP-4 inhibitor modification, which could lead to its scope in the diabetes treatment.

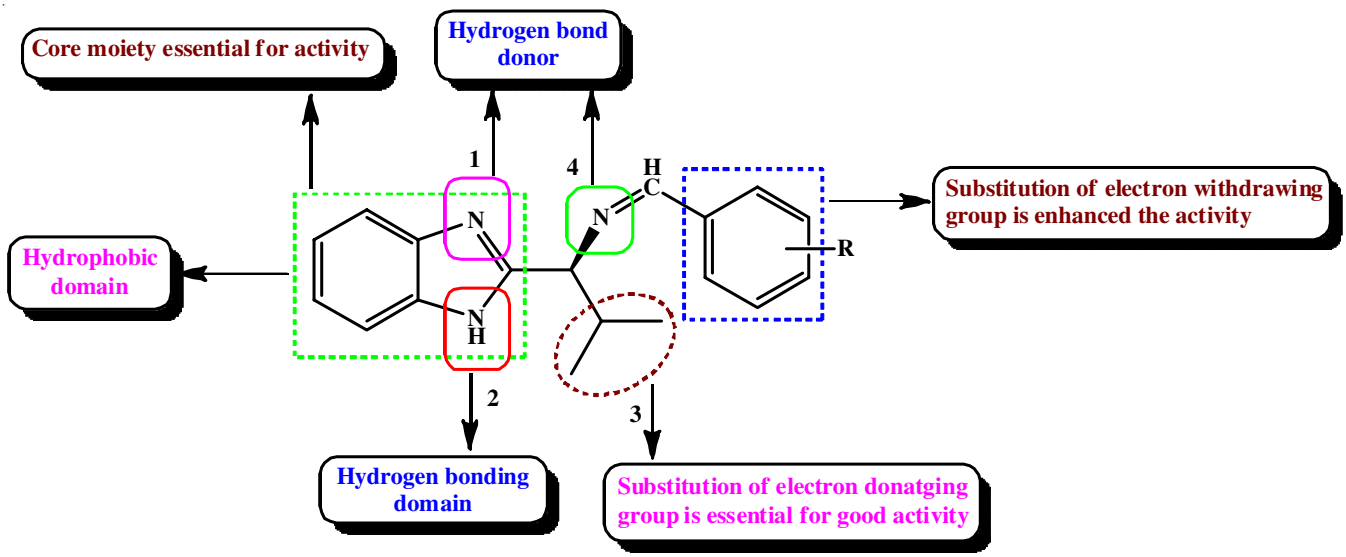


Fig. 3. Structure activity relationship of newly synthesized benzimidazole analogs

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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