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Synthesis, Structural Characterization and *in vitro* DPP-4 Inhibitory Evaluation of N-(1,3-Dioxoisoindolin-2-yl)benzamide Derivatives

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This study reports the synthesis, structural characterization and experimental *in vitro* DPP-4 inhibitory evaluation of N-(1,3-dioxoisoindolin-2-yl)benzamide derivatives (**P1-12**) that had previously been investigated computationally for DPP-4 binding. The derivatives were prepared through esterification, hydrazide formation and condensation with phthalic anhydride, affording products in 65-83% yield. Structural identities were supported by IR, ^{1}H NMR, ^{13}C NMR, ESI mass spectrometry and elemental analysis. DPP-4 inhibitory activity was evaluated using a continuous fluorometric Gly-Pro-AMC assay. Compounds **P5** (3,5-dihydroxy; IC_{50} 0.88 ± 0.04 nM) and **P10** (3,5-dinitro; IC_{50} 0.73 ± 0.03 nM) showed the lowest IC_{50} values in the series compared with alogliptin (IC_{50} 6.82 ± 0.21 nM). The experimental SAR did not follow a simple electron-donating/electron-withdrawing trend as both **P5** and **P10** derivatives showed high activity, whereas the unsubstituted **P1** and **P4** (2,4-dichloro) derivatives were less active. These results provide experimental chemical and enzyme-inhibition validation of a compound series previously examined by molecular docking.

Keywords: DPP-4 inhibitors, Type 2 diabetes, Benzamide hybrids, Isoindolinone, Gliptins.

INTRODUCTION

2-Methyl-N-(1,3-dioxoisoindolin-2-yl)benzamide scaffold contains a methyl-substituted benzamide group linked to a phthalimide ring, which provide an important framework for exploring structure-activity relationships (SARs) in medicinal chemistry [1,2]. Type 2 diabetes mellitus (T2DM) is associated with insulin resistance and progressive pancreatic β-cell dysfunction, making dipeptidyl peptidase-4 (DPP-4) an important target for glucose-lowering therapy [3-6]. DPP-4 inhibitors prolong the activity of endogenous incretin hormones, which support the glucose-dependent insulin secretion and improved glycemic control. Several isoindolin-2-yl-benzamide derivatives are investigated as potential DPP-4 inhibitors based on their chemical structures and reported computational binding studies [7,8]. Their structures combine a methyl-substituted benzamide unit with a phthalimide ring containing two carbonyl groups. These features provide sites for exploring the effects of steric, hydrophobic and polar interactions on ligand recognition within the DPP-4 binding pocket [9,10]. The substituent dependent changes in molecular geometry and electronic properties may influence binding affinity and inhibitory activity, although such effects require experimental or validated computational evidences [11-13].

In our previous study [7], *N*-(1,3-dioxoisoindolin-2-yl)-benzamide derivatives was evaluated by molecular docking against DPP-4 (PDB ID: 2OQV), which predicted favourable binding interactions for several derivatives. These computational findings provided an initial basis for investigating their potential as DPP-4 inhibitors, but the experimental enzyme inhibition had not been established. The present study extends this work through the synthesis and detailed structural characterization of the derivatives followed by direct *in vitro* assessment of their DPP-4 inhibitory activity using a fluorometric assay based on recombinant human DPP-4 and Gly-Pro-AMC substrate. This approach enables the predicted inhibitory potential to be examined experimentally and provides an initial assessment of the relationship between chemical structure and enzyme inhibition. The main contribution of the present work lies in bridging the gap between the earlier computa-

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tional predictions and experimental findings, which establish a preliminary SAR to investigate these derivatives as potential candidates for antidiabetic drug discovery.

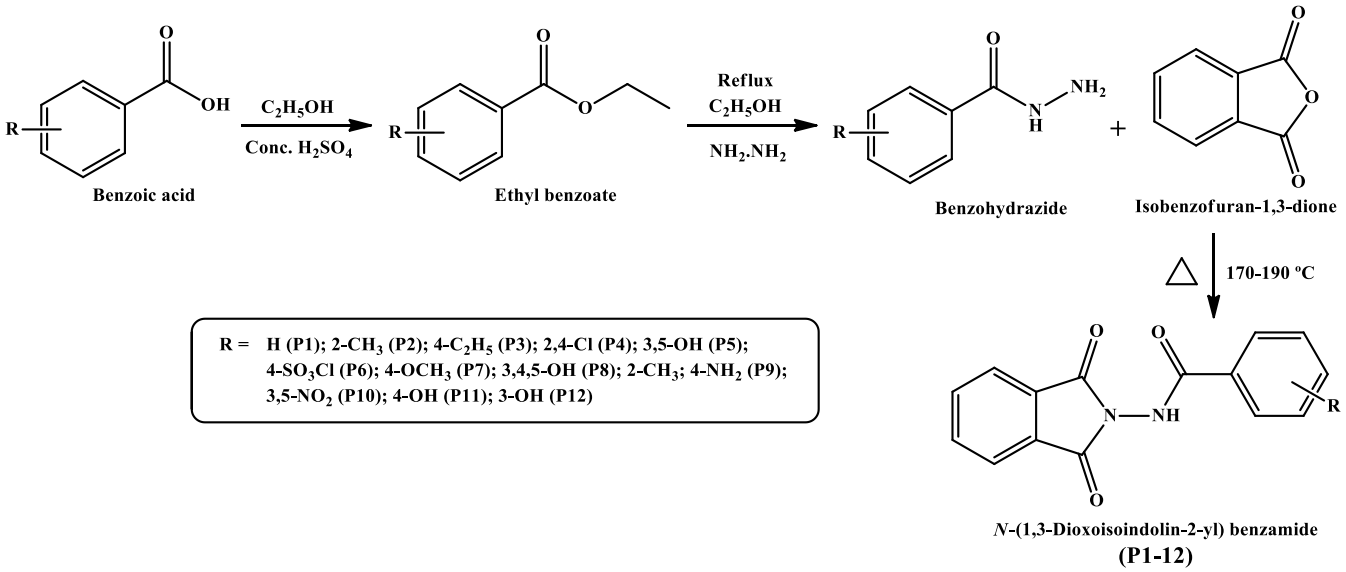
EXPERIMENTAL

Benzoic acid, ethanol, concentrated sulphuric acid and dichloromethane were purchased from Sigma-Aldrich, USA. Hydrazine hydrate (≥ 50 wt.% aq. solution) and phthalic anhydride were purchased from Sigma-Aldrich and TCI Chemicals, respectively. The recombinant human DPP-4 enzyme used in pharmacological experiments was provided by R&D Systems and the substrate, Gly-Pro-AMC and bovine serum albumin (BSA) were supplied by Sigma-Aldrich. HEPES buffer and sodium chloride were supplied by Merck chemicals Ltd., India. Melting points recorded on a digital melting point apparatus and are uncorrected. The synthesized compounds were characterized by infrared (IR) spectroscopy using the KBr pellet method (Bruker ALPHA FTIR spectrometer), ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectroscopy in DMSO-d_6 (Bruker Avance III 500 MHz NMR spectrometer), electrospray ioni-ation mass spectrometry (ESI-MS; Agilent 6120 Quadrupole LC/MS) and elemental analysis for carbon, hydrogen and nitrogen (Perkin-Elmer 2400 Series II CHNS/O Elemental Analyzer) within ±0.3% of theoretical values.

Synthesis of *N*-(1,3-dioxoisoindolin-2-yl)benzamide (P1-12): Substituted benzoic acid (1 equiv.) was mixed with 20 mL of ethanol followed by the addition of a few drops of conc. H_2SO_4. The reaction mixture was refluxed for 4 h and the progress of the reaction was monitored by TLC using 5% ethyl acetate in hexane. After completion, the solvent was evaporated under reduced pressure and the residue was dissolved in ethyl acetate. The organic layer was washed with 1 N NaOH, dried over anhydrous sodium sulphate, filtered and evaporated under reduced pressure to obtain substituted ethyl benzoate as a transparent, oily liquid. The ester (1 equiv.) was dissolved in 15 mL of ethanol and hydrazine hydrate (5 equiv.) was added in portions. The reaction mixture was heated at 60 ºC for 12 h, with progress monitored by TLC using a 1:1 mixture of ethanol and hexane. After complete consumption of the ester, the mixture was again concentrated under reduced pressure and washed with ethyl acetate and dichloromethane (DCM) to obtain substituted benzohydrazide as a dull white solid. Phthalic anhydride (4 equiv.) was then treated with substituted benzohydrazide (1 equiv.) in a 100 mL round-bottom flask fitted with a calcium chloride drying tube. The reaction mixture was heated under fusion conditions at 160-190 ºC for 30 min and the reaction progress was monitored by TLC using 50% ethyl acetate in hexane (**Scheme-I**). The resulting solid was washed several times with DCM and recrystallized to obtain the final product (**P1-12**).

***N*-(1,3-Dioxoisoindolin-2-yl)benzamide (P1):** White crystalline solid; yield: 82%; m.p.: 201-203 ºC; Elemental analysis of $C_{15}H_{10}N_2O_3$ (m.w.: 266.25 g/mol): calcd. (found) %: C, 67.66 (67.51); H, 3.79 (3.74); N, 10.52 (10.38); IR (KBr, ν_{max}, cm^{-1}): 3248 (N–H *str*., amide), 3072, 3048 (Ar C–H *str*.), 1778, 1726 (imide C=O asym. & sym. *str*.), 1664 (amide C=O *str*.), 1608, 1492, 1452 (Ar C=C *str*.), 1382 (C–N *str*., imide), 1284 (C–N *str*., amide), 1072 (C–H in-plane *bend*.), 768, 714 (monosubstituted Ar C–H out-of-plane *bend*.), 692 (Ar C–H out-of-plane *bend*.); ^{1}H NMR (500 MHz, DMSO-d_6, δ ppm): 10.76 (s, 1H, NH), 8.02 (dd, *J* = 8.4, 1.3 Hz, 2H, H-2′, H-6′, *ortho* Ar-H, benzoyl), 7.96-7.92 (m, 2H, H-4, H-7, isoindole Ar-H), 7.88-7.84 (m, 2H, H-5, H-6, isoindole Ar-H), 7.68 (tt, *J* = 7.5, 1.3 Hz, 1H, H-4′, *para* Ar-H, benzoyl), 7.58 (t, *J* = 7.8 Hz, 2H, H-3′, H-5′, *meta* Ar-H, benzoyl); ^{13}C NMR (125 MHz, DMSO-d_6, δ ppm): 166.8 (C=O, amide), 166.1 (2C, C=O, imide), 135.8 (2C, C-5, C-6, isoindole ring), 134.7 (2C, C-3a, C-7a, isoindole ring), 134.2 (C-4′, *para* Ar-C, benzoyl), 132.6 (C-1′, *ipso* Ar-C, benzoyl), 129.4 (2C, C-3′, C-5′, *meta* Ar-C, benzoyl), 128.8 (2C, C-2′, C-6′, *ortho* Ar-C, benzoyl), 123.6 (2C, C-4, C-7, isoindole ring). MS (ESI): *m/z* 267.07 $[M+H]^+$.

2-Methyl-*N*-(1,3-dioxoisoindolin-2-yl)benzamide (P2): Off-white crystalline solid; yield: 76%; m.p.: 195-197 ºC; Elemental analysis of $C_{16}H_{12}N_2O_3$ (m.w.: 280.28 g/mol):



Scheme-I: Synthetic route of *N*-(1,3-dioxo-isoindolin-2-yl)benzamide analogue

calcd. (found) %: C, 74.79 (74.81); H, 5.78 (5.82); N, 3.49 (3.42); O, 15.94 (15.98); IR (KBr, ν_{max}, cm^{-1}): 3242 (N–H *str*., amide), 3080 (Ar C–H), 2938 (aliphatic C–H, CH_3), 1774, 1722 (imide C=O asym. & sym. *str*.), 1668 (amide C=O *str*.), 1605, 1487 (Ar C=C), 1385 (C–N *str*., imide), 1281 (C–N *str*., amide), 748 (*ortho*-disubstituted Ar C–H out-of-plane *bend*.); ^{1}H NMR (500 MHz, DMSO-d_6, δ ppm): 10.82 (s, 1H, NH), 7.93-7.89 (m, 2H, H-4, H-7, isoindole Ar-H), 7.86-7.82 (m, 2H, H-5, H-6, isoindole Ar-H), 7.52 (dd, *J* = 7.5, 1.2 Hz, 1H, H-3′, benzoyl Ar-H), 7.44 (td, *J* = 7.5, 1.2 Hz, 1H, H-5′, benzoyl Ar-H), 7.39 (td, *J* = 7.5, 1.2 Hz, 1H, H-4′, benzoyl Ar-H), 7.32 (d, *J* = 7.6 Hz, 1H, H-6′, benzoyl Ar-H), 2.38 (s, 3H, CH_3); ^{13}C NMR (125 MHz, DMSO-d_6, δ ppm): 167.4 (C=O, amide), 166.2 (2C, C=O, imide), 137.8 (C-1′, *ipso*, benzoyl ring), 135.9 (2C, C-5, C-6, isoindole ring), 134.6 (C-3a, C-7a, isoindole ring), 132.7 (C-4′), 131.5 (C-3′), 130.9 (C-6′), 130.4 (C-5′), 129.2 (C-2′, *ipso*-CH_3), 123.6 (2C, C-4, C-7, isoindole ring), 19.8 (CH_3); MS (ESI): *m/z* 281.09 $[M+H]^+$.

4-Ethyl-*N*-(1,3-dioxoisoindolin-2-yl)benzamide (P3): Pale yellow crystalline solid; yield: 78%; m.p.: 189-191 ºC; Elemental analysis of $C_{17}H_{14}N_2O_3$ (*m.w*.: 294.31 g/mol): calcd. (found) %: C, 69.38 (69.22); H, 4.79 (4.74); N, 9.52 (9.38); O, 16.31 (16.35); IR (KBr, ν_{max}, cm^{-1}): 3244 (N–H *str*., amide), 3074, 3044 (Ar C–H *str*.), 2968 (asym. C–H *str*., –CH_2–), 2934 (sym. C–H *str*., –CH_2–), 2876 (C–H *str*., –CH_3), 1776, 1724 (imide C=O asym. & sym. *str*.), 1662 (amide C=O *str*.), 1606, 1494, 1458 (Ar C=C *str*.), 1384 (C–N *str*., imide), 1282 (C–N *str*., amide), 1116 (C–C *str*., ethyl chain), 838 (*para*-disubstituted Ar C–H out-of-plane *bend*.), 766 (isoindole Ar C–H out-of-plane *bend*.); ^{1}H NMR (500 MHz, DMSO-d_6, δ ppm): 10.72 (s, 1H, NH), 7.96-7.92 (m, 2H, H-4, H-7, isoindole Ar-H), 7.90 (d, *J* = 8.3 Hz, 2H, H-2′, H-6′, *ortho* Ar-H, 4-ethylbenzoyl), 7.87-7.83 (m, 2H, H-5, H-6, isoindole Ar-H), 7.38 (d, *J* = 8.3 Hz, 2H, H-3′, H-5′, *meta* Ar-H, 4-ethylbenzoyl), 2.68 (q, *J* = 7.6 Hz, 2H, –CH_2–), 1.22 (t, *J* = 7.6 Hz, 3H, –CH_3); ^{13}C NMR (125 MHz, DMSO-d_6, δ ppm): 166.9 (C=O, amide), 166.2 (2C, C=O, imide), 152.4 (C-4′, *ipso*-ethyl, Ar-C), 135.8 (2C, C-5, C-6, isoindole ring), 134.7 (2C, C-3a, C-7a, isoindole ring), 129.6 (C-1′, *ipso* Ar-C, 4-ethylbenzoyl), 129.2 (2C, C-3′, C-5′, *meta* Ar-C), 127.4 (2C, C-2′, C-6′, *ortho* Ar-C), 123.6 (2C, C-4, C-7, isoindole ring), 28.4 (–CH_2–), 15.2 (–CH_3); MS (ESI): *m/z* 295.11 $[M+H]^+$.

2,4-Dichloro-N-(1,3-dioxoisoindolin-2-yl)benzamide (P4): Pale cream crystalline solid; yield: 74%; m.p.: 210-212 ºC; Elemental analysis of $C_{15}H_8Cl_2N_2O_3$ (*m.w*.: 335.14 g/mol); C, 53.75 (53.59); H, 2.41 (2.37); N, 8.36 (8.22); Cl, 21.16 (21.02); O, 14.32 (14.36); IR (KBr, ν_{max}, cm^{-1}): 3252 (N–H *str*., amide), 3086, 3058 (Ar C–H *str*.), 1780, 1728 (imide C=O asym. & sym. *str*.), 1672 (amide C=O *str*.), 1588, 1484, 1446 (Ar C=C *str*.), 1386 (C–N *str*., imide), 1286 (C–N *str*., amide), 1104 (C–Cl *str*.), 872, 824 (Ar C–H out-of-plane *bend*.), 768 (isoindole Ar C–H out-of-plane *bend*.), 692 (C–Cl *str*., arom.); ^{1}H NMR (500 MHz, DMSO-d_6, δ ppm): 11.08 (s, 1H, NH), 7.98-7.94 (m, 2H, H-4, H-7, isoindole Ar-H), 7.91-7.87 (m, 2H, H-5, H-6, isoindole Ar-H), 7.82 (d, *J* = 2.1 Hz, 1H, H-3′, Ar-H, 2,4-dichlorobenzoyl), 7.68 (dd, *J* = 8.3, 2.1 Hz, 1H, H-5′, Ar-H, 2,4-dichlorobenzoyl), 7.58 (d, *J* = 8.3 Hz, 1H, H-6′, Ar-H, 2,4-dichlorobenzoyl); ^{13}C NMR (125 MHz, DMSO-d_6, δ ppm): 165.2 (C=O, amide), 165.9 (2C, C=O, imide), 136.8 (C-4′, *ipso*-Cl, Ar-C), 136.2 (2C, C-5, C-6, isoindole ring), 135.4 (C-1′, *ipso* Ar-C, 2,4-dichlorobenzoyl), 134.9 (2C, C-3a, C-7a, isoindole ring), 132.8 (C-5′, Ar-C), 131.6 (C-6′, Ar-C), 130.2 (C-3′, Ar-C), 128.4 (C-2′, *ipso*-Cl, Ar-C), 123.8 (2C, C-4, C-7, isoindole ring); MS (ESI): *m/z* 336.99 $[M+H]^+$, 338.99 $[M+2+H]^+$.

3,5-Dihydroxy-*N*-(1,3-dioxoisoindolin-2-yl)benzamide (P5): Light brown crystalline solid; yield: 69%; m.p.: 225-227 ºC; Elemental analysis of $C_{15}H_{10}N_2O_5$ (m.w.: 298.25 g/mol): calcd. (found) (%): C, 60.40 (60.24); H, 3.38 (3.33); N, 9.39 (9.24); O, 26.83 (26.92); IR (KBr, ν_{max}, cm^{-1}): 3486, 3412 (O–H *str*., phenolic, broad), 3238 (N–H *str*., amide), 3068, 3044 (Ar C–H *str*.), 1774, 1722 (imide C=O asym. & sym. *str*.), 1654 (amide C=O *str*.), 1608, 1584, 1476 (Ar C=C *str*.), 1382 (C–N *str*., imide), 1288 (C–N *str*., amide), 1248 (C–O *str*., phenolic), 1152 (C–O–H in-plane *bend*.), 882 (1,3,5-trisubstituted Ar C–H out-of-plane *bend*.), 768 (isoindole Ar C–H out-of-plane *bend*.); ^{1}H NMR (500 MHz, DMSO-d_6, δ ppm): 10.62 (s, 1H, NH), 9.72 (s, 2H, 2×OH, phenolic, exchange with D_2O), 7.94-7.90 (m, 2H, H-4, H-7, isoindole Ar-H), 7.86-7.82 (m, 2H, H-5, H-6, isoindole Ar-H), 7.18 (d, *J* = 2.2 Hz, 2H, H-2′, H-6′, Ar-H, 3,5-dihydroxybenzoyl), 6.68 (t, *J* = 2.2 Hz, 1H, H-4′, Ar-H, 3,5-dihydroxybenzoyl); ^{13}C NMR (125 MHz, DMSO-d_6, δ ppm): 167.2 (C=O, amide), 166.0 (2C, C=O, imide), 158.6 (2C, C-3′, C-5′, Ar-C–OH), 135.8 (2C, C-5, C-6, isoindole ring), 134.6 (2C, C-3a, C-7a, isoindole ring), 134.2 (C-1′, *ipso* Ar-C), 123.6 (2C, C-4, C-7, isoindole ring), 108.8 (C-4′, Ar-C), 107.4 (2C, C-2′, C-6′, Ar-C); MS (ESI): *m/z* 299.06 $[M+H]^+$.

4-(1,3-Dioxoisoindolin-2-ylcarbamoyl)benzene-1-sulphonyl chloride (P6): Pale yellow crystalline solid; yield: 65%; m.p.: 215-217 ºC; Elemental analysis of $C_{15}H_9ClN_2O_5S$ (m.w.: 368.76 g/mol): calcd. (found) (%): C, 48.85 (48.68); H, 2.46 (2.41); N, 7.60 (7.46); Cl, 9.61 (9.48); S, 8.69 (8.54); IR (KBr, ν_{max}, cm^{-1}): 3258 (N–H *str*., amide), 3082, 3052 (Ar C–H *str*.), 1782, 1730 (imide C=O asym. & sym. *str*.), 1674 (amide C=O *str*.), 1596, 1488, 1456 (Ar C=C *str*.), 1388 (C–N *str*., imide), 1368 (S=O asym. *str*., SO_2Cl), 1286 (C–N *str*., amide), 1182 (S=O sym. *str*., SO_2Cl), 1088 (S–Cl *str*.), 924 (S–Cl *str*., *sec*.), 856 (*p*-disubstituted Ar C–H out-of-plane *bend*.), 768 (isoindole Ar C–H out-of-plane *bend*.), 612 (C–S *str*., arom.); ^{1}H NMR (500 MHz, DMSO-d_6, δ ppm): 11.24 (s, 1H, NH), 8.18 (d, *J* = 8.6 Hz, 2H, H-3′, H-5′, Ar-H, *meta* to C=O), 8.06 (d, *J* = 8.6 Hz, 2H, H-2′, H-6′, Ar-H, *ortho* to C=O), 7.98-7.94 (m, 2H, H-4, H-7, isoindole Ar-H), 7.92-7.88 (m, 2H, H-5, H-6, isoindole Ar-H); ^{13}C NMR (125 MHz, DMSO-d_6, δ ppm): 164.8 (C=O, amide), 165.8 (2C, C=O, imide), 141.6 (C-4′, *ipso*-SO_2Cl, Ar-C), 136.4 (2C, C-5, C-6, isoindole ring), 135.2 (C-1′, *ipso* Ar-C), 134.9 (2C, C-3a, C-7a, isoindole ring), 130.8 (2C, C-2′, C-6′, *ortho* Ar-C), 129.6 (2C, C-3′, C-5′, *meta* Ar-C), 123.9 (2C, C-4, C-7, isoindole ring); MS (ESI): *m/z* 369.99 $[M+H]^+$, 371.98 $[M+2+H]^+$.

4-Methoxy-*N*-(1,3-dioxoisoindolin-2-yl)benzamide (P7): White crystalline solid; yield: 83%; m.p.: 203-205 ºC; Elemental analysis of $C_{16}H_{12}N_2O_4$ (m.w.: 296.28 g/mol): C, 64.86 (64.71); H, 4.08 (4.03); N, 9.45 (9.31); O, 21.61

(21.65); IR (KBr, ν_{max}, cm^{-1}): 3246 (N–H *str.*, amide), 3076, 3046 (Ar C–H *str.*), 2968, 2842 (C–H *str.*, $–OCH_3$), 1776, 1724 (imide C=O asym. & sym. *str.*), 1658 (amide C=O *str.*), 1608, 1512, 1458 (Ar C=C *str.*), 1384 (C–N *str.*, imide), 1284 (C–N *str.*, amide), 1254 (asym. C–O–C *str.*, aryl methoxy), 1176 (sym. C–O–C *str.*, aryl methoxy), 1028 (C–O *str.*, OCH_3), 842 (*p*-disubstituted Ar C–H out-of-plane *bend.*), 768 (isoindole Ar C–H out-of-plane *bend.*); ^{1}H NMR (500 MHz, DMSO-d_6, δ ppm): 10.68 (s, 1H, NH), 7.96-7.92 (m, 2H, H-4, H-7, isoindole Ar-H), 7.94 (d, *J* = 8.8 Hz, 2H, H-2′, H-6′, *ortho* Ar-H, 4-methoxybenzoyl), 7.87-7.83 (m, 2H, H-5, H-6, isoindole Ar-H), 7.10 (d, *J* = 8.8 Hz, 2H, H-3′, H-5′, *meta* Ar-H, 4-methoxybenzoyl), 3.84 (s, 3H, OCH_3); ^{13}C NMR (125 MHz, DMSO-d_6, δ ppm): 167.4 (C=O, amide), 166.1 (2C, C=O, imide), 163.2 (C-4′, *ipso*-OCH_3, Ar-C), 135.8 (2C, C-5, C-6, isoindole ring), 134.6 (2C, C-3a, C-7a, isoindole ring), 130.4 (2C, C-2′, C-6′, *ortho* Ar-C), 126.8 (C-1′, *ipso* Ar-C), 123.6 (2C, C-4, C-7, isoindole ring), 114.2 (2C, C-3′, C-5′, *meta* Ar-C), 55.8 ($–OCH_3$); MS (ESI): *m/z* 297.09 $[M+H]^+$.

3,4,5-Trihydroxy-*N*-(1,3-dioxoisoindolin-2-yl)benzamide (P8): Light brownish-yellow crystalline solid; yield: 66%; m.p.: 229-231 ºC; Elemental analysis of $C_{15}H_{10}N_2O_6$ (*m.w.*: 314.25 g/mol): 57.33 (57.16); H, 3.21 (3.16); N, 8.91 (8.76); O, 30.55 (30.64); IR (KBr, ν_{max}, cm^{-1}): 3496, 3448, 3386 (O–H *str.*, three phenolic –OH groups, br. and over-lapping), 3236 (N–H *str.*, amide), 3066, 3042 (Ar C–H *str.*), 1772, 1720 (imide C=O asym. & sym. *str.*), 1648 (amide C=O *str.*), 1612, 1582, 1478 (Ar C=C *str.*), 1380 (C–N *str.*, imide), 1286 (C–N *str.*, amide), 1244, 1186 (C–O *str.*, phenolic, multiple bands), 1148 (C–O–H in-plane *bend.*), 876 (Ar C–H out-of-plane *bend.*), 768 (isoindole Ar C–H out-of-plane *bend.*); ^{1}H NMR (500 MHz, DMSO-d_6, δ ppm): 10.58 (s, 1H, NH), 9.82 (s, 1H, C-4′–OH, phenolic, exchanges with D_2O), 9.14 (s, 2H, C-3′–OH, C-5′–OH, phenolic, exchanges with D_2O), 7.94-7.90 (m, 2H, H-4, H-7, isoindole Ar-H), 7.86-7.82 (m, 2H, H-5, H-6, isoindole Ar-H), 7.08 (s, 2H, H-2′, H-6′, Ar-H, 3,4,5-trihydroxybenzoyl); ^{13}C NMR (125 MHz, DMSO-d_6, δ ppm): 167.6 (C=O, amide), 166.0 (2C, C=O, imide), 148.4 (2C, C-3′, C-5′, Ar-C–OH), 145.8 (C-4′, Ar-C–OH), 135.8 (2C, C-5, C-6, isoindole ring), 134.6 (2C, C-3a, C-7a, isoindole ring), 124.8 (C-1′, *ipso* Ar-C), 123.6 (2C, C-4, C-7, isoindole ring), 109.4 (2C, C-2′, C-6′, Ar-C); (ESI): *m/z* 315.06 $[M+H]^+$.

4-Amino-2-methyl-*N*-(1,3-dioxoisoindolin-2-yl)benzamide (P9): Orange-yellow crystalline solid; yield: 72%; m.p.: 185.00-187 ºC; Elemental analysis of $C_{16}H_{13}N_3O_3$ (m.w.: 295.30 g/mol): C, 65.08 (64.92); H, 4.44 (4.39); N, 14.23 (14.08); O, 16.25 (16.28); IR (KBr, ν_{max}, cm^{-1}): 3468, 3374 (N–H *str.*, primary aromatic amine, $–NH_2$, asym. & sym.), 3244 (N–H *str.*, amide), 3068, 3042 (Ar C–H *str.*), 2934 (aliphatic C–H *str.*, $–CH_3$), 1774, 1722 (imide C=O asym. & sym. *str.*), 1652 (amide C=O *str.*), 1614 (N–H scissoring, 1º amine), 1596, 1508, 1462 (Ar C=C *str.*), 1382 (C–N *str.*, imide), 1286 (C–N *str.*, amide), 1178 (C–N *str.*, aromatic amine), 878 (Ar C–H out-of-plane *bend.*), 768 (isoindole Ar C–H out-of-plane *bend.*); ^{1}H NMR (500 MHz, DMSO-d_6, δ ppm): 10.54 (s, 1H, NH, amide), 7.94-7.90 (m, 2H, H-4, H-7, isoindole Ar-H), 7.86-7.82 (m, 2H, H-5, H-6, isoindole Ar-H), 7.18 (d, *J* = 8.2 Hz, 1H, H-6′, Ar-H), 6.52 (d, *J* = 2.4 Hz, 1H, H-3′, Ar-H), 6.46 (dd, *J* = 8.2, 2.4 Hz, 1H, H-5′, Ar-H), 5.48 (s, 2H, $–NH_2$, aromatic amine, exchanges with D_2O), 2.26 (s, 3H, $–CH_3$); ^{13}C NMR (125 MHz, DMSO-d_6, δ ppm): 168.2 (C=O, amide), 166.2 (2C, C=O, imide), 152.6 (C-4′, *ipso*-NH_2, Ar-C), 137.4 (C-1′, *ipso* Ar-C), 135.8 (2C, C-5, C-6, isoindole ring), 134.6 (2C, C-3a, C-7a, isoindole ring), 131.8 (C-6′, Ar-C), 128.6 (C-2′, *ipso*-CH_3, Ar-C), 123.6 (2C, C-4, C-7, isoindole ring), 112.4 (C-5′, Ar-C), 110.8 (C-3′, Ar-C), 19.6 ($–CH_3$); MS (ESI): *m/z* 296.10 $[M+H]^+$.

3,5-Dinitro-*N*-(1,3-dioxoisoindolin-2-yl)benzamide (P10): Bright yellow crystalline solid; yield: 68%; m.p.: 233-235 ºC); Elemental analysis of $C_{15}H_8N_4O_7$ (*m.w.*: 356.25 g/mol; IR (KBr, ν_{max}, cm^{-1}): 3268 (N–H *str.*, amide), 3092, 3062 (Ar C–H *str.*), 1784, 1736 (imide C=O asym. & sym. *str.*), 1692 (amide C=O *str.*), 1612 (Ar C=C *str.*), 1538, 1348 (asym. & sym. N=O *str.*, aromatic nitro group), 1388 (C–N *str.*, imide), 1292 (C–N *str.*, amide), 1176 (C–N *str.*, nitro-aryl), 924 (Ar C–H out-of-plane *bend.*), 768 (isoindole Ar C–H out-of-plane *bend.*), 716 (Ar C–H out-of-plane *bend.*); ^{1}H NMR (500 MHz, DMSO-d_6, δ ppm): 11.46 (s, 1H, NH), 9.12 (t, *J* = 2.1 Hz, 1H, H-4′, Ar-H, *para* to C=O, 3,5-dinitrobenzoyl), 8.96 (d, *J* = 2.1 Hz, 2H, H-2′, H-6′, Ar-H, nitro groups), 7.99-7.95 (m, 2H, H-4, H-7, isoindole Ar-H), 7.93-7.89 (m, 2H, H-5, H-6, isoindole Ar-H); ^{13}C NMR (125 MHz, DMSO-d_6, δ ppm): 163.4 (C=O, amide), 165.6 (2C, C=O, imide), 148.8 (2C, C-3′, C-5′, *ipso*-NO_2, Ar-C), 136.6 (2C, C-5, C-6, isoindole ring), 135.8 (C-4′, Ar-C, *para* to C=O), 135.2 (2C, C-3a, C-7a, isoindole ring), 130.2 (C-1′, ipso Ar-C), 124.2 (2C, C-4, C-7, isoindole ring), 122.6 (2C, C-2′, C-6′, Ar-C). MS (ESI): *m/z* 357.04 $[M+H]^+$.

4-Hydroxy-*N*-(1,3-dioxoisoindolin-2-yl)benzamide (P11): Off-white crystalline solid; yield: 79%; m.p.: 213-215 ºC; Elemental analysis of $C_{15}H_{10}N_2O_4$ (m.w.: 282.25 g/mol): C, 63.83 (63.67); H, 3.57 (3.52); N, 9.92 (9.78); O, 22.68; IR (KBr, ν_{max}, cm^{-1}): 3468 (O–H *str.*, phenolic, broad), 3242 (N–H *str.*, amide), 3072, 3046 (Ar C–H *str.*), 1776, 1724 (imide C=O asym. & sym. *str.*), 1656 (amide C=O *str.*), 1606, 1514, 1456 (Ar C=C *str.*), 1384 (C–N *str.*, imide), 1282 (C–N *str.*, amide), 1246 (C–O *str.*, phenolic), 1168 (C–O–H in-plane *bend.*), 1114 (C–O *str.*, *sec.*), 846 (*p*-disubstituted Ar C–H out-of-plane *bend.*), 768 (isoindole Ar C–H out-of-plane *bend.*). ^{1}H NMR (500 MHz, DMSO-d_6, δ ppm): 10.64 (s, 1H, NH, amide), 10.18 (s, 1H, OH, phenolic, exchanges with D_2O), 7.95-7.91 (m, 2H, H-4, H-7, isoindole Ar-H), 7.88 (d, *J* = 8.7 Hz, 2H, H-2′, H-6′, *ortho* Ar-H, 4-hydroxybenzoyl), 7.86-7.82 (m, 2H, H-5, H-6, isoindole Ar-H), 6.92 (d, *J* = 8.7 Hz, 2H, H-3′, H-5′, *meta* Ar-H, 4-hydroxybenzoyl); ^{13}C NMR (125 MHz, DMSO-d_6, δ ppm): 167.6 (C=O, amide), 166.1 (2C, C=O, imide), 162.4 (C-4′, *ipso*-OH, Ar-C), 135.8 (2C, C-5, C-6, isoindole ring), 134.6 (2C, C-3a, C-7a, isoindole ring), 130.6 (2C, C-2′, C-6′, *ortho* Ar-C), 124.4 (C-1′, *ipso* Ar-C), 123.6 (2C, C-4, C-7, isoindole ring), 115.4 (2C, C-3′, C-5′, *meta* Ar-C); MS (ESI): *m/z* 283.07 $[M+H]^+$.

3-Hydroxy-*N*-(1,3-dioxoisoindolin-2-yl)benzamide (P12): Pale pink crystalline solid; yield: 77%; m.p.: 209-211 ºC; Elemental analysis of $C_{15}H_{10}N_2O_4$ (m.w.: 282.25 g/mol); C, 63.83 (63.69); H, 3.57 (3.51); N, 9.92 (9.77); O, 22.68 (22.75); IR (KBr, ν_{max}, cm^{-1}): 3472 (O–H *str.*, phenolic, br),

3246 (N–H *str*., amide), 3074, 3048 (Ar C–H *str*.), 1778, 1726 (imide C=O asym. & sym. *str*.), 1660 (amide C=O *str*.), 1604, 1584, 1488, 1454 (Ar C=C *str*.), 1386 (C–N *str*., imide), 1284 (C–N *str*., amide), 1252 (C–O *str*., phenolic), 1164 (C–O–H in-plane *bend*.), 1082 (C–H in-plane *bend*.), 878 (*m*-substituted Ar C–H out-of-plane *bend*.), 788, 768 (isoindole Ar C–H out-of-plane *bend*.), 694 (*m*-substituted Ar C–H out-of-plane *bend*.); ^{1}H NMR (500 MHz, DMSO-d_6, δ ppm): δ 10.68 (s, 1H, NH, amide), 9.88 (s, 1H, OH, phenolic, exchanges with D_2O), 7.96-7.92 (m, 2H, H-4, H-7, isoindole Ar-H), 7.88-7.84 (m, 2H, H-5, H-6, isoindole Ar-H), 7.46 (ddd, *J* = 7.8, 1.6, 1.2 Hz, 1H, H-4′, Ar-H), 7.42 (t, *J* = 2.0 Hz, 1H, H-2′, Ar-H), 7.38 (t, *J* = 7.8 Hz, 1H, H-5′, Ar-H), 7.08 (ddd, *J* = 7.8, 2.6, 1.2 Hz, 1H, H-6′, Ar-H); ^{13}C NMR (125 MHz, DMSO-d_6, δ ppm): 167.2 (C=O, amide), 166.1 (2C, C=O, imide), 157.8 (C-3′, *ipso*-OH, Ar-C), 135.8 (2C, C-5, C-6, isoindole ring), 134.6 (2C, C-3a, C-7a, isoindole ring), 133.4 (C-1′, *ipso* Ar-C), 130.2 (C-5′, Ar-C), 123.6 (2C, C-4, C-7, isoindole ring), 120.8 (C-6′, Ar-C), 118.4 (C-4′, Ar-C), 114.6 (C-2′, Ar-C); MS (ESI): *m/z* 283.07 $[M+H]^+$.

***In vitro* DPP-4 inhibition assay:** Continuous fluorometric experiments were used to monitor DPP-4-mediated cleavage of Gly-Pro-AMC and release of fluorescent 7-amino-4-methylcoumarin (AMC) [14]. Fluorescence was monitored at 360 nm excitation and 460 nm emission using a microplate reader. The reaction mixture contained varying concentrations of the synthesized compounds (**P1-12**), recombinant human DPP-4 (20 μU/μL), Gly-Pro-AMC (10 μM) and assay buffer containing 150 mM NaCl, 25 mM HEPES and 0.12 mg/mL BSA. Alogliptin was used as the reference inhibitor. Measurements were performed in four independent experiments. Concentration-response data were analyzed in GraphPad Prism 10 to estimate IC_{50} values [14].

Statistical analysis: Data are presented as mean ± SD (n = 4 independent experiments). Where group comparisons were performed, one-way ANOVA was applied using GraphPad Prism version 10, with $p < 0.05$ considered statistically significant. The value of n should be verified against the original raw experimental records before final submission.

RESULTS AND DISCUSSION

The synthesis of *N*-(1,3-dioxoisoindolin-2-yl)benzamides (**P1-12**) involves a three-step transformation of substituted benzoic acid into the target heterocyclic compound (**Scheme-I**). Initially, substituted benzoic acid undergoes Fischer esterification with ethanol under acidic conditions to form substituted ethyl benzoate. The reaction involves protonation of the carbonyl group, nucleophilic attack by ethanol, formation of a tetrahedral intermediate and elimination of water. Then, substituted ethyl benzoate was refluxed with hydrazine hydrate in ethanol to form substituted benzohydrazide through nucleophilic substitution of the ester group. Finally, substituted benzohydrazide reacts with phthalic anhydride (isobenzofuran-1,3-dione) at 170-190 ºC to form the target isoindole-derived compounds. Structural identities of the 12 derivatives were supported by complementary spectroscopic and analytical methods before biological evaluation. Across the series, IR spectra showed characteristic imide and amide carbonyl absorptions, while ^{1}H and ^{13}C NMR spectra supported the expected aromatic, carbonyl and substituent groups. ESI-MS and elemental analysis further supported the assigned molecular compositions.

***In vitro* DPP-4 inhibition activity:** The present study extends the previous molecular docking investigation of the **P1-12** series against DPP-4 (PDB ID: 2OQV) [7] by providing experimental evidence of enzyme inhibition. The Gly-Pro-AMC fluorometric assay was performed in four independent experiments [15]. All tested derivatives inhibited DPP-4 under the assay conditions used, with IC_{50} values varying across the series (Table-1). Compounds **P10** (3,5-dinitro) and **P5** (3,5-dihydroxy) showed the lowest IC_{50} values of 0.73 ± 0.03 nM and 0.88 ± 0.04 nM, respectively, compared with 6.82 ± 0.21 nM for alogliptin tested under the same conditions. These values correspond to approximately 9.34-fold and 7.75-fold lower IC_{50} values than the reference inhibitor. The activity of compounds **P10** and **P5** shows two distinct substitution patterns associated with strong inhibition. The difference between **P1** and several substituted analogues supports a role for substituent modification in activity, since IC_{50} values can vary with the experimental conditions [16-18].

TABLE-1
DPP-4 INHIBITORY ACTIVITY (IC_{50}) OF THE SYNTHESIZED COMPOUNDS (**P1-P12**)

Compounds	DPP-4 IC_{50} (nM)*
P1	18.56 ± 1.27*
P2	2.52 ± 0.11
P3	3.18 ± 0.14
P4	15.62 ± 0.82*
P5	0.88 ± 0.04
P6	12.25 ± 0.36*
P7	4.87 ± 0.23
P8	4.62 ± 0.82
P9	3.18 ± 0.14
P10	0.73 ± 0.03
P11	8.35 ± 0.36
P12	2.42 ± 0.11
Standard alogliptin	6.82 ± 0.21

Data are presented as mean ± SD (n = 4 independent experiments); *$p < 0.05$ where indicated.

The observed activity differences can be considered alongside established structural studies of DPP-4 inhibitors, which report interactions with the S1/S2 regions and residues such as Arg125, Glu205, Glu206, Tyr547, Tyr662 and Tyr666, depending on the inhibitor scaffold and binding mode [19,20]. In particular, the high activity of the electron withdrawing 3,5-dinitro derivative **P10**, alongside the 3,5-dihydroxy derivative **P5**, indicates that electronic character alone cannot account for the observed structure–activity relationship (SAR).

Conclusion

The present study extends the previously published molecular docking investigation of the compounds **P1-12** by integrating synthesis, structural characterization and *in vitro* evaluation of recombinant human DPP-4 inhibition. The synthesized derivatives were characterized using IR, ^{1}H NMR,

^{13}C NMR, ESI-MS and elemental analysis. Among the tested compounds, **P5** (3,5-dihydroxy) and **P10** (3,5-dinitro) exhibited the lowest IC_{50} values of 0.88 and 0.73 nM, respectively, compared with 6.82 nM for alogliptin. The observed SAR indicates that DPP-4 inhibition cannot be explained by electronic effects alone, as substituent position, steric properties and hydrogen-bonding potential may also influence activity. These findings identify compounds **P5** and **P10** as promising candidates for further investigation.

CONFLICT OF INTEREST

The authors declare that there is no conflicts of interest regarding the publication of this article.

DECLARATION OF AI-ASSISTED TECHNOLOGIES

During the preparation of this manuscript, the authors used an AI-assisted tool(s) to improve the language and no data, results or scientific conclusions were generated by AI. All analyses, interpretations and conclusions are the authors' own. The authors reviewed and edited the content and take full responsibility for the published work.

REFERENCES

1. F. Doraghi, M.H. Morshedsolouk, N.A. Zahedi, B. Larijani and M. Mahdavi, *RSC Adv.*, **14**, 22809 (2024); https://doi.org/10.1039/D4RA03859B
2. V.M. Milovanović, Z.D. Petrović, S. Novaković, G.A. Bogdanović, V.P. Petrović and D. Simijonović, *Tetrahedron*, **76**, 131456 (2020); https://doi.org/10.1016/j.tet.2020.131456
3. E. Młynarska, W. Czarnik, N. Dzieża, W. Jędraszak, G. Majchrowicz, F. Prusinowski, M. Stabrawa, J. Rysz and B. Franczyk, *Int. J. Mol. Sci.*, **26**, 1094 (2025); https://doi.org/10.3390/ijms26031094
4. A. Singh, S. Shadangi, P.K. Gupta and S. Rana, *Compr. Physiol.*, **15**, e70003 (2025); https://doi.org/10.1002/cph4.70003
5. M. Bayanati, M. Ismail Mahboubi Rabbani, S. Sirous Kabiri, B. Mir, E. Rezaee and S.A. Tabatabai, *Iran. J. Pharm. Res.*, **23**, e151581 (2024); https://doi.org/10.5812/ijpr-151581
6. M. Lotfy, J. Singh, H. Kalász, K. Tekes and E. Adeghate, *Open Med. Chem. J.*, **5(Suppl 2)**, 82 (2011); https://doi.org/10.2174/1874104501105010082
7. P. Singh, N. Srivastava and A. Ved, *Res. J. Pharm. Technol.*, **18**, 4389 (2025); https://doi.org/10.52711/0974-360X.2025.00629
8. N. Kato, M. Oka, T. Murase, M. Yoshida, M. Sakairi, M. Yakufu, S. Yamashita, Y. Yasuda, A. Yoshikawa, Y. Hayashi, M. Shirai, Y. Mizuno, M. Takeuchi, M. Makino, M. Takeda and T. Kakigami, *Org. Med. Chem. Lett.*, **1**, 7 (2011); https://doi.org/10.1186/2191-2858-1-7
9. V. Mathur, O. Alam, N. Siddiqui, M. Jha, A. Manaithiya, N. Sharma, S. Bawa, S. Alshehri, P. Alam and F. Shakeel, *Molecules*, **28**, 5860 (2023); https://doi.org/10.3390/molecules28155860
10. R. Dutta, S. Khatun, P. Ghosh, P.K. Dey, S. Gayen and S.A. Amin, *Bioorg. Chem.*, **165**, 108947 (2025); https://doi.org/10.1016/j.bioorg.2025.108947
11. V. Kairys, L. Baranauskiene, M. Kazlauskiene, A. Zubriene, V. Petrauskas, D. Matulis and E. Kazlauskas, *Expert Opin. Drug Discov.*, **19**, 649 (2024); https://doi.org/10.1080/17460441.2024.2349169
12. A. Mazurek, *Acta Pol. Pharm.*, **73**, 269 (2016).
13. X. Du, Y. Li, Y.-L. Xia, S.-M. Ai, J. Liang, P. Sang, X.-L. Ji and S.-Q. Liu, *Int. J. Mol. Sci.*, **17**, 144 (2016); https://doi.org/10.3390/ijms17020144
14. N. da Silva, M. Oliveira, H. Filho, L. Pinheiro, R. Kolb and O.M. Bruno, *Bio Protoc.*, **7**, e2184 (2017); https://doi.org/10.21769/BioProtoc.2630
15. S.-H. Kim, E. Jung, M.K. Yoon, O.H. Kwon, D.-M. Hwang, D.-W. Kim, J. Kim, S.-M. Lee and H.J. Yim, *Eur. J. Pharmacol.*, **788**, 54 (2016); https://doi.org/10.1016/j.ejphar.2016.06.016
16. A.A.E. Mourad, A.E. Khodir, S. Saber and M.A.E. Mourad, *Pharmaceuticals*, **14**, 144 (2021); https://doi.org/10.3390/ph14020144
17. K. Saini, S. Sharma and Y. Khan, *Front. Mol. Biosci.*, **10**, 1130625 (2023); https://doi.org/10.3389/fmolb.2023.1130625
18. X.J. Zhou, L. Ding, J.X. Liu, L.Q. Su, J.J. Dong and L. Liao, *Endocr. Connect.*, **8**, 318 (2019); https://doi.org/10.1530/EC-18-0523
19. S.P. Upadhyay, P. Thapa, R. Sharma and M. Sharma, *Fitoterapia*, **146**, 104722 (2020); https://doi.org/10.1016/j.fitote.2020.104722
20. Y. Miyamoto, Y. Banno, T. Yamashita, T. Fujimoto, S. Oi, Y. Moritoh, T. Asakawa, O. Kataoka, K. Takeuchi, N. Suzuki, K. Ikedo, T. Kosaka, S. Tsubotani, M. Funami, M. Amano, Y. Yamamoto, K. Aertgeerts, A. Tani, J. Yano and H. Maezaki, *Bioorg. Med. Chem.*, **19**, 172 (2011); https://doi.org/10.1016/j.bmc.2010.11.038