



In Silico Design, Molecular Docking and Evaluation of α -Amylase Inhibition of Ethyl Indole-3-Carboxylate Mannich Base Derivatives as Potential Antidiabetic Agents

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ABSTRACT

Introduction: Type 2 diabetes mellitus (T2DM) is characterised by chronic hyperglycaemia, and inhibition of pancreatic α -amylase is an established approach to blunt postprandial glucose excursions. The indole nucleus is a privileged scaffold in medicinal chemistry, and ethyl indole-3-carboxylate Mannich bases were designed in the present work as potential α -amylase inhibitors.

Materials and Methods: Five ethyl 2-[1-(substituted amino)ethyl]-1H-indole-3-carboxylate derivatives (C-1 to C-5) were designed. Physicochemical properties, pharmacokinetic behaviour and drug-likeness were predicted with SwissADME (Lipinski, Ghose, Veber, Egan and Muegge filters), and toxicity risk with OSIRIS Property Explorer. Molecular docking was performed against human pancreatic α -amylase (PDB ID: 1B2Y) with PyRx (AutoDock Vina), and interactions were analysed in BIOVIA Discovery Studio. The *in vitro* α -amylase inhibitory activity was evaluated by the starch-iodine method at 10, 20, 30, 40 and 50 μ g/mL, using acarbose as the standard.

Results and Discussion: All five compounds obeyed Lipinski's rule of five with no violation (molecular weight 260.33–336.38 g/mol; consensus Log P 2.21–3.55; TPSA 45.33–71.19 Å²), were predicted to have high gastrointestinal absorption, and showed no mutagenic, tumorigenic, irritant or reproductive-toxicity risk in OSIRIS. Docking scores ranged from –7.2 to –7.9 kcal/mol, with C-3 showing the best affinity (–7.9 kcal/mol); the active-site residues Asp197, Glu233 and Asp300 of the catalytic triad, together with Gly306, Tyr62, Trp58/59, His201 and Ile235, were involved in binding. In the *in vitro* assay, inhibition was concentration dependent; C-1 and C-3 were the most active, giving 89.0% and 87.9% inhibition at 50 μ g/mL, followed by C-2 (82.5%), C-5 (64.0%) and C-4 (36.0%).

Conclusion: The ethyl indole-3-carboxylate Mannich bases, particularly the phenylamino (C-1) and benzamido (C-3) derivatives, combine favourable drug-likeness, good predicted binding to the α -amylase active site and marked *in vitro* enzyme inhibition, and may be taken forward as lead molecules for antidiabetic drug development.

Keywords: α -amylase inhibition, antidiabetic activity, drug design, indole, in silico studies, Mannich bases, molecular docking, type 2 diabetes mellitus.

INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder defined by persistent hyperglycaemia resulting from defects in insulin secretion, insulin action, or both, and type 2 diabetes mellitus (T2DM) accounts for the large majority of cases [1]. The global burden of diabetes has risen steadily and is projected to continue to rise through 2050 [2]. Control of blood glucose, including the postprandial component, is central to preventing micro- and macrovascular complications [3], and in T2DM the handling of dietary carbohydrate is altered [4].

Starch digestion begins with α -amylase, a glycoside hydrolase that cleaves the internal α -(1 $\rightarrow$ 4) glycosidic bonds of starch to give maltose, maltotriose and limit dextrins, which are then converted to glucose by intestinal α -glucosidases [5]. In human pancreatic α -amylase the catalytic machinery is formed by Asp197, Glu233 and Asp300, with further residues lining the subsites of the active-site cleft [6,7]. Blocking this enzyme slows carbohydrate digestion and glucose absorption, and therefore lowers postprandial hyperglycaemia.

Acarbose, miglitol and voglibose are clinically used inhibitors of carbohydrate-digesting enzymes, but their use is limited by gastrointestinal adverse effects such as flatulence, bloating and diarrhoea [8,9]. This has driven the search for new, safer and more selective α -amylase inhibitors based on diverse molecular scaffolds [10].

The indole nucleus is a privileged heterocycle that occurs in many natural products and marketed drugs and is associated with a wide range of pharmacological activities [11,12]. Indole-based molecules have been reported as dual inhibitors of α -glucosidase and α -amylase [13]. Computer-aided drug design allows candidate molecules to be prioritised for drug-likeness, pharmacokinetic profile and target binding before synthesis, saving time and resources [14]. In the present study, five ethyl indole-3-carboxylate Mannich bases were therefore designed, evaluated *in silico* for drug-likeness, ADME and toxicity, docked against human pancreatic α -amylase, and assessed for *in vitro* α -amylase inhibitory activity.

MATERIALS AND METHODS

Design of the test compounds

Ethyl indole-3-carboxylate was selected as the lead scaffold. Indole scaffolds are widely exploited in drug design [11,12]. Substitution at the C-2 position through a Mannich-type condensation with acetaldehyde and a primary or secondary amine (aniline, 2-aminopyridine, benzamide, diethylamine and dimethylamine) gave the five designed derivatives C-1 to C-5 (Table 1). The compounds were prepared and characterised by IR, NMR and mass spectroscopy, and their structures were confirmed before biological testing.

Physicochemical, ADME and drug-likeness prediction

The structures were drawn in ChemSketch and their SMILES were submitted to the SwissADME web tool [18]. Molecular weight, number of hydrogen-bond acceptors and donors, rotatable bonds, molar refractivity, topological polar surface area (TPSA), lipophilicity (consensus Log P), aqueous solubility (ESOL), gastrointestinal absorption, blood–brain barrier (BBB) permeation, P-glycoprotein substrate status and bioavailability score were recorded. Drug-likeness was assessed with the filters of Lipinski [15], Ghose [16] and Veber [17], together with the Egan and Muegge filters available in SwissADME. Lipinski's rule of five requires a molecular weight below 500, Log P below 5, not more than 5 hydrogen-bond donors and not more than 10 hydrogen-bond acceptors [18].

Toxicity prediction

Toxicity risks (mutagenicity, tumorigenicity, irritant and reproductive effects), together with cLogP, solubility, drug-likeness and overall drug-score, were predicted with the OSIRIS Property Explorer [19]. In this programme a red indicator denotes high risk of the undesired effect, whereas green denotes a safe profile.

Molecular docking

The crystal structure of human pancreatic α -amylase (PDB ID: 1B2Y) [20] was retrieved from the Protein Data Bank and prepared in BIOVIA Discovery Studio [21] by removing water molecules and adding hydrogen atoms. The three-dimensional structures of C-1 to C-5 were prepared in PyRx [22], including hydrogen addition and energy minimisation, and docked into the active site using AutoDock Vina [23] as implemented in PyRx [grid-box centre, grid-box size and exhaustiveness to be inserted]. The pose with the best (most negative) binding affinity was retained for each compound. Hydrogen bonds and hydrophobic, π - π and electrostatic contacts between the ligands and amino-acid residues were visualised in two and three dimensions with BIOVIA Discovery Studio.

In vitro α -amylase inhibitory assay

The assay was based on the starch–iodine method [24]. Intact starch forms a blue-black complex with iodine, and the intensity of the blue colour falls as α -amylase hydrolyses starch; an inhibitor preserves starch and hence the colour, so a higher absorbance indicates greater inhibition.

Reagents. Phosphate buffer (20 mM, pH 6.9) containing 6.7 mM NaCl; 1% (w/v) soluble starch solution prepared in warm distilled water by gentle boiling; iodine reagent (1% KI and 0.1% I₂); and α -amylase solution prepared by dissolving 27.5 mg of porcine pancreatic α -amylase (HiMedia) in 100 mL of the phosphate buffer, freshly before use. Stock solutions of the test compounds were prepared in buffer and serially diluted; acarbose was used as the standard inhibitor.

Procedure. To each test tube, 250 μ L of test solution (10, 20, 30, 40 or 50 μ g/mL) and 250 μ L of α -amylase solution (1 U/mL) were added and pre-incubated at 37 °C for 20 min. The reaction was started by adding 250 μ L of 1% starch solution and incubated at 37 °C for 10 min. It was stopped by adding 1 mL of iodine reagent, and the absorbance was read immediately at 580 nm. A blank (all reagents except enzyme), a control (buffer, enzyme and starch without inhibitor), a positive control (standard inhibitor) and a sample colour control (test compound, starch and iodine without enzyme) were run in parallel.

Calculation. The percentage inhibition was calculated as: % Inhibition = $[(A_{\text{sample}} - A_{\text{control}}) / (A_{\text{blank}} - A_{\text{control}})] \times 100$, where A_{blank} is the absorbance of starch–iodine without enzyme, A_{control} that of enzyme with starch and iodine without inhibitor, and A_{sample} that of the reaction mixture containing the test compound.

RESULTS AND DISCUSSION

Designed compounds

The five designed derivatives share the ethyl indole-3-carboxylate core and differ in the nitrogen substituent on the C-2 side chain, which carries an aromatic ring in C-1 to C-3 (anilino, pyridinylamino and benzamido) and a dialkylamino group in C-4 and C-5 (Table 1).

Table 1: Designed ethyl indole-3-carboxylate Mannich base derivatives

S. No.	Code	Amine component	IUPAC name
1.	C-1	Aniline	Ethyl 2-[1-(phenylamino)ethyl]-1H-indole-3-carboxylate
2.	C-2	2-Aminopyridine	Ethyl 2-[1-(pyridin-2-ylamino) ethyl]-1H-indole-3-carboxylate
3.	C-3	Benzamide	Ethyl 2-[1-(benzamido) ethyl]-1H-indole-3-carboxylate
4.	C-4	Diethylamine	Ethyl 2-[1-(diethylamino) ethyl]-1H-indole-3-carboxylate
5.	C-5	Dimethylamine	Ethyl 2-[1-(dimethylamino) ethyl]-1H-indole-3-carboxylate

Physicochemical properties, ADME and drug-likeness

The predicted properties are summarised in Table 2. All five compounds satisfied Lipinski's rule of five with zero violations [15]: molecular weight ranged from 260.33 to 336.38 g/mol (limit 500), consensus Log P from 2.21 to 3.55 (limit 5), hydrogen-bond donors from 1 to 2 (limit 5) and hydrogen-bond acceptors from 2 to 3 (limit 10). TPSA values of 45.33–71.19 Å² and 5–7 rotatable bonds are within the Veber limits [17], and all compounds also passed the Ghose, Egan and Muegge filters [16,18]. The bioavailability score was 0.55 for every compound, no PAINS alert was raised [18], and all compounds were predicted to be soluble or moderately soluble (ESOL Log S –3.07 to –4.47). C-1 was the only compound flagged for a lead-likeness violation (XLOGP3 above 3.5).

Table 2: Predicted physicochemical, pharmacokinetic and drug-likeness parameters (SwissADME)

Parameter	C-1	C-2	C-3	C-4	C-5
Molecular formula	C ₁₉ H ₂₀ N ₂ O ₂	C ₁₈ H ₁₉ N ₃ O ₂	C ₂₀ H ₂₀ N ₂ O ₃	C ₁₇ H ₂₄ N ₂ O ₂	C ₁₅ H ₂₀ N ₂ O ₂
Molecular weight (g/mol)	308.37	309.36	336.38	288.38	260.33
H-bond acceptors	2	3	3	3	3
H-bond donors	2	2	2	1	1
Rotatable bonds	6	6	7	7	5
Molar refractivity	92.98	90.78	96.68	80.28	76.67
TPSA (Å ²)	54.12	67.01	71.19	45.33	45.33
Consensus Log P	3.55	2.62	3.10	2.91	2.21
Log S (ESOL)	–4.47	–3.80	–4.10	–3.54	–3.07
Solubility class	Moderately soluble	Soluble	Moderately soluble	Soluble	Soluble
GI absorption	High	High	High	High	High
BBB permeant	Yes	Yes	Yes	Yes	Yes

Parameter	C-1	C-2	C-3	C-4	C-5
P-gp substrate	No	No	No	No	No
Lipinski violations	0	0	0	0	0
Ghose / Veber / Egan / Muegge	Yes	Yes	Yes	Yes	Yes
Bioavailability score	0.55	0.55	0.55	0.55	0.55
PAINS alerts	0	0	0	0	0
Lead-likeness	No (1 violation)	Yes	Yes	Yes	Yes

Because α -amylase acts in the lumen of the small intestine, systemic absorption is not a prerequisite for activity, and the predicted high gastrointestinal absorption and BBB permeation mainly indicate that these molecules are not expected to be limited by permeability if systemic exposure is required. Like acarbose, orally acting α -amylase inhibitors need not be absorbed [8,9]. Whether systemic exposure is desirable or not should be examined in later pharmacokinetic and safety studies.

Toxicity prediction

The OSIRIS results are given in Table 3. None of the five compounds was flagged for mutagenic, tumorigenic, irritant or reproductive-toxicity risk [19]. The computed drug-likeness was negative for C-1 (−1.75) and C-2 (−0.54) and positive for C-3 (0.99), C-4 (1.93) and C-5 (1.69), while the overall drug-score ranged from 0.50 to 0.88, with C-5 (0.88) and C-3 (0.75) scoring highest.

Table 3: Toxicity risk and predicted properties (OSIRIS Property Explorer)

Parameter	C-1	C-2	C-3	C-4	C-5
Mutagenic	None	None	None	None	None
Tumorigenic	None	None	None	None	None
Irritant	None	None	None	None	None
Reproductive effect	None	None	None	None	None
cLogP	3.42	2.42	3.22	2.79	1.98
Solubility (Log S)	−4.06	−3.26	−4.00	−2.79	−2.19
Molecular weight	308.38	309.37	336.39	288.39	260.34
TPSA (Å ²)	54.12	67.01	71.19	45.33	45.33
Drug-likeness	−1.75	−0.54	0.99	1.93	1.69
Drug-score	0.50	0.63	0.75	0.69	0.88

Molecular docking

The five compounds were docked into the active site of human pancreatic α -amylase (PDB ID: 1B2Y) [20]. The binding affinities, in order of strength, were C-3 (−7.9 kcal/mol), C-1 (−7.4), C-2 (−7.3), C-4 (−7.3) and C-5 (−7.2 kcal/mol) (Table 4), indicating favourable binding of all compounds in the substrate-binding cleft lined by the catalytic residues Asp197, Glu233 and Asp300 [6,7]. The interactions are shown in Figure 1.

C-1 was oriented so that the ester carbonyl accepted a hydrogen bond from Gly306, the indole N–H and the side-chain N–H formed hydrogen bonds with Glu233 and Asp197 respectively, and Asp300 contributed an electrostatic (π -anion) contact, so that all three catalytic residues were engaged [6,7]. The

phenyl ring made a π - π contact with Tyr62, and the indole ring was held by hydrophobic contacts with Ile235, Leu162 and Ala198. C-3, which had the best docking score, showed hydrogen bonds with Gly306 and Glu233, a π -anion interaction with Asp300, a π - π contact between its benzoyl ring and Trp59, and π -sigma/ π -alkyl contacts with Ile235, Ala198, Leu162 and Leu165. C-2 bound through a hydrogen bond with Glu233, a π -anion contact with Asp300, π - π interactions with Trp58 and His201, and hydrophobic contacts with Ile235, Leu162, Lys200 and Ala198. C-5 interacted with Glu233 and, through weaker carbon-hydrogen bonds, with Asp197 and Asp300, along with π - π and hydrophobic contacts with His201, Ile235, Lys200, Ala198 and Leu162. In contrast, C-4 made no contact with the catalytic triad and was held mainly by a carbon-hydrogen bond with His101 and hydrophobic contacts with Tyr62, Leu162 and Leu165.

Table 4: Docking results of C-1 to C-5 against human pancreatic α -amylase (PDB ID: 1B2Y)

Code	Binding affinity (kcal/mol)	Hydrogen-bond / polar interactions	Hydrophobic, π - π and π -anion interactions
C-1	-7.4	Gly306, Glu233, Asp197	π -anion: Asp300; π - π : Tyr62; π -sigma: Ile235; π -alkyl: Leu162, Ala198
C-2	-7.3	Glu233	π -anion: Asp300; π - π : Trp58, His201; π -sigma: Ile235, Leu162; π -alkyl: Lys200, Ala198
C-3	-7.9	Gly306, Glu233	π -anion: Asp300; π - π : Trp59; π -sigma: Ile235; π -alkyl: Ala198, Leu162, Leu165
C-4	-7.3	His101 (carbon-hydrogen bond)	π -sigma / π -alkyl: Tyr62, Leu165, Leu162
C-5	-7.2	Glu233; carbon-hydrogen bonds: Asp197, Asp300	π - π : His201; π -sigma: Ile235; π -alkyl: Lys200, Ala198, Leu162

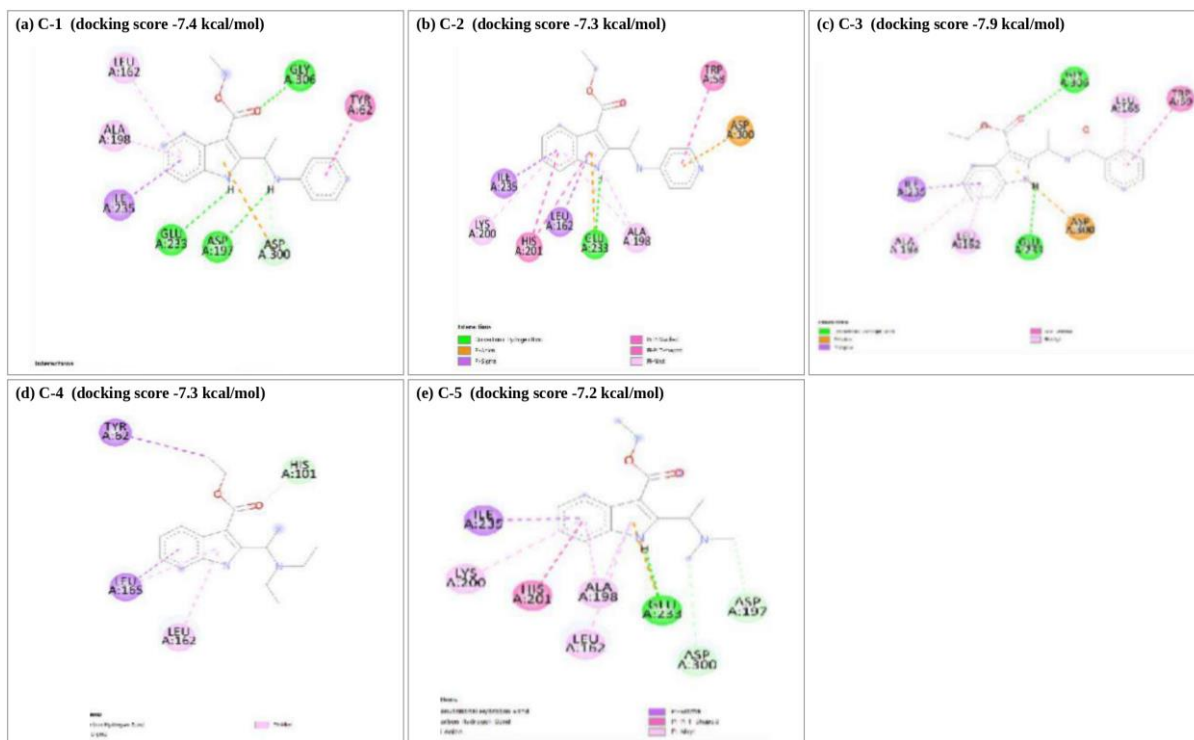


Figure 1: Two-dimensional interaction diagrams of (a) C-1, (b) C-2, (c) C-3, (d) C-4 and (e) C-5 in the active site of human pancreatic α -amylase (PDB ID: 1B2Y)

In vitro α -amylase inhibitory activity

The baseline readings of the assay are given in Table 5. The blank (starch and iodine without enzyme) gave a deep blue colour (absorbance 0.580), the uninhibited control gave a yellow colour owing to complete starch digestion (0.100), and the acarbose-treated standard [8,9] retained the blue colour (0.520), showing that the assay discriminates between active and inhibited enzyme [24].

Table 5: Baseline absorbance of the α -amylase inhibitory assay at 580 nm

Tube	Contents	Absorbance	Colour
Blank	Starch + iodine (no enzyme)	0.580	Blue
Control	Enzyme + starch + iodine (no inhibitor)	0.100	Yellow
Acarbose (standard)	Enzyme + starch + iodine + acarbose	0.520	Blue

All the compounds produced a concentration-dependent increase in inhibition (Table 6, Figure 2). At 50 $\mu\text{g/mL}$, C-1 and C-3 gave the highest inhibition (89.0% and 87.9%), which is of the same order as the acarbose-treated standard in the assay. C-3 was the most effective at low concentration, giving 42.2% inhibition at 10 $\mu\text{g/mL}$ and 62.9% at 20 $\mu\text{g/mL}$, whereas C-1 rose more steeply from 24.0% at 10 $\mu\text{g/mL}$ to 84.5% at 40 $\mu\text{g/mL}$. C-2 was also potent (82.5% at 50 $\mu\text{g/mL}$), C-5 showed moderate activity (64.0%), and C-4 was the weakest (36.0%).

Table 6: Percentage inhibition of α -amylase by C-1 to C-5

Concentration ($\mu\text{g/mL}$)	C-1	C-2	C-3	C-4	C-5
10	24.0%	21.2%	42.2%	2.0%	4.3%
20	48.0%	43.1%	62.9%	8.3%	16.6%
30	68.0%	57.2%	81.6%	17.1%	31.8%
40	84.5%	73.1%	85.0%	24.0%	46.0%
50	89.0%	82.5%	87.9%	36.0%	64.0%

Figure 2: Concentration-dependent inhibition of α -amylase by compounds C-1 to C-5

Correlation of docking with enzyme inhibition

The two compounds with the highest docking affinities that also engaged the catalytic residues, C-3 (−7.9 kcal/mol) and C-1 (−7.4 kcal/mol), were the most active in the enzyme assay, which supports the binding mode proposed by docking and agrees with the central role of the catalytic triad in α -amylase inhibition [6,7,13]. C-1 contacted all three residues of the catalytic triad, and C-3 contacted Glu233 and Asp300 as well as Gly306 and Trp59. The compounds that carry an aromatic ring on the side chain (C-1 to C-3) were considerably more active than those with a dialkylamino side chain (C-4 and C-5), in agreement with the additional π – π contacts of the former with Tyr62, Trp58/59 and His201. C-4, which did not interact with Asp197, Glu233 or Asp300 and relied on hydrophobic contacts, was the least active compound even though its docking score was identical to that of C-2 (−7.3 kcal/mol), which illustrates that docking scores alone do not rank inhibitory potency and that interaction with the catalytic residues appears to be an important determinant. The side-chain N–H of C-1, which hydrogen bonds to Asp197, is absent in C-4 and C-5, and may contribute to the difference. The modestly lower activity of C-2 compared with C-1 suggests that replacing the phenyl ring by a pyridine ring is less favourable for inhibition. These structure–activity observations are preliminary and need to be tested with a larger series.

The docking was carried out on human pancreatic α -amylase, whereas the *in vitro* assay used the porcine pancreatic enzyme, a closely related homologue that is widely used for screening [5,24]. Docking scores are predictions of binding and should be confirmed by kinetic studies.

CONCLUSION

Five ethyl indole-3-carboxylate Mannich base derivatives were evaluated as potential antidiabetic agents through *in silico* design, molecular docking and *in vitro* α -amylase inhibition. All compounds complied with Lipinski's rule of five and the other drug-likeness filters, were predicted to have favourable ADME properties and showed no toxicity risk flags in OSIRIS. Docking against human pancreatic α -amylase (PDB ID: 1B2Y) gave binding affinities of -7.2 to -7.9 kcal/mol with interactions at the catalytic residues Asp197, Glu233 and Asp300. In the enzyme assay, inhibition was concentration dependent, with the phenylamino derivative C-1 (89.0%) and the benzamido derivative C-3 (87.9%) showing the highest inhibition at 50 μ g/mL. These two compounds may serve as lead molecules for further antidiabetic investigation, in line with reports of indole-based α -amylase inhibitors [10,13], including determination of IC_{50} values, enzyme kinetic studies, structure–activity relationship analysis, selectivity against α -glucosidase, toxicity evaluation and *in vivo* studies in diabetic models.

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