

In Silico ADME Profiling, Synthesis, Characterization and In Vitro Acetylcholinesterase Inhibitory Evaluation of Ethyl 1H-Indole-3-carboxylate Mannich Base Derivatives as Potential Anti-Alzheimer's Agents

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Abstract: Alzheimer's disease (AD) is a progressive neurodegenerative disorder in which impaired cholinergic neurotransmission contributes to cognitive decline, and acetylcholinesterase (AChE) remains a validated target for symptomatic therapy. The indole nucleus is a privileged scaffold in medicinal chemistry, yet Mannich-type derivatives of ethyl indole-3-carboxylate have received little attention as AChE-directed agents. Five derivatives (compounds 1–5) were designed and screened in silico for drug-likeness and pharmacokinetics (SwissADME, Lipinski's rule of five) and toxicity risk (OSIRIS Property Explorer). All five complied with Lipinski's rule with no violations, were predicted to show high gastrointestinal absorption and blood–brain barrier permeation, and carried no mutagenic, tumorigenic or reproductive-toxicity alerts. Docking against human AChE (PDB ID: 4EY7) with PyRx/AutoDock Vina gave binding affinities of –7.1 to –10.3 kcal/mol, the best being ethyl 2-[1-(phenylamino) ethyl]-1H-indole-3-carboxylate (compound 1, –10.3 kcal/mol). The compounds were synthesized from ethyl indole-3-carboxylate, acetaldehyde and a primary or secondary amine in ethanol (yields 75–84%), recrystallized, and characterized by melting point, TLC, FT-IR, ¹H NMR and mass spectrometry. In Ellman's assay, compound 1 inhibited AChE in a concentration-dependent manner (6.76–56.41% at 0.2–1.0 µg/mL; IC₅₀ ≈ 0.97 µg/mL. Compound 1 is a promising starting point for AChE-directed optimization; assay of the remaining derivatives, selectivity and in vivo studies are still required.

Keywords: indole; Mannich base; acetylcholinesterase; Alzheimer's disease; SwissADME; molecular docking; Ellman's method.

INTRODUCTION

Alzheimer's disease (AD) is the leading cause of dementia and is characterized by progressive memory loss and cognitive decline associated with amyloid-β plaques and neurofibrillary tangles.[1,2] A loss of cholinergic neurotransmission in the basal forebrain is an early and consistent feature, which underlies the cholinergic hypothesis of the disease.[3,4] Inhibitors of acetylcholinesterase (AChE) raise synaptic acetylcholine levels and remain the mainstay of symptomatic treatment, but they do not halt neurodegeneration, benefit

declines as the disease advances, and gastrointestinal adverse effects are common.[5,6] These limitations sustain interest in new small-molecule AChE inhibitors.

AChE hydrolyses acetylcholine in a narrow, aromatic-lined active-site gorge that contains the catalytic triad (Ser203, His447, Glu334), the choline-binding residues Trp86 and Tyr337, and, near the gorge entrance, the peripheral anionic site (PAS).[7] The crystal structure of human AChE (PDB ID: 4EY7) provides a well-resolved template for structure-based inhibitor design.[8]

The indole ring is a privileged heterocyclic scaffold present in numerous natural products and drugs and is associated with a broad range of pharmacological activities, including neuroprotective effects.[9,10] Mannich-type amino alkylation introduces basic or aryl-amine side chains that can engage the aromatic residues of the AChE gorge, and Mannich bases are widely used in drug design.[11] We therefore designed five ethyl 1H-indole-3-carboxylate derivatives carrying a 1-(substituted amino)ethyl side chain at C-2, predicted their drug-likeness, pharmacokinetics and toxicity in silico, docked them against human AChE, synthesized and characterized them, and evaluated the top-ranked compound (compound 1) for AChE inhibition by Ellman's method.

2. Materials and Methods

2.1 In silico ADME and toxicity prediction

Structures were drawn in ChemSketch and converted to SMILES. Drug-likeness was assessed with Lipinski's rule of five (molecular weight ≤ 500 Da, $\log P \leq 5$, hydrogen-bond donors ≤ 5 , acceptors ≤ 10 ; not more than one violation).[12] Physicochemical descriptors, topological polar surface area (TPSA), consensus log P, water solubility, gastrointestinal (GI) absorption, blood–brain barrier (BBB) permeation, P-glycoprotein (P-gp) substrate status, Ghose, Veber, Egan and Muegge filters, bioavailability score, PAINS and Brenk alerts were obtained from the SwissADME web tool,[13] with the Veber criteria considered for oral bioavailability[14] and GI absorption/BBB permeation interpreted with the BOILED-Egg model.[15] Toxicity risks (mutagenic, tumorigenic, irritant, reproductive effects), drug-likeness and drug score were predicted with OSIRIS Property Explorer.[16]

2.2 Molecular docking

The crystal structure of human AChE (PDB ID: 4EY7) was retrieved from the RCSB Protein Data Bank. The protein was prepared by removing water and heteroatoms, adding polar hydrogens and charges, and saving in PDBQT format. Ligands were energy-minimized in PyRx,[17] a grid box enclosing the active-site gorge was defined, and docking was carried out with AutoDock Vina[18] the pose with the lowest binding affinity was taken as the best pose. Interactions were visualized in BIOVIA Discovery Studio Visualizer.

2.3 Synthesis

Step 1: ethyl indole-3-carboxylate. Indole-3-carboxylic acid (1.0 g, 6.2 mmol) in absolute ethanol (20–25 mL) with concentrated H₂SO₄ (1–2 mL) was refluxed at 75–80 °C for 4–6 h, cooled, poured into ice-cold water and neutralized with aqueous NaHCO₃. The precipitated ester was filtered, washed with cold water and recrystallized from ethanol–water. Step 2: Mannich bases (compounds 1–5). Ethyl indole-3-carboxylate was reacted with acetaldehyde and the respective amine (aniline, 2-aminopyridine, benzamide, diethylamine or dimethylamine) in ethanol with glacial acetic acid as catalyst under reflux (Table 1; Figure 1), following the Mannich amino alkylation approach.[11] Reaction progress was followed by TLC. The mixture was poured into ice-cold water, the precipitate was filtered, and the product was recrystallized from ethanol.

Table 1. Reaction parameters for compounds 1–5.

Cpd	Amine	Indole / amine (mmol)	Ethanol (mL)	Temp. (°C)	Time (h)
1	Aniline	3.0 / 3.0	30	80–85	5
2	2-Aminopyridine	3.0 / 3.0	35	85–90	4
3	Benzamide	3.0 / 3.0	30	75–85	5
4	Diethylamine	2.0 / 2.0	30	80–85	3
5	Dimethylamine	2.0 / 2.0	30	80–85	3

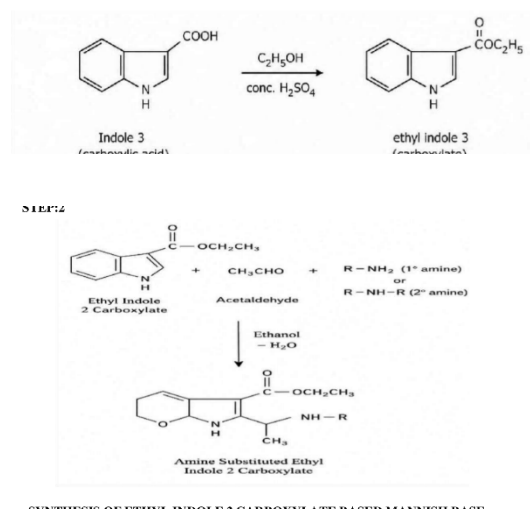


Figure 1. Synthetic route: (a) Fischer esterification of indole-3-carboxylic acid; (b) Mannich-type condensation of ethyl

indole-3-carboxylate with acetaldehyde and a primary (R–NH₂) or secondary (R–NH–R) amine.

2.4 Characterization

Colour, state, texture and solubility were recorded, and melting points were determined by the open-capillary method on a digital apparatus. TLC was run on precoated silica gel GF plates with hexane: ethyl acetate (7:3) and UV visualization, and R_f was calculated as distance travelled by the compound divided by that of the solvent front. IR spectra were recorded as KBr pellets on an ABB IR spectrophotometer; ¹H NMR spectra were recorded on a Bruker Topspin 500 MHz spectrometer in CDCl₃ with tetramethyl silane as internal standard; and mass spectra were recorded to establish molecular-ion and fragment peaks.[20]

2.5 In vitro AChE inhibition assay

AChE inhibition was measured by a modified Ellman's method[19] using electric eel AChE and acetylthiocholine iodide (ATCI) as substrate. Test sample (10 μL), AChE solution (20 μL, 0.09 U/mL) and 0.1 M sodium phosphate buffer (pH 8.0) were pre-incubated for 15 min at 25 °C; DTNB (10 μL, 10 mM) was added and the reaction was started with ATCI (10 μL, 14 mM). The yellow 5-thio-2-nitrobenzoate anion formed from thiocholine and DTNB was measured at 410 nm after 10 min. Compound 1 was tested at 0.2, 0.4, 0.6, 0.8 and 1.0 μg/mL. Physostigmine, dissolved in ethanol, was the positive control. Percentage inhibition was calculated as (A_{control} – A_{sample})/A_{control} × 100, and IC₅₀ was obtained by linear regression of percentage inhibition against concentration.

3. Results and Discussion

3.1 In silico drug-likeness, ADME and toxicity

All five compounds satisfied Lipinski's rule of five with zero violations (Table 2): molecular weights were 260–336 Da, consensus log P was about 2.0–3.5, TPSA was 45–71 Å², and hydrogen-bond donors and acceptors were well within limits. SwissADME predicted high GI absorption, BBB permeation and no P-gp efflux for every compound, and all passed the Ghose, Veber, Egan and Muegge filters with a bioavailability score of 0.55 and no PAINS or Brenk alerts (Table 3). Compound 1 was the only one to breach a lead-likeness

criterion (XLOGP3 > 3.5). These properties are favorable for a CNS-directed target.

Table 2. SwissADME physicochemical and Lipinski parameters.

Cpd	Formula	MW (Da)	TPSA (Å ²)	HBA	HBD	Cons. log P	Rot. bonds	Lipinski viol.
1	C ₁₉ H ₂₀ N ₂ O ₂	308.37	54.12	2	2	3.5	6	0
2	C ₁₈ H ₁₉ N ₃ O ₂	309.36	67.01	3	2	2.6	6	0
3	C ₂₀ H ₂₀ N ₂ O ₃	336.38	71.19	3	2	3.1	7	0
4	C ₁₇ H ₂₄ N ₂ O ₂	288.38	45.33	3	1	2.9	7	0
5	C ₁₅ H ₂₀ N ₂ O ₂	260.33	45.33	3	1	2.2	5	0

Table 3. Predicted pharmacokinetic and toxicity profile (SwissADME and OSIRIS).

Cpd	GI abs.	BBB	P-gp subs.	Biovail. score	OSIRIS toxicity alerts	OSIRIS drug-likeness	Drug score
1	High	Yes	No	0.55	None	–1.75	0.50
2	High	Yes	No	0.55	None	–0.54	0.63
3	High	Yes	No	0.55	None	0.99	0.75
4	High	Yes	No	0.55	Irritant (medium risk)	1.93	0.69
5	High	Yes	No	0.55	None	1.69	0.88

OSIRIS Property Explorer flagged no mutagenic, tumorigenic or reproductive-effect risk for any compound; compound 4 showed a medium-risk irritant alert. Drug scores ranged from 0.50 to 0.88, with compounds 3–5 showing positive drug-likeness values. These are computational estimates and do not replace experimental ADME and toxicity testing.

3.2 Molecular docking against AChE

All five derivatives docked within the AChE gorge with binding affinities of –7.1 to –10.3 kcal/mol (Table 4, Figure 2a). The N-aryl amine derivatives were the strongest binders: compound 1 (phenylamino, –10.3 kcal/mol) and compound 2 (pyridinyl amino, –9.6 kcal/mol), followed by the diethylamino (4, –8.5), dimethylamino (5, –8.0) and benzamido (3, –7.1) derivatives. The two-dimensional interaction map of compound 1 (Figure 2b) shows a hydrogen bond to Tyr124 and π-type contacts with Trp86, Tyr337, Tyr341, Trp286, Phe295, Phe297, Phe338 and His447, together with contacts to Ser203, Glu202, Gly121 and Gly448 near the catalytic triad. Contacts with Trp86 and Tyr337 (choline-binding site) together with Tyr124 and Trp286 (peripheral site) indicate that the ligand spans the gorge, consistent with its elongated aromatic-rich shape. Compound 1 was therefore selected for the enzyme assay.

Table 4. Docking scores against human AChE (PDB ID: 4EY7).

Cpd	Side chain at C-2 (1-substituted-aminoethyl)	Binding affinity (kcal/mol)
1	Phenylamino	-10.3
2	Pyridinyl amino	-9.6
3	Benzamido	-7.1
4	Diethylamino	-8.5
5	Dimethylamino	-8.0

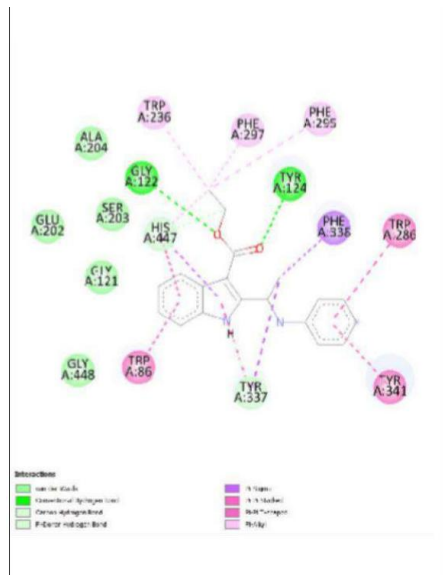
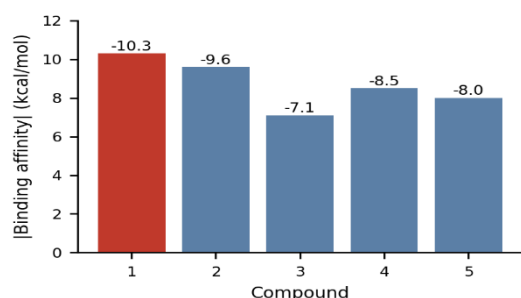


Figure 2. (a) Docking scores of compounds 1–5 against AChE (4EY7); compound 1 (red) is highest. (b) Two-dimensional protein–ligand interaction map of compound 1.

3.3 Synthesis and physical characterization

Cpd	Colour	State	Yield (%)	M.P. (°C)	R _f
1	Pale orange	Powder	80	180–186	0.47
2	Greyish yellow	Powder	75	191–200	0.53
3	Amber yellow	Powder	77	181–188	0.42
4	Falu red	Crystalline	84	187–195	0.52
5	Pale yellow	Crystalline	82	193–205	0.45

3.4 Spectral characterization

The IR spectra of all compounds showed N–H stretching at 3255–3280 cm⁻¹, a carbonyl band at 1664–1684 cm⁻¹ (ester, and amide for compound 3), aromatic C=C at 1531 cm⁻¹, and C–O (1052–1271 cm⁻¹) and C–N (1109–1313 cm⁻¹) bands (Table 6). The ¹H NMR spectra showed a downfield singlet at δ 10.1–10.3 for the indole N–H, aromatic multiplets at δ 7.0–8.5, ester O–CH₂ signals at δ 3.5–4.8 and alkyl methyl signals at δ 1.0–2.0 (Table 7); compound 1 additionally showed an aliphatic methine multiplet at δ 5.37. The molecular-ion peaks agreed with the calculated masses for compounds 1, 2 and 4 (Table 8), and the spectra of compound 1 are shown in Figure 3.

Table 6. Key IR bands (KBr, cm⁻¹).

Cpd	N–H	C=O	Ar C=C	C–O (ester)	C–N (indole)
1	3280	1684	1531	1195, 1053	1313, 1128
2	3265	1664	1531, 1442	1260, 1195, 1109, 1052	1260, 1250, 1110
3	3257	1665	1531	1271, 1196, 1053	1313, 1271
4	3255	1665	1531	1195, 1052	1313, 1126
5	3256	1664	1531	1126, 1052	1126

Table 7. ¹H NMR data (500 MHz, CDCl₃, δ ppm).

Cpd	N–H (s)	Ar–H (m)	O–CH ₂	CH ₃
1	10.27	7.10–7.74	4.84–4.68	1.32–1.02
2	10.10	7.0–8.5	4.10	1.20
3	10.1	7.0–8.5	3.5	1.00
4	10.6	7.0–8.5	4.10	1.20–2.0
5	10.1	7.0–8.5	4.80–4.70	1.35

Table 8. Mass spectral data (m/z).

Cpd	Formula	Calcd. [M]	Found	Base peak
1	C ₁₉ H ₂₀ N ₂ O ₂	308.15	308	143
2	C ₁₈ H ₁₉ N ₃ O ₂	309.15	309	144
3	C ₂₀ H ₂₀ N ₂ O ₃	336.15	360	144.1
4	C ₁₇ H ₂₄ N ₂ O ₂	288.18	288.2	130.1
5	C ₁₅ H ₂₀ N ₂ O ₂	260.15	259	130.1

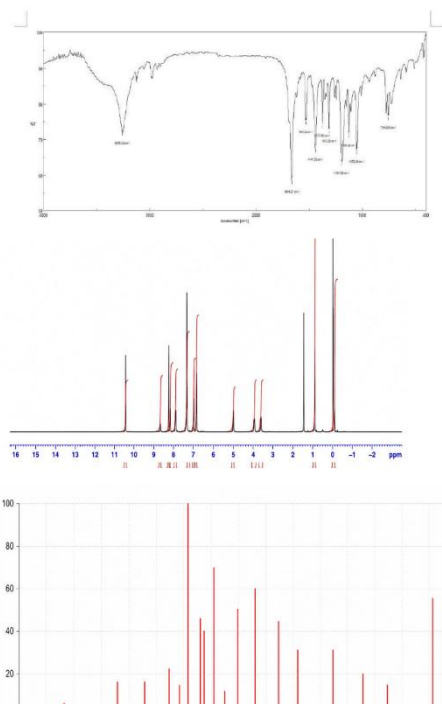


Figure 3. Spectra of compound 1: IR (KBr), ¹H NMR (500 MHz, CDCl₃) and mass spectrum.

3.5 In vitro AChE inhibitory activity of compound 1

Compound 1 inhibited AChE in a concentration-dependent manner, from 6.76% at 0.2 µg/mL to 56.41% at 1.0 µg/mL (Table 9, Figure 4). Linear regression of these values ($y = 60.32x - 7.35$; $R^2 = 0.977$) gives $IC_{50} \approx 0.98$ µg/mL, and physostigmine gave 100% inhibition under the same conditions. The potent docking score of compounds 1 is thus supported by a measurable enzymatic effect, although the assay used electric eel AChE whereas docking used the human enzyme.

Table 9. In vitro AChE inhibition by compound 1.

Concentration (µg/mL)	Inhibition (%)
0.2	6.76
0.4	17.16
0.6	25.38
0.8	38.49
1.0	56.41

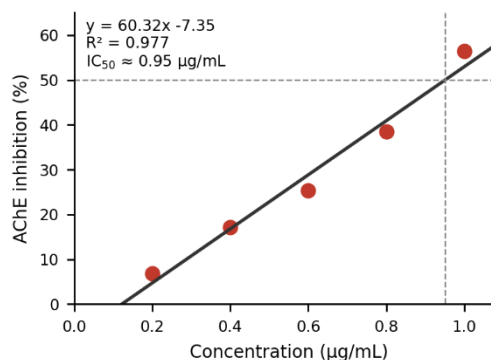


Figure 4. Concentration–response plot of AChE inhibition by compound 1 with linear fit and IC_{50} .

3.6 Discussion

The ranking of the docking scores indicates that an N-aryl amine at the C-2 side chain (compounds 1 and 2) favours binding to the aromatic gorge, whereas the bulkier benzamido group of compounds 3 gave the weakest affinity (−7.1 kcal/mol). Docking was predictive for the one compound tested: compound 1, the top-scoring derivative, inhibited AChE with sub-µg/mL potency. The synthesis is a simple one-pot condensation from inexpensive reagents and gives good yields, which makes the scaffold attractive for analogue libraries, and the in-silico profile (zero Lipinski violations, high GI absorption, predicted BBB permeation, no P-gp efflux) is favorable for a centrally acting agent. Several limitations should be noted. Only compound 1 was assayed, so no experimental structure–activity relationship across the series can be drawn; physostigmine was run at a single level, so relative potency cannot be ranked; docking scores and ADME/toxicity values are predictions; and ¹³C NMR, high-resolution mass spectrometry, replicate statistics and cytotoxicity data are lacking. Future work should include IC_{50} determination for all derivatives and the standard, enzyme

kinetics, selectivity against butyrylcholinesterase, molecular dynamics, cytotoxicity and in vivo evaluation.

4. Conclusions

Five ethyl 1H-indole-3-carboxylate Mannich bases were designed, profiled in silico, synthesized in 75–84% yield and characterized. All satisfied Lipinski's rule with favorable predicted absorption and brain penetration. Compound 1, ethyl 2-[1-(phenylamino)ethyl]-1H-indole-3-carboxylate, showed the best docking affinity (–10.3 kcal/mol) against human AChE and inhibited the enzyme in vitro in a concentration-dependent manner ($IC_{50} \approx 0.95 \mu\text{g/mL}$). It represents a promising indole-based starting point for further investigation as a potential anti-Alzheimer's agent, pending pharmacological, toxicological and in vivo validation.

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Author Contributions:

ArchanaA: Conceptualization, drug design, Molecular docking, in silico ADME and toxicity analysis, Synthesis, characterization and biological data analysis, visualization and writing—original draft. Ajin T.S, Hameedhash R, Priya M: editing of the manuscript. All authors contributed to the interpretation of the results, reviewed the manuscript critically, and approved the final version.

Conflicts of Interest: The authors declare no conflict of interest.

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