

Original Research Article

Design, molecular docking and biological evaluation of colchicine binding site inhibitors as possible anticancer agents

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Abstract

Purpose: To design novel colchicine binding site (topoisomerase) inhibitors and evaluate their anti-cancer effectiveness against different cancer cell lines.

Methods: Monoamine oxidase A inhibitors (MAO-AIs), clorgyline, as well as colchicine inhibitors: N-(4-acetylphenyl)-1H-indole-2-carboxamide (N1), N-(4-(1-hydroxyethyl)phenyl)-1H-indole-2-carboxamide (N2), N-(3-acetylphenyl)-1H-indole-2-carboxamide (N3) and N-(3-(1-hydroxyethyl)-1H-indole-2-carboxamide (N4), were created using pharmacophore modeling and possible inhibitory effect was investigated. PerkinElmer ChemDraw Ultra 16.0 software was used to generate ligand structures. Autodock was used to optimize the ligands. Rotable bonds and Gasteiger charges were assigned to the suggested compounds. Clorgyline was used as the control ligand and retrieved from PubChem database. A viability test was performed using increasing concentrations of Colchicine inhibitors on A549, H292, and H460 cells for 24, 48, and 72 h.

Results: Docking data revealed that Colchicine binding domains were accommodated by clorgyline and N series (N1, N2, N3, and N4). Structure-based binding of N1, N2, N3, and N4 create hydrogen-bond with Gln245, Leu 246 CYS239, ALA248, ASN256, ALA348, VAL313, ASN256, and fitting the 5CB4 binding domain. Vitality of cells treated with Clorgyline and N1 - N4 was lower compared to untreated control cells. The IC₅₀ of Clorgyline for A549, H460, and H292 were 208.99, 217, and 313 μ M, respectively, indicating strong antiproliferative action. Compound N4 showed the highest antiproliferative effect against A549, H460, and H292, with IC₅₀ values of 147.2, 89 and 130 μ M, respectively, after treatment for 72 h.

Conclusion: Colchicine inhibits the proliferation of cancer cells. Furthermore, tested compounds (N1 – N4) exhibited promising anticancer action.

Keywords: Cancer, Topoisomerase, Docking, Microtubules, Colchicine

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INTRODUCTION

Studies have reported a wide variety of chemicals targeting colchicine sites [1]. Agents that target the colchicine site were reported to have certain advantages, including low molecular

weight, lipophilicity, and the ability to overcome drug resistance. Clorgyline hydrochloride, which is structurally similar to pargyline, is a selective and irreversible inhibitor of monoamine oxidase A (MAO-A) widely used in scientific research with promising anticancer activity [1].

One approach that provides details on the binding capacity and affinity of a molecule for its target protein is molecular docking. It studies binding-orientation predictions and forecasts the optimal ligand concentration that will fit energetically and geometrically at the target molecule's binding site to create a stable compound [2]. It is one of the most utilized strategies in structure-based drug design (SBDD) by reasonably predicting the conformation of small molecule ligands within proper target binding site [3]. Three most popular SBDD techniques are molecular docking, structure-based virtual screening (SBVS), and molecular dynamics (MD) which are widely applied in molecular analysis including binding energy, molecular interactions, and induced conformational changes [4].

Earlier attempts to use molecular docking were hampered by limited computer resources, but with recent advancements in high-performance computing, virtual screening techniques have progressively improved [5,6]. Several medications and therapeutic candidates have been developed using these techniques and have progressed to clinical trials. Lead compounds for human immunodeficiency virus (HIV), myocardial infarction, Alzheimer's disease, rheumatoid arthritis, and numerous other illnesses have been developed [7,8] including cancer [9].

Two steps are required to identify the most likely binding conformations. One is the force field used to estimate the complex's free energy of binding, computed based on a specific bound conformation (scoring function), and evaluation of the large conformational space that represents possible potential binding modes available to the ligand and target (search algorithm) [10]. This study therefore investigated a molecular docking approach to design colchicine-binding-site (topoisomerase) inhibitors and evaluate their anti-cancer effectiveness against lung cancer cell lines.

EXPERIMENTAL

The A549, H460 and H292 lung cancer cells were purchased from American Type Culture Collection (ATCC, USA). Cells were grown in Roswell Park Memorial Institute (RPMI) 1640 (RPMI) media with 10 % fetal bovine serum (FBS), 2 mM L-glutamine, 100 U/mL penicillin, and 0.1 mg/mL streptomycin. The cells were kept in a controlled temperature, humidified incubator at 37 °C, 95 % humidity, and 5% carbon dioxide (CO₂). Monoamine oxidase A inhibitors (MAO-

Als), and Clorgyline were purchased from Sigma-Aldrich, Darmstadt, Germany. MAO -A inhibitors, as well as colchicine inhibitors (N1, N2, N3, and N4), were created using a pharmacophore modeling from previous study [11,13].

Composition of chemicals derived from 1H-indole-2-carboxamide

Perkin Elmer ChemDraw Ultra 16.0 software was used to generate the ligand structures which were subsequently converted into 2D structures (Figure 1). The MM2 force field minimization algorithm was used to minimize the energy of the ligands.

Docking

The ligands that were designed and synthesized were optimized appropriately, and notable bonds and Gasteiger charges were assigned. Clorgyline was the control ligand, retrieved from the PubChem database, and optimized. The enzyme (5CB4) was obtained from the Protein Data Bank (PDB) (<https://www.rcsb.org/structure/5CB4>) in pdb format and utilized in molecular docking for receptor refinement (enzyme structure preparation).

Preparation of the protease enzyme structure

Autodock Tools 1.5.6 was used for protein processing. The undesirable and water molecules were eliminated. Furthermore, the protein structure was assigned hydrogen and Kollman charges. For further docking, the optimized protein structure was saved as a pdbqt file.

Protein-ligand molecular docking with Autodock 4.2

Autodock 4.2 program was used to perform the ligand molecular docking with protein 5CB4. To hide full enzyme's binding site, a grid box measuring 60 x 60 x 60 points and 0.375 Å (grid spacing) was used. The aforementioned action was taken to enable unrestricted ligand mobility. The docking computation was performed using the Lamarckian Genetic Algorithm (LGA) approach. With all parameters set to default, 100 search runs were conducted. Ultimately, clustering histogram analysis was completed following the end of the docking search. Ki selection was stored for further data analysis, along with the optimal conformation (lowest binding energy, inhibition constant estimation) [14,15].

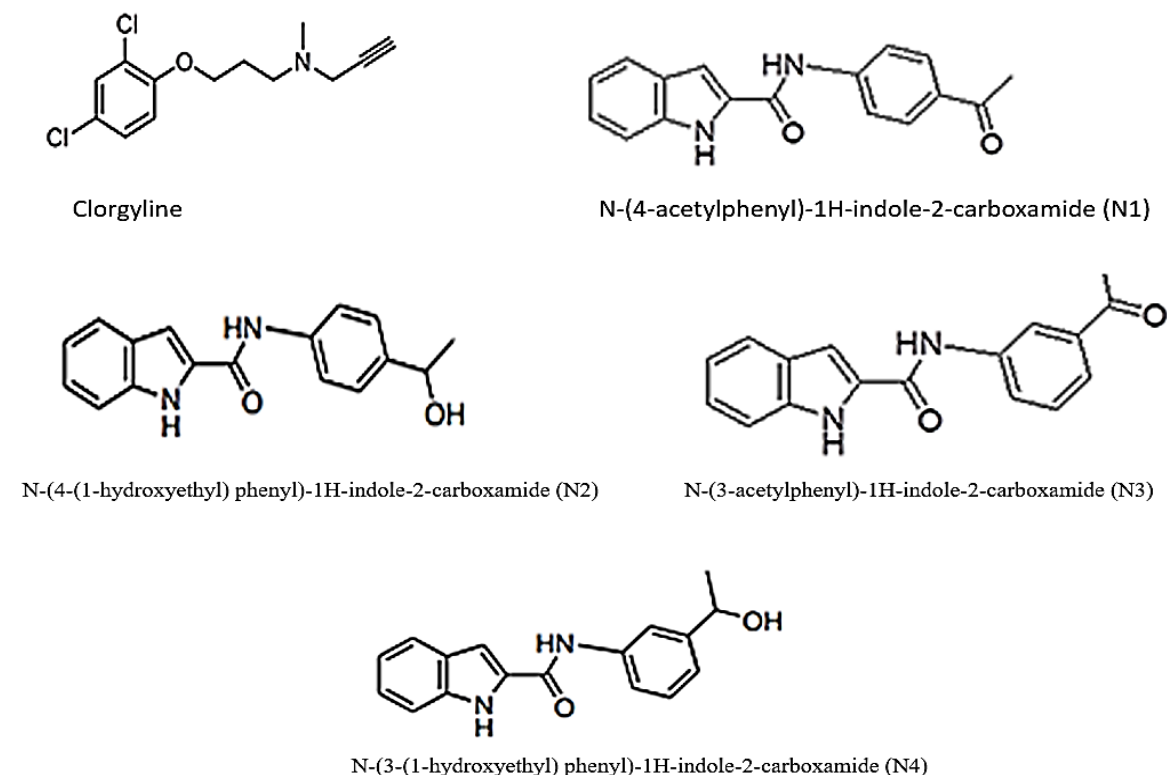


Figure 1: 2D Ligands structures using ChemDraw Ultra 16.0 software

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay

The viability test (MTT assay) was performed using increasing concentrations of Colchicine inhibitors on A549, H292, and H460 cells for 24, 48, and 72 h.

Computational techniques

The Swiss absorption, distribution, metabolism and elimination (ADME) server was used to set the ADME parameters. The server was used to forecast the physicochemical characteristics of the compounds under investigation, in addition to ADME parameters. Chemical structures and simplified molecular input line entry system (SMILES) notations of the compounds under investigation, clorgyline and N1 - N4, were drawn using ChemAxon's Marvin JS. Potential for passive gastrointestinal absorption, polarity, lipophilicity, and ultimately brain penetration were assessed using the open-access program (BOILED EGG).

RESULTS

Docking interactions

Colchicine binding domains were accommodated by Clorgyline and the N series (N1, N2, N3, and

N4) (Table 1). The results showed that Van der Waals, hydrophobic, and hydrogen bonding were all involved in the interaction. Furthermore, N1, N2, N3, and N4 created hydrogen-bond with Gln245, Leu 246 CYS239, ALA248, ASN256, ALA348, VAL313, ASN256, and fit the 5CB4 binding domain (Figure 2).

Drug likeness parameters

The results demonstrated that the compounds met all requirements and that there were no infractions of Lipinski's rule (Table 2). Furthermore, there were variations in characteristics according to chemical structures. The compounds exhibited good penetration across the blood-brain barrier (BBB), high passive oral absorption, and good bioavailability (Table 3 and Table 4).

Boiled egg representation of Clorgyline and compounds N1 – N4

While compounds N1, N2, N3, and N4 demonstrated potential predictive inhibitory actions, Clorgyline did not exhibit predictive inhibitory activity to any of the cytochrome P (CYP) enzymes tested in this investigation (Figure 3, Tables 5 – 9).

Table 1: Docking interactions

Compound		Estimated free energy of binding (kcal/mol)	Estimated Inhibition constant (μM)	Hydrogen bond interaction Close contact residues
Linear tetrapeptides	N1	-8.86	0.320	ASN247, GLY244, GLN245*, LEU246*, LEU253, VAL313, VAL181, THR312, ASN348, ALA314, MET257, ASN256, LYS350, ALA352, CYS239, ALA248
	N2	-8.01	1.21	CYS239*, ALA248*, ALA352, LEU246, ASN256*, ALA314, LYS350, LEU253, MET257, VAL181, VAL313, ASN347, ASN348, THR312, GLY244, GLN245
	N3	-8.61	0.490	VAL181, THR312, VAL313, ALA314, MET257, GLN245, GLY244, ASN247, ALA248*, LEU245, CYS239, ASN256, LEU253, LYS350, ASN348
	N4	-9.43	0.122	ALA352, ALA314, VAL313*, VAL349, THR312, ASN348, LYS350, MET257, VAL181, ALA180, LEU253, ASN256*, ALA248, LEU246, THR350.
Clorgyline		-6.92	8.53	ASN347, ASN348, SER178, THR312, THR179, LEU246, LEU253, ALA248, ASN101, ASN256, MET257, VAL181, ALA180, LYS350.

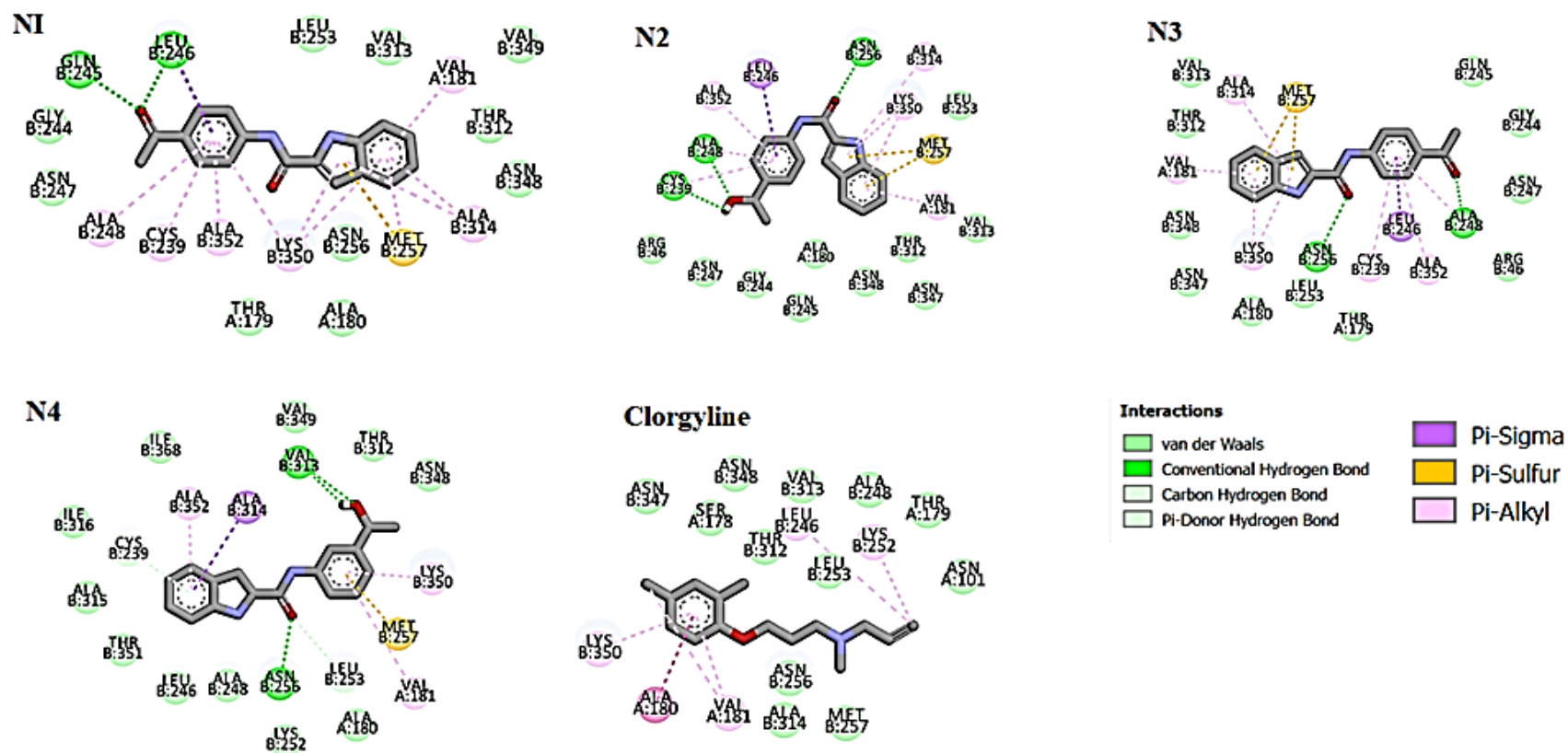


Figure 2: Picture visualized by the AutoDock 4.2 program of $\alpha\beta$ -tubulin/ligand complex of N1-N4, and Clorgyline. Green colour indicated hydrogen bond interactions

Table 2: Drug likeness parameters for Clorgyline and compounds N1-4

Compound	Drug likeness characteristics				
	Lipinski	Ghose	Veber	Egan	Muegge
Clorgyline	Yes	Yes	Yes	Yes	Yes
N1	Yes	Yes	Yes	Yes	Yes
N2	Yes	Yes	Yes	Yes	Yes
N3	Yes	Yes	Yes	Yes	Yes
N4	Yes	Yes	Yes	Yes	Yes

Table 3: Pharmacokinetic properties of Clorgyline and compounds N1 - N4

Parameter	Compounds				
	Clorgyline	N1	N2	N3	N4
Passive GI absorption	High	High	High	High	High
BBB permeation	Yes	Yes	Yes	Yes	Yes
P-gp substrate	No	No	No	No	Yes
CYP1A2 inhibitor	No	Yes	Yes	Yes	Yes
CYP2C19 inhibitor	No	Yes	Yes	Yes	Yes
CYP2C9 inhibitor	No	Yes	Yes	Yes	Yes
CYP2D6 inhibitor	No	Yes	Yes	Yes	Yes
CYP3A4 inhibitor	No	Yes	Yes	Yes	No
Log K_p (skin permeation) cm/s	-4.63	-5.65	-5.79	-5.65	-6.07

Table 4: Output parameters of drug-likeness and ADME

Molecule no.	Compound	nRB	nHBA	nHBD	TPSA ($\text{\AA}^2$)	Bioavailability score	iLOGP
1	Clorgyline	6	2	0	12.47	0.55	3.43
2	N1	4	2	2	61.96	0.55	2.24
3	N2	4	2	3	65.12	0.55	2.29
4	N3	4	2	2	61.96	0.55	2.33
5	N4	4	2	3	65.12	0.55	2.57

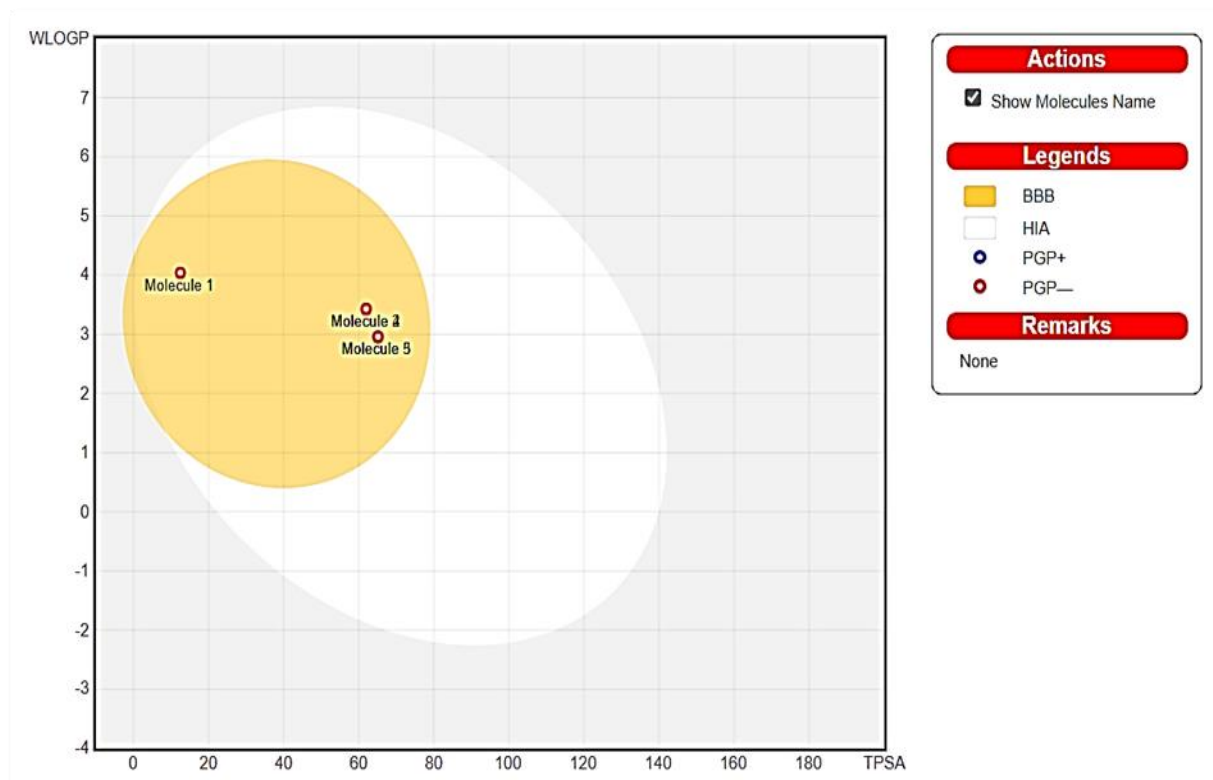
**Figure 3:** The BOILED EGG figure of Clorgyline and compounds N1 - N4

Table 5: Boiled egg data analysis of N1

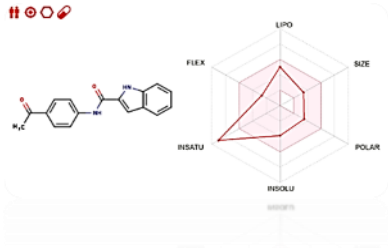
Category	Property	Value / Explanation
General Info	Formula	C ₁₇ H ₁₄ N ₂ O ₂
Lipophilicity		
	Molecular weight	278.31 g/mol
	Heavy atoms	21
	Aromatic heavy atoms	15
	Fraction Csp ³	0.06
	Rotatable bonds	4
	H-bond acceptors	2
	H-bond donors	2
	Molar Refractivity	82.71
	TPSA	61.96 Å ²
	Log P _{ow} (iLOGP)	2.24
	Log P _{ow} (XLOGP3)	3.20
	Log P _{ow} (WLOGP)	2.24
	Log P _{ow} (MLOGP)	3.35
	Log P _{ow} (SILICOS-IT)	3.57
Water Solubility	Consensus Log P _{ow}	2.90
	Log S (ESOL)	-3.91 → Soluble (3.43e-02 mg/ml; 1.23e-04 mol/L)
	Log S (Ali)	-4.28 → Moderately soluble (1.47e-02 mg/mL; 5.29e-05 mol/l)
Pharmacokinetics	Log S (SILICOS-IT)	-6.13 → Poorly soluble (2.04e-04 mg/mL; 7.33e-07 mol/L)
	GI absorption	High
	BBB permeant	Yes
	BBB Criteria (general)	Molecules with LogP between 2–4 and TPSA ≤ 90 Å ² are more likely to cross the BBB. High polarity or large size reduces permeability.
	P-gp substrate	No
	CYP1A2 inhibitor	Yes
	CYP2C19 inhibitor	Yes
	CYP2C9 inhibitor	Yes
	CYP2D6 inhibitor	Yes
	CYP3A4 inhibitor	Yes
Drug-likeness (Rules)	Log K _p (skin permeation)	-5.65 cm/s
	Lipinski's Rule of Five	MW ≤ 500; LogP ≤ 5; ≤ 5 H-bond donors; ≤ 10 H-bond acceptors. Predicts oral bioavailability.
	Ghose Filter	MW 160–480; LogP -0.4 to +5.6; molar refractivity 40–130; atoms 20–70. Screens for drug-like chemical space.
	Veber's Rule	≤ 10 rotatable bonds; TPSA ≤ 140 Å ² . Focuses on flexibility and polarity for oral bioavailability.
	Egan's Rule	TPSA ≤ 132 Å ² ; LogP -1 to +6. Identifies compounds with good intestinal absorption.
	Muegge's Rule	MW 200–600; LogP -2 to +5; ≤ 7 rings; ≤ 15 rotatable bonds; TPSA ≤ 150 Å ² . Broad drug-likeness filter.
	Bioavailability Score	0.55
Medicinal Chemistry	PAINS	0 alert
	Brenk	0 alert
	Leadlikeness	Yes
Interpretation	Synthetic accessibility	1.90
	Summary	Molecule 2 is drug-like (passes Lipinski, Ghose, Veber, Egan, Muegge), shows high GI absorption, is BBB permeant (potential CNS activity), not a P-gp substrate, but inhibits multiple CYP enzymes. Overall: promising oral bioavailability with CNS penetration, though CYP inhibition suggests possible drug–drug interactions.

Table 6: Boiled egg data analysis of N2

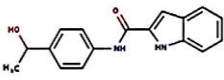
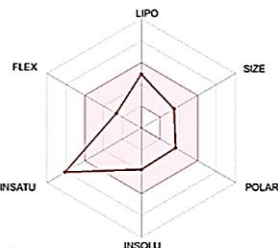
Category	Property	Value / Explanation
Lipophilicity	 	<chem>C17H16N2O2</chem> Molecular weight 280.32 g/mol Heavy atoms 21 Aromatic heavy atoms 15 Fraction Csp3 0.12 Rotatable bonds 4 H-bond acceptors 2 H-bond donors 2 Molar Refractivity 83.45 TPSA 65.12 Å ²
	Log P _{ow} (iLOGP)	2.29
	Log P _{ow} (XLOGP3)	3.25
	Log P _{ow} (WLOGP)	2.02
	Log P _{ow} (MLOGP)	3.25
	Log P _{ow} (SILICOS-IT)	3.25
	Consensus Log P _{ow}	2.73
	Log S (ESOL)	−3.81 → Soluble (4.30×10^{-2} mg/mL; 1.53×10^{-4} mol/L)
	Log S (Ali)	−4.17 → Moderately soluble (1.91×10^{-2} mg/mL; 6.81×10^{-5} mol/L)
	Log S (SILICOS-IT)	−5.66 → Moderately soluble (6.15×10^{-4} mg/mL; 2.19×10^{-6} mol/L)
	GI absorption	High
	BBB permeant	Yes
	BBB Criteria (general)	Molecules with LogP 2 and TPSA ≤ 90 Å ² are likely to cross the BBB; high polarity reduces permeability.
	P-gp substrate	No
	CYP1A2 inhibitor	Yes
Drug-likeness (Rules)	CYP2C19 inhibitor	Yes
	CYP2C9 inhibitor	Yes
	CYP2D6 inhibitor	Yes
	CYP3A4 inhibitor	Yes
	Log K _p (skin permeation)	−5.79 cm/s
	Lipinski's Rule of Five	MW ≤ 500; LogP ≤ 5; ≤ 5 H-bond donors; ≤ 10 H-bond acceptors → predicts oral bioavailability.
	Ghose Filter	MW 160–480; LogP −0.4 to +5.6; molar refractivity 40–130; atoms 20–70 → drug-like chemical space.
	Veber's Rule	≤ 10 rotatable bonds; TPSA ≤ 140 Å ² → flexibility and polarity for oral bioavailability.
	Egan's Rule	TPSA ≤ 132 Å ² ; LogP −1 to +6 → good intestinal absorption.
	Muegge's Rule	MW 200–600; LogP −2 to +5; ≤ 7 rings; ≤ 15 rotatable bonds; TPSA ≤ 150 Å ² → broad drug-likeness filter.
Medicinal Chemistry	Bioavailability Score	0.55
	PAINS	0 alert
	Brenk	Yes
	Leadlikeness	Yes
Interpretation	Synthetic accessibility	2.42
	Summary	Molecule 3 is drug-like (passes Lipinski, Ghose, Veber, Egan, Muegge), shows high GI absorption and BBB permeability → potential CNS activity. It is not a P-gp substrate but inhibits multiple CYP enzymes, suggesting possible drug–drug interactions. Overall: promising oral bioavailability with CNS penetration.

Table 7: Boiled egg data analysis of N3

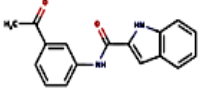
Category	Property	Value
 	SMILES	<chem>O=C(c1ccc2c((nH)1)ccc2)Nc1ccc(C(=O)C)cc1</chem>
	Formula	C ₁₇ H ₁₄ N ₂ O ₂
Lipophilicity	Molecular weight	278.31 g/mol
	Heavy atoms	21
	Aromatic heavy atoms	15
	Fraction Csp ³	0.06
	Rotatable bonds	4
	H-bond acceptors	2
	H-bond donors	1
	Molar Refractivity	82.71
	TPSA	61.96 Å ²
	Log Po/w (iLOGP)	2.33
Water Solubility	Log Po/w (XLOGP3)	3.30
	Log Po/w (WLOGP)	3.23
	Log Po/w (MLOGP)	3.33
	Log Po/w (SILICOS-IT)	2.53
	Consensus Log Po/w	2.92
	Log S (ESOL)	-3.91
	Solubility (ESOL)	3.43e-02 mg/mL; 1.23e-04 mol/L
	Class (ESOL)	Soluble
	Log S (Ali)	-4.28
	Solubility (Ali)	1.47e-02 mg/mL; 5.29e-05 mol/L
Pharmacokinetics	Class (Ali)	Moderately soluble
	Log S (SILICOS-IT)	-6.13
	Solubility (SILICOS-IT)	2.04e-04 mg/ml; 7.33e-07 mol/L
	Class (SILICOS-IT)	Poorly soluble
	GI absorption	High
	BBB permeant	Yes
	P-gp substrate	No
	CYP1A2 inhibitor	Yes
	CYP2C19 inhibitor	Yes
	CYP2C9 inhibitor	Yes
Druglikeness	CYP2D6 inhibitor	Yes
	CYP3A4 inhibitor	Yes
	Log Kp (skin permeation)	-5.65 cm/s
	Lipinski	Yes; 0 violation
	Ghose	Yes
	Veber	Yes
	Egan	Yes
	Muegge	Yes
	Bioavailability Score	0.55
	PAINS alerts	0
Medicinal Chemistry	Brenk alerts	0
	Leadlikeness	Yes
	Synthetic accessibility	2.02
Summary	Overall assessment	Drug-like molecule with high absorption and CNS penetration; moderate lipophilicity; limited solubility; broad CYP inhibition may cause drug–drug interactions but otherwise promising profile

Table 8: Table represents the boiled egg data analysis of N4

Category	Property	Value / Explanation
General Info	Formula	C ₁₇ H ₁₆ N ₂ O ₂
Lipophilicity		
	Molecular weight	280.32 g/mol
	Heavy atoms	21
	Aromatic heavy atoms	15
	Fraction Csp ³	0.12
	Rotatable bonds	4
	H-bond acceptors	4
	H-bond donors	1
	Molar Refractivity	83.45
	TPSA	65.12 Å ²
Water Solubility	Log P _{ow} (iLOGP)	2.57
	Log P _{ow} (XLOGP3)	2.70
	Log P _{ow} (WLOGP)	2.96
	Log P _{ow} (MLOGP)	2.20
	Log P _{ow} (SILICOS-IT)	3.25
	Consensus Log P _{ow}	2.70
Pharmacokinetics	Log S (ESOL)	-3.56 → Soluble (7.68 × 10 ⁻² mg/mL; 2.74 × 10 ⁻⁴ mol/L)
	Log S (Ali)	-3.75 → Soluble (4.97 × 10 ⁻² mg/mL; 1.77 × 10 ⁻⁴ mol/L)
	Log S (SILICOS-IT)	-5.66 → Moderately soluble (6.15 × 10 ⁻⁴ mg/mL; 2.19 × 10 ⁻⁶ mol/L)
	GI absorption	High
	BBB permeant	Yes
	BBB Criteria (general)	Molecules with LogP 2–4 and TPSA ≤ 90 Å ² are likely to cross the BBB; high polarity reduces permeability.
	P-gp substrate	Yes
	CYP1A2 inhibitor	Yes
	CYP2C19 inhibitor	Yes
	CYP2C9 inhibitor	Yes
Drug-likeness (Rules)	CYP2D6 inhibitor	Yes
	CYP3A4 inhibitor	No
	Log K _p (skin permeation)	-6.07 cm/s
	Lipinski's Rule of Five	MW ≤ 500; LogP ≤ 5; ≤ 5 H-bond donors; ≤ 10 H-bond acceptors → predicts oral bioavailability.
	Ghose Filter	MW 160–480; LogP -0.4 to +5.6; molar refractivity 40–130; atoms 20–70 → drug-like chemical space.
	Veber's Rule	≤ 10 rotatable bonds; TPSA ≤ 140 Å ² → flexibility and polarity for oral bioavailability.
Medicinal Chemistry	Egan's Rule	TPSA ≤ 132 Å ² ; LogP -1 to +6 → good intestinal absorption.
	Muegge's Rule	MW 200–600; LogP -2 to + 5; ≤ 7 rings; ≤ 15 rotatable bonds; TPSA ≤ 150 Å ² → broad drug-likeness filter
	Bioavailability Score	0.55
	PAINS	0 alert
	Brenk	0 alert
	Leadlikeness	Yes
Interpretation	Synthetic accessibility	2.52
	Summary	Molecule 6 is drug-like (passes Lipinski, Ghose, Veber, Egan, Muegge), shows high GI absorption and BBB permeability → potential CNS activity. It is a P-gp substrate and inhibits multiple CYP enzymes except CYP3A4, suggesting possible drug interactions. Overall: promising oral bioavailability with CNS penetration.

Table 9: Boiled egg data analysis of Clorgyline

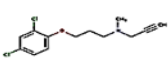
Category	Property	Value / Explanation
General Info		
Lipophilicity	 	Formula: $C_{13}H_{15}Cl_2NO$ Molecular weight: 272.17 g/mol Heavy atoms: 17 Aromatic heavy atoms: 6 Fraction Csp3: 0.38 Rotatable bonds: 6 H-bond acceptors: 2 H-bond donors: 0 Molar Refractivity: 72.85 TPSA: 12.47 Å²
	Log P _{ow} (iLOGP)	3.43
	Log P _{ow} (XLOGP3)	4.14
	Log P _{ow} (WLOGP)	3.41
	Log P _{ow} (MLOGP)	3.18
	Log P _{ow} (SILICOS-IT)	3.87
	Consensus Log P _{ow}	3.71
	Log S (ESOL)	−4.03 → Moderately soluble (2.56×10^{-2} mg/mL; 9.42×10^{-5} mol/L)
	Log S (Ali)	−4.15 → Moderately soluble (1.92×10^{-2} mg/mL; 7.07×10^{-5} mol/L)
	Log S (SILICOS-IT)	−4.79 → Moderately soluble (4.36×10^{-3} mg/mL; 1.60×10^{-5} mol/L)
Water Solubility		
Pharmacokinetics	GI absorption	High
	BBB permeant	Yes
	BBB Criteria (general)	Molecules with LogP 2 and TPSA ≤ 90 Å ² are likely to cross the BBB; high polarity reduces permeability.
Druglikeness (Rules)	P-gp substrate	No
	CYP1A2 inhibitor	Yes
	CYP2C19 inhibitor	No
	CYP2C9 inhibitor	No
	CYP2D6 inhibitor	No
	CYP3A4 inhibitor	No
	Log K _p (skin permeation)	−4.99 cm/s
	Lipinski's Rule of Five	MW ≤ 500; LogP ≤ 5; ≤ 5 H-bond donors; ≤ 10 H-bond acceptors → predicts oral bioavailability.
	Ghose Filter	MW 160–480; LogP −0.4 to +5.6; molar refractivity 40–130; atoms 20–70 → drug-like chemical space.
	Veber's Rule	≤ 10 rotatable bonds; TPSA ≤ 140 Å ² → flexibility and polarity for oral bioavailability.
Medicinal Chemistry	Egan's Rule	TPSA ≤ 132 Å ² ; LogP −1 to +6 → good intestinal absorption.
	Muegge's Rule	MW 200–600; LogP −2 to +5; ≤ 7 rings; ≤ 15 rotatable bonds; TPSA ≤ 150 Å ² → broad drug-likeness filter.
	Bioavailability Score	0.55
	PAINS	0 alert
	Brenk	1 alert (triple bond)
Interpretation	Leadlikeness	No (1 violation: XLOGP3 > 3.5)
	Synthetic accessibility	2.24
Summary		Molecule 7 is drug like (passes Lipinski, Ghose, Veber, Egan, Muegge), shows high GI absorption and BBB permeability → potential CNS activity. It is not a Pgp substrate and only inhibits CYP1A2, suggesting lower interaction risk. However, its high lipophilicity (XLOGP3 > 3.5) and presence of a triple bond may affect lead likeness. Overall: promising bioavailability with moderate solubility and CNS penetration.

Table 9: IC₅₀ of Clorgyline and N1 – N4 (mean ± SD)

Treatment time (h)	Cell lines		
	A549	H460	H292
Clorgyline IC ₅₀ (μM)			
24	367±12.68	245±2.65	323±0.45
48	208.99± 1.25	217±0.5	313±0.56
72	198.2±1.63	230±0.8	150±0.26
N-(4-acetylphenyl)-1H-indole-2-carboxamide (N1) IC ₅₀ (μM)			
24	>1000± 0.82	>1000±1.2	>1000±0.87
48	307.7± 1.04	298±1.1	>1000±0.73
72	255.9±1.95	159±0.8	360±0.33
N-(4-(1-hydroxyethyl) phenyl)-1H-indole-2-carboxamide (N2) IC ₅₀ (μM)			
24	>1000±2	>1000±1.2	>1000±0.35
48	>1000±0.83	765±0.7	>1000±0.54
72	>1000±2.08	119±0.3	>1000±0.68
N-(3-acetylphenyl)-1H-indole-2-carboxamide (N3) IC ₅₀ (μM)			
24	>1000±1.16	>1000±0.9	>1000±0.49
48	>1000±2.52	167±0.4	689±0.64
72	357±2.08	379±0.6	350±0.39
N-(3-(1-hydroxyethyl) phenyl)-1H-indole-2-carboxamide (N4) IC ₅₀ (μM)			
24	>1000±5.57	>1000±0.9	>1000±0.59
48	147.2±1.71	89±0.3	130±0.88
72	143±2.08	35±0.7	128±0.83

Experiments were done in triplicate. Micromolar (μM)

Inhibitory concentration

Clorgyline and compounds N1 - N4 demonstrated strong antiproliferative effects, and vitality of the treated cells was lower compared to untreated control cells. The IC₅₀ values of Clorgyline for A549, H460, and H292 were 208.99, 217, and 313 μM, respectively, indicating strong antiproliferative action. Among the compounds that were produced, N4 demonstrated stronger antiproliferative effect against A549, H460, and H292 (Table 9).

DISCUSSION

Numerous investigations have demonstrated the connection between topoisomerase and cancer [1,6]. This study investigated the antiproliferative effect of colchicine inhibitors against various lung cancer cell lines utilizing molecular docking techniques. Lung cancer is the second most common cancer in women, behind breast cancer, and the most common in males. Treatment for lung cancer has several side effects that are challenging to overcome. Chemotherapy resistance and the high cost of treatment are additional challenges to the negative effects of drugs [6].

Findings of this study revealed that clorgyline and colchicine inhibitors (N1, N2, N3, and N4) formed H-bonds and occupy 5CB4 binding sites. Surprisingly, inhibitors of colchicine have similar binding affinities to 5CB4. It is important to note

that, except for N4, these reported colchicine inhibitors showed identical binding affinities against 5CB4. The MTT test was used to assess the impact of colchicine inhibitors on lung cancer cell line viability. The MTT test measures the capacity of the mitochondrial oxidoreductase to convert yellow tetrazolium salt (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, or MTT), into purple formazan crystals. Cell viability is thus colorimetrically indicated by MTT test [17]. The investigation was founded on previously documented anti-proliferative effects of clorgyline, a common MAO-A inhibitor, on cell lines from prostate and glioma [17-19]. The pharmacophoric and docking data were used to screen MAO-A inhibitors because clorgyline was the reference molecule for this investigation.

The current investigation demonstrated that colchicine inhibitors (N1, N2-4) strongly decreased the proliferation of A549, H292, and H460 cells in a concentration and time-dependent manner compared to untreated control cells. Previous studies have reported that clorgyline significantly inhibited prostate cancer growth [20,21]. The aforementioned chemicals were evaluated on normal cells (human fibroblasts) to see if colchicine inhibitors had harmful effects on healthy cells. The results showed great promise in terms of selectivity. The IC₅₀ of clorgyline against fibroblast cells was three times higher than its IC₅₀ against lung cancer cells. However, the produced compounds (N1 and N4) did not exhibit any harmful effects

on healthy cells, indicating that the synthetic inhibitors had a respectable safety margin.

CONCLUSION

Colchicine inhibits the proliferation of cancer cells. Furthermore, tested compounds (N1 – N4) exhibit promising antiproliferative activity, and colchicine inhibition was the main cause of cancer cell inhibition.

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Ethical approval

None provided.

Use of Artificial intelligence/Large language models

The authors confirm that AI-based tools were used solely for language editing and manuscript polishing purposes (e.g., grammar, phrasing, and clarity). No AI tools were used to generate content, interpret data, or influence study design, results, or conclusions. All intellectual contributions, study analyses, and interpretations reported in the manuscript are the original work of the authors.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the author(s) named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

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