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Synthesis, in vitro cytotoxicity, and molecular docking study of novel 3,4-dihydroisoquinolin-1(2*H*)-one based piperlongumine analogues

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With the aim of expanding the scope of SAR on piperlongumine (PL), a naturally occurring anticancer molecule, we have designed a novel hybrid molecule bearing 3,4-dihydroisoquinolin-1(2*H*)-one and trans-cinnamic acids. The structure, based on hybridization strategy, is used for hybridization of naturally occurring scaffolds. We have synthesized 14 hybrid molecules by coupling 3,4-dihydroisoquinolin-1(2*H*)-one core with cinnamic acids using the mix anhydride approach. The newly synthesized inhibitors were evaluated for cell viability against breast cancer *MCF-7* and cervical cancer *HeLa* cell lines. Furthermore, the active compounds were screened for their potential in breast cancer *MDA-MB-231,* cervical cancer *C33A* cell lines, prostate cancer *DU-145, PC-3*, and normal *VERO* cells. From the series, compound **10g** was seen to inhibit *MCF-7* cell growth significantly with $GI_{50} < 0.1$ μM along with growth inhibition in *MDA-MB-231* ($GI_{50} = 20$ μM) and *C33A* ($GI_{50} = 3.2$ μM). While the inhibitor **10i** inhibits *MCF-7* breast cancer cell growth $GI_{50} = 3.42$ μM along with inhibition of cell growth in *MDA-MB-231* ($GI_{50} = 30$ μM), *HeLa* ($GI_{50} = 7.67$ μM), *C33A* ($GI_{50} = 13$ μM), *DU-145* ($GI_{50} = 6.45$ μM), *PC-3* ($GI_{50} = 8.68$ μM), and *VERO* ($GI_{50} = 2.93$ μM), respectively. Furthermore, molecular docking study demonstrated these compounds could bind tightly to the colchicine domain of tubulin through a network of favorable steric and electrostatic interactions and thus act as a tubulin polymerization inhibitor.

1 | INTRODUCTION

Piperlongumine (PL) is a natural product belonging to the amide alkaloid family isolated from long piper (*Piper longum L.*). It was isolated in 1961 but the structure was assigned in 1984 and also known by the name Piplartine.[1] Traditionally, PL is used in Ayurvedic medicine for the treatment of tumors, cough, asthma, malaria, respiratory infections, etc.,[2] and it is equally economically important due to its use in spices (Pepper seed).[3] Over the last decade, PL was widely studied for its biological potential and reported to possess a range of bioactivities, including anti-inflammatory, antiplatelet, antiseptic, antimicrobial, antidepressant, anticancer, etc.[4] PL was extensively studied for its anticancer potential and it possesses a good range of cytotoxicity in a broad range of human cancer cell lines.[4–6] PL selectively inhibits cancer cell growth by enhancement of reactive oxygen species (ROS) level and induces apoptosis but the exact mechanism of anticancer effect was not clear. In the recent years, PL was reported as an inhibitor of STAT3, proteasome, involved in modulation of NF-κB and NF-κB-regulated gene products.[6]

The 3,4-dihydroisoquinolin-1(2*H*)-one (DHIQ) belongs to a class of nitrogen containing heterocyclic compound with six-membered lactam ring fused together with a benzene ring. It resembles to the rigidly fixed amino acid,

which gives characteristics of biomimetic.[7] DHIQ core is more common in natural products and it belongs to the isoquinolinone alkaloid family. DHIQ core containing compounds possess a wide range of biological activities such as anti HIV,[8] antidepressant,[9] anticancer,[10] H3 receptor antagonist for the treatment of neuropathic pain,[11] EZH2 inhibitor,[12] PARP inhibitor,[13] steroidomimetic,[14] cyclin-dependent kinase inhibitor,[10] antihypertension,[15] anti-arrhythmia,[16] antioxidant,[15] inhibitor of cholesterol biosynthesis,[16] antithrombotic,[17] anti-inflammatory,[9] antibacterial,[18] PET imaging of s2 receptors,[19] etc. Moreover, many natural products and synthetic analogues containing DHIQ core have been studied extensively as an anticancer agent. The structure of the representative anticancer natural and synthetic DHIQ is illustrated in Figure 1.

Due to abundance in nature, broad range of biological activities, and easy synthesis, the cinnamic acid analogues have gained much attention in drug discovery. In search of the anticancer drug, cinnamic acid and its derivatives have received much attention. Trans-cinnamic acid and other substituted analogues such as ferulic acid, caffeic acid, and cinnapic acid have been studied for anticancer potential, and these compounds possess anticancer activity.[20] The natural product PL, which is a cinnamic acid derivative, has also been extensively studied for anticancer potential.

On careful review of Piperlongumine and DHIQ, it was observed that "DHIQ" and "PL" share the same skeleton, that is, six-membered dihydro lactam ring. Like PL, in the designed hybrid, DHIQ ring linked to aryl ring with α,β-unsaturated carbonyl chain, which resembles with the chalcone type molecules. A number of the chalcone's and its analogues are widely studied for antiproliferative activity in a panel of cancer cell lines, including breast cancer cell lines.[21] Moreover, M. J. Meegan et al demonstrated that PL exhibits tubulin depolymerization potential in *MCF-7* breast cancer cells, and M. P.

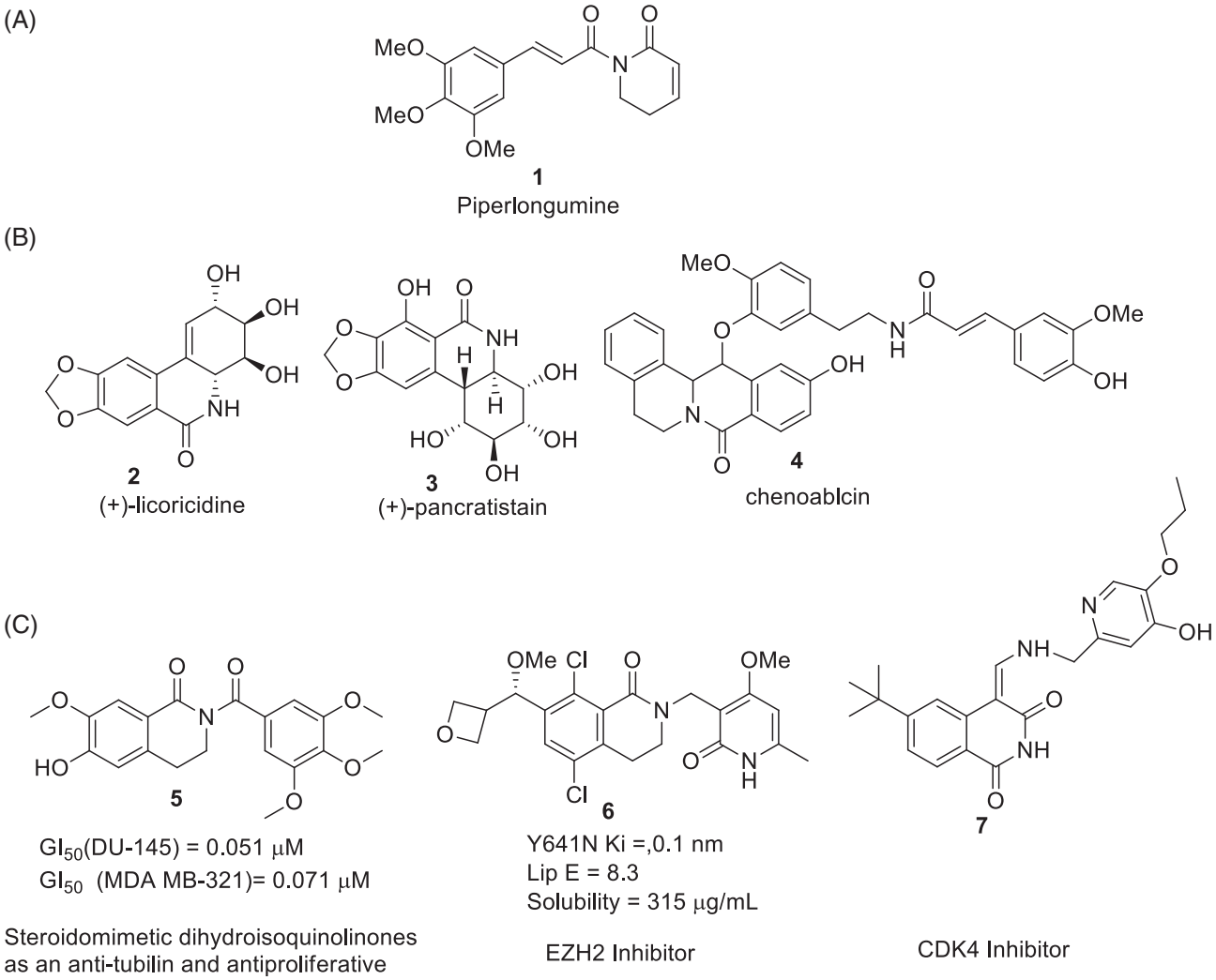


FIGURE 1 Molecular structures of A, Piperlongumine; B, representative natural anticancer DHIQ; C, synthetic anticancer DHIQ

Leese et al synthesized DHIQ derivatives (Figure 1, **5**) with antiproliferative activity with sub-micromolar to nanomolar concentration for growth inhibition in the range of prostate and breast cancer cell lines. Furthermore, the reported DHIQ compound functions as microtubule disruptors, having the ability to inhibit tubulin polymerization potential by competing at colchicine binding site. Accordingly, we proposed a hybrid of PL with DHIQ via replacement of lactam ring with DHIQ ring, which results in novel PL analogues that may be a potent anticancer agent.[14] To test our hypothesis, we have synthesized novel DHIQ hybrids and evaluated anticancer potentials of synthesized compounds in breast cancer and cervical cancer cell lines.

2 | RESULTS AND DISCUSSION

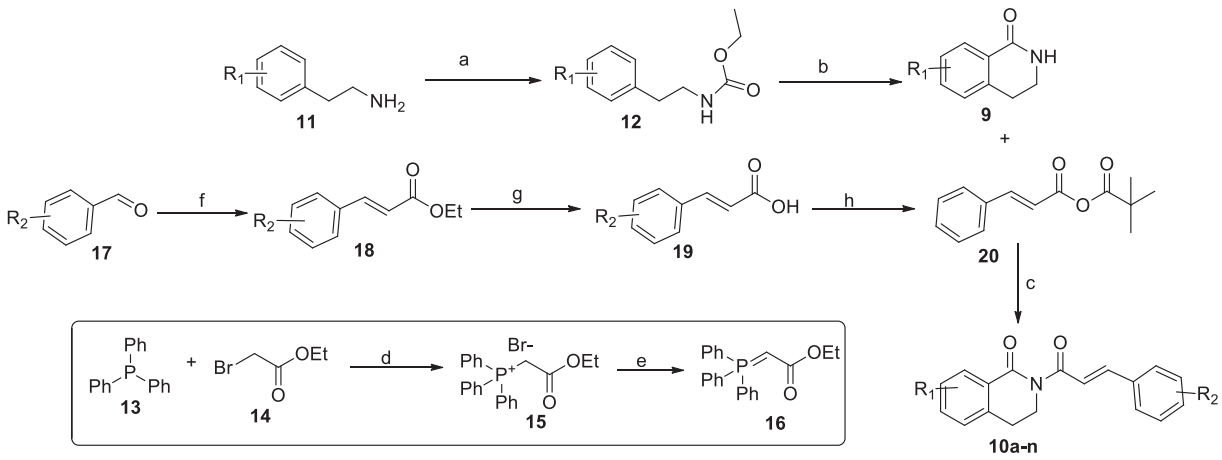
2.1 | Chemistry

The synthesis of the designed hybrid molecule is achieved as per the scheme depicted in the following scheme 1.

The designed DHIQ analogues (**10a-n,** Figure 2) were synthesized by coupling of the 3,4-dihydroisoquinolin-1(2*H*)-one **9** and anhydride **20** as per the modification of previously reported procedure.[4f] The compound **9** on treatment with *n*-BuLi at −78°C produces anion, which on reaction with the mixed anhydride of cinnamic acid (**20**) at −78°C to 25°C yields DHIQ **10a-n**. The key intermediate that is DHIQ core **9** was synthesized from the commercially available phenylethylamine **11** in two steps.[22] In the first step, phenylethylamine **11** was treated with ethyl chloroformate in the presence of triethylamine to get carbamate derivative **12** in good yield. The carbamate **12** is cyclized to 3,4-dihydroisoquinolin-1(2*H*)-one (**9**) upon heating in the presence of PPA at 130°C for 2.0 hours. The cinnamic acid mix anhydride **20** was prepared from substituted cinnamic acids **19**. The trans-cinnamic acid's **19** on reactions with pivaloyl chloride in the presence of triethylamine at 0°C in THF gave anhydride **20** and the anhydride solution as such used for the next step without isolation. The structure of all the newly synthesized DHIQs (Figure 2, **10a-n**) was in full agreement with spectral data.

2.2 | Antiproliferative activity

Cell viability of all the synthesized DHIQ hybrid derivatives was determined by SRB assay using *HeLa* cervical cancer cells and *MCF-7* breast cancer cells. The cancer cells were treated with various concentrations of experimental drugs and the positive control, Adriamycin (Doxorubicin, reference standard) for 48 hours. The biological results are presented in Table 1. The results of the biological assay demonstrated inhibition of *HeLa* cell and *MCF-7* cell growth in a dose-dependent manner by some of the newly synthesized compounds. Of these, **10g**, **10i** reduced the *MCF-7* cell growth significantly (Table 1). At the higher concentration, all the compounds exhibited cell death as compared to the starting population. Out of the 14 tested compounds, **10g** and **10i** were found to have significant antiproliferative activity against *MCF-7* cancer cells. No particular trends in activity with an effect of substituent are observed. However, the **10g** bearing 3-Cl



SCHEME 1 Synthesis of 3,4-dihydroisoquinolin-1(2*H*)-one hybrid **10a-n**. [a]**Reaction conditions**: a) Ethyl chloroformate, triethylamine, THF, 0°C to 5°C, 15 minutes; b) PPA, 140°C, 2.0 hours; c) *n*-BuLi, THF, −78°C to 25°C, 2.0 to 4.0 hours; d) Ethyl acetate, 25°C to 30°C, 16 hours; e) 30% aqueous NaOH, 0.5 hours; f) **16**, DCM, 25°C to 30°C, 2 to 6 hours; g) THF: MeOH: H_2O, NaOH, 0.5 to 2 hours; h) Pivaloyl chloride, triethylamine, THF, 0°C to 5°C, 0.5 hours

TABLE 1 Inhibition of growth of *MCF-7* and HeLa, cells by DHIQ

	Human breast cancer cell line *MCF-7*					Human cervical cancer cell line *HeLa*						
	% Control growth					% Control growth						
	Molar drug concentrations					Molar drug concentrations						
	Average values of three expt.					Average values of three expt.						
Comp. No.	**10^{-7} M**	**10^{-6} M**	**10^{-5} M**	**10^{-4} M**	**GI_{50} (μM)[a]**	**10^{-7} M**	**10^{-6} M**	**10^{-5} M**	**10^{-4} M**	**GI_{50} (μM)[a]**	**Glide score**	**Glide interaction energy (kcal/mole)**
10a	99.6	148.2	137.9	25.0	>100	110.6	121.3	99.6	−15.0	>100	−6.609	−39.762
10b	101.6	163.2	154.8	35.7	>100	104.0	121.7	97.3	8.4	>100	−6.773	−40.475
10c	90.8	148.7	120.1	33.8	>100	94.6	113.8	81.5	−7.8	>100	−6.612	−39.854
10d	110.4	175.5	157.5	39.8	>100	102.8	117.9	82.5	−6.7	>100	−6.331	−39.758
10e	91.4	154.7	147.6	50.2	>100	113.2	116.8	114.1	−8.6	>100	−6.022	−38.614
10f	79.6	110.8	114.1	39.5	>100	116.4	128.1	107.4	4.7	>100	−6.724	−39.849
10g	**50.1**	**23.6**	**47.0**	**35.4**	**<0.01**	119.0	121.7	115.9	76.9	>100	−8.988	−49.975
10h	94.4	90.2	91.0	35.8	>100	112.8	123.2	125.4	48.1	NE	−6.825	−39.882
10i	**90.9**	**91.3**	**43.7**	**−20.6**	**3.42**	**84.4**	**133.2**	**59.1**	**−18.0**	**7.67**	−8.834	−48.825
10j	103.6	99.7	96.2	50.0	>100	123.0	149.7	135.3	65.5	>100	−6.593	−39.322
10k	114.7	111.8	107.5	59.4	>100	127.5	157.8	148.6	61.8	>100	−6.347	−38.323
10l	116.2	102.0	105.0	47.7	>100	119.0	155.7	90.5	7.1	37.47	−6.299	−39.367
10m	115.2	119.9	98.7	51.6	>100	95.0	150.7	79.0	−2.2	22.06	−6.032	−37.117
10n	132.3	124.6	117.6	59.9	>100	113.3	131.4	115.4	−8.0	31.69	−6.471	−36.718
PL	94.0	91.2	40.1	20.6	8.61	81.9	74.1	56.7	38.5	20.01	−8.805	−48.171
ADR.	−10.3	−12.6	12.2	2.6	<0.1	69.1	19.9	−34.7	−43.0	0.2	−9.587	−51.522

Note: Bold values indicates the activity. GI50 = Concentration of drug causing 50% inhibition of cell growth. ADR indicates adriamycin (Doxorubicin), reference standard.

[a]Values represent the average of three independent experiments and calculated from graph.

substitution on cinnamic acid and **10i** with 4-pyridyl cinnamic acid reduced significantly *MCF-7* cell growth with $GI_{50} < 0.1$ and 3.42 μM, respectively. The inhibitor **10i** inhibits the *HeLa* cell growth with $GI_{50} = 7.67$ μM, which is a significant inhibitor of HeLa cell growth from the tested series.

We have selected **10g** and **10i** and evaluated cell viability in additional cancer cell lines, that is, breast cancer *MDA-MB-231,* cervical cancer *C33A,* prostate cancer *DU-145*, *PC-3*, and *VERO* cell lines, and the results are presented in Table 2. Inhibitor **10g** inhibits cell growth with $GI_{50} = 20$ μM and 3.2 μM in *MDA-MB-231* cell line and *C33A* cell lines, respectively. Moreover, the inhibitor **10i**, hybrid bearing 3,4-dihydroisoquinolin-1(2*H*)-one 4-pyridyl cinnamic acid has reduced the cancer cell growth in *MCF-7, HeLa, MDA-MB-231, C33A, DU-145,* and *PC-3* cancer cell lines with $GI_{50} = 3.62$, 3.67, 30, 13, 6.45, and 8.68 μM, respectively.

The newly synthesized hybrid compound exhibits similar activity compared to the parent piperlongumine on the tested cancer cell lines. The hybrids **10d** and **10m** bearing trimethoxyphenyl group showed unfavorable cytotoxicity. Moreover, the other synthesized DHIQ-hybrid bearing dimethoxyl, methoxy, fluoro, trifluoromethyl, and methyl substitutions also found to be unfavorable for the activity. From the synthesized hybrid compounds, inhibitor **10g** bearing 3-Chloropheyl and **10i** having 4-pyridyl group inhibit cancer cell growth in a dose-dependent manner on the tested cell lines. The DHIQ hybrid **10i** showed good activity in all tested cell lines and showed that the designed modification has potential for development of the anticancer molecule.

2.3 | Molecular modeling

In an effort to elucidate the plausible mechanism by which the 3,4-Dihydroisoquinolin-1(2*H*)-one(DHIQ) based piperlongumine analogues can induce anticancer activity and to gain better understanding on their potency of the synthesized for guiding further SAR studies, we proceeded to examine the interaction of these novel compounds with tubulin crystal structure (PDB code: 1SA0) through molecular docking simulations. All the 3,4-Dihydroisoquinolin-1(2*H*)-one(DHIQ) based piperlongumine analogues were found to snugly fit into the active site cavity of the tubulin receptor with varying degrees of affinities and at the co-ordinates close to the co-crystallized ligand through a network of prominent interactions (Table 1). However, compounds **10g** and **10i** exhibit excellent affinity for tubulin, which could rationally prove the reason why these compounds also possess effective antitumor activity profile. Furthermore, in order to gain an insight into the residues and their associated types of thermodynamic interactions guiding the anchoring of these molecules to the tubulin receptor, a detailed per-residue interaction analysis between the tubulin and these two compounds was carried out.

The lowest energy docked conformation of **10g (ESI,** Figure 1**)** revealed that the molecule is anchored into the active site of tubulin through an extensive network of balanced steric and electrostatic interactions. The molecule is seen to be stabilized through a chain of favorable van der Waals interactions observed with Lys: B352 (−1.768 kcal/mol), Asn: B258(−1.546 kcal/mol), Ala: B256(−1.153 kcal/mol), Lys: B254(−4.603 kcal/mol), Gln: B249(−1.027 kcal/mol), Leu: B248(−4.445 kcal/mol), Gln: B247(−1.274 kcal/mol), Cys: B241(−1.943 kcal/mol), Arg: B48(−1.019 kcal/mol), Arg: B2(−1.437 kcal/mol), Tyr: A224(−1.093 kcal/mol), Glu: A183(−1.245 kcal/mol), Val: A182(−1.094 kcal/mol), Val: A181(−1.181 kcal/mol), Ala: A180(−1.408 kcal/mol), Thr: A179(−1.188 kcal/mol), Ser: A178(−1.46 kcal/mol), Gly: A144(−1.042 kcal/mol), Gly: A143(−1.091 kcal/mol), Gly: A142(−1.038 kcal/mol), Asn: A101(−1.988 kcal/mol), Asp: A98(−1.093 kcal/mol), Glu: A71(−1.055 kcal/mol), and Gln: A11(−1.158 kcal/mol) residues through the 3,4-Dihydroisoquinolin-1(2*H*)-one nucleus while the acryloyl side chain containing meta-chloro phenyl moiety was found to be engaged in similar van der Waals interactions with Ile: B378(−1.133 kcal/mol), Phe: B377(−1.109 kcal/mol), Thr: B376(−1.12 kcal/mol), Ala: B354(−1.786 kcal/mol), Thr: B353(−1.308

TABLE 2 GI_{50} values of experimental compounds in comparison with PL and adriamycin

	GI_{50} (μM)[a]						
Compound no.	**MCF-7**	**MDA-MB-231**	**HeLa**	**C33A**	**DU-145**	**PC-3**	**VERO**
10g	<0.01	20	>100	3.20	NE	NE	NE
10i	3.42	30	7.67	13.0	6.45	8.68	2.93
PL	8.61	10.9	20.01	1.3	1.56	2.25	21.03
ADR.	<0.1	1.1	0.2	0.058	0.004	0.014	0.0001

[a]Results are mean of triplicate analysis; NE, Non-evaluable data. The experiment needs to be repeated using different set of drug concentrations. ADR, Adriamycin (Doxorubicin), reference standard.

kcal/mol), Val: B318(−1.984 kcal/mol), Ala: B317(−1.759 kcal/mol), Ala: B316(−1.533 kcal/mol), Val: B315(−1.125 kcal/mol), Leu: B255(−3.619 Kcal/mol), Asp: B251 (−1.053 kcal/mol), Ala: B250(−2.135 kcal/mol), Leu: B242(−1.57 kcal/mol), Thr: B240(−1.195 kcal/mol), Thr: B239(−1.3 kcal/mol), Val: B238(−1.896 kcal/mol), and Gly: B237(−1.109 kcal/mol). The best docked conformation of **10i** as well was seen to be involved in favorable van der Waals interactions with Lys: B352(−1.204 kcal/mol), Asn: B258(−2.135 kcal/mol), Ala: B256(−1.179 kcal/mol), Lys: B254(−5.908 kcal/mol), Leu: B248(−2.131 kcal/mol), Gln: B247(−1.115 kcal/mol), Leu: B242(−1.128 kcal/mol), Arg: B48(−1.042, Tyr: A224(−1.06 kcal/mol), Glu: A183 (−1.22 kcal/mol), Val: A182(−1.117 kcal/mol), Val: A181 (−1.244 kcal/mol), Ala: A180(−1.488 kcal/mol), Thr: A179 (−1.11 kcal/mol), Ser: A178(−1.357 kcal/mol), Gly: A144 (−1.035 kcal/mol), Gly: A143(−1.072 kcal/mol), Gly: A142 (−1.03 kcal/mol), Asn: A101(−1.803 kcal/mol), Asp: A98 (−1.073 kcal/mol), Glu: A71(−1.039 kcal/mol), and Gln: A11(−1.088 kcal/mol) residues via 3,4-Dihydroisoquinolin-1(2*H*)-one nucleus while the pyridine containing acryloyl side chain was engaged in this type of favorable interactions with Ile: B378(−1.013 Kcal/mol), Phe: B377(−1.118 Kcal/mol), Thr: B376(−1.117 Kcal/mol), Ala: B354(−1.816 Kcal/mol), Thr: B353(−1.438 kcal/mol), Val: B318 (−1.114 kcal/mol), Ala: B317(−1.499 kcal/mol), Ala: B316(−2.072 kcal/mol), Val: B315(−1.184 kcal/mol), Leu: B255(−3.753 kcal/mol), Asp: B251(−1.96 kcal/mol), Ala: B250(−1.772 kcal/mol), Gln: B249(−1.331 kcal/mol), Cys: B241(−1.328 kcal/mol), Thr: B240 (−1.111 kcal/mol), Thr: B239(−1.112 kcal/mol), Val: B238(−1.381 kcal/mol), Gly: B237(−1.066 kcal/mol), and Arg: B2(−1.03 kcal/mol) residues in the active site. The enhanced binding affinity of **10g** is also attributed to a network of significant electrostatic interactions observed with Lys: B254 (−6.372 Kcal/mol), Arg: B253 (−1.253 Kcal/mol), Ala: B250(−2.35 kcal/mol), Leu: B48 (−1.01 kcal/mol), Arg: B2(−1.321 kcal/mol), Glu: A183 (−1.102 kcal/mol), Asn: A101(−1.345 kcal/mol) while for **10i (ESI,** Figure 3**)** it was observed with Lys: B254 (−5.564 kcal/mol), Arg: B253(−1.09 kcal/mol), Ala: B250 (−2.439 kcal/mol), Leu: B48(−1.01 kcal/mol), Arg: B2(−1.321 kcal/mol), Glu: A183(−1.102 kcal/mol), and Asn: A101(−1.322 kcal/mol) residues lining active site cavity. Overall, it is observed that the major driving force required for the mechanical interlocking of these molecules into the active site of tubulin receptor is steric complementarity as reflected by the relatively larger contribution of steric over electrostatic interactions with active site residues. Such a per-residue interaction analysis provides an opportunity to carry out site-specific modification in the molecule to enhance the binding affinity toward the receptor.

3 | CONCLUSION

In summary, we have designed the novel PL analogues by hybridization of 3,4-dihydroisoquinolin-1(2*H*)-one and

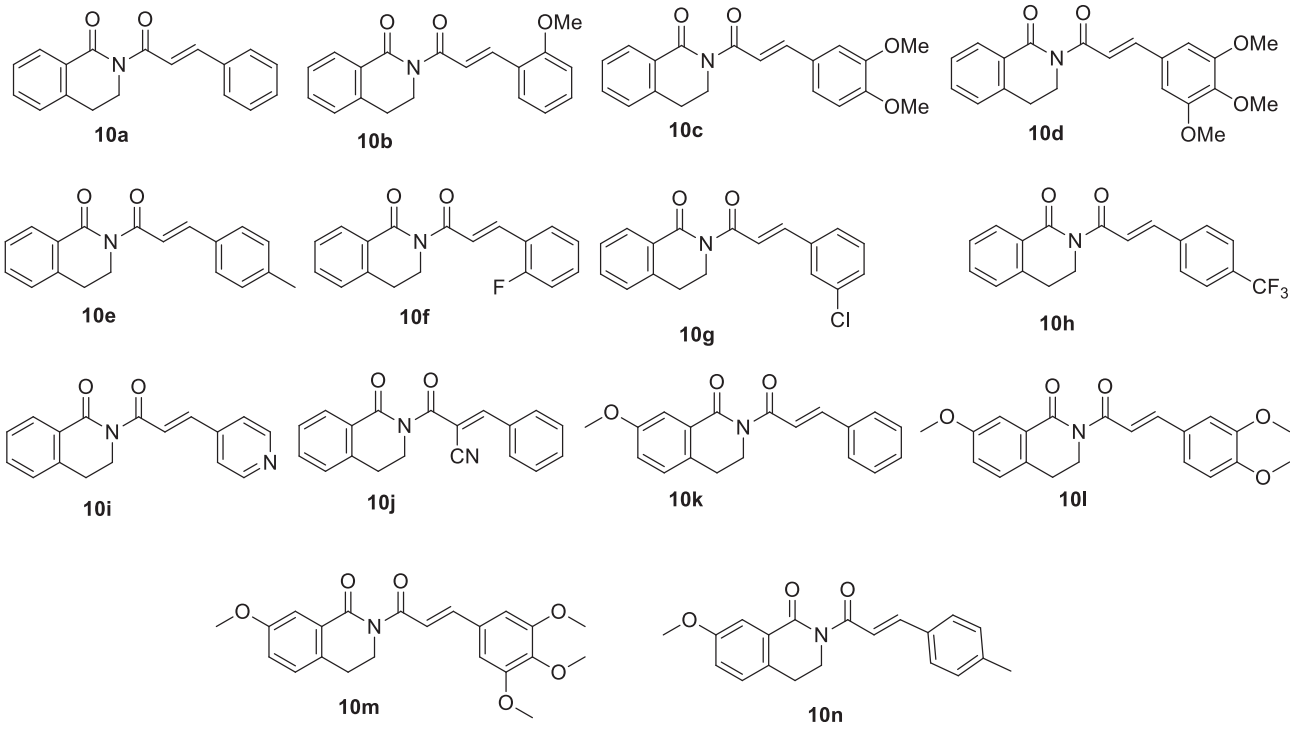


FIGURE 2 Structures of newly synthesized hybrid 3,4-dihydroisoquinolin-1(2H)-one

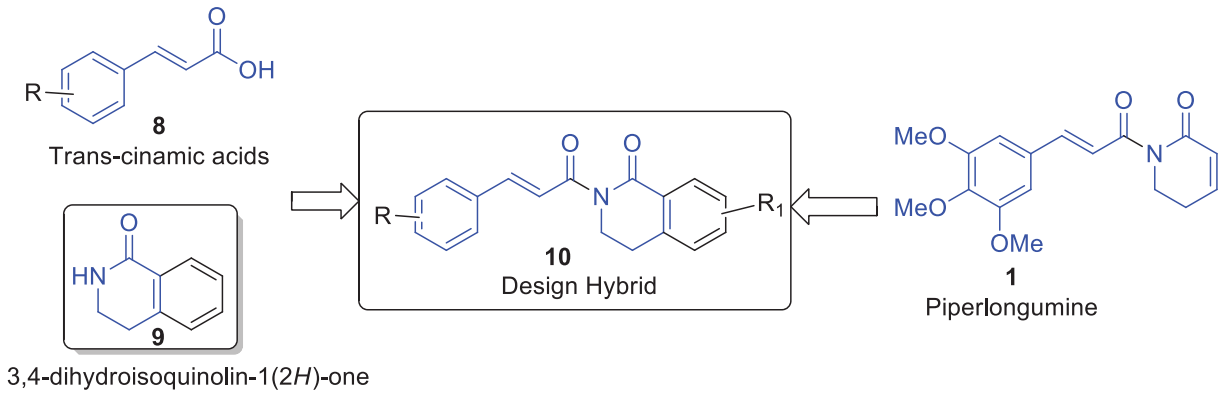


FIGURE 3 Structure-based hybridization and scaffold hopping of novel 3,4-dihydroisoquinolin-1(2*H*)-one-piperlongumine hybrids

trans-cinnamic acids and synthesized a small library of hybrid molecules of biological interests. The newly synthesized hybrids were evaluated for anticancer activity in breast cancer and cervical cancer cell lines. From the tested series, inhibitor **10i** inhibits significantly cancer cell growth in micro-molar to sub-micromolar range. The inhibitor **10i** bearing pyridyl group inhibits cell growth with $GI_{50} < 0.1$, 20, 7.67, 3.2, 6.45, and 8.67 μM in *MCF-7*, *MDA-MB-231*, *HeLa, C33A, DU-145*, and *PC-3* cancer cell lines, respectively. Inhibitor **10i** is considered as the initial hit for the extensive medicinal chemistry program in search of anticancer drug. Moreover, molecular docking studies could provide valuable insight into binding mode, and the associated thermodynamic interactions in the colchicine-binding site of tubulin suggest that these compounds have the potential to inhibit tubulin polymerization to elicit anticancer activity. These results pave the way for further exploration of 3,4-Dihydroisoquinolin-1(2*H*)-one(DHIQ) based piperlongumine analogues as anticancer agents.

4 | EXPERIMENTAL SECTION

4.1 | General methods

All commercial chemicals and solvents are of reagents grade and were used without further purification. The thin-layer chromatography was performed on Merck precoated silica gel 60 F_{254} plates, with visualization under UV light. ^{1}H NMR spectra were recorded with Bruker 400 MHz AVANCE instrument, and *J* values are in Hertz, and chemical shifts (δ) are reported in ppm relative to internal tetramethylsilane. Mass spectral (MS) data were obtained on a Bruker Daltonics spectrometer using an electrospray ionization quadrupole-time of flight (ESI-QTOF) analyzer. Benzylic alcohols were purchased either from commercially available sources or synthesized from commercially available sources. Purification of the reaction products was carried out by flash column chromatography using silica gel (60–120) mesh.

4.2 | Chemistry protocols and experimental data for all compounds

4.2.1 | Synthesis of ethyl phenethylcarbamate 12

Triethylamine (11.5 mL, 82.6 mmol) was added to a stirred solution of 2-phenylethanamine **11** (5.0 g, 41.30 mmol) in dichloromethane (50 mL) at 0°C to 5°C and stirred for 15 minutes. Ethyl chloroformate was added dropwise (4.12 mL, 43.3 mmol) and allowed to stir at the same temperature for 0.5 hours. Ice bath was removed, and the reaction mixture was allowed to warm to 25°C to 30°C and continued stirring for 4 hours. The progress of the reaction was monitored by TLC, and upon completion of the reaction, the reaction mixture was transferred to a separating funnel and washed with water (2 × 50 mL) and brine (50 mL). Dried organic layer over anhydrous sodium sulfate and filtered. The filtrate was evaporated on rotavapor under reduced pressure to get crude. The crude was purified by silica gel column chromatography using 0% to 30% ethyl acetate in *n*-hexane to get ethyl phenethylcarbamate **2.29** (7.51 g, 94.22%) as a colorless oil.

4.2.2 | Synthesis of 3,4-dihydroisoquinolin-1(2*H*)-one (9a)

Ethyl phenethylcarbamate **12** (7.5 g, 38.8 mmol) was mixed with PPA (11.4 g, 116.4 mmol) and heated to 130°C for 2 hours. The resulting reaction mixture was cooled to 25°C to 30°C and diluted with water (150 mL). The reaction mixture was extracted with ethyl acetate (3 × 75 mL), and combined organic layers were washed with saturated sodium bicarbonate solution (100 mL), water (100 mL), and brine (100 mL). Organic layers were dried over anhydrous sodium sulfate and filtered. The filtrate was concentrated under reduced pressure to get the

crude, which was purified by silica gel column chromatography in 0% to 40% ethyl acetate in *n*-hexane to get 3,4-dihydroisoquinolin-1(2*H*)-one (4.2 g, 73.35%) as a colorless oil.

Oil; Yield: 4.2 g (73.55%); ^{1}H-NMR (400 MHz, $CDCl_3$): δ 3.05 (t, J = 6.4 Hz, 2H), 3.61 (t, J = 6.0 Hz, 2H), 6.06 (s, 1H), 7.26 (d, J = 7.2 Hz, 1H), 7.40 (t, J = 7.2 Hz, 1H), 7.49 (dt, J = 7.2 Hz, 0.8 Hz, 1H), 8.11 (d, J = 7.2 Hz, 1H); MS (ESI) *m/z*: 148.0$[M + H]^+$.

4.2.3 | Synthesis of 7-methoxy-3,4-dihydroisoquinolin-1(2*H*)-one (9b)

Oil; Yield: 0.7 g (81.22%); ^{1}H-NMR (400 MHz, $CDCl_3$): δ 2.87 (t, J = 6.8 Hz, 2H), 3.41 (t, J = 6.8 Hz, 2H), 3.81 (s, 3H), 6.85 to 6.89 (m, 2H), 7.71 (s, 1H), 7.77 (d, J = 8.4 Hz, 1H); MS (ESI) *m/z*: 178.0$[M + H]^+$.

4.2.4 | General procedure for synthesis of DHIQ hybrid 10a-n

To a solution of substituted cinnamic acid **19** (1.50 mmol) in a freshly distilled THF (5 mL) was added triethyl amine (2.0 mmol). To this reaction mixture, pivaloyl chloride (1.50 mmol) was added at 0°C to 5°C, and the reaction was allowed to stir at the same temperature for 45 minutes. After completion of the reaction, (monitored by TLC) this mixture was used for the next step without purification and work up. To a solution of 3,4-dihydroisoquinolin-1(2*H*)-one **9** (1.0 mmol) in freshly distilled THF (5 mL) was added 1.2 equivalent *n*-BuLi at −78°C under inert atmospheric conditions, and the reaction was allowed to stir for 0.5 hours. Then, added the anhydride (**20**) prepared from the above step. The reaction mixture was allowed to stir for 1 hour at −78°C and then at 25°C to 30°C for 1 to 2 hours. Then, the reaction mixture was quenched with saturated ammonium chloride (2 mL) and extracted with ethyl acetate (15 mL). The organic layer was separated and washed with brine (2 × 15 mL). Organic layers were dried over anhydrous sodium sulfate and filtered. The filtrate was concentrated under reduced pressure to get the crude product. The crude was purified by silica gel column chromatography using 0% to 50% ethyl acetate in *n*-hexane to get the title compound in moderate to good yield.

4.2.5 | Synthesis of 2-cinnamoyl-3,4-dihydroisoquinolin-1(2*H*)-one (10a)

Off-white solid; Yield: 0.1 g (53.07%); mp: 59°C to 61°C; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 3.11 (t, J = 5.2 Hz, 2H), 4.22 (t, J = 6.0 Hz, 2H), 7.30 to 7.34 (m, 1H), 7.42 to 7.47 (m, 4H), 7.58 (t, J = 7.2 Hz, 1H), 7.66 to 7.69 (m, 3H), 7.84 (d, J = 16.0 Hz, 1H), 8.24 (d, J = 6.8 Hz, 1H); ^{13}C-NMR(100 MHz, DMSO-d_6): δ 28.39, 42.71, 122.09, 127.46, 127.52(2C), 128.41, 128.66, 128.82, 128.96, 129.63, 130.12, 133.57, 135.14, 140.34, 143.67, 166.10, 169.59; MS (ESI) *m/z*: 278.4$[M + H]^+$; HRMS (ESI): *m/z* $[M + H]^+$calcd for $C_{18}H_{15}NNaO_2$:300.0995; found: 300.1000.

4.2.6 | Synthesis of (*E*)-2-(3-(2-methoxyphenyl)acryloyl)-3,4-dihydroisoquinolin-1(2*H*)-one (10b)

Off-white solid; Yield: 0.149 g (71.13%); mp: 111°C to 116°C; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 3.10 (t, J = 6.0 Hz, 2H), 3.94 (s, 3H), 4.21 (t, J = 6.4 Hz, 2H), 6.94 to 7.05 (m, 2H), 7.32 (t, J = 8.0 Hz, 1H), 7.33 (td, J = 7.2 Hz, 1.8 Hz, 1H), 7.50 (t, J = 7.6 Hz, 1H), 7.55 to 7.60 (m, 1H), 7.68 to 7.73 (m, 2H), 8.19 (d, J = 15.6 Hz, 1H), 8.24 (d, J = 7.6 HZ, 1H); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 27.95, 42.93, 56.14, 112.50, 121.25, 123.29, 123.52, 127.61, 128.30, 128.52, 128.88, 129.24, 132.21, 134.03, 136.95, 141.33, 158.28, 165.85, 169.62; MS (ESI) *m/z*: 308.3$[M + H]^+$.

4.2.7 | Synthesis of (*E*)-2-(3-(3,4-dimethoxyphenyl)acryloyl)-3,4-dihydroisoquinolin-1(2*H*)-one (10c)

Off-white solid; Yield: 0.2 g (58.12%); mp: 114°C to 117°C; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 3.07 (t, J = 5.2 Hz, 2H), 3.34 (s, 3H), 3.82 (s, 3H), 4.05 (t, J = 6.0 Hz, 2H), 7.03 (d, J = 8.8 Hz, 1H), 7.27 (d, J = 7.2 Hz, 2H), 7.43 (t, J = 6.8 Hz, 1H), 7.48 (d, J = 7.2 Hz,1H), 7.61 to 7.63 (m, 2H), 8.07 (d, J = 7.2 Hz, 1H); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 28.01, 43.00, 56.99, 57.02, 111.00, 112.13, 120.76, 122.81, 127.60, 127.97, 128.30, 128.74, 128.90, 129.24, 134.00, 142.76, 149.37, 151.25, 165.82, 169.58; MS (ESI) *m/z*: 338.2$[M + H]^+$.

4.2.8 | Synthesis of (*E*)-2-(3-(3,4,5-trimethoxyphenyl)acryloyl)-3,4-dihydroisoquinolin-1(2*H*)-one (10d)

Off-white solid; Yield: 0.16 g (51.23%); mp: 110°C to 112°C; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 3.10 (t, J = 6.0 Hz, 2H), 3.94 (s, 9H), 4.12 (t, J = 6.0 Hz, 2H), 6.87 (s, 2H), 7.32 (t, J = 6.4 Hz, 1H), 7.45 (t, J = 7.6 Hz, 1H), 7.56 (d, J = 2.4 Hz, 1H), 7.59 (d, J = 4.8 Hz,1H), 7.77 (d, J = 15.6 HZ, 1H), 8.24 (d, J = 7.6 Hz, 1H); ^{13}C-NMR

(100 MHz, DMSO-d_6): δ 28.03, 43.07, 56.46(2C), 60.60, 106.12(2C), 122.57, 127.62, 128.34, 128.81, 129.28, 130.84, 134.07, 139.77, 141.32, 142.47, 153.58(2C), 165.83, 169.57; MS (ESI) *m/z*: 368.61[M + H]$^+$; HRMS (ESI): *m/z* [M + H]$^+$calcd for $C_{21}H_{21}NO_5$+ Na^+: 390.1311; found: 390.1326.

4.2.9 | Synthesis of (*E*)-2-(3-(*p*-tolyl) acryloyl)-3,4-dihydroisoquinolin-1(2*H*)-one (10e)

Off-white solid; Yield: 0.141 g (71.13%); mp: 106°C to 107°C; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 2.42 (s, 3H), 3.10 (t, J = 6.0 Hz, 2H), 4.21 (t, J = 6.4 Hz, 2H), 7.23 (d, J = 7.6 Hz, 2H), 7.31 (t, J = 7.2 Hz, 1H), 7.45 (t, J = 7.6 Hz, 1H), 7.55 to 7.59 (m, 3H), 7.64 (d, J = 15.6 Hz, 1H), 7.83 (d, J = 15.6 Hz,1H), 8.24 (d, J = 7.6 HZ, 1H); ^{13}C-NMR(100 MHz, DMSO-d_6): δ 21.50, 27.96, 42.91, 122.16, 127.61, 128.30, 128.59 (2C), 128.91, 129.24, 130.08 (2C), 130.49, 134.03, 140.60, 141.32, 142.29, 165.89, 169.37; MS (ESI) *m/z*: 292.42[M + H]$^+$.

4.2.10 | Synthesis of (*E*)-2-(3-(2-fluorophenyl)acryloyl)-3,4-dihydroisoquinolin-1(2*H*)-one (10f)

Off-white Solid; Yield: 0.155 g, (77.22%); mp: 94°C to99°C; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 3.11 (t, J = 6.0 Hz, 2H), 4.22 (t, J = 6.4 Hz, 2H), 7.11–7.25 (m, 2H), 7.35 (d, J = 10.4 Hz, 2H), 7.41 (q, J = 6.0 Hz, 1H), 7.45 (t, J = 7.6 Hz, 1H), 7.58(t, J = 7.2 Hz, 1H), 7.69–7.42 (m, 2H), 7.97(d, J = 16.0 Hz,1H), 8.24 (d, J = 7.6 HZ, 1H); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 27.91, 42.95, 116.50, 116.72, 122.84, 122.95, 125.57, 125.94, 127.64, 129.78, 132.47, 133.78, 134.13, 141.38, 159.84, 162.33, 165.92, 169.15; MS (ESI) *m/z*: 296.4[M + H]$^+$; HRMS (ESI): *m/z* [M + H]$^+$calcd for $C_{18}H_{14}FNO_2$+ Na^+:318.0900; found: 318.0908.

4.2.11 | Synthesis of (*E*)-2-(3-(3-chlorophenyl)acryloyl)-3,4-dihydroisoquinolin-1(2*H*)-one (10g)

Off-white solid; Yield: 0.168 g (79.11%); mp: 119°C to 123°C; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 3.11 (t, J = 6.4 Hz, 2H), 4.21 (t, J = 6.4 Hz, 2H), 7.30 to 7.38 (m, 3H), 7.46 (t, J = 7.2 Hz, 1H), 7.51 (d, J = 6.8 Hz, 1H), 7.59 (t, J = 7.6 Hz, 1H), 7.64 (t, J = 7.2 Hz, 2H), 7.74 (d, J = 15.6 Hz,1H), 8.24 (d, J = 7.6 HZ, 1H); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 27.94, 42.94, 125.08, 127.06, 127.66, 128.13, 128.37, 128.73, 129.26, 131.29, 134.16, 134.22, 137.55, 139.55, 141.36, 165.95, 169.17; MS (ESI) *m/z*: 312.1[M + H]$^+$.

4.2.12 | Synthesis of (*E*)-2-(3-(4-(trifluoromethyl)phenyl)acryloyl)-3,4-dihydroisoquinolin-1(2*H*)-one (10h)

Off-white solid; Yield: 0.12 g (46.49%); mp: 134°C to 137°C; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 3.12 (t, J = 6.4 Hz, 2H), 4.22 (t, J = 6.4 Hz, 2H), 7.34 (t, J = 7.6 Hz, 1H), 7.46 (t, J = 7.6 Hz, 1H), 7.59 (t, J = 7.2 Hz, 1H), 7.68 (d, J = 8.4 Hz, 2H), 7.73 to 7.82 (m, 3H), 8.24 (d, J = 8.0 HZ, 1H); ^{13}C-NMR(100 MHz, DMSO-d_6): δ 27.91, 42.93, 125.91, 126.18, 126.26, 126.29, 126.33, 126.37, 127.67, 128.37, 128.70, 129.26, 129.65, 134.19, 139.32, 139.63, 141.39, 165.96, 169.05; MS (ESI) *m/z*: 493.1[M + H]$^+$.

4.2.13 | Synthesis of (*E*)-2-(3-(pyridin-4-yl)acryloyl)-3,4-dihydroisoquinolin-1(2H)-one (10i)

Off-white solid; Yield: 0.171 g (73.07%); mp: 77°C to 82°C; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 3.09 (t, J = 6.0 Hz, 2H), 4.07 (t, J = 6.0 Hz, 2H), 7.43 to 7.49 (m, 2H), 7.55 (d, J = 16 Hz, 1H), 7.63 to 7.73 (m, 4H), 8.07 (d, J = 7.6 Hz, 1H), 8.65 (d, J = 5.6 Hz, 2H); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 27.76, 44.76, 109.62, 116.33, 127.46, 127.54, 127.80, 128.66, 129.32, 129.77, 130.64, 133.15, 134.65, 141.35, 151.16, 165.64, 167.60; MS (ESI) *m/z*: 280.27[M + H]$^+$.

4.2.14 | Synthesis of (*E*)-2-(1-oxo-1,2,3,4-tetrahydroisoquinoline-2-carbonyl)-3-phenylacrylonitrile (10j)

Off-white solid; Yield: 0.155 g (75.73%); mp: 117°C to 123°C; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 3.21 (t, J = 6.0 Hz, 2H), 4.13 (t, J = 6.8 Hz, 2H), 7.35 (d, J = 7.6 Hz, 1H), 7.46 (t, J = 7.2 Hz, 1H), 7.52 to 7.63 (m, 5H), 7.99 9 s, 1H), 7.04 (d, J = 6.8 Hz, 1H), 8.26 (d, J = 8.0 HZ, 1H); ^{13}C-NMR(100 MHz, DMSO-d_6): δ 27.88, 42.91, 122.40(2C), 127.12, 127.47, 128.39, 128.61, 128.76, 129.14, 129.85, 134.34, 138.39, 141.40, 142.46, 150.89(2C), 165.93, 168.96; MS (ESI) *m/z*: 303.43[M + H]$^+$.

4.2.15 | Synthesis of 2-cinnamoyl-7-methoxy-3,4-dihydroisoquinolin-1(2*H*)-one (10k)

Off-white solid; Yield: 0.218 g (83.74%); mp: 76°C to 83°C; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 3.05

(t, J = 6.0 Hz, 2H), 3.87 (s, 3H), 4.04 (t, J = 6.8 Hz, 2H), 7.01 (d, J = 14.4 Hz, 2H), 7.49 to 7.53 (m, 3H), 7.61–7.68 (m, 4H), 8.01 (d, J = 7.6 Hz, 1H); ^{13}C-NMR(100 MHz, DMSO-d_6): δ 28.37, 42.92, 56.12, 112.5, 114.15, 121.37, 123.40, 128.54 (2C), 129.46 (2C), 130.56, 131.62, 135.28, 141.72, 143.86, 162.82, 165.59, 169.22; MS (ESI) m/z: 308.26$[M + H]^+$, 330.22$[M + Na]^+$; HRMS (ESI): m/z $[M + H]^+$calcd for $C_{19}H_{17}NO_3$+ Na^+: 330.1100; found: 330.1133.

4.2.16 | Synthesis of (*E*)-2-(3-(3,4-dimethoxyphenyl)acryloyl)-7-methoxy-3,4-dihydroisoquinolin-1(2*H*)-one (10l)

Off-white solid; Yield: 0.165 g (79.44%); mp: 142°C to 145°C; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 3.03 (t, J = 5.6 Hz, 2H), 3.81 (s, 6H), 3.86 (s, 3H), 4.02 (t, J = 5.6 Hz, 2H), 6.97 to 7.03 (m, 3H), 7.24 to 7.26 (m, 2H), 7.38 (d, J = 14.8 Hz, 1H), 7.60 (d, J = 15.2 Hz, 1H), 8.01 (d, J = 7.6 Hz, 1H); ^{13}C-NMR(100 MHz, DMSO-d_6): δ 28.43, 43.00, 56.00 (2C), 56.09, 111.00, 112.13, 112.45, 114.08, 120.87, 121.44, 122.73, 128.03, 131.60, 142.44, 143.78, 149.37, 151.19, 167.73, 165.51, 169.48; MS (ESI) m/z: 368.41$[M + H]^+$.

4.2.17 | (*E*)-7-methoxy-2-(3-(3,4,5-trimethoxyphenyl)acryloyl)-3,4-dihydroisoquinolin-1(2*H*)-one (10m)

Off-white solid; Yield: 0.174 g, 73.72%; mp: 147°C to 149°C; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 3.04 (t, J = 6.0 Hz, 2H), 3.71 (s, 3H), 3.85 (s, 3H), 3.86 (s, 3H), 3.87(s, 3H), 4.03 (t, J = 6.8 Hz, 2H), 6.98 to 7.02 (m, 4H), 7.41 (d, J = 15.6 Hz, 1H), 7.58 (d, J = 15.2 Hz, 1H), 8.01 (d, J = 8.8 Hz, 1H); ^{13}C-NMR(100 MHz, DMSO-d_6): δ 28.45, 43.08, 56.12, 56.46(2C), 60.60, 106.08(2C), 112.51, 114.12, 121.36, 122.68, 130.89, 131.65, 139.70, 142.16, 143.83, 151.58(2C), 163.78, 165.52, 169.47; MS (ESI) m/z: 398.34$[M + H]^+$; HRMS (ESI): m/z $[M + H]^+$calcd for $C_{22}H_{23}NO_6$+ Na^+: 420.1417; found: 420.1429.

4.2.18 | Synthesis of (*E*)-7-methoxy-2-(3-(*p*-tolyl)acryloyl)-3,4-dihydroisoquinolin-1(2*H*)-one (10n)

Off-white solid; Yield: 0.152 g (83.74%); mp: 66°C to 71°C; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 2.33 (s, 3H), 3.02 (t, J = 6.0 Hz, 2H), 3.85 (s, 3H), 4.02 (t, J = 6.0 Hz, 2H), 6.96 to 7.00 (m, 2H), 7.25 (d, J = 8.0 Hz, 2H), 7.45 (d, J = 16 Hz, 1H), 7.56 (dd, J = 8.0 Hz, 2.4 Hz, 2H), 7.99 (d, J = 8.4 Hz, 1H); ^{13}C-NMR(100 MHz, DMSO-d_6): δ 21.48, 28.37, 42.91, 56.10, 112.47, 114.12, 124.40, 122.23, 128.55 (2C), 130.05 (2C), 131.59, 132.51, 140.51, 141.94, 143.83, 163.77, 165.57, 169.28; MS (ESI) m/z: 322.42 $[M + H]^+$.

4.2.19 | Synthesis of (*E*)-1-(3-(3,4,5-trimethoxyphenyl)acryloyl)-5,6-dihydropyridin-2(1H)-one (Piperlongumine)

Off-white solid; Yield: 0.303 g (82.22%); ^{1}H-NMR (400 MHz, DMSO-d_6): δ 2.50 (d, J = 6.0 Hz, 2H), 3.89 (s, 3H), 3.90 (s, 6H), 4.06 (t, J = 6.4 Hz, 2H), 6.06 (d, J = 13.6 Hz, 1H), 6.82 (s, 2H), 6.97 (t, J = 5.2 Hz, 2H), 7.44 (d, J = 15.6 Hz, 1H), 7.70 (d, J = 15.2 Hz, 1H); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 24.82, 41.62, 56.23(2C), 60.94, 105.7 (2C), 121.15, 125.89, 130.68, 140.21, 143.80, 145.41, 153.42, 165.82, 165.86;MS (ESI) m/z: 318.42$[M + H]^+$.

4.3 | Experimental procedure for SRB assay

The cell lines were grown in RPMI 1640 medium containing 10% fetal bovine serum and 2 mM L-glutamine. For the present screening experiment, the cells were inoculated into 96-well microtiter plates in 90 µL at 5000 cells per well. After cell inoculation, the microtiter plates were incubated at 37°C, 5%CO_2, 95% air, and 100% relative humidity for 24 hours prior to addition of experimental drugs. Experimental drugs were solubilized in appropriate solvent to prepare a stock of 10–2 concentration. At the time of experiment, four 10-fold serial dilutions were made using the complete medium. Aliquots of 10 µL of these different drug dilutions were added to the appropriate microtiter wells already containing 90 µL of medium, resulting in the required final drug concentrations.

After compound addition, the plates were incubated at standard conditions for 48 hours and assay was terminated by the addition of cold TCA. The cells were fixed in situ by the gentle addition of 50 µL of cold 30% (w/v) TCA (final concentration, 10% TCA) and incubated for 60 minutes at 4°C. The supernatant was discarded; the plates were washed five times with tap water and air dried. Sulforhodamine B (SRB) solution (50 µL) at 0.4% (w/v) in 1% acetic acid was added to each of the wells, and the plates were incubated for 20 minutes at room temperature. After staining, unbound dye was recovered and the residual dye was removed by washing five times with 1% acetic acid. The plates were air dried. Bound stain was subsequently

eluted with 10 mM trizma base, and the absorbance was read on an Elisa plate reader at a wavelength of 540 nm with 690 nm reference wavelength.

Percent growth was calculated on a plate-by-plate basis for test wells relative to control wells. Percent growth was expressed as the ratio of average absorbance of the test well to the average absorbance of the control wells * 100. Using the six absorbance measurements [time zero (Tz), control growth (C), and test growth in the presence of drug at the four concentration levels (Ti)], the percentage growth was calculated at each of the drug concentration levels. The dose–response parameters were calculated for each test article. Growth inhibition of 50% (GI50) was calculated from $[(Ti\text{-}Tz)/(C\text{-}Tz)] \times 100 = 50$, which is the drug concentration, resulting in a 50% reduction in the net protein increase (as measured by SRB staining) in control cells during the drug incubation. The drug concentration resulting in total growth inhibition (TGI) was calculated from Ti = Tz. The LC50 (concentration of drug resulting in a 50% reduction in the measured protein at the end of the drug treatment as compared to that at the beginning) indicating a net loss of cells following treatment is calculated from $[(Ti\text{-}Tz)/Tz] \times 100 = -50$.

The values were calculated for each of these three parameters if the level of activity was reached; however, if the effect was not reached or was exceeded, the values for that parameter were expressed as greater or less than the maximum or minimum concentration tested.

4.4 | Molecular docking (procedure)

Molecular docking study was performed to identify the putative binding modes of the 3,4-Dihydroisoquinolin-1(2*H*)-one(DHIQ) based piperlongumine analogues using the *Glide* (Grid-Based Ligand Docking With Energetics) program[2] integrated in the Schrödinger molecular modeling suite (Schrödinger, LLC, New York, NY, 2018), which involves selection of target and its preparation, grid generation to define the active site, ligand preparation, docking, and analysis of binding modes. For this, the crystal structure of tubulin/DAMA-colchicine complex (PDB ID: 1SA0) was obtained from the protein data bank (PDB) and refined using the *protein preparation wizard*. This involves removing crystallographically observed water molecules as there are no reported conserved interactions with the target, adding missing hydrogen/side chain atoms corresponding to pH 7.0, considering the appropriate ionization states for the acidic and basic amino acids, assignment of appropriate protonation states and charge. Finally, the obtained structure was subject to energy minimization until the average r.m.s.d. for the heavy atoms reached 0.3 Å. The shape and properties of the active site for docking were defined using the receptor grid generation protocol for which a grid box of 10×10×10Å dimensions around the centroid of the co-crystallized ligand was generated, which was sufficient to explore a larger space of the target cavity. The receptor cavity of this tubulin structure is present between the two monomers of the tubulin dimer structure (Chain A and B). Next, the 3D structures of the 3,4-Dihydroisoquinolin-1(2*H*)-one (DHIQ) based piperlongumine analogues were sketched using the *build* panel in Maestro and refined using the *Ligand Preparation* protocol to correctly assign the protonation states and atom types of the molecule, and final minimization was done using OPLS-2005 force field to generate single low energy 3D structure for each input structure. Using this setup, the ligands were subjected to molecular docking against tubulin structure with extra precision (i.e., GlideXP) scoring function, which generates favorable poses for ligands in the active site of the tubulin. The docking poses thus obtained were visualized to examine the spatial fit of the ligand in the active site and quantitatively analyzed for the thermodynamic interactions with the amino acid residues lining the active site using the *Maestro's* Pose Viewer utility.

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CONFLICT OF INTEREST

The authors declare no financial involvement or conflict.

DATA AVAILABILITY STATEMENT

Supplementary data scan spectra available

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