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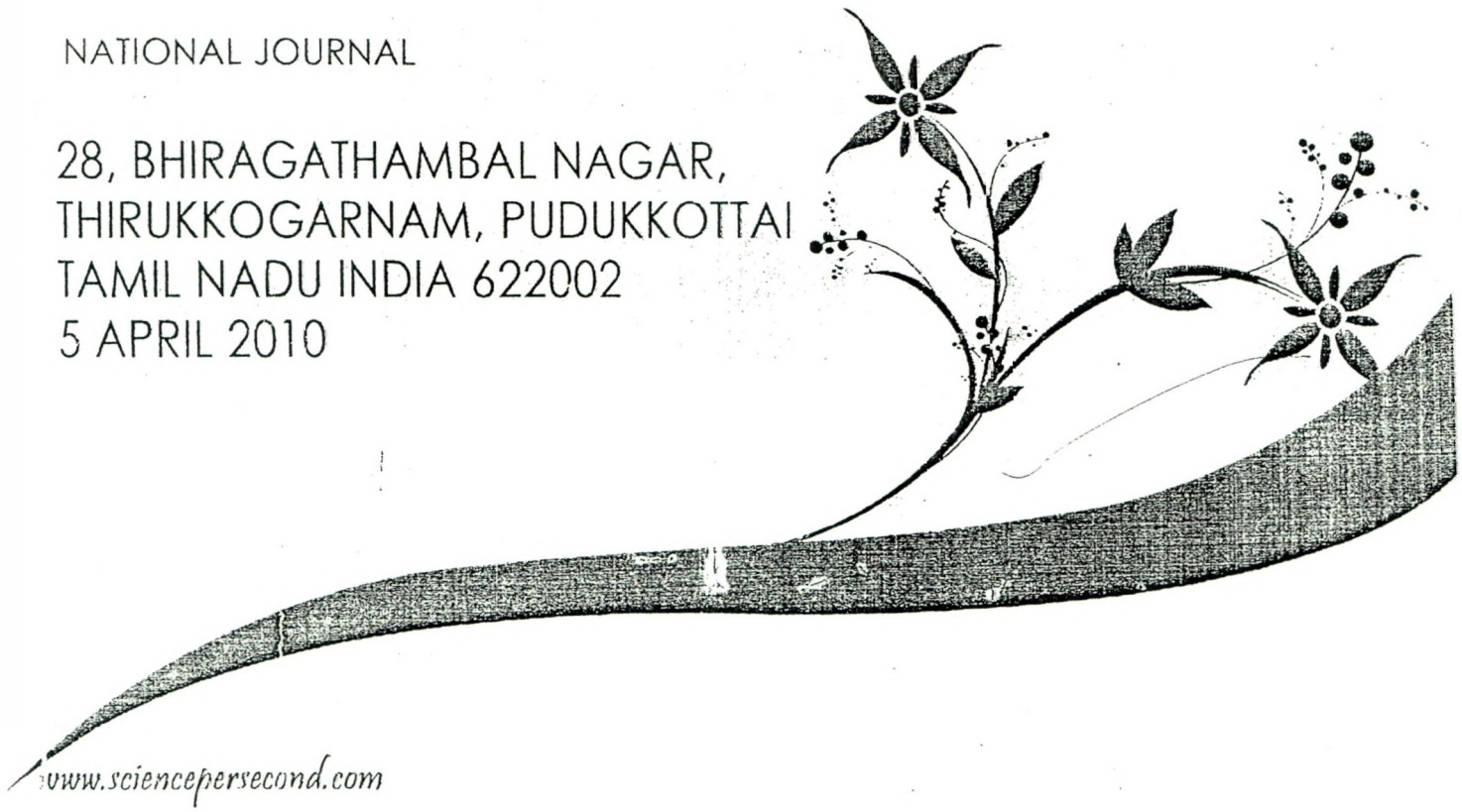

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IN SILICO AND PHARMOKINETIC (ADME) ANALYSIS FOR SELECTIVE INHIBITORS OF TUBULIN AS POTENTIAL CURE FOR CANCER

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ABSTRACT:

Microtubules are extremely important in the process of **mitosis**, during which the duplicated chromosomes of a cell are separated into two identical sets before cleavage of the cell into two daughter cells. Their importance in mitosis and cell division makes microtubules an **important target for anticancer drugs.** A new class of simple synthetic antimitotic compounds based on **2-aroylindoles** was drawn using **MAESTRO and CHEMSKETCH**. The crystal structure of **TUBULIN** was used as a target structure. It was obtained from RCSB Protein Data Bank with the PDB ID: **1Z2B.** High Throughput Virtual Screening, and Induced Fit Docking studies were carried out using **GLIDE.** Docking studies was also carried out using **GOLD**. Interactions were noted for both ligand and synthetic compounds with the target protein. Energy values, GLIDE and GOLD scores were calculated. **ADME** studies were carried out for those compounds and its activity was predicted using **PASS prediction.** Based on this, **3-Benzyloxyphenyl- (5-methoxy-1-phenylsulfonyl-1H-2-indolyl)-methanol** was found to be potent inhibitor for tubulin. We conclude, that 2-aroylindoles constitute an interesting new class of antitubulin agents with the potential to be clinically developed for cancer treatment.

KEYWORDS: tubulin, antimitotic compounds, 2-aroylindoles, 3-Benzyloxyphenyl- (5-methoxy-1-phenylsulfonyl-1H-2-indolyl)-methanol

INTRODUCTION

Cancer (medical term: malignant neoplasm) is a class of diseases in which a group of cells display **uncontrolled growth, invasion and sometimes metastasis** (spread to other locations in the body via lymph or blood). These three malignant properties of cancers differentiate them from benign tumors, which are self-

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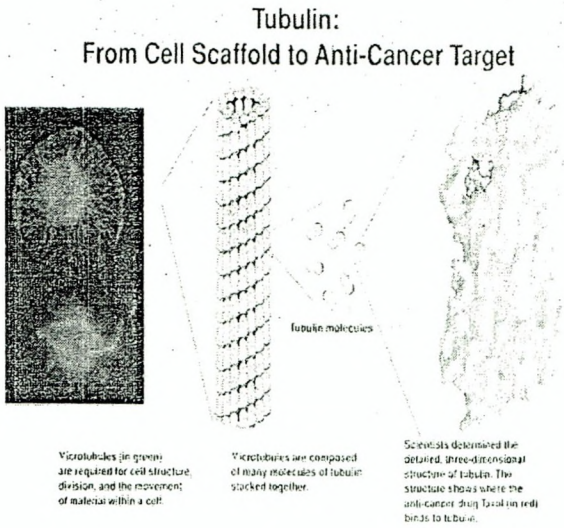
limited, do not invade or metastasize. Most cancers form a tumor but some, like leukemia, do not. The branch of medicine concerned with the study, diagnosis, treatment, and prevention of cancer is **oncology.**

Cancer may affect people at all ages, even fetuses, but the risk for most varieties increases with age. Cancer causes about **13%** of all deaths. According to the American Cancer Society, **7.6 million** people died from cancer in the world during 2007.

Nearly all cancers are caused by abnormalities in the genetic material of the transformed cells. These abnormalities may be due to the effects of carcinogens, such as **tobacco smoke, radiation, chemicals, or infectious agents**. Other cancer-promoting genetic abnormalities may be randomly acquired through **errors in DNA replication**, or are inherited, and thus present in all cells from birth. The heritability of cancers are usually affected by complex interactions between carcinogens and the host's genome.

Genetic abnormalities found in cancer typically affect two general classes of genes. Cancer-promoting ***oncogenes*** and ***tumor suppressor genes.***

TARGET PROTEIN-TUBULIN



Tubulin is one of several members of a small family of **globular proteins**. The most common members of the tubulin family are **α-tubulin and β-tubulin**, the proteins that make up **microtubules (MTs).** Each has a molecular weight of approximately **55 kilo Daltons.** Microtubules are assembled from dimers of α- and β-tubulin. These subunits are slightly acidic with an isoelectric point between 5.2 and 5.8. . Tubulins are **guanosine triphosphate (GTP)-binding proteins.** Beta-tubulin is a GTPase, whereas alpha-tubulin has no enzyme activity.

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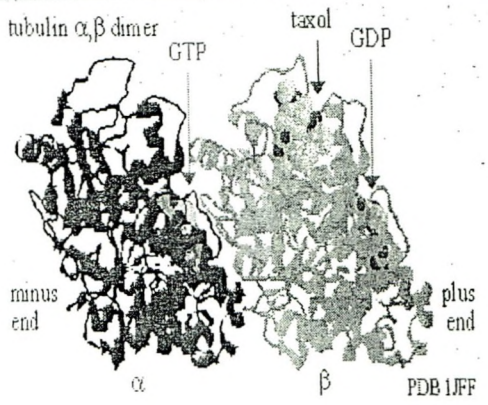
FIG: 1 STRUCTURE OF TUBULIN



It is a compact molecular structure with **three functional components or domains**:

- One that binds to nucleotides,
- One that binds to drugs like taxol, and
- One that looks to be a binding site for other proteins.

TARGETING MICROTUBULES FOR CANCER HEMOTHERAPY

During eukaryotic cell division, in order for each daughter cell to inherit one and only one copy of each chromosome, the mother cell must replicate its chromosomes **exactly once** in the synthetic phase, and then must separate the replicated chromosomes evenly at the end of the mitotic phase to the two daughter cells. Defects in the coordination of chromosome replication and chromosome segregation can have severe consequences leading to genetic instability and aneuploidy, and eventually fostering **tumor malignancy**.

Microtubules are extremely important in the process of **mitosis**, during which the duplicated chromosomes of a cell are separated into two identical sets before cleavage of the cell into two daughter cells. Their importance in mitosis and cell division makes microtubules an **important target for anticancer drugs.**

To ensure faithful transmission of chromosomes during cell division, eukaryotic cells have evolved cellular regulatory mechanisms termed cell cycle checkpoints.

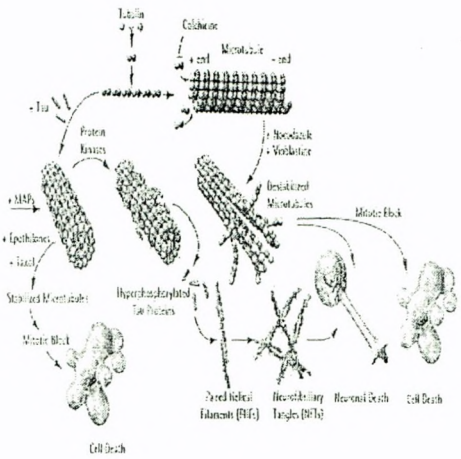
The checkpoints prevent or delay cell cycle progression if certain cellular processes or proteins are disrupted, to gain time to repair the damage before cell division occurs. When the damage is irreparable, the cell undergoes **apoptosis** through the triggering of specific biochemical pathways.

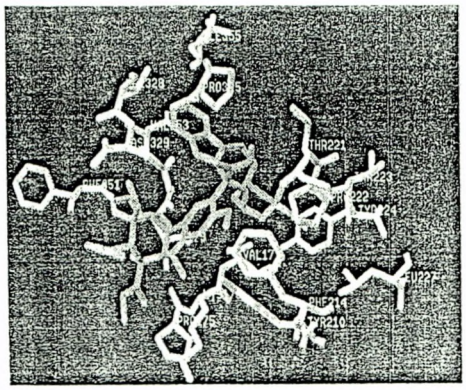
However, cancer cells often harbor defective cell cycle checkpoints allowing for uncontrolled cell proliferation, even when cell division does not occur properly.

Therefore, effective cancer treatment can be achieved by drugs that target certain

OF FIG2: MECHANISM MITOTIC BLOCK

processes or proteins impinging on the cell cycle machinery. In particular, chemical compounds that target microtubules and inhibit the normal function of the mitotic spindle, have proven to be one of the best classes of cancer chemotherapeutic drugs available to date





MATERIALS AND ETHODS (GOLD)

TARGET

The protein molecule chosen for the docking studies is **TUBULIN**. There are different forms of tubulin. The crystal structure of TUBULIN was used as a target structure in current study. It was obtained from RCSB Protein Data Bank with the PDB ID: **1Z2B**

LIGANDS

About **150 compounds** were used for docking studies, which were selected using literature studies. The canonical structure or PDB files of the compounds were used for docking.

PREPARATION OF PROTEIN MOLECULE

The experimental structure of human tubulin was retrieved from the RCSB Protein Data Bank as a pdb file. The PDB code of the protein was **1Z2B**. The protein molecules were prepared mainly by using the software Swiss-pdb viewer. Active site residues with in a range of 4Å were selected and saved in pdb format. Later, the active site residues were minimized in Argus lab after adding hydrogen bonds. The list of atoms in active site, were saved separately as a list file in text document format, which will be used as an input for GOLD.

PREPARATION OF LIGAND

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The ligand compounds (2-aroylindole derivatives) were taken from literature. The ligand structures were drawn using Chemsketch and saved in mol format. The saved ligand compounds were later import and minimized in Argus lab after adding hydrogen bonds. The molecules thus obtained were saved in pdb format.

SETTING UP GOLD PARAMETER

The protein molecule was imported into GOLD. The ligands were also imported. GOLD was run in a particular way such that a particular atom number was given from the identified active site. The GOLD was setup to run at an active site radius of 4Å. The output folder was also specified. All the other fitness function parameters and the genetic algorithm parameters were kept in default mode. The GOLD was run and the output was viewed using Silver and pymol.

MATERIALS AND METHODS

(GLIDE)DOCKING METHOD – GLIDE (GRID BASED LIGAND DOCKING WITH ENERGETICS)

Glide searches for favourable interactions between one or more typically small ligand molecules and a typically larger receptor molecule usually a protein. Each ligand must be a single molecule, while the receptor may include more than one molecule **E.g.**: a protein and a cofactor. GLIDE can be run in rigid or flexible docking modes; the later automatically generates conformation for each input ligand. The combination of positions and orientation of the ligand relative to the receptor, along with its conformation in flexible docking, is referred to as a ligand pose. The ligand poses that GLIDE generates pass through a series of hierarchical filters that evaluate the ligand interaction with the receptor. The initial filters test the spatial fit of the ligand to the defined active site, and examine the complimentarity of ligand–receptor interactions using the GRID based method patterned after the empirical ChemScore function.

Poses that pass these initial screens enter the final stage of the algorithm, which involves evaluation and minimization of a grid approximation to the OPLS-AA nonbonded ligand–receptor interaction energy. Final scoring is then carried out on the energy-minimized poses. Schrödinger's proprietary GLIDE Score multi ligand scoring function is used to score the poses.

If Glide Score was selected as the scoring function, a composite Emodel score is then used to rank the poses of each ligand and to select the poses to report to the user. Emodel combines Glide Score non-bonded interaction energy, and for flexible docking, the excess internal energy of the generated energy conformation.

SCHEMATIC REPRESENTATION OF LIGAND CONSTRUCTION

Glide

↓

Tubulin inhibitors structure was drawn in Maestro and saved in Maestro format

↓

The structures was saved in the specific folder

SCHEMATIC REPRESENTATION OF TARGET AND LIGAND FITTING

1Z2B target structure was loaded into Glide software

↓

Tubulin inhibitors structure was loaded

↓

Binding site specified

↓

Ligand was docked with tubulin

↓

Results and docking score was saved

RESULTS & DISCUSSION

TABLE: 1 BEST COMPOUNDS RESULT (GOLD)

S.NO	COMPOUNDS	INTERACTION (D-H...A)	DISTANCE BETWEEN DONOR HYDROGEN & ACCEPTOR(Å)	GOLD SCORE
1	CO-CRYSTAL LIGAND	(O-H...O)PRO222 (O-H...O)THR220 ASN329(N-H...O)	2.302 2.447 2.443	55.62
2	COMPOUND 24	ASN329 (N-H...O)	2.370	44.29
3	COMPOUND 26	THR220 (C-H...O) THR221 (O-H...O) ASP179 (N-H...O)	2.714 2.320 2.661	46.41
4	COMPOUND 3b	TYR210 (O-H...O) ASN329 (C-H...N) (C-H...O) TYR220	2.313 2.749 2.570	57.44
5	COMPOUND 4b	(O-H...O) PRO222	2.300	56.15
6	COMPOUND 15b	(C-H...O) THR220 (O-H...O)PRO222 ASN329(N-H...O)	2.702 2.661 2.330	63.31

FIG: 1 INTERACTION OF CO-CRYSTAL LIGAND WITH THE ACTIVE SITE RESIDUES OF TARGET PROTEIN TUBULIN

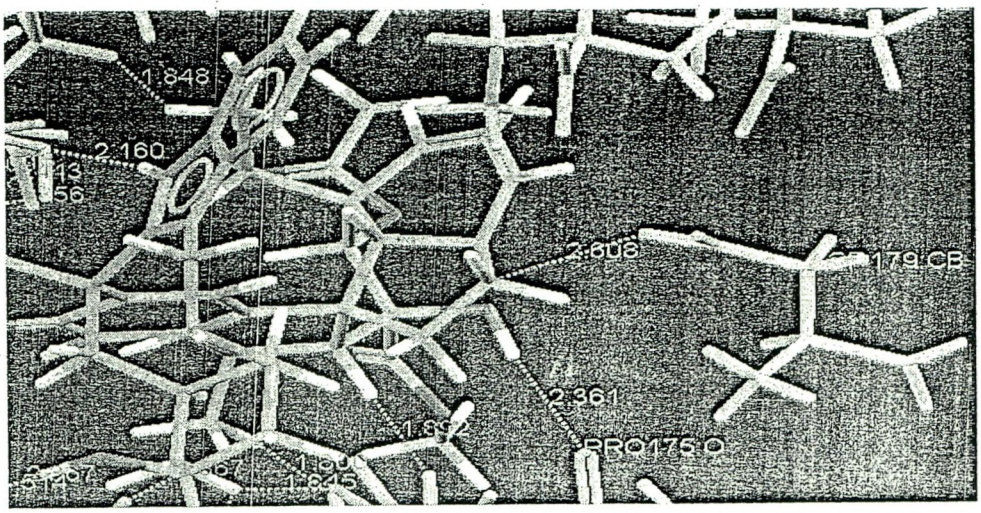

FIG: 2 INTERACTION OF COMPOUND 15b WITH THE ACTIVE SITE RESIDUES OF TARGET PROTEIN TUBULIN

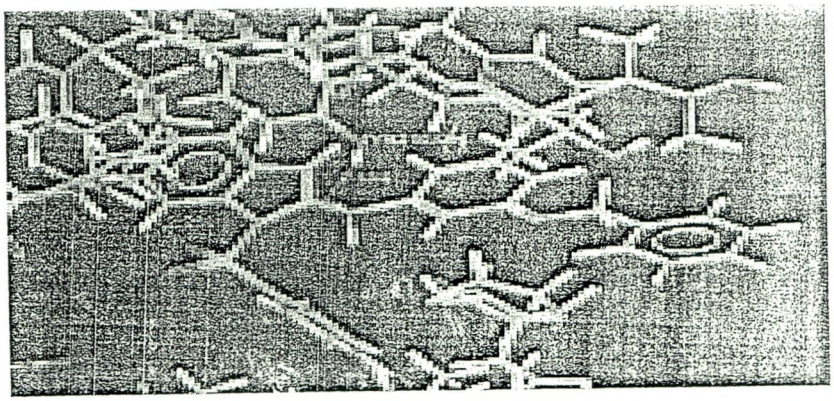

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TABLE: 2 VIRTUAL SCREENING RESULTS (GLIDE)

S.NO	COMPOUND	GLIDE SCORE	GLIDE ENERGY (Kcal/mol)	INTERACTION (D-H...A)	DISTANCE BETWEEN DONOR HYDROGEN & ACCEPTOR (Å)
1	CO-CRYSTAL LIGAND	-0.747180	-2.691400	THR349(O-H...O) ASN329 (N-H...O)	3.260 2.915
2	COMPOUND 2	-4.236866	-25.294179	(N-H...O)ASN329	3.072
3	COMPOUND 22	-3.976076	-24.394056	ASN329(N-H...O)	3.063
4	COMPOUND 23	-4.467714	-25.415357	(N-H...O)ASN329	3.004
5	COMPOUND 39	-3.936288	-25.194167	ASN329(N-H...F)	3.132
6	COMPOUND 3b	-4.477194	-35.438452	(O-H...O)ASN329	3.208
7	COMPOUND 4b	-4.492242	-35.998313	(O-H...O)ASN329	3.045
8	COMPOUND 15b	-4.623950	-40.411585	(O-H...O)ASN329	3.277
9	COMPOUND 74	-4.075947	-29.284022	(N-H...O)ASN329	3.116

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TABLE: 3 QM DOCKING RESULTS OF BEST COMPOUNDS

S.NO	COMPOUND	GLIDE SCORE	GLIDE ENERGY(kcal/mol)	INTERACTION (D-H...A)	DISTANCE BETWEEN DONOR HYDROGEN &ACCEPTOR(Å)
1	CO-CRYSTAL LIGAND	-1.792988	-9.406519	ASN329(N-H...O) LYS326(N-H...O) (O-H...N)LYS326	3.391, 2.619, 3.001
2	COMPOUND 2	-4.386934	-26.496112	ASN249 (N-H...O) ASN329 (N-H...O)	3.108 3.468
3	COMPOUND 22	-4.597493	-28.980123	(N-H...O)ASN329	3.471
4	COMPOUND 23	-4.367992	-26.222764	(N-H...O)PRO325 (N-H...O)ASN329	2.756 3.236
5	COMPOUND 39	-3.890119	-24.645005	ASN249(N-....O) (N-H...O)ASN329	3.492 3.273
6	COMPOUND 3b	-4.774163	-35.425202	(O-H...O)ASN329	2.994
7	COMPOUND 4b	-3.425930	-17.685971	(O-H...O)ASN329 ASN249(N-H...O) (O-H...O)PRO325	2.404 3.155 3.368
8	COMPOUND 15b	-4.338399	-40.799936	(O-H...O)ASN329 (O-H...N)LYS326 ASN329(N-H...O)	2.888 2.756 3.210
9	COMPOUND 74	-4.284291	-29.755978	ASN329(N-H...O) ASN249(N-H....O)	3.148 2.925

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TABLE: 4 ADME RESULTS FOR THE BEST COMPOUNDS

	Co-Crystal	Compounds			
		2	22	23	39
Mol wt (130.0 to725.0)	810.986	281.310	296.282	266.299	303.720
SASA-Solvent ccessible surface area in square angstroms (300.0-00.0)	1039.041	539.723	536.933	512.387	531.732
Volume (500.0-2000.0)	2231.118	923.090	913.658	868.278	900.203
Donor HB (0.0-6.0)	2.000	1.000	1.000	2.000	1.000
Hb accpt HB (2.0-20.0)	12.250	3.500	3.750	2.750	2.750
QP logS (-6.5-0.5)	-5.395	-4.208	-4.074	-3.791	-4.886
QPlogBB (3.0-1.2)	-0.383	-0.268	-0.986	-0.686	0.076
QPlogKp (8.0 to -1.0)	-6.474	-1.261	-2.670	-2.108	-1.281
Percentage Human oral absorption (>80% is high & <25% is poor)	40.991	100.000	91.072	96.764	100.000
Rule of five	3	0	0	0	0

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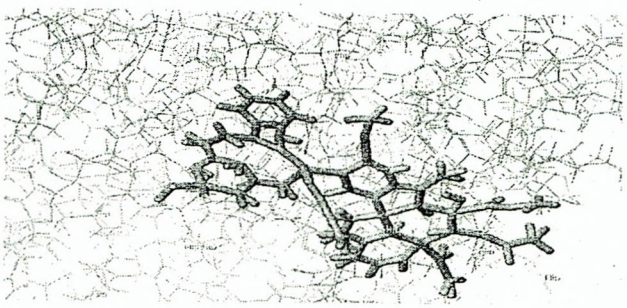
FIG:8 INTERACTION OF CO-CRYSTAL LIGAND WITH THE ACTIVE SITE RESIDUES OF TARGET PROTEIN TUBULIN

FIG:8 INTERACTION OF COMPOUND 15b WITH THE ACTIVE SITE RESIDUES OF TARGET PROTEIN TUBULIN



	Compounds			
	3b	4b	15b	74
Mol wt (130.0 to 725.0)	423.483	423.483	499.583	337.380
SASA-Solvent Accessible surface area in square angstroms (300.0-1000.0)	675.909	679.334	789.823	613.387
Volume (500.0-2000.0)	1233.887	1239.541	1468.867	1069.186
Donor HB (0.0-6.0)	1.000	1.000	1.000	1.000
Hb accpt HB (2.0-20.0)	7.700	7.700	7.700	6.750
QP logS (-6.5-0.5)	-4.597	-4.656	-6.251	-4.679
QPlogBB (3.0-1.2)	-0.579	-0.653	-0.739	-0.567
QPlogKp (8.0 to -1.0)	-0.758	-0.907	0.045	-2.233
Percentage Human oral absorption (>80% is high & <25% is poor)	100.000	100.000	100.000	96.497
Rule of five	0	0	1	0

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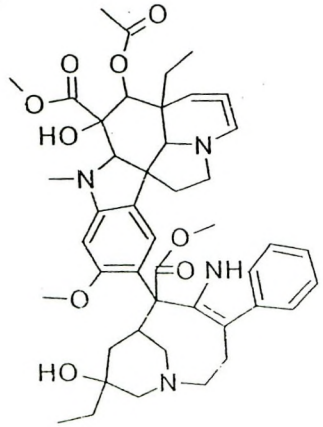
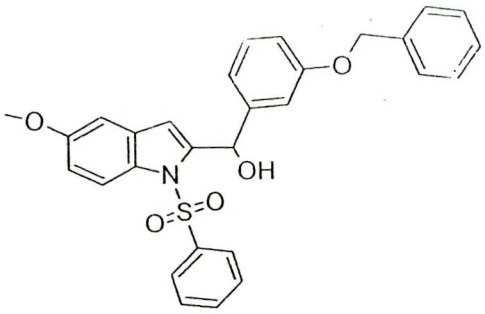
TABLE: 5 STRUCTURE AND IUPAC NAME OF THE LIGAND AND THE BEST COMPOUND

S.NO	COMPOUNDS	STRUCTURES	IUPAC NAME
1.	CO-CRYSTAL LIGAND		dimethyl (2β,3β,4β,5α,12β,19α)-15-[(5*S*,9*S*)-5-ethyl-5-hydroxy-9-(methoxycarbonyl)-1,4,5,6,7,8,9,10-octahydro-2*H*-3,7-methanoazacycloundecino[5,4-*b*]indol-9-yl]-3-hydroxy-16-methoxy-1-methyl-6,7-didehydroaspidospermidine-3,4-dicarboxylate
2	COMPOUND 15b		3-Benzyloxyphenyl-(5-methoxy-1-phenylsulfonyl-1H-2-indolyl)-methanol

DISCUSSION

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Microtubules, major structural components in cells, are the target of a large and diverse group of natural product anticancer drugs. Microtubules are highly dynamic assemblies of the protein **tubulin.** They readily polymerize and depolymerize in cells. These dynamic behaviors are crucial to mitosis, the process of chromosomal division to form new cells. Microtubule dynamics are highly regulated during the cell cycle by endogenous cellular regulators. In addition, many antitumor drugs and natural compounds alter the polymerization dynamics of microtubules, blocking mitosis, and consequently, inducing cell death by apoptosis.

The ability of molecular docking methods to locate selective inhibitors reinforces our view of structure-based drug discovery as a valuable strategy, not only for identifying lead compounds, but also for addressing receptor specificity. This study focuses on series of ligands that are further screened for a successful candidate drug using rational drug design.

In the present work we proposed and evaluated the interaction of **2-aroylindole derivatives** with TUBULIN as target by using the docking programs GOLD and GLIDE.

To study the molecular basis of interaction and binding affinity of **2-aroylindole** and its derivatives, these compounds were docked into active site of TUBULIN receptor using GLIDE. The best 10 compounds were screened out using high throughput virtual screening. These 10 best compounds were further subjected to QM Polarized Docking studies.

Based on ADME results, the clinically available drug had a Percentage human oral absorption of only **40.991%,** but Compound **15b** has a Percentage human oral absorption of **100%.** It is found to satisfy all the necessary parameters to act as a Drug molecule.

Based on overall studies, we can conclude that **compound 15b** to be more potent inhibitor based on glide score, glide energy and interaction with residues in the active site of the TUBULIN. In future this can be taken as an effective drug candidate for the second – generation drug discovery.

Binding affinity of 2-AROYLINDOLE derivatives, were docked into active site of TUBULIN receptor using GOLD. Among the compounds which were docked, **compound 15b** has given higher fitness score compared to other compounds (including co-crystallized ligand). The **compound 15b** has given highest score of **63.31** by showing strong interaction with the residues **(C-H...O) THR220** having hydrogen bonds of length **2.702** Å respectively.

Based on pass prediction, **compound 15b** found to contain **antineoplastic** and **antiangiogenic** activities satisfying the needs for a drug molecule.

The type of interaction it exhibits and the residues with which it interacts convey that they are good inhibitors of TUBULIN as they exhibit drug like activity. The results of the current project suggest that the compounds **(2-aroylindole derivatives)** herewith proposed are showing orientation close to active site and these compounds can be used as a lead for designing future pharmaceuticals that may be used as inhibitors of TUBULIN.

SUMMARY AND CONCLUSION

Nature has presented us with a validated, highly successful anticancer target in the microtubule.

Future challenges in the use of microtubule-targeted agents lie in increasing the understanding of their basic mechanisms and improving their clinical effectiveness. For example, microtubule targeted drugs would be used in combination therapy at much lower doses than are now used at their biologically effective doses (that suppress microtubule dynamics) rather than at their maximum tolerated doses.

Furthermore, relatively weak microtubule-targeted drugs that suppress dynamics (for example, griseofulvin, coumarins and benomyl) could be used as adjuvants in chemotherapy to attain efficacy with decreased toxicity.

In future, microtubule-targeting drugs may be effectively used in combination with:

- other microtubule-targeting drugs;
- other classes of cancer chemotherapeutic agents; or
- other treatment options such as immunotherapy.

Stay tuned. Many more remain to be discovered.

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