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Synthesis and evaluation of analogues Indole-based agents as Tubulin inhibitors using Cell lines

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Abstract:
Back ground : Combretastatin is a natural stilbene compound. These molecules generally have three similar features: the trimethoxy ring "A", the ring "B", which usually has substitutions between C3' and C4', and the ethylene bridge between the two rings to give the necessary rigidity to the structure. Members of the combretastatin family have different abilities to act on vascular tumors. Combretastatin binds to the colchicine binding site of the tubulin beta subunit. Despite the similar name, combretastatin is not related to the statin family of cholesterol-lowering drugs.

Results: A novel combretastatin 2-(1-acetyl-1H-indol-3-yl)-3-(phenyl)acrylic acid analog was synthesized from the A ring position of combretastatin (CA4) (2a to 2j). is an indole moiety and has been evaluated for immunogenicity against various cancer cells such as THP-1 (leukemia), A-549 (cancer), IGROV-1 (ovarian), HEP-2 (liver), MCF-7 (breast) and DU. -145 (prostate). Compound 2d has anti-cancer activity against THP-1 and MCF-7 with IC50 of 0.80 and 0.37 μM, respectively.

Summary: Immunofluorescence technology confirmed that the target binding to the colchicine binding site is located at the α and β interface of tubulin, preventing polymerization. Molecular docking further confirmed that the active 2d product binds to the colchicine binding site at the α and β interfaces of tubulin. Hot Tags: colchicine binding site, indolyl combretastatin, molecular docking, tubulin inhibitor.

KEYWORDS: Material and methods for molecular docking

Introduction

Background : Combretastatin A-4 (CA-4, Figure 1) was first described by Pettit et al. Defined by. It is, by definition, a natural product. Bark of South African windmill tree. In 1989[1]. As a potential cause of vascular injury (VDA), CA-4 can distinguish between normal blood vessels and vascular lesions and selectively open the tumor vasculature, causing vascular rupture [24] . Additionally, CA-4 is effective in preventing cancer from affecting cells. Regarding tubulin dynamics, CA-4 inhibits tubulin polymerization and binds to colchicine sites, causing mitotic arrest [5]. However, the trans isomer of CA-4 is no better than CA-4 (Figure 1). For example, CA-4 exhibits cytotoxicity at low nanomolar concentrations, while the trans-isomer inhibits cell growth in the micromolar concentration range [6]. CA-4 has recently become an important therapeutic target, and various CA-4 analogs and derivatives have been developed and evaluated for their in vitro antitumor activity. Based on these derivatives, several specific interactions (SARs) of CA-4 have been identified, as shown in Figure 2. In these SARs, the A ring is replaced by 3,4,5-trimethoxy and stilbene linked A and B rings. Both cis configurations are required for the protective activity of the compound [7-9]. The 3′-hydroxyl group of ring B has been shown to interact with tubulinated CA-4, and this particular moiety can be replaced by further modifications (10). Various CA-4 derivatives with different properties, such as halogen atoms (such as fluorine or bromine), amines, boric acids, nitro groups, amide groups, alkoxy groups or acyl group, coexist at the 3' position of the B ring. and evaluate antitumor activity. [11-13]. Among these derivatives, CA-4 phosphate (CA-4P, Figure 1) is a water-soluble CA-4 prodrug that has been identified and entered clinical trials for its ability to stimulate blood

vessels. It will be used in combination with cytotoxic drugs used in cancer treatment [14]. Vincristine and vinblastine are anti-inflammatory drugs extracted from Catharanthus roseus and used in cancer treatment, and this effect is due to the interaction of these drugs with tubulin (15).

It was found that to form combretastatin it needed to combine another ring with indole and replace one ring with an indole moiety. Because vincristine, rosette [16] and bromoindirubin have an indole moiety and have good tubulin polymerization inhibitory properties [17]. As with many chemotherapeutic drugs, drug resistance through changes in microtubule dynamics is still a significant problem in paclitaxel treatment [18]. Our goal is to develop tubulin inhibitors of the indole ring.

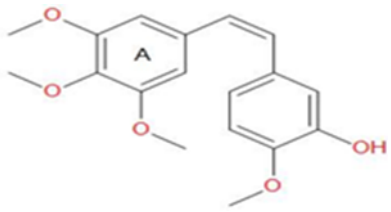
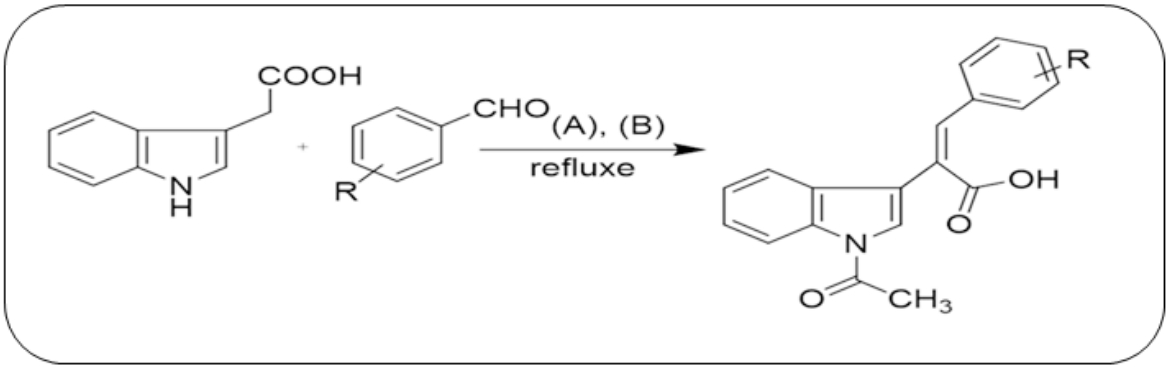


Fig 1 Structure of Combretastatin

Methods: Combretastatin 2-(1-acetyl-1H-indol-3-yl)-3-(phenyl) propene analogues (2a to 2y) were synthesized by condensation of indole-3-acetic acid and different products. Tri ethylamine and acetic anhydride (shown in Scheme 1) . For prevention, seven types of cancer such as lung cancer (A-549), ovarian (IGROV-1), prostate (DU-145), breast cancer (HEP-2), leukemia (THP-1) and breast cancer (using MCF- 7).

Materials and methods for Immunofluorescence Confocal Microscopy:

THP1 cells (8 x 106 [4] cells/well) were seeded on 18 mm square coverslips in six plates. Cells were allowed to adhere for 24 h and 2 days as needed before injection. Use 1 µM paclitaxel as a positive control. After the fixation period, cells were fixed with 4% paraformaldehyde for 10 min at room temperature and permeabilized with 0.5% Triton-X (Sigma-Aldrich, MO, USA) in PBS for 5 min. Cells are closed. 10% goat serum is left at room temperature for 20 minutes. The microbiome was detected using a monoclonal α-tubulin antibody (Sigma Corporation, Cream Ridge, USA) diluted 1:100 in 0.1% Triton X-100 and Alexa in PBS at 37°C. Tube 1 hr Fluorine 488-conjugated secondary antibody (Invitrogen, Carlsbad, USA), diluted 1:1,000 in PBS, 1 hr at room temperature. Cells were washed three times in PBS and stained with DAPI diluted 1:1,000 in PBS [19] . Coverslips were placed on glass slides and cells were visualized using a confocal microscope and an Olympus Fluoview FV1000 laser scanning microscope (Olympus Inc., Center Valley, USA).



Scheme 1: Reagents and conditions. (A) Triethylamine; (B) acetic anhydride, refluxed for 6 to 10 h.

Material and methods for molecular docking:

(a) Ligand preparation : Tubulin regulation can be found in the Protein Data Bank (PDB ID: 1SA0) [18,19]. The selected ligands were validated before preparing for analysis, and the second assignment required slight modification. 2D models of the ligands were created using Chem Draw Pro 12.0 and converted to 3D models. All 3D models were reduced using the ff MMF94 force field method and then the open babel 3.1.1 program was used to generate the data required for fitting.

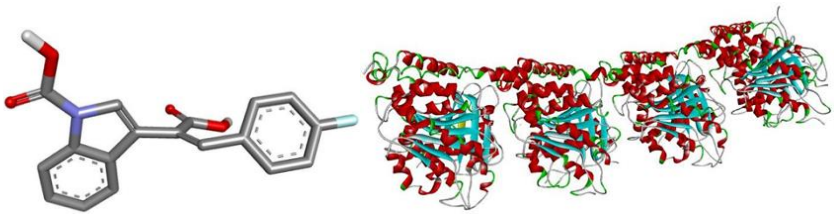


Fig 2 (a) Structure of ligand (b) Binding pocket.

(b)Protein Preparation: After all the different structures were considered, targets were selected for the protein database [PDB:1SA0] using the RCSB database. Remove normal ligands, water, and heteroatoms from the protein crystal structure in preparation for docking. The program used is "BIOVIA Discover Visualizer 2021".

Results and Discussion:

Chemistry: The current work involves the synthesis of combretastatin analogues specifically having free carboxyl functionality at the bridgehead as part of the target compound. This change should provide a rotation constraint that could help increase synthetic power for the need to combat disease. Scheme 1 shows the strategy for preparing target molecules by condensing phenylacetic acid with various substituted benzaldehyde. Ten combretastatin analogs (2a to 2j) were prepared via indole condensation. 3-acetic acid (1) was prepared from various substituted aldehydes using triethylamine and acetic anhydride as previously described (scheme 1). [twenty three]. Monitor the reaction by TLC.

Compounds were purified by column chromatography using 60 to 120 pore size silica gel (48 to 63% yield). All synthesized compounds (Figure 3) were purified. Characterization was performed by spectroscopic methods such as infrared spectroscopy (IR), mass spectrometry (MS), and nuclear magnetic resonance spectroscopy (NMR) (1H NMR, 13CNMR, NMR) (Figure 4 and Table 1). N-acetyl protects the cones. H-3 is not protected because it is close to the N-acetylcarbonyl group. H-3 is not protected due to its carbonyl position at the β position, but is no less protected than H-7 because it is not affected by the magnetic anisotropy of the carbonyl group. group. No change in the incorporation of H-3 was observed when H-2′ was subjected to double irradiation. These results show that H-2' and H-3' are not very close. This is only possible if the two rings are relative to each other. If the two rings are in the cis configuration, two light beams of H-2 will cause the H-3 signal to be higher due to the nuclear Overhauser effect (nOe). It has also been confirmed that free OH (2") when combined with a 2" hydroxyl group is used to form lactone because the geometry is as follows: The proximity of OH and C=O facilitates lactone formation. H-3 is less protected than H-7 because the guard cone of the carbonyl group and the guard cone of the benzene ring plane are within the guard cone. Due to lactone formation, the IR value of the carbonyl group changes from 1.710 to 1.763. Combining phenylacetic acid with an aldehyde successfully completed the PSchorr addition reaction , producing combretastatin in the cis configuration. Based on the above analysis, it can be obtained that the composite material has two bell trans configurations.

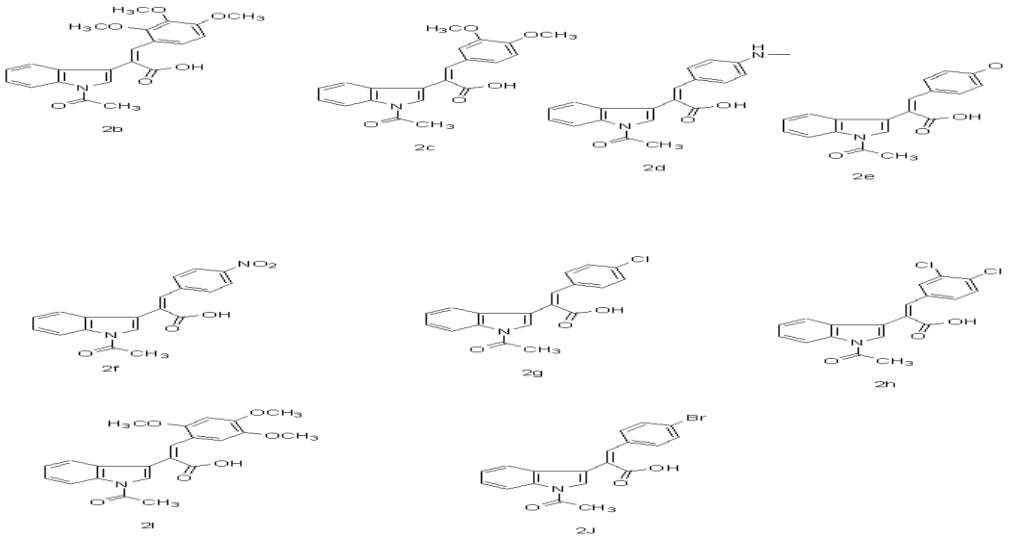


Fig 3: Chemical structure of all synthesized compounds (2a- 2j)

Anticancer activity: The immunogenicity of each combination was evaluated (Table 2): lung (A-549), ovarian (IGROV-1), prostate (PC-3), and breast (MCF-7) cancer cells. Compounds 2j, 2k and 2r showed good activity against THP-1, DU-145 and MCF-7 cell lines. However, compounds 2d (IC50 = 0.80 and 0.37 µM) and 2y (IC50 = 3.6 µM) showed significant inhibitory activity. When the synthesized compound has a simple indole moiety, it binds to the colchicine binding site. The vinblastine binding site opens a space between the binding sites, as both vincristine and vinblastine molecules are very large molecules, and additional binding material is incorporated, as the actual small, vinblastine rolls into place. The corresponding IC50 values were calculated and listed in Table 1.

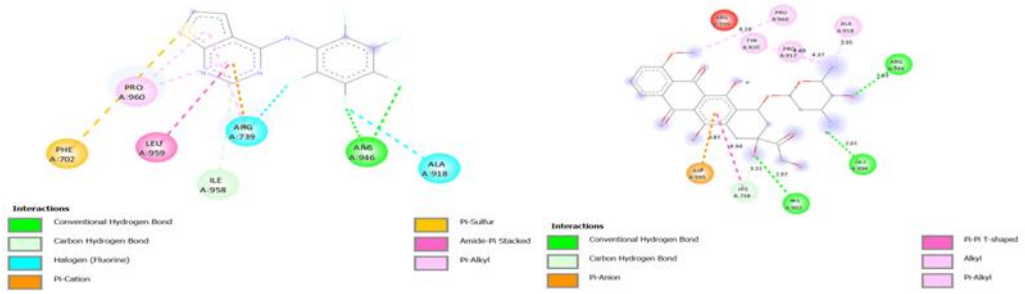
Cell line	PC-3	IGROV-1	A-549	MCF-7
TISSUE	Prostate	Ovary	Lungs	Breast
Compound code				
2a	12.29	8.00	7.68	693
2b	7.26	8.38	14.78	486
2c	7.77	7.59	14.88	476
2d	2.30	1.32	<2.50	180
2e	7.94	10.04	14.90	309
2f	7.94	11.29	8.76	330
2g	6.68	10.39	9.42	435
2h	6.40	12.46	12.28	316
2i	4.39	7.09	8.85	265
2j	7.27	10.85	9.65	735
Standard	6.27	10.49	5.75	428

Table 1 In vitro cytotoxicities against four different tumor cell lines.

As shown in Table 1, all of the synthesized compounds showed good in vitro cytotoxicity against all four tumor cell lines tested; IC50 values were below 800 nM in all cases except the compound. Compound (2d) shows all compounds tested. It is the most cytotoxic against all four cell lines, with IC50 values ranging from 1 to 180 nM. Importantly, 2d showed lower cytotoxicity than the standard. Compared to the product, compound 2a had strong cytotoxicity only on MCF-7 cells. Compounds 2a, 2b, 2c, 2e, 2f, 2g and 2i showed good cytotoxicity against parent model MDA-MB-231, MCF-7 and A549 cells. However, compounds 2a and 2b, especially 2e, showed lower cytotoxicity than CA-4, and this lower cytotoxicity was generally attributed to the large 3'-O-substituted carbon ether moiety due to long side chains that prevent binding. Tubulin is the active part of the colchicine domain. These results therefore indicate that mutation of CA-4 with a large mutation at the C-3' position may lead to decreased activity.

Molecular Docking of 2d:

The highest binding energy shown by compound 2d which is having ΔG = -8.8 kcal/mol and forming a two hydrogen bond with ARG946 amino acid which is having bond distance of 2.30 and 2.37Å. enlisted in Table 2



(a) 2d interaction of 2d **(b) 2d interaction of standard**

Fig 4(a) 2d interaction of 2d (b) 2d interaction of standard

Comp code	MW	RB	HBA	HBD	LogP	GI Abs	BBB permiability	P-gp Substrate	Log K	LD 50
2a	**341.17**	**3**	**4**	**1**	**1.84**	High	No	No	-5.49	260
2b	**317.28**	**4**	**6**	1	**1.63**	Low	No	No	-5.96	250
2c	**430.07**	**3**	**4**	1	**2.10**	High	No	No	-5.94	240
2d	**263.27**	**2**	**4**	1	**2.41**	High	Yes	Yes	-5.64	260
2e	**317.24**	**2**	**7**	1	**2.39**	High	No	Yes	-5.76	245
2f	**329.24**	**3**	**5**	1	**2.92**	High	No	Yes	-5.12	260
2g	**329.73**	**3**	**5**	1	**1.53**	High	No	No	-6.68	275
2h	**272.28**	**2**	**2**	1	**1.72**	High	Yes	Yes	-5.10	245
2i	**296.18**	**2**	**2**	1	**2.71**	High	Yes	Yes	-5.10	230
2j	296.19	2	2	1	2.95	High	Yes	Yes	-5.10	230

Table 2 Drug likeness, ADME and Toxicity properties of synthesized compound.

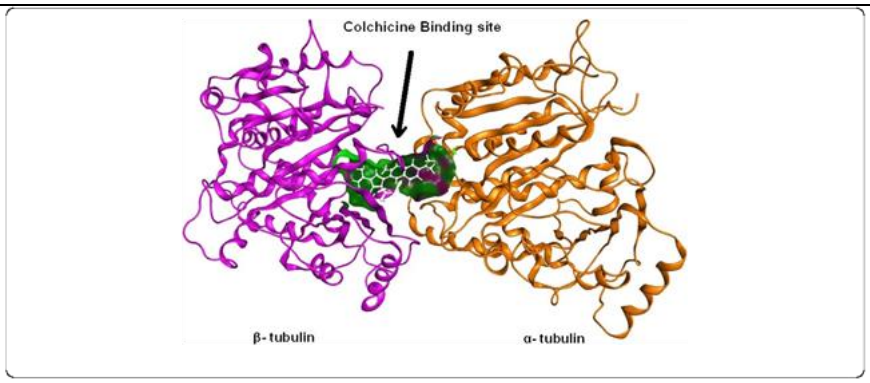


Fig 5: Colchicine binding site at the junction of tubulin α and β subunits. Inhibitor 2d is shown in silver.

Experimental : Reagents were purchased from Sigma-Aldrich (Missouri, USA), Loba Chemie (Mumbai, India) and Central Drug House (New Delhi, India) and used without further purification. All yields refer to the product isolated after purification. The product contains spectroscopic data (IR, [1]H NMR and [13]C NMR spectra).

Spectra measured by TMS (0.00 ppm) in DMSO-d6. Infrared (KBr tray) spectra were recorded on a Fourier transform infrared thermal spectrophotometer (Thermo Fisher Scientific Inc., Waltham, USA).

Typical experimental procedure for synthesis (2a to 2j): Add 2 millilitres of triethylamine and 4 millilitres of acetic anhydride to a combination of substituted benzaldehyde and indole acetic acid. After six to ten hours of

reflux, chill the mixture and add thirty-five percent of the six millilitre HCL. Let it sit for the whole evening. When the ice cubes are added, mix them well to create crystals. After that, sift them and store them till they dry. **Scheme 1**

Characterization data: As mentioned in the previous scheme total synthesized compound from 2a to 2j But 2d showed positive results as compared to other compounds so we are showing only interpretation of 2d compound.

IR Spectra of 2d: FTIR range: 3113.78 cm-1(Ar. C-H str), 3105 cm-1(Ali. C-H str), 1552.35 cm-1 (C=N str), 1508.96 cm-1 (C=C), 1416.37 cm-1 (N-H), 1262.06 cm-1(C-N str), 716.19 cm-1 (C-S-C str), 574.41 cm-1(C-Clstr).Fig 6

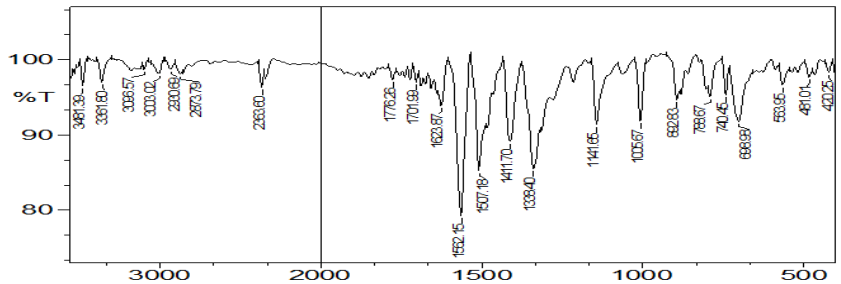


Fig 6 IR Spectra of 2d

NMR Spectra of 2d:

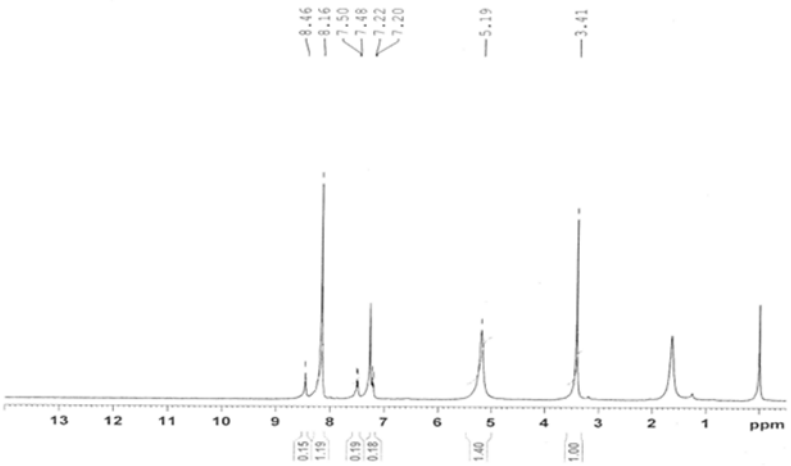


Fig 7 NMR spectra of 2d

Interpretation of 2d:
1H-NMR (500MHz,DMSO,TMS=0): 2.5 (3H, s, OAc), 4.69(1H,d,J=12.5Hz,β H-7), 5.83 (1H,d,J=6.0 Hz, H-2), 3.76 (1H,dd,J=4.3, 3.6Hz,H-4), 4.1(1H,d,J=4.5 Hz,H-6),4.27 (1H,d,J=12.5Hz, αH-7), 7.57 (2H, d, J=7.0, 7.3, aromatic),4.16 (1H,dd, J=4.3, 4.5Hz, H-5), 7.9 (2H, d, j=7.0, aromatic), 4.98 (1H,dd,J=3.6, 3.7Hz, H-3), 3.2, 3.4, 5.2,5.3, 5.5 (5H, OH, D2O exchangeable), 7.7 (1H, d, J=7.3,aromatic).

Biological evaluation
Cytotoxicity by MTT assay
It is now well established that tetrazolium salt reduction is a reliable method to assess cell proliferation. Metabolically active cells reduce the amount of yellow tetrazolium MTT (3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide), thereby decreasing the balance of NADPH and NADH, due in part to the dehydrogenase action. Plasma formazan obtained using spectrophotometric methods can be solubilized and measured. This measurement evaluates the rate of cell division and, on the other hand, the decrease in cell viability due to metabolic processes leading to apoptosis or necrosis.

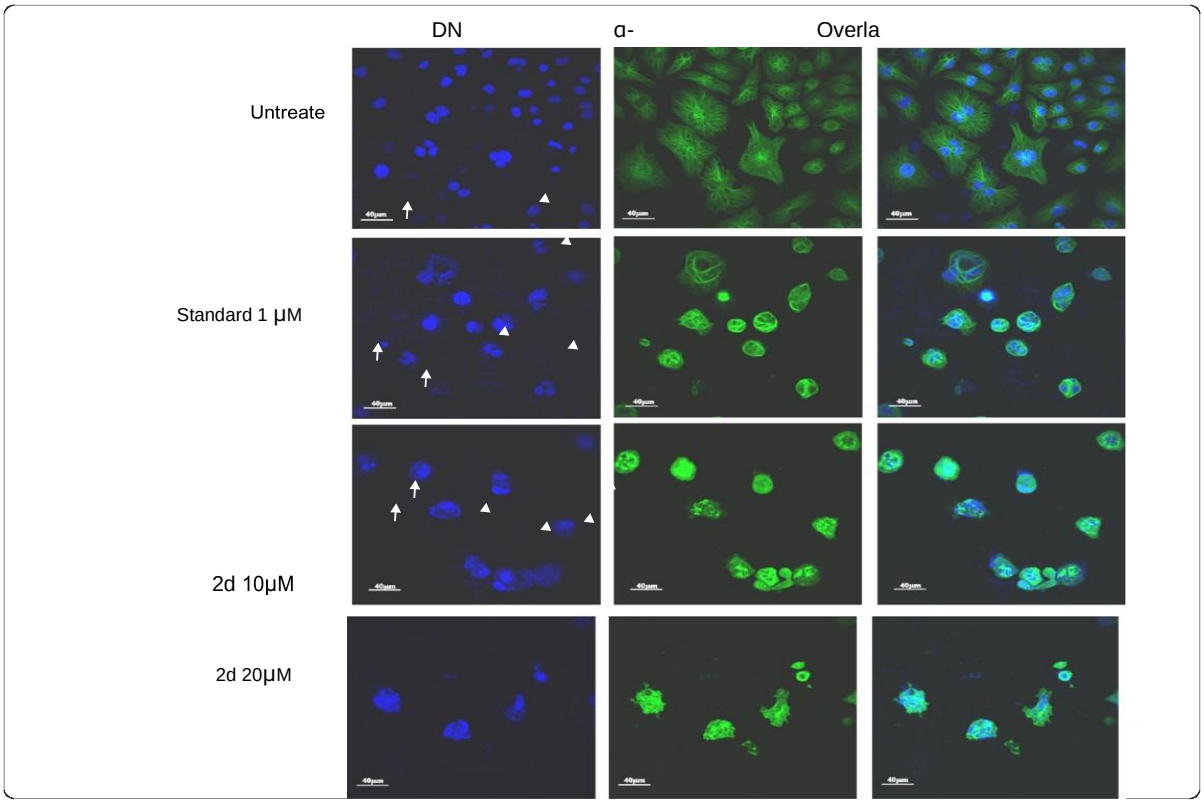


Fig 8 Effect of 2d on the microtubules of A-549 cells

Cell culture media– 10% fetal bovine serum (FBS), 1X Dulbecco phosphate buffered saline (DPBS), Germany, DMEM medium supplemented with MTT reagents, MP Biomedicals, MP Biomedicals, 0.25% trypsin-EDTA solution, Germany Germany DMSO, Thermo Fisher Scientific' It was purchased from. , USA, Germany Cell culture treatment T-25 flask, cell culture grade, from Biolite, Merck, Thermo Fisher Scientific Inc., USA, 10 mL serological pipette and 96-well plates, 1.5 mL, from Sunny Instruments, Nunc, 2 mL and 5 mL tubes, Tarsons, India.

Procedure : Trypsinization and aspiration of cells grown in T-25 flasks into a 5 mL centrifuge tube were performed. A cell pellet weighing 300 x g was produced after centrifugation.With DMEM-HG media, the cell count was modified to ensure 200µl of suspension contained around 10,000 cells. 200µl of the cell suspension was added to each well of the 96-well microlitre plate. The plate was then incubated at 37°C with five percent carbon dioxide in the air for a full twenty-four hours. The used medium was aspirated after a 24-hour period. 200µl of various test drug concentrations were introduced into their corresponding wells. After that, the plate was left to incubate for twenty-four hours at 37 °C in an environment 5% CO2. The moment the plate was removed from the incubator, the drug-containing media was aspirated. Following the addition of 200 µl of media comprising 10% MTT reagent to each well to achieve the final concentration of 0.5 mg/mL, the plate was incubated for three hours at 37 °C in an environment of 5 % CO_2. Without causing any disruption to the crystals that had grown, the culture media was fully withdrawn. After adding 100 µl of DMSO solubilization solution, the plate was mildly agitated in a gyratory shaker to dissolve the formazan that had formed. A device called a microplate reader was used to determine the absorbance at 630 and 570 nm wavelengths. Upon subtracting the background and blank, the growth inhibition % was calculated. The amount of test drug needed to impede cell growth by fifty percent was found using the dose-response graph for the cell line (IC_{50}).

Conclusions:

A series of new combretastatin analogues were produced by condensation of 3-indoleacetic acid with differently substituted aldehydes, and their structures were determined by various spectroscopic techniques such as infrared, mass spectrometry, and nuclear magnetic resonance. [1]H, [13]C, HMBC and HSQC). When there is a hydroxyl group in the second row of the aldehyde, a lactone forming arrangement is formed. Moreover, this is confirmed by nOe (doubling the irradiation intensity of H-3 and H-4' increases). The compounds have been evaluated as antibacterial agents. Confocal microscopy dose-dependent disruption was used to determine the

effect on microtubule structure; microtubule loss indicates apoptosis after 2 days. Molecular docking studies were performed to determine the conformation of the colchicine binding site. This will help prevent inhibition by inhibitors. The carbonyl oxygen (carbonyl group of indole) forms a hydrogen bond with the sulfhydryl group of Cys241β. This is similar to the interaction of colchicine with tubulin. Another hydrogen bond forms between the hydroxyl group of the carboxylic acid moiety of 2d and the nitrogen of Leu255β. These two hydrogen bonds play an important role in stabilizing both sides of the colchicine binding site. The effects of synthetic products suggest they may be promising for anti-cancer drugs. To confirm its effectiveness, more in vivo experiments are needed to address the immune system in cancer treatment.

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