62P Pyrrole-based carboxamides exhibit potent cytotoxic activities against epithelial cancer cell lines via targeting tubulin polymerization

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Introduction

Microtubule targeting agents (MTAs) that interfere with the dynamic state of the mitotic spindle are well-known and effective chemotherapeutic agents. These agents interrupt the microtubule network via polymerization or depolymerization, halting the cell cycle progression and leading to apoptosis. In our efforts to discover novel tubulin inhibitors, we developed novel pyrrole-based analogs targeting the colchicine binding site on tubulin and thereby interfering with tubulin polymerization.

Figure 1: Computer modeling of the CAs binding site on tubulin. (A, C) 3D diagram illustrating the proposed binding mode of interactions of CA-61 (A) and CA-84 (C) with the tubulin (B, D) 2D Ligand interaction diagrams of the CA-61 (B), and CA-84 (D) with significant amino acid residues.

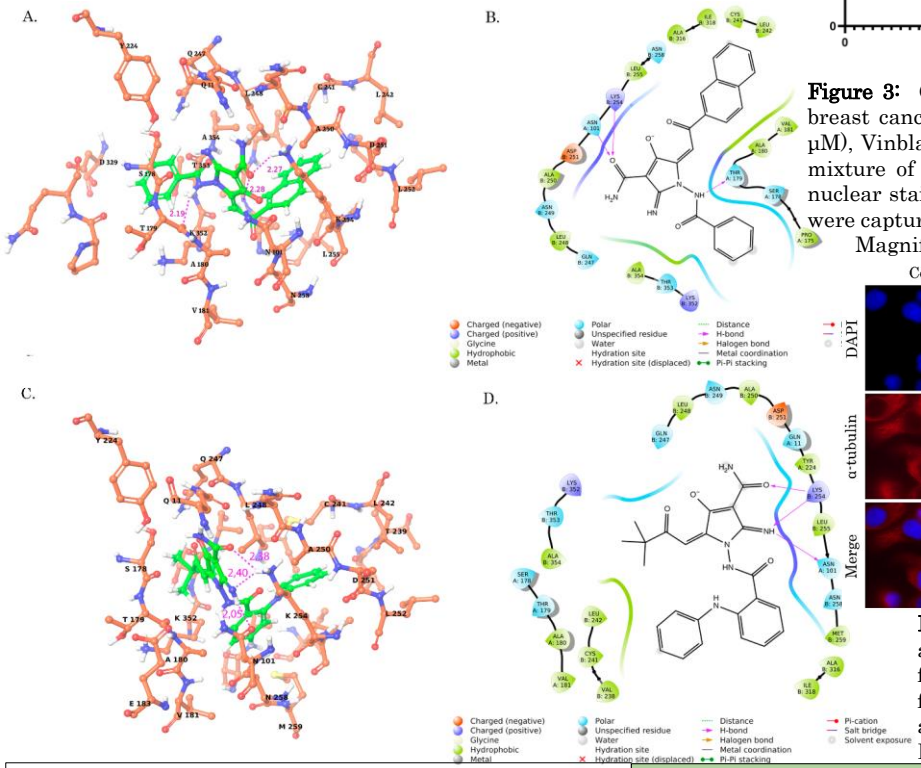


Figure 2: Dynamics of tubulin polymerization in samples treated with CA-61 and 84, DMSO (control), Paclitaxel (PTX), Vinblastine (Vin), and CaCl2 at 37 °C. Absorbance was assessed every min for 1 h. A shift of the curve to the upper left of the control (DMSO) represents an increase of polymerized microtubules. A shift to the downright reflects the decrease in the rate of tubulin polymerization.

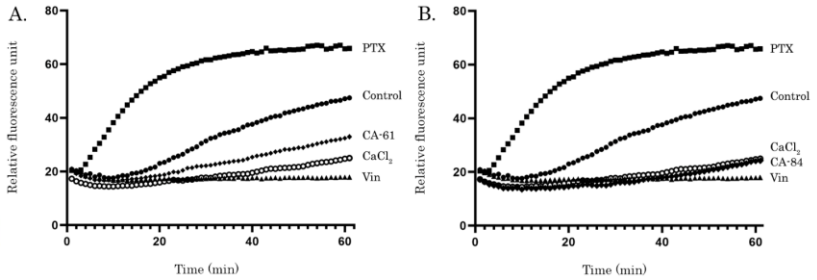


Figure 3: CAs disrupt the microtubules network in cancer cells. HCC1806 breast cancer cells were treated with DMSO (control), Paclitaxel (PTX- 0.5 µM), Vinblastine (Vin - 0.01 µM), CA-61 and 84 (10 µM) for 9h, fixed with a mixture of methanol and acetone, and stained with α-tubulin (red). DAPI nuclear staining (blue) was used to outline an equal number of cells. Images were captured by using a fluorescence Olympus BX63 microscope. Magnification 100x, scale bars 10 µm.

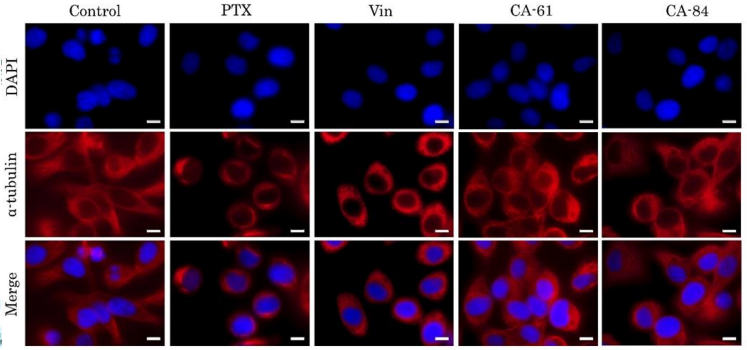
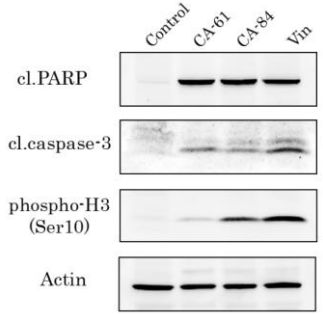


Figure 4: CA-61 and 84 induce apoptosis. Immunoblot analysis for apoptosis markers (cleaved forms of PARP and caspase-3), and pospho-H3 (Ser10) in HCC1806 breast cancer cells.



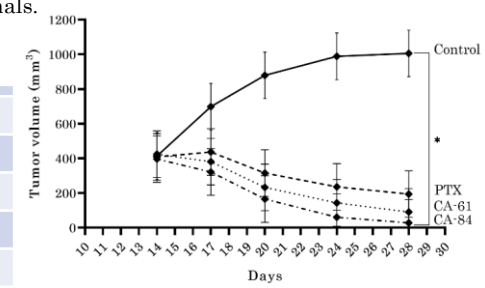
Methods and Materials

To identify the potential binding sites of CAs on the tubulin, the molecular docking procedure was performed by using Schrodinger molecular modeling software (Schrödinger, Inc, New York, NY, 2021). The impact of the CAs on the tubulin polymerization dynamic state was assessed by using the Tubulin Polymerization kit (Cytoskeleton Inc., Denver, Colorado, USA). Primary antibodies raised against the following proteins were used for westernblot: Cell Cycle and Apoptosis WB Cocktail (pHH3(Ser10)/Actin/Cleaved PARP and caspase 3); for immunofluorescence staining – α-tubulin. The distribution of cell cycle phases in HCC1806 cells was analyzed by Guava Muse Cell Analyzer using Cell Cycle Kit. Subcutaneous human tumor xenografts were generated via s.c. inoculation in flank areas of 5-to 8-week-old female nu/nu mice with 100 µl of 5 × 106 HCC1806 breast cancer cells/ml suspensions in Dulbecco's phosphate-buffered saline. The animal experimental protocols were approved by the Committee for Ethics of Animal Experimentation.

Table 1: Quantitative analysis of cell-cycle distribution in HCC1806 breast cancer cells as measured by flow cytometry. Normally distributed data is presented as mean ± standard deviation, non-normally distributed data are shown as median (interquartile range); * p<0.05, ** p<0.01.

	G0/G1	S	G2/M
Control	37.04±2.17	18.80 (18.5-18.9)	42.26±3.03
CA-61	22.84±2.35**	23.38±2.68*	51.26±3.65*
CA-84	24.10 (24-24.5)**	23.14±0.69*	68.30 (67.6-69)*
PTX	1.90±0.85**	20.40±2.47	68.24±1.80*
Vin	30.48±1.54**	19.56±0.74	47.12±1.34*

Figure 5: Antitumor effects of CAs and PTX in a nude mice xenograft tumor model. After HCC1806 breast cancer xenografts reached ~400 mm3 volumes (day 14), nude mice were randomized into 4 groups and administered i.p. 150 µl of vehicle (negative control), CA-61, -84 (2 mg/kg), or PTX (10 mg/kg). Results are expressed as mean volume of tumors (mean ± SD, n = 4; *: p < 0.001, compared to control (vehicle-treated) animals.



Results

We report here 2 novel pyrrole-based carboxamides (CAs) (CA-61 and -84) as the compounds exhibiting potent anti-cancer properties against a broad spectrum of epithelial cancer cell lines, including breast, lung, and prostate cancer. The anti-cancer activity of CAs was due to their ability to interfere with the microtubules network and inhibit tubulin polymerization. Molecular docking demonstrated an efficient binding between these ligands and the colchicine-binding site on the tubulin. CA-61 (Fig.1A-B) formed 2 hydrogen bonds interaction with THR 179 (B) and THR 353 (B), whereas 2 hydrogen bonds with LYS 254 (B) and 1 with ASN 101 (A) were identified for CA-84 (Fig.1C-D) . The binding energy for CA-84 and CA-61 was -9.910 kcal/mol and -9.390 kcal/mol. Tubulin polymerization assay revealed a strong inhibition of tubulin polymerization induced by CA-61 and -84 (Fig.2). Immunofluorescence data revealed the disruption of the tubulin assembly in CA-treated cancer cells (Fig.3). As an outcome of the tubulin inhibition, these compounds halted the cell cycle progression in the G2/M phase (Table 1), leading to the accumulation of the mitotic cells and further inducing apoptosis (Fig.4). Lastly, the in vivo study indicated that CAs significantly inhibited the HCC1806 breast cancer xenograft tumor growth in a nude mouse model (Fig.5).

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Conclusion:
Collectively, we identified the novel CAs as the potent MTAs, inhibiting tubulin polymerization via binding to the colchicine-binding site, disrupting the microtubule network, and exhibiting potent pro-apoptotic activities against the epithelial cancer cell lines both in vitro and in vivo.