MPC-6827: A Small Molecule Inhibitor of Microtubule Formation That is Not a Substrate for Multi-Drug Resistance Pumps

Vijay Baichwal[1], Shailaja Kasibhatla, Bruce Roth[1], Nicki English[2], Nilantha Sirisoma[2], Sui Xiong Cai[2], Ben Tseng[2], Adrian Hobden[1]and Christopher M. Pleiman[1]

[1]Myriad Pharmaceuticals Inc, 320 Wakara Way, Salt Lake City, UT 84108 and [2]Maxim Pharmaceuticals, 6650 Nancy Ridge Drive, San Diego, CA 92121

ABSTRACT

MPC-6827 was discovered as a result of an extensive medicinal chemistry effort. The initial lead compound was discovered in a proprietary caspase-based high-throughput screen. This molecule displays pro-apoptotic activity, with potency at low nanomolar concentrations in multiple cancer types including pancreatic, prostate, breast, colorectal, non-small cell lung, small cell lung, melanoma, ovarian and leukemia (Tseng et al., 95th Annual Meeting of the AACR, 2004). Additionally, statistically significant ($p<0.05$) inhibition of tumor growth was observed in human breast (MCF-7, MX-1, MB-MDA-435), colon (HT-29), pancreas (MiaPACA), ovarian (OVCAR-3), and prostate (LNCaP) xenografts and a mouse melanoma (B16) allograft implanted subcutaneously into athymic nude mice (Pleiman et al., 95th Annual Meeting of the AACR, 2004).

In order to determine the molecular target of MPC-6827, three structurally related photoaffinity and radiolabeled analogs of MPC-6827 were synthesized (data for MPI-0441264 is shown). All three were found to bind a 55 kDa protein and were competed with MPC-6827, paclitaxel and colchicine, but not vincristine. MPC-6827 effectively inhibited the polymerization of tubulin *in vitro* and disrupted the formation of microtubules, but not actin filaments in intact A549, NIH-3T3, NRK, CHO-K1 and MDCK cells. Treatment of either MCF-7 or Jurkat cells led to pronounced G2/M cell cycle arrest, followed by nearly complete apoptotic response (not shown).

Unlike the tubulin binding vinca alkaloids (vincristine, vinblastine, and navelbine) and taxanes (paclitaxel and docetaxel), MPC-6827 was equipotent for induction of apoptosis in cancer cell lines, regardless of the expression levels for the multidrug resistance ABC transporters MDR-1 (Pgp-1), MRP-1, and BCRP-1.

These studies demonstrate that MPC-6827 acts through tubulin, the drug target of vinca alkaloids and the taxanes Taxol® and Taxotere®.

MATERIALS AND METHODS

MPC-6827 competes with radiolabeled and photo-reactive MPI-0441264 for binding to a 55kDa protein

Jurkat cells were pre-treated with MPC-6827 or the low-potency analog, MPI-0441136, then treated with 100 nM tritiated, photo-affinity MPI-0441264. Cells were subjected to *UV* radiation to activate the photo-affinity label thus irreversibly conjugating the labeled compound to the target protein. Cellular lysates were run on a gel and processed for autoradiography.

MPC-6827 disrupts tubulin polymerization *in vitro*

Lyophilized tubulin (Cytoskeleton #ML113, 1 mg, MAP-rich) was assayed for the effect of MPC-6827 on tubulin polyermization according to the recommended procedure of the manufacturer. To 1 µL of each experimental compound (from a 100x stock) in a 96-well was added 99 µL of supplemented tubulin supernatant. Incubation was done in a plate reader at 37 °C, and absorbance readings at 340 nm were recorded every minute for an hour.

MPC-6827, Taxol and Colchicine compete for binding of MPI-0441264 to tubulin

Jurkat were pre-treated with DMSO (vehicle control), 1 µM MPC-6827, 1 µM MPI-0441136, 1 µM or 10 µM colchicine, 1 µM or 10 µM vinblastine, or 1 µM or 10 µM paclitaxel and then labeled with 100nM tritiated MPI-0441264. Cell were subjected to *UV* radiation to activate the photo-affinity label thus irreversibly conjugating the labeled compound to the target protein. Cellular lysates were run on a gel and processed for autoradiography.

Disruption of microtubules in intact cells

A549 cells were treated with saline or 10nM MPC-6827 on cover-slips. After one or three hours, the cover slips were removed from media, fixed in formaldehyde and permeabilized with 0.1% Triton-X 100. Following blocking, cover slips were stained for tubulin, actin and nuclei.

MPC-6827 doesn't disrupt actin filaments

Rat NRK cells were treated with saline, 10 nM MPC-6827 or 100 nM vincristine for three hours. Treated cells were fixed on cover-slips, permeabilized and stained for actin, tubulin and nuclei.

Cytotoxicity of MPC-6827 in MDR-1, MRP-1 and BCRP-1 over-expressing cell-lines

The MCF-7 human breast cancer cells, NCI/ADR-RES (over-express MDR-1), MCF-7/MX (over-express BCRP) and MCF-7/VX (over-express MRP-1) cells were then exposed to various final concentrations of MPC-6827 and various chemotherapeutics know to be substrates for ABC transporters The inhibition of cell growth due to exposure to the drug was determined using the ATP-Lite assay.

RESULTS

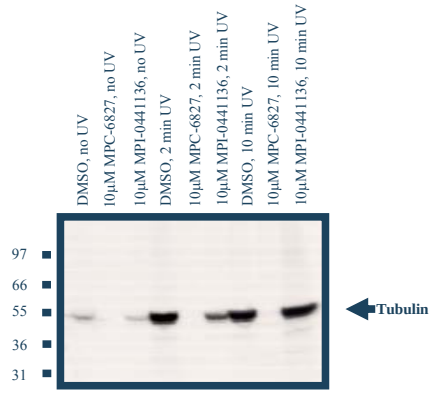

Figure 1. MPC-6827 competes with radiolabeled and photo-reactive MPI-0441264 for binding to a 55kDa protein

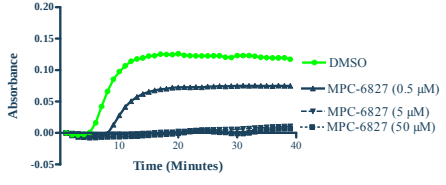

Figure 2. MPC-6827 disrupts tubulin polymerization *in vitro*

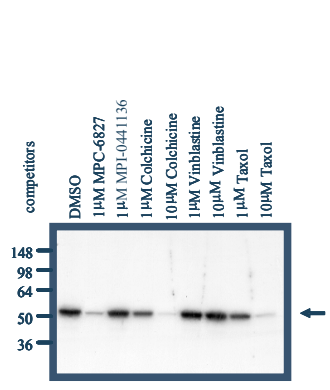

Figure 3. MPC-6827, Taxol and Colchicine compete for binding of MPI-0441264 to tubulin

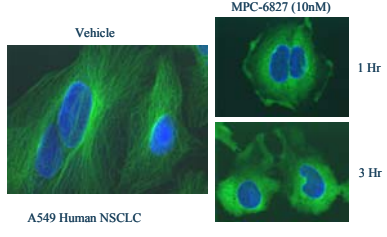

Figure 4. MPC-6827 disrupts microtubules in intact cells

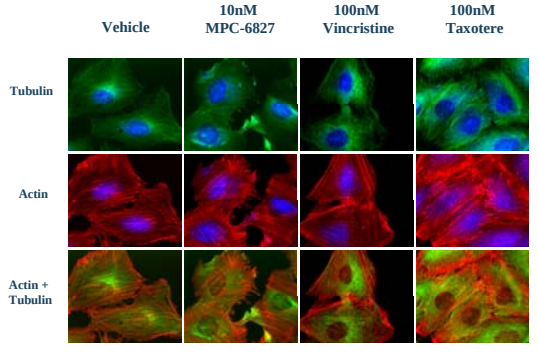

Figure 5. MPC-6827 doesn't disrupt actin filaments

Multi - Drug Resistance	Fold Change in IC_{50}				
	MPC - 6827	Vinblastine	Docetaxel	Epirubicin	Irinotecan
MDR - 1	1.0	930	245	856	N.D.
MRP - 1	1.3	6.4	N.D.	6.4	5
BCRP - 1	1.8	N.D.	N.D.	7.4	14

Figure 6. MPC-6827 is not a substrate for MDR-1, MRP-1 and BCRP-1 transporters

CONCLUSIONS

- MPC-6827 binds to tubulin
- Competition studies suggested that MPC-6827 shares a common binding site with the tubulin binding agents colchicine and taxol, but not vinblastine
- MPC-6827 disrupts microtubule formation
- MPC-6827 is not a substrate for the ABC transporters MDR-1, MRP-1 and BCRP-1

MPC-6827 is currently in Phase 1 clinical trials. See posters 3413 and 3420 at the Targeting Microtubules session to learn more

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