Supplementary Experimental Methods

Animal Studies:

B16/F10 mouse melanoma model: cells were resuspended in PBS and adjusted 0.5 x 10^7 cells.mL^{-1} for implantation. Cells were implanted intradermally on the mid-dorsal area in 0.1 mL (0.5 x 10^6 cells /mouse) and allowed to grow for 24 hr until visually detected before beginning TR100 drug treatment. Control (DMSO alone) and TR100 treatment doses (20 and 30 mg.kg^{-1} in DMSO) were administered via intraperitoneal injection (IP) daily for 5 days/week for 3 weeks or until tumor size reached 1cm in diameter. The tumor sizes were measured by the two most different perpendicular diameter three times a week using digital callipers. Tumor volume was determined according to the formula, $V=D \times d^2 \times 0.4$, where V is the tumor volume, D is the larger diameter, and d is the smaller diameter (1). Upon termination of the experiment heart, bloods and tumors were harvested for further analysis. All procedures were approved by the University of Sydney Animal Ethics Committee.

1205Lu human melanoma xenograft model: The flanks of 6-week old CB17 NOD/SCID male mice were injected subcutaneously with 2x10^6 1205Lu cells in 100 µl complete medium. Eight days post-engraftment, the mice were treated with 20 mg.kg^{-1}TR100 in DMSO or DMSO only, daily for 21 days or until sacrificed. Mice were weighed daily and tumor growth measured three times per week with digital calipers. Mice were sacrificed when tumors reached 1cm^3 volume or became ulcerated. Experiments were approved by the University of Sydney Animal Ethics Committee.

CHP134 human neuroblastoma xenograft model: 4.2x10^6 CHP134 cells in Matrigel were injected bilaterally in the flanks of female, athymic nude (nu/nu) mice. When the tumors reached ~200 mm^3 on average, 18 individual tumors were selected and randomized to either control or experimental group. The mice were then injected intraperitoneally once daily with control mice receiving 10% DMSO in PBS at 10 mL.kg^{-1}, and experimental mice receiving

TR100 at 25 $mg.kg^{-1}$. Mice were weighed and tumors measured twice weekly with tumor volume calculated by the formula: $length*width^2 *\pi/6$. Mice were sacrificed when tumors reached a volume of 1500 mm^3. Experiments were approved by the CCHMC Institute of Animal Care and Use Committee (IACUC).

Tropomyosin compound modeling

In order to determine the binding mode of TR100 to the LMW tropomyosin, the homology model of TM-Gamma-1 was first built based on the solution NMR structure of the junction between tropomyosin molecules (2). The coordinates for the NMR structures were obtained from the PDB databank (www.pdb.org) (3). A representative structure from the deposited ten conformations was chosen as the template sequence. After sequence alignment, a homology model was created using SWISS-MODEL (4). The model was refined to optimize side chain packing and to explore the conformational space of the helix pair using a simulated annealing procedure based on Smith *et al*. (5), however, using the NAMD2 software (6).

The binding of TR100 to the dimer of tropomyosin was firstly examined using Multi-Copy Simultaneous Search (MCSS) method (7). The several hundred fragments of indole which is similar to the scaffold of TR100 were minimized simultaneously around the C-terminus of tropomyosin. Ten indole minima were found to be located inside the cavity of the C-terminus formed between two helices. These minima served as guidance for docking of TR100 into the C-terminus. However, the long aliphatic chain of TR100 is very flexible, presenting significant challenge to the standard docking methodology. Therefore, we firstly dock the scaffold of TR100 () into the cavity.

The in-house developed software Qu-Cbit (version 1.01, Qubist Molecular Design,

Melbourne, Australia, 2009) was used and the calculations were performed using CHARMM22 force field. In total, forty seven docked conformations were obtained and after scoring based on its overlay with the indole minima, three conformations were selected followed by further refinement at quantum mechanical level. During the QM calculations using GAMESS program (http://www.msg.ameslab.gov/gamess), the system consists of the TR100 scaffold and five residues of tropomyosin surrounding the ligand and was geometry optimized with the residues fixed. Density Functional Theory method was used with B3LYP functional (8, 9) and 6-31G(d) basis (10). The conformation with lowest total energy was selected as the best binding mode of the TR100 scaffold to the tropomyosins. Using the optimized structure of the scaffold, the missing part of TR100 was added, followed by the QM refinement of geometry optimization of the full TR100 with the fixed residues of tropomyosins. The final optimized structure of TR100 presents its best binding mode to the dimer of tropomyosin (Figure 2D).

Supplementary References

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