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Stable and orally bio-available pro-drugs of CPS11

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ABSTRACT

Stable and orally bio-available pro-drugs of CPS11 were synthesized. They are active on human umbilical vein endothelial cell proliferation assay and tube formation assay. The therapeutic efficacy and safety of **4** as a single agent or combined with Taxol in the treatment of MX-1 human breast cancer xenograft were evaluated. Compound **4** as a single agent failed to produce an anti-tumor activity, while it significantly enhanced antitumor potency of Taxol.

Thalidomide (**1**) was originally marketed as a sedative during the 1950s.[1–3] It was found to be effective to treat leprosy (erythema nodosum leprosum, ENL). FDA approved thalidomide to treat ENL in 1998.[4] Thalidomide was found effective to inhibit angiogenesis. When combined with dexamethasone, thalidomide demonstrated impressive efficacy to treat multiple myeloma (MM). Nonetheless, thalidomide was reported only limited efficacy against other cancers, especially solid tumors.

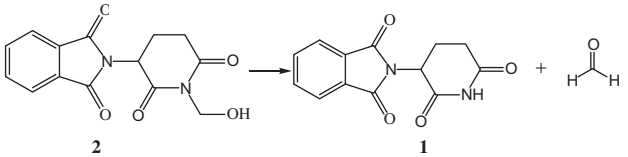
N-Hydroxymethylthalidomide (CPS11, **2**) repressed NF-κB while stimulating nuclear factor of activated T cells. Principal component analysis of cytokine showed that CPS11 had different collective targeting profiles compared with that of thalidomide.[5] Further studies revealed that CPS11 significantly inhibited angiogenesis in rat aortic ring assay. CPS11 is also a potent inhibitor in human umbilical vascular endothelial cell (HUVEC) proliferation tube formation assay. Used as a single agent, CPS11 significantly inhibited tumor growth by 90% on the human prostate cancer PC3 xenograft model, while thalidomide did not show efficacy in the same experiment. In lung cancer metastasis model, CPS11 significantly reduced the number of lung metastases by 87%, while thalidomide did not show efficacy in the same experiment.[6] CPS11 is well tolerated (maximum tolerated dose of 100 mg/kg).[2] Therefore, CPS11 is an attractive drug candidate for further development.

However, CPS11 is unstable during storage. CPS11 decomposes into thalidomide and formaldehyde even in low temperature as shown in Scheme 1, making the further development of CPS11 impossible. Thus stable CPS11 pro-drug with proper bioavailability is needed.

Here we wish to report a set of pro-drugs of CPS11 with good stability and oral bioavailability. Their activities to inhibit angiogenesis and their synergistic effects with cell toxin anticancer agent to inhibit tumor growth are also presented.

The pro-drugs of CPS11 were prepared as illustrated in Scheme 2. Thalidomide reacted with formaldehyde aqueous solution to form CPS11. When reaction was complete, the mixture was cooled to room temperature. Some CPS11 crystals were added and the mixture was cooled with ice bath. The solid form was collected and dried for next step reaction. The best purity of CPS11 is around 90% and the main impurity is thalidomide. Re-crystallization could not further improve purity.

CPS11 reacted with picolinoyl chloride, nicotinoyl chloride, or isonicotinoyl chloride to give **5**, **6**,[7] and **7**, respectively. Compounds **5**, **6**, and **7** could be purified by aqueous work-up and re-crystallization. These are stable and have proper pharmaceutical property



Scheme 1. Decomposition of CPS11.

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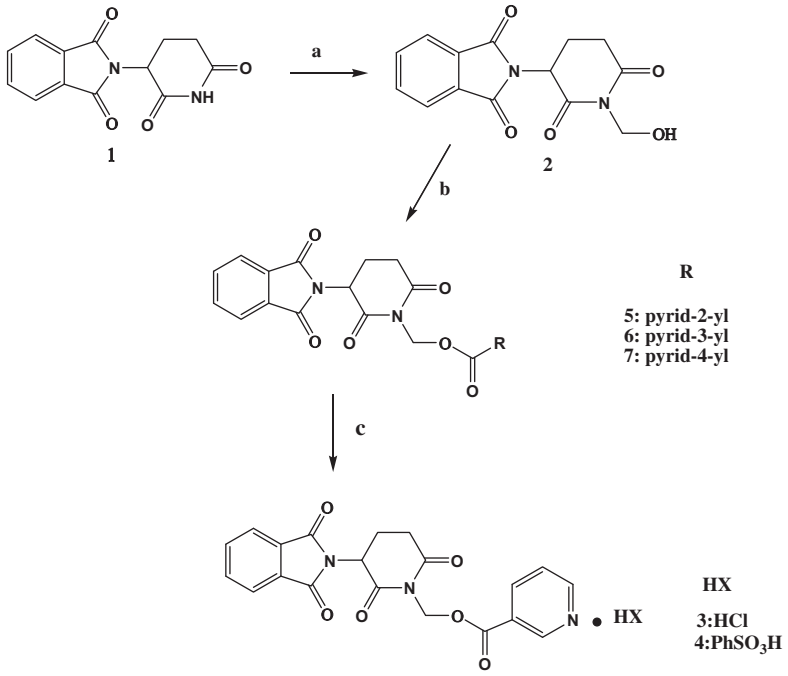

Scheme 2. Synthesis of CPS11 pro-drugs. *Reagents and conditions:* (a) 30% CH_2O aqueous solution, reflux, 1 h, yield: 53.9%; (b) RC(O)Cl, TEA, DCM, rt, overnight, yields: 80–90%; (c) dilute hydrochloric acid, or $PhSO_3H$, DCM, 30 min.

(results will be published elsewhere). They all are metabolized into CPS11 in the preliminary pharmacokinetic screening in rats and meet requirement for further evaluation. Compound **6** was chosen for efficacy study and salt form screening because its another metabolite except CPS11 generated in vivo is the endogenous nicotinic acid which is not toxic.

The stability of CPS11, **3**, and **4** was determined by HPLC. The purity of CPS11 decreased to 58.9% from 91.6% when stored at room temperature for 1 month. While after 2 months storage, the purity of **3** and **4** remained unchanged as their initial purity of 99.3% and 98.9%, respectively. The water solubility of **3** and **4**, determined using UV, is 1.48 and 0.84 mg/mL, respectively. Compounds **3** and **4** are stable more than 8 h in aqueous solution. They are more stable than compounds reported by Hess.[3] After single intragastric administration to rats, the bioavailability of **4** is 60.7%. When intravenously administered, **4** is rapidly metabolized into CPS11, indicating that **4** behaves as pro-drug of CPS11 in vivo.

Compounds **5**, **6**, and **7** inhibit ECV-304 cell growth in a concentration dependent fashion.[8] Their IC_{50}s to inhibit the growth of cell line ECV-304 were 0.1, 0.12, and 0.18 μM, respectively. The maximum inhibition rate for thalidomide in the same assay was around 40%.

HUVEC proliferation was significantly decreased (88–94%) by **5**, **6**, and **7** at 195 μM.[9] Thalidomide was found to inhibit 53% tube formation at 195 μM in the same assay.

Many studies showed that inhibitors of angiogenesis like thalidomide can enhance the anti-tumor efficacy of cell toxin anticancer agents and Taxol is one of the most effective anti-tumor agents currently used for the therapy of breast cancer with microtubule stabilizing properties.[10] Anti-cancer efficacy of compound **4** and the synergic anti-cancer effect of **4** and Taxol were carried out on MX-1 human breast cancer xenograft model.[11] Compound **4** was administered daily at a dosage of 65 mg/kg ip to mice with established tumors from day 7 after inoculation for five consecutive days. Taxol was administered at a dosage of 5 mg/kg iv on day 7 after inoculation. No significant efficacy on tumor growth in group treated by **4** was observed. Tumor growth inhibition rates in Taxol and **4** combined with Taxol treated groups were 33.1% and 74.3%, respectively, suggesting that **4** could enhance anti-cancer efficacy of Taxol in this experiment. Compound **4** did not increase the toxicity of Taxol. All mice survived and no significant body weight loss was observed.

In summary, novel pro-drugs of CPS11, **3**, and **4** are stable, water soluble, and oral bio-available. The in vitro anti-angiogenic efficacy of **6**, the free base form of **4**, is better than that of thalidomide as demonstrated in ECV-304 cell growth assay and HUVEC tube formation assay. Significant synergic anti-cancer effect of **4** and Taxol is observed on MX-1 human breast cancer xenograft model. Efficacy of **4** to inhibit cancer metastasis is being evaluated on lung metastasis model and will be reported on due date. Compound **4** is well tolerated when administered as a single agent or with Taxol. These results warrant further evaluation of **4** as a novel anti-cancer agent.

Supplementary data

Supplementary data associated (general procedures and spectral data) with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.09.026.

References and notes

1. Warfel, N. A.; Lepper, E. R.; Zhang, C. Y.; Figg, W. D.; Dennis, P. A. *Cancer Ther.: Preclinical* **2006**, *12*, 3502.
2. Ng, S. S. W.; Gütschow, M.; Weiss, M.; Hauschildt, S.; Teubert, U.; Hecker, T. K.; Luzzio, F. A.; Kruger, E. A.; Eger, K.; Figg, W. D. *Cancer Res.* **2003**, *63*, 3189.
3. Hess, S.; Akermann, M. A.; Wnendt, S.; Zwingenberger, K.; Eger, K. *Bioorg. Med. Chem.* **2001**, *9*, 1279.
4. Nightingale, S. L. *JAMA* **1998**, *280*, 872.
5. Ge, Y.; Montano, I.; Rustici, G.; Freebern, W. J.; Haggerty, C. M.; Cui, W. M.; Ponciano-Jackson, D.; Chandramouli, G. V. R.; Gardner, E. R.; Figg, W. D.; Abu-Asab, M.; Tsokos, M.; Jackson, S. H.; Gardner, K. *Blood* **2006**, *108*, 4126.
6. Ng, S. S. W.; MacPherson, G. R.; Gütschow, M.; Eger, K.; Figg, W. D. *Clin. Cancer Res.* **2004**, *10*, 4192.
7. ^{1}H NMR of compound **6** ($CDCl_3$, ppm) δ 9.2(s, 1H), 8.78(d, 1H, J = 4.0 Hz), 8.29(d, 1H, J = 8.0 Hz), 7.87–7.90(m, 2H), 7.75–7.78(m, 2H), 7.41(dd, 1H, J = 4.0, 8.0 Hz), 6.17(d, 1H, J = 9.6 Hz), 6.09(d, 1H, J = 9.6 Hz), 5.09–5.14(m, 1H), 3.02–3.17(m, 1H), 2.80–2.95(m, 2H), 2.17–2.28(m, 1H); MS (M+H) 394.

8. *Cell proliferation assay:* ECV-304 was cultured for several days in RPMI 1640 containing 10% of bovine serum, collected and suspended in RPMI 1640, placed into a 96-well cell culture plate at a density of 6000 cells/well and allowed to attach overnight at 37 °C and 5% CO_2. The culture medium were changed to fresh culture medium containing 1% fetal bovine serum and either the vehicle (DMSO), thalidomide, compound **5**, **6**, or **7**, then cultured for 72 h under the condition of 5% CO_2 and 37 °C. Subsequently, 20 μL of MTT solution was added to each well of the 96-well cell culture plate and cultured for another 4 h under the condition of 5% CO_2 and 37 °C. Absorbance was read at 535 nm wavelength. All experiments were done in duplicate and repeated three times.
9. *HUVEC proliferation and tube formation assay:* 300 μL of Matrigel was added into 24-well cell plate and allowed to gel at 37 °C and 5% CO_2 for 30 min. HUVECs were suspended in M199 containing 20% of bovine serum and 20 ng/mL bFGF, and placed into 24-well cell plate at a density of 20,000 cells/well and allowed to attach overnight at 37 °C and 5% CO_2. The culture medium were changed to fresh culture medium containing either the vehicle (0.5% DMSO), thalidomide, compound **5**, **6**, or **7** (0.195 μmol/mL), then cultured for 24 h under the condition of 5% CO_2 and 37 °C. Subsequently, each well was photographed and counted.
10. Toshiyuki, F.; Mitsuo, T.; Dhar, D. K.; Shuhei, U.; Shoichi, K.; Hiroshi, Y.; Hitoshi, K.; Naofumi, N. *Anticancer Res.* **2003**, *23*, 2405.
11. *In vivo effect study:* Each mouse was inoculated subcutaneously at the right flank with the MX-1 tumor fragment of 1–2 mm^3. The treatments were started at day 7 after tumor inoculation when the tumor size reached approximately 200 mm^3. Each group consisted of five tumor-bearing mice and treated with vehicle, compd **4** (65 mg/kg, once a day for five consecutive days), and compd **4** (65 mg/kg, once a day for five consecutive days) combined with Taxol (5 mg/kg, once at day 7 after tumor inoculation).