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Development of MBRI-001, a Deuterium-substituted Plinabulin Derivative as a Potent Anti-cancer Agent

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Abstract: Plinabulin, a drug targeting microtubule of cancer cells, has been currently tried in its phase III clinical study. However, low efficacy caused by poor pharmacokinetic (PK) properties has been considered to be the main obstacle to approved by the Food and Drug Administration. Herein, we introduced a deuterium atom as an isostere in its structure to become a new compound named (MBRI-001, No. 9 in a series of deuterium-substituted compounds). The structure of MBRI-001 was characterized by HRMS, NMR, IR and a single crystal analysis. MBRI-001 exhibited better pharmacokinetic characteristics than that of plinabulin. Additionally, its antitumor activity is in a low nanomolar level for a variety of cancer cell lines and high activity against human NCI-H460 xenograted in mice intravenous administration. Importantly, continuous administration of MBRI-001 exhibited lower toxicity compared to docetaxel. We thus suggest that MBRI-001 could be developed as a promising

anti-cancer agent in near future.

Key words: Plinabulin; Deuterium substituted; Microtubule; Pharmacokinetic properties; Docetaxel

Microtubules, composing of α/β-tubulin heterodimers, are a component of the cytoskeleton essential function in maintenance shape, motility, and transmission of cell signaling and mitosis.[1-3] Microtubules also play an important role in eukaryotic cells as a target for anticancer drugs.[2,4] Anti-microtubule agents are currently classified into two groups: microtubule-stabilizing agents which bind to the tubulin to stable microtubule, and microtubule-destabilizing agents which locate in the tubulin dimers to destabilize the microtubules.[5,6] Anti-microtubule agents, namely the microtubule stabilizing taxanes such as paclitaxel and docetaxel, and the microtubule depolymerizing vinca alkaloids such as vinblastine, vincristine and vinorelbine, have been used as first-line drugs for cancer therapy.[7-11] However, colchicine, a microtubule destabilizing agent, has not been approved for its extreme toxicity, such as neurological and bone marrow toxicity.[6,12] Further, cancer cells development of resistance to colchicine might also the main reason for limited use of the drug.[6] Development of new drug with high efficacy and lower toxicity of microtubule inhibitors have still needed in the great market prospects.

Plinabulin is developed from the marine natural “phenylahistin” namely as (-)-1, possessing a dehydropiperazine Structure (Figure 1).[13,14] The bind of Plinabulin was found at the interfacial region between α- and β- tubulins near the colchicine binding site, but not inside the colchicine binding cavity.[15-17] It has multi-faceted activities reported as anti-cancer activity through the anti-angiogenesis, interruption of tumor blood flow and induction of cancer apoptosis via the c-Jun N-terminal kinase (JNK) pathway.[18] To date, Plinabulin has been under phase III clinical trial for treatment of lung cancers.[10,19] In this study, we developed some Plinabulin derivatives, a series of deuterium-substituted plinabulin derivatives, demonstrating potent anti-microtubule, lower toxicity and better pharmacokinetic properties evaluated in vitro and in vivo

assays.

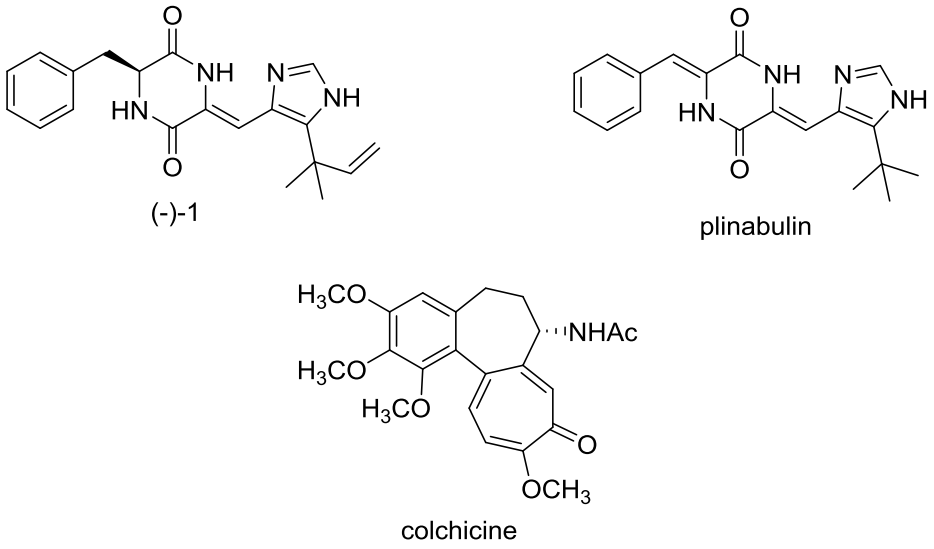


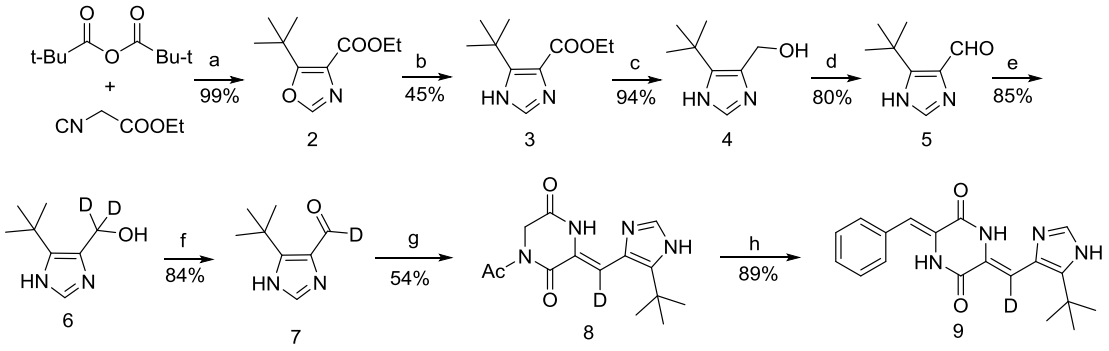
Figure 1. Structures of natural colchicine-site binder (−)- **1**, plinabulin and colchicine.

Deuterium atom is the stable isotope of hydrogen atom. The volume of deuterium atom is similar to that of hydrogen atom with nearly identical physiochemical properties, but the carbon-deuterium bond is more stable than the carbon-hydrogen bond. We thus suggest that it is the best nonradioactive bioisosteric replacement.[20,21] In addition, a common feature is breaking the carbon-hydrogen in the process of drug metabolism. Thus, if hydrogen atom of drug molecules is replaced by deuterium, it might obtain the beneficial because of improving the metabolism such as less-frequent dosing, lower toxicity.[22-24] Many advantages have been reported with deuterium replacing hydrogen as drugs, such as reducing gene toxicity of D_1-halothane, and increasing half-life of D_6-nifedipine.[25] Currently, a series of deuterated drug candidates, such as SD809 as a vesicular amine transporter 2 (VMAT2) inhibitor and CTP-347 as a selective serotonin reuptake inhibitor, have been developed in clinical trial. SD809, is a deuterated version of tetrabenazine, can attenuate CYP2D6 metabolism to increase metabolite half-lives in patients with Huntington disease. And CTP-347, is a deuterium substituted of paroxetine, can decrease inactivation of CYP2D6 and reduce drug-drug interactions for improving the metabolism profiles.[26-28]

We report the synthesis of a deuterated plinabulin derivative (compound **9**) and the evaluation of its pharmacokinetic properties assayed by in vitro and in vivo assays, including human NCI-H460 lung carcinoma xenograted in mice.

The deuterium-plinabulin derivative was synthesized via a sequence of eight linear steps (Scheme 1). The optimized synthesis route has been utilized to the preparation of more than 30 g of the compound 9. First, to obtain oxazole, ethyl isocyanoacetate and pivalic anhydride was condensed in the presence of 1, 8-diazabicyclo[5.4.0]undec-7-ene (DBU) for 48 h at room temperature. After purification using silica gel column chromatography, the oxazole ester was converted into the imidazole ester by the solvolysis reaction in formamide at 175^{o}C. Then, $LiAlH_4$ was used to reduce imidazole ester to alcohol, and MnO_2 was used for the oxidation to prepare aldehyde. The imidazole aldehyde was further converted to the deuterium-imidazole aldehyde by reduction reaction with using $NaBD_4$ at -20^{o}C and again oxidation with using MnO_2. Total yield to get deuterium-imidazolealdehyde was 24% by six steps.

Scheme 1. Synthesis of deuterium-plinabulin derivative compound 9.



Reagents, conditions and yield: (a) DBU, THF, rt, 48 h, 99%; (b) formamide, 175 °C, 36 h, 45%; (c) $LiAlH_4$, THF, 0 ^{o}C to rt, 6 h, 94%; (d) MnO_2, acetone, rt, 48 h, 80%; (e) $NaBD_4$, EtOH, 5 h, -20 ^{o}C, 85% (f) MnO_2, acetone, rt, 48 h, 84%; (g) 1,4-diacetylpiperazine-2,5-dione, Cs_2CO_3, DMF, rt, 20 h, 54%; (h) benzaldehyde, Cs_2CO_3, DMF, 50 °C, 24 h, 89%.

Then a tandem aldol condensation with two different kinds of aldehydes onto the diacetyl-2,5-piperazinedione ring was observed in the presence of Cs_2CO_3 in N, N-dimethylformamide (DMF). Namely, diacetyl-2,5-piperazinedione was reacted with the deuterium-imidazole aldehyde for 20 h at room temperature. The low

reactivity of the deuterium-imidazolealdehyde probably caused by the high steric hindrance with a bulky tert-butyl group at the 5-position.[13] The second condensation reaction was completed between compound 8 and benzaldehyde at 50 ^{o}C in DMF under an N_2 atmosphere. The degassed condition was utilized to avoid probable oxidation of the activated methylene in the core of diketopiperazine (DKP) ring in the presence of Cs_2CO_3.[13,29] During last condensation reaction, the dark condition had to be used to avoid probable yield E configuration of deuterium plinabulin. The total yield of synthesis of compound **9** was 11% in the eight steps. The chemical structures of compound **9** was characterized by high resolution mass spectrometer (HRMS), infrared radiation (IR), nuclear magnetic resonance (NMR) spectroscopies (see the Supporting Information) and a single crystal analysis (Figure 2).

From analysis of the single crystal of the compound 9 (CCDC: 1509116), there is a hydrogen bonding between NH of the DKP-amide core and imine of the imidazole rings to form a pseudo tricyclic structure (Figure 2). A dihedral angle of -29^{o} was found in the left side phenyl ring and the core of the DKP structure, which indicated that it is not a coplanar (Figure 2). As shown in Figure 2, a Z configuration was observed for the double bond of C13-C2, which can be transformed into E configuration in ultraviolet light.

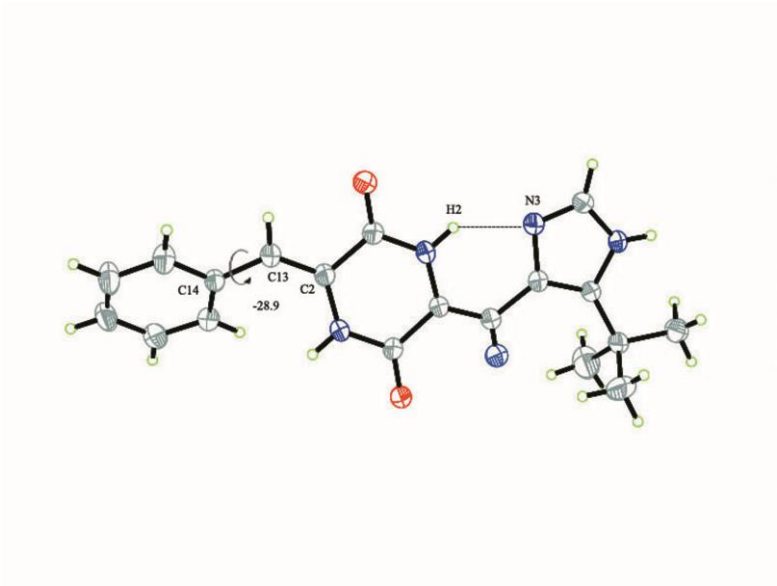


Figure 2. ORTEP drawing of compound **9**

The pharmacokinetic properties of compound **9** and plinabulin have been evaluated in the plasma and in the liver microsomal of rat. The results showed that both compound **9** and plinabulin were stable in the plasma from 0-100 mins. Both compound **9** and plinabulin exhibited similar stability in rat liver microsomes. The amide bond of the DKP structures were not hydrolyzed by the esterase in rat. (See the Supporting Information)

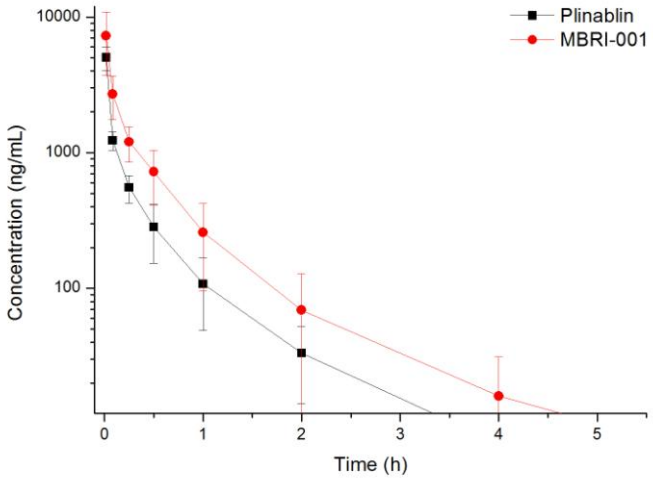


Figure 3: Plasma concentration-curve of compound **9** and plinabulin (5 mg·kg^{-1}) after single intravenous injection to Wistar rats(n=5). Each time point is the mean± SD from five individual mice.

Table 1: Pharmacokinetic parameters of compound **9** and plinabulin after single intravenous injection at 5 mg·kg^{-1}

Parameter(unit)	Plinabulin	Compound **9**
k_e (h^{-1})	0.68	0.50
$t_{1/2}$ (h)	1.13	1.47
T_{max} (h)	0.02	0.02
C_{max} (ng·mL^{-1})	5037.50	7276.00
AUC_{last} (h·ng·mL^{-1})	720.76	1519.32
AUC_{INF_obs}(h·ng·mL^{-1})	723.08	1520.52

$Vz_{\_obs}$ ($L \cdot kg^{-1}$)	11.39	7.72
$CL_{\_obs}$ ($L \cdot h^{-1} \cdot kg^{-1}$)	7.26	3.53
MRT_{last} (h)	0.50	1.68

k_e = elimination rate constant; $t_{1/2}$ = halflife; T_{max} = time to maximal plasma concentration; C_{max} = maximal concentration; AUC_{last} = area under the plasma concentration–time curve from 0 h to last quantifiable concentration; AUC_{INF_obs} = area under the plasma concentration–time curve from 0 h to infinity; $Vz_{\_obs}$ = apparent volume of distribution; $CL_{\_obs}$ = clearance; MRT = mean residence time.

The pharmacokinetic behaviors were further evaluated in the vivo assay. No oral absorption was observed for both compound **9** and plinabulin in the Wistar rats. After single intravenous injection with dosage 5 $mg \cdot kg^{-1}$, the concentration-curves of plinabulin and compound **9** were shown in Figure 3. The pharmacokinetic parameters were further calculated and summarized in Table 1. Compound **9** was showed to display a better PK property compared with plinabulin at the dosage of 5.0 mg/kg for a signal administration. Compound **9** had longer $T_{1/2}$ at 1.47 h compared with plinabulin at 1.13 h, and exhibited over 2-fold increase in the area under the plasma concentration-time curve $AUC_{0-\infty}$ at 1520.52 $h \cdot ng \cdot mL^{-1}$ compared with plinabulin at 723.08 $h \cdot ng \cdot mL^{-1}$. The peak concentration (C_{max}) of compound **9** at 7276 $ng \cdot mL^{-1}$ was closed to 1.5-fold higher than that of plinabulin at 5037 $ng \cdot mL^{-1}$. Compound **9** exhibited an obviously decrease in the clearance (CL) at 3.53 $L \cdot h^{-1} \cdot kg^{-1}$ compared with plinabulin at 7.26 $L \cdot h^{-1} \cdot kg^{-1}$, and also exhibited a longer total body mean residence time (MRT) at 1.68 h compared with plinabulin at 0.50 h. These results indicated that compound **9** exhibited better pharmacokinetic parameters than plinabulin in rats.

Cytotoxic effect was determined in the cell lines of NCI-H460 (human lung carcinoma), NCI-H446 (small cell lung cancer) and Jurkat (human T lymphocyte carcinoma). As shown in Table 2，compound **9** and plinabulin have the similar effect of cytotoxic activities against cancer cell growth.

Table 2. Cytotoxicity of compound **9** and plinabulin against human cancer cell lines.

Compound	Cytotoxicity IC_{50}[a](nM)		
	NCI-H460	NCI-H446	Jurkat
Plinabulin	33.9 ± 0.5	44.2 ± 1.1	3.3 ± 1.3
9	33.2 ± 0.5	22.0 ± 2.4	4.3 ± 1.6

[a] Values represent mean ± SD from at least three independent dose response curves.

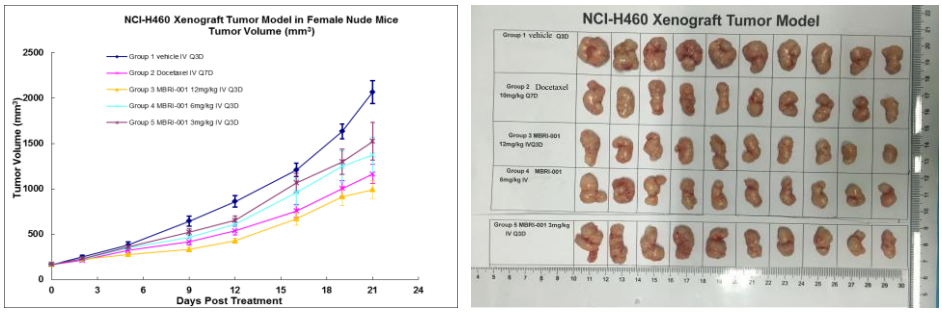


Figure 4: Antitumor activity in vivo. A. The volumes of NCI-H460 xenografted in nude mice after administrated with compound 9 by intravenous injection with Q3D7. The data were presented as the mean ± SD. B. The images of excised tumors of xenograft.

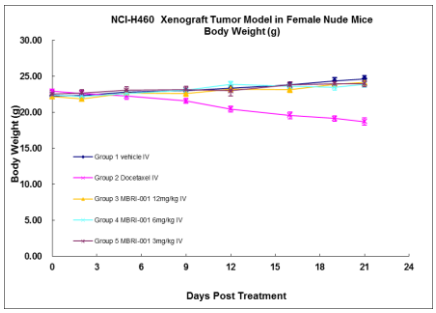


Figure 5 : The body weights of nude mice after administrated with compound 9 by intravenous injection with Q3D7. Data were presented as mean ± SD.

The anticancer activity of the compound **9** (MBRI-001) was further evaluated against NCI-H460 xenograft models by intravenous injection with a dose at 3 mg/kg, 6 mg/kg, or 12 mg/kg every two days for consecutive 21 days. As a microtubule-stabilizing agent with first-line therapy for lung cancers,[5,30] docetaxel was used as a positive control at a dose of 10 mg/kg. At the end of administration

period, the mean of final tumor volumes of vehicle, docetaxel and compound **9** at 3 mg/kg, 6 mg/kg, or 12 mg/kg were 2065.87, 1163.16, 1523.29, 1372.27 and 991.78 mm^3 (Figure 4A), respectively. Average excised tumor weights of the corresponding groups were 1.25 g, 0.75 g, 0.90 g, 0.69 g and 0.66 g, respectively. The response calculated inhibitory rates were 40.0%, 28.8%, 44.8% and 48.0%, respectively (Figure 4B). Overall, antitumor activity of compound **9** showed doses dependent in the concentrations range of 3 to 12 mg/mL, which was agreement with measured concentration of compound **9** with different doses at anatomic tumors. The tissue concentration of compound **9** was 316.45 ng/g, 809.11 ng/g, 2493.72 ng/g at doses of 3 mg/kg, 6 mg/kg, 12 mg/kg, respectively. Compared with docetaxel at 10 mg/kg, compound **9** at 6 mg/kg and 12 mg/kg caused a considerable suppression of the tumor growth (Figure 4B). Significant decrease of body weights were not observed in all groups of compound **9** treated mice (Figure 5). However, docetaxel caused a decrease of body weights by 18.3% (Figure 5).

In summary, a deuterium-substituted plinabulin, compound **9** (MBRI-001) was synthesized as an anti-microtubule agent. In comparison with plinabulin, its pharmacokinetic properties were improved with better AUC. The deuterium-substituted plinabulin compound **9** exhibited a considerable efficacy against cancer growth and lower toxicity in xenografted mice compared with docetaxel. Thus, Compound **9** (MBRI-001) has been suggested to be promising agent in future clinical therapy.

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Plinabulin

MBRI-001 (9)

obviously improve pharmacokinetic properties
retain a potent cytotoxic activity in vito
exhibite a significant efficient against NCI-H460 in vivo

1) MBRI-001 is a deuterium-substituted plinabulin derivative.
2) MBRI-001 exhibited better pharmacokinetic characteristics than plinabulin.
3) MBRI-001 showed high activity against human NCI-H460 xenograted in mice.
4) MBRI-001 and plinabulin have the similar cytotoxicity against cancer cell growth.
5) MBRI-001 exhibited lower toxicity compared to docetaxel as control.