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Synthesis of the analogs of plocabulin and their preliminary structure-activity relationship study

Leiming Wang[a,b,#], Xin Li[c,#], Hong Cui[b], Xinsheng Lei[b], Hongchun Liu[c,*], Quanrui Wang[a,*] and Yingxia Li[b,*]

[a] *Department of Chemistry, Fudan University, 2005 Songhu Road, Shanghai 200438, China*

[b] *Department of Medicinal Chemistry, School of Pharmacy, Fudan University, Shanghai, 201203, China*

[c] *Division of Anti-tumor Pharmacology, State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai, 201203, China*

[#] *These authors contributed equally to this work.*

ABSTRACT

Plocabulin, a marine natural polyketide isolated from the sponge *Lithoplocamia lithistoides*, is a novel and potent microtubule-destabilizing agent. Guided by the reported binding mode, several new analogs of plocabulin have been designed through removing the right aliphatic chain and further modifying on the carbamate group and the enamide unit. The preliminary results indicate that the right aliphatic chain in plocabulin is allowed to remove with a little loss of activity, the carbamate group plays a role in the activity, and particularly, the enamide unit has an important effect on the activity. This new finding will aid the design of novel potent tubulin-binding agents based on plocabulin.

Keywords: marine nature products, plocabulin, PM060184, synthesis, antiproliferation

Marine organisms have been viewed as an unignorable source of bioactive natural products besides terrestrial plants and other nonmarine microorganisms. Harsh marine conditions drive marine organisms to evolve in distinctive manners from nonmarine microorganisms, favorably producing structurally unique and diverse secondary metabolites. These metabolites are prolific sources of products with a wide range of significant and novel bioactivity.[1-3] This is supported by the fact that eight approved therapeutic agents have been developed from about 30000 marine natural products (MNPs) obtained so far, and at least 23 marine-derived compounds are in clinical trials.[4-6] Therefore, the MNPs are more attractive candidates for drug discovery.

The recent example is plocabulin, a novel and potent microtubule-destabilizing agent which was isolated from the sponge *Lithoplocamia lithistoides* collected in Madagascar (Figure 1)[7] and identified to target a unique site on β-tubulin different from that of vinca alkaloids, taxanes or eribulin.[8-10] The exact site where plocabulin binds to tubulin is the maytansine site, and the agents that bind to this site include maytansine, rhizoxin, disorazole and spongistatin, which are all cyclic molecules

* Corresponding author: For H. L. email: hchliu@simm.ac.cn; For Q. W. email: qrwang@fudan.edu.cn; For Y. L. email: liyx417@fudan.edu.cn

except plocabulin.[9,11] Based on its potent antimitotic activity and distinct biochemical mechanism, plocabulin is currently undergoing phase II clinical trials for the treatment of several tumors and has shown superior antitumor efficacy in patients with advanced solid tumors.[11,12]

As one new member of polyketides, plocabulin possesses some intriguing structural features, including a chiral α,β-unsaturated δ-lactone, a conjugated triene unit and L-*tert*-leucine linked *via* a (*Z*)-enamide to a diene moiety containing a carbamate subunit (Figure 1). This complex structure requires about 18 linear steps (totally 30 steps) for the synthesis of plocabulin.[7,13] Therefore, it is desirable to explore novel and simplified microtubule-destabilizing agents based on this marine natural product. Recently, we have explored an efficient copper-catalyzed cross-coupling reaction of amino acid-derived amides with (*Z*)-vinyl iodides, and through this reaction we have developed an improved method for the preparation of plocabulin.[14] As a continuation of the project, we herein describe a preliminary result on the synthesis of several simplified analogs of plocabulin and their antiproliferation towards three tested cells.

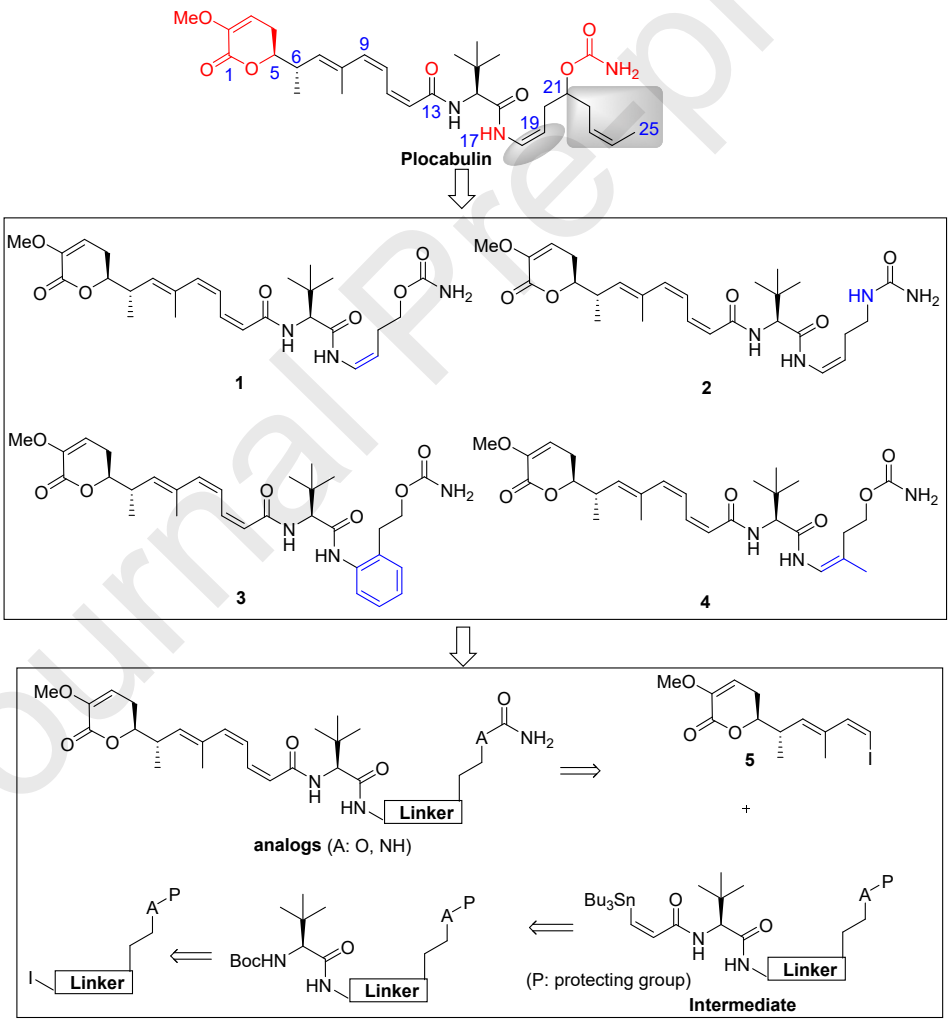


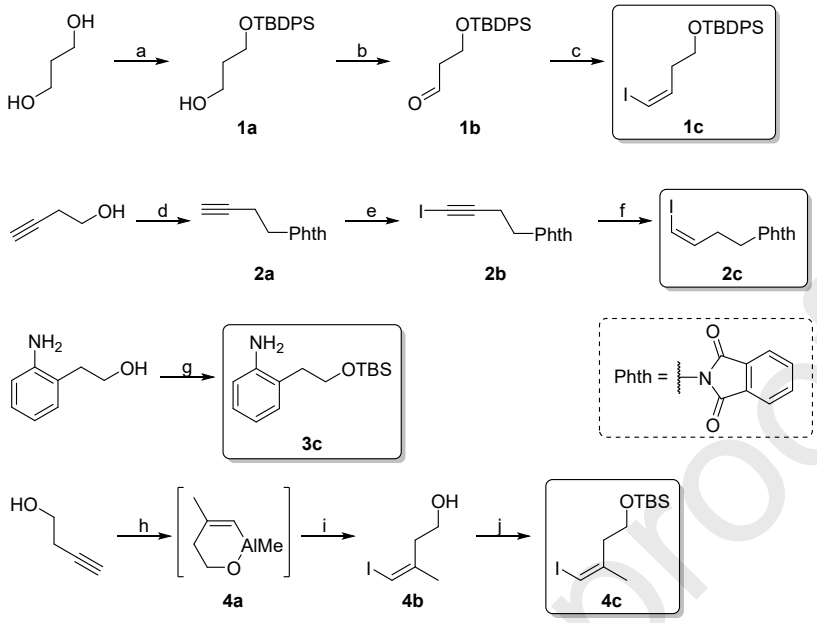
Figure 1. The design of the analogs of plocabulin and their retrosynthetic analysis

Based on the X-ray crystal complex of plocabulin and the tubulin (PDB: 4TV9), the binding mode has been disclosed.[9] It indicates that the lactone moiety contributes to two key hydrogen–bond interactions with the tubulin, and the oxygen atom of the carbonyl at C13 position, the nitrogen atom at N17 position and the carbamate group at C21 position also provide three important hydrogen–bond interactions, respectively. Meanwhile, the conjugated triene moiety provides the rigid scafold and controls those heteroatoms in the favorable orientations. Notably, the right aliphatic chain from C22 to C25 appears to be unnecessary pharmacophore. Thus, we designed the simplified analog **1** (Figure 1) by removing the right aliphatic chain, and furthermore, the carbamate group of **1** is replaced by more rigid aminoformamidoyl group resulted in compound **2**. Considering the potentially unstable (*Z*)-enamide unit of plocabulin, we designed the compound **3** by the replacement of the double bond in enamide unit with a benzene ring. As for compound **4**, the purpose of introduction of methyl group at C19 position is to compensate the effect of the right aliphatic chain and to attempt to offer the favorable orientation of the carbamate group.

As can be seen from Figure 1, the left fragment is commonly shared by all the designed analog, and therefore the similar strategy was adopted for the synthesis of those analogs, according to the previous preparation of plocabulin.[7,13] The analogs could be assembled in the late-stage by the Still coupling reaction between the *Z*-iodoalkene **5** and the organostanane intermediates (Figure 1), and the subsequent functional group transformations produced the targets (**1**-**4**). The in-turn intermediates were formed via amidation between the α,β-unsaturated acid bearing Bu_3Sn group and the corresponding *L*-*tert*-leucine amides bearing the different linker fragments. The amides bearing the linker would be connected *via* the coupling reaction between *L*-Boc-*tert*-leucine amide with (*Z*)-vinyl iodides, or via the condensation between *L*-Boc-*tert*-leucine and the corresponding aryl amine. In addition, the iodide compound **5** could be obtained from commercially 1,3-propanediol in 14 steps according to the known method.[7,13]

Keep these in mind, we set out to prepare the right fragments for those analogs, as illustrated in Scheme 1. At first, 1,3-propanediol was selectively protected in the presence of TBDPSCl, giving the mono-protected **1a** in 85% yield. In the presence of Dess-Martin reagent, **1a** was oxidized to the aldehyde **1b** with a yield of 67%. Under the Stork-Zhao olefination condition, **1b** could be conveniently transform to the (*Z*)-vinyl iodide **1c** in 84% yield. Next, starting from but-3-yn-1-ol, **2a** was prepared from phthalimide in 88% yield through the Mitsunobu reaction.[15] Iodination of the terminal triple bond of **2a** was carried out with *N*-iodosuccinimide (NIS) and $AgNO_3$ in acetone, giving **2b** in 86% yield.[16] After reduction by tosyl hydrazide (TSH) under the basic condition, **2b** was selectively reduced into the (*Z*)-vinyl iodide **2c** in 64% yield.[17] As for compound **3c**, it was easily available from the commercial 2-(2-aminophenyl)ethan-1-ol through the protection of the hydroxyl group. In addition, treatment of but-3-yn-1-ol with Me_3Al in the presence of Cp_2ZrCl_2 as a catalyst,[18,19] followed by thermally isomerization to the (*Z*)-methylaluminated species (**4a**) due to the chelation effect of the terminal hydroxy group, and trapping of **4a** by I_2 stereoselectively produced (*Z*)-

vinyl iodide **4b** in 44% yield. Subsequently the hydroxyl group was protected by TBS, giving **4c** in an almost quantitative yield.



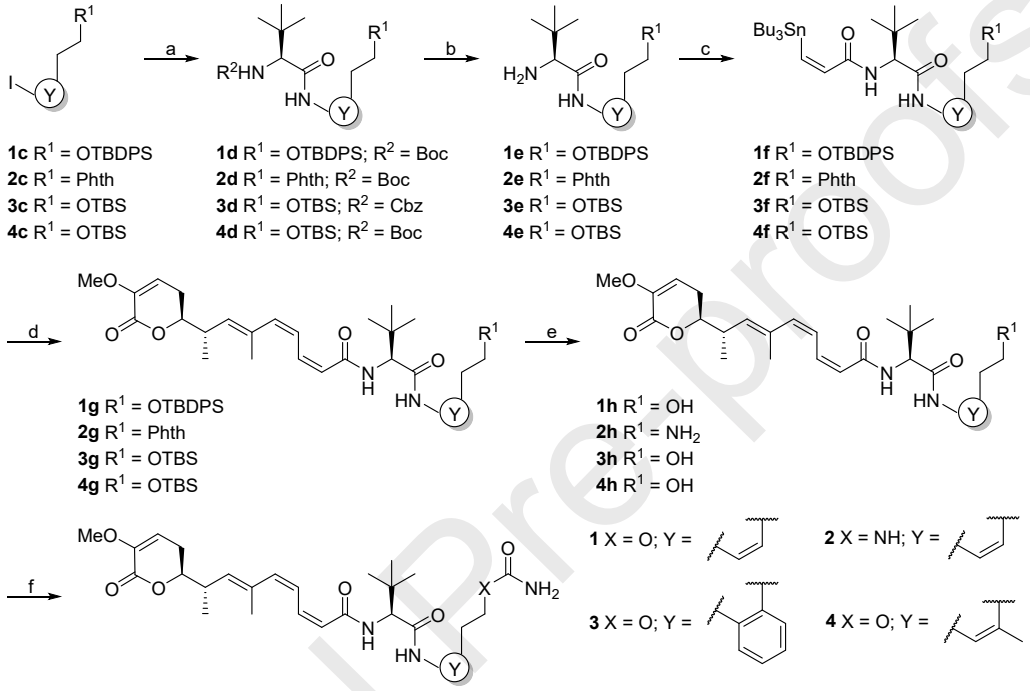
Scheme 1. The preparation of the intermediates (**1c**-**4c**)

Reagents and conditions: (a) TBDPSCl, Im, DMF, THF, rt, 85%; (b) Dess-Martin Reagent, DCM. rt., 67%; (c) $(Ph_3PCH_2I)^+ I^-$, NaHMDS, THF, −78 °C, 84%; (d) PPh_3, phthalimide, DIAD, THF, rt., 88%; (e) NIS, $AgNO_3$, acetone, rt, 86%; (f) TSH, AcONa,THF, H_2O, 65 °C, 64%; (g) TBSCl, Im, DMF, rt., 91%; (h) $ZrCp_2Cl_2$, $AlMe_3$, DCE, 0 °C, reflux; (i) I_2, THF, −30 °C, 44%; (j) TBSCl, Im, DMF, rt., 98%;

With the right fragments in hand, we then carried out the assembly work to prepare the designed analogs (Scheme 2). Initially, under the standard condition (CuI, K_2CO_3, DMEDA, DMF, 90 °C), **1c** was coupled with *L*-Boc-*tert*-leucine amide to produce **1d**, giving a poor yield (39%). Thus, both **2c** and **4c** were subjected to our optimized condition (CuI, Cs_2CO_3, DMEDA, DME, 55 °C), giving **2d** and **4d** in much improved yields (45% and 93%), respectively. On the other hand, **3c** and Cbz-*L*-*tert*-leucine could be condensed under the routine amidation condition, providing **3d** in 79% yield.

Considering the acidic sensitivity of TBDPS group in the compounds (**1d**, **3d** and **4d**), the removal of the Boc group was performed under the pyrolysis conditions (up to 200 °C for about 15 min in ethyleneglycol), leading to the corresponding free amines in satisfying yields (**1e**: 53%; **3e**: 83%; **4e**: 76%;). As for **2e**, the Cbz protective group could be removed in an almost quantitative yield by the routine hydrogenation. Next, easily available (*Z*)-3-(tributylstannyl) acrylic acid was condensed with **1-4e** under the routine amidation conditions, resulting in **1-4f** in reasonable yields ranged from 63% to 82%. Subsequently, the Stille coupling reaction of the vinyl iodides (**1-4f**) with the fragment **5** (the structure shown in Figure 1) was catalyzed by copper (I) thiophene-2-carboxylate (CuTC) in 1-methyl-2-pyrrolidinone (NMP) at 0 °C, affording the corresponding trienes (**1-4g**) in acceptable yields (50-75%). Removal of TBDPS group

in the resultant intermediates (**1g**, **3g** and **4g**) with TBAF smoothly furnished the alcohols (**1h**: 75%; **3h**: 57%; **4h**: 55%). The free amine (**2h**) was fortunately prepared from **2g** by the treatment of $N_2H_4 \cdot H_2O$.[20] Finally, the free alcohols (**1**, **3-4h**) were reacted with trichloroacetyl isocyanate (TCAI), leading to the trichloroacetyl carbomates, respectively. Followed by the treatment **of** Al_2O_3, those carbomates were finally transformed into the analogs (**1**: 69%; **3**: 41%; **4**: 44%). On the other hand, the free amine (**2h**) were treated with KCNO in the presence of diluted HCl aqueous solution,[21] giving the analog **2** in 3% yield over 2 steps.



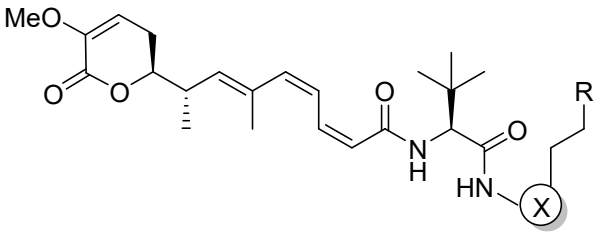
Scheme 2. The synthesis of the analogs (**1-4**)

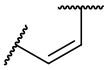
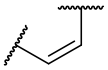
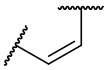
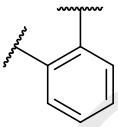
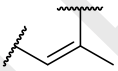
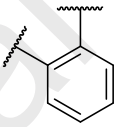
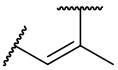
Reagents and conditions: (a) **1d** (39%): *L*-Boc-*tert*-leucine amide, CuI, K_2CO_3, DMEDA, DMF, 90 °C; **2d** (45%), **4d** (93%): *L*-Boc-*tert*-leucine amide, CuI, Cs_2CO_3, DMEDA, DME, 55 °C; **3d** (79%): *L*-Cbz-*tert*-leucine, DIPEA, HOAT, HATU, DCM, DMF, 0 °C; (b) **1e** (53%), **2e** (83%), **4e** (76%): ethylenglycol, 200 °C; **3e** (95%): Pd/C, H_2, MeOH, rt; (c) **1f** (63%), **2f** (64%), **3f** (82%), **4f** (74%), (*Z*)-3-(tributylstannyl) acrylic acid, DIPEA, HOAT, HATU, DCM, DMF, 0 °C; (d) **1g** (57%), **2g** (57%), **3g** (50%), **4g** (75%): **5**, CuTC, NMP, 0 °C; (e) **1h** (75%), **3h** (57%), **4h** (55%): TBAF, THF, rt; **2h** $N_2H_4 \cdot H_2O$, THF, rt; (f) **1** (69%), **3** (41%), **4** (44%): (1) TCAI, DCM, 0 °C; (2) Al_2O_3, rt; **2** (3%, 2 steps): KCNO, HCl aq., MeOH, H_2O, 45 °C.

After analog **1** was obtained, the in vitro antiproliferative activities (A549, HT-29 and HepG2 cells) were at once assessed using the CCK-8 method. The results were shown in Table 1, and plocabulin previously obtained[14] was used as the positive compound. As expected, the IC_{50} (nM) values of plocabulin in this assay were 0.72 (A549), 0.27 (HT-29) and 1.64 (HepG2), respectively, which were similar to the literature.[11] To our pleasure, the analog **1** also showed potent cytotoxicities towards the tested cells with IC_{50} values around nanomolar concentration (A549: 2.4 nM; HT-29: 1.1 nM; HepG2: 5.8 nM), and its cytotoxicities were generally about one third of

those of plocabulin. The comparable activities suggested that the right aliphatic moiety in plocabulin was permitted to remove, demonstrating our initial design hypothesis.

Table 1. The in vitro antiproliferative activity of plocabulin and its analogs (IC_{50} ± SEM, nM).



Compound	R	X	IC_{50} ± SEM (nM)[a]		
			A549	HT-29	HepG2
1	-$OCONH_2$		2.4 ± 0.3[b]	1.1 ± 0.1[b]	5.8 ± 1.1[b]
2	-$NHCONH_2$		> 100[c]	25 ± 5.3[c]	> 100[c]
2g	-Phth		686 ± 256[c]	622 ± 125[c]	5190 ± 1590[c]
3	-$OCONH_2$		5370 ± 3590[c]	4960 ± 3430[c]	**>** 10000[c]
4	-$OCONH_2$		127 ± 8[c]	78 ± 1[c]	469 ± 76[c]
3h	-OH		> 10000[c]	> 10000[c]	> 10000[c]
4h	-OH		384 ± 29[c]	287 ± 45[c]	2310 ± 13[c]
taxol	-	-	4.7 ± 0.2[c]	3.8 ± 0.3[c]	10.1 ± 0.7[c]
plocabulin	-	-	0.72 ± 0.07[b] (< 0.20)[c]	0.27 ± 0.02[b] (< 0.20)[c]	1.64 ± 0.12[b] (0.36 ± 0.06)[c]

[a] Compounds were tested in at least 8-dose IC_{50} mode in duplicate with 5-fold serial dilution.

[b] Data was obtained in the first round.

[c] Data was obtained in the secondary round.

Encouraged by the above results, in the secondary round we evaluated the activities of the other analogs (**2**, **2g**, **3**, **4, 3h** and **4h**), and their results were compiled in Table 1. Notably, the IC_{50} values for plocabulin coming from the same sample were lower than those values in the first round, while those in two rounds were up to sub-nanomolar level. Despite that, we believed that both of the data in the two rounds are relevant and comparable.

Based on the results in the secondary round, we found **2** dramatically lost the cytotoxic activity towards both A549 and HepG2 except HT-29 cell (IC_{50}: 25 nM), compared with **1**. Furthermore, **2g** just exhibited marginal cytotoxic activities towards the tested cells. These results suggested that it seemed to be unbeneficial to replace the carbamate group with a more rigid bioisostere.

In contrast to **1**, **3** also almost lost the cytotoxic activity towards the tested cells (IC_{50}: > 4960 nM), indicating that the double bond in enamide unit could not be replaced by the benzene ring with the same rigidity. The reason might be that a limited space was required for the binding interaction between the enamide unit domain and tublin, and the benzene ring was larger than the double bond in the enamide. This statement was also supported to some extent by the results of analog **4**, because **4** displayed obviously reduced activities towards the tested cells (IC_{50}: from 78 nM to 469 nM). Compared with **1**, **4** was installed with a methyl group on the double bond in the enamide unit, but the related cytotoxic activities were generally reduced with a range of 7- to 60-fold. Interestingly, **4h** exhibited about 5-fold decreased cytotoxic activities with IC_{50} values ranging from 287 nM to 2320 nM, as compared with **4**. This result implied that the carbamate group cannot be simplified to hydroxyl group because of the obviously decreased activity.

Next, the in vitro assay of the tubulin polymerization was carried out with the representative compound **4** and plocabulin, using Combretastatin A-4 (CA-4) as a positive control. The result indicated that the IC_{50} value of **4** was 16.0+2.6 μM, lower than those of plocabulin and CA-4 (3.1+0.5 μM and 1.2+0.3 μM, respectively). The result confirmed the interaction mechanism of the analogs with tubulin.

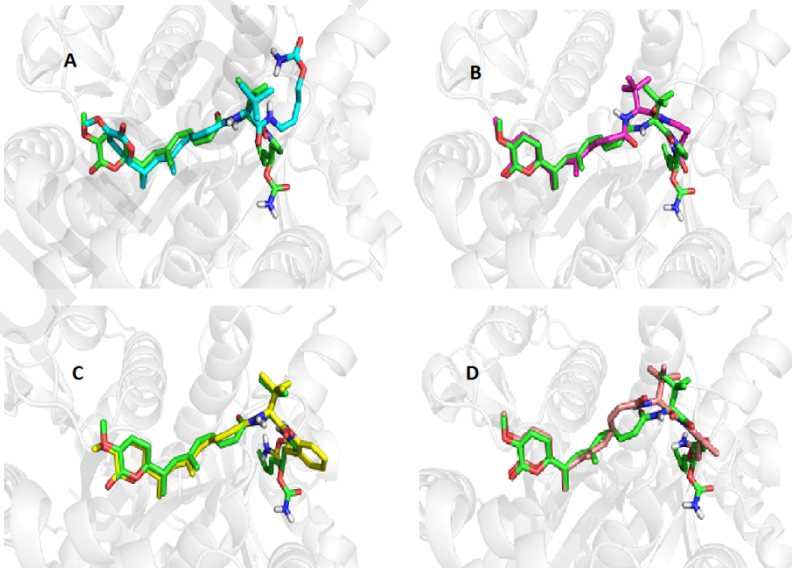


Figure 2. The best docking poses for Compound **1**-**4** with tubulin (PDB: 4TV9). **A**: the overlap of compound **1** (Gyan) and plocabulin (Green); **B**: the overlap of compound **2** (Magenta) and plocabulin (Green); **C**: the overlap of compound **3** (Yellow) and plocabulin (Green); **D**: the overlap of compound **4** (Pink) and plocabulin (Green).

Then, the above results were further analyzed by the molecular modelling study according to the reported X-ray crystal complex structure.[9] The result suggested that compound **1**-**4** could interact with tubulin in a similar manner to plocabulin, respectively (Figure 2). Notably, the carbamate moiety in **1** adopted a different orientation from that in plocabulin due to loss of the side aliphatic chain, which resulted in partially decreased activity (Figure 2A). Upon the replacement of the carbamate group with the aminoformamidoyl group, **2** adopted a more similar molecular pose to plocabulin, but the *L*-tert-leucine amide moiety was in an obviously different orientation, leading to an evident loss of activity (Figure 2B). In addition, when the double bond in the enamide moiety of **1** was replaced with the benzene ring, **3** appeared to overlap well with plocabulin, but its carbamate moiety was forced to stretch in a different direction, making a dramatical loss of activity (Figure 2C). In contrast to **1**, the installment of methyl group at the double bond made the linker of **4** adopt a twisted posture, having an adverse effect (Figure 2D).

In summary, guided by the disclosed binding mode, a set of analogs of plocabulin were designed through removing the right aliphatic chain and further modifying on the carbamate group and the enamide unit in plocabulin. All the designed analogs were synthesized and evaluated for their cytotoxic activity. Most of them showed significantly reduced cytotoxic activity towards A549, HT-29 and HepG2 cells, however, the analog (**1**) without the right aliphatic chain remained potent cytotoxicities towards the tested cells with IC_{50} values around nanomolar level, and furthermore, the replacement of the carbamate group with hydroxyl group (**4** *vs* **4h**) could result in a decrease of the activity to an evident extent. This new finding will assist the design of novel potent tubulin-binding agents based on plocabulin.

Acknowledgements

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Supplementary data

Supplementary data is available on the publishers' web site along with the published article.

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