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Design, synthesis and biological evaluation of paclitaxel-mimics possessing only the oxetane D-ring and side chain structures

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ABSTRACT

Two spiro paclitaxel-mimics consisting only of an oxetane D-ring and a C-13 side chain were designed and synthesized on the basis of analysis of structure–activity relationships (SAR) of paclitaxel. In vitro microtubule-stabilizing and antiproliferative assays indicated a moderate weaker activity of the mimics than paclitaxel, but which still represented the first example of simplified paclitaxel analogues with significant anti-tumor biological activity.

1. Introduction

The natural diterpenoid paclitaxel (Taxol®), and its semi-synthetic derivatives docetaxel (Taxotere®) and cabazitaxel (Jevana®) (Fig. 1), are important chemotherapeutics in current clinical treatment of breast cancer, ovarian cancer, non-small cell lung cancer and prostate cancer [1]. They accelerate microtubule polymerization through a combination with tubulin and stabilize cell division cycle at G2/M phase, stimulating the apoptosis of tumor cells [2]. Due to their potent anti-cancer activities, medicinal chemists have conducted in-depth research on the SAR (Structure–Activity Relationship) of paclitaxel and have developed a new generation of anti-cancer taxoids with higher activity [3].

Current structural modification research on taxoids is mainly focused on changes to the taxane core or the side chain. On this basis, a large number of paclitaxel derivatives have been synthesized, and the resulting SAR have been summarized [4]. Despite the limited number of studies on the simplification of paclitaxel, some interesting results (Fig. 2) have been reported. Ojima et al. reported several paclitaxel-mimics with a simplified structure, in which the taxane core was substituted by an indolizidine scaffold, and the C-13 side chain was retained. These compounds expressed modest cytotoxic activities, but lost the activity to stabilize microtubules [5,6]. Kingston et al. designed and synthesized macrocyclic paclitaxel-mimics, which exhibited both cytotoxic and microtubule polymerization activity, based on the configuration study of T-Taxol [7,8]. Guenard et al. synthesized a series of novel compounds combining steroids with the side-chain of docetaxel, in which the taxane skeleton was replaced by a steroid scaffold. These compounds exhibited weak cytotoxic activities and showed no microtubule disassembly inhibitory activity [9]. Beau et al. synthesized a series of paclitaxel-mimics via click chemistry based on the study of the active three-dimensional conformation of paclitaxel, but without bioactivity data [10]. Although researchers have not yet prepared strongly bioactive paclitaxel-mimics, the bioactivities observed indicate their potential for SAR research of paclitaxel as well as the discovery of more active paclitaxel analogues [11–15].

Current SAR research on paclitaxel demonstrated that the unique oxetane D-ring and side chains of paclitaxel were indispensable active groups. A-seco-paclitaxel analogues synthesized by Ojima et al. showed moderate activity, although

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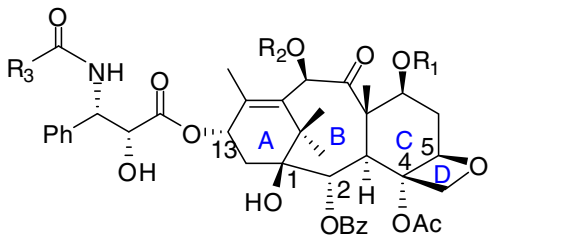


Fig. 1. Structures of paclitaxel, docetaxel and cabazitaxel.

less than paclitaxel [16]. C-seco-paclitaxel analogues, which were also obtained by Ojima et al., showed favorable activities against paclitaxel-resistant tumor cell lines [17]. In contrast, paclitaxel analogues without a D-ring or a side chain showed major reductions or even loss of activities [18–20]. In current research, two novel paclitaxel-mimics consisting only of oxetane D-rings and side chains, in which the taxane diterpenoid core was replaced by 2-oxa-6-azaspiro[3.3]heptane, were synthesized and their biological activities were evaluated.

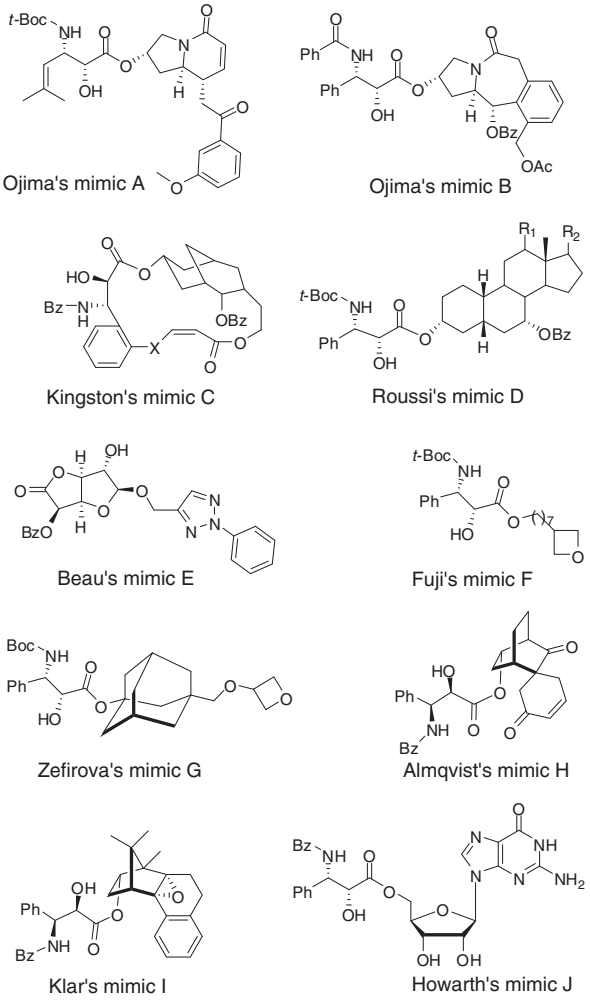


Fig. 2. Structures of representative paclitaxel-mimics.

2. Experimental

2.1. General

^{1}H and ^{13}C NMR spectra were recorded on a Varian Unity INOVA 400/54NMR spectrometer in $CDCl_3$ with tetramethylsilane (TMS) as the internal standard. Chemical shifts are given as δ value and are referenced to residual solvent proton carbon pick. Mass spectra were obtained on a VG Auto spec 3000 or on a Finnigan MAT 90 instrument. Optical rotations were measured on a Perkin-Elmer 341. Silica gel H (Qingdao Sea Chemical Factory, Qingdao, PR China) was used for column chromatography. Spots on TLC (silica gel GF_{254}) were detected with H_2SO_4–EtOH or UV. Commercially available reagents and solvents were used without further purification. Biological activity evaluation was carried out according to the protocols described previously [21].

2.2. Preparation of N-tosyl-2-oxa-6-azaspiro[3.3]heptane (5)

To a solution of KOH (179 g, 3.2 mol) and *p*-tosylamide (205 g, 1.2 mol) in 1500 mL ethanol, 3-bromo-2,2-bis (bromomethyl)propan-1-ol (324 g, 1.0 mol) was added at room temperature and the reaction mixture was heated to reflux for 90 h. The solvent was removed by evaporation, 2000 mL 1 M KOH was added and the white suspension was left to stir for another 2 h at room temperature. The mixture was filtered and the white filter cake was rinsed with water until the washing water was neutral. The filter cake was dried under high vacuum to give N-tosyl-2-oxa-6-azaspiro[3.3]heptane (5, 192 g, yield 76%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.69 (d, 2H, J = 8.4 Hz), 7.47 (d, 2H, J = 8.4 Hz), 4.42 (s, 4H), 3.85 (s, 4H), 2.41 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 144.4, 130.9, 130.2, 128.5, 79.1, 59.5, 37.3, 21.3.

2.3. Preparation of 2-oxa-6-azaspiro[3.3]heptane oxalate (6)

A solution of N-tosyl-2-oxa-6-azaspiro[3.3]heptane (7.30 g, 0.0288 mol, 1.00 equiv) in MeOH (1000 mL) was sonicated, and Mg powder (5.2 g, 0.2166 mol, 9.00 equiv) was added in potions over 1 h. The crude mixture was concentrated in vacuum, and suspended in Et_2O (1000 mL) and $Na_2SO_4 \cdot 10H_2O$ (50 g) was added. The suspension was vigorously stirred at room temperature for 1 h, then filtered, the filtrate dried (Na_2SO_4), and filtered. To the filtrate was added under stirring a solution of anhydrous oxalic acid (4.5 g, 0.05 mol) in EtOH (10 mL), which immediately formed a white precipitate. The solid was filtered and dried under reduced pressure to give pure 2-oxa-6-azaspiro[3.3]heptane (3.22 g, 73%) of oxalate mono-salt as an amorphous white powder. ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.65 (s, 4H), 4.12 (s, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 79.2, 54.1, 39.3.

2.4. Preparation of compounds 9 and 10

Commercial (4S,5R)-3-benzoyl-2-(4-methoxyphenyl)-4-phenyl-5-oxazolidinecarboxylic acid (7, paclitaxel side chain, 2 g, 0.005 mol, 1.00 equiv) and HATU (3 g, 0.0079 mol, 1.50 equiv) were added to a solution of compound 6 (1 g, 0.0052 mol, 1.00 equiv) in THF (50 mL), and the reaction mixture was stirred at room temperature for 24 h. Then 150 mL water and CH_2Cl_2 was added. The organic layer was washed with brine, dried over $MgSO_4$, and evaporated to give a residue which was chromatographed over silica gel (Petroleum ether:EtOAc = 1:3) to afford compound 9 as a white amorphous powder (2.252 g, 86%) as a pair of diastereoisomers.

Compound 10 was prepared through the coupling of compounds 6 and commercial (4S,5R)-3-tert-butoxycarbony-2-(4-methoxpheny)-4-phenyl-5-oxazolidinecarboxylic acid (8, docetaxel side chain) in 95% yields by similar procedures. Compound 10 is also a pair of diastereoisomers.

2.4.1. (4S,5R)-3-benzoyl-2-(4-methoxyphenyl)-4-phenyloxazolidin-5-yl)(2-oxa-6-azaspiro[3.3]heptan-6-yl) methanone (9)

White amorphous powder, ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.88–7.31 (14H, Ar–H), 5.51 (1H, s),4.74 (4H, m), 4.69 (2H, s), 4.69 (2H, d, J = 10.0 Hz), 4.48 (2H, d, J = 10.0 Hz), 4.20 (2H, s), 3.81 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.5, 159.7, 135.4, 130.2, 129.5, 128.3, 128.3, 127.9, 127.6, 126.8, 113.4, 90.2, 80.4, 80.2, 60.8, 57.9, 55.0, 38.3.

2.4.2. 4S,5R)-tert-butyl-2-(4-methoxyphenyl)-4-phenyl-5-(2-oxa-6-azaspiro[3.3]heptane-6-carbonyl)oxazolidine-3-carboxylate (10)

White amorphous powder, ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.89–7.37 (9H, Ar–H), 5.71 (1H, s),4.61 (1H, d, J = 7.2 Hz), 4.56 (1H, d, J = 7.2 Hz), 4.46 (1H, s), 4.43 (1H, d, J = 6.8 Hz), 4.27 (1H, d, J = 6.8 Hz), 3.99 (2H, t, J = 12.0 Hz), 3.81 (3H, s),3.72(2H, t, J = 12.0 Hz);^{13}C NMR (100 MHz, $CDCl_3$) δ 160.0, 131.2, 128.3, 127.9, 127.9, 127.4, 126.1, 113.3, 91.1, 80.4, 80.3, 80.1, 61.9, 60.6, 57.7, 55.1, 37.38, 27.6.

2.5. Preparation of compounds 11 and 12

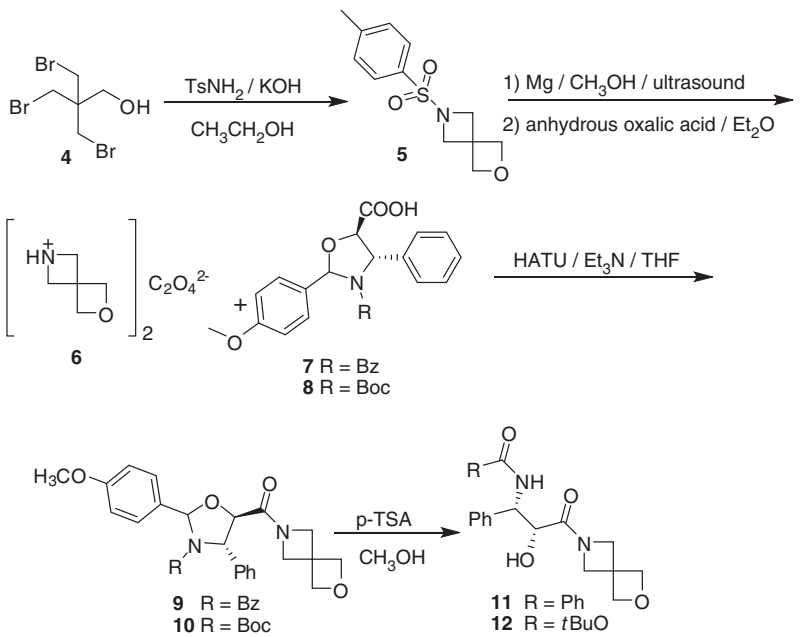
p-Toluenesulfonic acid powder (0.15 g, 0.871 mmol) was added in portions to a solution of compound 9 (400 mg, 0.826 mmol) in CH_3OH (100 mL) at room temperature over 12 h. $NaHCO_3$ was added to adjust PH to neutral. After addition of water, the mixture was concentrated under reduced pressure and extracted with AcOEt, the organic layer was washed with brine, dried over MgSO4, and evaporated to give compound 11 (90 mg, 30.0%) after purification by column chromatography over silica gel (chloroform:methanol = 20:1). Compound 12 was prepared in 36.0% isolated yield by similar procedures.

2.5.1. N-((1S,2R)-2-hydroxy-3-oxo-1-phenyl-3-(2-oxa-6-azaspiro[3.3]heptan-6-yl)propyl)-benzamide (11)

White amorphous powder, $[\alpha]_D^{25}$ −15.6 ($CHCl_3$, c 1.0);^{1}H NMR (400 MHz, $CDCl_3$): δ 7.26–7.77 (10H, Ar–H), 5.49 (1H, br. s), 4.52 (1H, br. s), 3.94 (1H, br.s), 3.68 (4H, m), 3.50 (4H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.1, 167.7, 138.2, 133.6, 130.0, 128.6, 128.6, 127.8, 127.1, 127.1, 80.2, 71.8, 63.5, 54.8, 39.5; MS (ESI, MeOH) m/z 367 $[M + H]^+$; HR-ESI-MS: 367.1634 $[M + H]^+$, calcd. for $C_{21}H_{23}N_2O_4$ 367.1657.

2.5.2. Tert-butyl(1S,2R)-2-hydroxy-3-oxo-1-phenyl-3-(2-oxa-6-azaspiro[3.3]heptan-6-yl)-propylcarbamate (12)

White amorphous powder, $[a]_D^{25}$ −22.6 ($CHCl_3$, c 1.0);^{1}H NMR (400 MHz, $CDCl_3$): δ 7.32 (5H, Ar–H), 5.58 (1H, br. s),



Scheme 1. Synthesis of paclitaxel-mimics.

4.86 (1H, br. s), 4.60 (4H, m), 4.21 (1H, br. s), 3.95 (4H, m), 1.38 (9H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.6, 155.4, 128.1, 127.4, 126.7, 80.2, 79.6, 72.0, 59.7, 57.6, 38.1, 28.0; MS (ESI, MeOH) *m/z* 363 $[M + H]^+$; HR-ESI-MS: 363.1902 $[M + H]^+$, calcd. for $C_{19}H_{27}N_2O_5$ 363.1919.

3. Results and discussion

The simplified scaffold 2-oxa-6-azaspiro[3.3]heptane, which would replace the taxane core, was synthesized firstly. The paclitaxel-mimics were then prepared by coupling different side chains with the nitrogen atom using a known semi-synthetic method of paclitaxel. Lastly, the paclitaxel-mimics were subjected to stabilizing-microtubule and antiproliferative activity assays.

Following the synthetic method for spirocyclic oxetanes reported by Burkhard et al.[22], 6-tosyl-2-oxa-6-azaspiro [3.3]heptane (5) was conveniently obtained by refluxing tribromopentaerythritol (4) and p-toluenesulfonamide for 72 h in anhydrous ethanol under KOH. The protecting group of tosyl was removed from 5 via ultrasonic-assisted reduction with Mg powder, and the corresponding salt was formed with oxalic acid to yield 2-oxa-6-azaspiro[3.3]heptane (6). Paclitaxel oxetane D-ring analogues 9 and 10 were prepared by a coupling reaction with the side chains of paclitaxel and docetaxel (Scheme 1).

In most semi-synthesis of paclitaxel, the coupling reaction of 10-DAB (10-deacetylbacatin III) with different side chains was conducted in the presence of DCC (dicyclohexylcarbodiimide) and DMAP (4-dimethylamiopryidine) [20]. However, under these conditions, the coupling reaction of 2-oxa-6-azaspiro[3.3] heptane with iso-oxazolidine acid side chains (7 and 8) proceeded slowly. After testing various condensing agents, we found that when DCC and DMAP were replaced by HATU (2-(7-aza-1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate) and triethylamine respectively, the coupling reaction proceeded rapidly and gave high yields of the corresponding products 9 and 10. The designed paclitaxel-mimics 11 and 12 were prepared by removal of the p-methoxybenzylidene protecting group with *p*-TSA (p-toluenesulfonic acid). The two paclitaxel-mimics proved to have good aqueous solubility, in contrast with the poor aqueous solubility of anti-cancer taxoids (Scheme 1).

Mimics 11 and 12 were tested to determine the concentrations required to inhibit the rate of cold-induced microtubule disassembly by 50% [23]. The ratios of IC_{50} (11 or 12): IC_{50} (paclitaxel) gave the activities with 200 and 120 to paclitaxel respectively (Table 1). These values, although low, represent the most significant microtubule disassembly inhabitant activity in the reported paclitaxel-mimics. Interestingly, compound 12 possessing the docetaxel side chain was more active in tubulin assays than compound 11 consisting of the paclitaxel side chain.

Assays for anti-proliferative activity were also conducted and indicated that paclitaxel-mimics 11 and 12 exhibited moderate anti-proliferative activities (Table 2). It is particularly worth mentioning that these two compounds showed favorable activities against paclitaxel-sensitive tumor cell lines, such as A2780 and BGC-823, and showed comparatively low activities against paclitaxel-insensitive tumor cell lines, such as Bel-7402, which suggests a probably similar anti-cancer mechanism to that of paclitaxel. Of course, more pharmacological research should be done to interpret this interesting result.

In summary, two paclitaxel-mimics 11 and 12 bearing only an oxetane D-ring and C-13 side chain were designed on the SAR of paclitaxel, and were readily prepared through four simple reactions. In vitro stabilizing-microtubule and antiproliferative assays indicated that the two mimics 11 and 12 showed moderate anti-tumor activities. Interestingly, their antiproliferative activity was consistent with that of paclitaxel, i.e., exhibiting favorable activities against paclitaxel-sensitive tumor cell lines while showing similar drug-resistance against paclitaxel-insensitive tumor cell lines, which indicated a probably anti-cancer mechanism of mimics 11 and 12 with paclitaxel. More interestingly, the varying trend stabilizing-microtubule activity of mimics 11 and 12, which consisted of paclitaxel's and docetaxel's side chain respectively, was also consistent with those of paclitaxel and docetaxel, i.e., paclitaxel and mimic 11 showed lower tubulin inhibit activity than docetaxel and mimic 12, respectively. In future research, further optimizing the 2-oxa-6-azaspiro[3.3]heptane core will be conducted in the hope of getting more active paclitaxel-mimics with simpler structures.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.fitote.2013.10.015.

Table 1
Microtubule disassembly inhibitor activity of paclitaxel, docetaxel, paclitaxel-mimics 11 and 12.

Compound	Microtubule disassembly inhibitor activity
	IC_{50}:IC_{50} (Paclitaxel)
Paclitaxel	1
Docetaxel	0.5
11	200
12	120

Table 2
Antiproliferative activity of paclitaxel and paclitaxel-mimics 11 and 12.

	IC_{50} (nM)				
Compd.	HCT-8	Bel-7402	BGC-823	A549	A2780
Paclitaxel	37	100	7	19	6.3
11	128	768	75	85	32
12	135	538	64	76	45

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