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Original article

Investigation of amino acid conjugates of (S)-1-[1-(4-aminobenzoyl)-2,3-dihydro-1*H*-indol-6-sulfonyl]-4-phenyl-imidazolidin-2-one (DW2282) as water soluble anticancer prodrugs

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ABSTRACT

The amino acid-conjugates (**1a**–**k**) with eleven amino acids attached to primary amine of (S)-1-[1-(4-aminobenzoyl)-2,3-dihydro-1*H*-indol-6-sulfonyl]-4-phenyl-imidazolidin-2-one (DW2282, **1**) were prepared and studied for their prodrug characteristics and anti-cancer activity against SW620 cell line. All the amino acid derivatives showed not only improved water solubility but also displayed potent anti-cancer activity *in vitro*. Among these amino acid-conjugates the compounds, DW2282-L-Ala (**1b**), DW2282-L-Phe (**1e**), DW2282-L-Leu (**1g**) and DW2282-L-Met (**1h**) showed good reconversion within 8 h (104.76%, 84.03%, 95.02% and 78.34%, respectively) to the parent drug in human plasma. In addition, the compounds **1e**, **1g** and **1j** also showed good bioavailability profile along with potent *in vivo* anticancer activity.

1. Introduction

The drug delivery system such as prodrug is an important aspect of drug discovery and development. A prodrug serves as a precursor to the anticipated drug [1] and also helps in improving its absorption, distribution, metabolism and excretion or in other words improves its physicochemical and pharmacokinetic properties [2,3]. This system is effective especially in case of the drugs which are unable to perform *in vivo* despite proven *in vitro* activity.

Since more than a decade, among the prodrug and promoieties, amino acid conjugates are one of the crucial ones, having improved aqueous solubility of various drugs containing alcohol and amine functional groups [4–12]. The presence of ionized carboxylate anion or ammonium cations is the main reason for this improved solubility. Several amino acid ester prodrugs have been reported and investigated by the researchers as water soluble derivatives for oral administration [13–16]. In addition, these conjugates are not only proven for structural diversity and chemical stability but can also endure the rapid and quantitative bio-reversion to parent drug by the action of enzymes such as esterases or peptidases [17].

The amino acid conjugates with amide linkage of dapsone, a primary aromatic amine, have been synthesized as highly water-soluble and chemically stable prodrugs that target peptidase enzymes for cleavage to the parent drug *in vivo* [18,19]. Another water soluble prodrug of an anti-tumor agent 3-[(3-amino-4-methoxy) phenyl]-2-(3,4,5-trimethoxyphenyl)cyclopent-2-ene-1-one [20], a novel analog of combretastatin A-4 (CA-4), has also been reported. It was observed that all the prodrug analogs improve the water solubility as well as anti-tumor activity without exhibiting significant toxicities in mice [20]. These studies are proven examples of amino acid conjugates as worthy carrier of drug *in vivo* and have potential role in new drug discovery system.

As we previously discovered a novel and promising anticancer candidate, (S)-1-[1-(4-aminobenzoyl)-2,3-dihydro-1*H*-indol-6-sulfonyl]-4-phenyl-imidazolidin-2-one (DW2282, Fig. 1) [21–25] which not only showed the broad and potent growth inhibitory activity against various cancer cell lines but also proved its worth in the *in vivo* test, by exhibiting approximately 45%, 42%, and 94% suppression of tumor growth against murine colon adenocarcinoma (colon 26), Lewis lung carcinoma (3LL), and human colon cancer (SW620) at a dose of 65 mg/kg (p.o., q2d × 5), respectively [26]. One of our previous study on the mechanism of action of arylsulfonyl-4,5-dihydroimidazolones proved them as tubulin inhibitors [25]. These compounds were not only the potent inhibitors of tubulin polymerization but also maintained activity against multi-drug resistant tumor cell lines, which implies that they are not a substrate for p-glycoprotein mediated transport like taxanes and vinca alkaloids derivatives. Despite the therapeutic potential of

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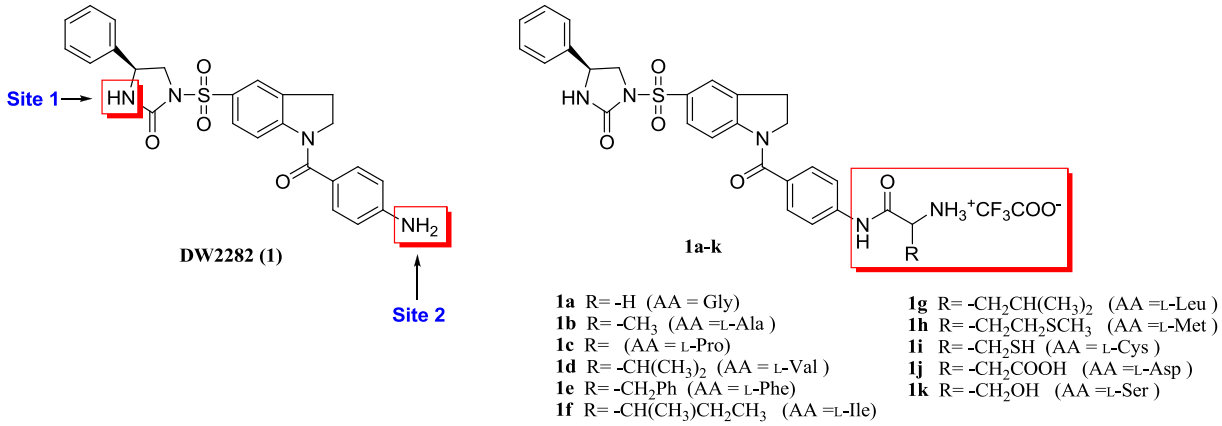
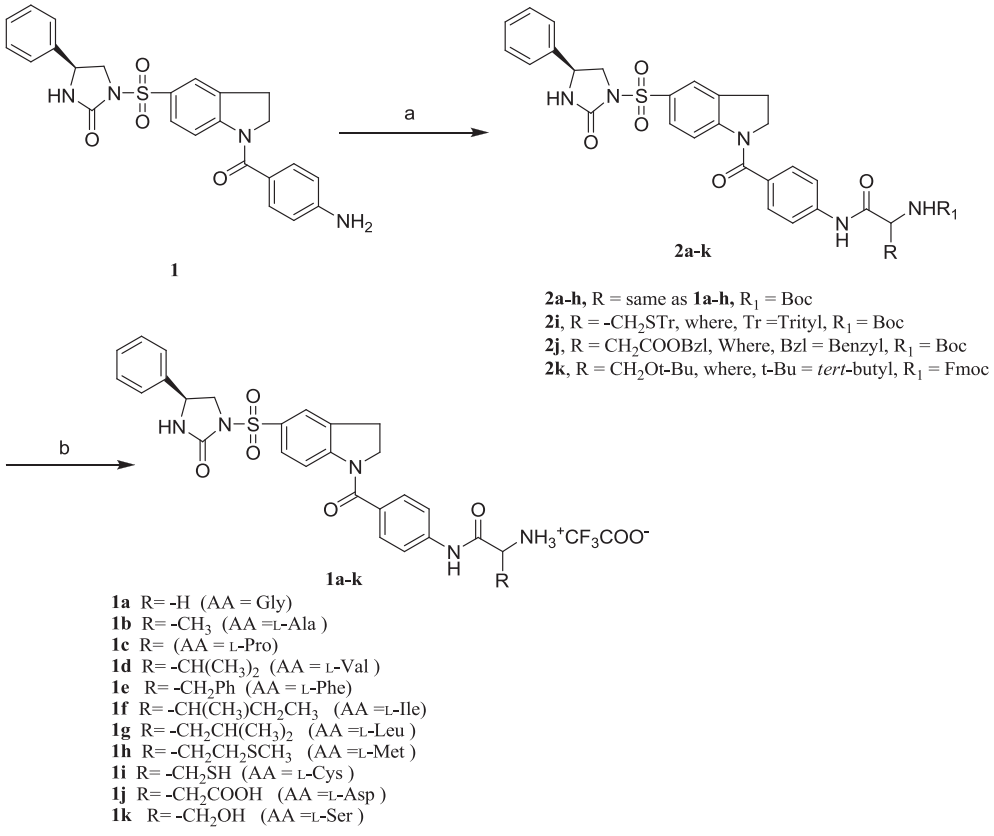


Fig. 1. Structure of DW2282 (**1**) and designed prodrugs (**1a**–**k**).

DW2282, in preclinical trial on beagle dog, the severe gastrointestinal (GI) toxicity was observed. The biopsy revealed considerable accumulation of drug on the intestinal wall after five day oral administration, which might be resulted due to its poor water solubility (0.0235 mg/mL) (Data not published). Hence, its solubility was considered as a critical parameter amenable to manipulation *via* a prodrug strategy. As we have discussed above, the presence of amino acid conjugates with parent drug improves its aqueous solubility and delivery to the target site. Bearing this in mind, we designed and synthesized amino acid conjugates of DW2282. The chemical structure of parent drug DW2282 has two possible linkable sites. One is the amide nitrogen (Site 1 in Fig. 1) of heterocyclic imidazolidin-2-one and the other one is a basic aromatic amine (Site 2 in Fig. 1) attached at indoline moiety. Our preliminary studies clearly indicated that for the synthesis of prodrug, the primary amine is more suitable to collaborate with amino acid conjugates. Thus, amino acid conjugates of DW2282 with various amino acids attached to primary amine were designed, synthesized and evaluated for their aqueous solubility, cytotoxicity against SW620 human colon cancer cell, reconversion test as well as the anti-tumor activity.

2. Chemistry

Target compounds **1a**–**k** were prepared using the synthetic pathway shown in Scheme 1. Condensation of DW2282 (**1**) with



Scheme 1. Synthesis of prodrugs **1a**–**k**. Reagents and conditions; (a) protected amino acid (*N*-Boc-Gly for **2a**, *N*-Boc-L-Ala for **2b**, *N*-Boc-L-Pro for **2c**, *N*-Boc-L-Val for **2d**, *N*-Boc-L-Phe for **2e**, *N*-Boc-L-Ile for **2f**, *N*-Boc-L-Leu for **2g**, *N*-Boc-L-Met for **2h** *N*-Boc-*S*-trityl-L-Cysteine for **2i,** *N*-Boc-L-Asp(*O*-Bzl)-OH for **2j**, *N*-Fmoc-*O*-*tert*-Bu-L-Ser for **2k**) DCC, HOBt/H_2O, DMF; (b) TFA, CH_2Cl_2 (for **1a**–**h**), TFA, Triethylsilane, CH_2Cl_2 for **1i**, Pd/C in MeOH then TFA, CH_2Cl_2 (for **1j**), Piperidine, CH_2Cl_2 then 4*N*-HCl–dioxane, reflux (for **1k**).

protected amino acids was carried out in the presence of dicyclohexylcarbodiimide (DCC) and 1-hydroxybenzotriazole hydrate (HOBt·H_2O) as coupling reagents. Deprotection of *tert*-butyloxylcarbonyl (Boc) group of **2a**–**h** was performed by the treatment with trifluoroacetic acid (TFA) in dichloromethane to obtain the TFA salt of **1a**–**h**. Compound **1i** was prepared by the deprotection of **2i** treated with TFA at room temperature until the color of reaction turned into brown and then treated with triethylsilane until the reaction is colorless. To obtain the compound **1j** the protected compound **2j** was first deprotected by the treatment of TFA in dichloromethane to remove the Boc group, followed by hydrogenolysis of the benzyl ester group using hydrogen gas and Pd/C. The hydrochloride salt of compound **1k** was obtained by the deprotection of Fmoc group of **2k** using piperidine followed by refluxing in 4 *N* HCl–dioxane. All final products were obtained in sufficient purity after purification through a silica gel column eluting with 5–10% MeOH in CH_2Cl_2 and/or recrystallization from appropriate solvents.

3. Results and discussion

3.1. Water solubility

The solubility of all the amino acid conjugates **1a**–**k** was measured in distilled water at 25 °C as reported earlier [27]. The results showed that all the amino acid conjugates have much better solubility in water compared to **1** (Table 1). Especially, compounds **1c** (L-Pro, 115 times), **1d** (L-Val, 64 times), **1g** (L-Leu, 65 times), **1h** (L-Met, 90 times) and **1k** (L-Ser, 50 times) had remarkably increased water solubility compared to **1**. Conversely, the measurement of solubility is not correlated to hydrophilic properties of side chain of amino acids.

3.2. In vitro anti-cancer activity

In the next set of experiment growth inhibitory activity of **1a**–**k** against human colorectal adenocarcinoma cell (SW620) line using MTT assay [28,29] was measured (Table 2). Interestingly, compounds **1b** (IC_{50} = 0.020 μM), **1d** (IC_{50} = 0.038 μM) and **1e** (IC_{50} = 0.024 μM) were three times more active than **1** while compounds **1a** (IC_{50} = 0.068 μM), **1f** (IC_{50} = 0.095 μM) and **1h** (IC_{50} = 0.095 μM) showed comparable activity to **1** (IC_{50} = 0.057 μM). On the other hand compounds **1c** (IC_{50} = 0.648 μM), **1g** (IC_{50} = 0.198 μM), **1i** (IC_{50} = 0.415 μM), **1j** (IC_{50} = 0.383 μM) and **1k** (IC_{50} = 0.992 μM) showed lesser activity than **1**. In general, amino acid conjugates with hydrophobic side chain increase the activity, with exception of proline (**1c**) and leucine (**1g**) conjugates whereas amino acid conjugates (**1i**, **1j**, **1k**) with hydrophilic side chain decrease the activity.

Table 1
Water solubility of prodrugs **1a**–**k**.

Compound	Solubility (mg/mL)[a]	Compound	Solubility (mg/mL)[a]
1a (Gly)	0.064	**1g** (L-Leu)	1.620
1b (L-Ala)	0.214	**1h** (L-Met)	2.176
1c (L-Pro)	2.758	**1i** (L-Cys)	0.353
1d (L-Val)	1.541	**1j** (L-Asp)	0.732
1e (L-Phe)	0.865	**1k** (L-Ser)	1.201
1f (L-Ile)	0.953	**1** (DW2282)	0.024[b]

[a] Solubility was measured in distilled water at 25 °C as reported previously [27].
[b] Solubility values are taken as a mean from 3 experiments.

Table 2
The *in vitro* anticancer activity of prodrugs (**1a**–**k**) against human cancer cell line (SW620).

Compound	Cytotoxicity[a] (IC_{50}[b] μM)	Compound	Cytotoxicity[a] (IC_{50}[b] μM)
1a (Gly)	0.068	**1g** (L-Leu)	0.198
1b (L-Ala)	0.020	**1h** (L-Met)	0.095
1c (L-Pro)	0.648	**1i** (L-Cys)	0.415
1d (L-Val)	0.038	**1j** (L-Asp)	0.383
1e (L-Phe)	0.024	**1k** (L-Ser)	0.992
1f (L-Ile)	0.095	**1** (DW2282)	0.057

[a] Cancer cell line; SW620 (Human colorectal adenocarcinoma cell).
[b] All experiments were performed at least in triplicate using the MTT assay [28,29] according to the previously reported procedure with a minor modification.

3.3. In vitro reconversion test of *1a*–*k* to *1* in human plasma

In order to predict the rate of reconversion from **1a**–**k** to **1** in body, the reconversion of amino acid conjugates **1a**–**k** to **1** in human plasma in 24 h were measured as shown in Fig. 2. The maximum amounts of conversion of **1a**–**k** to **1** were obtained in 8 h and were not changed much after that (Table 3). Compounds **1b** (104.76%, $T_{1/2}$ = 8.0 min), **1e** (84.03%, $T_{1/2}$ = 135.5 min), **1g** (95.02%, $T_{1/2}$ = 50.1 min) and **1h** (78.34%, $T_{1/2}$ = 154.3 min) are highly susceptible to hydrolytic conversion to **1** in human plasma. These results suggest that these analogs might be considered as prodrugs.

3.4. Pharmacokinetic studies

Compounds for pharmacokinetic study in Balb/c mice were selected based on relatively two acceptable parameters among water solubility, cytotoxicity and reconversion rate. Thus compounds **1d**–**g** were selected and additionally **1j** was also selected due to its carboxylic acid moiety, even though these properties were not much improved. Since compounds were expected to form compound **1** *in vivo* system, the blood concentration was measured by HPLC after oral administration of 70 mg/kg in 1% CMC solution. The pharmacokinetic results are shown in Table 4 and Fig. 3. The data clearly indicated that **1e** (AUC, 344.33 μg min/mL), **1g** (AUC, 379.05 μg min/mL) and **1j** (AUC, 672.23 μg min/mL) exhibit good exposure by the oral route. The pharmacokinetic profiles of **1e** and **1g** are well correlated to their reconversion rate (**1e**: 84.03% and **1g**: 95.02%) in human plasma. However, good pharmacokinetic profile of **1j** was unexpected since its reconversion rate was very poor in human plasma. This result obviously indicates species different metabolism. Compounds **1d** (AUC, 19.95 μg min/mL) and **1f** (AUC, 44.24 μg min/mL) have very poor pharmacokinetic profiles as expected in the reconversion test. Therefore, the compounds **1e**, **1g** and **1j** were subjected for animal study.

3.5. Anticancer activity

Such significant bioavailability of compounds **1e**, **1g** and **1j** led us to investigate their antitumor activities *in vivo* against human colon carcinoma (SW620) xenograft tumor models in mice (Table 5) [30]. Compound **1e** (Dose: 70 mg/kg, b.i.d., 5 days, p.o.) showed 99% TGI against SW620 xenograft tumor models in mice with 9.5% decrease in body weight. Compound **1g** (Dose: 70 mg/kg, b.i.d., 5 days, p.o.) showed 86% TGI against SW620 xenograft tumor models in mice with 10.5% decrease in body weight. Although there are some body weight changes, antitumor activities of these compounds are remarkable at this single dose experiment. However, compound **1j** (70 mg/kg, b.i.d., 5 days, p.o.) showed 63% TGI with markedly decrease in body weight as shown in Table 5, which indicates significant toxicity.

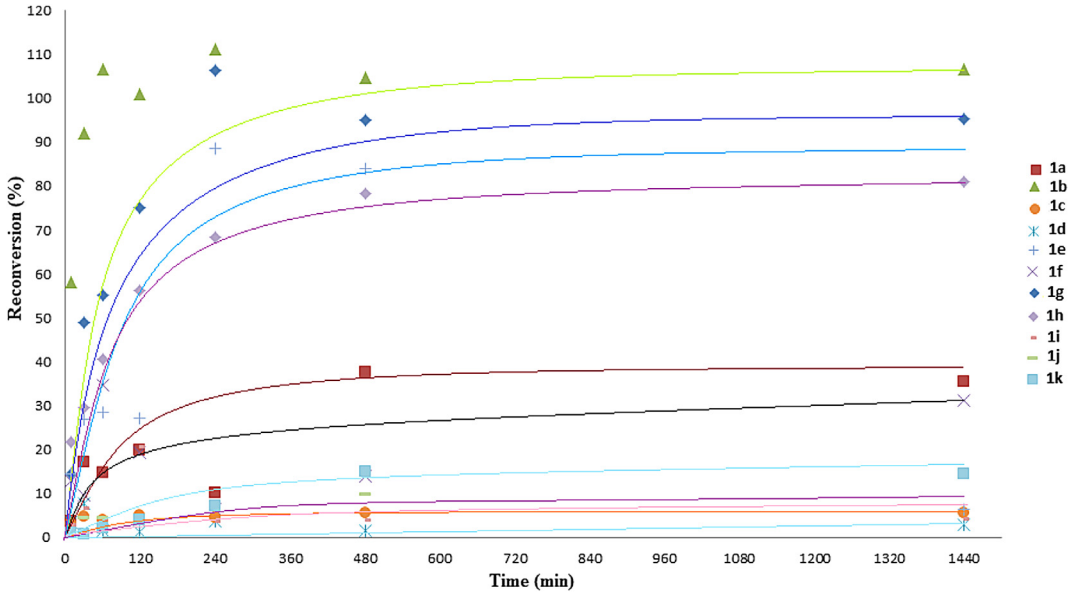


Fig. 2. Reconversion curve in human plasma of prodrugs (**1a**–**k**) to **1** (DW2282).

4. Conclusion

For finding a possible prodrug candidate of **1**, its eleven amino acid conjugates were synthesized and tested for their water solubility, reconversion rate in human plasma, pharmacokinetic profile and *in vitro* and *in vivo* anticancer activity.

The results indicated improved solubility by all the amino acid derivatives. Amino acid conjugation to **1** varied the activity of these analogs. In general, amino acid conjugates with hydrophobic side chain increase the *in vitro* activity with exception of proline (**1c**) and leucine (**1g**) conjugates. Compounds **1b** (L-Ala), **1e** (L-Phe), **1g** (L-Leu) and **1h** (L-Met) showed good reconversion rate in human plasma. The pharmacokinetic study obviously indicated that **1e** (AUC, 344.33 μg min/mL), **1g** (AUC, 379.05 μg min/mL) and **1j** (AUC, 672.23 μg min/mL) exhibited good exposure by the oral route in mice as compound **1**. Compounds **1e** (99% TGI) and **1g** (86% TGI) showed remarkable tumor growth inhibition against SW620 xenograft tumor model in mice at single dose experiment (Dose: 70 mg/kg, b.i.d., 5 days, p.o.). Therefore, compounds **1e** and **1g** can be considered as potential prodrug candidates.

5. Experimental

5.1. Chemistry

All reagents and solvents were dried prior to use according to the standard methods. Commercial reagents were used without further purification unless otherwise stated. Melting points were measured on an Electrothermal melting point apparatus. IR spectra were obtained on KBr disks using a JASCO Report 100 spectrophotometer. ^{1}H NMR and ^{13}C NMR spectra were recorded on JEOL, JNM-AL-400 (Alice) 400 FT-NMR spectrometer and the chemical shift (δ) were reported in parts per million downfield from tetramethylsilane (TMS) and from solvent references. High resolution mass spectra (HRMS) were measured by using Shimadzu LCMS-IT-TOF spectrometer. Chromatographic separations were performed on silica gel columns by flash (Kieselgel 40, 0.040–0.063 mm; Merck) or gravity column (Kieselgel 60, 0.063–0.200 mm; Merck). The reactions were monitored by thin-layer chromatography (TLC) on Merck glass silica gel (Kieselgel 60 F_{254}) plates that were visualized under a UV lamp. HPLC was performed using a Shimadzu liquid chromatograph model Class-vp version 6.12, equipped with a SPD-10A UV–vis detector (Shimadzu). The HPLC analysis was performed using a Symmetry TM® C-18 column (3.9 × 150 mm, Part No. WAT054205, Waters, Milford, MA). The elution condition was a linear gradient of 31–95% acetonitrile in 0.1% TFA for 30 min at a flow rate of 1 mL/min with UV detection at 254 nm. All solvents for HPLC were filtered through a 0.45 μm membrane filter (Waters, USA).

5.1.1. Synthesis of amino acid prodrugs *2a*–*k*

*5.1.1.1. DW2282-N-Boc-Gly (**2a**).* 1-Hydroxybenzotriazole hydrate (HOBt·H_2O) (0.436 g, 3.23 mmol) and *N,N'*-Dicyclohexylcarbodiimide (DCC) (0.532 g, 2.58 mmol) were added to solution

Table 3
Reconversion rate of prodrugs (**1a**–**k**) to **1** (DW2282) in human plasma.

Compound	Reconversion rate[a] (%)	Half life (min)	Compound	Reconversion rate[a] (%)	Half life (min)
1a (Gly)	37.56	–	**1g** (L-Leu)	95.02	50.1
1b (L-Ala)	104.76	8.0	**1h** (L-Met)	78.34	154.3
1c (L-Pro)	5.74	–	**1i** (L-Cys)	4.09	–
1d (L-Val)	1.59	–	**1j** (L-Asp)	10.00	–
1e (L-Phe)	84.03	135.5	**1k** (L-Ser)	15.09	–
1f (L-Ile)	13.84	–			

[a] Reconversion rate: measured at 8 h after initiation of reaction.

Table 4
Pharmacokinetic Parameters for prodrug (**1d**–**g and 1j**) tested *in vivo*.

PK parameters	**1d** (L-Val)	**1e** (L-Phe)	**1f** (L-Ile)	**1g** (L-Leu)	**1j** (L-Asp)
C_{max} (μg/mL)[a]	0.95	6.61	0.83	6.24	9.09
T_{max} (min)[b]	60.00	30.00	120.00	30.00	60.00
$T_{1/2}$ (min)[c]	X	69.94	X	60.00	32.81
AUC_{0-240} (μg min/ml)[d]	19.95	344.33	44.25	379.05	672.23
$AUC_{0-\infty}$ (μg min/ml)	X	412.94	X	426.66	680.27

[a] C_{max} is a maximum blood concentration of DW2282.
[b] T_{max}: Time when the blood concentration of DW2282 is maximum.
[c] $T_{1/2}$ is half life time.
[d] AUC: 0 → 240 is a integral value of area under the concentration–time curve from 0 min to 240 min.

Table 5
Antitumor activity of prodrugs (**1e**, **1g** and **1j**).

Compound	Schedule	% TGI	Body weight change (%)
1e	70 mg/kg, b.i.d., 5 days, p.o.	99	−9.5
1g	70 mg/kg, b.i.d., 5 days, p.o.	86	−10.5
1j	70 mg/kg, b.i.d., 5 days, p.o.	63	−28.6
1	70 mg/kg, 1 time/2days, 3 times, p.o.	86	1.5

of DW2282 (**1**, 1.0 g, 2.16 mmol) in anhydrous dimethylformamide (DMF) (30 mL) at 0 °C. The reaction mixture was kept under continuous stirring for 15 min. *N*-Boc-Gly (0.452 g, 2.58 mmol) in the solid form was the added at once, and the reaction mixture was stirred for additional 24 h while it was gradually allowed to reach ambient temperature. The solvent was then removed under reduced pressure. The crude product was dissolved in ethyl acetate (50 mL), washed with water (50 mL), dried (Na_2SO_4) and evaporated. Chromatography of crude product on silica gel (hexane:ethyl acetate = 1:1 → hexane:ethyl acetate:methanol = 10:10:1) gave DW2282-*N*-Boc-Gly (**2a**) as a white solid.

Yield: 0.635 g (47.7%); R_f 0.43 (10:10:1 ethyl acetate/hexane/methanol); ^{1}H NMR (DMSO-d_6) δ 1.39 (s, 9H), 3.15 (t, J = 8.0 Hz, 2H), 3.49 (m, 1H), 3.76 (d, J = 6.0 Hz, 2H), 4.13 (m, 2H), 4.27 (m. 1H), 4.76 (t, J = 7.6 Hz, 1H), 7.20–7.40 (m, 5H), 7.59–7.61 (m, 2H), 7.69–7.78 (m, 5H), 8.21 (s, 1H).

*5.1.1.2. DW2282-N-Boc-L-Ala (**2b**).* The compound **2b** was prepared by the same procedure described for DW2282-*N*-Boc-Gly (**2a**) using *N*-Boc-L-Ala instead of *N*-Boc-Gly.

Yield: 40.1%; R_f 0.34 (3:1 ethyl acetate/hexane); ^{1}H NMR (, DMSO-d_6) δ 1.26 (d, J = 7.2 Hz, 3H), 1.38 (s, 9H), 3.15 (m, 2H), 3.49 (m, 1H), 4.10–4.19 (m, 3H), 4.25 (m, 1H), 4.79 (t, J = 7.4 Hz, 1H), 5.77 (s, 1H), 6.58 (d, J = 8.4 Hz, 1H) 7.22–7.38 (m, 7H), 7.59–7.61 (m, 2H), 7.69–7.78 (m, 3H), 8.21 (s, 1H), 10.21 (s, 1H).

*5.1.1.3. DW 2282-N-Boc-L-Pro (**2c**).* Compound (**2c**) was prepared by the same procedure described for DW2282-*N*-Boc-Gly (**2a**) using *N*-Boc-L-Pro instead of *N*-Boc-Gly.

Yield: 36.5%; R_f 0.40 (10:10:1 ethyl acetate/hexane/methanol); ^{1}H NMR (DMSO-d_6) δ 1.39 (s, 9H), 1.79–1.89 (m, 3H), 2.20 (m, 1H), 3.15 (m, 2H), 3.49 (m, 1H), 4.11–4.29 (m, 4H), 4.79 (t, J = 7.4 Hz, 1H), 7.22–7.38 (m, 6H), 7.60–7.62 (m, 2H), 7.71–7.79 (m, 4H), 8.21 (s, 1H), 10.26 (s, 1H).

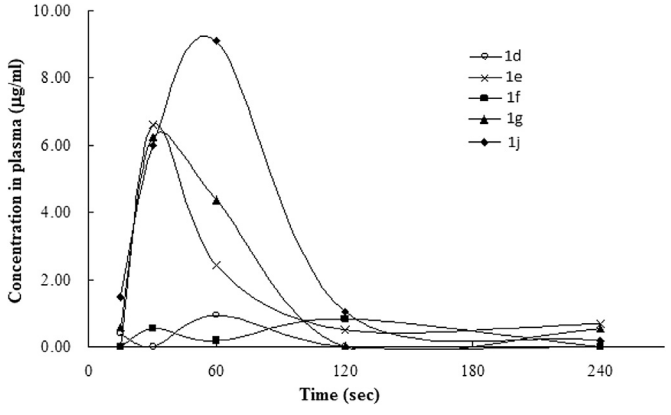


Fig. 3. The blood concentration–time curve of DW2282 (**1**) in the female Balb/c mice soon after the administration of prodrugs (**1d**–**g** and **1j**), respectively.

*5.1.1.4. DW 2282-N-Boc-L-Val (**2d**).* The compound (**2d**) was prepared by the same procedure described for DW2282-*N*-Boc-Gly (**2a**) using *N*-Boc-L-Val instead of *N*-Boc-Gly.

Yield: 23.8%; R_f 0.45 (3:1 ethyl acetate/hexane); ^{1}H NMR ($CDCl_3$-d_6) δ 1.03 (q, J = 9.2, 6.8 Hz, 6H), 1.36 (s, 9H), 2.11 (m, 1H), 3.14 (m, 2H), 3.80 (m, 1H), 4.07–4.17 (m, 3H), 4.31 (t, J = 8.8 Hz, 1H), 4.80 (t, J = 8.4 Hz, 1H), 5.28 (d, J = 8.8 Hz, 1H), 7.26–7.56 (m, 12H), 7.86 (m, 2H), 9.23 (s, 1H).

*5.1.1.5. DW 2282-N-Boc-L-Phe (**2e**).* The compound (**2e**) was prepared by the same procedure described for DW2282-*N*-Boc-Gly (**2a**) using *N*-Boc-L-Phe instead of *N*-Boc-Gly.

Yield: 46.0%; R_f 0.24 (2:1 ethyl acetate/hexane); ^{1}H NMR (DMSO-d_6) δ 1.32 (s, 9H), 2.86–3.05 (m, 2H), 3.15 (m, 2H), 3.51 (m, 1H), 4.14 (m, 3H), 4.28–4.30 (m, 2H), 4.80 (m, 1H), 5.57 (d, J = 8.0 Hz, 1H), 7.20–7.40 (m, 13H), 7.61–7.80 (m, 6H), 10.35 (s, 1H).

*5.1.1.6. DW 2282-N-Boc-L-Ile (**2f**).* The compound (**2f**) was prepared by the same procedure described for DW2282-*N*-Boc-Gly (**2a**) using *N*-Boc-L-Ile instead of *N*-Boc-Gly.

Yield: 28.3%; R_f 0.35 (3:1 ethyl acetate/hexane); ^{1}H NMR (DMSO-d_6) δ 0.80–0.88 (m, 6H), 1.23 (m, 1H), 1.39 (s, 9H), 1.62 (m, 1H), 1.72 (m, 1H), 3.16 (m, 2H), 3.50 (m, 2H), 3.97 (m, 1H), 4.15 (m, 2H), 4.28 (t, J = 8.8 Hz, 1H), 4.80 (t, J = 7.2 Hz, 1H), 5.57 (d, J = 8.0 Hz, 1H), 7.22–7.40 (m, 5H), 7.59–7.80 (m, 6H), 10.29 (s, 1H).

*5.1.1.7. DW 2282-N-Boc-L-Leu (**2g**).* The compound (**2g**) was prepared by the same procedure described for DW2282-*N*-Boc-Gly using *N*-Boc-L-Leu instead of *N*-Boc-Gly.

Yield: 60.1%; R_f 0.44 (2:1 ethyl acetate/hexane); ^{1}H NMR ($CDCl_3$-d_1) δ 0.95 (m, 6H), 1.36 (s, 9H), 1.69 (m, 3H), 3.17 (m, 2H), 3.51 (m, 1H), 3.96 (m, 1H), 4.09–4.32 (m, 3H), 4.80 (m, 1H), 5.23 (m, 1H), 7.21–7.86 (m, 12H).

*5.1.1.8. DW 2282-N-Boc-L-Met (**2h**).* The compound (**2h**) was prepared by the same procedure described for DW2282-*N*-Boc-Gly (**2a**) using *N*-Boc-L-Met instead of *N*-Boc-Gly.

Yield: 41.6%; R_f 0.35 (3:1 ethyl acetate/hexane); ^{1}H NMR ($CDCl_3$-d_1) δ 1.35 (s, 9H), 1.87–2.10 (m, 2H), 2.12 (s, 3H), 3.20 (m, 2H), 3.67 (m, 2H), 4.29 (m, 3H), 4.80 (m, 1H), 5.55 (m, 1H), 7.27–7.85 (m, 12H).

*5.1.1.9. DW2282-N-Boc-S-trityl-L-Cys (**2i**).* The compound (**2i**) was prepared by the same procedure described for DW2282-*N*-Boc-Gly (**2a**) using *N*-Boc-*S*-trityl-L-Cys instead of *N*-Boc-Gly.

Yield: 46.3%; R_f 0.65 (1:1 ethyl acetate/hexane); ^{1}H NMR (DMSO-d_6) δ 1.38 (s, 9H), 2.62 (m, 1H), 3.13 (m, 2H), 3.67 (m, 1H), 4.12–4.18 (m, 3H), 4.25 (m, 1H), 4.75 (m, 1H), 7.18–8.33 (m, 28H).

*5.1.1.10. DW2282-N-Boc-L-Asp(OBzl) (**2j**).* The compound (**2j**) was prepared by the same procedure described for **DW**2282-*N*-Boc-Gly (**2a**) using *N*-Boc-L-Asp(OBzl)-OH instead of *N*-Boc-Gly.

Yield: 57.7%; R_f 0.50 (2:1 ethyl acetate/hexane); ^{1}H NMR ($CDCl_3$-d_6) δ 1.48 (s, 9H), 2.80–3.10 (m, 2H), 3.17 (m, 2H), 3.44 (m, 1H), 3.70 (q, J = 9.2, 7.2 Hz, 1H), 4.17 (t, J = 8.4 Hz, 2H), 4.30 (t, J = 8.8 Hz, 1H), 4.78 (t, J = 7.6 Hz, 1H), 5.18 (d, J = 5.2 Hz, 2H), 5.90 (m, 1H), 7.22–7.37 (m, 10H), 7.54–7.57 (m, 4H), 7.60–7.92 (m, 4H).

*5.1.1.11. DW2282-N-Fmoc-O-tert-Bu-L-Ser (**2k**).* The compound (**2k**) was prepared by the same procedure described for **DW**2282-*N*-Boc-Gly (2a) using *N*-Fmoc-*O*-*tert*-Bu-L-Ser instead of *N*-Boc-Gly.

Yield: 38.0%; R_f 0.31 (3:1 ethyl acetate/hexane); ^{1}H NMR ($CDCl_3$-d_6) δ 1.28 (s, 9H), 3.19 (t, J = 8.4 Hz, 2H), 3.49 (t, J = 8.8 Hz, 1H), 3.69 (q, J = 9.6, 6.8 Hz, 1H), 4.16–4.46 (m, 8H), 4.78 (t, J = 8.0 Hz, 1H), 7.20–7.92 (m, 22H).

*5.1.2. Synthesis of TFA salt of amino acid prodrugs **1a**–**k***

*5.1.2.1. DW2282-Gly.TFA (**1a**).* DW2282-*N*-Boc-Gly (0.5g, 0.8 mmol) was dissolved in TFA/CH_2Cl_2 (20 mL + 40 mL) and stirred at room temperature for 3 h. After removal of solvent, chromatography of crude product on silica gel (dichloromethane:methanol = 10:1) gave DW2282-Gly **(1a)** as a white solid.

Yield: 0.62 g (90.3%); mp 167.0–168.0 °C; R_f 0.22 (10:1 dichloromethane:methanol); FT-IR (cm^{-1}) 3254, 2360, 1723, 1381, 1156; ^{1}H NMR (DMSO-d_6) δ 3.16 (m, 2H), 3.48 (q, J = 9.2, 6.0 Hz 1H), 3.83 (s, 2H), 4.12 (t, J = 8.4 Hz, 2H), 4.27 (t, J = 9.2 Hz, 1H), 4.79 (t, J = 7.2 Hz, 1H), 7.22–7.37 (m, 5H), 7.64–7.79 (m, 6H), 8.16 (s, 3H), 8.21 (s, 1H), 10.73 (s, 1H); ^{13}C NMR (400 MHz, DMSO-d_6) δ. 170.7, 169.2, 168.8, 168.5, 158.6, 147.5, 140.9, 140.4, 134.3, 131.5, 131.4, 128.9, 128.1, 127.8, 126.0, 124.3, 117.7, 116.0, 57.8, 51.3, 50.9, 48.9, 27.6. HRMS Calcd for $C_{28}H_{26}F_3N_5O_7S$ m/z 633.1505, found 633.1501.

*5.1.2.2. DW2282-Ala. TFA (**1b**).* The compound (**1b**) was prepared by the same procedure described for DW2282-Gly.TFA (**1a**) using DW2282-*N*-Boc-Ala instead of DW2282-*N*-Boc-Gly.

Yield: 70.6%; mp 238.0–239.0 °C; R_f 0.25 (10:1 dichloromethane/methanol); FT-IR (cm^{-1}) 3254, 2358, 1746, 1404, 1156; ^{1}H NMR (DMSO-d_6) δ 1.44 (d, J = 6.8 Hz, 3H), 3.16 (m, 2H), 3.49 (q, J = 9.2, 6.0 Hz, 1H) 4.01 (m, 1H), 4.12 (t, J = 8.0 Hz, 2H), 4.27 (t, J = 8.8 Hz, 1H), 4.80 (m, 1H), 7.22–7.24 (m, 2H), 7.30–7.39 (m, 3H), 7.64–7.79 (m, 6H), 8.21 (s, 1H), 10.73 (s, 1H); ^{13}C NMR (400 MHz, DMSO-d_6) δ. 170.5, 169.2, 168.6, 168.3, 157.8, 154.5, 147.5, 140.7, 140.1, 134.0, 131.5, 131.4, 128.6, 128.4, 128.1, 127.8, 125.8, 124.3, 118.8, 115.8, 52.1, 51.3, 50.9, 48.9, 27.2, 16.9. HRMS Calcd for $C_{29}H_{28}F_3N_5O_7S$ m/z 647.1662, found 647.1656.

*5.1.2.3. DW2282-Pro. TFA (**1c**).* The compound (**1c**) was prepared by the same procedure described for DW2282-Gly (**1a**) using DW2282-*N*-Boc-Pro instead of DW2282-*N*-Boc-Gly.

Yield: 60.7%; mp 142.0–143.0 °C; R_f 0.28 (10:1 dichloromethane/methanol); FT-IR (cm^{-1}) 3105, 2359, 1673, 1381, 1130; ^{1}H NMR (CD_3OD-d_4) δ 2.10–2.17(m, 3H), 2.54 (m, 1H), 3.21 (m, 2H), 3.36–3.50 (m, 2H), 3.60 (q, J = 10.0, 6.4 Hz, 1H), 4.20 (t, J = 8.4 Hz, 2H), 4.32 (t, J = 9.6 Hz, 1H), 4.44 (m, 1H), 4.80 (m, 1H), 7.20–7.23 (m, 2H), 7.30–7.37 (m, 4H), 7.63–7.65 (m, 2H), 7.76–7.78 (m, 4H) 7.85 (m, 1H); ^{13}C NMR (DMSO-d_6) δ. 176.9, 169.3, 167.5, 162.5, 158.3, 152.8, 147.7, 140.9, 140.4, 134.3, 131.5, 131.4, 128.9, 128.6, 128.1, 127.8, 126.0, 124.3, 119.1, 115.7, 61.1, 59.9, 53.8, 45.9, 35.8, 32.2, 30.8, 29.6 HRMS Calcd for $C_{31}H_{30}F_3N_5O_7S$ m/z 673.1818, found 673.1814.

*5.1.2.4. DW2282-Val. TFA (**1d**).* The compound (**1d**) was prepared by the same procedure described for DW2282-Gly.TFA (**1a**) using DW2282-*N*-Boc-Val instead of DW2282-*N*-Boc-Gly.

Yield: 45.1%; mp 184.0–185.0 °C; R_f 0.24 (10:1 dichloromethane/methanol); FT-IR (cm^{-1}) 3251, 2357, 1528, 1201; ^{1}H NMR (CD_3OD-d_4) δ 1.11 (q, J = 13.6, 6.8 Hz, 6H), 2.30 (m, 1H), 3.21 (m, 2H), 3.60 (q, J = 9.6, 6.0 Hz, 1H), 3.84 (d, J = 5.6 Hz, 1H), 4.20 (t, J = 8.4 Hz, 2H), 4.31 (t, J = 9.6 Hz, 1H), 4.79 (q, J = 8.8, 5.6 Hz, 1H), 7.20–7.22 (m, 2H), 7.30–7.36 (m, 3H), 7.63–7.65 (m, 2H), 7.77–7.80 (m, 3H), 7.85 (s, 1H); ^{13}C NMR (DMSO-d_6) δ. 178.0, 169.2, 168.5, 164.9, 158.5, 154.7, 147.5, 141.0, 140.3, 135.3, 134.3, 131.7, 128.9, 128.4, 128.1, 127.8, 126.0, 124.6, 119.2, 118.8, 58.3, 51.5, 51.1, 48.9, 36.1, 30.0, 18.4. HRMS Calcd for $C_{31}H_{32}F_3N_5O_7S$ m/z 675.1975, found 675.1969.

*5.1.2.5. DW2282-Phe. TFA (**1e**).* The compound (**1e**) was prepared by the same procedure described for **DW**2282-Gly.TFA (**1a**) using DW2282-*N*-Boc-Phe instead of DW2282-*N*-Boc-Gly.

Yield: 75.5%; mp 169.0–170.0 °C; R_f 0.20 (10:1 dichloromethane/methanol); FT-IR (cm^{-1}) 3254, 2359, 1745, 1659, 1383, 1156; ^{1}H NMR (CD_3OD-d_4) δ 3.15–3.52 (m, 4H), 3.60 (q, J = 9.6, 6.0 Hz, 1H), 4.19 (t, J = 8.4 Hz, 2H), 4.24 (t, J = 7.2 Hz, 1H), 4.31 (t, J = 9.2 Hz, 1H), 4.79 (q, J = 8.8, 6.0 Hz, 1H), 7.20–7.22 (m, 2H), 7.29–7.36 (m, 9H), 7.60–7.84 (m, 6H); ^{13}C NMR (DMSO-d_6) δ. 174.0, 169.1, 168.5, 164.9, 158.5, 154.7, 147.5, 140.9, 140.3, 135.3, 134.7, 134.1, 131.7, 130.2, 129.6, 128.9, 128.7, 128.4, 128.1, 127.8, 126.0, 124.6, 119.2, 118.8, 54.4, 52.3, 51.5, 48.9, 37.0, 27.4. HRMS Calcd for $C_{35}H_{32}F_3N_5O_7S$ m/z 723.1975, found 723.1971.

*5.1.2.6. DW2282-Ile. TFA (**1f**).* The compound (**1f**) was prepared by the same procedure described for DW2282-Gly.TFA (**1a**) using DW2282-*N*-Boc-Ile instead of DW2282-*N*-Boc-Gly.

Yield: 62.3%; mp 114.0–115.0 °C; R_f 0.22 (10:1 dichloromethane/methanol); FT-IR (cm^{-1}) 3254, 2359, 1724, 1667, 1381, 1156; ^{1}H NMR (CD_3OD-d_4) δ 1.00 (m, 6H), 1.10 (d, J = 6.8 Hz, 3H), 1.28 (m, 1H), 1.65 (m, 1H), 2.05 (m, 1H), 3.20 (m, 2H), 3.60 (q, J = 9.6, 6.0 Hz, 1H), 3.86 (d, J = 5.6 Hz, 1H), 4.20 (t, J = 8.0 Hz, 2H), 4.31 (t, J = 9.6 Hz, 1H), 4.79 (q, J = 8.8, 6.0 Hz, 1H), 7.20–7.22 (m, 2H), 7.30–7.37 (m, 3H), 7.63–7.65 (m, 2H), 7.77–7.80 (m, 3H), 7.85 (s, 1H); ^{13}C NMR (DMSO-d_6) δ. 174.4, 169.3, 168.6, 168.1, 158.6, 154.7, 147.8, 141.0, 140.3, 134.3, 131.9, 131.6, 128.9, 128.4, 128.1, 127.8, 126.0, 124.6, 119.2, 118.8, 57.6, 52.3, 51.5, 51.1, 36.4, 27.5, 24.0, 14.7, 11.0. HRMS Calcd for $C_{32}H_{34}F_3N_5O_7S$ m/z 689.2131, found 689.2125.

*5.1.2.7. DW2282-Leu. TFA (**1g**).* The compound (**1g**) was prepared by the same procedure described for **DW**2282-Gly.TFA (**1a**) using **DW**2282-*N*-Boc-Leu instead of **DW**2282-*N*-Boc-Gly.

Yield: 93.0%; mp 142.0–143.0 °C; R_f 0.28 (10:1 dichloromethane/methanol); FT-IR (cm^{-1}) 3262, 2360, 1724, 1654, 1381, 1156; ^{1}H NMR (DMSO-d_6) δ 0.95(d, J = 3.6 Hz, 6H), 1.69 (m, 3H), 3.17 (t, J = 8.0 Hz, 2H), 3.51 (q, J = 8.8, 6.4 Hz 1H), 3.96 (m, 1H), 4.13 (t, J = 8.0 Hz, 2H), 4.28 (t, J = 9.2 Hz, 1H), 4.80 (t, J = 7.2 Hz, 1H), 7.23–7.25 (m, 2H), 7.31–7.40 (m, 3H), 7.66–7.68 (m, 2H), 7.73–7.81(m, 4H), 8.28 (s, 2H), 10.76 (s, 1H); ^{13}C NMR (DMSO-d_6) δ. 176.6, 169.3, 168.6, 162.5, 158.2, 154.7, 147.7, 140.9, 140.3, 134.2, 131.7, 131.6, 128.9, 128.5, 128.1, 127.7, 126.0, 124.5, 119.5, 119.2, 52.3, 51.9, 51.5, 51.2, 35.8, 30.7, 22.6, 21.8. HRMS Calcd for $C_{32}H_{34}F_3N_5O_7S$ m/z 689.2131, found 689.2126.

*5.1.2.8. DW2282-Met. TFA (**1h**).* The compound (**1h**) was prepared by the same procedure as described for the preparation of DW2282-Gly.TFA (**1a**) using DW2282-*N*-Boc-Met instead of **DW**2282-*N*-Boc-Gly.

Yield: 75.5%; mp 147.5–148.5 °C; R_f 0.20 (10:1 dichloromethane/methanol); FT-IR (cm^{-1}) 3254, 2359, 1724, 1665, 1382, 1156; ^{1}H NMR (CD_3OD-d_4) δ 1.87–2.10 (m, 2H), 2.08 (s, 3H), 2.62 (m, 2H), 3.20 (m, 2H), 3.60 (m, 2H), 4.20 (t, J = 8.4 Hz, 2H), 4.31 (t, J = 9.6 Hz, 1H), 4.55 (s, 1H), 4.76 (m, 1H), 7.20–7.22 (m, 3H), 7.30–7.34 (m, 3H), 7.60–7.62 (m, 2H), 7.76–7.84 (m, 5H); ^{13}C NMR (DMSO-d_6) δ. 178.0, 169.2, 168.6, 167.6, 162.4, 154.7, 147.7, 140.9, 140.3, 134.3, 131.8, 131.6, 128.9, 128.5, 128.1, 127.7, 126.0, 124.6, 119.5, 112.9, 52.6, 52.3, 51.5, 51.1, 35.8, 30.7, 28.3, 14.5. HRMS Calcd for $C_{31}H_{32}F_3N_5O_7S_2$ m/z 707.1695, found 707.1691.

*5.1.2.9. DW2282-Cys. TFA (**1i**).* DW2282-*N*-Boc-*S*-trityl-L-Cys (0.5 g, 0.56 mmol) was dissolved in trifluoroacetic acid/dichloromethane (20mL + 40 mL) and stirred at room temperature for 3 h, the color of the solution turned brown. Triethylsilane was added until the solution became colorless. Stirring was continued for 1 h. After removal of solvent, the solid residue was washed several times with n-hexane to remove most of the triphenylmethane. Then, the solid was dissolved in a minimal amount of methanol and excess of diethyl ether. The precipitate was collected and washed with dry diethyl ether to give the compound (**1i**).

Yield: 92.6%; mp 174.0–175.0 °C; R_f 0.30 (10:1 dichloromethane/methanol); FT-IR (cm^{-1}) 3105, 2360, 1673, 1383, 1157; ^{1}H NMR (DMSO-d_6) δ 1.39 (s, 1H), 3.06 (m, 1H), 3.16 (m, 3H), 3.50 (q, J = 8.8,

6.8 Hz, 1H), 4.12–4.18 (m, 3H), 4.28 (t, J = 8.8 Hz, 1H), 4.78 (t, J = 7.8 Hz, 1H), 7.23–7.37 (m, 6H), 7.62–7.80 (m, 7H), 8.20 (s, 1H), 8.43 (s, 1H), 10.78 (s, 1H); ^{13}C NMR (DMSO-d_6) δ. 177.9, 169.3, 168.5, 164.9, 157.8, 154.7, 147.5, 141.9, 140.8, 135.3, 134.2, 131.7, 128.8, 128.4, 128.1, 127.8, 126.0, 124.6, 119.3, 116.0, 52.3, 51.5, 51.1, 48.9, 27.4, 20.7. HRMS Calcd for $C_{29}H_{28}F_3N_5O_7S_2$ m/z 679.1382, found 679.1375.

*5.1.2.10. DW2282-Asp. TFA (**1j**).*

DW2282-*N*-Boc-L-Asp(OBzl)-OH (0.5 g, 0.65 mmol) was dissolved in trifluoroacetic acid/dichloromethane (20 mL + 40 mL) and stirred at room temperature for 3 h. After removal of solvent, the solid residue was washed several times with diethyl ether. Then, the solid was dissolved in methanol (30 mL), 10% Pd/C (about 50 mg) was added, and the mixture was hydrogenated at room temperature using a hydrogen balloon. After 2 h, the mixture was filtered through Celite and evaporated under vacuum. Then, the solid was dissolved in a minimal amount of methanol and excess of diethyl ether. The precipitate was collected and washed with dry diethyl ether to get the compound (**1j**).

Yield: 68.8%; mp 164.0–165.0 °C; R_f 0.24 (10:1 dichloromethane/methanol); FT-IR (cm^{-1}) 3254, 2360, 1742, 1665, 1383, 1157; ^{1}H NMR (DMSO-d_6) δ 2.82–3.02 (m, 2H), 3.22 (m, 2H), 3.51 (q, J = 9.2, 6.4 Hz, 1H), 4.13 (t, J = 8.4 Hz, 2H), 4.28 (m, 2H), 4.80 (t, J = 7.6 Hz, 1H), 7.23–7.40 (m, 5H), 7.65–7.95 (m, 6H), 8.28 (s, 1H), 10.72 (s, 1H); ^{13}C NMR (DMSO-d_6) δ. 177.8, 171.2, 169.2, 168.3, 164.7, 157.8, 154.5, 147.5, 140.7, 140.1, 134.0, 131.8, 131.4, 128.6, 128.4, 128.1, 127.8, 126.0, 125.1, 119.1, 118.5, 52.8, 51.3, 49.8, 48.9, 38.9, 35.3. HRMS Calcd for $C_{30}H_{28}F_3N_5O_9S$ m/z 691.1560, found 691.1553.

*5.1.2.11. DW2282-Ser. HCl (**1k**).*

DW2282-*N*-Fmoc-*O*-*tert*-Bu-L-Ser (0.5 g, 0.60 mmol) was dissolved in piperidine/dichloromethane (20mL + 80 mL) and stirred at room temperature for 3 h. After removal of solvent, the mixture was dissolved in 4-*N* hydrochloric acid/dioxane (50mL + 50 mL) and refluxed for 3 h. After removal of solvent, chromatography of crude product on silica gel (dichloromethane:methanol = 10:1) gave the compound **(1k)** as a white solid.

Yield: 36.7%; mp 152.3–153.0 °C; R_f 0.28 (10:1 dichloromethane/methanol); FT-IR (cm^{-1}) 3119, 2803, 2358, 1702, 1665, 1382, 1156; ^{1}H NMR (CD_3OD-d_4) δ 3.21 (m, 2H), 3.60 (q, J = 9.6, 6.0 Hz, 1H), 4.01 (m, 2H), 4.10 (m, 1H), 4.20 (t, J = 8.4 Hz, 2H), 4.31 (t, J = 9.6 Hz, 1H), 4.80 (m, 1H), 7.20–7.23 (m, 2H), 7.30–7.34 (m, 4H), 7.61–7.64 (m, 2H), 7.70–7.85 (m, 4H); ^{13}C NMR (DMSO-d_6) δ. 178.1, 169.0, 168.5, 164.8, 154.5, 148.9, 140.7, 140.3, 134.0, 131.6, 131.4, 128.9, 128.4, 128.1, 127.8, 126.0, 125.1, 119.0, 110.8, 79.9, 76.0, 52.3, 44.3, 32.8, 27.1. HRMS Calcd for $C_{29}H_{28}F_3N_5O_8S$ m/z 663.1611, found 663.1607.

5.2. Procedure for the measurement of water solubility of **1a–k**

Standard sample solutions of 1 and **1a–k** were prepared by dissolving sample **1** and **1a–k** (1 mg) in HPLC grade methanol (10 mL) and the absorbance was measured [27]. Saturated solutions were prepared by adding **1** and **1a–k** (1 mg) in water (250 μL). The mixture was vortexed for one hour at room temperature and then filtered using 0.45 μm syringe filter. Absorbance of saturated water solutions of **1** and **1a–k** was measured. Solubility of **1** and **1a–k** was calculated using equation (1).

$$C' = A' \times C/A \quad (1)$$

where C = concentration of standard solution (mg/mL), A = absorbance of standard solution, A' = absorbance of saturated sample solution, C' = concentration of water solution of **1** and **1a–k** (mg/mL).

5.3. Procedure for in vitro plasma reconversion kinetics studies of **1a–k**

Human plasma was obtained from Sigma (P9523). The stock solution (1 mg/mL) of compounds **1a–k** in dimethylsulfoxide (DMSO) was diluted 10 times with the plasma and the mixture was maintained at 37 ± 0.5 °C in a Precision shaking water bath. No plasma protein precipitation was observed at this concentration of DMSO. The plasma was sampled at selected times (0, 0.5, 1, 2, 4, 8 and 24 h), and then it was later subjected to the sample preparation method described below and then analyzed with the modified HPLC assay (Table 3).

Sample Preparation for RPHPLC: Plasma samples (40 μL) were diluted with a sufficient volume (3 mL) of methylene chloride to precipitate the plasma proteins as well as to extract the compounds of interest. After vortexing for 5 min and centrifuging at 3000 × g for 10 min, the organic layer was carefully taken out and dried. The plasma extracts were reconstituted in 160 μL of methanol with vortexing. After syringe filtration (0.45 μm, Waters), the filtrate was analyzed using RPHPLC.

5.4. Procedure for the in vivo anti-tumor activity of **1e**, **1g** and **1j**

Each syngeneic host mice were intradermally implanted with SW620 tumor cells on day zero. When tumor volume reached proper size, thirty animals were distributed into five groups. Compounds **1e**, **1g** and **1j** (70 mg/kg, b.i.d., 5 days, p.o.) were fed to the animals from the following day after grouping. Compound **1** (70 mg, every other day, 3 times, p.o.) was used as a positive control. Control group received vehicle only. Anti-tumor activities were evaluated by comparing the tumor volume of treated group with that of control group. Tumor diameter was serially measured with vernier calipers and tumor volume (V) was calculated as follows:

$V = ½ \times a \times b^2$ [a: long axis (mm), b: short axis (mm)]

Tumor wet weight of treated (T_w) and control (C_w) group was measured at the day 10 of each experiment and the percentage of tumor growth inhibition was calculated as follows:

Inhibition rate (%) = $[1 - (T_w/C_w)] \times 100$

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2014.04.048.

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