J Ind Microbiol Biotechnol (2012) 39:1563–1568
DOI 10.1007/s10295-012-1157-2

SHORT COMMUNICATION

Biological activities of an engineered tautomycetin analogue via disruption of *tmcR*-encoding hydroxylase in *Streptomyces* sp. CK4412

Dongju Kim · Ji-Hye Nah · Si-Sun Choi · Hwa Sung Shin · David H. Sherman · Eung-Soo Kim

Received: 14 April 2012 / Accepted: 31 May 2012 / Published online: 26 June 2012
© Society for Industrial Microbiology and Biotechnology 2012

Abstract Tautomycetin (TMC), originally isolated from *Streptomyces griseochromogenes,* has been reported to possess biological functions including T cell-specific immunosuppressive and anticancer activities through a mechanism of differential inhibition of protein phosphatases such as PP1, PP2A, and SHP2. Independently isolated *Streptomyces* sp. CK4412 was also reported to produce a structurally identical TMC compound. Previously, we isolated and characterized the entire TMC biosynthetic gene cluster from *Streptomyces* sp. CK4412. In silico database comparison revealed a 1,359-bp *tmcR* as a putative bacterial Cytochrome P450 hydroxylase gene in the TMC biosynthetic gene cluster. Through targeted gene disruption and complementation, the *tmcR* mutant was confirmed to produce a C5-deoxy-TMC, the same analogue produced by the *S. griseochromogenes ttnI* mutant, implying that TmcR behaves as a regiospecific C5-oxygenase in the TMC biosynthetic pathway in *Streptomyces* sp. CK4412. In particular, the C5-deoxy-TMC from the *tmcR* mutant exhibited 3.2-fold higher inhibition activity toward SHP2 with significantly reduced inhibition activities toward PP1, and human Vero and lung cancer cells. These results suggested that C5 regiospecific modification of the TMC polyketide moiety may result in a drug development target for use in preferentially enhancing immunosuppressive activity while minimizing its undesirable biological activities.

Keywords Tautomycetin · *Streptomyces* · *tmcR* · P450 hydroxylase · Immunosuppressant

Introduction

Tautomycetin (TMC) is an unusual linear secondary metabolite with a structurally unique ester bond linkage between a terminal cyclic anhydride moiety and a linear polyketide chain. It was originally isolated from *Streptomyces griseochromogenes* for use as a new antifungal antibiotic ([8], Fig. 1). *Streptomyces* sp. CK4412, independently isolated from Jeju Island, Korea, produces the same TMC compound with a new mode of pharmacological action, and functions as an activated T cell-specific immunosuppressant as demonstrated in both in vivo and in vitro studies [16]. TMC was proposed to specifically block tyrosine phosphorylation of intracellular signal mediators downstream of Src tyrosine kinases in a T cell-specific manner via selective inhibition of protein phosphatase 1 (PP1) and PP2A, leading to apoptosis resulting from the cleavage of Bcl-2, caspase-9 and caspase-1 [16]. TMC was also reported to inhibit growth of colorectal cancer cells through p21$^{cip/WAF1}$ induction via the extracelluar signal-regulated kinase pathway [9] and suppress the growth of medullary thyroid cancer cells via inhibition of glycogen synthase kinase [1], implying that TMC is a potentially valuable natural product containing multiple biological activities. In particular, the Src homology-2 domain containing protein tyrosine phosphatase-2 (SHP2) was recently

Electronic supplementary material The online version of this article (doi:10.1007/s10295-012-1157-2) contains supplementary material, which is available to authorized users.

D. Kim · J.-H. Nah · S.-S. Choi · H. S. Shin · E.-S. Kim (✉)
Department of Biological Engineering,
Inha University, Incheon 402-751, Korea
e-mail: eungsoo@inha.ac.kr

D. H. Sherman
Life Sciences Institute and Departments of Medicinal Chemistry, Chemistry, and Microbiology and Immunology, University of Michigan, Ann Arbor, MI 48109-2216, USA

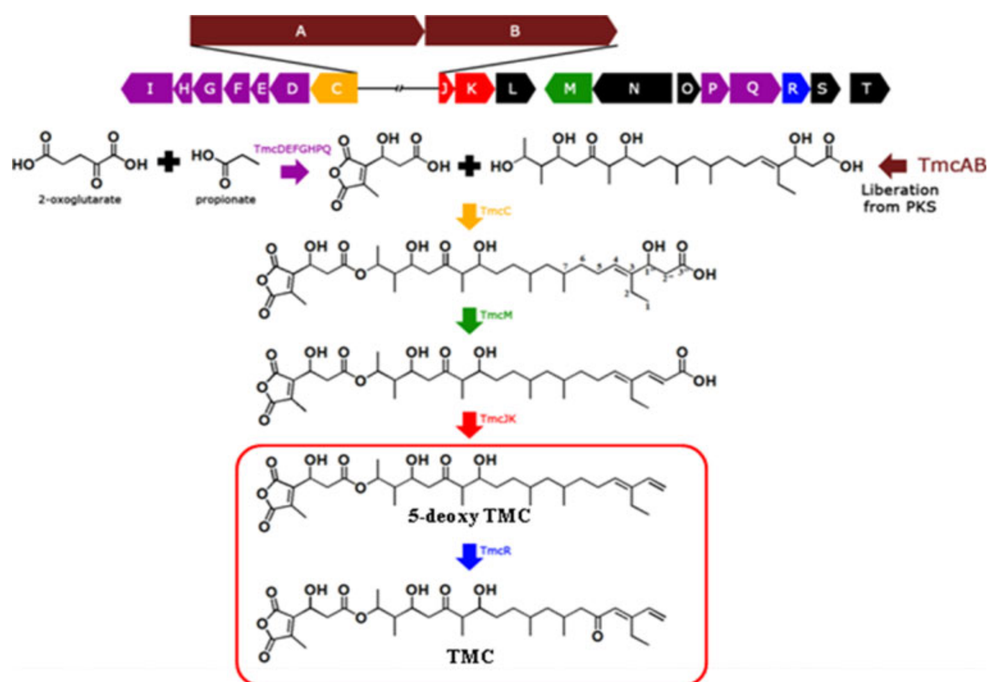

Fig. 1 Proposed steps of the post-PKS modification in the TMC biosynthetic pathway. The anhydride moiety is synthesized by TmcDEFGHPQ (*purple arrow*) and linear polyketide is liberated from TmcB-TE after synthesis by TmcAB (*brown arrow*). Esterification between anhydride moiety and linear polyketide is believed to be catalyzed by TmcC (*yellow arrow*), the following tailoring steps such as dehydration, decarboxylation, and oxidation are catalyzed by TmcM (*green arrow*), TmcJK (*red arrow*), and TmcR (*blue arrow*), respectively. *Red box* highlights the C5 oxidation step carried out by TmcR (color figure online)

proposed to be a major target of the immunosuppressive activity of TMC [11].

Previously, we isolated and characterized the entire TMC biosynthetic and regulatory pathway gene cluster from *Streptomyces* sp. CK4412 and demonstrated its identity by gene disruption and complementation analysis [5, 7]. The TMC biosynthetic gene cluster revealed two modular polyketide synthetase (PKS) genes as well as 18 ORFs located at both flanking regions, the deduced functions of which were consistent with TMC biosynthesis. The TMC biosynthetic gene cluster isolated from *S. griseochromogenes* was later proven to be nearly identical to that previously characterized in *Streptomyces* sp. CK4412 [10]. Moreover, a global antibiotic down-regulatory gene, *S. coelicolor wblA* ortholog (*wblA*$_{tmc}$) was also isolated from *Streptomyces* sp. CK4412, and proven to play a critical role in the TMC biosynthetic regulatory cascade system through differential expression of two TMC pathway-specific regulatory genes, *tmcT*, and *tmcN* [14].

Tautomycetin is known to be the most selective PP1 inhibitor, showing nearly a 40-fold preference over PP2A, and unlike the structurally similar non-immunosuppressive tautomycin, the observed PP1 inhibition selectivity associated with TMC is believed to be a key factor in its immunosuppressive activity [12]. However, SHP2 was recently proposed to be a putative target of TMC leading to immunosuppression because TMC was observed to block T cell receptor–mediated tyrosine phosphorylation [11]. Attempts to generate several TMC mutants via post-PKS tailoring modification of genes in *S. griseochromogenes* [8, 12] and linear polyketide-specific thioesterase domain characterization in *Streptomyces* sp. CK4412 [15] revealed how the multiple biological activities of TMC correlated to specific moieties in its structure. Here, we characterized a *Streptomyces* sp. CK4412 post-PKS gene, *tmcR* encoding a putative bacterial Cytochrome P450 hydroxylase involved in regiospecific C5-oxygenase in TMC. The significantly altered biological activities of the engineered TMC analogue produced by the *Streptomyces* sp. CK4412 *tmcR*-disrupted mutant strain suggested that the C5 regiospecific modification of the TMC polyketide moiety is a potential target for the enhancement of immunosuppressive activity while leaving other undesirable biological activities minimized.

Materials and methods

Bacterial strains and culture conditions

The *Escherichia coli* DH5α strain was used as a host for the construction of the recombinant plasmids. *E. coli* ET12567/pUZ8002 was used as donor strain in the conjugation experiments. All *E. coli* strains were cultured at 37 °C in LB broth, or on LB agar supplemented with the appropriate antibiotics as needed. *Streptomyces* sp. CK4412 was used as a TMC-producing strain [5]. For production of TMC and its analogues, *Streptomyces* sp. CK4412 wild-type and its mutants were cultured for 6 days in MS solid medium [5].

Gene disruption and complementation of *tmcR*

The *tmcR* was inactivated using a PCR targeted gene disruption system [6]. Briefly, an apramycin resistance gene (*aac(3)IV)/oriT* cassette was used to replace an internal region of *tmcR*. This cassette was introduced into *E. coli* BW25113/pIJ790 containing pTMC2290, thus forming pTMC2290*ΔtmcR* whereby *tmcR* was replaced by the *aac(3)IV/oriT*. The pTMC2290*ΔtmcR* cosmid was introduced into *Streptomyces* sp. CK4412 by conjugation with *E. coli* ET12567/pUZ8002. *Streptomyces* sp. CK4412 spores (conjugation recipient) were mixed with *E. coli* ET12567/pUZ8002 harboring the pTMC2290*ΔtmcR* (conjugation donor) and then spread onto modified ISP-4 medium plates. After incubation at 28 °C for 16 h, each plate was overlaid with 1 ml of sterile water containing apramycin and nalidixic acid at final concentrations of 50 and 25 µl/ml until exconjugants appeared. The desired double crossover mutants, selected by their apramycin-resistant and kanamycin-sensitive phenotype, were isolated as *Streptomyces* sp. CK4412-006 to form the *tmcR*-disrupted strain. Their genotypes were verified by PCR analysis.

For complementation of the *ΔtmcR* mutant, the entire *tmcR* was amplified by PCR using pTMC2290 as a template cosmid. The amplified PCR product was analyzed by electrophoresis in 1 % (w/v) agarose gel, purified via a DNA extraction kit, and ligated into pGEM®-T Easy vectors. The ligated vector was completely sequenced in order to ensure its integrity (Macrogen, Korea). The *tmcR* fragment, digested with *Bgl*II and *Xba*I, was cloned into pSET152 [4] and followed by spectinomycin resistance gene cloning into the *BamH*I and *Xba*I restriction sites of pSET152 in order to yield pSETSPE*tmcR*. Conjugation of pSETSPE*tmcR* into the mutants strain CK4412-006 produced strain CK4412-006/*tmcR,* which was complemented by the constitutive expression of a functional copy of *tmcR* under the control of the e*rmE** promoter. All primer sequences are listed in the supplementary Table 1.

Cell culture and structural confirmation of TMC analogues from each mutant

For HPLC analysis of the TMC and TMC analogues production, *Streptomyces* sp. CK4412, CK4412-006 and CK4412-006/*tmcR* were streaked onto MS solid medium and incubated at 28 °C for 6 days. The MS solid medium was blended with an equal volume of distilled water (pH 4.0 with 10 N HCl), centrifuged, and then extracted twice with an equal volume of ethyl acetate. The combined ethyl acetate extracts were concentrated using a rotary evaporator and dissolved in MeOH, filtered through a 0.2 µm filter, and subjected to HPLC analysis. Analytical HPLC was carried out on a Genesis C18 5 µm column at a flow rate of 1 ml/min and UV detection at 273 nm. The identity of TMC and TMC analogues produced by the wild-type *Streptomyces* sp. CK4412 and CK4412-006 were confirmed using LC–MS. For purification of TMC and TMC analogues, semi-preparative HPLC was carried out on an X-bridge C18 5 µm at a flow rate of 3 ml/min and monitored by UV detection at 273 nm.

Protein phosphatase inhibition assays

The protein phosphatase inhibition assays were carried out in 96-well multiwell plates. The assay included three groups including the sample (TMC and TMC analogues), positive control, and blank. Each treatment was performed in duplicate. The reaction mixture contained 30 µl of H_2O, 10 µl of PMP buffer (New England BioLabs), 10 µl of 1 mM $MnCl_2$ and either 4 µl of PP1 enzyme (0.1 U per assay, New England BioLabs), PP2A enzyme (0.1 U per assay, Millipore) or GST-fused SHP2 (0.1 U per assay, expressed and purified in *E. coli* BL21, supplementary Fig. 1) and were added into 96-well plates and pre-warmed at 37 °C for 10 min. The 1 mM $MnCl_2$ was used only in the PP1 and PP2A inhibition assays, and the enzyme storage buffer was added into the blank well instead of

Table 1 Strains and plasmids used in this study

Strain	Relevant characteristics	Source
Streptomyces sp.		
CK4412	Wild-type TMC-producing strain	4
CK4412-006	*tmcR*-disrupted strain (replaced with apr^R/*oriT*)	This work
CK4412-006/*tmcR*	*Streptomyces* sp. CK4412-006 (*tmcR*-disruptant) containing pSETSPE*tmcR*	This work
Plasmid		
pTMC2290	Cosmid vector including *tmcR*	4
pTMC2290*ΔtmcR*	pTMC2290 mutant cosmid vector containing *tmcR* disrupted with apr^R/*oriT*	This work
pSETSPE*tmcR*	pSET152-based insertion vector which was replaced the selection marker spectinomycin-resistance (Spe^R) gene including single copy of *tmcR* and constitutive promoter, *ermE**	This work

enzyme. After pre-warming, 10 µl of various concentrations of the TMC and TMC analogues diluted in methanol were added into each well. For the control and blank wells, 10 µl of methanol was added instead of inhibitor. The reaction was initiated by the addition of substrate (100 µm fluorescein diphosphate at final concentration), which was pre-warmed together with the reaction mix at 37 °C. Incubation was conducted at the same temperature for 1 h. The hydrolysis of FDP was determined using fluorescence detection on a microplate reader (Tecan, Switzerland) at 492 nm. The inhibitory activities of the test samples was determined by the activity ratio of the sample to the control and expressed as a percentage (%). The correlation curves were plotted based on the percentage activity of the TMC and TMC analogues relative to their compared concentration. The IC50 value (concentration required to inhibit 50 % of enzyme activity) was calculated [13].

Evaluation of cytotoxicities and antifungal activity

The cell viability assay exploited the use of NCI-H1229 human lung cancer and Vero cell lines. Each cell line was pre-cultured and then ~4,000 cells plated into each well of a 96-well plate. The cells were then incubated at 37 °C under an atmosphere of 5 % CO_2 for 1 day in order to allow the cells to attach in their wells. The TMC and its analogues were dissolved in DMSO, administered into each well and the cells were incubated at same condition for 2 days. MTT (3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) solution was added to each well containing incubated cells. After 4 h of incubation, lysis buffer was added into each well. The resulting absorbance of formazan was determined on a microplate reader at 540 nm. The assay was performed in duplicate. Evaluation of the antifungal activities of the TMC and its analogues was carried out by the agar disc diffusion method. The discs (6-mm diameter) were saturated with 15 µl of the extracts dissolved in methanol and then placed on ME medium inoculated with *Aspergillus niger*. The plates were incubated at 30 °C for 48 h. The diameter of the inhibition zone surrounding the discs, resulting from the diffusion of bacterial compounds, was then measured.

Results and discussion

Construction of *tmcR* disruption mutant, *Streptomyces* sp. CK4412-006

Previously, we reported that a 1,350-bp *tmcR* may encode a TMC-specific oxygenases due to their chromosomal location within *tmc* cluster, as well as identified its homology to

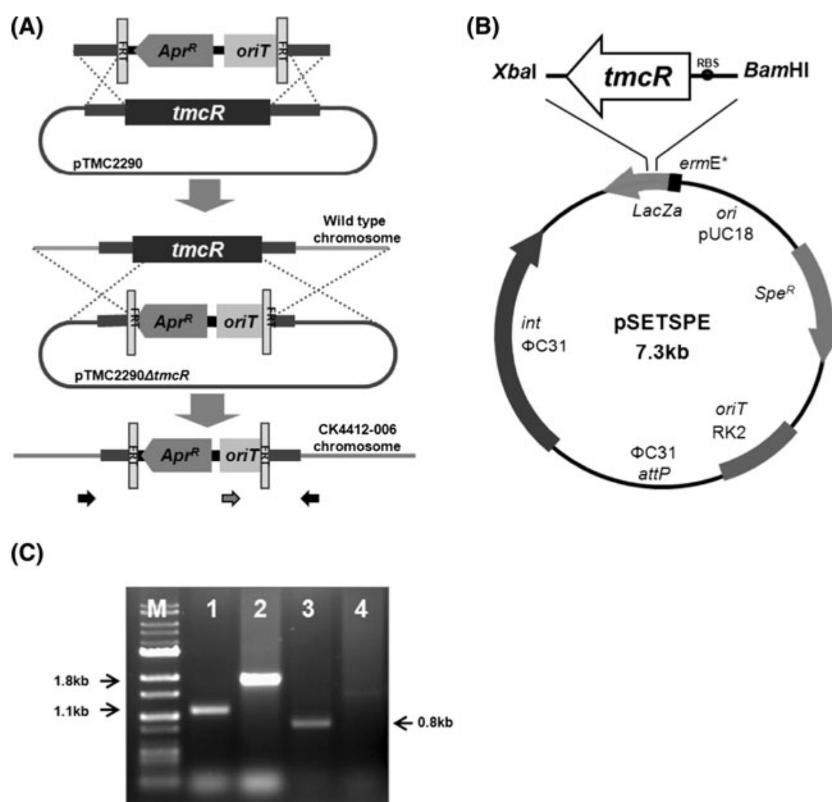

Fig. 2 **a** Schematic representation of PCR targeted *tmcR* disruption and apramycin-resistance (apr^R)/*oriT*. **b** Construct of integrative plasmid for complementation of △*tmcR*. **c** Confirmation of constructed △*tmcR* mutant. *Lanes*: *M*, 1 kb ladder; *1* and *4*, *Streptomyces* sp. CK4412 wild-type genomic DNA; *2* and *3*, *Streptomyces* sp. CK4412-006 genomic DNA; *1* and *2*, *tmcR* check F and R primer (CHtmcR-F and R); *3* and *4*, *oriT* and *tmcR* check R primers (CHtmcR-R)

other bacterial Cytochrome P450 hydroxylase (CYP) genes [5]. Characterization of the *tmcR* gene product, via database-assisted in silico analysis, revealed that it encodes protein that comprises 449 a.a, and shows 44 % identity to CYP in *Mycobacterium vanbaalenii* [3]. In order to confirm the in vivo function of *tmcR,* inactivation of *tmcR* was performed and confirmed by PCR analysis (Fig. 2a, c). The expected size of the PCR amplified bands was observed in the genomic DNA samples isolated from both *Streptomyces* sp. CK4412 (1.1 kb) and the *tmcR* mutant named CK4412-006 (1.8 kb) as shown in Fig. 2c. Using an alternative PCR primer pair designed to detect an apramycin resistance gene/*oriT* cassette, the expected size (0.8 kb) of the PCR amplified fragment was observed only in *Streptomyces* sp. CK4412-006 (Fig. 2c), implying that the *tmcR* was specifically disrupted as expected.

Functional complementation and structural confirmation of TMC analogue produced by *Streptomyces* sp. CK4412-006

In order to prove that the inactivation of *tmcR* was responsible for TMC production, we performed genetic complementation of *Streptomyces* sp. CK4412-006 by expressing *tmcR* under the control of the e*rmE** promoter (Fig. 2b). For this purpose, we used an integrating conjugative vector pSET152 [2] into which the coding region of *tmcR* was cloned, resulting in pSETSPE*tmcR* (Fig. 2b). Both HPLC and the antifungal bioassay results confirmed that TMC productivity was restored to significant levels in the *Streptomyces* sp. CK4412-006 mutant strain carrying pSETSPE*tmcR* (Fig. 3a), implying that the absence of TMC in the *Streptomyces* sp. CK4412-006 strain was due to the lack of TMC-specific CYP gene, *tmcR*.

The *Streptomyces* sp. CK4412-006 culture was further examined in order to detect any structurally related TMC analogues. While no TMC production was observed in the *tmcR*-deleted CK4412-006 culture, new peaks with a different HPLC retention time showing a UV spectrum similar to TMC was detected in the *tmcR*-deleted CK4412-006 culture. Through LC–MS analysis (Supplementary Fig. 2), these new peaks were determined to be C5-deoxy-TMC (Fig. 1). This structural elucidation supported the conclusion that *tmcR* encodes a functional TMC C5-specific CYP enzyme in *Streptomyces* sp. CK4412, a result which correlated well with the recently reported analysis of *ttnI (tmcR* counterpart)-deleted *S. griseochromogenes* mutant [17].

Biological activities of C5-deoxy-TMC produced in CK4412-006

Fermentation broths of *Streptomyces* sp. CK4412 and CK4412-006 grown under conditions optimal for TMC production were extracted with ethyl acetate, and followed by an antifungal bioassay and HPLC quantitative analysis for the C5-deoxy-TMC. Very weak antifungal activity against *A. niger* was detected in extracts of the CK4412-006 strain under the same concentration with TMC, implying that the regiospecific TMC modification of 5-deoxydation dramatically reduced antifungal activity (Fig. 3b).

Since TMC was reported to be the most selective PP1 inhibitor, displaying nearly a 40-fold preference over PP2A [12], PP1 inhibition assay was performed in order to

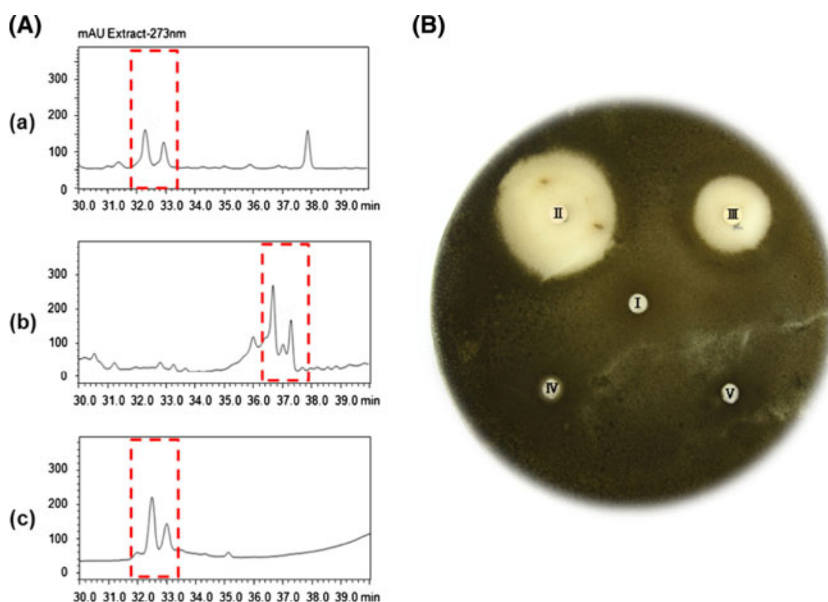

Fig. 3 **A** HPLC chromatograms of metabolite profiles from *Streptomyces* sp. CK4412 wild-type and recombinant strains: **a** *Streptomyces* sp. CK4412 wile-type, **b** CK4412-006, **c** CK4412-006/*tmcR*. **B** Antifungal activities of TMC and TMC analogues in 1 mM concentration: (*I*) Methanol, (*II*) TMC, (*III*) 5-deoxy-TMC, (*IV*) 3′-dehydroxy-TMC (unpublished data), and (*V*) 1″-dehydroxy-carboxylic TMC (unpublished data)

Table 2 Summary of in vitro PP inhibition and cytotoxicity data (IC_{50} in μm) for TMC and 5-deoxy-TMC against various protein phosphatases, human lung cancer cell (NCI-H1299) and Vero cell

	TMC	5-deoxy-TMC	TMC/5-deoxy-TMC
PP1	$3.2 \pm 0.3 \times 10^{-3}$	$10.2 \pm 0.4 \times 10^{-3}$	1:3.18
PP2A	$136.5 \pm 0.8 \times 10^{-3}$	$38.3 \pm 0.5 \times 10^{-3}$	1:0.28
SHP2	3.23 ± 0.03	0.9 ± 0.07	1:0.27
NCI-H1299	4.04 ± 0.06	28.7 ± 0.03	1:7.10
Vero	0.68 ± 0.01	2.01 ± 0.02	1:2.95

evaluate biological activity change of the engineered analogue, C5-deoxy-TMC, produced by *Streptomyces* sp. CK4412-006. The IC_{50} values of PP1 inhibition activity associated with the TMC and C5-deoxy-TMC as measured in this study were 0.003 μm and 0.010 μm, respectively (Table 2), with the result that the IC_{50} value for C5-deoxy-TMC inhibition against PP1 increased about 3.4-fold as compared the TMC. However, the IC_{50} values of PP2A inhibition activity by C5-deoxy-TMC decreased from 0.136 to 0.038 μm, resulting in selectivity for PP1 over PP2A with just 3.7-fold preference with C5-deoxy-TMC (Table 2).

Interestingly, the most important indicator for SHP2 inhibition activity, the IC_{50} value, improved by 3.5-fold in the C5-deoxy-TMC compared to the original TMC (Table 2). Moreover, the cytotoxicity assays using both TMC and C5-deoxy-TMC revealed that the latter exhibited approximately 7-fold and 3-fold reduced activities toward lung cancer and Vero cells, respectively (Table 2). These results suggest that C5 regiospecific modification of the TMC polyketide moiety produces a novel drug candidate, preferentially enhancing immunosuppressive activity while leaving other undesirable biological activities minimized.

Acknowledgments This work was supported by grant No. D00040 from the Global Research Network (GRN) Program of the National Research Foundation of Korea.

References

1. Adler JT, Cook M, Luo Y (2009) Tautomycetin and tautomycin suppress the growth of medullary thyroid cancer cells via inhibition of glycogen synthase kinase-3*β*. Mol Cancer Ther 8:914–920
2. Bierman M, Logan R, O'Brien K, Seno ET, Nagaraja Rao R, Schoner BE (1992) Plasmid cloning vectors for the conjugal transfer of DNA from *Escherichia coli* to *Streptomyces* spp. Gene 116:43–49
3. Brezna B, Kweon O, Stingley RL, Freeman JP, Khan AA, Polek B, Jones RC, Cerniglia CE (2006) Molecular characterization of cytochrome P450 genes in the polycyclic aromatic hydrocarbon degrading *Mycobacterium vanbaalenii* PYR-1. Appl Microbiol Biotechnol 71:522–532
4. Cheng XC, Kihara T, Ying X, Uramoto M, Osada H, Kusakabe H, Wang BN, Kobayashi Y, Ko K, Yamaguchi I, Shen YC, Isono K (1989) A new antibiotic, tautomycetin. J Antibiot 42:141–144
5. Choi SS, Hur YA, Sherman DH, Kim ES (2007) Isolation of the biosynthetic gene cluster for tautomycetin, a linear polyketide T cell-specific immunomodulator from *Streptomyces* sp. CK4412. Microbiology 153:1095–1102
6. Gust B, Challis GL, Fowler K, Kieser T, Chater KF (2003) PCR-targeted *Streptomyces* gene replacement identifies a protein domain needed for biosynthesis of the sesquiterpene soil odor geosmin. Proc Natl Acad Sci USA 100:1541–1546
7. Hur YA, Choi SS, Sherman DH, Kim ES (2008) Identification of TmcN as a pathway-specific positive regulator of tautomycetin biosynthesis in *Streptomyces* sp. CK4412. Microbiology 154: 2912–2919
8. Ju J, Li W, Yuan Q, Peters NR, Hoffmann FM, Rajski SR, Osada H, Shen B (2009) Functional characterization of *ttmM* unveils new tautomycin analogs and insight into tautomycin biosynthesis and activity. Org Lett 11:1639–1642
9. Lee JH, Lee JS, Kim SE, Moon BS, Kim YC, Lee SK, Lee SK, Choi KY (2006) Tautomycetin inhibits growth of colorectal cancer cells through $p21^{cip/WAF1}$ induction via the extracellular signal-regulated kinase pathway. Mol Cancer Ther 5:3222–3231
10. Li W, Luo Y, Ju J, Rajski SR, Osada H, Shen B (2009) Characterization of the tautomycetin biosynthetic gene cluster from *Streptomyces griseochromogenes* provides new insight into dialkylmaleic anhydride biosynthesis. J Nat Prod 72:450–459
11. Liu S, Yu Z, Yu X, Huang SX, Luo Y, Wu L, Shen W, Yang Z, Wang L, Gunawan AM, Chan RJ, Shen B, Zhang ZY (2011) SHP2 is a target of the immunosuppressant tautomycetin. Chem Biol 18:101–110
12. Luo Y, Li W, Ju J, Yuan Q, Peters NR, Hoffmann FM, Huang SX, Bugni TS, Rajski S, Osada H, Shen B (2010) Functional characterization of TtnD and TtnF, unveiling new insights into tautomycetin biosynthesis. J Am Chem Soc 132:6663–6671
13. Mountfort DO, Kennedy G, Garthwaite I, Quilliam Michael, Truman P, Hannah DJ (1999) Evaluation of the fluorometric protein phosphatase inhibition assay in the determination of okadaic acid in mussels. Toxicon 37:909–922
14. Nah JH, Park SH, Yoon HM, Choi SS, Lee CH, Kim ES (2012) Identification and characterization of *wblA*-dependent *tmcT* regulation during tautomycetin biosynthesis in *Streptomyces* sp. CK4412. Biotechnol Adv 30:202–209
15. Scaglione JB, Akey DL, Sullivan R, Kittendorf JD, Rath CM, Kim ES, Smith JL, Sherman DH (2010) Biochemical and structural characterization of the tautomycetin thioesterase: analysis of a stereoselective polyketide hydrolase. Angew Chem Int Ed 49:5726–5730
16. Shim JH, Lee HK, Chang EJ, Chae WJ, Han JH, Han DJ, Morio T, Yang JJ, Bothwell A, Lee SK (2002) Immunosuppressive effects of tautomycetin in vivo and in vitro via T cell-specific apoptosis induction. Proc Natl Acad Sci USA 99:10617–10622
17. Yang D, Li W, Huang SX, Shen B (2012) Functional characterization of *ttnI* completing the tailoring steps for tautomycetin biosynthesis in *Streptomyces griseochromogenes*. Org Lett 14:1302–1305