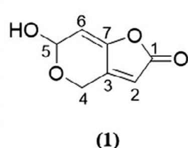
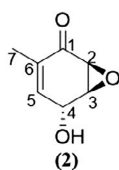
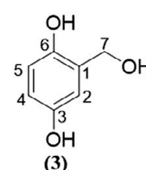
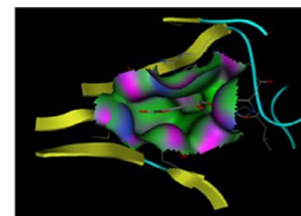
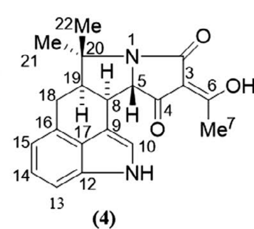
cogent chemistry

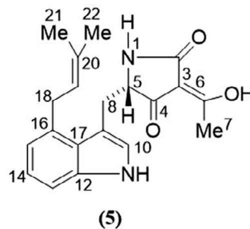


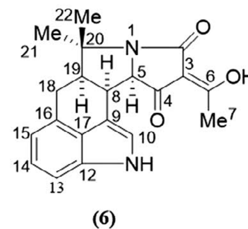


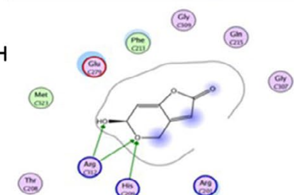












MEDICINAL CHEMISTRY | RESEARCH ARTICLE

Small-molecule inhibitors of the tuberculosis target, phenylalanyl-tRNA synthetase from Penicillium griseofulvum CPCC-400528

Li-Ning Wang, Wen-Jing Di, Zhi-Ming Zhang, Li-li Zhao, Tao Zhang, Yan-Ru Deng and Li-Yan Yu

Cogent Chemistry (2016), 2: 1181536

cogent chemistry

MEDICINAL CHEMISTRY | RESEARCH ARTICLE

Small-molecule inhibitors of the tuberculosis target, phenylalanyl-*t*RNA synthetase from *Penicillium griseofulvum* CPCC-400528

Li-Ning Wang[1,2,§], Wen-Jing Di[2,§], Zhi-Ming Zhang[1], Li-li Zhao[1], Tao Zhang[1], Yan-Ru Deng[2] and Li-Yan Yu[1*]

Received: 22 February 2016
Accepted: 19 April 2016
Published: 11 May 2016

*Corresponding author: Li-Yan Yu, Institute of Medicinal Biotechnology, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100050, China
E-mail: yly@cpcc.ac.cn

Reviewing editor:
John Spencer, University of Sussex, UK

Additional information is available at the end of the article

Abstract: Phenylalanyl-tRNA synthetase (PheRS), a member of aminoacyl-tRNA synthetase family, was the new target of anti-tubercular drug discovery. In an attempt to fully exploit the new potential anti-tuberculosis drugs presented in micro-organisms, we developed a high-throughput screening assay against *Mycobacterium tuberculosis* (*Mtb*) PheRS and then screened a library consisting of 32,000 strains and 1500 natural product-derived compounds. One potent hit extract of *Penicillium griseofulvum* CPCC-400528 was identified. In this study, isopatulin (**1**), (+)-epiepoformin (**2**) and gentisyl alcohol (**3**), three patulin-producing intermediates, together with three indole-tetramic acids, α-cyclopiazonic acid (**4**), β-cyclopiazonic acid (**5**) and iso-α-cyclopiazonic acid (**6**), were isolated and identified as bioactive constituents from *P. griseofulvum* CPCC-400528. Their structures were elucidated on the basis of spectroscopic data. Compounds **1**, **3**, **4**, and **5** exhibited *Mtb* PheRS-inhibiting activities, as well as moderate to weak anti-tuberculosis activities against *Mtb* H37Rv.

Subjects: Infectious Diseases; Medicinal & Pharmaceutical Chemistry; Pulmonary Medicine

Keywords: phenylalanyl-tRNA synthetase; isopatulin; cyclopiazonic acid; *Mycobacterium tuberculosis*; *Penicillium griseofulvum*

ABOUT THE AUTHOR

Born in China in 1981, Li-Ning Wang is a lecturer of the Tianjin University of Traditional Chinese Medicine since 2013. From 2011 to 2013, he was a research assistant at the Institute of Medicinal Biotechnology Chinese Academy of Medical Sciences. After having obtained his academic degrees in pharmacy, he gained his PhD at the Shandong University in 2011. From that date on, he worked in the field of natural medicine chemistry. From 2013 to 2016, Li-Ning Wang was responsible for two research programs on microbial natural products at the Tianjin University of Traditional Chinese Medicine and at the Institute of Medicinal Biotechnology Chinese Academy of Medical Sciences. He has authored more than 30 scientific works, mainly in the field of natural products. For more formal details about Li-Ning Wang, see the website https://www.researchgate.net/profile/Li-Ning_Wang.

PUBLIC INTEREST STATEMENT

Tuberculosis is an infectious bacterial disease caused by *Mycobacterium tuberculosis (Mtb*), which most commonly affects the lungs. Tuberculosis occurs in every part of the world. According to a WHO Global Tuberculosis Report (2015), about 19% of reported Tuberculosis cases occurred in China in 2014. The emergence of multidrug resistant and extensively drug-resistant strains of *Mtb*, have amplified the incidence of tuberculosis. There is an urgent need to develop new, effective, and inexpensive anti-tubercular agents. We thus selected Phenylalanyl-tRNA synthetase (PheRS) as a potential target for the development of the *Mtb*-specific inhibitor. Subsequently, six small molecules were isolated and identified as bioactive constituents from the active strain CPCC-400528. At last, Compounds **1**, **3**, **4**, and **5** exhibited the enzymatic inhibiting activity of PheRS *in vitro*, as well as moderate to weak anti-tuberculosis activities against *Mtb* H37Rv.

cogent • oa

© 2016 The Author(s). This open access article is distributed under a Creative Commons Attribution (CC-BY) 4.0 license.

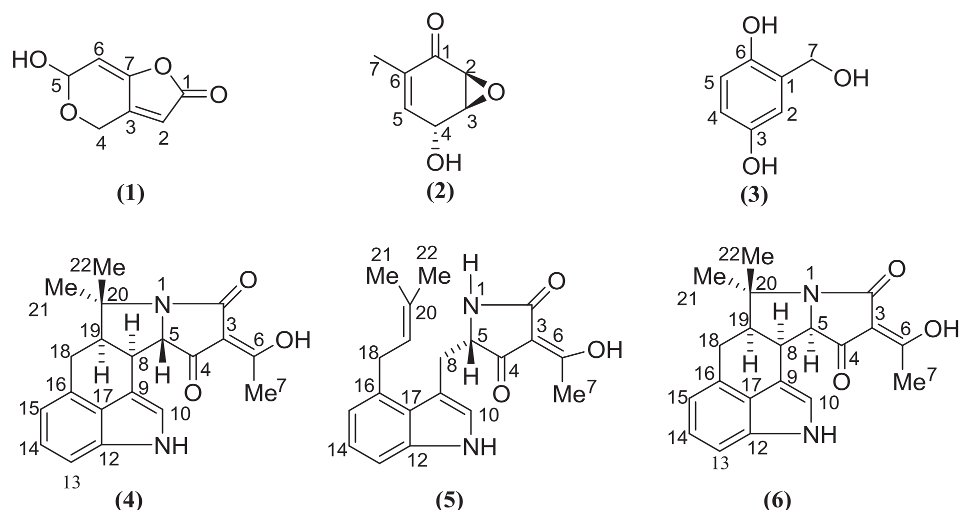
1. Introduction

Mycobacterium tuberculosis (*Mtb*), the etiologic agent of tuberculosis (TB), continues to be the leading cause of death due to bacterial infection worldwide (Gandhi et al., 2010; Lienhardt et al., 2012; World Health Organization, 2013). The emergence of multidrug-resistant and extensively drug-resistant strains of *Mtb*, have amplified the incidence of TB. There isan urgent need to develop new, effective, and inexpensive anti-tubercular agents (Falzon et al., 2013; Lemos & Matos, 2013; Lynch, 2013; Migliori et al., 2013; Skrahina et al., 2013; Zhao et al., 2012).

Aminoacyl-tRNA synthetases (AaRSs) are being pursued as targets for new drugs (Delarue, 1995). Blocking AaRS function inhibits the proper charging of tRNAs leading to disruption of translation. This leads to protein synthesis inhibition, which in turn causes cell growth arrest (Tao & Schimmel, 2000). With the aim of developing a new anti-tuberculosis drug, we selected Phenylalanyl-tRNA synthetase (PheRS) as a potential target. PheRS is a member of subclass IIc of the AaRSs which have been considered as promising targets due to their central role in cell metabolism, the significant sequence differences between the prokaryotic and eukaryotic enzymes, the availability of enzyme material and access to structural information (Delarue, 1995; Gallant, Finn, Keith, & Wendler, 2000; Mosyak, Reshetnikova, Goldgur, Delarue, & Safro, 1995; Schimmel, Tao, & Hill, 1998). Fortunately, Mupirocin, one of AaRS-specific inhibitor, has been used as a currently topical antibiotic (Sutherland et al., 1985).

Recently, we have reported the establishment and application of a high throughput screening (HTS) assay for inhibitors of *Mtb* PheRS (Zhang et al., 2012). During our campaign to discover PheRS inhibitors, the culture broth of *Penicillium griseofulvum* CPCC-400528 showed PheRS inhibitory activity and thus was selected for further study of chemical components. Bioassay guided purification of the extract of *P. griseofulvum* CPCC-400528 afforded isopatulin (**1**), (+)-epiepoformin (**2**), and gentisyl alcohol (**3**), three patulin-producing metabolites, together with three indole-tetramic acids, α-cyclopiazonic acid (**4**), β-cyclopiazonic acid (**5**), and iso-α-cyclopiazonic acid (**6**) (Figure 1). Four of them exhibited activity in a range of 8–256 μg/ml against *Mtb*. Although these patulins and cyclopiazonic acids were recognized as mycotoxins and limited by some governments, investigations of their bioactivity and structural characteristics continue owing to their significant biological activities. To our knowledge, a comprehensive evaluation of the anti-tuberculosis effects of these compounds has not been systematically studied. Herewith, we reported the isolation, structure elucidation, PheRS inhibitory activity of these small-molecule inhibitors **1–6**.

Figure 1. Chemical structures of compounds 1–6.



2. Results and discussion

Though HTS assay for inhibitors of *Mtb* PheRS (Zhang et al., 2012), we have identified six active strains, which can inhibit enzymatic activity of PheRS *in vitro* and growth of *Mycobacterium smegmatis* with low cytotoxicity. Among them, fungal strain CPCC-400528 was isolated from soil collected in Kanas lake, Sinkiang, China. Based on the morphological and molecular analysis, the producing strain CPCC-400528 was identified as *P. griseofulvum Dierckx*. The ITS rRNA sequence of the strain CPCC-400528 obtained in this study was deposited in GenBank under the accession number AB733349.

Compounds **1–6** were all obtained from fermentation of the fungus *P. griseofulvum* CPCC-400528 by processes involving silica gel column chromatography followed by reversed-phase HPLC. The lactone isopatulin (**1**) was isolated as very unstable colorless oil, which was deduced to have the molecular formula $C_7H_8O_4$ from the HREIMS of it and NMR data (Table 1). The UV spectrum of **1** showed the presence of an α, β, γ, δ unsaturated carbonxyl in the two system (274 nm). The ^{1}H and ^{13}C NMR spectra of **1** show signals attributable to one lactone (δ 171.6, C-1), two trisubstituted double bonds [(δ 6.06, H-2), (δ 110.5, C-2), (δ 154.3, C-3), (δ 6.04, H-6), (δ 111.8, C-6), (δ 148.7, C-7)], one oxygenated methylene [(δ 4.67, 4.00, H-4), (δ 61.1, C-4)], and one hemiacetal group [(δ 5.98, H-5), (δ 90.7, C-5)]. Isopatulin (**1**) is an isomer of the well-known fungal toxin, patulin (Mikami et al., 1996; Moss & Long, 2002). Finally, Interpretation of 2D NMR (COSY, HMQC, and HMBC) confirmed the presence of functional groups noted above and led to structure **1**.

ESIMS and NMR data established the molecular formula of (+)-epiepoformin (**2**), $[\alpha]^{20}$ = +310.0 (*c* 0.27, ethanol), as $C_7H_8O_3$ was isolated. Analysis of ^{1}H and ^{13}C NMR data for **2** (Table 1) revealed the characteristic resonances for one lactone (δ 194.0, C-1), one 2,3-disubstituted oxirane [(δ 3.78, H-2), (δ 53.3, C-2), (δ 3.52, H-3), (δ 57.6, C-3)], one oxygenated methyne [(δ 4.67, H-4), (δ 63.4, C-4)], one trisubstituted double bonds [(δ 6.46, H-5), (δ 134.7, C-5), (δ 138.6, C-6)], and one methyl group [(δ 1.86, H-7), (δ 15.9, C-7)]. Interpretation of 2D NMR (COSY, HMQC, and HMBC) confirmed the presence of functional groups noted above and led to structure **2**. Compound **2** has the positive optical rotation consistent with (+)-epiepoformin, whereas (−)-epiepoformin has the negative one in ethanol (Gloer & Truckenbrod, 1988). Therefore, **2** was determined to be (+)-type.

The structures of three indole-tetramic acids, α-cyclopiazonic acid (**4**), β-cyclopiazonic acid (**5**), and iso-α-cyclopiazonic acid (**6**) were also unequivocally identified by ESIMS, 1D NMR and 2D NMR spectroscopic analysis (Table 2), and comparisons with data reported in the literatures (Beyer, 2011; Losito, Monaci, Aresta, & Zambonin, 2002; Nolte, Steyn, & Wessels, 1980). The 3-acylpyrrolidine-2,4-diones (tetramic acids) existed in solution as pairs of internal tautomers and as a pair of acylated external tautomers (**4α** and **4β**), (**5α** and **5β**). The disproportionate integration area was easily observed in the 1D NMR spectra of **4** and **5** (Table 2). Since Iso-α-cyclopiazonic acid (**6**) was isolated in only quite small amounts, sharp resonances in the ^{1}H-NMR spectrum were not obtained.

Table 1. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR spectroscopic data for compounds 1–3

No.	1		2		3	
	δH (J/Hz)	δC	δH (J/Hz)	δC	δH (J/Hz)	δC
1		171.6		184.2		129.4
2	6.06 (*s*)	111.8	3.50 (dd, 3.6, 1.2)	53.4	6.71 (*d*, 3.0)	115.8
3		148.7	3.78–3.80 (*m*)	57.6		151.0
4	5.98 (*s*)	90.7	4.67 (t, 6.0)	63.4	6.49 (dd, 8.4, 3.0)	115.5
5	4.67 (dd, 17.3, 2.5)	61.1	6.46–6.47 (*m*)	138.8	6.56 (*d*, 8.4)	116.7
	4.40 (dd, 17.3, 3.6)					
6	6.04 (*m*)	110.5		134.6		148.9
7		154.3	1.86 (*s*)	15.9	4.54 (*s*)	60.0

Notes: Recorded in $CDCl_3$ and chemical shifts are expressed as δ ppm. *s*, singlet; *d*, doublet; *t*, triplet; *m*, multiplet.

Table 2. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR spectroscopic data for compounds 4–6

No.	4				5				6
	4*α*		4*β*		5*α*		5*β*		
	*δ*H (J/Hz)	*δ*C	*δ*H (J/Hz)	*δ*C	*δ*H (J/Hz)	*δ*C	*δ*H (J/Hz)	*δ*C	*δ*H (J/Hz)
1	8.09 (brs)		8.09 (brs)		8.20 (brs)		8.23 (brs)		
2		175.2		175.2		174.7		168.9	
3		105.6		105.6		101.6		104.6	
4		195.2		195.2		194.5		200.2	
5	4.08 (*d*, 10.8)	71.8	4.25 (*d*, 6.6)	71.8	4.08 (dd, 10.8, 2.4)	63.5	4.25 (*d*, 9.0)	60.5	4.25 (*d*, 10.8)
6		184.4		184.4		185.4		189.3	
7	2.46 (3H, *s*)	19.7	2.54 (3H, *s*)	19.7	2.49 (3H, *s*)	19.6	2.51 (3H, *s*)	20.5	2.46 (3H, *s*)
8	3.68 (brs)	53.0	3.68 (brs)	53.5	3.65(dd, 15.0, 1.8) 2.85(dd, 15.0, 10.8)	32.2	3.65(dd, 15.0, 1.8) 2.86(dd, 15.0, 10.8)	32.3	3.73 (dd, 10.8, 5.6)
9		110.0		110.0		111.4		110.9	
10	7.27 (*s*)	120.8	7.27 (*s*)	120.8	7.00 (brs)	123.0	7.00 (brs)	123.3	7.07 (brs)
12		133.4		133.4		137.2		137.2	
13	7.20 (*o*)	108.7	7.20 (*o*)	108.7	7.21 (*d*, 7.8)	109.5	7.21 (*d*, 7.8)	109.6	6.88 (*d*, 6.6)
14	7.20 (*o*)	123.0	7.20 (*o*)	123.0	7.12 (dd, 7.8, 7.2)	122.6	7.12 (dd, 7.8, 7.2)	122.6	6.75 (dd, 8.4, 3.0)
15	6.93(*s*)	116.5	6.88 (*s*)	116.5	6.91 (*d*, 7.2)	120.4	6.91 (*d*, 7.2)	120.5	6.84 (*d*, 8.4)
16		128.6		128.6		134.6		134.4	
17		125.9		125.9		124.6		124.5	
18	3.08 (2H, brs)	26.5	3.10 (2H, brs)	26.5	3.75 (ddd, 22.8, 15.6, 6.6)	30.0	3.75 (ddd, 22.8, 15.6, 6.6)	30.1	3.05(2H, d, 9.0)
19	2.66 (*s*)	36.1	2.66 (*s*)	36.4	5.31 (*t*, 6.6)	123.2	5.31 (*t*, 6.6)	123.3	2.66–2.22(*m*)
20		63.4		63.4		132.8		132.8	
21	1.70 (3H, *s*)	26.3	1.70 (3H, *s*)	26.1	1.76 (3H, *s*)	25.7	1.76 (3H, *s*)	25.7	1.71 (3H, *s*)
22	1.53 (3H, *s*)	24.4	1.67 (3H, *s*)	24.8	1.74 (3H, *s*)	18.1	1.74 (3H, *s*)	18.1	1.69 (3H, *s*)

Notes: Recorded in $CDCl_3$ and chemical shifts are expressed as δ ppm. *s*, singlet; *d*, doublet; *t*, triplet; *m*, multiplet, *o*, overlap.

The structures of the bacterial PheRSs are well conserved but differ significantly from their eukaryotic counterparts (Ling et al., 2012). Compounds **1**, **3–5** were evaluated for inhibition of the aminoacylation activity of PheRS derived from both *Mtb* and human mitochondria (*Hm*). They were further tested for antibacterial activity against replicating *Mtb* H37Rv strain using a microplate Alamar blue assay (MABA) method, following our previously validated protocol (Zhang et al., 2012). Table 3 shows the enzyme and whole cell activities. The patulin-producing metabolite **1** yielded with Ic_{50}s (0.161 mM) for *Mtb* PheRS with high selectivity over *Hm* PheRS (Ic_{50} = 5.503 mM). While the other patulin-producing compound **3** showed lower enzymatic activity for *Mtb* PheRS (Ic_{50} = 0.262 mM) than **1**, whereas higher activity was observed for *Hm* PheRS (Ic_{50} = 0.648 mM). The two indole-tetramic acids **4** and **5** showed no significant differences with both enzymes (Table 3). *In vitro* antibacterial activity against *Mtb* H37Rv assays were done to assess those compounds potencies (Table 3). Compounds **1** and **3** were effective in antibacterial activity against *Mtb* H37Rv with minimum inhibitory concentration (MIC) of 8 and 16 μg/mL, respectively, as compared with compounds **4** and **5** (MIC = 128 and 64 μg/mL). As mentioned above, it appeared that the patulin-producing metabolites were more potent *Mtb* PheRS inhibitors than the indole-tetramic acids.

Table 3. Inhibition of *Mtb* PheRS, *Hm* PheRS and minimum inhibitory concentration (MIC) of the isolates (1, 3–5)

Compound	Ic_{50} (mM)[a] (*Mtb* PheRS)	Ic_{50} (mM) (*Hm* PheRS)	MIC(μg/ml) (*Mtb* H37Rv)
1	0.161	5.503	8
3	0.262	0.648	16
4	1.345	0.967	128
5	1.338	0.193	64
Rifampin	–	–	0.125

To understand the interactions between the most active compound **1** and *Mtb* PheRS, we performed molecular docking calculations using the molecular operating environment (MOE) program. Structural models of *Mtb* PheRS A and B were built from the *Escherichia coli* PheRS (PDB code 3PCO) crystal structure. Compound **1** was found to favorably interact with *Mtb* PheRS A (S-value −10.0437), occupying the phenylalanyl-adenylate binding site, as shown in Figure 2. **1** formed three hydrogen bonding interactions with the amino acid residues Arg 312 and His 209, electronic interactions with Glu 279, Thr 208, Arg 201, Gly 307, Gln 215, and Gly 309, and hydrophobic interactions with Phe 213 and Met 323. Compound **1** has no interact with *Mtb* PheRS B.

Docking test of *Mtb* PheRS showed that the binding mode was well conserved in prokaryotes. *Mtb* PheRS inhibitors were thus suspected to have the broad antibacterial activities. The patulin-producing compounds **1** and **3** with better activities against *Mtb* PheRS were evaluated *in vitro* antibacterial activities against 37 other important human pathogens, followed our previously validated protocol (Ling et al., 2012). The obtained results were represented in Table 4, where it could be noticed that the tested compounds showed mild activity in comparison to the reference drug levofloxacin.

3. Experimental

3.1. General experiment

NMR spectra were measured on a Bruker Avance DRX-600 spectrometer operating at 600 (^{1}H) and 150 (^{13}C) MHz with TMS as internal standard. HREIMS spectra were obtained on a Waters GCT system mass spectrometer. HRESIMS were carried out on a LTQ-Orbitrap XL. HPLC was performed on an Agilent 1200 liquid chromatography with a ZORBAXSB-C18 Column (9.4, 250, and 5 mm). All solvents used were of analytical grade. Silica gel (200–300 mesh, Qingdao Haiyang Chemical Co., Ltd., Qingdao, People's Republic of China and Sephadex LH-20 (25–100 mm; Pharmacia Biotek, Denmark) were used for column chromatography. TLC was carried out with high-performance TLC plates precoated with silica gel GF_{254} (Qingdao Haiyang Chemical Co., Ltd.). Spots of TLC were visualized within UV light or by spraying with H_2SO_4–EtOH (1:9) followed by heating.

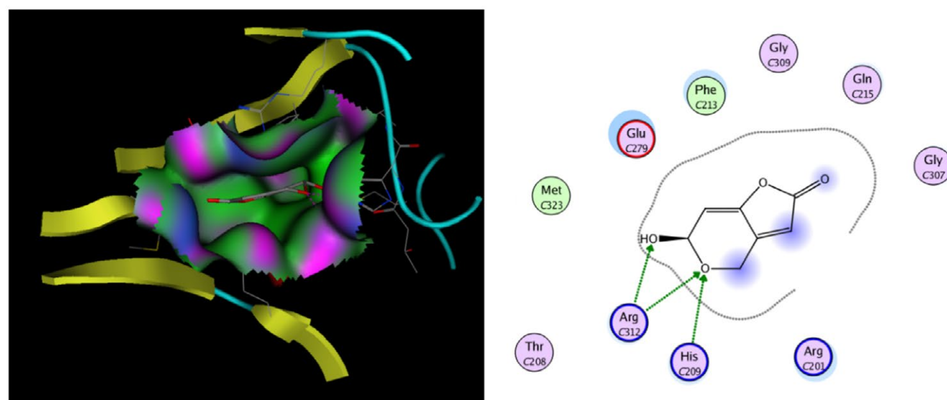


Figure 2. Docking of compound 1 with *Mtb* PheRS A.

Notes: Docked positions of compound **1** obtained by Dock module inside the ***Mtb*** PheRS A gorge (PDB ID: 4EY7), showing 3D (left) and 2D (right) images.

Table 4. *In vitro* antibacterial activity of the isolates 1 and 3 (MIC:μg/ml)

Strain	Strain number	Resistance/enzyme-producing	1	3	Levofloxacin
Staphylococcus aureus	ATCC 29213	MSSA	64	32	0.125
	ATCC 33591	MRSA	64	32	0.125
	15	MSSA	64	32	0.125
	09–6	MSSA	64	32	0.125
	09–13	MRSA	64	32	32
Staphylococcus epidermidis	ATCC 12228	MSSE	32	32	0.125
	09–3	MRSE	64	32	8
	09–9	MSSE	64	32	16
Enterococcus faecalis	ATCC 29212	VSE	128	128	0.5
	ATCC 51299	VRE	128	128	0.5
	09–8	VSE	128	128	2
	09–9	VRE	128	64	128
Enterococcus faecium	ATCC 700221	VRE	128	64	64
	09–10	VSE	128	64	64
	05–8	VRE	128	64	128
Escherichia coli	ATCC 25922	ESBLs(−)	16	128	≤0.03
	1515	ESBLs(−)	32	128	≤0.03
	09–1	ESBLs(+)	16	>128	16
	09–20	ESBLs(−)	32	128	0.5
Klebsiella pneumoniae	ATCC 700603	ESBLs(+)	32	128	0.5
	7	ESBLs(−)	64	128	≤0.03
	ATCC BAA-2146	NDM-1(+)	128	128	>128
	09–8	ESBLs(−)	32	128	≤0.03
	09–25	ESBLs(+)	32	128	4
Pseudomonas aeruginosa	ATCC 27853		128	64	0.5
	PAO1		128	128	4
	09–14		64	64	2
Acinetobacter calcoacetious	ATCC 19606		32	64	0.125
Enterobacter cloacae	ATCC 45301		32	128	0.06
Enterobacter aerogenes	ATCC 45102		32	128	0.06
Serratia marcescens	ATCC 41002		32	128	1
Morganella morganii	ATCC 25830		32	64	0.5
Providencia rettgeri	ATCC 31052		16	64	≤0.03
Proteus vulgaris	ATCC 29905		16	64	0.125
Proteus mirabilis	09–1		32	64	2
Salmonella typhi	H901		8	64	≤0.03
Citrobacter freundii	ATCC 43864		16	64	≤0.03

3.2. Fungi

Fungal strain CPCC-400528 was isolated from soil collected in Kanas lake, Sinkiang, China. Based on the morphological and molecular analysis, the producing strain CPCC-400528 was identified as *P. griseofulvum Dierckx*. The ITS rRNA sequence of the strain CPCC-400528 obtained in this study was deposited in GenBank under the accession number AB733349.

3.3. Cultures of P. corylophilum *stressed with* P. griseofulvum Dierckx

Made the medium with the ingredients (per liter), 4 g of malt extract, 10 g of malt extract, 4 g of glucose, 18 g of agar, and turned the pH to 6.0. Then dispensed the medium into test tubes, sterilized at the condition of 121°C, 30 min, made Slant medium while it was still hot.

3.4. Extraction and isolation

Fermentation of the fungus *P. griseofulvum* CPCC-400528 was carried out at 26°C for 96 h under agitation speed 150 rpm. The whole fractionation was guided by a bioassay for PheRS inhibitory and antibacterial activities. The cultured broth was separated into supernatant and mycelium by centrifugation. About 3 liters of supernatant was chromatographed on an RP-18 silica gel column (Merck, 70–150μm, MeOH/H_2O, 0:1 to 1:0) to fractions 1–50. Two active fractions, 2 and 48 were collected and concentrated in *vacuo* to yield *ca.* 2.0 g and 0.2 g of brownish oils, respectively. Fraction 2 (1.8 g) was further purified over HPLC [Agilent 1100 isopump, Agilent 1200 VWD detector (270 nm), and Phenomenex Luna 5 μm C_{18} column (250 × 4.60 mm), MeOH/H_2O 2.5:97.5, 0.8 mL/min] to yield **1** (1.5 g), **2** (2.3 mg), and **3** (23.0 mg). Fraction 48 (180 mg) was further purified over HPLC [Agilent 1100 isopump, Agilent 1200 VWD detector (230 nm), and Phenomenex Luna 5 *μ*m C18 column (250 × 4.60 mm), MeOH/H_2O 76.5:23.5, 0.8 mL/min] to yield 4 (23.0 mg), 5 (12 mg) and 6 (0.3 mg). The structures of compounds **1–6** were identified as isopatulin (**1**), (+)-epiepoformin (**2**), gentisyl alcohol (**3**), α-cyclopiazonic acid (**4**), β-cyclopiazonic acid (**5**) and iso-α-cyclopiazonic acid (**6**), respectively, by comparing the spectral (MS, NMR) data with the values reported in the literature (Beyer, 2011; Gloer & Truckenbrod, 1988; Losito et al., 2002; Nolte et al., 1980).

3.5. Strains and antibiotic

Mtb H37Rv is clinical isolates (purified and identified according to standard procedures) from clinical. All other antibiotics used in this study were bought from sigma.

3.6. Molecular docking

MOE was used for ligand and protein preparation, docking calculations and molecular structure viewing. The crystal structure was obtained from the PDB with the accession code 3PCO.

3.7. PheRS and M. tuberculosis *inhibition studies*

The PheRS inhibition determined using a luciferase chemiluminescence-based assay. In this assay, Phenylalanyl, bulk *E. coli* tRNA, and ATP are incubated with PheRS enzyme in the presence of test compounds. MICs against replicating *Mtb* were determined by the MABA. The fluorescence was read at an excitation of 530 nm and an emission of 590 nm.

Funding
We emphasize our appreciation for excellent technical assistance by financial supports from the National Natural Science Foundation of China [grant number 81202444] and [grant number 81373452] are gratefully acknowledged.

Author details
Li-Ning Wang[1,2]
E-mail: liningwang@aliyun.com
Wen-Jing Di[2]
E-mail: dw12112@163.com
Zhi-Ming Zhang[1]
E-mail: zhang6637419@126.com
Li-li Zhao[1]
E-mail: zll-616@163.com
Tao Zhang[1]
E-mail: zhangt@cpcc.ac.cn
Yan-Ru Deng[2]
E-mail: dyanru@sina.com
Li-Yan Yu[1]
E-mail: yly@cpcc.ac.cn
[1] Institute of Medicinal Biotechnology, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100050, China.
[2] College of Herbal Medicine, Tianjin University of Traditional Chinese Medicine, Tianjin 300193, China.
[§] The authors Li-Ning Wang and Wen-Ding Di contributed equally to this work.

Citation information
Cite this article as: Small-molecule inhibitors of the tuberculosis target, phenylalanyl-tRNA synthetase from Penicillium griseofulvum CPCC-400528, Li-Ning Wang, Wen-Jing Di, Zhi-Ming Zhang, Li-li Zhao, Tao Zhang, Yan-Ru Deng & Li-Yan Yu, *Cogent Chemistry* (2016), 2: 1181536.

Cover image
Source: Authors.

References

Beyer, C. (2011). *Synthese des indolalkaloids α-cyclopiazonic acid* (Chemie Dissertationen). Halle: Bergischen Universität Wuppertal.

Delarue, M. (1995). Aminoacyl-tRNA synthetases. *Current Opinion in Structural Biology, 5*, 48–55. doi:10.1016/0959-440X(95)80008-O

Falzon, D., Jaramillo, E., Wares, F., Zignol, M., Floyd, K., & Raviglione, M. C. (2013). Universal access to care for multidrug-resistant tuberculosis: An analysis of surveillance data. *Journal of the Lancet Infectious Diseases, 13*, 690–697. doi:10.1016/S1473-3099(13)70130-0

Gallant, P., Finn, J., Keith, D., & Wendler, P. (2000). The identification of quality antibacterial drug discovery targets: A case study with aminoacyl-tRNA synthetases. *Emerging Therapeutic Targets,* 4(1), 1–9. doi:10.1517/14728222.4.1.1

Gandhi, N. R., Nunn, P., Dheda, K., Schaaf, H. S., Zignol, M., Van Soolingen, D., ... Bayona, J. (2010). Multidrug-resistant and extensively drug-resistant tuberculosis: A threat to global control of tuberculosis. *The Lancet, 375*, 1830–1843. doi:10.1016/S0140-6736(10)60410-2

Gloer, J. B., & Truckenbrod, S. (1988). Interference competition among coprophilous fungi: Production of (+)-isoepoxydon by *Poronia punctata*. *Applied and Environmental Microbiology, 54*, 861–864. PMCID: PMC202564.

Lemos, A. C. M., & Matos, E. D. (2013). Multidrug-resistant tuberculosis. *The Brazilian Journal of Infectious Diseases, 17*, 239–249. doi:10.1016/j.bjid.2013.01.007

Lienhardt, C., Glaziou, P., Uplekar, M., Lönnroth, K., Getahun, H., & Raviglione, M. (2012). Global tuberculosis control: Lessons learnt and future prospects. *Journal of Nature Reviews Microbiology, 10*, 407–416. doi:10.1038/nrmicro2797

Ling, Z. Q., Wang, L. N., Hu, X. X., Dong, G. X., Wei, Y. Z., Liu, H. Y., ... Yu, L. Y. (2012). Purification and characterization of anti multi-drug-resistant bacterial constituents from fermentation broth of *Actinoplanes sp.* PDF-1. *Chinese Journal of Antibiotics, 37*, 366–371. doi:10.13461/j.cnki.cja.004995

Losito, I., Monaci, L., Aresta, A., & Zambonin, C. G. (2002). LC-ion trap electrospray MS-MS for the determination of cyclopiazonic acid in milk samples. *Analyst, 127*, 499–502. doi:10.1039/B200394P

Lynch, J. B. (2013). Multidrug-resistant tuberculosis. *Journal of Medical Clinics of North America, 97*, 553–579. doi:10.1016/j.mcna.2013.03.012

Migliori, G. B., Sotgiu, G., Gandhi, N. R., Falzon, D., DeRiemer, K., Centis, R., ... Vargas, M. H. (2013). Resistance to fluoroquinolones and second-line injectable drugs: Impact on MDR-TB outcomes. *Journal of European Respiratory, 42*, 169–179. doi:10.1183/09031936.00136312

Mikami, A., Okazaki, T., Sakai, N., Ichihara, T., Hanada, K., & Mizoue, K. (1996). A new isopatulin derivative pintulin produced by *Penicillium vulpinum* F-4148 taxonomy, isolation, physico-chemical properties, structure and biological properties. *The Journal of Antibiotics, 49*, 985–989. doi:10.7164/antibiotics.49.985

Moss, M., & Long, M. (2002). Fate of patulin in the presence of the yeast *Saccharomyces cerevisiae*. *Journal of Food Additives and Contaminants, 19*, 387–399. doi:10.1080/02652030110091163

Mosyak, L., Reshetnikova, L., Goldgur, Y., Delarue, M., & Safro, M. G. (1995). Structure of phenylalanyl-tRNA synthetase from *Thermus thermophilus*. *Journal of Nature Structural Biology, 2*, 537–547. doi:10.1038/nsb0795-537

Nolte, M. J., Steyn, P. S., & Wessels, P. L. (1980). Structural investigations of 3-acylpyrrolidine-2, 4-diones by nuclear magnetic resonance spectroscopy and X-ray crystallography. *Journal of Chemical Society, Perkin Transaction 1,* 1057. doi:10.1039/P19800001057

Schimmel, P., Tao, J., & Hill, J. (1998). Aminoacyl tRNA synthetases as targets for new anti-infectives. *Journal of the FASEB, 12*, 1599–1609.

Skrahina, A., Hurevich, H., Zalutskaya, A., Sahalchyk, E., Astrauko, A., Hoffner, S., ... Dara, M. (2013). Multidrug-resistant tuberculosis in Belarus: The size of the problem and associated risk factors. *Journal of Bulletin of the World Health Organization, 91*, 36–45. doi:10.2471/BLT.12.104588

Sutherland, R., Boon, R., Griffin, K., Masters, P., Slocombe, B., & White, A. (1985). Antibacterial activity of mupirocin (pseudomonic acid), a new antibiotic for topical use. *Antimicrobial Agents and Chemotherapy, 27*, 495–498. doi:10.1128/AAC.27.4.495

Tao, J., & Schimmel, P. (2000). Inhibitors of aminoacyl-tRNA synthetases as novel anti-infectives. *Expert Opinion on Investigational Drugs, 9*, 1767–1775. doi:10.1517/13543784.9.8.1767

World Health Organization. (2013). *Global tuberculosis report 2013*. Geneva: Author.

Zhang, Z. M., Sun, Y., Zhao, L. L., Wang, L. N., Wei, Y. Z., Su, J., ... Yu, L. Y. (2012). The establishment and application of a high throughput screening assay for inhibitors of *Mycobacterium tuberculosis* phenylalanyl-tRNA synthetase. *Microbiology China* 39, 1437–1446. doi:10.13344/j.microbiol.china.2012.10.007

Zhao, Y., Xu, S., Wang, L., Chin, D. P., Wang, S., Jiang, G., ... Ou, X. N. (2012). National survey of drug-resistant tuberculosis in China. *New England Journal of Medicine, 366*, 2161–2170. doi:10.1056/NEJMoa1108789

© 2016 The Author(s). This open access article is distributed under a Creative Commons Attribution (CC-BY) 4.0 license.

You are free to:
Share — copy and redistribute the material in any medium or format
Adapt — remix, transform, and build upon the material for any purpose, even commercially.
The licensor cannot revoke these freedoms as long as you follow the license terms.

Under the following terms:
Attribution — You must give appropriate credit, provide a link to the license, and indicate if changes were made.
You may do so in any reasonable manner, but not in any way that suggests the licensor endorses you or your use.
No additional restrictions
You may not apply legal terms or technological measures that legally restrict others from doing anything the license permits.