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Original article

Design, synthesis and antibacterial activity of novel pleuromutilin derivatives with 4*H*-pyran-4-one and pyridin-4-one substitution in the C-14 side chain

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ABSTRACT

A series of novel pleuromutilin derivatives with 4*H*-pyran-4-one and pyridin-4-one substitution in the C-14 side chain have been designed and synthesized. *In vitro* antibacterial activity evaluation showed that most of the derivatives exhibited potent antibacterial activity against drug resistant Gram-positive strains. Compounds **12a**, **12d**, and **28** are the most active derivatives in this series, displaying activity comparable to that of retapamulin and BC-3781. As the metabolic stability of this series is not satisfactory, further modifications are going on to improve their pharmacokinetic profile.

1. Introduction

The emergence and spread of multi-drug resistant bacteria have become a serious threat to public health [1]. In particular, multi-drug resistant Gram-positive bacteria including methicillin-resistant *Staphylococcus aureus* (MRSA) [2], penicillin-resistant *Streptococcus pneumoniae* (PRSP) [3], and vancomycin-resistant *Enterococci* (VRE) [4] represent major concerns. There is, therefore, an urgent need to identify and develop novel antibiotics with modes of action that are distinct from those of established classes.

Pleuromutilin (**1**, Fig. 1), a diterpenoid natural product with a fused 5–6–8 tricyclic skeleton, was first isolated in 1951 from two basidiomycete species [5]. It displays modest *in vitro* antibacterial activity against Gram-positive pathogens and mycoplasmas. Further studies have shown that the pleuromutilin class of antibiotics selectively inhibits bacterial protein synthesis by specifically targeting the 50s subunit of the bacterial ribosome and displays no cross-resistance with antibiotics currently in clinical use [6]. The distinct mode of action of pleuromutilin has made it an attractive target in the development of novel antibiotics for the treatment of multi-drug resistant bacterial infections.

Since the 1960s, numerous semisynthetic pleuromutilin analogs have been prepared and evaluated, and it was recognized that pleuromutilin derivatives containing a sulfide linkage in the C-14 side chain demonstrated superior antibacterial activity [7]. Of the derivatives generated, tiamulin [8] (**2**, Fig. 1) and valnemulin [9] (**3**, Fig. 1) were successfully developed as veterinary medicine. In 2007, retapamulin [10] (**4**, Fig. 1), a novel pleuromutilin derivative, was first approved for human use as a topical antimicrobial agent to treat skin infections. Other pleuromutilin derivatives in clinical trials include Nabriva Therapeutics' BC-7013 [11] (**5**, Fig. 1) and BC-3781 [12] (**6**, Fig. 1). Especially, BC-3781 has completed phase II clinical trial for the systemic treatment of acute bacterial skin and skin structure infections (ABSSSI) and acquired Qualified Infectious Disease Product (QIDP) as well as fast track status designation.

4*H*-Pyran-4-one and pyridin-4-one ring systems are very attractive moieties in medicinal chemistry because of their occurrence in a variety of bioactive compounds [13]. Existing studies have proved their wide range of biological activities such as antiproliferative, antibacterial, and antimalarial activity [14–16]. Based on these interesting biological activity and previous SAR of pleuromutilins, a series of novel thioether pleuromutilin

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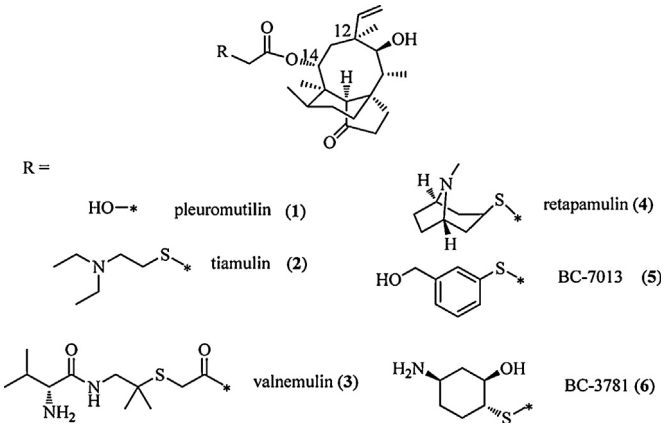


Fig. 1. Structures of pleuromutilin and its derivatives.

derivatives bearing 4*H*-pyran-4-one and pyridin-4-one moieties in the C-14 side chain were designed, synthesized, and evaluated for their antibacterial activity. Herein, we described the details of this study.

2. Experimental

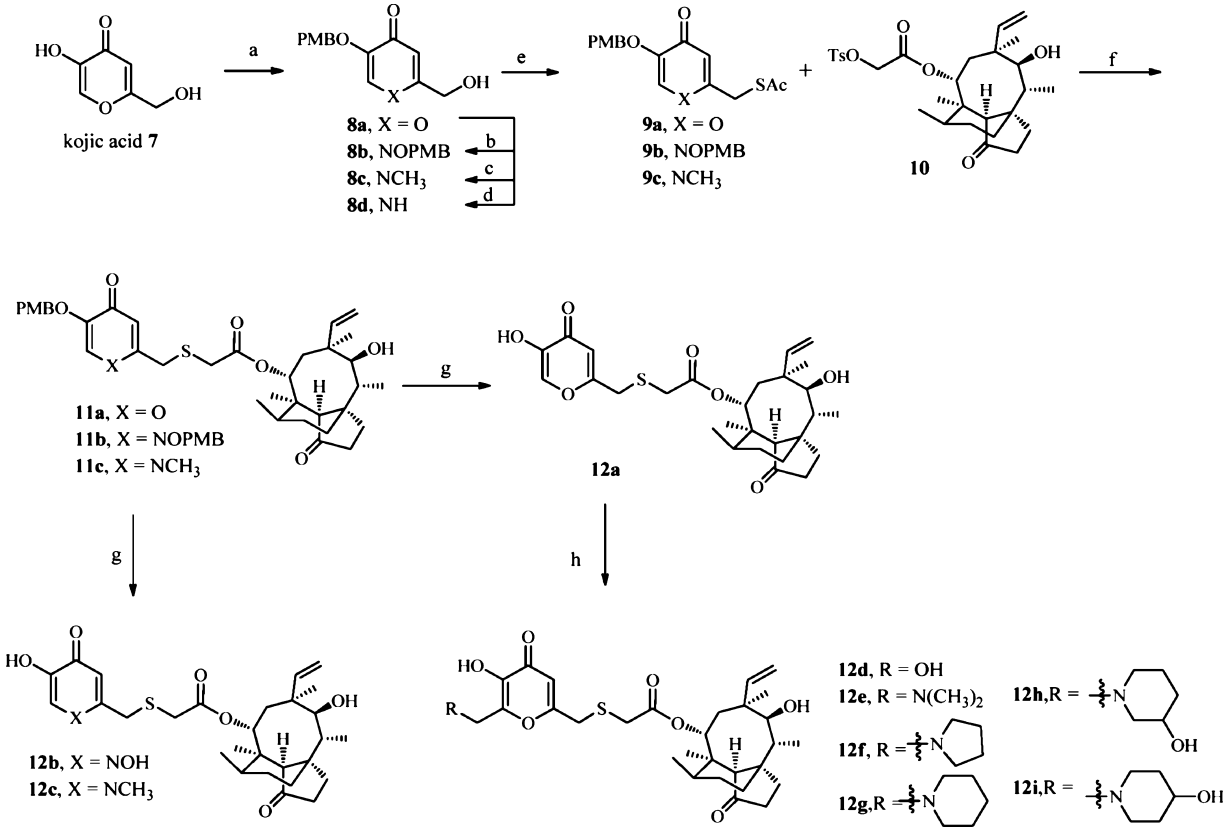
The synthesis of compounds **12a–12i** is shown in Scheme 1. Commercially available kojic acid was protected with 4-methoxybenzyl group at C-5 position to give compound **8a**, which was transformed to **8b–8d** by reported method [17]. Mesylation of the 2-hydroxymethyl group in compounds **8a–8c** followed by nucleophilic substitution with potassium thioacetate gave compounds **9a–9c** in 55%–62% yield over 2 steps. Hydrolysis of the thioacyl groups in compounds **9a–9c** revealed the corresponding thiol anions that reacted with mutilin 14-tosyloxyacetate **10** under basic condition to give compounds **11a–11c** in 57%–68% yield. Deprotection of the 4-methoxybenzyl group in compounds **11a–11c** by trifluoroacetic acid yielded compounds **12a–12c** in 55%–70% yield. Treatment of compound **12a** with formaldehyde solution furnished compound **12d**. Mannich reaction of compound **12a** with corresponding amines afforded compounds **12e–12i** in 46%–59% yield.

As shown in Scheme 2, compound **16** was prepared from compound **8d**. Reaction of compound **8d** with PMBCl and K_2CO_3 in DMF at 70 °C afforded compound **13** in 68% yield and *N*-4-methoxybenzylated byproduct was isolated in 12% yield. Compound **13** was then converted to compound **16** by the same procedures as described for compounds **12a**–**12c**.

As shown in Scheme 3, the synthesis of compound **22** started from maltol (compound **17**), which was converted to compound **19** by TBDMS-protection and subsequent bromination. Nucleophilic substitution of compound **19** with potassium thioacetate followed by hydrolysis of the thioacyl group and reaction with **10** gave compound **21**. Deprotection of the TBDMS group in compound **21** afforded compound **22** in quantitative yield.

The synthesis of compound **28** is illustrated in Scheme 4. The primary alcohol of kojic acid (compound **7**) was protected with the tetrahydropyranyl group to give compound **23**, which was treated with formaldehyde solution to yield compound **24**. Protection of the C-5 hydroxyl group afforded compound **25** which was converted to compound **28** following procedures similar to those described for compounds **12a–12c**.

As shown in Scheme 5, compound **31** was synthesized from compound **29** by the same procedures as described for compounds **12a–12c**.



Scheme 1. Synthesis of compounds **12a**–**12i**. Reagents and conditions: (a) PBMCl, K_2CO_3, DMF, 65 °C, 8 h; (b) (i) $NH_2OH{\cdot}HCl$, CH_3COONa, *N*-methyl pyrrolidone, 70 °C, 12 h; (ii) PBMCl, K_2CO_3, DMF, 65 °C, 6 h; (c) CH_3NH_2/alcohol solution, 70 °C, 6 h; (d) 25% aqueous NH_4OH, 60 °C, 12 h; (e) (i) MsCl, TEA, CH_2Cl_2, 0 °C–r.t., 12 h; (ii) KSAc, DMF, 40 °C, 2–4 h; (f) 5% aqueous NaOH, MeOH/CH_2Cl_2, r.t.; (g) CF_3COOH, CH_2Cl_2, 0 °C–r.t., 4–6 h; (h) 37% aqueous CH_2O, NaOH, MeOH, r.t. or 37% aqueous CH_2O, R_1R_2NH, MeOH, r.t.

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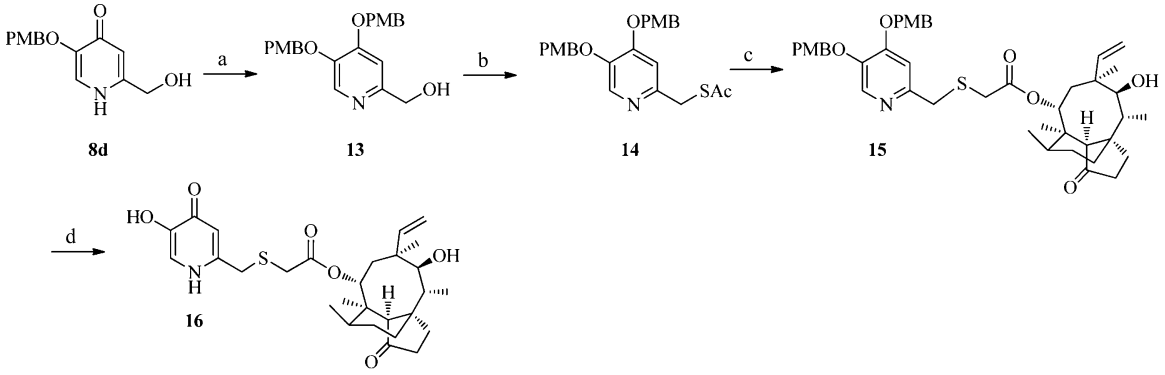
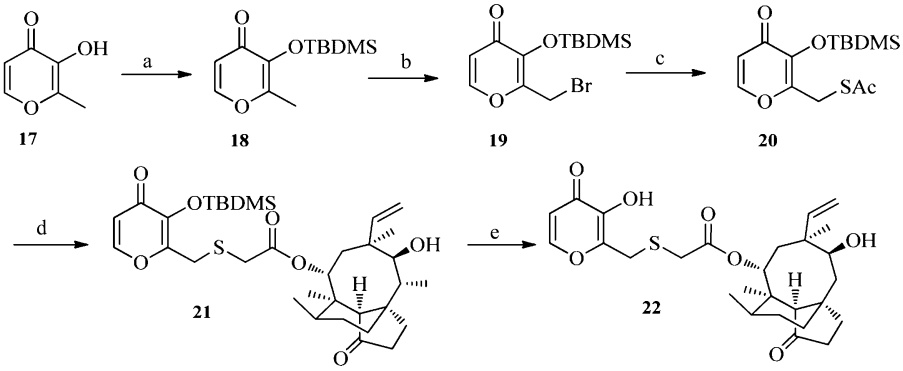
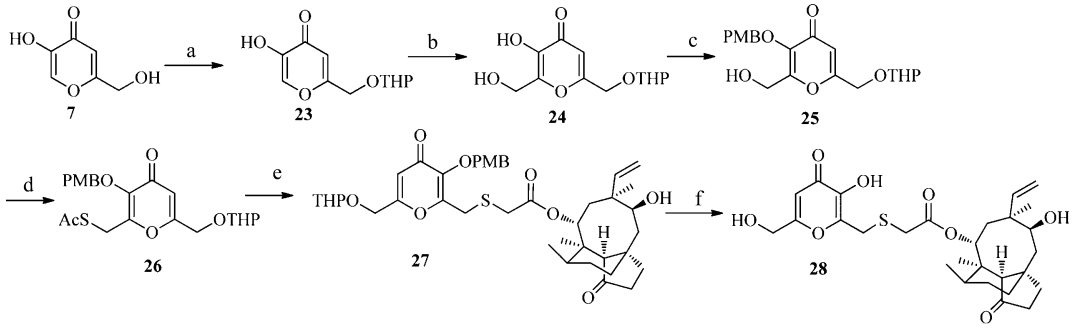


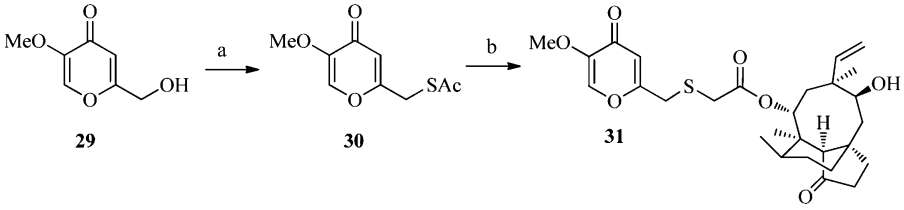
Scheme 2. Synthesis of compound **16**. Reagents and conditions: (a) PBMCl, K_2CO_3, DMF, 65 °C, 8 h; (b) (i) MsCl, TEA, CH_2Cl_2, 0 °C–r.t., 12 h; (ii) KSAc, DMF, 40 °C, 4 h; (c) 5% aqueous NaOH, mutilin 14-tosyloxyacetate **10**, MeOH/CH_2Cl_2, r.t.; (d) CF_3COOH, CH_2Cl_2, 0 °C–r.t., 6 h.



Scheme 3. Synthesis of compound **22**. Reagents and conditions: (a) TBDMSCl, imidazole, CH_2Cl_2, 0 °C–r.t., 6 h; (b) NBS, AIBN, CCl_4, 60 °C, N_2, 8 h; (c) KSAc, DMF, 40 °C, 5 h; (d) mutilin 14-tosyloxyacetate **10**, 5% aqueous NaOH, MeOH/CH_2Cl_2, r.t.; (e) TBAF/THF, 0 °C–r.t., 5 h.



Scheme 4. Synthesis of compound **28**. Reagents and conditions: (a) 3,4-dihydro-2*H*-pyran, 0.1% PTSA·H_2O, CH_2Cl_2, r.t., 4 h; (b) 37% aqueous CH_2O, NaOH, MeOH, r.t.; (c) PBMCl, K_2CO_3, DMF, 65 °C, 8 h; (d) (i) MsCl, TEA, CH_2Cl_2, 0 °C–r.t., 12 h; (ii) KSAc, DMF, 40 °C, 4 h; (e) 5% aqueous NaOH, mutilin-14-tosyloxyacetate **10**, MeOH/CH_2Cl_2, r.t.; (f) CF_3COOH, CH_2Cl_2, 0 °C–r.t., 6 h.



Scheme 5. Synthesis of compound **31**. Reagents and conditions: (a) (i) MsCl, TEA, CH_2Cl_2, 0 °C–r.t., 12 h; (ii) KSAc, DMF, 35 °C, 4 h; (b) 5% aqueous NaOH, mutilin 14-tosyloxyacetate **10**, MeOH/CH_2Cl_2, r.t.

3. Results and discussion

The *in vitro* antibacterial activities of the target compounds were tested against clinically isolated Gram-positive strains with retapamulin and BC-3781 as positive controls. The minimal inhibitory concentration (MIC) values were determined using agar dilution method according to CLSI guidelines [18]. The results were summarized in Table 1. It was interesting that compound **12a** exhibited very potent antibacterial activity (MIC = 0.031–0.25 μg/mL), whereas compound **12b**, bearing

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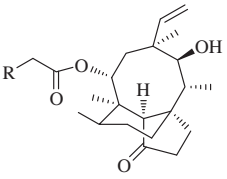
Table 1
Minimum inhibitory concentration of pleuromutilin derivatives.



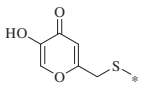
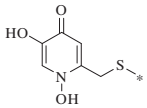
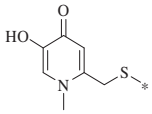
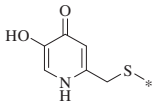
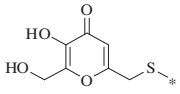
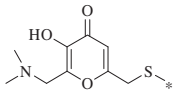
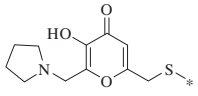
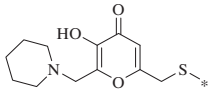
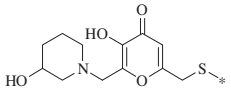
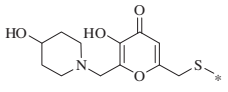
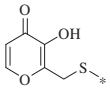
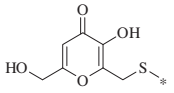
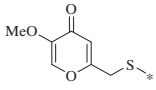
Compd.	R	MIC range (μg/mL)[a]				
		MSSA	MRSA	MSSE	MRSE	PRSP
12a		0.063–0.25	0.063–0.25	0.031–0.25	0.063–0.125	0.063–0.25
12b		>16	>16	>16	>16	>16
12c		2–4	2–4	2–4	1–4	2–4
16		1–4	1–4	1–4	1–4	1–4
12d		0.063–0.5	0.063–0.125	0.125–0.5	0.125–0.25	0.25–0.5
12e		0.125–0.25	0.125–0.5	0.25–1	0.25–0.5	0.5
12f		0.5–2	0.5–2	0.25–1	0.25–1	0.5–2
12g		0.25–1	0.25–1	0.25–1	0.5–1	0.5–2
12h		0.5–1	0.25–1	0.25–0.5	0.5	0.5–1
12i		0.5–2	0.5–2	0.5–1	0.5–1	0.5–1
22		0.25–0.5	0.25–0.5	0.25	0.25–0.5	0.25–1
28		0.063–0.5	0.063–0.5	0.25–0.5	0.25–0.5	0.5
31		0.25–0.5	0.25–0.5	0.125–0.5	0.25	0.25–0.5

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Table 1 (*Continued*)

Compd.	R	MIC range (μg/mL)[a]				
		MSSA	MRSA	MSSE	MRSE	PRSP
Retapamulin		0.125	0.125	0.125–0.5	0.125–0.25	0.063–0.125
BC-3781		0.125–0.25	0.125–0.25	0.063–0.125	0.125–0.25	0.125–0.25

[a] Abbreviations are as follows: MSSA, methicillin-sensitive *Staphylococcus aureus*, 5 strains; MRSA, methicillin-resistant *Staphylococcus aureus*, 5 strains; MSSE, methicillin-sensitive *Staphylococcus epidermidis*, 5 strains; MRSE, methicillin-resistant *Staphylococcus epidermidis*, 5 strains; PRSP, penicillin-resistant *Streptococcus pneumoniae*, 5 strains.

Table 2
Pharmacokinetic parameters of compounds **12a**, **12d**, **12e**, **28** and **BC-3781** by cassette dosing at 2 mg/kg in rats (i.v., $n = 3$).

Compd.	C_{max} (ng/mL)	AUC_{0-t} (h ng/mL)	MRT (h)	$T_{1/2}$ (h)	CL (L/h/kg)	V_{ss} (L/kg)
12a	644 ± 46	195 ± 11	0.16 ± 0.01	0.15 ±0.01	10.3 ± 0.6	1.7 ± 0.2
12d	308 ± 35	87 ± 9	0.13 ± 0.01	0.11 ± 0.01	23.2 ± 2.3	2.9 ± 0.5
12e	472 ± 33	170 ± 13	0.25 ± 0.02	0.24 ± 0.05	11.7 ± 1.0	2.9 ± 0.3
28	377 ± 34	112 ± 9	0.17 ± 0.02	0.27 ± 0.14	17.9 ± 1.5	3.0 ± 0.4
BC-3781	279 ± 24	296 ± 23	2.44 ± 0.11	2.51 ± 0.27	6.3 ± 0.5	15.5 ± 1.8

All values are represented as mean ± SD; AUC_{0-t}, area under the serum concentration–time curve up to last sampling time; MRT, mean residence time; $T_{1/2}$, elimination half-life; V_{ss}, volume of distribution at steady-state.

N-hydroxylpyridin-4-one moiety, almost lost activity. Replacement of the *N*-hydroxyl group in compound **12b** with a methyl group or hydrogen atom restored some of the activity (compounds **12c** and **16** *vs.* compound **12b**). This suggested that 4*H*-pyran-4-one moiety is very important for maintaining antibacterial activity. Further modifications were then focused on compound **12a**. Introduction of hydroxymethyl or amino groups onto the pyranone ring of compound **12a** gave compounds **12d**–**12i**, which displayed moderate to good activity. Compound **22**, the regioisomer of compound **12a**, showed a 2–4-fold decreased antibacterial activity, whereas compound **28** exhibited comparable activity to its regioisomer, compound **12d**. *O*-Methylation of the hydroxyl group in the 4*H*-pyran-4-one moiety of compound **12a** gave compound **31** with 2–4-fold reduced activity, indicating the hydroxyl group was essential for potent antibacterial activity. Among all the derivatives, compounds **12a**, **12d**, and **28** are most potent in this series, exhibiting antibacterial activity comparable to or slightly better than retapamulin and BC-3781.

Compounds **12a**, **12d**, **12e** and **28** were then selected for pharmacokinetic study. Cassette-dosing experiments were performed on these compounds with clinical investigational drug BC-3781 included for comparison. Results of this study are summarized in Table 2.

As shown in Table 2, while all the target compounds displayed a favorable maximum plasma concentration (C_{max}), their plasma exposure levels (AUC_{0-t}) were much lower than that of BC-3781. An elimination half-life ($T_{1/2}$) and a mean residence time (MRT) of below 15 min suggested that these compounds underwent a rapid clearance from plasma after intravenous administration. As the PK profiles were not favorable, further investigation and structural optimization are still ongoing.

4. Conclusion

By incorporation of 4*H*-pyran-4-one and pyridin-4-one moieties into the C-14 side chain, a series of novel pleuromutilin derivatives were designed and synthesized. Structure–activity relationship studies showed that 4*H*-pyran-4-one moiety is favored over pyridin-4-one. Among the series, compounds **12a**, **12d**, and **28** exhibited excellent antibacterial activity against clinically isolated Gram-positive strains including MRSA, MRSE, and PRSP. Cassette-dosing experiments showed that theses novel pleuromutilin derivatives underwent a rapid clearance after intravenous administration. Further investigation and structural modifications are needed to improve their PK profiles.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.cclet.2015.09.019.

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