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The synthesis and antistaphylococcal activity of dehydroabietic acid derivatives: Modifications at C-12

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ABSTRACT

A series of 12-oxime and *O*-oxime ether derivatives of dehydroabietic acid were synthesized and investigated for the antibacterial activity against *Staphylococcus aureus* Newman strain and five multidrug-resistant strains (NRS-1, NRS-70, NRS-100, NRS-108, and NRS-271). The aromatic oximate derivative **11a** showed the highest activity with MIC of 0.39–0.78 μg/mL against *S. aureus* Newman. Of note, compounds **10b**, **11** and **14** showed the most potent antibacterial activity against five multidrug-resistant *S. aureus* with MIC values of 1.25–3.13 μg/mL. These results offered useful information for further strategic optimization in search of the antibacterial candidates against infection of multidrug-resistant Gram-positive bacteria.

Hospital acquired infections with multidrug-resistant bacteria represent a global public health threat. In particular, methicillin-resistant *Staphylococcus aureus* (MRSA) acquired resistance against many different antibiotics, including β-lactam, cephalosporin, fluoroquinolone, aminoglycoside, tetracycline, macrolide, trimethoprim-sulfamethoxazole, and vancomycin.[1] The current situation has stimulated an urgent need to develop more effective antistaphylococcal agents with novel chemical structures and mechanisms. Natural products (NPs) are biologically validated starting points for drug discovery and have played a dominant role in drug discovery efforts for treatment of human diseases.[2] The abietanes are diterpenoids possessing an ABC ring system and have demonstrated to show a broad range of biological properties particularly antibacterial activity.[3–6] For example, the well-known natural diterpenoids totarol and ferruginol (Fig. 1) and related NPs, as well as tricyclic diterpene analogues have shown antibacterial activity against Gram-positive bacteria[7–9]and in particular MRSA.[10,11]

Dehydroabietic acid (DHAA, **1**) is an abietane diterpene that can be readily obtained by disproportionation of rosin or abietic acid. DHAA and its derivatives have been reported to possess a broad spectrum of biological properties such as antiulcer, antitumor, antiviral, antimicrobial, anti-inflammatory, and BK channel-opening activities.[12–17] Interestingly, DHAA and its derivatives inhibited the formation of bacterial biofilm during infections of *S. aureus*, which demonstrated its ability not only to prevent bacterial colonization, but also to inhibit existing biofilms.[18,19] These findings indicate the potential usage of the DHAA analogues as novel biological probes and pharmaceutical agents. In addition to the several series of DHAA derivatives that displayed potent antimicrobial activities[20–22], there are still interesting modifications to be exploited, especially for antibiotic-resistant Gram-positive bacteria.

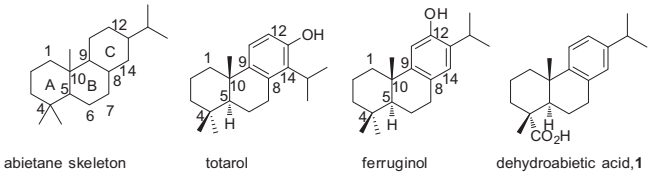

Figure 1.

Oximes and derivatives are found in a variety of drugs on the market, including fluvoxamine (antidepressant), oxiconazole (antifungal), and antibacterial drugs gemifloxacin and cefetamet (Fig. 2). In our continued efforts against *S. aureus* infection,[23]

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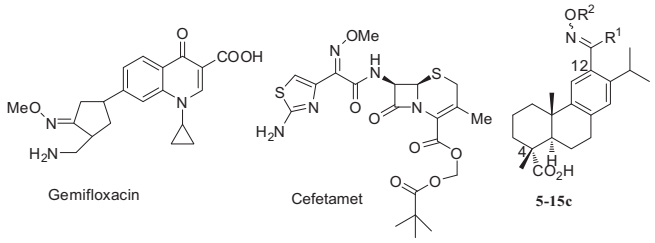
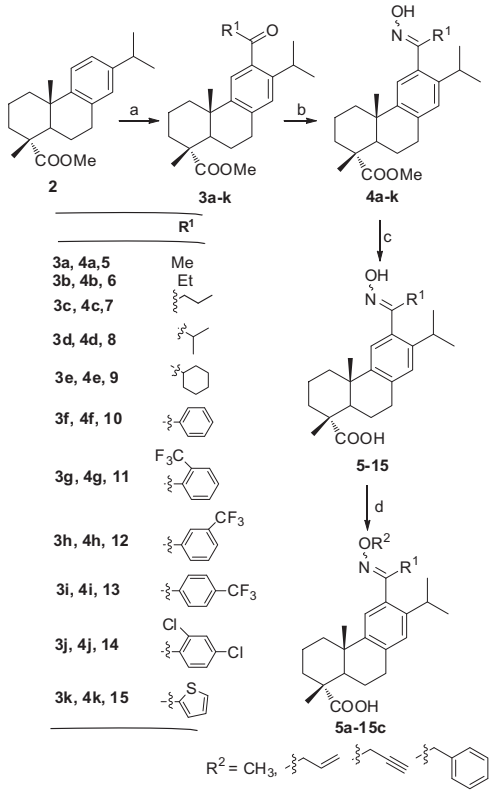


Figure 2.

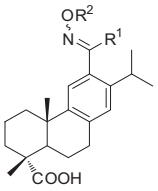


Scheme 1. Reagents and conditions: (a) R^1COCl, $AlCl_3$, CCl_4, −20 °C, overnight, 56–99%; (b) NH_2OH–HCl, Py, EtOH, reflux, 4 h, 51–86%; (c) $KOBu^t$, DMSO, rt, 2 h, 73–91% (d) R^2Br or R^2I, NaI, KOH, EtOH, 40–70 °C, 6 h, 44–95%.

herein, we report the synthesis of a series of new 12-oxime or oxime ether derivatives **5**–**15c** starting from DHAA. The in vitro antibacterial activities were evaluated against *S. aureus* Newman strain and five multidrug-resistant *S. aureus* in order to define the influence of structural features on the aromatic ring upon antibacterial potency of these diterpenes.

The synthetic procedures for the target compounds are outlined in Scheme 1. Friedel–Crafts acylation of dehydroabietic acid methyl ester (**2**) with various acyl chloride in the presence of $AlCl_3$ in CCl_4 at −20 °C afforded the 12-keto derivatives **3a**–**k** in 56–99% yields. Treatment of **3a**–**k** with hydroxylamine hydrochloride in EtOH under reflux afforded the corresponding oximes **4a**–**k** in 51–86% yields. Then esterolysis of **4a**–**k** by $KOBu^t$ in DMSO afforded **5**–**15** in 73–91% yields. The target oxime ether derivatives **5a**–**15c** (Tables 1 and 2) were correspondingly prepared by *O*-Alkylation of **5**–**15** with various alkyl or aryl halides. Almost all the oximes **4a**–**k** obtained in the coupling reactions were mixtures of *E* and *Z* isomers and, in most cases, not resolved in the two components when their separation proved to be impossible by crystallization or flash chromatography. Compound **5**, the

Table 1
Preliminary MIC measurements for aliphatic oximate derivatives against *S. aureus* Newman

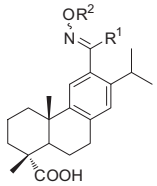


Compound	R^1	R^2	MIC (μg/mL)
Vancomycin	—	—	0.78–1.56
Tetracycline	—	—	<0.2
DHAA (**1**)	—	—	12.5–20
5	CH_3	H	>50
5a	CH_3		30–40
5b	CH_3		>50
5c	CH_3		>50
6		H	>50
6a		CH_3	>50
6b			>50
6c			20–25
6d			>50
7		H	40–50
7a		CH_3	40–50
7b			10–12.5
7c			15–20
7d			>50
8		H	>50
8a		CH_3	30–40
8b			10–12.5
8c			10–12.5
8d			>50
9		H	12.5–25
9a		CH_3	>50
9b			12.5–25
9c			>50

stereo-configuration of which was determined by X-ray analysis[24], and its derivatives **5a**–**c** were isolated as *E* isomers. All remaining oximes **6**–**15c** were used in the pharmacological tests as mixtures of *E* and *Z* isomers.

The in vitro antimicrobial activities of all the synthesized DHAA derivatives were evaluated for Gram-positive bacteria, *S. aureus* Newman, and five multidrug-resistant *S. aureus* including NRS-1 (resistant to aminoglycosides and tetracycline), NRS-70 (resistant to erythromycin), NRS-100 (resistant to oxacillin and tetracycline), NRS-108 (resistant to gentamicin), and NRS-271 (linezolid resistant, containing phage type E-MRSA 15). All target compounds were evaluated at the concentrations ranging from 0.39 μg/mL to 50 μg/mL and assessed in terms of minimum inhibitory concentrations (MICs), which are defined as the lowest concentrations of compound at which microbial growth was inhibited. Vancomycin and tetracycline were included as positive controls. Experiments were performed in triplicate for each condition. The antibacterial data are shown in Tables 1 and 2.

Table 2
MICs of synthetic aromatic oximate derivatives against the growth of strains of *S. aureus* Newman, NRS-1, NRS-70, NRS-100, NRS-108, and NRS-271



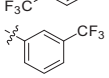
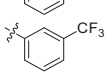
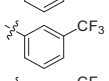
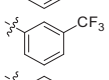
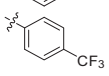
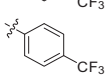
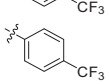
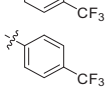
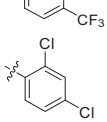
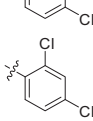
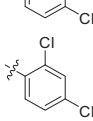
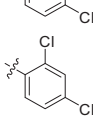
Compound	R^1	R^2	MIC (μg/mL)					
			Newman	NRS-1	NRS-70	NRS-100	NRS-108	NRS-271
Vancomycin	—	—	0.78–1.56	1.56–3.13	0.2–0.39	0.39–0.78	0.39–0.78	0.39–0.78
Tetracycline	—	—	<0.2	>25	<0.20	<0.20	<0.20	<0.20
DHAA (**1**)	—	—	12.5–20	12.5–25	12.5–25	12.5–25	12.5–25	12.5–25
10	—	—	10–12.5	—	—	—	—	—
10a		CH_3	5.0	—	—	—	—	—
10b			1.25–2.5	2.5–3.13	1.25–1.56	1.56–2.5	1.56–2.5	1.56–2.5
10c			2.5	6.25–12.5	1.56–2.5	3.13	3.13–6.25	3.13–6.25
10d			>50	—	—	—	—	—
11		H	1.56–3.13	1.56–3.13	1.56–3.13	1.56–3.13	1.56–3.13	1.56–3.13
11a		CH_3	0.39–0.78	3.13–6.25	1.56–3.13	3.13–6.25	6.25–12.5	1.56–3.13
11b			0.78–1.56	12.5–25	1.56–3.13	6.25–12.5	6.25–12.5	>50
11c			1.56–3.13	>25	>25	>25	>25	>25
12		H	1.56–3.13	3.13–6.25	1.56–3.13	3.13–6.25	3.13–6.25	3.13–6.25
12a		CH_3	1.56–3.13	>25	>25	>25	>25	>25
12b			>50	—	—	—	—	—
12c			1.56–3.13	>25	>25	>25	>25	>25
13		H	>50	—	—	—	—	—
13a		CH_3	>50	—	—	—	—	—
13b			>50	—	—	—	—	—
13c			>50	—	—	—	—	—
14		H	1.56–3.13	3.13	1.56–3.13	3.13	1.56–3.13	1.56–3.13
14a		CH_3	1.56–3.13	>25	1.56–3.13	>25	>25	>25
14b			1.56–3.13	>25	1.56–3.13	12.5–25	12.5	>25
14c			1.56–3.13	>25	1.56–3.13	12.5–25	12.5	12.5–25

Table 2 (*continued*)

Compound	R^1	R^2	MIC (μg/mL)					
			Newman	NRS-1	NRS-70	NRS-100	NRS-108	NRS-271
15		H	6.25–12.5	–	–	–	–	–
15a		CH_3	6.25–12.5	–	–	–	–	–
15b			25–50	–	–	–	–	–
15c			1.56–3.13	>25	3.13–6.25	>25	>25	>25

As shown in Table 1, DHAA exhibited moderate antibacterial activity against Newman with MIC in the range of 12.5–20 μg/mL. Most of the aliphatic oximate derivatives **5**–**9c** showed weak activities at 50 μg/mL against *S. aureus* Newman. Introduction of a small saturated substituent such as methyl, small unsaturated substituents or benzyl group (compounds **5a**–**c**, **6a**–**d**, **7a**–**d**, **8a**–**d**) slightly impacted antistaphylococcal activity when compared to the corresponding *O*-unsubstituted oximes (**5**, **6**, **7**, **8**). Among these derivatives, only the butyraldehyde oxime analogues **7b**, **8b**–**c** exhibited comparable activity (10–12.5 μg/mL) with DHAA.

We also made a series of aromatic oximate derivatives **10**–**15c**. The activities of these compounds are dependent on the positions and properties of the substituents (Table 2). 12-((Hydroxyimino)(phenyl)methyl)-dehydroabietic acid **10** exhibited comparable activity (10–12.5 μg/mL) with DHAA. Introduction of small unsaturated substituents to the oxime functionality (compounds **10b** and **10c**) increased the activity significantly, with MICs in the range of 1.25–2.5 μg/mL. However, introduction of a phenyl-substituted carbon chain such as benzyl group decreased the activity, for example the inactive compound **10d**. In the series of oxime derivatives **11**, **12**, and **13**, *ortho*- or *meta*-substitution of an electron-withdrawing trifluoromethyl group on the aromatic ring increased the activity, and compounds **11** and **12** showed MICs values of 1.56–3.13 μg/mL. On the contrary, the *para*-CF_3 isomer **13** and its oxime ether derivatives **13a**–**c** exhibited no in vitro activity against any of the Gram-positive strains at 50 μg/mL. For the *o*-CF_3 series of compounds, introduction of a small saturated substituent such as methyl (**11a**) or small unsaturated substituents (**11b**, **11c**) retained or increased the activities as compared with the *O*-unsubstituted oxime **11**. Compound **11a** showed the highest activity among the synthesized compounds with MIC value of 0.39–0.78 μg/mL. However, for the *m*-CF_3 series of compounds, introduction of a small unsaturated allyl substituent (**12b**) resulted in a loss in activity. The 2,4-dichloro oxime derivative **14** and its oxime ether derivatives **14a**–**c** exhibited similar activity with MICs in the range of 1.56–3.13 μg/mL. At the same time, we synthesized several aromatic heterocycle (thiophene) containing derivatives (**15**–**15c**), only compound **15c** showed much improved activity than DHAA.

The most active compounds against Newman were further evaluated on five multidrug-resistant *S. aureus* (Table 2). For NRS-1, NRS-70, NRS-100, NRS-108, and NRS-271, DHAA itself showed moderate antibacterial activity with MICs in the range of 12.5–25 μg/mL, compounds **10b**, **11** and **14** showed the most potent activity with MICs of 1.25–3.13 μg/mL. Oxime derivative **12** also showed good inhibitory activity (MIC, 1.56–6.25 μg/mL). Except **10b**, all of **11**, **12**, and **14** have the *O*-unsubstituted oxime functionality. In general, to these multidrug-resistant *S. aureus*, alkylation of the oxime group (compounds **11c**, **12a** and **12c**) showed dramatically decreased antimicrobial activity, (MICs > 25 μg/mL) compared to compounds **11** and **12**, the trend of which was different for *S. aureus* Newman. Similarly, for the 2,4-dichloro series of compounds, alkylation of the oxime group (**14a**–**c**) decreased the activity except for NRS-70 strain. The SARs of these compounds suggest the importance of the hydroxyloxime group in ring C, especially for the activity against multidrug-resistant *S. aureus*.

In conclusion, we have described here the design, synthesis, and the evaluation of antibacterial activity against *S. aureus* of novel derivatives of dehydroabietic acid with modifications at C12 position. Analysis of SARs indicates that the 12-oxime structure provides good opportunity for designing novel antimicrobial agents against multidrug-resistant *S. aureus*. The aromatic oximate derivative **11a** showed the highest activity with MIC of 0.39–0.78 μg/mL against *S. aureus* Newman, while compounds **10b**, **11**, and **14** showed the most potent antibacterial activity with MIC values of 1.25–3.13 μg/mL against five multidrug-resistant *S. aureus* (NRS-1, NRS-70, NRS-100, NRS-108 and NRS-271). The results highlight these new oxime and oxime ether derivatives as potential leads for further investigation of new antimicrobial agents. Further SAR study in depth of the active scaffolds and the mechanism are yet to be investigated.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2016.10.018.

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