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Research paper

Discovery of novel piperonyl derivatives as diapophytoene desaturase inhibitors for the treatment of methicillin-, vancomycin- and linezolid-resistant *Staphylococcus aureus* infections

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ABSTRACT

Inhibition of *S. aureus* diapophytoene desaturase (CrtN) could serve as an alternative approach for addressing the tricky antibiotic resistance by blocking the biosynthesis of carotenoid pigment which shields the bacterium from host oxidant killing. In this study, we designed and synthesized 44 derivatives with piperonyl scaffold targeting CrtN and the structure-activity relationships (SARs) were examined extensively to bring out the discovery of **21b** with potent efficacy and better hERG safety profile compared to the first class CrtN inhibitor benzocycloalkane derivative **2**. Except the excellent pigment inhibitory activity against wild-type *S. aureus*, **21b** also showed excellent pigment inhibition against four pigmented MRSA strains. In addition, H_2O_2 killing and human whole blood killing assays proved **21b** could sensitize *S. aureus* to be killed under oxidative stress conditions. Notably, the murine study *in vivo* validated the efficacy of **21b** against pigmented *S. aureus* Newman, vancomycin-intermediate *S. aureus* Mu50 and linezolid-resistant *S. aureus* NRS271.

1. Introduction

Staphylococcus aureus (*S. aureus*) is a pathogen that has been evolving multi-resistance to many antibiotics and poses a major menace to human health. The diseases typically present as skin infections and more serious cases, like sepsis, pneumonia and bloodstream infections [1]. The mortality related to methicillin-resistant *S. aureus* (MRSA) bloodstream infection remains as high as 30% [2]. It's estimated by the U.S. Centers for Disease Control and Prevention (CDC) that in the U.S. alone, almost half of the total documented fatalities caused by antibiotic resistant infections was from MRSA invasive infections [3]. To cope with MRSA infections, vancomycin and linezolid have been taken as the most reliable therapeutic agents [4,5]. However, the increasing emergence of vancomycin-intermediate *S. aureus* (VISA) and linezolid-resistant *S. aureus* (LRSA) makes it a great challenge in response to this treatment failure [6,7]. These issues emphasize the urgency to find novel structures and mechanism of action to withstand antibiotic resistant bacteria.

Antivirulence is an alternative approach to revitalize the drug-development pipeline with new targets and new chemical entities to treat infections caused by resistant bacteria [8]. Anti-virulence agents target bacterial products that promote disease by either damaging the host or evading the host immune system, and allow bacterial multiplication in the host rather than kill or halt pathogen growth, thus generating much weaker selection for resistance than traditional antibiotics [8–11]. As a novel target for antivirulence therapy against *S. aureus*, diapophytoene desaturase (CrtN) is an

Abbreviations used: S. aureus, *Staphylococcus aureus*; MRSA, methicillin-resistant *Staphylococcus aureus*; STX, staphyloxanthin; NTF, naftifine hydrochloride; CrtM, dehydrosqualene synthase; CrtN, diapophytoene desaturases; ROS, reactive oxygen species; SAR, structure-activity relationship; IC_{50}, half maximal inhibitory concentration; HPLC, high-performance liquid chromatography; MS, mass chromatography; CFU, colony-forming unit; PBS, phosphate-buffered saline; DMF, *N,N*-dimethylformamide; DMSO, dimethyl sulfoxide; EtOH, ethanol; EtOAc, ethyl acetate; MeOH, methanol; THF, tetrahydrofuran; CH_2Cl_2, dichloromethane; Et_3N, triethylamine.

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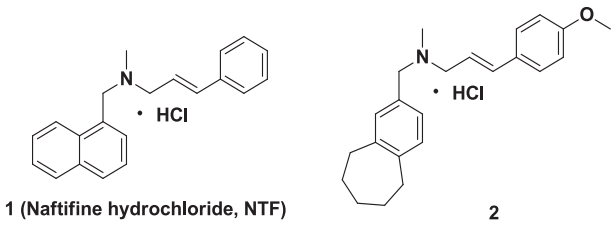


Fig. 1. Structures of Naftifine and benzocycloalkane derivative **2**.

established essential enzyme for staphyloxanthin (STX) biosynthesis, which shields golden carotenoid pigmented *S. aureus* from host oxidant killing [12–15]. Naftifine (Fig. 1), a US Food and Drug Administration (FDA)-approved antifungal drug, was proved efficient blocking biosynthesis of carotenoid pigment at nanomolar concentrations and decreasing bacteria survival rate in murine abscess formation model without directly killing the pathogen [16]. Based on this, our research group has designed and synthesized a series of CrtN inhibitors with novel scaffolds as potent drug candidates against MRSA infections [17,18]. Benzocycloalkane derivative **2** (Fig. 1) exhibited potent anti-infectious activity *in vitro* and *in vivo* [18]. However, the further exploration was suspended for the unsatisfactory hERG safety profile ($IC_{50} = 3.2\ \mu M$) and poor water solubility (4.24 mg/mL). In an attempt to increase hydrophilicity of the core ring and gain novel CrtN inhibitors with better hERG safety profiles, we designed and synthesized a collection of piperonyl derivatives by inserting two oxygen atoms into the cycloalkane ring and through rational structure modification to find better CrtN inhibitors. As an extension to that study, our work was aimed at the design, synthesis and evaluation of potent CrtN inhibitors with novel scaffold plus superior physical property and safety profile. Moreover, the effectiveness of the synthesized compound on attenuating the virulence of *S. aureus* strains including vancomycin-intermediate MRSA Mu50 and linezolid-resistant MRSA NRS271 [19,20] was assessed in a murine abscess formation model from the aspect of dosage and the mode of administration with linezolid and vancomycin as positive control drugs.

2. Results and discussion

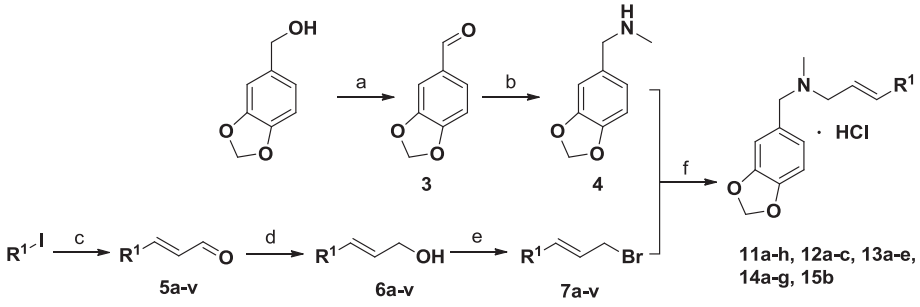
2.1. Chemistry

The general synthetic route of derivatives **11a-h, 12a-c, 13a-e, 14a-g** and **15b** are outlined in Scheme 1. Commercial acquired piperonol was oxidized by manganese dioxide to yield piperonyl aldehyde **3**. Compound **4** was prepared by reductive amination of **3** with methylamine. Iodide compounds reacted with acrolein diethyl acetal through Heck reaction and acidification to produce cinnamic aldehyde derivatives. The latter was subsequently deoxidized by sodium borohydride and then brominated by phosphorus tribromide to generate compounds **7a-v**. **4** reacted with **7a-v** via nucleophilic substitution to afford target derivatives.

Another route in Scheme 2 was applied to prepare cinnamyl alcohol derivatives and target compounds **12d, 12e, 14h-m, 15a** and **15c-e** due to the availability of starting materials. Commercial acquired benzaldehyde derivatives reacted with trimethyl phosphonoacetate to give methyl cinnamate **8a-i** via Wittig-Horner reaction. Cinnamyl alcohol derivatives **9a-i** were prepared by ester reduction with diisobutylaluminum hydride and then brominated by phosphorus tribromide to generate compounds **10a-i**. **4** reacted with **10a-i** via nucleophilic substitution to produce target derivatives **12d, 12e, 14h-m, 15a** and **15c-e**.

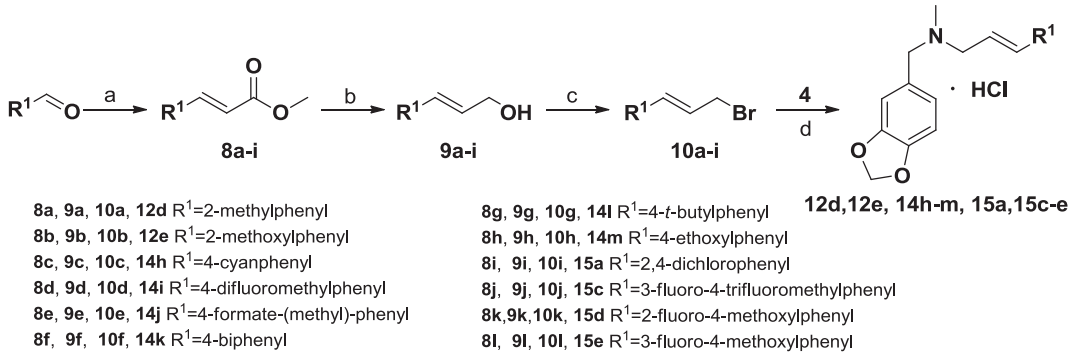
As shown in Scheme 3, to prepare derivatives **16a-c**, piperonylamine was coupled with intermediate **7v** via nucleophilic substitution to afford derivative **16a**, followed by substitution with an ethyl group or isopropyl group to yield target derivatives **16b** and **16c** respectively.

Scheme 4 depicts the synthetic route for derivatives **21a-e**. Intermediate **17** was obtained from 3-fluoro-4-iodobenzotrifluoride via Sonogashira coupling, followed by deprotonation of O-TBDMS to give key intermediate **19a**. Intermediate **5v** reacted with triethyl phosphonoacetate to produce **18a** via Wittig-Horner reaction and then reduced by diisobutylaluminium hydride to yield key

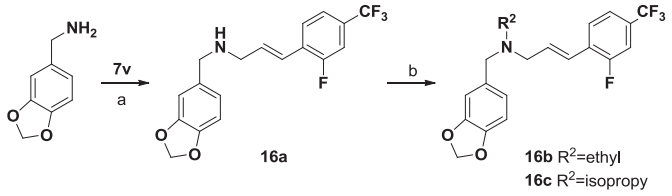


11a R^1 = phenyl
11d R^1 = H
5a, **6a**, **7a**, **11b** R^1 = furan-2-yl
5b, **6b**, **7b**, **11c** R^1=2-thienyl
5c, **6c**, **7c**, **11e** R^1=cyclopentyl
5d, **6d**, **7d**, **11f** R^1=cyclohexyl
5e, **6e**, **7e**, **11g** R^1=1-naphthyl
5f, **6f**, **7f**, **11h** R^1=2-naphthyl
5g, **6g**, **7g**, **12a** R^1=2-fluorophenyl
5h, **6h**, **7h**, **12b** R^1=2-chlorophenyl
5i, **6i**, **7i**, **12c** R^1=2-trifluoromethylphenyl
5j, **6j**, **7j**, **13a** R^1=2-trifluoromethylphenyl
5k, **6k**, **7k**, **13b** R^1=3-chlorophenyl
5l, **6l**, **7l**, **13c** R^1=3-trifluoromethylphenyl
5m, **6m**, **7m**, **13d** R^1=3-methylphenyl
5n, **6n**, **7n**, **13e** R^1=3-methoxylphenyl
5o, **6o**, **7o**, **14a** R^1=4-fluorophenyl
5p, **6p**, **7p**, **14b** R^1=4-chlorophenyl
5q, **6q**, **7q**, **14c** R^1=4-bromophenyl
5r, **6r**, **7r**, **14d** R^1=4-trifluoromethylphenyl
5s, **6s**, **7s**, **14e** R^1=4-nitrophenyl
5t, **6t**, **7t**, **14f** R^1=4-methylphenyl
5u, **6u**, **7u**, **14g** R^1=4-methoxylphenyl
5v, **6v**, **7v**, **15b** R^1=2-fluoro-4-trifluoromethylphenyl

Scheme 1. Synthesis of compounds **11a-h, 12a-c, 13a-e, 14a-g** and **15b**. Reagents and conditions: (a) MnO_2, DCM, r.t., overnight, 95%; (b) methylamine (30%–33% in methanol), MeOH, r.t., 5 h; then $NaBH_4$, MeOH, 0 °C to r.t., 30 min, 90%, (2 steps); (c) acrolein diethyl acetal, tetrabutylammonium acetate, palladium diacetate, K_2CO_3, KCl, DMF, 90 °C, 2 h; then HCl (33 wt. % in water), 62–85% (2 steps); (d) $NaBH_4$, MeOH, 0 °C to r.t., 30min, 90%; (e) PBr_3, Et_2O, 0 °C to r.t., overnight, under N_2, 50–85%; (f) K_2CO_3, DMF, r.t., overnight, 42–65%, then bubbled into hydrogen chloride gas.



Scheme 2. Synthesis of compounds **12d, 12e, 14h-m, 15a** and **15c-e**. Reagents and conditions: (a) trimethyl phosphonoacetate, NaOMe (28% in methanol), DMF, r.t., 1 h, 95%; (b) diisobutylaluminum hydride (1.0 M in hexane), DCM, 1.5 h, 0 °C to r.t., 65–90%; (c) PBr_3, Et_2O, 0 °C to r.t., overnight, under N_2, 50–85%; (d) K_2CO_3, DMF, r.t., overnight, 42–65%, then bubbled into hydrogen chloride gas.



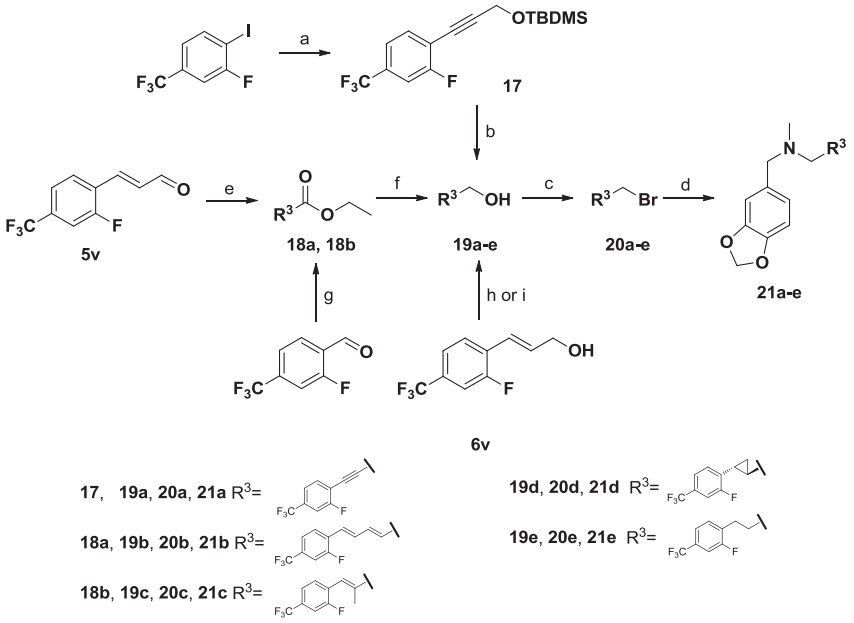
Scheme 3. Synthesis of compounds **16a-c**. Reagents and conditions: (a) K_2CO_3, DMF, r.t., overnight, 55%; (b) iodoethane or 2-iodopropane, NaH, DMF, 0 °C to r.t, overnight, under N_2, 50%; hydrogen chloride gas.

intermediate **19b**. 2-Fluoro-4-(trifluoromethyl)benzaldehyde reacted with Wittig salt to give **18b** and further reduced as **18a** to afford key intermediate **19c**. Intermediate **6v** was converted to key intermediate **19d** and **19e** through reduction by hydrogen and carbene reaction respectively. The five key intermediates were brominated by phosphorus tribromide producing **20a-e** and were finally reacted with **4** to give the final products **21a-e**.

2.2. Biological activity

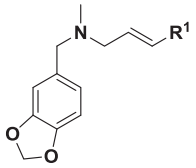
2.2.1. In vitro pigment inhibitory activities

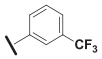
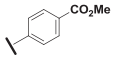
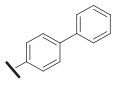
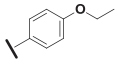
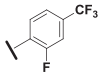
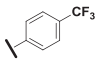
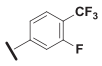
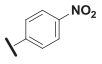
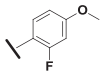
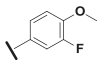
To preliminarily assess the activities of synthesized compounds *in vitro*, the half maximal inhibitory concentration (IC_{50}) values for golden carotenoid pigment inhibition of 44 target compounds were tested against wild-type *S. aureus* Newman. As shown in Table 1, compounds bearing unsubstituted phenyl ring or methyl, cycloalkyls, heteroaryls (**11a-f**) showed a loss of the pigment inhibitory activities. Replacement of phenyl ring at para position with various substituents, including electron-donating (**14f-g**) and electron-withdrawing groups (**14a-b**, **14d**), showed superior activity to their isomers (**12a-e** and **13a-e**), which demonstrated that the substituted positions on the phenyl ring substantially affected the activity. Moreover, there is no obvious relation between pigment inhibitory activity and the electronic effect of substituents (comparing the compounds **14a-14g**), indicating that both electron-donating and electron-withdrawing groups were both tolerable at the phenyl ring. Based on this, more para-substitution



Scheme 4. Synthesis of compounds **20a-e**. Reagents and conditions: (a) *tert*-butyldimethyl(2-propynyloxy)silane, $Pd(PPh_3)_4$, CuI, Et_3N, 0 °C to 60 °C, 30 min, 89%; (b) TBAF, THF, r.t., 30 min, 98%; (c) PBr_3, Et_2O, 0 °C to r.t., overnight, under N_2, 62–76%; (d) K_2CO_3, DMF, r.t., overnight, 25–57%; hydrogen chloride gas; (e) triethyl phosphonoacetate, NaOMe (28% in methanol), DMF, r.t., 1 h, 95%; (f) $AlH(Bu\text{-}i)_2$ (1.0 M in hexane), DCM, 1.5 h, 0 °C to r.t., 70%; (g) (carbethoxyethylidine)triphenylphosphorane, PhMe, reflux, 1 h, 85%; (h) TFA, Et_2Zn (1.0 M in hexane), CH_2I_2, 0 °C to r.t., 2 h; 86% for **19d**; (i) Pd/C, MeOH, H_2, r.t., overnight; 94% for **19e**.

Table 1
Chemical structures and their pigment inhibitory activities against *S. aureus* Newman for derivatives **11a-h**, **12a-e**, **13a-e**, **14a-m** and **15a-e**.



Cpd.	R^1	*S. aureus* Newman IC_{50}(nM)[a]	Cpd.	R^1	*S. aureus* Newman IC_{50}(nM)[a]	Cpd.	R^1	*S. aureus* Newman IC_{50}(nM)[a]
11a		>1000	**12e**		>1000	**14g**		9.1 ± 0.6
11b		>1000	**13a**		412.3 ± 12.4	**14h**		12.5 ± 1.8
11c		>1000	**13b**		575.5 ± 11.4	**14i**		4.1 ± 0.1
11d		>1000	**13c**		523.3 ± 136.9	**14j**		7.9 ± 0.5
11e		>1000	**13d**		372.4 ± 16.3	**14k**		3.9 ± 0.2
11f		>1000	**13e**		>1000	**14l**		5.2 ± 0.4
11g		>1000	**14a**		58.4 ± 9.8	**14m**		4.2 ± 0.4
11h		4.0 ± 0.1	**14b**		4.2 ± 0.4	**15a**		17.6 ± 4.1
12a		121.2 ± 6.9	**14c**		4.6 ± 0.2	**15b**		1.8 ± 0.0
12b		>1000	**14d**		3.5 ± 0.6	**15c**		57.0 ± 0.5
12c		28.8 ± 4.6	**14e**		82.6 ± 10.5	**15d**		3.8 ± 0.2
12d		>1000	**14f**		15.7 ± 0.3	**15e**		112.0 ± 20.6

[a] All the data represent mean values ± S.D.

(bromo, nitro, cyan, difluoromethyl, formate, biphenyl, tertiary butyl and ethyoxyl) and disubstituted compounds (**15a-e**) were synthesized and evaluated for obtaining better inhibitors. Comparison of IC_{50} values exhibited by **14a-m**, the sterically hindered substituents exerted a beneficial influence on the activity (**14a** vs **14d**, **14f** vs **14l** and **14g** vs **14m**), indicating that the size of substituents may favor the pigment inhibitory activity. Moreover, the activity of mono para-substituted derivatives were higher than that of 3-fluoro-4-disubstitute but lower than that of 2-fluoro-4-disubstitute derivative (**14d** vs **15c** vs **15b** and **14g** vs **15e** vs **15d**).

For further investigating the drug-like properties of these compounds, two important assays were introduced into the study, including water solubility and cell toxicity. First, 9 compounds with IC_{50} values lower than 10 nM were selected to evaluate the cytotoxicity against two mammalian cell lines of hepatic carcinoma cell HepG-2 and normal human embryonic kidney cell HEK-293T by determining their half cytotoxic concentrations (CC_{50}). Since solubility is a critical property of druggability, UV assay was applied to determine the water solubility of these compounds in parallel. As shown in Table 2, all selected compounds displayed good solubility, and six (**14b**, **14c**, **14d**, **14m**, **15b** and **15d**) out of these nine tested compounds demonstrated excellent water solubility (over 10 mg/mL). Moreover, compounds **14b**, **14m**, **15b** and **21b** exhibited outstanding cytotoxicity profile against these two cell lines at comparable levels ($CC_{50} > 80\ \mu M$). Especially, **15b** possessed balanced cytotoxicity profiles against cell lines HepG-2 and HEK-293T, compared with **14b** and **14m**. Considering the pigment inhibitory activity, water solubility and cytotoxicity profile, **15b** thus far emerged as the most attractive candidate for further structural modification.

Table 2
Pigment (*S. aureus* Newman, IC_{50}), cytotoxicity (CC_{50}) and water solubility results of representative compounds.

Cpd.	*S. aureus* Newman IC_{50}(nM)[a]	Cytotoxicity CC_{50} (μM)[a]		Water Solubility (mg/mL)[b]
		HepG2	HEK293T	
11h	4.02 ± 0.13	60.48 ± 2.04	48.32 ± 0.96	4.56
14b	4.24 ± 0.38	140.51 ± 10.98	83.83 ± 6.01	18.71
14c	4.55 ± 0.19	104.40 ± 8.96	68.33 ± 4.87	17.46
14d	3.51 ± 0.61	69.23 ± 5.52	133.10 ± 15.90	13.50
14i	4.10 ± 0.12	137.82 ± 8.84	67.99 ± 6.06	9.03
14k	3.87 ± 0.24	53.55 ± 1.63	59.12 ± 5.32	5.02
14m	4.22 ± 0.38	82.61 ± 5.07	122.45 ± 12.45	24.25
15b	1.81 ± 0.04	94.67 ± 3.85	111.88 ± 9.58	14.03
15d	3.84 ± 0.21	120.45 ± 7.42	76.83 ± 3.98	19.22
21b	4.02 ± 0.23	90.33 ± 4.73	92.64 ± 6.68	8.69
Amphotericin B	–	28.62 ± 4.22	36.49 ± 5.08	–

[a] All the data represent mean values ± S.D.
[b] The values given are the solubility in water.

Further structural modification was carried out on **15b**. Firstly, *N*-methyl group was replaced by hydrogen, ethyl and isopropyl (**16a**, **16b** and **16c**) to confirm the steric interference of *N*-substituents matters. And comparing with **15b**, the *N*-methyl substituent was necessary for pigment inhibitory activity. Then, various linker substituents were synthesized and tested, and only the diene linker derivative displayed the single-digit nanomolar pigment inhibitory activity.

The analysis of the data shown in Tables 1 and 3 revealed some noteworthy observations for the SAR study: (1) In the studied set of R^1 group (Table 1), removing the substituents from the phenyl ring or replacing with methyl, cycloalkyls or heteroaryls resulted in the loss of potency. *Para*-position of phenyl ring is the best location of substituents compared with *ortho*- or *meta*-position. The electronic effect of the substituents for the potency was finite, and both electron-donating and electron-withdrawing groups were tolerable at the phenyl ring. Moreover, various types of substituted group on the phenyl could remarkably affect the pigment inhibitory activities, and the structure and activity relationships were unambiguous. (2) *N*-methyl fragment was essential for the potency (Table 3), for *N*-substituents variation resulted in the elimination of potency. (3) For the allyl linker (Table 3), inserting a vinyl retained its potency, whereas the allyl linker was irreplaceable. (4) Introduction of oxygen atoms to the benzocycloalkane was tolerable for achieving high potency.

Before choosing the final compound, which would be subjected to further biological tests, the water solubility and cytotoxicity of **21b** were tested (Table 2). Besides, hERG potassium channel inhibition test was performed to evaluate the safety profile of **15b** and **21b** (Table 4). As shown in Table 2, both **15b** and **21b** exhibited good water solubility, along with lower cytotoxicity than Amphotericin B. Moreover, finding compound with better hERG safety profile is one major task of our modification. Considering the superior hERG safety profile of **21b** (~3-fold higher than **15b**), we finally chose **21b** for further *in vitro*/*in vivo* assessments.

To assess the pigment inhibitory activities against antibiotic resistant *S. aureus*, **21b** together with **15b** was incubated with two community-acquired MRSAs (USA300 LAC and USA400 MW) [21,22] and two hospital-acquired MRSAs (vancomycin-intermediate MRSA Mu50 and linezolid-resistant MRSA NRS271) to examine the pigment inhibitory activity against multidrug-resistant strains. The IC_{50} values are presented in Table 4, indicating that **15b** and **21b** are efficient to a variety of pigmented MRSA strains.

Table 3
Chemical structures and their pigment inhibitory activities against *S. aureus* Newman for derivatives **16a-c** and **21a-e**.

Cpd.	*S. aureus* Newman IC_{50}(nM)[a]	Cpd.	*S. aureus* Newman IC_{50}(nM)[a]
16a	>1000	**21b**	4.0 ± 0.2
16b	>1000	**21c**	>1000
16c	>1000	**21d**	>1000
21a	17.9 ± 2.3	**21e**	>1000

[a] All the data represent mean values ± S.D.

2.2.2. Determination of the target enzyme

To verify whether our derivatives targeted the same enzyme as NTF (the first class CrtN inhibitor [16]), an HPLC experiment was performed at 286 nm to analysis the 4,4′-diapophytoene, which is the product of CrtM and the substrate of CrtN. As shown in Fig. 2, the expression of CrtM in *Escherichia coli* displayed the peak of 4,4′-diapophytoene (Fig. 2B, arrow character) similar to that of wild-type *S. aureus* Newman (Fig. 2C), including retention time and UV absorption spectra in the HPLC chromatogram. Subsequently, this peak disappeared in the carotenoid extract of *CrtM* mutant (Fig. 2D) and augmented in *CrtN* mutant (Fig. 2E), which further proved that this peak belonged to 4,4′-diapophytoene. Lastly, either NTF-treated or **21b**-treated Newman (Fig. 2G) both had the HPLC peak similar to that of the *CrtN* mutant, which led to the accumulation of 4,4′-diapophytoene in the *S. aureus* Newman. These results indicated that CrtN is the enzyme target of **21b**.

To further prove the piperonyl derivatives targeting CrtN and measure the efficacy of enzymatic inhibition directly, CrtN enzymatic inhibitory activities *in vitro* of **21b** and **15b** were tested using the previous protocol [9]. The results exhibited in Table 4 demonstrated that **21b** and **15b** could inhibit the CrtN enzymatic activity with IC_{50} values of 407.8 nM and 430.0 nM, respectively.

Table 4
Pigment inhibition (*S. aureus* Newman, Mu50, NRS271, USA400 MW2 and USA300 LAC, IC_{50}), enzyme (CrtN IC_{50}), and hERG results of **15b** and **21b**.

Cpd.	Mu50 IC_{50} (nM)[a]	NRS271 IC_{50} (nM)[a]	USA400 MW2 IC_{50} (nM)[a]	USA300 LAC IC_{50} (nM)[a]	CrtN IC_{50} (nM)[a]	hERG IC_{50}(μM)
15b	2.2 ± 0.2	2.0 ± 0.2	6.1 ± 0.3	11.0 ± 0.8	430.0 ± 32.0	4.22
21b	2.5 ± 0.1	4.1 ± 0.3	7.6 ± 0.1	12.9 ± 1.4	407.8 ± 40.3	14.00

[a] The data represent mean values ± S.D.

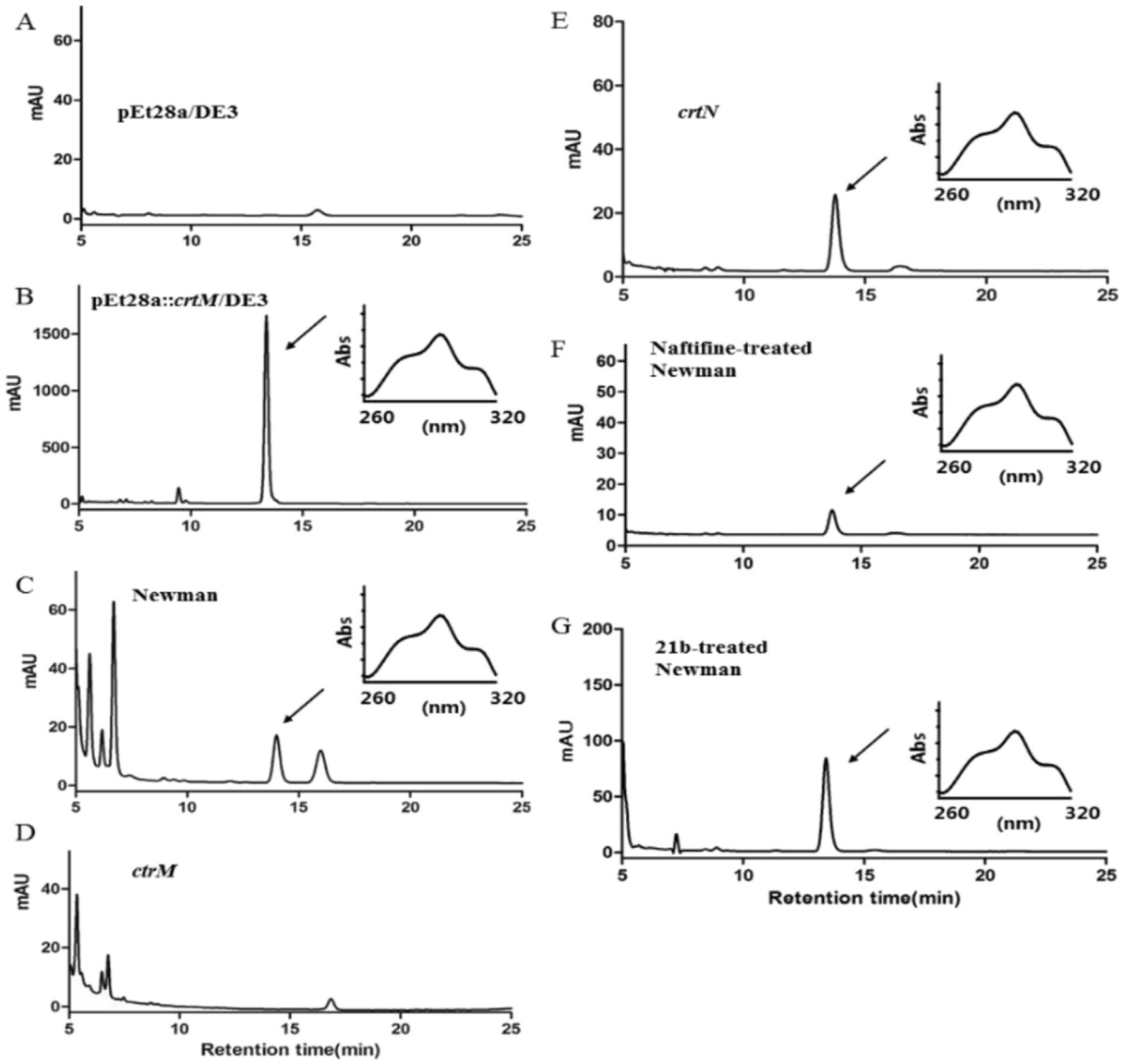


Fig. 2. 21b treatment resulted in the accumulation of 4,4′-diapophytoene. (A–G) HPLC chromatograms (absorption at 286 nm) of the carotenoid extracts from *E. coli* (A), *E. coli* expressing *S. aureus crtM* (B), wild-type *S. aureus* Newman (C), *CrtM* mutant (D), *CrtN* mutant (E), NTF-treated wild-type *S. aureus* Newman (F), **21b**-treated wild-type *S. aureus* Newman strains (G). Insets on the right show the absorbance spectra of the indicated HPLC peaks. mAu, milli-absorbance units. Absorbance (Abs) represents the amount of light absorbed by the sample.

In addition, compound **21b** did not affect the growth of *S. aureus* strains (Newman strain and three MRSA strains) at 0.2 mM, indicating that **21b** did not function as traditional antibiotic (Fig. S1, Supporting Information).

2.2.3. *In vitro oxidative killing assay*

Two independent experiments of hydrogen peroxide killing and human whole blood killing assays were carried out to assess the effects of the most promising compound **21b** on sensitizing *S. aureus* Newman and three MRSA strains (Mu50, USA300 LAC, and USA400 MW2) to immune clearance. The results were shown in Fig. 3. In hydrogen peroxide killing assay, the survival rate of **21b**-treated *S. aureus* Newman was reduced significantly than that of untreated *S. aureus* Newman by a factor of ~11.7 (survival, 2.33% vs 26.67%), which survived worse than the antioxidant Nacetylcysteine (NAC)-treated *S. aureus* Newman cells (survival, 26.67% vs 57.33%) for NAC served as an oxidant scavenger. Moreover, incubation with **21b** also sensitized Mu50, USA300 LAC, and USA400 MW2 to killing by H_2O_2 (2.00% vs 23.33%, 2.67% vs 26.33%, 2.33% vs 29.67%, respectively), and the addition of NAC promoted the survival rates of all three MRSA strains. The results above illustrated that the oxidizability of H_2O_2 could reduce the survival of *S. aureus*, and the pigment served as protective antioxidant. Fig. 3E indicates that **21b**-treated *S. aureus* Newman dramatically reduced bacteria survival in fresh human whole blood compared to vehicle control (1.83% vs 20.0%, reduced by a factor of ~10.9). Similarly, the other three **21b**-treated MRSA strains (and Mu50, USA300 LAC, and USA400 MW2) also presented less survivors than untreated *S. aureus* strains (0.28% vs 8.66%%, 1.57% vs 23.33%, 0.93% vs 14.33, respectively). These results demonstrated that through inhibiting protective pigment biosynthesis, **21b** could render *S. aureus* to be more susceptible to immune clearance and lower the survival rate *in vitro*.

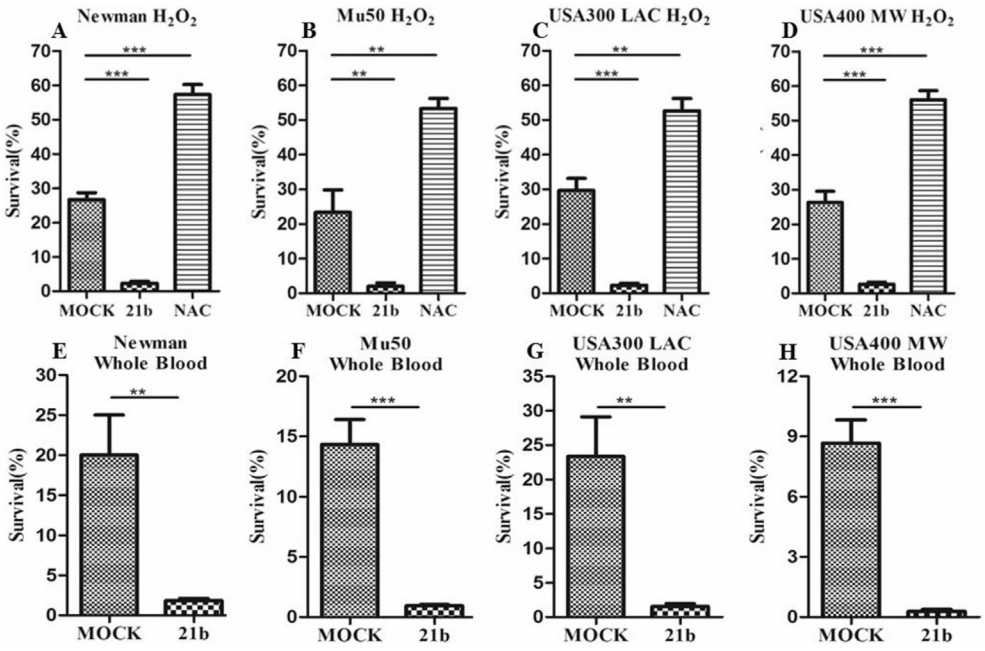


Fig. 3. Effects of **21b** on susceptibility of *S. aureus* to killing by hydrogen peroxide and human whole blood; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$via a two-tailed *t*-test (n = three biological replicates, each with two technical replicates).

*2.2.4. In vivo effects of **21b** on attenuating the virulence of Newman, Mu50 and NRS271*

Since derivative **21b** possessed potent pigment inhibition activity *in vitro* and superior hERG safety profile, the *in vivo* efficacy of **21b** against wild-type *S. aureus* Newman, MRSA Mu50 and LRSA NRS271 was evaluated in a murine abscess formation model where four regimens were established to systemically assess the impacts of dosage as well as the mode of administration. The four regimens included two dose levels (0.4 mg/bid/4.5 d and 0.1 mg/bid/4.5 d) twice per day for two different administration modes of pre-infection and post-infection administration. The pre-treatment and post-treatment groups were injected intraperitoneally with derivative **21b** 24 h before and 6 h after retro-orbital inoculation with *S. aureus* strains, respectively. The two positive control groups were vancomycin and linezolid at a dosage of 0.4 mg/bid/4.5 d via pre-infection administration. The results are summarized in Fig. 4.

In the Newman model, the mice were inoculated with 2.2×10^7 colony-forming units (CFU) of *S. aureus* Newman bacteria. Upon euthanatizing the mice at 108 h, the bacterial survivals in the livers and kidneys were counted. In the kidneys (Fig. 4A), staphylococcal loads of the treatment groups (high dosage with pre-treatment, high dosage with post-treatment, low dosage with pre-treatment and low dosage with post-treatment) were significantly lower than those of the vehical control group, with statistical significance (P = 0 .0017, 0.0006, 0.0010, 0.0014, respectively). There was no significant statistical difference with respect to either dosage or treatment mode.

In the livers (Fig. 4B), the high-dose for pre-treatment group and low-dose for post-treatment group significantly decreased the bacterial survival by 1.90 $\log_{10}$ CFU and 1.53 $\log_{10}$ CFU, respectively (equal to 98.74% and 97.06% decrease in surviving bacteria), slightly higher than linezolid-treatment group, which led to a reduction by 1.41 $\log_{10}$ CFU, but there was no significant difference, so the same for vancomycin-treatment group (2.13 $\log_{10}$ CFU reduction) which also demonstrated no significant difference. On the whole, all of the four regimens reduced the Newman staphylococcal loads in the livers by at least 95% decrease in surviving bacteria. The results showed that the dose-dependence effect of the **21b** was not obvious and the effects of treatment mode was limited.

In the Mu50 model, all the four treatment groups decreased bacterial loads in both the kidneys and the livers with significance level of P value less than 0.001 (Fig. 4C–D). Especially in the liver, all the treatment groups except low-dosage with post-treatment group showed comparative clearance relative to that of linezolid-treatment group.

In the NRS271 model, bacterial populations were counted in the livers (Fig. 4E). The therapeutic effect of two pre-treatment groups were comparable with that of linezolid-treatment, besides, the post-treatment group showed lower staphylococcal loads than linezolid-treatment group with statistical significance. However, the four treatment groups displayed no statistical significance. In this regard, **21b** have an advantage over linezolid.

3. Conclusions

In summary, 44 piperonyl derivatives were synthesized and evaluated to obtain potent CrtN inhibitors with excellent pigment inhibition against *S. aureus* Newman. Through structure optimization, we found that the diene linker possessed better hERG cardiac safety profile, coupled with fair aqueous solubility. Taken together, safety and efficacy as well as physical property allowed **21b** to be more appealing than other derivatives. The screened compounds were then evaluated for *in vitro* pigment inhibitory against four MRSA strains. The HPLC experiment and the enzymatic inhibitory activity confirmed that **21b** targets the enzyme CrtN. In the oxidative killing assay, **21b** was proved capable of sensitizing *S. aureus* strains to be killed by H_2O_2 or human whole blood *in vitro*. To evaluated the efficacy *in vivo* as well as systemically examine the suitable dosage as well as the mode of administration, four treatment regimen and two positive control were set in a murine abscess formation model. In this model, compound **21b** validated its therapeutic efficacy against wild-type *S. aureus* Newman, Mu50 and NRS271, which could significantly alleviate the staphylococcal loads in host organs. The study *in vivo* also indicated that the

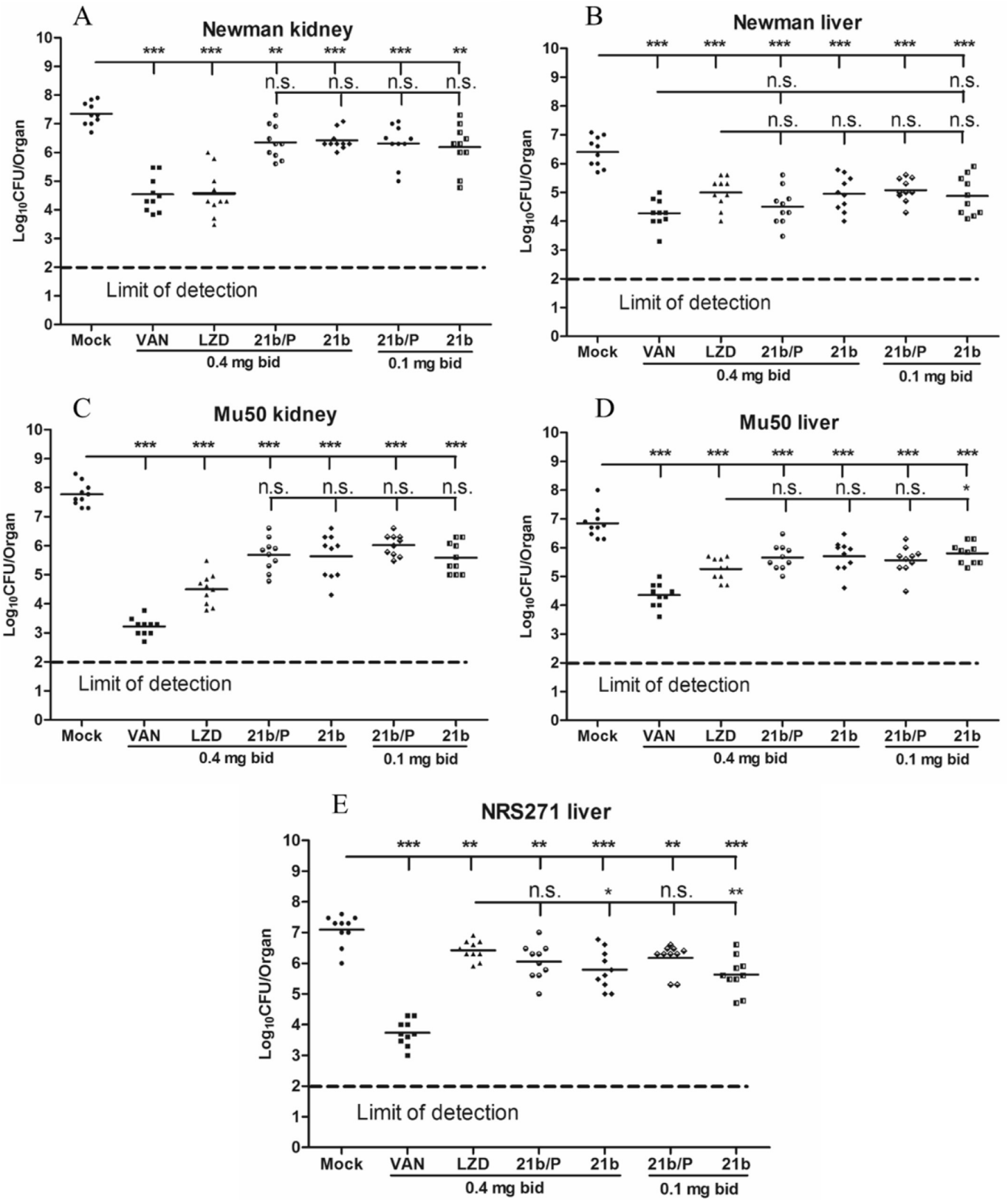


Fig. 4. *In vivo* effect of **21b** on reducing bacteria survival. Bacteria survival in the kidneys and livers of mice challenged with Newman bacteria (A and B). Bacteria survival in the kidneys and livers of mice challenged with Mu50 bacteria (C and D). Bacteria survival in the livers of mice challenged with NRS271 bacteria (E). **P** = pretreatment, drugs or compounds were intraperitoneally administered 24 h before infection, compared with the post-treatment (compound was administered 6 h after infection). Statistical significance was determined by the Mann-Whitney test (two-tailed): * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, n.s. indicates no significant difference. Each symbol represents the value for an individual mouse. Horizontal bars indicate the observation means, and dashed lines mark the limits of detection.

administration of **21b** after occurrence of infection could achieve comparative effects with prophylactical treatment, and moreover, **21b** may possess considerably broad therapeutic window concentration against pigmented *S. aureus* strains.

4. Experimental section

4.1. General chemistry

TLC was performed on HSGF 254 (150–200 μm thickness; Yantai Huiyou Co., China). UV light and also, I_2 and DNP were used to monitor synthetic progress. Column chromatography was performed on silica gel (200–300 mesh) and eluted with ethyl acetate and petroleum ether. NMR spectra data were recorded on a Bruker AMX-400 NMR. Chemical shifts were reported in parts per million (ppm). NMR signals were described from the aspect of multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, q = quartet, m = multiplet and br = broad), coupling constant (Hz) and integrated value. Infrared spectra were recorded in potassium bromide disks on a Nicolet FT-IR 6700 spectrometer, and ν_{max} are partially reported in cm^{-1}. The mass spectral (MS) data were acquired with electron spray ionization (ESI) produced by a Finnigan MAT-95 and LCQ-DECA spectrometer. Each reported compound had >95% purity, as determined by HPLC analysis on an Agilent 1100 with a quaternary pump and diode-array detector (DAD) (Supporting Information). Melting points of each compound were determined on an SGW X-4 melting point apparatus. All starting reagents and solutions were commercially available and were used without further purification.

4.1.1. Synthesis of benzo[d](1,3)dioxole-5-carbaldehyde (**3**)

To a stirred solution of 1-hydroxymethyl-3,4-methylenedioxybenzene (304 mg, 2.0 mmol) in DCM (25 mL) was added MnO_2 (20 mmol, 1.74 g), and the reaction mixture was stirred at room temperature overnight. Subsequently, the mixture was filtrate over Celite, and the organic solvent was evaporated under reduced pressure to produce **3** as a colorless oil without further purification. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.81 (s, 1H), 7.55 (d, J = 8.0 Hz, 1H), 7.33 (s, 1H), 7.15 (d, J = 8.0 Hz, 1H), 6.18 (s, 2H).

4.1.2. Synthesis of 1-(benzo[d](1,3)dioxol-5-yl)-N-methylmethanamine (**4**)

To a stirred solution of **3** (300 mg, 2.0 mmol) in anhydrous methanol (10 mL) was added methylamine (601 mg, 33% in methanol) at room temperature. After stirring for 5 h, the solvent was evaporated under reduced pressure to remove the excessive methylamine and the residue was re-dissolved in methanol (15 mL) with stirring at 0 °C. $NaBH_4$ (76 mg, 2.0 mmol) was added afterwards and then the reaction was transferred to r.t for 30 min. The solvent was removed under reduced pressure and extracted with EtOAc and water. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and condensed under reduced pressure. The residue was then purified by silica gel column chromatography (eluent, EtOAc/petroleum ether, 1/1, V/V) to afford **4** as a colorless oil. ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.83 (s, 1H), 6.78–6.74 (m, 2H), 5.95 (s, 2H), 4.06 (s, 1H), 3.65 (s, 2H), 2.43 (s, 3H).

4.1.3. Synthesis of (E)-3-(furan-2-yl)acrylaldehyde (**5a**)

A mixture of 2-iodofuran (388 mg, 2.0 mmol), acrolein diethyl acetal (729 mg, 5.6 mmol), tetrabutylammonium acetate (1.20 g, 4.0 mmol), palladium diacetate (14 mg, 0.06 mmol), K_2CO_3 (553 mg, 4.0 mmol), and KCl (209 mg, 2.8 mmol) in anhydrous DMF (10 mL) was stirred for 2 h at 90 °C. The resulting dark brown mixture was quenched with 3% (w/w) hydrochloric acid, and then poured into water. The mixture was extracted with EtOAc for three times. The combined extracts were washed with brine, dried over anhydrous $MgSO_4$, filtered and condensed. The residue was then purified by silica gel column chromatography (eluent, EtOAc/petroleum ether, 1/15, V/V) to afford **5a** as a yellowish oil.

4.1.4. Synthesis of (E)-3-(furan-2-yl)prop-2-en-1-ol (**6a**)

To a solution of **5a** (244.0 mg, 2.0 mmol) in methanol (10 mL) was added $NaBH_4$ (76 mg, 2.0 mmol) at 0 °C. The reaction mixture was then stirred at room temperature for 30 min and concentrated. The residue was poured into water and extracted with EtOAc. The combined extracts were washed with brine, dried over anhydrous Na_2SO_4, filtered, and condensed to afford **6a** as a colorless oil. The crude was used for the next step without further purification.

4.1.5. Synthesis of (E)-2-(3-bromoprop-1-en-1-yl)furan (**7a**)

To a solution of **6a** (248 mg, 2.0 mmol) in anhydrous ether (10 mL) was added phosphorus tribromide (75 μL, 0.8 mmol) at 0 °C under a nitrogen atmosphere. The reaction mixture was stirred at room temperature overnight and poured into ice water containing saturated sodium bicarbonate. The mixture was then extracted with EtOAc. The combined extracts were washed with brine, dried over anhydrous Na_2SO_4, filtered, and condensed at 30 °C to afford **7a** as a yellow oil. The crude was used for the next step without further purification.

4.1.6. Synthesis of methyl (E)-4-(o-tolyl)but-3-enoate (**8a**)

Sodium methoxide (28% in MeOH, 386 mg, 2.0 mmol) was added to a stirred solution of 2-methylbenzaldehyde (253 mg, 2.1 mmol) and trimethyl phosphonoacetate (364 mg, 2.0 mmol) in dimethylformamide (10 mL) at 0 °C. After the mixture was stirred for 2 h, the reaction mixture was poured into water to give crystals. Then the crystals were filtered to give **8a** as colorless needles. The crude was used for the next step without further purification. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.88 (d, J = 15.9 Hz, 1H), 7.73 (d, J = 7.6 Hz, 1H), 7.32 (d, J = 6.8 Hz, 1H), 7.29–7.24 (m, 2H), 6.53 (d, J = 15.9 Hz, 1H), 3.74 (s, 3H), 2.40 (s, 3H).

4.1.7. Synthesis of (E)-3-(o-tolyl)prop-2-en-1-ol (**9a**)

Diisobutylaluminum hydride (1.0 M solution in hexane, 4 mL, 4.0 mmol) was added dropwise to a stirred solution of **8a** (296 mg, 2.0 mmol) in dichloromethane (10 mL) at 0 °C. After the mixture was stirred at room temperature for 1.5 h, methyl (0.4 mL) and water (0.4 mL) were added to the mixture with cooling. The reaction mixture was acidified with concentrated hydrochloric acid and extracted with dichloromethane. The extract was washed with brine, dried over $MgSO_4$, and condensed to give the **9a** as a colorless oil. The crude was used for the next step without further purification. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.47 (d, J = 8.7 Hz, 1H), 7.19–7.12 (m, 3H), 6.76 (d, J = 15.8 Hz, 1H), 6.24 (dt, J = 15.8, 5.0 Hz, 1H), 4.90 (t, J = 5.5 Hz, 1H), 4.14 (td, J = 5.3, 1.5 Hz, 2H), 2.29 (s, 3H).

4.1.8. Synthesis of (E)-N-(benzo[d][1,3]dioxol-5-ylmethyl)-N-methyl-3-phenylprop-2-en-1-amine hydrochloride (**11a**)

A mixture of intermediate **4** (182 mg, 1.1 mmol), (*E*)-(3-bromoprop-1-en-1-yl)benzene (197 mg, 1.0 mmol) and K_2CO_3 (276 mg, 2.0 mmol) in DMF (10 mL) was stirred at room temperature overnight. The reaction mixture was then poured into water and extracted with ethyl acetate. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, and then filtered and condensed. The residue was then purified by silica gel column chromatography (eluent, EtOAc/petroleum ether, 1/5, V/V) to give the free base of **11a** as a yellow oil. Subsequently, the free base was dissolved in EtOAc (10 mL) and inflated with hydrogen chloride gas until the solids stopped showing. Then the solvent was evaporated

in vacuo and the residue was suspended in a mixture of ethyl acetate and petroleum ether under ultrasonic. The precipitate was filtrated and washed with ethyl ether to afford the final compound **11a** in the form of hydrochloride as a white solid. Spectroscopic data given below are in their hydrochloride form. Yield: 59%; m.p.: 139–141 °C. ^{1}H NMR (400 MHz, CD_3OD) δ 7.52 (d, J = 7.1 Hz, 2H), 7.42–7.30 (m, 3H), 7.06–6.99 (m, 2H), 6.94 (s, 1H), 6.91 (d, J = 9.4 Hz, 1H), 6.35 (dt, J = 15.2, 7.5 Hz, 1H), 6.02 (d, J = 7.6 Hz, 2H), 4.40 (d, J = 13.0 Hz, 1H), 4.18 (d, J = 13.1 Hz, 1H), 4.03 (dd, J = 13.2, 7.1 Hz, 1H), 3.86 (dd, J = 13.2, 7.9 Hz, 1H), 2.79 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 148.54, 147.90, 138.79, 135.98, 129.21 (2C), 129.06, 127.27 (2C), 125.85, 124.24, 118.73, 111.64, 108.83, 101.90, 57.80, 56.57, 38.26; HRMS (ESI) m/z calcd for $C_{18}H_{20}NO_2$ $[M+H]^+$ 282.1494, found 282.1492.

4.1.9. Synthesis of (E)-N-(benzo[d][1,3]dioxol-5-ylmethyl)-3-(furan-2-yl)-N-methylprop-2-en-1-amine hydrochloride (**11b**)

Compound **11b** was prepared by the experimental procedure depicted for compound **11a** as a white solid. Yield: 44%; m.p.: 114–115 °C. ^{1}H NMR (400 MHz, CD_3OD) δ 7.53 (s, 1H), 7.05–6.87 (m, 3H), 6.75 (d, J = 15.7 Hz, 1H), 6.57–6.43 (m, 2H), 6.15 (dt, J = 15.4, 7.6 Hz, 1H), 6.02 (s, 2H), 4.37 (d, J = 13.1 Hz, 1H), 4.14 (d, J = 13.1 Hz, 1H), 4.00 (dd, J = 13.2, 7.1 Hz, 1H), 3.81 (dd, J = 13.0, 8.3 Hz, 1H), 2.76 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 151.31, 148.54, 147.89, 144.18, 127.04, 125.85, 124.16, 116.57, 112.36, 111.63, 110.85, 108.83, 101.90, 57.86, 56.51, 38.06; HRMS (ESI) m/z calcd for $C_{16}H_{18}NO_3$ $[M+H]^+$ 272.1287, found 272.1289.

4.1.10. Synthesis of (E)-N-(benzo[d][1,3]dioxol-5-ylmethyl)-N-methyl-3-(thiophen-2-yl)prop-2-en-1-amine hydrochloride (**11c**)

Compound **11c** was prepared by the experimental procedure depicted for compound **11a** as a white solid. Yield: 42%; m.p.: 113–115 °C. ^{1}H NMR (400 MHz, CD_3OD) δ 7.40 (d, J = 5.0 Hz, 1H), 7.18 (d, J = 3.3 Hz, 1H), 7.10–6.87 (m, 5H), 6.08 (dt, J = 15.5, 7.6 Hz, 1H), 6.01 (s, 2H), 4.38 (d, J = 13.0 Hz, 1H), 4.16 (d, J = 13.0 Hz, 1H), 4.00 (dd, J = 13.3, 7.1 Hz, 1H), 3.82 (dd, J = 13.2, 8.1 Hz, 1H), 2.77 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 148.56, 147.91, 140.55, 132.14, 128.37, 128.34, 127.07, 125.84, 124.19, 117.69, 111.60, 108.85, 101.91, 57.95, 56.62, 38.20; HRMS (ESI) m/z calcd for $C_{16}H_{18}NO_2S$ $[M+H]^+$ 288.1058, found 288.1057.

4.1.11. Synthesis of (E)-N-(benzo[d][1,3]dioxol-5-ylmethyl)-N-methylbut-2-en-1-amine hydrochloride (**11d**)

Compound **11d** was prepared by the experimental procedure depicted for compound **11a** as a yellow oil. Yield: 45%. ^{1}H NMR (400 MHz, CD_3OD) δ 6.97 (d, J = 8.4 Hz, 2H), 6.92 (d, J = 7.7 Hz, 1H), 6.17–5.96 (m, 3H), 5.63 (dt, J = 15.1, 7.4 Hz, 1H), 4.13 (s, 2H), 3.68 (s, 2H), 2.71 (s, 3H), 1.79 (d, J = 6.4 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 148.50, 147.92, 136.58, 125.78, 124.18, 119.93, 111.57, 108.82, 101.90, 57.67, 56.33, 38.00, 18.33; HRMS (ESI) m/z calcd for $C_{13}H_{18}NO_2$ $[M+H]^+$ 220.1338, found 220.1337.

4.1.12. Synthesis of (E)-N-(benzo[d][1,3]dioxol-5-ylmethyl)-3-cyclopentyl-N-methylprop-2-en-1-amine hydrochloride (**11e**)

Compound **11e** was prepared by the experimental procedure depicted for compound **11a** as a white solid. Yield: 48%; m.p.: 97–98 °C. ^{1}H NMR (400 MHz, CD_3OD) δ 7.00–6.89 (m, 3H), 6.09–5.98 (m, 3H), 5.66–5.51 (m, 1H), 4.28 (s, 1H), 4.09 (s, 1H), 3.78 (s, 1H), 3.63 (s, 1H), 2.70 (s, 3H), 2.62–2.51 (m, 1H), 1.92–1.77 (m, 2H), 1.75–1.57 (m, 4H), 1.43–1.28 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 148.50, 147.88, 145.99, 125.78, 124.18, 117.39, 111.58, 108.80, 101.89, 57.67, 56.50, 42.89, 38.00, 32.65 (2C), 25.12 (2C); HRMS (ESI) m/z calcd for $C_{17}H_{24}NO_2$ $[M+H]^+$ 274.1807, found 274.1808.

4.1.13. Synthesis of (E)-N-(benzo[d][1,3]dioxol-5-ylmethyl)-3-cyclohexyl-N-methylprop-2-en-1-amine hydrochloride (**11f**)

Compound **11f** was prepared by the experimental procedure depicted for compound **11a** as a white solid. Yield: 52%; m.p.: 147–150 °C. ^{1}H NMR (400 MHz, CD_3OD) δ 7.00–6.88 (m, 3H), 6.06–5.95 (m, 3H), 5.55 (dt, J = 14.8, 7.4 Hz, 1H), 4.30 (d, J = 13.1 Hz, 1H), 4.09 (d, J = 13.1 Hz, 1H), 3.79 (dd, J = 13.1, 6.7 Hz, 1H), 3.61 (dd, J = 13.0, 7.9 Hz, 1H), 2.69 (s, 3H), 2.10 (s, 1H), 1.83–1.64 (m, 5H), 1.41–1.08 (m, 5H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 148.52, 147.89, 147.05, 125.78, 124.16, 116.98, 111.57, 108.82, 101.90, 57.67, 56.60, 38.02, 32.33 (2C), 26.02, 25.85, 25.75 (2C); HRMS (ESI) m/z calcd for $C_{18}H_{26}NO_2$ $[M+H]^+$ 288.1964, found 288.1963.

4.1.14. Synthesis of (E)-N-(benzo[d][1,3]dioxol-5-ylmethyl)-N-methyl-3-(naphthalen-1-yl)prop-2-en-1-amine hydrochloride (**11g**)

Compound **11g** was prepared by the experimental procedure depicted for compound **11a** as a white solid. Yield: 58%; m.p.: 173–176 °C. ^{1}H NMR (400 MHz, CD_3OD) δ 8.17 (d, J = 8.3 Hz, 1H), 7.90 (dd, J = 7.6, 5.5 Hz, 2H), 7.78 (d, J = 7.5 Hz, 1H), 7.75 (s, 1H), 7.61–7.47 (m, 3H), 7.05 (d, J = 8.2 Hz, 2H), 6.95 (d, J = 7.7 Hz, 1H), 6.39 (dt, J = 15.2, 7.4 Hz, 1H), 6.02 (s, 2H), 4.46 (d, J = 13.1 Hz, 1H), 4.25 (d, J = 13.1 Hz, 1H), 4.17 (dd, J = 13.2, 7.1 Hz, 1H), 4.00 (dd, J = 13.1, 8.0 Hz, 1H), 2.86 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 148.57, 147.92, 136.26, 133.67, 133.43, 130.98, 129.20, 128.97, 126.99, 126.56, 126.18, 125.92, 124.60, 124.20, 124.05, 121.56, 111.71, 108.85, 101.91, 57.89, 56.64, 38.34; HRMS (ESI) m/z calcd for $C_{22}H_{22}NO_2$ $[M+H]^+$ 332.1651, found 332.1649.

4.1.15. Synthesis of (E)-N-(benzo[d][1,3]dioxol-5-ylmethyl)-N-methyl-3-(naphthalen-2-yl)prop-2-en-1-amine hydrochloride (**11h**)

Compound **11h** was prepared by the experimental procedure depicted for compound **11a** as a white solid. Yield: 56%; m.p.: 174–176 °C. ^{1}H NMR (400 MHz, CD_3OD) δ 7.90 (s, 1H), 7.89–7.82 (m, 3H), 7.73 (d, J = 8.6 Hz, 1H), 7.56–7.44 (m, 2H), 7.08 (d, J = 15.8 Hz, 1H), 7.03 (d, J = 7.5 Hz, 2H), 6.94 (d, J = 8.2 Hz, 1H), 6.53–6.39 (m, 1H), 6.02 (s, 2H), 4.43 (d, J = 13.1 Hz, 1H), 4.21 (d, J = 13.1 Hz, 1H), 4.13–4.02 (m, 1H), 3.96–3.86 (m, 1H), 2.82 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 148.56, 147.92, 138.85, 133.56, 133.48, 133.38, 128.78, 128.52, 128.10, 127.54, 127.08, 126.96, 125.87, 124.27, 124.05, 119.20, 111.65, 108.85, 101.91, 57.82, 56.65, 38.32; HRMS (ESI) m/z calcd for $C_{22}H_{22}NO_2$ $[M+H]^+$ 332.1651, found 332.1651.

4.1.16. Synthesis of (E)-N-(benzo[d][1,3]dioxol-5-ylmethyl)-3-(2-fluorophenyl)-N-methylprop-2-en-1-amine hydrochloride (**12a**)

Compound **12a** was prepared by the experimental procedure depicted for compound **11a** as a white solid. Yield: 55%; m.p.: 152–154 °C. ^{1}H NMR (400 MHz, CD_3OD) δ 7.65 (t, J = 7.6 Hz, 1H), 7.39 (dd, J = 13.7, 6.9 Hz, 1H), 7.23 (t, J = 7.6 Hz, 1H), 7.20–7.13 (m, 1H), 7.07 (d, J = 16.0 Hz, 1H), 7.03 (d, J = 7.8 Hz, 2H), 6.95 (d, J = 7.7 Hz, 1H), 6.51–6.41 (m, 1H), 6.04 (s, 2H), 4.39 (s, 1H), 4.24 (s, 1H), 4.08 (s, 1H), 3.94 (s, 1H), 2.82 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.09 (d, $J_{C\text{-}F}$ = 248.5 Hz), 148.54, 147.89, 130.91 (d, $J_{C\text{-}F}$ = 10.0 Hz), 130.66, 128.45, 125.86, 125.22, 124.21, 123.56 (d, $J_{C\text{-}F}$ = 11.8 Hz), 121.90, 116.30 (d, $J_{C\text{-}F}$ = 21.9 Hz), 111.65, 108.80, 101.89, 57.88, 56.66, 38.30; HRMS (ESI) m/z calcd for $C_{18}H_{19}NO_2F$ $[M+H]^+$ 300.1400, found 300.1400.

4.1.17. Synthesis of (E)-N-(benzo[d][1,3]dioxol-5-ylmethyl)-3-(2-chlorophenyl)-N-methylprop-2-en-1-amine hydrochloride (**12b**)

Compound **12b** was prepared by the experimental procedure depicted for compound **11a** as a white solid. Yield: 47%; m.p.: 162–165 °C. ^{1}H NMR (400 MHz, CD_3OD) δ 7.72–7.67 (m, 1H), 7.41 (dd, J = 6.5, 3.0 Hz, 1H), 7.32 (dd, J = 4.8, 3.8 Hz, 2H), 7.27 (d, J = 15.8 Hz, 1H), 7.02–6.96 (m, 2H), 6.91 (d, J = 7.8 Hz, 1H),

6.40–6.30 (m, 1H), 5.99 (s, 2H), 4.38 (d, $J = 13.0$ Hz, 1H), 4.18 (d, $J = 13.1$ Hz, 1H), 4.05 (dd, $J = 13.0$, 7.0 Hz, 1H), 3.90 (dd, $J = 13.2$, 7.9 Hz, 1H), 2.79 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 148.56, 147.90, 134.13, 133.92, 132.58, 130.60, 130.12, 128.12, 128.05, 125.87, 124.17, 122.51, 111.65, 108.82, 101.90, 57.87, 56.33, 38.39; HRMS (ESI) m/z calcd for $C_{18}H_{19}NO_2Cl$ $[M+H]^+$ 316.1104, found 316.1104.

4.1.18. Synthesis of (E)-N-(benzo[d][1,3]dioxol-5-ylmethyl)-N-methyl-3-(2-(trifluoromethyl)phenyl)prop-2-en-1-amine hydrochloride (**12c**)

Compound **12c** was prepared by the experimental procedure depicted for compound **11a** as a white solid. Yield: 49%; m.p.: 159–161 °C. ^{1}H NMR (400 MHz, CD_3OD) δ 7.81 (d, $J = 7.9$ Hz, 1H), 7.73 (d, $J = 7.7$ Hz, 1H), 7.66 (t, $J = 7.6$ Hz, 1H), 7.53 (t, $J = 7.6$ Hz, 1H), 7.27 (d, $J = 15.0$ Hz, 1H), 7.05–6.89 (m, 3H), 6.34 (dt, $J = 15.4$, 7.6 Hz, 1H), 6.02 (s, 2H), 4.38 (s, 1H), 4.21 (s, 1H), 4.08 (s, 1H), 3.95 (s, 1H), 2.74 (s, 3H). ^{13}C NMR (100 MHz, DMSO-*d6*) δ 148.58, 147.92, 134.79 (q, $J_{C\text{-}F} = 1.6$ Hz), 133.89, 133.39, 129.26, 128.71, 126.40 (q, $J_{C\text{-}F} = 28.4$ Hz), 126.23 (q, $J_{C\text{-}F} = 5.8$ Hz), 125.87, 124.68 (q, $J_{C\text{-}F} = 272.1$ Hz), 124.37, 124.12, 111.64, 108.82, 101.91, 57.92, 56.23, 38.28; HRMS (ESI) m/z calcd for $C_{19}H_{19}NO_2F_3$ $[M+H]^+$ 350.1368, found 350.1370.

4.1.19. Synthesis of (E)-N-(benzo[d][1,3]dioxol-5-ylmethyl)-N-methyl-3-(o-tolyl)prop-2-en-1-amine hydrochloride (**12d**)

Compound **12d** was prepared by the experimental procedure depicted for compound **11a** as a white solid. Yield: 44%; m.p.: 179–181 °C. ^{1}H NMR (400 MHz, CD_3OD) δ 7.33 (s, 1H), 7.30 (d, $J = 7.8$ Hz, 1H), 7.25 (t, $J = 7.5$ Hz, 1H), 7.16 (d, $J = 7.2$ Hz, 1H), 7.05–6.99 (m, 2H), 6.93 (dd, $J = 7.6$, 0.8 Hz, 1H), 6.87 (d, $J = 15.7$ Hz, 1H), 6.37–6.25 (m, 1H), 6.01 (s, 2H), 4.37 (d, $J = 12.9$ Hz, 1H), 4.17 (d, $J = 13.1$ Hz, 1H), 4.01 (dd, $J = 12.9$, 6.8 Hz, 1H), 3.85 (dd, $J = 13.3$, 8.1 Hz, 1H), 2.78 (s, 3H), 2.35 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 148.55, 147.90, 136.92, 136.04, 135.05, 130.81, 128.86, 126.65, 126.32, 125.87, 124.19, 119.84, 111.66, 108.83, 101.90, 57.80, 56.69, 38.23, 19.80; HRMS (ESI) m/z calcd for $C_{19}H_{22}NO_2$ $[M+H]^+$ 296.1651, found 296.1648.

4.1.20. Synthesis of (E)-N-(benzo[d][1,3]dioxol-5-ylmethyl)-3-(2-methoxyphenyl)-N-methylprop-2-en-1-amine hydrochloride (**12e**)

Compound **12e** was prepared by the experimental procedure depicted for compound **11a** as a white solid. Yield: 42%; m.p.: 149–151 °C. ^{1}H NMR (400 MHz, CD_3OD) δ 7.51 (dd, $J = 7.7$, 1.6 Hz, 1H), 7.38–7.29 (m, 1H), 7.17 (d, $J = 15.9$ Hz, 1H), 7.05–6.98 (m, 3H), 6.98–6.90 (m, 2H), 6.40–6.28 (m, 1H), 6.01 (s, 2H), 4.38 (d, $J = 12.9$ Hz, 1H), 4.17 (d, $J = 13.2$ Hz, 1H), 4.01 (d, $J = 13.1$ Hz, 1H), 3.87 (s, 3H), 3.83 (d, $J = 12.7$ Hz, 1H), 2.77 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 156.97, 148.53, 147.90, 133.48, 130.44, 127.50, 125.83, 124.39, 124.25, 121.04, 119.09, 111.95, 111.62, 108.82, 101.90, 57.84, 57.06, 55.97, 38.26; HRMS (ESI) m/z calcd for $C_{19}H_{22}NO_3$ $[M+H]^+$ 312.1600, found 312.1601.

4.1.21. Synthesis of (E)-N-(benzo[d][1,3]dioxol-5-ylmethyl)-3-(3-fluorophenyl)-N-methylprop-2-en-1-amine hydrochloride (**13a**)

Compound **13a** was prepared by the experimental procedure depicted for compound **11a** as a white solid. Yield: 50%; m.p.: 170–172 °C. ^{1}H NMR (400 MHz, CD_3OD) δ 7.40 (dd, $J = 8.0$, 5.9 Hz, 1H), 7.36–7.27 (m, 2H), 7.13–6.99 (m, 3H), 6.92 (dd, $J = 11.7$, 9.2 Hz, 2H), 6.45–6.35 (m, 1H), 6.02 (s, 2H), 4.40 (d, $J = 13.1$ Hz, 1H), 4.19 (d, $J = 13.0$ Hz, 1H), 4.03 (dd, $J = 13.3$, 7.1 Hz, 1H), 3.87 (dd, $J = 13.3$, 7.9 Hz, 1H), 2.80 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 162.93 (d, $J_{C\text{-}F} = 243.3$ Hz), 148.55, 147.90, 138.61 (d, $J_{C\text{-}F} = 7.9$ Hz), 137.44 (d, $J_{C\text{-}F} = 2.1$ Hz), 131.17 (d, $J_{C\text{-}F} = 8.5$ Hz), 125.86, 124.22, 123.71 (d, $J_{C\text{-}F} = 2.4$ Hz), 120.49, 115.72 (d, $J_{C\text{-}F} = 21.2$ Hz), 113.49 (d, $J_{C\text{-}F} = 21.9$ Hz), 111.63, 108.83, 101.90, 57.77, 56.22, 38.37; HRMS (ESI) m/z calcd for $C_{18}H_{19}NO_2F$ $[M+H]^+$ 300.1400, found 300.1403.

4.1.22. Synthesis of (E)-N-(benzo[d][1,3]dioxol-5-ylmethyl)-3-(3-chlorophenyl)-N-methylprop-2-en-1-amine hydrochloride (**13b**)

Compound **13b** was prepared by the experimental procedure depicted for compound **11a** as a white solid. Yield: 48%; m.p.: 123–124 °C. ^{1}H NMR (400 MHz, CD_3OD) δ 7.56 (s, 1H), 7.43 (d, $J = 6.8$ Hz, 1H), 7.41–7.31 (m, 2H), 7.09–6.94 (m, 2H), 6.95–6.85 (m, 2H), 6.44–6.33 (m, 1H), 6.02 (s, 2H), 4.37 (s, 1H), 4.20 (s, 1H), 4.00 (s, 1H), 3.87 (s, 1H), 2.78 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 148.54, 147.90, 138.27, 137.14, 134.01, 131.05, 128.72, 126.72, 126.12, 125.86, 124.23, 120.65, 111.64, 108.83, 101.90, 57.75, 56.25, 38.38; HRMS (ESI) m/z calcd for $C_{18}H_{19}NO_2Cl$ $[M+H]^+$ 316.1104, found 316.1104.

4.1.23. Synthesis of (E)-N-(benzo[d][1,3]dioxol-5-ylmethyl)-N-methyl-3-(3-(trifluoromethyl)phenyl)prop-2-en-1-amine hydrochloride (**13c**)

Compound **13c** was prepared by the experimental procedure depicted for compound **11a** as a white solid. Yield: 61%; m.p.: 149–151 °C. ^{1}H NMR (400 MHz, CD_3OD) δ 7.82 (s, 1H), 7.78 (d, $J = 7.5$ Hz, 1H), 7.67–7.56 (m, 2H), 7.01 (d, $J = 7.1$ Hz, 2H), 6.94 (t, $J = 8.1$ Hz, 2H), 6.52–6.41 (m, 1H), 6.02 (s, 2H), 4.37 (s, 1H), 4.22 (s, 1H), 4.02 (s, 1H), 3.91 (s, 1H), 2.80 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 148.63, 147.95, 137.47(q, $J_{C\text{-}F} = 3.9$ Hz), 137.00, 131.22, 130.35, 130.03 (q, $J_{C\text{-}F} = 31.7$ Hz), 125.87, 125.49 (q, $J_{C\text{-}F} = 2.4$ Hz),124.55 (q, $J_{C\text{-}F} = 270.6$), 124.03, 123.71 (q, $J_{C\text{-}F} = 3.8$ Hz), 120.76, 111.46, 108.94, 101.93, 58.24, 56.67, 38.84; HRMS (ESI) m/z calcd for $C_{19}H_{19}NO_2F_3$ $[M+H]^+$ 350.1368, found 350.1369.

4.1.24. Synthesis of (E)-N-(benzo[d][1,3]dioxol-5-ylmethyl)-N-methyl-3-(m-tolyl)prop-2-en-1-amine hydrochloride (**13d**)

Compound **13d** was prepared by the experimental procedure depicted for compound **11a** as a white solid. Yield: 46%; m.p.: 136–137 °C. ^{1}H NMR (400 MHz, CD_3OD) δ 7.57–7.52 (m, 1H), 7.29–7.11 (m, 4H), 7.06–6.99 (m, 2H), 6.94 (d, $J = 8.0$ Hz, 1H), 6.20 (dt, $J = 15.3$, 7.4 Hz, 1H), 6.01 (s, 2H), 4.40 (d, $J = 13.1$ Hz, 1H), 4.18 (d, $J = 13.0$ Hz, 1H), 4.05 (dd, $J = 13.3$, 7.1 Hz, 1H), 3.88 (dd, $J = 13.2$, 7.9 Hz, 1H), 2.80 (s, 3H), 2.38 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 148.53, 147.90, 138.92, 138.33, 135.92, 129.73, 129.09, 127.72, 125.85, 124.56, 124.25, 118.47, 111.64, 108.83, 101.90, 57.76, 56.60, 38.22, 21.41; HRMS (ESI) m/z calcd for $C_{19}H_{22}NO_2$ $[M+H]^+$ 296.1651, found 296.1652.

4.1.25. Synthesis of (E)-N-(benzo[d][1,3]dioxol-5-ylmethyl)-3-(3-methoxyphenyl)-N-methylprop-2-en-1-amine hydrochloride (**13e**)

Compound **13e** was prepared by the experimental procedure depicted for compound **11a** as a white solid. Yield: 45%; m.p.: 150–152 °C. ^{1}H NMR (400 MHz, CD_3OD) δ 7.28 (t, $J = 7.9$ Hz, 1H), 7.08 (d, $J = 7.6$ Hz, 1H), 7.06 (s, 1H), 7.01 (d, $J = 7.6$ Hz, 2H), 6.96–6.84 (m, 3H), 6.39–6.26 (m, 1H), 6.03 (s, 2H), 4.39 (d, $J = 12.9$ Hz, 1H), 4.17 (d, $J = 13.1$ Hz, 1H), 4.01 (dd, $J = 13.0$, 7.0 Hz, 1H), 3.91–3.74 (m, 4H), 2.79 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 159.99, 148.54, 147.90, 138.70, 137.42, 130.26, 125.85, 124.25, 119.75, 119.05, 114.63, 112.59, 111.63, 108.83, 101.90, 57.80, 56.50, 55.61, 38.30; HRMS (ESI) m/z calcd for $C_{19}H_{22}NO_3$ $[M+H]^+$ 312.1600, found 312.1602.

4.1.26. Synthesis of (E)-N-(benzo[d][1,3]dioxol-5-ylmethyl)-3-(4-fluorophenyl)-N-methylprop-2-en-1-amine hydrochloride (**14a**)

Compound **14a** was prepared by the experimental procedure depicted for compound **11a** as a white solid. Yield: 60%; m.p.: 158–161 °C. ^{1}H NMR (400 MHz, CD_3OD) δ 7.60–7.49 (m, 2H), 7.17–7.07 (m, 2H), 7.04–6.86 (m, 4H), 6.28 (dt, $J = 15.2$, 7.5 Hz, 1H), 6.02 (s, 2H), 4.39 (d, $J = 12.9$ Hz, 1H), 4.17 (d, $J = 13.0$ Hz, 1H), 4.08–3.91 (m, 1H), 3.90–3.77 (m, 1H), 2.73 (s, 3H). ^{13}C NMR

(100 MHz, DMSO-d_6) δ 162.64 (d, J_{C-F} = 245.8 Hz), 148.55, 147.91, 137.62, 132.60 (d, J_{C-F} = 3.1 Hz), 129.33 (d, J_{C-F} = 8.3 Hz, 2C), 125.84, 124.22, 118.57, 116.11 (d, J_{C-F} = 21.6 Hz, 2C), 111.62, 108.84, 101.91, 57.79, 56.48, 38.29; HRMS (ESI) *m/z* calcd for $C_{18}H_{19}NO_2F$ $[M+H]^+$ 300.1400, found 300.1399.

4.1.27. Synthesis of (E)-N-(benzo[d][1,3]dioxol-5-ylmethyl)-3-(4-chlorophenyl)-N-methylprop-2-en-1-amine hydrochloride (**14b**)

Compound **14b** was prepared by the experimental procedure depicted for compound **11a** as a white solid. Yield: 58%; m.p.: 160–162 °C. ^{1}H NMR (400 MHz, CD_3OD) δ 7.51 (d, *J* = 8.5 Hz, 2H), 7.38 (d, *J* = 8.4 Hz, 2H), 7.05–6.99 (m, 2H), 6.93 (d, *J* = 7.8 Hz, 1H), 6.89 (d, *J* = 16.0 Hz, 1H), 6.42–6.29 (m, 1H), 6.01 (s, 2H), 4.39 (d, *J* = 13.0 Hz, 1H), 4.18 (d, *J* = 12.9 Hz, 1H), 4.02 (dd, *J* = 13.3, 7.0 Hz, 1H), 3.86 (dd, *J* = 13.3, 8.0 Hz, 1H), 2.79 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 148.55, 147.90, 137.42, 134.94, 133.45, 129.21 (2C), 129.01 (2C), 125.85, 124.20, 119.71, 111.62, 108.84, 101.91, 57.82, 56.42, 38.34; HRMS (ESI) *m/z* calcd for $C_{18}H_{19}NO_2Cl$ $[M+H]^+$ 316.1104, found 316.1104.

4.1.28. Synthesis of (E)-N-(benzo[d][1,3]dioxol-5-ylmethyl)-3-(4-bromophenyl)-N-methylprop-2-en-1-amine hydrochloride (**14c**)

Compound **14c** was prepared by the experimental procedure depicted for compound **11a** as a white solid. Yield: 58%; m.p.: 162–164 °C. ^{1}H NMR (400 MHz, CD_3OD) δ 7.54 (d, *J* = 8.5 Hz, 2H), 7.48–7.36 (m, 2H), 7.01 (d, *J* = 8.9 Hz, 2H), 6.93 (d, *J* = 7.7 Hz, 1H), 6.88 (d, *J* = 15.8 Hz, 1H), 6.43–6.31 (m, 1H), 6.02 (s, 2H), 4.39 (d, *J* = 13.1 Hz, 1H), 4.18 (d, *J* = 13.0 Hz, 1H), 4.01 (dd, *J* = 13.1, 6.9 Hz, 1H), 3.85 (dd, *J* = 13.0, 7.8 Hz, 1H), 2.78 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 148.55, 147.91, 137.48, 135.28, 132.13 (2C), 129.29 (2C), 125.84, 124.20, 122.11, 119.79, 111.62, 108.84, 101.91, 57.83, 56.43, 38.35; HRMS (ESI) *m/z* calcd for $C_{18}H_{19}NO_2Br$ $[M+H]^+$ 360.0599, found 360.0601.

4.1.29. Synthesis of (E)-N-(benzo[d][1,3]dioxol-5-ylmethyl)-N-methyl-3-(4-(trifluoromethyl)phenyl)prop-2-en-1-amine hydrochloride (**14d**)

Compound **14d** was prepared by the experimental procedure depicted for compound **11a** as a white solid. Yield: 65%; m.p.: 160–162 °C. ^{1}H NMR (400 MHz, CD_3OD) δ 7.72 (d, *J* = 8.7 Hz, 2H), 7.69 (d, *J* = 8.7 Hz, 2H), 7.07–6.96 (m, 3H), 6.94 (d, *J* = 7.8 Hz, 1H), 6.57–6.46 (m, 1H), 6.02 (s, 2H), 4.42 (d, *J* = 13.0 Hz, 1H), 4.21 (d, *J* = 13.1 Hz, 1H), 4.07 (dd, *J* = 13.3, 6.9 Hz, 1H), 3.91 (dd, *J* = 13.2, 7.8 Hz, 1H), 2.81 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 148.56, 147.91, 140.05, 137.08, 128.97 (d, J_{C-F} = 31.6 Hz), 127.93 (2C), 126.09 (q, J_{C-F} = 3.6 Hz, 2C), 125.10 (q, J_{C-F} = 272.4 Hz) 124.18, 123.30, 122.07, 111.64, 108.83, 101.91, 57.88, 56.30, 38.42; HRMS (ESI) *m/z* calcd for $C_{19}H_{19}NO_2F_3$ $[M+H]^+$ 350.1368, found 350.1368.

4.1.30. Synthesis of (E)-N-(benzo[d][1,3]dioxol-5-ylmethyl)-N-methyl-3-(4-nitrophenyl)prop-2-en-1-amine hydrochloride (**14e**)

Compound **11c** was prepared by the experimental procedure depicted for compound **11a** as a brown solid. Yield: 57%; m.p.: 200–203 °C. ^{1}H NMR (400 MHz, CD_3OD) δ 8.24 (d, *J* = 8.6 Hz, 2H), 7.75 (d, *J* = 8.7 Hz, 2H), 7.02 (d, *J* = 8.1 Hz, 2H), 7.00 (d, *J* = 3.7 Hz, 1H), 6.92 (d, *J* = 7.8 Hz, 1H), 6.62–6.53 (m, 1H), 6.01 (s, 2H), 4.41 (d, *J* = 13.0 Hz, 1H), 4.21 (d, *J* = 13.1 Hz, 1H), 4.07 (dd, *J* = 13.4, 6.9 Hz, 1H), 3.92 (dd, *J* = 13.4, 7.7 Hz, 1H), 2.81 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 148.57, 147.91, 147.45, 142.59, 136.43, 128.34 (2C), 125.89, 124.46 (2C), 124.16, 123.96, 111.65, 108.84, 101.91, 57.93, 56.23, 38.51; HRMS (ESI) *m/z* calcd for $C_{18}H_{19}N_2O_4$ $[M+H]^+$ 327.1345, found 327.1348.

4.1.31. Synthesis of (E)-N-(benzo[d][1,3]dioxol-5-ylmethyl)-N-methyl-3-(p-tolyl)prop-2-en-1-amine hydrochloride (**14f**)

Compound **14f** was prepared by the experimental procedure depicted for compound **11a** as a white solid. Yield: 48%; m.p.: 169–171 °C. ^{1}H NMR (400 MHz, CD_3OD) δ 7.40 (d, *J* = 8.0 Hz, 2H), 7.19 (d, *J* = 7.9 Hz, 2H), 7.04–6.98 (m, 2H), 6.93 (d, *J* = 7.7 Hz, 1H), 6.87 (d, *J* = 15.6 Hz, 1H), 6.32–6.23 (m, 1H), 6.03 (s, 2H), 4.27 (s, 2H), 3.91 (s, 2H), 2.77 (s, 3H), 2.34 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 148.50, 147.89, 138.72, 138.55, 133.24, 129.76 (2C), 127.21 (2C), 125.78, 124.37, 117.63, 111.58, 108.82, 101.89, 57.84, 56.72, 38.28, 21.32; HRMS (ESI) *m/z* calcd for $C_{19}H_{22}NO_2$ $[M+H]^+$ 296.1651, found 296.1648.

4.1.32. Synthesis of (E)-N-(benzo[d][1,3]dioxol-5-ylmethyl)-3-(4-methoxyphenyl)-N-methylprop-2-en-1-amine hydrochloride (**14g**)

Compound **14g** was prepared by the experimental procedure depicted for compound **11a** as a white solid. Yield: 43%; m.p.: 122–124 °C. ^{1}H NMR (400 MHz, CD_3OD) δ 7.6 (d, *J* = 8.2 Hz, 2H), 7.07–6.97 (m, 2H), 6.94–6.90 (m, 3H), 6.84 (d, *J* = 15.7 Hz, 1H), 6.22–6.12 (m, 1H), 6.02 (s, 2H), 4.38 (d, *J* = 13.1 Hz, 1H), 4.16 (d, *J* = 13.1 Hz, 1H), 3.99 (dd, *J* = 12.9, 6.8 Hz, 1H), 3.89–3.74 (m, 4H), 2.77 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.08, 148.54, 147.91, 138.59, 128.69 (2C), 128.59, 125.82, 124.26, 115.93, 114.60 (2C), 111.60, 108.84, 101.90, 57.75, 56.78, 55.68, 38.19; HRMS (ESI) *m/z* calcd for $C_{19}H_{22}NO_3$ $[M+H]^+$ 312.1600, found 312.1603.

4.1.33. Synthesis of (E)-4-(3-((benzo[d][1,3]dioxol-5-ylmethyl)(methyl)amino)prop-1-en-1-yl)benzonitrile hydrochloride (**14h**)

Compound **14h** was prepared by the experimental procedure depicted for compound **11a** as a white solid. Yield: 55%; m.p.: 194–196 °C. ^{1}H NMR (400 MHz, CD_3OD) δ 7.75 (d, *J* = 8.4 Hz, 2H), 7.70 (d, *J* = 8.4 Hz, 2H), 7.06–7.01 (m, 2H), 6.98 (d, *J* = 15.0 Hz, 1H), 6.93 (d, *J* = 7.8 Hz, 1H), 6.59–6.47 (m, 1H), 6.03 (s, 2H), 4.41 (d, *J* = 13.0 Hz, 1H), 4.21 (d, *J* = 13.0 Hz, 1H), 4.07 (dd, *J* = 13.4, 7.0 Hz, 1H), 3.91 (dd, *J* = 13.3, 7.7 Hz, 1H), 2.81 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 148.56, 147.90, 140.62, 136.91, 133.15 (2C), 128.04 (2C), 125.88, 124.18, 122.98, 119.25, 111.64, 111.10, 108.83, 101.91, 57.88, 56.20, 38.48; HRMS (ESI) *m/z* calcd for $C_{19}H_{19}N_2O_2$ $[M+H]^+$ 307.1447, found 307.1445.

4.1.34. Synthesis of (E)-N-(benzo[d][1,3]dioxol-5-ylmethyl)-3-(4-(difluoromethyl)phenyl)-N-methylprop-2-en-1-amine hydrochloride (**14i**)

Compound **14i** was prepared by the experimental procedure depicted for compound **11a** as a white solid. Yield: 52%; m.p.: 154–157 °C. ^{1}H NMR (400 MHz, CD_3OD) δ 7.64 (d, *J* = 8.0 Hz, 2H), 7.56 (d, *J* = 8.1 Hz, 2H), 7.05–6.92 (m, 4H), 6.77 (t, *J* = 56.2 Hz, 1H), 6.49–6.36 (m, 1H), 6.02 (s, 2H), 4.38 (s, 1H), 4.21 (s, 1H), 4.01 (s, 1H), 3.91 (s, 1H), 2.74 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 148.57, 147.92, 138.50, 137.74, 134.35 (t, J_{C-F} = 22.2 Hz), 127.63 (2C), 126.60 (t, J_{C-F} = 6.0 Hz, 2C), 125.85, 124.19, 120.75, 115.22 (t, J_{C-F} = 235.7 Hz), 111.62, 108.85, 101.91, 57.89, 56.42, 38.40; HRMS (ESI) *m/z* calcd for $C_{19}H_{20}NO_2F_2$ $[M+H]^+$ 332.1462, found 332.1461.

4.1.35. Synthesis of (E)-methyl 4-(3-((benzo[d][1,3]dioxol-5-ylmethyl)(methyl)amino)prop-1-en-1-yl)benzoate hydrochloride (**14j**)

Compound **14j** was prepared by the experimental procedure depicted for compound **11a** as a white solid. Yield: 62%; m.p.: 167–170 °C. ^{1}H NMR (400 MHz, CD_3OD) δ 8.02 (d, *J* = 8.4 Hz, 2H), 7.63 (d, *J* = 8.3 Hz, 2H), 7.02 (s, 1H), 7.01–6.92 (m, 3H), 6.54–6.42 (m, 1H), 6.02 (s, 2H), 4.38 (s, 1H), 4.21 (s, 1H), 4.03 (s, 1H), 3.91 (s, 4H), 2.80 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.34, 148.54, 147.90, 140.61, 137.46, 130.06 (2C), 129.67, 127.51 (2C), 125.86,

124.22, 121.93, 111.64, 108.83, 101.90, 57.88, 56.39, 52.65, 38.39; HRMS (ESI) *m/z* calcd for $C_{20}H_{22}NO_4$ $[M+H]^+$ 340.1549, found 340.1548.

4.1.36. Synthesis of (E)-3-([1,1′-biphenyl]-4-yl)-N-(benzo[d][1,3]dioxol-5-ylmethyl)-N-methylprop-2-en-1-amine hydrochloride (**14k**)

Compound **14k** was prepared by the experimental procedure depicted for compound **11a** as a white solid. Yield: 53%; m.p.: 182–185 °C. ^{1}H NMR (400 MHz, CD_3OD) δ 7.73–7.52 (m, 6H), 7.44 (t, *J* = 7.6 Hz, 2H), 7.35 (t, *J* = 7.3 Hz, 1H), 7.07–6.89 (m, 4H), 6.49–6.31 (m, 1H), 6.02 (s, 2H), 4.41 (d, *J* = 13.0 Hz, 1H), 4.19 (d, *J* = 13.1 Hz, 1H), 4.05 (dd, *J* = 13.2, 7.1 Hz, 1H), 3.88 (dd, *J* = 13.2, 7.9 Hz, 1H), 2.80 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 148.55, 147.91, 140.61, 139.90, 138.33, 135.12, 129.47 (2C), 128.15, 127.91 (2C), 127.40 (2C), 127.03 (2C), 125.87, 124.23, 118.82, 111.64, 108.85, 101.91, 57.84, 56.65, 38.30; HRMS (ESI) *m/z* calcd for $C_{24}H_{24}NO_2$ $[M+H]^+$ 358.1807, found 358.1806.

4.1.37. Synthesis of (E)-N-(benzo[d][1,3]dioxol-5-ylmethyl)-3-(4-(tert-butyl)phenyl)-N-methylprop-2-en-1-amine hydrochloride (**14l**)

Compound **14l** was prepared by the experimental procedure depicted for compound **11a** as a white solid. Yield: 50%; m.p.: 220–222 °C. ^{1}H NMR (400 MHz, CD_3OD) δ 7.49–7.36 (m, 4H), 7.04–6.98 (m, 2H), 6.94 (d, *J* = 8.0 Hz, 1H), 6.88 (d, *J* = 15.8 Hz, 1H), 6.29 (dt, *J* = 15.2, 7.5 Hz, 1H), 6.01 (s, 2H), 4.37 (d, *J* = 13.0 Hz, 1H), 4.17 (d, *J* = 13.1 Hz, 1H), 4.01 (dd, *J* = 13.6, 6.8 Hz, 1H), 3.84 (dd, *J* = 13.2, 8.0 Hz, 1H), 2.78 (s, 3H), 1.32 (s, 9H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 151.72, 148.55, 147.91, 138.73, 133.25, 127.05 (2C), 125.95 (2C), 125.83, 124.23, 117.76, 111.62, 108.84, 101.90, 57.79, 56.68, 38.22, 34.86, 31.47 (3C); HRMS (ESI) *m/z* calcd for $C_{22}H_{28}NO_2$ $[M+H]^+$ 338.2120, found 338.2119.

4.1.38. Synthesis of (E)-N-(benzo[d][1,3]dioxol-5-ylmethyl)-3-(4-ethoxyphenyl)-N-methylprop-2-en-1-amine hydrochloride (**14m**)

Compound **14m** was prepared by the experimental procedure depicted for compound **11a** as a white solid. Yield: 49%; m.p.: 121–123 °C. ^{1}H NMR (400 MHz, CD_3OD) δ 7.44 (d, *J* = 8.7 Hz, 2H), 7.00 (d, *J* = 7.6 Hz, 2H), 7.04–6.90 (m, 3H), 6.84 (d, *J* = 15.7 Hz, 1H), 6.23–6.09 (m, 1H), 6.03 (s, 2H), 4.38 (d, *J* = 12.5 Hz, 1H), 4.16 (d, *J* = 11.6 Hz, 1H), 4.05 (q, *J* = 7.0 Hz, 2H), 3.97 (d, *J* = 6.4 Hz, 1H), 3.83 (d, *J* = 7.5 Hz, 1H), 2.77 (s, 3H), 1.38 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 159.35, 148.53, 147.90, 138.60, 128.69 (2C), 128.45, 125.81, 124.27, 115.84, 115.03 (2C), 111.60, 108.84, 101.90, 63.58, 57.74, 56.79, 38.18, 15.06; HRMS (ESI) *m/z* calcd for $C_{20}H_{24}NO_3$ $[M+H]^+$ 326.1756, found 326.1757.

4.1.39. Synthesis of (E)-N-(benzo[d][1,3]dioxol-5-ylmethyl)-3-(2,4-dichlorophenyl)-N-methylprop-2-en-1-amine hydrochloride (**15a**)

Compound **15a** was prepared by the experimental procedure depicted for compound **11a** as a white solid. Yield: 60%; m.p.: 188–190 °C. ^{1}H NMR (400 MHz, CD_3OD) δ 7.72 (d, *J* = 8.5 Hz, 1H), 7.53 (s, 1H), 7.38 (d, *J* = 8.5 Hz, 1H), 7.23 (d, *J* = 15.8 Hz, 1H), 7.01 (d, *J* = 7.9 Hz, 2H), 6.93 (d, *J* = 7.7 Hz, 1H), 6.44–6.33 (m, 1H), 6.03 (s, 2H), 4.37 (s, 1H), 4.21 (s, 1H), 4.04 (s, 1H), 3.94 (s, 1H), 2.81 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 148.56, 147.90, 134.11, 133.35, 132.99, 130.60, 129.54, 129.38, 128.28, 125.87, 124.15, 123.34, 111.64, 108.83, 101.91, 57.87, 56.20, 38.44; HRMS (ESI) *m/z* calcd for $C_{18}H_{18}Cl_2NO_2$ $[M+H]^+$ 350.0715, found 350.0714.

4.1.40. Synthesis of (E)-N-(benzo[d][1,3]dioxol-5-ylmethyl)-3-(2-fluoro-4-(trifluoromethyl)phenyl)-N-methylprop-2-en-1-amine hydrochloride (**15b**)

Compound **15b** was prepared by the experimental procedure depicted for compound **11a** as a white solid. Yield: 54%; m.p.: 165–166 °C. FTIR: 3422.6, 3027.5, 2905.6, 1627.2, 1609.1, 1334.8, 1251.2, 1170.4, 1128.7, 1040.9, 910.6, 878.7 cm-1^{1}H NMR (400 MHz, CD_3OD) δ 7.85 (t, *J* = 7.7 Hz, 1H), 7.65–7.42 (m, 2H), 7.09 (d, *J* = 16.0 Hz, 1H), 7.05–6.82 (m, 3H), 6.67–6.49 (m, 1H), 6.02 (s, 2H), 4.39 (s, 1H), 4.22 (s, 1H), 4.08 (s, 1H), 3.94 (s, 1H), 2.77 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 159.60 (d, J_{C-F} = 250.9 Hz), 148.57, 147.91, 130.56 (qd, J_{C-F} = 32.8, 8.6 Hz), 129.66 (d, J_{C-F} = 3.6 Hz), 129.21 (d, J_{C-F} = 2.1 Hz), 127.81 (d, J_{C-F} = 11.7 Hz), 125.88, 125.08 (q, J_{C-F} = 271.8 Hz), 124.13, 124.07 (d, J_{C-F} = 2.7 Hz), 122.06 (p, J_{C-F} = 3.7 Hz), 113.91 (dq, J_{C-F} = 21.8, 4.1 Hz), 111.64, 108.82, 101.90, 57.99, 56.42, 38.49.^{19}F NMR (376 MHz, DMSO-d_6/CF_3COOH) δ −61.17 (s, 3F), −115.52(dd, *J* = 9.4, 8.0 Hz, 1F); HRMS (ESI) *m/z* calcd for $C_{19}H_{18}NO_2F_4$ $[M+H]^+$ 368.1274, found 368.1272.

4.1.41. Synthesis of (E)-N-(benzo[d][1,3]dioxol-5-ylmethyl)-3-(3-fluoro-4-(trifluoromethyl)phenyl)-N-methylprop-2-en-1-amine hydrochloride (**15c**)

Compound **15c** was prepared by the experimental procedure depicted for compound **11a** as a white solid. Yield: 51%; m.p.: 167–170 °C. ^{1}H NMR (400 MHz, CD_3OD) δ 7.70 (t, *J* = 7.7 Hz, 1H), 7.54 (d, *J* = 11.8 Hz, 1H), 7.48 (d, *J* = 7.8 Hz, 1H), 7.02 (s, 1H), 7.01–6.87 (m, 3H), 6.60–6.47 (m, 1H), 6.02 (s, 2H), 4.37 (s, 1H), 4.22 (s, 1H), 4.05 (s, 1H), 3.92 (s, 1H), 2.74 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 159.62 (dq, J_{C-F} = 252.8, 3.7 Hz), 148.58, 147.92, 143.23 (d, J_{C-F} = 7.7 Hz), 136.01–135.85 (m), 128.18 (q, J_{C-F} = 3.8 Hz), 125.87, 124.16, 123.94 (d, J_{C-F} = 2.5 Hz), 123.78 (d, J_{C-F} = 1.4 Hz), 123.09 (q, J_{C-F} = 270.3 Hz), 116.64–115.94 (m), 115.15 (d, J_{C-F} = 21.1 Hz), 111.62, 108.85, 101.91, 57.85, 55.95, 38.55.^{19}F NMR (376 MHz, DMSO-d_6/CF_3COOH) δ −59.84 (d, *J* = 12.0 Hz, 3F), −115.86 to −116.16 (m, 1F); HRMS (ESI) *m/z* calcd for $C_{19}H_{18}NO_2F_4$ $[M+H]^+$ 368.1274, found 368.1276.

4.1.42. Synthesis of (E)-N-(benzo[d][1,3]dioxol-5-ylmethyl)-3-(2-fluoro-4-methoxyphenyl)-N-methylprop-2-en-1-amine hydrochloride (**15d**)

Compound **15d** was prepared by the experimental procedure depicted for compound **11a** as a white solid. Yield: 49%; m.p.: 132–134 °C. ^{1}H NMR (400 MHz, CD_3OD) δ 7.53 (t, *J* = 8.8 Hz, 1H), 7.07–6.88 (m, 4H), 6.78 (d, *J* = 8.8 Hz, 1H), 6.74 (dd, *J* = 12.9, 2.1 Hz, 1H), 6.34–6.21 (m, 1H), 6.02 (s, 2H), 4.38 (d, *J* = 12.4 Hz, 1H), 4.16 (d, *J* = 12.6 Hz, 1H), 4.00 (d, *J* = 6.1 Hz, 1H), 3.91–3.73 (m, 4H), 2.73 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.32 (d, J_{C-F} = 11.4 Hz), 160.93 (d, J_{C-F} = 247.0 Hz), 148.54, 147.90, 130.75, 129.14 (d, J_{C-F} = 5.1 Hz), 125.83, 124.23, 118.88, 115.91 (d, J_{C-F} = 12.3 Hz), 111.61, 111.58, 108.82, 101.98 (d, J_{C-F} = 26.0 Hz), 101.90, 57.82, 56.89, 56.25, 38.22.^{19}F NMR (376 MHz, DMSO-d_6/CF_3COOH) δ −115.65 (dd, *J* = 12.5, 9.1 Hz, 1F); HRMS (ESI) *m/z* calcd for $C_{19}H_{21}NO_3F$ $[M+H]^+$ 330.1505, found 330.1504.

4.1.43. Synthesis of (E)-N-(benzo[d][1,3]dioxol-5-ylmethyl)-3-(3-fluoro-4-methoxyphenyl)-N-methylprop-2-en-1-amine hydrochloride (**15e**)

Compound **15e** was prepared by the experimental procedure depicted for compound **11a** as a white solid. Yield: 48%; m.p.: 152–154 °C. ^{1}H NMR (400 MHz, CD_3OD) δ 7.34 (dd, *J* = 12.5, 2.1 Hz, 1H), 7.24 (d, *J* = 8.5 Hz, 1H), 7.09 (t, *J* = 8.6 Hz, 1H), 7.04–6.98 (m, 2H), 6.93 (d, *J* = 7.8 Hz, 1H), 6.82 (d, *J* = 15.7 Hz, 1H), 6.27–6.15 (m, 1H), 6.02 (s, 2H), 4.32 (s, 1H), 4.21 (s, 1H), 3.93 (s, 1H), 3.89 (s, 4H), 2.77 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 152.02 (d, J_{C-F} = 243.8 Hz), 148.52, 147.89, 147.80, 137.50, 129.32 (d, J_{C-F} = 6.6 Hz), 125.82, 124.42 (d, J_{C-F} = 3.0 Hz), 124.28, 117.63, 114.22, 113.98 (d, J_{C-F} = 18.5 Hz), 111.62, 108.82, 101.89, 57.66, 56.52, 56.36, 38.26.^{19}F NMR (376 MHz, DMSO-d_6/CF_3COOH) δ −135.30 (dd, *J* = 12.6, 9.0 Hz, 1F); HRMS (ESI) *m/z* calcd for $C_{19}H_{21}NO_3F$ $[M+H]^+$ 330.1505, found

330.1507.

*4.1.44. Synthesis of (E)-N-(benzo[d][1,3]dioxol-5-ylmethyl)-3-(2-fluoro-4-(trifluoromethyl)phenyl)prop-2-en-1-amine hydrochloride (**16a**)*

A solution of intermediate **7v** (1.42 g, 5.0 mmol) was added slowly to a mixture of benzo[*d*][1,3]dioxol-5-ylmethanamine (831 mg, 5.5 mmol) and K_2CO_3 (1.38 g, 10.0 mmol) in DMF (10 mL) with stirring at room temperature overnight. The reaction mixture was then poured into water and extracted with ethyl acetate. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, and then filtered and condensed. The residue was then purified by silica gel column chromatography (eluent, EtOAc/petroleum ether, 1/5, V/V) to give the free base of **16a** as a yellow oil. The final compound **16a** in the form of hydrochloride was prepared by the salification procedure described for compound **11a** as a white solid. Yield: 60%; m.p.: 104–105 °C. ^{1}H NMR (400 MHz, CD_3OD) δ 7.80 (t, J = 7.8 Hz, 1H), 7.51 (t, J = 7.9 Hz, 2H), 7.04 (d, J = 16.1 Hz, 1H), 7.01–6.97 (m, 2H), 6.90 (dd, J = 7.7, 0.5 Hz, 1H), 6.55 (dt, J = 16.0, 7.1 Hz, 1H), 6.00 (s, 2H), 4.17 (s, 2H), 3.89 (d, J = 7.1 Hz, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 159.58 (d, $J_{C\text{-}F}$ = 250.7 Hz), 148.12, 147.75, 130.36 (qd, $J_{C\text{-}F}$ = 32.9, 8.5 Hz), 129.52 (d, $J_{C\text{-}F}$ = 3.7 Hz), 127.94 (d, $J_{C\text{-}F}$ = 12.0 Hz), 127.41 (d, $J_{C\text{-}F}$ = 2.6 Hz), 126.97 (d, $J_{C\text{-}F}$ = 4.9 Hz), 125.91, 123.72 (qd, $J_{C\text{-}F}$ = 270.6, 2.6 Hz), 124.67, 122.09 (p, $J_{C\text{-}F}$ = 3.6 Hz), 113.90 (dq, $J_{C\text{-}F}$ = 25.6, 3.8 Hz), 110.87, 108.70, 101.75, 49.55, 48.03; HRMS (ESI) *m/z* calcd for $C_{18}H_{16}F_4NO_2$ $[M+H]^+$ 354.1117, found 354.1118.

*4.1.45. Synthesis of (E)-N-(benzo[d][1,3]dioxol-5-ylmethyl)-N-ethyl-3-(2-fluoro-4-(trifluoromethyl)phenyl)prop-2-en-1-amine hydrochloride (**16b**)*

To a solution of **16a** (424 mg, 1.2 mmol) in DMF (10 mL) was added sodium hydride (48 mg, 1.2 mmol) at 0 °C under a nitrogen atmosphere. The reaction mixture was stirred for 15 min, and iodoethane (0.2 mL, 2.4 mmol) was added into the solution. The mixture was stirred at room temperature overnight, poured into water, and extracted with EtOAc. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered, and condensed. The residue was then purified by silica gel column chromatography (eluent, EtOAc/petroleum ether, 1/5, V/V) to give the free base of **16b** as a yellowish oil. The final compound **16b** in the form of hydrochloride was prepared by the salification procedure described for compound **11a** as a white solid. Yield: 55%; m.p.: 150–152 °C. ^{1}H NMR (400 MHz, CD_3OD) δ 7.85 (t, J = 7.8 Hz, 1H), 7.52 (t, J = 8.0 Hz, 2H), 7.07 (d, J = 16.4 Hz, 1H), 7.03 (d, J = 8.2 Hz, 2H), 6.93 (d, J = 8.1 Hz, 1H), 6.62–6.51 (m, 1H), 6.02 (d, J = 2.6 Hz, 2H), 4.33 (d, J = 3.2 Hz, 2H), 4.01 (dd, J = 6.7, 4.3 Hz, 2H), 3.26 (dt, J = 7.4, 5.9 Hz, 2H), 1.40 (t, J = 7.3 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 159.55 (d, $J_{C\text{-}F}$ = 250.8 Hz), 148.50, 147.94, 130.56 (qd, $J_{C\text{-}F}$ = 32.9, 8.4 Hz), 129.61 (d, $J_{C\text{-}F}$ = 3.6 Hz), 128.81 (d, $J_{C\text{-}F}$ = 3.1 Hz), 127.87 (d, $J_{C\text{-}F}$ = 11.3 Hz), 125.81, 124.91 (d, $J_{C\text{-}F}$ = 4.2 Hz), 124.15, 123.74 (qd, $J_{C\text{-}F}$ = 273.0, 2.4 Hz), 122.03 (p, $J_{C\text{-}F}$ = 3.6 Hz), 113.88 (dq, $J_{C\text{-}F}$ = 25.4, 3.8 Hz), 111.63, 108.82, 101.89, 55.54, 52.92, 46.65, 8.93; HRMS (ESI) *m/z* calcd for $C_{20}H_{20}F_4NO_2$ $[M+H]^+$ 382.1430, found 382.1431.

*4.1.46. Synthesis of (E)-N-(benzo[d][1,3]dioxol-5-ylmethyl)-3-(2-fluoro-4-(trifluoromethyl)phenyl)-N-isopropylprop-2-en-1-amine hydrochloride (**16c**)*

Compound **16c** was prepared by the experimental procedure depicted for compound **16b** as a white solid. Yield: 30%; m.p.: 155–156 °C. ^{1}H NMR (400 MHz, CD_3OD) δ 7.74 (t, J = 7.8 Hz, 1H), 7.55–7.47 (m, 2H), 7.04–6.96 (m, 3H), 6.91 (d, J = 8.2 Hz, 1H), 6.46–6.36 (m, 1H), 5.99 (d, J = 3.7 Hz, 2H), 4.43 (d, J = 13.1 Hz, 1H), 4.18 (d, J = 13.2 Hz, 1H), 4.07 (dd, J = 14.5, 7.6 Hz, 1H), 3.95 (dd, J = 14.0, 7.4 Hz, 1H), 3.80–3.71 (m, 1H), 1.49 (d, J = 6.7 Hz, 3H), 1.44 (d, J = 6.7 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 159.51 (d, $J_{C\text{-}F}$ = 250.6 Hz), 148.52, 147.93, 130.56 (qd, $J_{C\text{-}F}$ = 32.8, 8.4 Hz), 129.65 (d, $J_{C\text{-}F}$ = 3.6 Hz), 128.54 (d, $J_{C\text{-}F}$ = 3.2 Hz), 127.90 (d, $J_{C\text{-}F}$ = 12.0 Hz), 125.83, 124.92 (d, $J_{C\text{-}F}$ = 4.2 Hz), 124.15, 123.52 (qd, $J_{C\text{-}F}$ = 271.8, 2.4 Hz), 122.07 (p, $J_{C\text{-}F}$ = 3.4 Hz), 113.86 (dq, $J_{C\text{-}F}$ = 25.6, 3.7 Hz), 111.62, 108.84, 101.92, 52.45, 51.22, 49.84, 15.47 (2C); HRMS (ESI) *m/z* calcd for $C_{21}H_{22}F_4NO_2$ $[M+H]^+$396.1587, found 396.1586.

*4.1.47. Synthesis of tert-butyl((3-(2-fluoro-4-(trifluoromethyl)phenyl)prop-2-yn-1-yl)oxy)dimethylsilane (**17**)*

To a mixture of the 2-fluoro-1-iodo-4-(trifluoromethyl)benzene (580 mg, 2.0 mmol), tetrakis(triphenylphosphine)palladium (231 mg, 0.2 mmol), and CuI (381 mg, 2.0 mmol) in triethylamine at 0 °C was added *tert*-butyldimethyl(2-propynyloxy)silane (341 mg, 2.0 mmol). The reaction mixture was heated to 60 °C for 30 min and then filtrated through a short pad of silica gel. The residue was washed with dichloromethane and the filtrate concentrated. The crude product was then purified by silica gel column chromatography (eluent, petroleum ether) to afford **17** as a brown oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.54 (t, J = 7.4 Hz, 1H), 7.35 (dd, J = 11.3, 8.9 Hz, 2H), 4.58 (s, 2H), 0.92 (d, J = 9.8 Hz, 9H), 0.17 (s, 6H).

*4.1.48. Synthesis of ethyl (2E,4E)-5-(2-fluoro-4-(trifluoromethyl)phenyl)penta-2,4-dienoate (**18a**)*

Sodium methoxide (28% in MeOH, 386 mg, 2.0 mmol) was added to a stirred solution of intermediate **5v** (253 mg, 2.1 mmol) and triethyl phosphonoacetate (448 mg, 2.0 mmol) in dimethylformamide (10 mL) at 0 °C. After the mixture was stirred for 2 h, the reaction mixture was poured into water to give crystals. Then the crystals were filtered to give **18a** as colorless needles. The crude was used for the next step without further purification. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.95 (t, J = 7.7 Hz, 1H), 7.74 (d, J = 10.5 Hz, 1H), 7.62 (d, J = 8.2 Hz, 1H), 7.51 (dd, J = 15.2, 10.5 Hz, 1H), 7.34 (dd, J = 15.5, 10.7 Hz, 1H), 7.25 (d, J = 15.7 Hz, 1H), 6.24 (d, J = 15.2 Hz, 1H), 3.98 (q, J = 8.0 Hz, 2H), 1.20 (t, J = 8.0 Hz, 3H).

*4.1.49. Synthesis of ethyl (E)-3-(2-fluoro-4-(trifluoromethyl)phenyl)-2-methylacrylate (**18b**)*

To a solution of 2-fluoro-4-(trifluoromethyl)benzaldehyde (384 mg, 2.0 mmol) in toluene (60 mL) was added (carbethoxyethylidine)triphenylphosphorane (1.05 g, 2.9 mmol) in one portion. The reaction mixture was heated to 100 °C for 1 h before being cooled to room temperature. The solvent was removed under reduced pressure and petroleum ether was added to the residue. The precipitated triphenylphosphine oxide was removed via filtration and the filtrate concentrated. Purification of the crude material by silica gel column chromatography (eluent, EtOAc/petroleum ether, 1/20, V/V) to afford **18b** as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.58 (s, 1H), 7.42–7.34 (m, 2H), 7.31 (d, J = 10.0 Hz, 1H), 4.23 (q, J = 7.1 Hz, 2H), 1.96 (t, J = 1.3 Hz, 3H), 1.29 (t, J = 7.1 Hz, 3H).

*4.1.50. Synthesis of 3-(2-fluoro-4-(trifluoromethyl)phenyl)prop-2-yn-1-ol (**19a**)*

To a solution of **17** (332 mg, 1,0 mmol) in tetrahydrofuran (10 mL) was added tetrabutylammonium fluoride (2.6 g, 10 mmol) at 0 °C for 30 min. The solvent was then evaporated *in vacuo*. The residue was extracted with water and EtOAc. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and condensed. The residue was then purified by silica gel column chromatography to afford **19a** as a colorless oil. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.81 (d, J = 9.6 Hz, 1H), 7.75 (t, J = 7.6 Hz, 1H), 7.61 (d, J = 8.1 Hz, 1H), 5.51 (t, J = 6.0 Hz, 1H), 4.38 (d, J = 6.0 Hz, 2H).

*4.1.51. Synthesis of (2E,4E)-5-(2-fluoro-4-(trifluoromethyl)phenyl) penta-2,4-dien-1-ol (**19b**)*

To a solution of **18a** (288 mg, 1.0 mmol) in dichloromethane (10 mL) was added diisobutyl aluminium hydride (1.0 M in hexane, 2 mL, 2.0 mmol) dropwise at 0 °C under nitrogen atmosphere. The reaction solution was stirred at room temperature for 1.5 h and quenched with methyl (0.2 mL) and water (0.2 mL) with cooling. The reaction mixture was acidified with concentrated hydrochloric acid and extracted with dichloromethane. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and condensed to generate **19b** as a colorless oil without further purification. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.88 (dd, J = 16.2, 8.3 Hz, 1H), 7.66 (d, J = 10.8 Hz, 1H), 7.55 (d, J = 8.2 Hz, 1H), 7.17 (dd, J = 15.8, 10.6 Hz, 1H), 6.69 (d, J = 15.6 Hz, 1H), 6.46 (dd, J = 15.2, 10.7 Hz, 1H), 6.12 (dt, J = 15.2, 5.0 Hz, 1H), 4.91 (t, J = 5.4 Hz, 1H), 4.08 (t, J = 4.9 Hz, 2H).

*4.1.52. Synthesis of ((1R,2R)-2-(2-fluoro-4-(trifluoromethyl)phenyl) cyclopropyl)methanol (**19d**)*

A solution of diethyl zinc (1.1 M, 2.72 mL, 3.0 mmol) in hexanes was added to 5 mL of dichloromethane under nitrogen. The resulting solution was cooled to 0 °C. Trifluoroacetic acid (0.23 mL, 3.0 mmol) was added slowly to the cooled diethylzinc solution. After the completion of addition, the resulting mixture was stirred for 20 min. A solution of diiodomethane, (0.24 mL, 3.0 mmol) in 5 mL of dichloromethane was added to the mixture. Subsequently, a solution of intermediate **6v** (330 mg, 1.5 mmol) in 5 mL of dichloromethane was added. After completing addition, the reaction mixture was warmed to room temperature and stirred for 2 h. Excess reagent was quenched by slow addition of 5 mL of 1 M hydrochloric acid. The resulting solution was partitioned with EtOAc and water. The combined organic extracts were washed with saturated ammonium chloride, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude was purified by silica gel column chromatography (eluent, EtOAc/petroleum ether, 1/5, V/V) to afford **19d** as a colorless oil. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.66 (d, J = 10.8 Hz, 1H), 7.46 (d, J = 7.9 Hz, 1H), 7.22 (t, J = 7.9 Hz, 1H), 4.70 (t, J = 5.6 Hz, 1H), 3.52 (dt, J = 11.1, 5.5 Hz, 1H), 3.41 (dt, J = 11.4, 5.8 Hz, 1H), 2.02 (dd, J = 8.9, 4.6 Hz, 1H), 1.51–1.39 (m, 1H), 1.08–0.95 (m, 2H).

*4.1.53. Synthesis of 3-(2-fluoro-4-(trifluoromethyl)phenyl)propan-1-ol (**19e**)*

A solution of intermediate **6v** (330 mg, 1.5 mmol) in methanol was subjected to hydrogenation at atmospheric pressure in the presence of 10% Pd/C (33 mg, 0.3 mmol) at ambient temperature overnight. The reaction solution was filtered and the filtrate was concentrated as a colorless oil to afford **19e** without further purification. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.59 (d, J = 10.1 Hz, 1H), 7.57–7.47 (m, 2H), 4.56 (t, J = 5.1 Hz, 1H), 3.42 (dd, J = 11.6, 6.2 Hz, 2H), 2.72 (t, J = 7.6 Hz, 2H), 1.70 (tt, J = 16.2, 8.1 Hz, 2H).

*4.1.54. Synthesis of 1-(3-bromoprop-1-yn-1-yl)-2-fluoro-4-(trifluoromethyl)benzene (**20a**)*

Intermediate **20a** was prepared by the experimental procedure depicted for intermediate **7a** as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.57 (t, J = 7.4 Hz, 1H), 7.39 (d, J = 8.3 Hz, 1H), 7.35 (d, J = 9.2 Hz, 1H), 4.18 (s, 2H).

*4.1.55. Synthesis of N-(benzo[d][1,3]dioxol-5-ylmethyl)-3-(2-fluoro-4-(trifluoromethyl)phenyl)-N-methylprop-2-yn-1-amine hydrochloride (**21a**)*

Compound **21a** was prepared by the experimental procedure depicted for compound **11a** as a white solid. Yield: 57%; m.p.: 138–140 °C. ^{1}H NMR (400 MHz, CD_3OD) δ 7.83 (t, J = 7.2 Hz, 1H), 7.65 (d, J = 9.4 Hz, 1H), 7.61 (d, J = 8.1 Hz, 1H), 7.05 (s, 2H), 6.96 (d, J = 8.4 Hz, 1H), 6.05 (s, 2H), 4.39 (s, 2H), 4.35 (s, 2H), 2.99 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 162.44 (d, $J_{C\text{-}F}$ = 252.7 Hz), 148.71, 147.91, 135.57, 131.89 (qd, $J_{C\text{-}F}$ = 33.1, 8.1 Hz), 125.93, 123.76, 123.43 (qd, $J_{C\text{-}F}$ = 273.6, 3.4 Hz), 122.34–122.08 (m), 114.25 (d, $J_{C\text{-}F}$ = 15.8 Hz), 113.91 (dq, $J_{C\text{-}F}$ = 24.4, 3.0 Hz), 111.64, 108.86, 101.94, 87.32, 81.55, 57.69, 44.13, 39.06; HRMS (ESI) m/z calcd for $C_{19}H_{16}F_4NO_2$ $[M+H]^+$ 366.1117, found 366.1118.

*4.1.56. Synthesis of (2E,4E)-N-(benzo[d][1,3]dioxol-5-ylmethyl)-5-(2-fluoro-4-(trifluoromethyl)phenyl)-N-methylpenta-2,4-dien-1-amine hydrochloride (**21b**)*

Compound **21b** was prepared by the experimental procedure depicted for compound **11a** as a white solid. Yield: 45%; m.p.: 168–170 °C. FTIR: 3421.7, 3015.5, 2904.1, 1622.1, 1494.3, 1374.1, 1331.8, 1260.3, 1164.7, 1128.0, 1040.7, 912.1, 883.1 cm-1^{1}H NMR (400 MHz, CD_3OD) δ 7.85 (t, J = 7.8 Hz, 1H), 7.50 (t, J = 9.3 Hz, 2H), 7.20 (dd, J = 15.8, 10.5 Hz, 1H), 7.08–6.99 (m, 2H), 6.99–6.89 (m, 2H), 6.79 (dd, J = 15.0, 10.6 Hz, 1H), 6.16–5.97 (m, 3H), 4.28 (s, 2H), 3.92 (s, 2H), 2.78 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 159.58 (d, $J_{C\text{-}F}$ = 250.6 Hz), 148.55, 147.89, 138.79, 133.39 (d, $J_{C\text{-}F}$ = 4.5 Hz), 129.85 (qd, $J_{C\text{-}F}$ = 32.8, 8.7 Hz), 129.05 (d, $J_{C\text{-}F}$ = 3.5 Hz), 128.73 (d, $J_{C\text{-}F}$ = 11.8 Hz), 125.85, 125.60, 125.00, 124.17, 123.80 (qd, $J_{C\text{-}F}$ = 270.3, 2.7 Hz), 122.10–121.86 (m), 113.82 (dq, $J_{C\text{-}F}$ = 25.5, 3.8 Hz), 111.64, 108.80, 101.90, 57.88, 56.34, 38.20.^{19}F NMR (376 MHz, DMSO-d_6/CF_3COOH) δ −61.13 (s, 3F), −115.82 (dd, J = 11.3, 8.0 Hz, 1F); HRMS (ESI) m/z calcd for $C_{21}H_{20}F_4NO_2$ $[M+H]^+$ 394.1430, found 394.1431.

*4.1.57. Synthesis of (E)-N-(benzo[d][1,3]dioxol-5-ylmethyl)-3-(2-fluoro-4-(trifluoromethyl)phenyl)-N,2-dimethylprop-2-en-1-amine hydrochloride (**21c**)*

Compound **21c** was prepared by the experimental procedure depicted for compound **11a** as a white solid. Yield: 55%; m.p.: 155–158 °C. ^{1}H NMR (400 MHz, CD_3OD) δ 7.63–7.57 (m, 1H), 7.53 (t, J = 9.1 Hz, 2H), 7.07–7.00 (m, 2H), 6.95 (d, J = 8.2 Hz, 1H), 6.81 (s, 1H), 6.04 (s, 2H), 4.40 (d, J = 13.1 Hz, 1H), 4.24 (d, J = 13.0 Hz, 1H), 4.03 (d, J = 13.0 Hz, 1H), 3.87 (d, J = 12.9 Hz, 1H), 2.83 (s, 3H), 1.93 (t, J = 1.2 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 159.61 (d, $J_{C\text{-}F}$ = 250.1 Hz), 148.56, 147.90, 138.85 (d, $J_{C\text{-}F}$ = 2.6 Hz), 130.50 (qd, $J_{C\text{-}F}$ = 32.0, 8.8 Hz), 129.20 (d, $J_{C\text{-}F}$ = 2.4 Hz), 127.77 (d, $J_{C\text{-}F}$ = 12.5 Hz), 125.86, 124.85 (d, $J_{C\text{-}F}$ = 3.6 Hz), 123.91 (q, $J_{C\text{-}F}$ = 270.5 Hz), 124.16, 122.98–122.43 (m), 113.95 (dq, $J_{C\text{-}F}$ = 22.4, 3.6 Hz), 111.60, 108.81, 101.90, 58.89, 54.62, 38.99, 15.42; HRMS (ESI) m/z calcd for $C_{20}H_{20}F_4NO_2$ $[M+H]^+$ 382.1430, found 382.1431.

*4.1.58. Synthesis of 1-(benzo[d][1,3]dioxol-5-yl)-N-(((1R,2R)-2-(2-fluoro-4-(trifluoromethyl)phenyl)cyclopropyl)methyl)-N-methylmethanamine hydrochloride (**21d**)*

Compound **21d** was prepared by the experimental procedure depicted for compound **11a** as a white solid. Yield: 25%; m.p.: 160–162 °C. ^{1}H NMR (400 MHz, CD_3OD) δ 7.49–7.40 (m, 2H), 7.27 (s, 1H), 7.00 (d, J = 8.7 Hz, 2H), 6.93 (d, J = 7.7 Hz, 1H), 6.03 (s, 2H), 4.45 (d, J = 11.6 Hz, 1H), 4.19 (d, J = 12.8 Hz, 1H), 3.50–3.35 (m, 1H), 3.28–3.10 (m, 1H), 2.86 (s, 3H), 2.31 (s, 1H), 1.63 (s, 1H), 1.38 (s, 1H), 1.23 (dd, J = 14.1, 6.9 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.08 (d, $J_{C\text{-}F}$ = 245.7 Hz), 148.53, 147.88, 133.81 (qd, $J_{C\text{-}F}$ = 34.4, 3.5 Hz), 128.88 (d, $J_{C\text{-}F}$ = 12.1 Hz), 128.31 (d, $J_{C\text{-}F}$ = 3.7 Hz), 125.83, 124.05, 123.93 (qd, $J_{C\text{-}F}$ = 270.1, 2.4 Hz), 121.98–121.75 (m), 112.85 (dq, $J_{C\text{-}F}$ = 25.9, 3.8 Hz), 111.62, 108.80, 101.89, 58.48, 57.80, 38.45, 17.04, 16.42, 14.19; HRMS (ESI) m/z calcd for $C_{20}H_{20}F_4NO_2$ $[M+H]^+$ 382.1430, found 382.1431.

*4.1.59. Synthesis of N-(benzo[d][1,3]dioxol-5-ylmethyl)-3-(2-fluoro-4-(trifluoromethyl)phenyl)-N-methylpropan-1-amine hydrochloride (**21e**)*

Compound **21e** was prepared by the experimental procedure depicted for compound **11a** as a white solid. Yield: 33%; m.p.: 188–190 °C. ^{1}H NMR (400 MHz, CD_3OD) δ 7.30 (d, $J = 9.6$ Hz, 1H), 7.01–6.89 (m, 2H), 6.67–6.47 (m, 3H), 5.95 (s, 2H), 4.33 (s, 2H), 3.15 (t, $J = 8.6$ Hz 2H), 2.70–2.54 (m, 5H), 2.22–2.01 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.59 (d, $J_{C\text{-}F} = 250.7$ Hz), 148.55, 147.87, 133.50 (qd, $J_{C\text{-}F} = 32.6$, 8.2 Hz), 132.43, 131.43 (d, $J_{C\text{-}F} = 12.8$ Hz), 125.84, 124.07, 123.90 (qd, $J_{C\text{-}F} = 270.0$, 2.6 Hz), 121.96–121.73 (m), 112.82 (dq, $J_{C\text{-}F} = 21.8$, 3.8 Hz), 111.58, 108.79, 101.91, 58.37, 53.78, 38.95, 25.61, 23.66; HRMS (ESI) m/z calcd for $C_{19}H_{20}F_4NO_2$ $[M+H]^+$ 370.1430, found 370.1431.

4.2. Biological methods

4.2.1. Pigment inhibition assay

S. aureus Newman bacteria were cultured in TSB (4 mL) medium at 37 °C for 48 h with 20 μL synthesized compounds dissolved in DMSO and diluted to a set of concentrations, in duplicate. The vehicle control was prepared by adding equal amount of DMSO. The positive control was using nonpigmented *CtrN* mutant of *S. aureus* Newman instead of Newman, with no compounds or DMSO added. 3 mL bacteria cultures were centrifuged and washed twice with 0.01 M phosphate-buffered saline (PBS) and resuspended in methanol to extract pigment. The absorbance value was determined at 450 nm on a NanoDrop 2000c (Thermo scientific) spectrophotometer. IC_{50} values were calculated by Graphpad Prism 5.0 software. The IC_{50} values of the MRSA strains NRS271, USA300 LAC, USA400 MW and Mu 50 were determined in the same way.

4.2.2. Cytotoxicity assay

The cytotoxic activity against HepG 2 and HEK-293T cell lines *in vitro* was determined using the CCK-8 assay. The cells were plated in 96-well plates at density of 5000 cells per well and incubated at 37 °C in 5% CO_2 atmosphere for 24 h. The tested compounds were dissolved in DMSO and diluted with culture medium (DMSO final concentration < 0.4%). The vehicle control was prepared by mixing the culture medium with the corresponding concentration of DMSO. Amphotericin B was set as reference drug. The various concentration of tested compounds and controls were treated with the cells for 72 h at 37 °C in a 5% CO_2 incubator. Then the supernatant liquor was removed and 100 μL of new media diluted CCK-8 solution (10% CCK-8) was added to each well with 1 h incubating. The cell survival was determined by measuring the absorbance at 450 nm. The assay was measured in triplicate.

4.2.3. CrtN enzyme inhibition assay

Diapophytoene was purified from diapophytoene-producing *E. coli* BL21 (DE3)/pET28a:*crtM* extracting with acetone. 8 mg diapophytoene was mixed with 24 mg of phosphatidylcholine (Sigma-Aldrich) in 200 μL $CHCl_3$ to prepare diapophytoene emulsion. The mixture was spun-dried and incubated with 2 mL 0.02 M HEPES buffer (pH 7.5) followed by sonicating in ice water to obtain the homogeneous emulsion. For the preparation of CrtN lysate, *E. coli* BL21 (DE3)/pET28a:*crtN* was sub-cultured into 1000 mL of LB broth supplemented with 50 μg/mL kanamycin to achieve an OD_{600} of ~0.1 and grown to an OD_{600} of ~0.5. The expression of 6His-CrtN protein was induced with 0.5 mM isopropyl-β-D-thiogalactoside (IPTG) at 16 °C overnight. The cells were harvested, and the pellets were suspended in 30 mL HEPES buffer and lysed at 4 °C by sonication.

The enzyme activity was determined in triplicate, with a total of 700 μL of the following: 50 μL diapophytoene emulsion, 70 μL different concentrations of test compounds (**15b** and **21b**) or mock (corresponding amount of ddH_2O), 3.5 μL FAD stock solution (10 mM), and 300 μL CrtN lysate (~1.41 mg CrtN, as estimated by western blot using a known concentration of the purified 6His-crtN protein), then 0.02 M HEPES buffer (pH 7.5) to 700 μL. The tests were proceeded under anaerobic atmosphere by adding a final concentration of 20 U/mL glucose oxidase (Sigma-Aldrich, G2133), 20000 U/mL catalase (Sigma-Aldrich, C1345), and 2 mM glucose as an oxygen-trapping system. The reaction mixture was started by adding the lysate and incubating overnight at 37 °C and then stopped by methanol. The pigments were extracted twice against 700 μL chloroform. The organic phase was combined, concentrated, and redissolved in 200 μL chloroform and OD_{450} was recorded. The IC_{50} values were obtained by fitting the OD data to a normal dose-response curve using Graphpad Prism 5.0 software.

4.2.4. Bacterial growth assays of S. aureus Newman and MRSA strains

21b was dissolved in DMSO to 20 mM as a stock solution and diluted with fresh TSB medium to produce a final concentration of 0.2 mM and 0.05 mM 100 μL of each dilution was distributed in 96-well plates, together with the growth controls (containing equal amount of DMSO). 60 μL paraffin wax was covered onto the dilutions to prevent the medium from evaporating. The dilutions were placed at 37 °C for 4 h to sufficiently dissolve the test compounds. Overnight cultured *S. aureus* strains were washed twice with PBS and diluted with fresh medium to obtain an optical density at 600 nm (OD_{600}) of ~1.0. Test and growth control wells were inoculated with 5 μL of a bacterial suspension (final $OD_{600} \approx 0.05$). The 96-well plates were incubated at 37 °C overnight, and the OD_{600} was recorded every half an hour with a Synergy 2 (Biotek) plate reader following the manufacturer's instructions.

4.2.5. Hydrogen peroxide killing and human whole blood killing assays

For H_2O_2 killing assay, four strains, including Newman, USA300 LAC, USA400 WM2 and Mu50, were cultured in TSB and grown at 37 °C for 24 h with 1 μM compound **21b** (40 μL in DMSO) or 40 μL DMSO as vehicle control or equal amount of *N*-Acetyl-Cysteine (NAC). The bacteria were washed twice in PBS and then diluted to a concentration of 4×10^6 CFU per 250 μL reaction mixture in a 2-mL Eppendorf tube. After H_2O_2 was added to a final concentration of 1.5%, the tubes were incubated for 30 min at 37 °C with shaking at 250 r.p.m. The reaction was terminated by the addition of 1000 U/mL exogenous catalase (Sigma-Aldrich). Bacterial survival was assessed by serial dilutions on TSA plates for enumeration of CFU.

For human whole blood killing assay, overnight cultured strains were centrifuged and suspended in sterile PBS to generate a suspension of 1×10^7 CFU/mL. Whole blood (360 μL) from healthy human volunteer was collected using a BD VACUTAINER PT tube and then mixed with 40 μL bacterial sample, which resulted in a concentration of 1×10^6 CFU/mL. The tubes were incubated at 37 °C for 6 h, and then the dilutions were plated on TSA agar plates for enumeration of the surviving CFUs.

4.2.6. S. aureus systemic infection models

6-8-week-old female BALB/c mice were obtained from JSJ Lab Animal, Ltd. and housed under specified pathogen-free conditions. The pre-treatment groups received intraperitoneal injections of **21b** at a total dose of 50 mg/kg or 200 mg/kg (in 12 intervals for 108 h) twelve hours before infection. Similarily, vancomycin, linezolid and ddH_2O were given as the pre-treatment group at a total dose of 200 mg/kg. The post-treatment groups received intraperitoneal injections at a total dose of 39 mg/kg or 156 mg/kg (in 12

intervals for 84 h) six hours after challenged with *S. aureus* strains. All the compounds were dissolved in sterile ddH_2O. For the mouse model of abscess formation, the mice were challenged with 100 μL of a bacterial suspension of either 2.2×10^7 CFU of *S. aureus* Newman, 1.1×10^9 CFU of *S. aureus* Mu50, or 1.8×10^8 CFU of *S. aureus* NRS271 via retro-orbital injection. Animals were euthanized 90 h after infection. Kidneys, and livers were isolated and homogenized in 1 mL PBS plus 0.01% Triton X-100 to obtain single-cell suspensions, and serial dilutions of each organ were plated on TSA (Difco) plates for the enumeration of CFUs. The statistical significance was determined by the Mann-Whitney Test (two-tailed).

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Appendix A. Supplementary data

HPLC analysis data of derivatives **11a-h**, **12a-e**, **13a-e**, **14a-m**, **15a-e**, **16a-c** and **21a-e**; bacterial growth assays of *S. aureus* Newman and MRSA strains, the hERG inhibition assay and MIC values of derivatives against MRSA strains.

Supplementary data related to this article can be found at https://doi.org/10.1016/j.ejmech.2017.12.090.

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