Supporting Information

Novel Staphyloxanthin Inhibitors with Improved Potency Against Multidrug Resistant *Staphylococcus aureus*

Shuaishuai Ni[a, †], Baoli Li[a, †], Feifei Chen[b, †], Hanwen Wei[a], Fei Mao[a], Yifu Liu[a], Yixiang Xu[a], Xiaoxia Qiu[a], Xiaokang Li[a], Wenwen Liu[a], Linghao Hu[a], Dazheng Ling[a], Manjiong Wang[a], Xinyu Zheng[a], Jin Zhu[a, *], Lefu Lan[b, *], Jian Li[a, *].

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Chemical

HPLC analysis data of compounds 11-48

Table S1. HPLC analysis data of compounds **11-48**. The purities of identified compounds were essential to the conclusions drawn in the text and determined by standard instrumentation with one system given in the following table. The peak purity was checked with UV spectra.

Equipment		Agilent 1100 with quaternary pump, diode-array detector (DAD)				
Column		Agilent Exlipse XDB-C18 (250*4.6 mm, 5 μm particle size)				
System condition		CH_3OH/H_2O (0.1% H_3PO_4), 95% (v/v) of CH_3OH gradient, flow rate: 0.5 mL/min, calculated the relative purity of each compound at 254 nm				
Results	Compound	Retention time (min)	Relative purity (%)	Compound	Retention time (min)	Relative purity (%)
	11	5.33	98.1	**30**	5.36	99.0
	12	5.87	98.5	**31**	5.74	99.5
	13	5.46	96.2	**32**	5.89	98.5
	14	5.36	95.9	**33**	5.14	96.7
	15	5.75	98.2	**34**	5.28	98.6
	16	5.48	99.0	**35**	5.47	96.2
	17	5.96	99.1	**36**	5.29	99.5
	18	5.79	99.4	**37**	5.34	98.9
	19	5.12	97.9	**38**	5.31	99.2
	20	5.86	99.9	**39**	5.37	96.8
	21	5.42	96.3	**40**	5.35	97.8
	22	5.69	98.5	**41**	5.15	97.6
	23	5.74	97.6	**42**	5.91	98.6
	24	5.33	98.6	**43**	5.52	98.9
	25	5.48	99.1	**44**	5.42	99.1
	26	5.26	99.0	**45**	5.31	99.0

	27	5.96	98.6	**46**	5.14	97.5
	28	5.41	98.1	**47**	5.42	98.9
	29	5.55	98.5	**48**	5.39	96.8

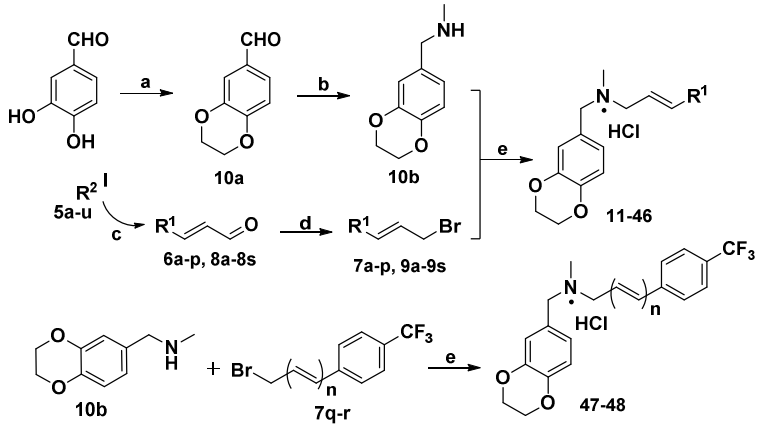
Determination of Water Solubility

The water solubilities of **15, 16, 47, 48, 4, and 4a** were determined by an HPLC method. Stock solutions (800 μg/mL) of the samples were prepared in methanol. Then, 10 μL dilute solutions with concentrations of 50, 100, 200, 400, and 800 μg/mL were injected into the HPLC system to assess linearity. Calibration curves were plotted as peak area versus concentration of the sample. Next, 10 mg of the sample was added into a 5 mL centrifuge tube, and 1 mL pure water was pipetted into the tube. If the solution was unsaturated and remained clear and transparent, more testing of the compound was needed. After stirring for 24 h, the solution was filtered with a syringe, and the HPLC system was injected with the same 10 μL. Water solubility was calculated by comparing the peak area of the tested compound to the calibration curves

Experimental procedures and characterizations of compounds 11-48

Derivatives **11-46** were synthesized by using the synthetic route outlined in Scheme 1. Treatment of 3, 4-dibromobenzaldehyde in ethylene glycol in the presence of triethylamine overnight provided **10a** in high yield, followed by the reductive amination reaction to yield **10b**. On the other hand, commercially available cinnamaldehydes **6a-p** underwent the reduction reaction, followed by bromination reaction with employing the phosphorous tribromide, generated intermediates **7a-p**. In parallel, cinnamaldehydes **8a-s** were afforded by Mizoroki-Heck reaction of commercially available **5a-u** with subsequent acid-catalyzed hydrolysis reactions, and followed by reduction and bromination to yield **9a-s**. Then, the target derivatives **11-46** were achieved by coupling **7a-p** or **9a-s** with **10b**. Subsequently, starting material 4-trifluoromethylbenzaldehyde was exchanged by Witting reaction, and followed by the reduction with DIBAL-H and the bromination to perform **7q** with phosphorous tribromide. In parallel, intermediate **7r** were prepared from **6h** using the same synthetic routes, as described above. The intermediates **7q-r** were reacted with **10b** according to a procedure described previously to obtain derivatives **47-48**.

Scheme S1. The Synthesis of Derivatives **11-48**[a]



7q (allyl bromide), **42** R^1 = H
6a, 7a, 44 R^1 = furan-2-yl
6b, 7b, 47 R^1 = 1-naphthyl
6c, 7c, 11 R^1 = phenyl
6d, 7d, 12 R^1 = 4-fluorophenyl
6e, 7e, 13 R^1 = 4-chlorophenyl
6f, 7f, 14 R^1=4-bromophenyl
6g, 7g, 15 R^1=4-methylphenyl
6h, 7h, 16 R^1=4-trifluoromethylphenyl
6i, 7i, 18 R^1=4-methoxylphenyl
6j, 7j, 20 R^1=4-nitrophenyl
6k, 7k, 24 R^1=2-fluorophenyl
6l, 7l, 25 R^1=2-chlorophenyl
6m, 7m, 30 R^1=3-fluorophenyl
6n, 7n, 31 R^1=3-chlorophenyl
6o, 7o, 32 R^1=3-methylphenyl
6p, 7p, 36 R^1=2,4-dichlorophenyl
8a, 9a, 42 R^1=R^2=cyclopentyl
8b, 9b, 43 R^1=R^2=cyclohexyl
8c, 9c, 45 R^1=R^2=2-thienyl
8d, 9d, 17 R^1=R^2=4-*t*-butylphenyl
8e, 9e, 19 R^1=R^2=4-ethoxylphenyl
8f, 9f, 20 R^1=R^2=4-cyanphenyl
8g, 9g, 22 R^1=R^2=4-formate-(methyl)-phenyl
8h, 9h, 23 R^1=R^2=4-biphenyl
8i, 9i, 24 R^1=R^2=2-methylphenyl
8k, 9k, 28 R^1=R^2=2-nitrophenyl
8l, 9l, 39 R^1= R^2=2-methoxylphenyl
8m, 9m, 33 R^1=R^2=3-trifluoromethylphenyl
8n, 9n, 34 R^1=R^2=3-nitrophenyl
8o, 9o, 35 R^1=R^2=3-methoxylphenyl
8p, 9p, 37 R^1=R^2=2-fluoro-4-trifluoromethylphenyl
8q, 9q, 38 R^1=R^2=3-fluoro-4-trifluoromethylphenyl
8r, 9r, 39 R^1=R^2=2-fluoro-4-methoxylphenyl
8s, 9s, 40 R^1=R^2=3-fluoro-4-methoxylphenyl
8j, 9j, 25 R^1=R^2=2-trifluoromethylphenyl
7q, 47 R^3=methyl, n=2
7r, 48 R^3=methyl, n=3

[a]Reagents and conditions: (a) ethylene glycol, K_2CO_3, DMF, 80 ºC, overnight; (b) (1) 30 % methylamine in methanol, r.t., 2 h; (2) NaBH4, methanol, 0 ºC, 1 h; (c) (1) 3, 3-dimethoxy-1-propene, K_2CO_3, KCl, $Pd(OAc)_2$, PPh_3, THF, 60 ºC,4 h; (2) 5% hydrochloric acid, r.t., 2 h; (d) PBr_3, Et_2O, 0 ºC, 10 min; (e) (1) K_2CO_3, DMF, r.t., 1 h; (2) HCl gas, Et_2O, r.t., 5 min.

General Synthetic Procedure.

Unless otherwise stated, all chemicals were purchased from commercial suppliers and used without further purification. Reaction progress was monitored by TLC on silica gel containing an inert binder and a fluorescent indicator (activated at 254 nm) coated flexible sheet. Chromatography was performed using silica gel columns with ethyl acetate/PE gradient solvent system. The purity of all final compounds was determined by Agilent 1100 with quaternary pump, diode-array detector (DAD) was performed on Agilent Exlipse XDB-C18 (250*4.6 mm, 5 μm particle size), eluting with a CH_3OH/H_2O (0.1% H_3PO_4), 90% (v/v) of CH_3OH gradient., flow rate: 0.5 mL/min, calculated the relative purity of each compound at 254 nm. All compounds were determined to be >95% pure by this method. The mass spectra were recorded with an Ion Trap Mass Spectrometer (Agilent, Santa Clara, CA). High- resolution mass spectral (MS) data were acquired with electron spray ionization (ESI) produced by a LCQ-DECA spectrometer. Melting points of each compound were determined on an SGW X-4 melting point apparatus. NMR spectra were recorded on Bruker 400 spectrometers at ambient temperature. Chemical shifts are reported in parts per million (δ) referenced to the internal standards (7.26 ppm for $CDCl_3$, 3.34 ppm for CD_3OD and 2.50 ppm for $(CD3)_2SO$) and coupling constants in Hz.

To a solution of (*E*)-3-(phenyl)acrylaldehyde (2.6 g, 20 mmol) in methanol (15 mL) was treated with $NaBH_4$ (0.84 g, 22 mmol) in batches at 0 ^oC. The reaction mixture was stirred at room temperature for 1h and concentrated. The residue was poured into water and extracted with ethyl acetate. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered, and condensed. The crude was used for the next step without further separation to give **6c** as yellow oil.

To a solution of **6c** (2.7 g, 20.0 mmol) in anhydrous ether (15 mL) was treated with phosphorus tribromide (0.65 mL, 7 mmol) at 0 °C. The reaction mixture was stirred at room temperature for 10 min and poured into ice water containing sodium bicarbonate. The mixture was partitioned between ethyl acetate and water. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered, and condensed at 30 °C. The crude was used for the next step without further purification to afford **7c** as red oil.

To a stirred mixture of 3, 4-dibromobenzaldehyde (26.2 g, 100 mmol) in ethylene glycol (150 ml) was added K_2CO_3 (48.3 g, 350 mmol) in portion under nitrogen gas in ambient temperature. The resulting dark minxture was stirred with reflux overnight. The residue was poured into water and extracted with ethyl acetate. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered, and condensed. The crude was used for the next step without further separation to give **10a** as a white solid. ^{1}H NMR (400 MHz, CDCl3) δ 9.82 (s, 1H), 7.41 (dd, J = 4.2, 2.4 Hz, 2H), 6.99 (d, J = 8.7 Hz, 1H), 4.36-4.28 (m, 4H).

Methanamine (10.16g, 33% in methanol) was added to a solution of 1, 4-benzodioxan-7-aldehyde (**10a**, 18.6 g, 30 mmol) in anhydrous methanol (15 mL) at room temperature. After stirring for 2 h, the solvent was removed under reduced pressure and the residue was re-dissolved in methanol (15 mL) with stirring at 0 ºC. $NaBH_4$ (1.28 g, 33.75 mmol) was added in small batches and then the reaction was moved to r.t for 1 h. The solvent was removed under reduced pressure and partitioned between ethyl acetate 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 via flash chromatography on silica gel. Eluting with ethyl acetate/petroleum ether (1/1, v/v) gave **10b** as pale yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.72 (dd, J = 16.0, 8.1 Hz, 3H), 4.16 (s, 4H), 3.56 (s, 2H), 2.35 (s, 3H).

A mixture of intermediate **7c** (98.0 mg, 0.5 mmol), **10b** (90.0 mg, 0.5 mmol) and K_2CO_3 (138.0 mg, 1 mmol) in DMF (5 mL) was stirred at room temperature for 1 h. The reaction mixture was poured into water and extracted thrice 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 flash chromatography, eluting with ethyl acetate /petroleum ether (1 / 5, v / v) to give the free base of **11** as yellow oil. To a solution of oily compound **11** (100 mg) in ethyl ether (10 mL) stirring at room temperature was bubbled hydrogen chloride gas for 1 min. After stirring for 15 min, the solvent was removed by rotary evaporation and the residue was suspended in ethyl acetate/petroleum ether (1:100, v/v, 10 mL) for additional one-hour agitation. The precipitate was filtrated and washed with ethyl ether to obtain the final compound in the form of hydrochloride. All other final compounds went through this salification process to give the amorphous solid form. Spectroscopic data given below are in their hydrochloride form a white solid (*E*)-*N*-(1, 4-Benzodioxine-7-methyl)-*N*-methyl-3-phenylprop-2-en-1-amine Hydrochloride **11** Yield = 61%. m.p. 164-166 ºC. ^{1}H NMR (400 MHz, MeOD) δ 7.51 (d, J = 7.1 Hz, 2H), 7.45-7.30 (m 3H), 7.09-6.84 (m, 4H), 6.42-6.26 (m, 1H), 4.36 (d, J = 13.0 Hz, 1H), 4.27 (s, 4H), 4.15 (d, J = 13.0 Hz, 1H), 4.01 (dd, J = 13.2, 7.0 Hz, 1H), 3.85 (dd, J = 13.0, 8.0 Hz, 1H), 2.78 (s, 3H); HRMS (ESI) m/z calcd for $C_{19}H_{21}NO_2$ $(M+H)^+$ 296.1651, found 296.1650.

(*E*)-*N*-(1, 4-Benzodioxine-7-methyl)-*N*-methyl-3-(4-chlorophenyl)prop-2-en-1-amine Hydrochloride (13).

Yield = 54%. **13** was synthesized by the general procedure of **11** given above as a white solid, m.p. 162-164 ºC. ^{1}H NMR (400 MHz, MeOD) δ 7.51 (d, J = 8.1 Hz, 2H), 7.37 (d, J = 8.0 Hz, 2H), 7.06 (s, 1H), 7.00 (d, J = 8.2 Hz, 1H), 6.91 (dd, J = 16.7, 12.0 Hz, 2H), 6.44-6.30 (m, 1H), 4.36 (d, J = 12.9 Hz, 1H), 4.26 (s, 4H), 4.16 (d, J = 12.8 Hz, 1H), 4.01 (dd, J = 12.8, 6.6 Hz, 1H), 3.85 (dd, J = 12.7, 7.8 Hz, 1H), 2.78 (s, 3H); ^{13}C NMR (101 MHz, DMSO) δ 144.76, 143.74, 137.37, 134.94, 133.44, 129.21, 129.21, 129.00, 129.00, 124.74, 123.49, 120.47, 119.76, 117.71, 64.54, 64.54, 57.52, 56.44, 38.37. HRMS (ESI) m/z calcd for $C_{19}H_{20}ClNO_2$ $(M+H)^+$ 330.1261, found 330.1259.

(*E*)-*N*-(1, 4-Benzodioxine-7-methyl)-*N*-methyl-3-(4-bromophenyl)prop-2-en-1-amine

Hydrochloride (14).

Yield = 46%. **14** was synthesized by the general procedure of **11** given above as a white solid, m.p. 157-159 ºC. ^{1}H NMR (400 MHz, MeOD) δ 7.53 (d, J = 8.3 Hz, 2H), 7.44 (d, J = 8.4 Hz, 2H), 7.05 (s, 1H), 6.99 (d, J = 8.3 Hz, 1H), 6.93 (d, J = 8.2 Hz, 1H), 6.87 (d, J = 15.8 Hz, 1H), 6.43-6.32 (m, 1H), 4.34 (m, 1H), 4.26 (s, 4H), 4.17 (m, 1H), 3.99 (m, 1H), 3.86 (m, 1H), 2.77 (s, 3H); ^{13}C NMR (101 MHz, DMSO) δ 144.76, 143.73, 137.43, 135.28, 132.13, 132.13, 129.29, 129.29, 124.75, 123.49, 122.10, 120.47, 119.86, 117.71, 64.54, 64.54, 57.52, 56.45, 38.37. HRMS (ESI) m/z calcd for $C_{19}H_{20}BrNO_2$ $(M+H)^+$ 374.0756, found 374.0753.

(*E*)-*N*-(1,

4-Benzodioxine-7-methyl)-*N*-methyl-3-(4-methylphenyl)prop-2-en-1-amine

Hydrochloride (15).

Yield = 48%. **15** was synthesized by the general procedure of **11** given above as a white solid, m.p. 154-156 ºC. ^{1}H NMR (400 MHz, MeOD) δ 7.40 (d, J = 7.1 Hz, 2H), 7.18 (d, J = 6.9 Hz, 2H), 7.05 (s, 1H), 7.02-6.91 (m, 2H), 6.87 (d, J = 15.2 Hz, 1H), 6.29 (s, 1H), 4.43-4.31 (m, 1H), 4.26 (s, 4H), 4.14 (d, J = 13.7 Hz, 1H), 3.99 (s, 1H), 3.83 (s, 1H), 2.72 (d, J = 35.7 Hz, 3H), 2.34 (s, 3H). HRMS (ESI) m/z calcd for $C_{20}H_{23}NO_2$ $(M+H)^+$ 310.1807, found 310.1808.

(*E*)-*N*-(1,

4-Benzodioxine-7-methyl)-*N*-methyl-3-(4-trifluorophenyl)prop-2-en-1-amine

Hydrochloride (16).

Yield = 43%. **16** was synthesized by the general procedure of **11** given above as a white solid, m.p. 165-167 ºC. ^{1}H NMR (400 MHz, MeOD) δ 7.73-7.65 (m, 4H), 7.05 (d, J = 1.9 Hz, 1H), 6.99 (d, J = 8.4 Hz, 1H), 6.97 (s, 1H), 6.94 (d, J = 8.3 Hz, 1H), 6.54-6.44 (m, 1H), 4.37 (d, J = 13.0 Hz, 1H), 4.27 (s, 4H), 4.18 (d, J = 13.0 Hz, 1H), 4.05 (dd, J = 13.1, 6.8 Hz, 1H), 3.89 (dd, J = 13.4, 7.8 Hz, 1H), 2.80 (s, 3H); ^{13}C NMR (101 MHz, DMSO) δ 144.75, 143.74, 140.05, 136.99, 134.75, 129.64, 127.92, 127.92, 127.56, 127.14, 126.64, 125.80, 124.72, 120.44, 117.71, 64.53, 64.53, 57.67, 56.39, 38.52. HRMS (ESI) m/z calcd for $C_{20}H_{20}F_3NO_2$ $(M+H)^+$ 364.1524, found 364.1521.

(*E*)-*N*-(1, 4-Benzodioxine-7-methyl)-*N*-methyl-3-(4-ethoxylphenyl)prop-2-en-1-amine Hydrochloride (19).

Yield = 43%. **19** was synthesized by the general procedure of **11** given above as a white solid, m.p. 164-166 ºC. ^{1}H NMR (400 MHz, MeOD) δ 7.35 (d, J = 8.7 Hz, 2H), 6.95 (d, J = 1.9 Hz, 1H), 6.89 (dd, J = 8.3, 1.9 Hz, 1H), 6.85 (d, J = 8.3 Hz, 1H), 6.82 (d, J = 8.7 Hz, 2H), 6.74 (d, J = 15.7 Hz, 1H), 6.14-6.02 (m, 1H), 4.26 (d, J = 13.0 Hz, 1H), 4.18 (s, 4H), 4.04 (d, J = 13.0 Hz, 1H), 3.96 (q, J = 7.0 Hz, 2H), 3.89 (dd, J = 13.1, 7.1 Hz, 1H), 3.72 (dd, J = 12.9, 8.0 Hz, 1H), 2.67 (s, 3H), 1.30 (t, J = 7.0 Hz, 3H); ^{13}C NMR (101 MHz, DMSO) δ 159.35, 144.75, 143.74, 138.58, 128.69, 128.69, 128.45, 124.70, 123.56, 120.42, 117.72, 115.89, 115.04, 115.04, 64.54, 64.54, 63.58, 57.67, 56.84, 38.26, 15.07. HRMS (ESI) m/z calcd for $C_{20}H_{25}NO_3$ $(M+H)^+$ 340.1913, found 340.1912.

(*E*)-*N*-(1, 4-Benzodioxine-7-methyl)-*N*-methyl-3-(4-formate-methylphenyl)prop-2-en-1-amine Hydrochloride (22).

Yield = 35%. **22** was synthesized by the general procedure of **11** given above as a white solid, m.p. 179-181 ºC. ^{1}H NMR (400 MHz, MeOD) δ 8.02 (d, J = 8.3 Hz, 2H), 7.63 (d, J = 8.4 Hz, 2H), 7.05 (d, J = 1.3 Hz, 1H), 7.0-6.97 (m, 1H), 6.94 (d, J = 8.3 Hz, 1H), 6.55-6.43 (m, 1H), 4.37 (d, J = 13.0 Hz, 1H), 4.26 (s, 4H), 4.18 (d, J = 13.0 Hz, 1H), 4.05 (dd, J = 12.7, 6.5 Hz, 1H), 3.91 (s, 3H), 3.87 (d, J = 8.1 Hz, 1H), 2.79 (s, 3H); ^{13}C NMR (101 MHz, DMSO) δ 166.34, 144.79, 143.75, 140.59, 137.53, 130.08, 130.8, 129.70, 127.54, 127.54, 124.74, 123.45, 121.86, 120.45, 117.74, 64.54, 64.54, 57.66, 56.45, 52.67, 38.49. HRMS (ESI) m/z calcd for $C_{21}H_{23}NO_4$ $(M+H)^+$ 354.1705, found 354.1706.

(*E*)-*N*-(1, 4-Benzodioxine-7-methyl)-*N*-methyl-3-(4-biphenyl)prop-2-en-1-amine Hydrochloride (23).

Yield = 46%. **23** was synthesized by the general procedure of **11** given above as a white solid, m.p. 173-175 ºC. ^{1}H NMR (400 MHz, MeOD) δ 7.73-7.59 (m, 6H), 7.47 (t, J = 7.6 Hz, 2H), 7.37 (dd, J = 8.3, 6.4 Hz, 1H), 7.07 (d, J = 1.9 Hz, 1H),

7.03-6.93 (m, 3H), 6.46-6.33 (m, 1H), 4.40 (d, J = 12.6 Hz, 1H), 4.29 (s, 4H), 4.19 (d, J = 12.2 Hz, 1H), 4.11-4.01 (m, 1H), 3.91 (d, J = 7.5 Hz, 1H), 2.82 (s, 3H); ^{13}C NMR (101 MHz, DMSO) δ 144.76, 143.75, 140.61, 139.91, 138.25, 135.14, 129.46, 129.46, 128.15, 127.89, 127.89, 127.40, 127.40, 127.03, 127.03, 124.77, 123.55, 120.49, 118.91, 117.72, 64.55, 64.55, 57.53, 56.65, 38.34. HRMS (ESI) m/z calcd for $C_{25}H_{25}NO_2$ $(M+H)^+$ 372.1964, found 372.1965.

(*E*)-*N*-(1, 4-Benzodioxine-7-methyl)-*N*-methyl-3-(4-fluorophenyl)prop-2-en-1-amine Hydrochloride (12).

Yield = 56%. **12** was synthesized by the general procedure of **11** given above as a white solid, m.p. 174-176 ºC. ^{1}H NMR (400 MHz, MeOD) δ 7.56 (s, 2H), 7.11 (d, J = 7.4 Hz, 2H), 7.07 (s, 1H), 7.01 (d, J = 7.0 Hz, 1H), 6.96-6.87 (m, 2H), 6.34 (s, 1H), 4.36 (s, 1H), 4.26 (s, 4H), 4.16 (d, J = 7.3 Hz, 1H), 4.01 (s, 1H), 3.86 (s, 1H), 2.78 (s, 3H); HRMS (ESI) m/z calcd for $C_{19}H_{20}FNO_2$ $(M+H)^+$ 314.1556, found 314.1557.

(*E*)-*N*-(1, 4-Benzodioxine-7-methyl)-*N*-methyl-3-(4-*t*-butylphenyl)prop-2-en-1-amine Hydrochloride (17).

Yield = 47%. **17** was synthesized by the general procedure of **11** given above as a white solid, m.p. 218-220 ºC. ^{1}H NMR (400 MHz, MeOD) δ 7.43 (q, J = 8.4 Hz, 4H), 7.04 (d, J = 1.1 Hz, 1H), 6.96 (dd, J = 18.9, 8.3 Hz, 2H), 6.87 (d, J = 15.8 Hz, 1H), 6.29 (dt, J = 15.3, 7.5 Hz, 1H), 4.35 (d, J = 13.0 Hz, 1H), 4.26 (s, 4H), 4.14 (d, J = 13.0 Hz, 1H), 4.00 (dd, J = 13.0, 6.9 Hz, 1H), 3.83 (dd, J = 13.1, 8.0 Hz, 1H), 2.77 (s, 3H), 1.31 (s, 9H); HRMS (ESI) m/z calcd for $C_{23}H_{29}NO_2$ $(M+H)^+$ 352.2277, found 352.2277.

(*E*)-*N*-(1, 4-Benzodioxine-7-methyl)-*N*-methyl-3-(4-methoxylphenyl)prop-2-en-1-amine Hydrochloride (18).

Yield = 56%. **18** was synthesized by the general procedure of **11** given above as a white solid, m.p. 185-187 ºC. ^{1}H NMR (400 MHz, MeOD) δ 7.39-7.20 (m, 4H), 7.15 (d, J = 6.7 Hz, 1H), 7.07 (s, 1H), 6.97 (d, J = 22.9 Hz, 2H), 6.36 (s, 1H), 4.35 (s,

1H), 4.26 (s, 4H), 4.16 (s, 1H), 4.00 (s, 1H), 3.85 (s, 1H), 3.32 (s, 3H), 2.77 (s, 3H); HRMS (ESI) m/z calcd for $C_{20}H_{23}NO_3$ $(M+H)^+$ 326.1756, found 326.1755.

(*E*)-*N*-(1, 4-Benzodioxine-7-methyl)-*N*-methyl-3-(4-nitrophenyl)prop-2-en-1-amine Hydrochloride (20).

Yield = 48%. **20** was synthesized by the general procedure of **11** given above as a brown solid, m.p. 172-174 ºC. ^{1}H NMR (400 MHz, MeOD) δ 8.24 (d, J = 8.7 Hz, 2H), 7.75 (d, J = 8.7 Hz, 2H), 7.06 (d, J = 1.9 Hz, 1H), 7.03 (d, J = 11.7 Hz, 1H), 7.01-6.97 (m, 1H), 6.94 (d, J = 8.3 Hz, 1H), 6.64-6.53 (m, 1H), 4.39 (d, J = 13.0 Hz, 1H), 4.26 (m, 4H), 4.18 (m, 1H), 4.07 (dd, J = 13.6, 6.1 Hz, 1H), 3.96-3.87 (m, 1H), 2.81 (s, 3H); HRMS (ESI) m/z calcd for $C_{19}H_{20}N_2O_4$ $(M+H)^+$ 341.1501, found 341.1503.

(*E*)-*N*-(1, 4-Benzodioxine-7-methyl)-*N*-methyl-3-(4-cyanophenyl)prop-2-en-1-amine Hydrochloride (21).

Yield = 47%. **21** was synthesized by the general procedure of **11** given above as a white solid, m.p. 186-188 ºC. ^{1}H NMR (400 MHz, MeOD) δ 7.91-7.84 (m, 2H), 7.71 (dd, J = 8.6, 1.6 Hz, 2H), 7.54-7.48 (m, 1H), 7.17 (d, J = 7.7 Hz, 1H), 7.06 (d, J = 15.8 Hz, 1H), 6.98-6.89 (m, 1H), 6.49-6.40 (m, 1H), 4.26 (m, 2H), 4.23-4.11 (s, 4H), 3.98 (m, 2H), 2.81 (s, 3H); HRMS (ESI) m/z calcd for $C_{20}H_{20}N_2O_2$ $(M+H)^+$ 321.1603, found 321.1601.

(*E*)-*N*-(1, 4-Benzodioxine-7-methyl)-*N*-methyl-3-(2-fluorophenyl)prop-2-en-1-amine Hydrochloride (24).

Yield = 58%. **24** was synthesized by the general procedure of **11** given above as a white solid, m.p. 176-178 ºC. ^{1}H NMR (400 MHz, MeOD) δ 7.74-7.68 (m, 1H), 7.46-7.41 (m, 1H), 7.37-7.31 (m, 2H), 7.28 (d, J = 15.8 Hz, 1H), 7.05 (d, J = 1.7 Hz, 1H), 7.00-6.95 (m, 1H), 6.94 (d, J = 8.3 Hz, 1H), 6.40-6.31 (m, 1H), 4.37 (d, J = 13.0 Hz, 1H), 4.27 (s, 4H), 4.17 (d, J = 13.0 Hz, 1H), 4.06 (dd, J = 13.4, 6.8 Hz, 1H), 3.91 (dd, J = 13.2, 8.0 Hz, 1H), 2.80 (s, 3H); HRMS (ESI) m/z calcd for $C_{19}H_{20}FNO_2$

$(M+H)^+$ 314.1556, found 314.1555.

(*E*)-*N*-(1,

4-Benzodioxine-7-methyl)-*N*-methyl-3-(2-chlorophenyl)prop-2-en-1-amine

Hydrochloride (25).

Yield = 61%. **25** was synthesized by the general procedure of **11** given above as a white solid, m.p. 201-203 ºC. ^{1}H NMR (400 MHz, MeOD) δ 7.72 (d, J = 12.8 Hz, 2H), 7.60 (dt, J = 15.2, 7.7 Hz, 2H), 7.12-6.90 (m, 4H), 6.54-6.44 (m, 1H), 4.38 (d, J = 11.9 Hz, 1H), 4.26 (s, 4H), 4.18 (d, J = 13.1 Hz, 1H), 4.04 (d, J = 10.3 Hz, 1H), 3.89 (s, 1H), 2.80 (d, J = 25.6 Hz, 3H). HRMS (ESI) m/z calcd for $C_{19}H_{20}ClNO_2$ $(M+H)^+$ 330.1261, found 330.1260

(*E*)-*N*-(1,

4-Benzodioxine-7-methyl)-*N*-methyl-3-(2-methylphenyl)prop-2-en-1-amine

Hydrochloride (26).

Yield = 53%. **26** was synthesized by the general procedure of **11** given above as a white solid, m.p. 155-157 ºC. ^{1}H NMR (400 MHz, MeOD) δ 7.54 (d, J = 6.6 Hz, 1H), 7.20 (d, J = 5.0 Hz, 2H), 7.15 (d, J = 3.5 Hz, 2H), 7.04 (s, 1H), 6.96 (dd, J = 16.7, 8.3 Hz, 2H), 6.25-6.14 (m, 1H), 4.36 (d, J = 13.1 Hz, 1H), 4.26 (s, 4H), 4.16 (d, J = 13.1 Hz, 1H), 4.03 (dd, J = 13.1, 6.4 Hz, 1H), 3.87 (dd, J = 13.3, 7.9 Hz, 1H), 2.79 (s, 3H), 2.28 (s, 3H); HRMS (ESI) m/z calcd for $C_{20}H_{23}NO_2$ $(M+H)^+$ 310.1807, found 310.1805.

(*E*)-*N*-(1,

4-Benzodioxine-7-methyl)-*N*-methyl-3-(2-trifluorophenyl)prop-2-en-1-amine

Hydrochloride (27).

Yield = 62%. **27** was synthesized by the general procedure of **11** given above as a white solid, m.p. 182-184 ºC. ^{1}H NMR (400 MHz, MeOD) δ 7.80 (d, J = 12.8 Hz, 2H), 7.60 (dt, J = 15.2, 7.7 Hz, 2H), 7.07 (s, 1H), 6.99 (t, J = 9.9 Hz, 2H), 6.93 (d, J = 8.2 Hz, 1H), 6.56-6.43 (m, 1H), 4.38 (d, J = 11.9 Hz, 1H), 4.26 (s, 4H), 4.18 (d, J = 13.1 Hz, 1H), 4.04 (s, 1H), 3.90 (s, 1H), 2.79 (s, 3H); HRMS (ESI) m/z calcd for $C_{20}H_{20}F_3NO_2$ $(M+H)^+$ 364.1524, found 364.1525.

(*E*)-*N*-(1,

4-Benzodioxine-7-methyl)-*N*-methyl-3-(2-nitrophenyl)prop-2-en-1-amine Hydrochloride (28).

Yield = 42%. **28** was synthesized by the general procedure of **11** given above as a brown solid, m.p. 172-174 ºC. ^{1}H NMR (400 MHz, MeOD) δ 8.05 (d, J = 8.1 Hz, 1H), 7.76 (dt, J = 14.7, 7.4 Hz, 2H), 7.60 (t, J = 7.5 Hz, 1H), 7.34 (d, J = 15.5 Hz, 1H), 7.09 (s, 1H), 6.99 (dd, J = 29.5, 8.2 Hz, 2H), 6.42 – 6.26 (m, 1H), 4.41 (d, J = 12.6 Hz, 1H), 4.28 (d, J = 5.2 Hz, 4H), 4.22 (t, J = 9.3 Hz, 1H), 4.07 (s, 1H), 3.95 (d, J = 6.9 Hz, 1H), 2.84 (s, 3H); HRMS (ESI) m/z calcd for $C_{19}H_{20}N_2O_4$ $(M+H)^+$ 341.1501, found 341.1502.

(*E*)-*N*-(1, 4-Benzodioxine-7-methyl)-*N*-methyl-3-(2-methoxylphenyl)prop-2-en-1-amine Hydrochloride (29).

Yield = 65%. **29** was synthesized by the general procedure of **11** given above as a white solid, m.p. 174-175 ºC. ^{1}H NMR (400 MHz, MeOD) δ 7.52 (d, J = 7.5 Hz, 1H), 7.31 (t, J = 7.8 Hz, 1H), 7.16 (d, J = 15.9 Hz, 1H), 7.05 (d, J = 1.2 Hz, 1H), 7.03-6.96 (m, 2H), 6.93 (dd, J = 7.9, 3.8 Hz, 2H), 6.42 – 6.26 (m, 1H), 4.34 (d, J = 13.0 Hz, 1H), 4.26 (s, 4H), 4.14 (d, J = 13.4 Hz, 1H), 4.07 (d, J = 7.5 Hz, 1H), 3.99 (dd, J = 13.1, 6.9 Hz, 1H), 3.87 (s, 3H), 2.77 (s, 3H); HRMS (ESI) m/z calcd for $C_{20}H_{23}NO_3$ $(M+H)^+$ 326.1756, found 326.1756.

(*E*)-*N*-(1, 4-Benzodioxine-7-methyl)-*N*-methyl-3-(3-fluorophenyl)prop-2-en-1-amine Hydrochloride (30).

Yield = 63%. **30** was synthesized by the general procedure of **11** given above as a white solid, m.p. 177-179 ºC. ^{1}H NMR (400 MHz, MeOD) δ 7.44-7.34 (m, 1H), 7.34-7.25 (m, 2H), 7.07 (d, J = 1.5 Hz, 1H), 7.05 (d, J = 1.7 Hz, 1H), 6.97 (d, J = 2.0 Hz, 1H), 6.92 (dd, J = 15.5, 12.1 Hz, 2H), 6.38 (d, J = 15.7 Hz, 1H), 4.36 (d, J = 13.0 Hz, 1H), 4.27 (s, 4H), 4.16 (d, J = 13.0 Hz, 1H), 4.01 (m, 1H), 3.86 (m, 1H), 2.78 (s, 3H); HRMS (ESI) m/z calcd for $C_{19}H_{20}FNO_2$ $(M+H)^+$ 314.1556, found 314.1555.

(*E*)-*N*-(1, 4-Benzodioxine-7-methyl)-*N*-methyl-3-(3-chlorophenyl)prop-2-en-1-amine

Hydrochloride (31).

Yield = 43%. **31** was synthesized by the general procedure of **11** given above as a white solid, m.p. 168-170 ºC. ^{1}H NMR (400 MHz, MeOD) δ 7.39 (dd, J = 13.9, 8.0 Hz, 1H), 7.34-7.26 (m, 2H), 7.06 (dd, J = 9.4, 1.6 Hz, 2H), 6.97 (dt, J = 17.8, 5.1 Hz, 2H), 6.90 (d, J = 15.8 Hz, 1H), 6.43-6.33 (m, 1H), 4.35 (s, 1H), 4.27 (s, 4H), 4.16 (d, J = 13.0 Hz, 1H), 4.02 (dd, J = 13.2, 7.1 Hz, 1H), 3.86 (dd, J = 13.1, 7.9 Hz, 1H), 2.78 (s, 3H); HRMS (ESI) m/z calcd for $C_{19}H_{20}ClNO_2$ $(M+H)^+$ 330.1261, found 330.1262.

(*E*)-*N*-(1,

4-Benzodioxine-7-methyl)-*N*-methyl-3-(3-methylphenyl)prop-2-en-1-amine

Hydrochloride (32).

Yield = 45%. **32** was synthesized by the general procedure of **11** given above as a white solid, m.p. 154-156 ºC. ^{1}H NMR (400 MHz, MeOD) δ 7.40 (m, 1H), 7.23-7.15(m, 3H), 7.05 (s, 1H), 7.00-6.79 (m, 4H), 6.29 (s, 1H), 4.35 (d, J = 11.8 Hz, 1H), 4.26 (,s 4H), 4.14 (d, J = 13.7 Hz, 1H), 3.99 (m, 1H), 3.83 (m, 1H), 2.77 (s, 3H), 2.28 (s, 3H); HRMS (ESI) m/z calcd for $C_{20}H_{23}NO_2$ $(M+H)^+$ 310.1807, found 310.1808.

(*E*)-*N*-(1,

4-Benzodioxine-7-methyl)-*N*-methyl-3-(3-trifluorophenyl)prop-2-en-1-amine

Hydrochloride (33).

Yield = 43%. **33** was synthesized by the general procedure of **11** given above as a white solid, m.p. 162-164 ºC. ^{1}H NMR (400 MHz, MeOD) δ 7.60 (d, J = 12.8 Hz, 2H), 7.50 (dt, J = 15.2, 7.7 Hz, 2H), 7.09-6.87 (m, 4H), 6.48-6.39 (m, 1H), 4.29 (d, J = 11.9 Hz, 1H), 4.24(s, 4H), 4.13 (d, J = 13.1 Hz, 1H), 3.99(d, J = 10.3 Hz, 1H), 3.92 (s, 1H), 2.78 (d, J = 25.6 Hz, 3H), HRMS (ESI) m/z calcd for $C_{20}H_{20}F_3NO_2$ $(M+H)^+$ 364.1524, found 364.1523.

(*E*)-*N*-(1,

4-Benzodioxine-7-methyl)-*N*-methyl-3-(3-nitrophenyl)prop-2-en-1-amine

Hydrochloride (34).

Yield = 39%. **34** was synthesized by the general procedure of **11** given above as a red solid, m.p. 182-184 ºC. ^{1}H NMR (400 MHz, MeOD) δ 8.10 (d, J = 7.6 Hz,1H), 7.75-7.65 (m, 2H), 7.37-7.28 (d, J = 8.4 Hz, 1H), 7.06 (s, 1H), 7.01-6.95 (m, 2H),

6.85 (s, 1H), 6.35-6.24 (m, 1H), 4.37 (d, *J* = 13.1 Hz, 1H), 4.26 (s, 4H), 4.16 (d, *J* = 12.9 Hz, 1H), 4.02 (m, 1H), 3.84 (m, 7.1 Hz, 1H), 2.78 (s, 3H); HRMS (ESI) m/z calcd for $C_{19}H_{20}N_2O_4$ $(M+H)^+$ 340.1423, found 340.1425.

(*E*)-*N*-(1, 4-Benzodioxine-7-methyl)-*N*-methyl-3-(3-methoxylphenyl)prop-2-en-1-amine Hydrochloride (35).

Yield = 52%. **35** was synthesized by the general procedure of **11** given above as a white solid, m.p. 169-171 ºC. ^{1}H NMR (400 MHz, MeOD) δ 7.56 (s, 1H), 7.43 (d, *J* = 7.1 Hz, 1H), 7.35 (d, *J* = 7.2 Hz, 2H), 7.05 (s, 1H), 7.01-6.93 (m, 2H), 6.85 (s, 1H), 6.45-6.30 (m, 1H), 4.36 (d, *J* = 12.7 Hz, 1H), 4.26 (s, 4H), 4.16 (d, *J* = 12.9 Hz, 1H), 4.02 (dd, *J* = 14.3, 8.3 Hz, 1H), 3.84 (dd, *J* = 20.6, 7.1 Hz, 1H), 3.31 (s, 3H), 2.78 (s, 3H); HRMS (ESI) m/z calcd for $C_{20}H_{23}NO_3$ $(M+H)^+$ 326.1756, found 326.1755.

(*E*)-*N*-(1, 4-Benzodioxine-7-methyl)-*N*-methyl-3-(2, 4-dichlorophenyl)prop-2-en-1-amine Hydrochloride (36).

Yield = 53%. **36** was synthesized by the general procedure of **11** given above as a yellow solid, m.p. 201-203 ºC. ^{1}H NMR (400 MHz, MeOD) δ 7.71 (d, *J* = 8.5 Hz, 1H), 7.52 (d, *J* = 2.1 Hz, 1H), 7.37 (dd, *J* = 8.5, 2.0 Hz, 1H), 7.22 (d, *J* = 15.8 Hz, 1H), 7.05 (d, *J* = 2.0 Hz, 1H), 6.97 (dt, *J* = 15.3, 5.2 Hz, 2H), 6.44-6.32 (m, 1H), 4.36 (d, *J* = 13.0 Hz, 1H), 4.29 – 4.24 (m, 4H), 4.17 (d, *J* = 13.0 Hz, 1H), 4.06 (dd, *J* = 13.5, 6.9 Hz, 1H), 3.91 (dd, *J* = 13.2, 7.8 Hz, 1H), 2.80 (s, 3H); HRMS (ESI) m/z calcd for $C_{19}H_{19}Cl_2NO_2$ $(M+H)^+$ 364.0871, found 364.0869.

(*E*)-*N*-(1, 4-Benzodioxine-7-methyl)-*N*-methyl-5-(4-trifluorophenyl)prop-2,4-dien-1-amine Hydrochloride (47).

Yield = 42%. **47** was synthesized by the general procedure of **11** given above as a white solid, m.p. 160-162 ºC. ^{1}H NMR (400 MHz, MeOD) δ 7.70-7.63 (m, 4H), 7.10 (dd, *J* = 15.7, 10.5 Hz, 1H), 7.04 (d, *J* = 2.0 Hz, 1H), 6.96 (dt, *J* = 13.9, 5.2 Hz, 2H), 6.83 (d, *J* = 15.7 Hz, 1H), 6.74 (dd, *J* = 15.1, 10.5 Hz, 1H), 6.00 (dt, *J* = 15.1, 7.6 Hz, 1H), 4.33 (d, *J* = 12.2 Hz, 1H), 4.27 (s, 4H), 4.13 (d, *J* = 11.7 Hz, 1H), 3.94 (d, *J* = 6.1 Hz, 1H), 3.81 (d, *J* = 7.7 Hz, 1H), 2.75 (s, 3H); ^{13}C NMR (101 MHz, DMSO) δ

147.23, 146.13, 143.43, 141.22, 135.33, 132.88, 130.55, 130.18, 129.32, 129.32 127.97, 127.97, 126.50, 126.08, 125.27, 122.33, 119.38, 64.79, 64.79, 57.55, 56.45, 37.78. HRMS (ESI) m/z calcd for $C_{22}H_{22}F_3NO_2$ $(M+H)^+$ 390.1681, found 390.1680.

(*E*)-*N*-(1, 4-Benzodioxine-7-methyl)-*N*-methyl-3-(2-fluoro-4-trifluorophenyl)prop-2-en-1-amine Hydrochloride (37).

Yield = 43%. **37** was synthesized by the general procedure of **11** given above as a white solid, m.p. 153-155 ºC. ^{1}H NMR (400 MHz, MeOD) δ 7.84 (t, J = 7.7 Hz, 1H), 7.58-7.48 (m, 2H), 7.16-6.89 (m, 4H), 6.64-6.51 (m, 1H), 4.37 (d, J = 12.9 Hz, 1H), 4.26 (s, 3H), 4.18 (d, J = 12.7 Hz, 1H), 4.08 (dd, J = 12.9, 6.9 Hz, 1H), 3.97-3.86 (m, 1H), 2.80 (s, 3H); HRMS (ESI) m/z calcd for $C_{20}H_{19}F_4NO_2$ $(M+H)^+$ 382.1430, found 382.1431.

(*E*)-*N*-(1, 4-Benzodioxine-7-methyl)-*N*-methyl-3-(3-fluoro-4-trifloromethylphenyl)prop-2-en-1-amine Hydrochloride (38).

Yield = 45%. **38** was synthesized by the general procedure of **11** given above as a white solid, m.p. 163-165 ºC. ^{1}H NMR (400 MHz, MeOD) δ 7.71 (d, J = 7.7 Hz, 1H), 7.56 (s, 1H), 7.47 (d, J = 7.9 Hz, 1H), 7.31 (d, J = 15.7 Hz, 2H), 7.06 (s, 1H), 6.96-6.91 (m, 2H), 6.35-6.23 (m, 1H), 4.38 (d, J = 12.9 Hz, 1H), 4.27 (s, 4H), 4.16 (s, 1H), 4.08-4.00 (m, 1H), 3.90 (dd, J = 12.8, 7.9 Hz, 1H), 2.82 (s, 3H); HRMS (ESI) m/z calcd for $C_{20}H_{19}F_4NO_2$ $(M+H)^+$ 382.1430, found 382.1432.

(*E*)-*N*-(1, 4-Benzodioxine-7-methyl)-*N*-methyl-3-(2-fluoro-4-methoxylphenyl)prop-2-en-1-amine Hydrochloride (39).

Yield = 47%. 39 was synthesized by the general procedure of **11** given above as a white solid, m.p. 178-180 ºC. ^{1}H NMR (400 MHz, MeOD) δ 7.57 (t, J = 8.7 Hz, 1H), 7.47 (d, J = 7.6 Hz, 1H), 7.43 (d, J = 8.6 Hz, 2H), 7.09 (s, 1H), 7.03 (d, J = 8.3 Hz, 1H), 6.92 (d, J = 8.3 Hz, 1H), 6.38-6.27 (m, 1H), 4.38 (d, J = 13.0 Hz, 1H), 4.26 (s, 4H), 4.18 (d, J = 13.0 Hz, 1H), 4.07-4.00 (m, 1H), 3.91-3.83 (m, 1H), 3.81 (s, 3H), 2.77 (s, 3H); HRMS (ESI) m/z calcd for $C_{20}H_{22}FNO_3$ $(M+H)^+$ 344.1662, found

344.1660.

(*E*)-*N*-(1, 4-Benzodioxine-7-methyl)-*N*-methyl-3-(3-fluoro-4-methoxylphenyl)prop-2-en-1-amine Hydrochloride (40).

Yield = 49%. **40** was synthesized by the general procedure of **11** given above as a white solid, m.p. 162-164 ºC. ^{1}H NMR (400 MHz, MeOD) δ 7.57 (d, J = 8.1 Hz, 1H), 7.48 (d, J = 7.5 Hz, 1H), 7.45 (d, J = 8.2 Hz, 2H), 7.03 (s, 1H), 6.94 (d, J = 5.1 Hz, 2H), 6.24-6.10 (m, 1H), 4.34 (d, J = 12.2 Hz, 1H), 4.27 (s, 5H), 4.12 (d, J = 12.3 Hz, 1H), 3.96 (s, 1H), 3.80 (s, 3H), 2.76 (s, 3H); HRMS (ESI) m/z calcd for $C_{20}H_{22}FNO_3$ $(M+H)^+$ 344.1662, found 344.1661.

(*E*)-*N*-(1, 4-Benzodioxine-7-methyl)-*N*-methylprop-2-en-1-amine Hydrochloride (41).

Yield = 48%. **41** was synthesized by the general procedure of **11** given above asas pale yellow oil. ^{1}H NMR (400 MHz, MeOD) δ 7.05 (s, 1H), 6.83-6.79 (m, 2H), 6.15 (dd, J = 14.9, 6.7 Hz, 1H), 5.83-5.68 (m, 1H), 4.26 (d, J = 5.4 Hz, 1H), 4.21 (s, 4H), 4.08 (dd, J = 13.8, 6.6 Hz, 1H), 3.82 (dd, J = 13.1, 7.6 Hz, 1H), 3.73 (dd, J = 13.2, 7.3 Hz, 1H), 2.82 (s, 3H); HRMS (ESI) m/z calcd for $C_{14}H_{19}NO_2$ $(M+H)^+$ 234.1494, found 234.1492.

(*E*)-*N*-(1, 4-Benzodioxine-7-methyl)-*N*-methyl-3-cyclopentylprop-2-en-1-amine Hydrochloride (42).

Yield = 42%. **42** was synthesized by the general procedure of **11** given above as pale yellow oil. ^{1}H NMR (400 MHz, MeOD) δ 6.75 (dd, J = 15.2, 9.5 Hz, 3H), 5.61 (dd, J = 15.2, 7.4 Hz, 1H), 5.55-5.40 (m, 1H), 4.21 (s, 4H), 3.38 (s, 2H), 2.95 (d, J = 6.7 Hz, 2H), 2.46 (dt, J = 15.7, 7.8 Hz, 1H), 2.14 (s, 3H), 1.79 (d, J = 6.8 Hz, 2H), 1.70-1.55 (m, 4H), 1.36-1.25 (m, 2H); HRMS (ESI) m/z calcd for $C_{18}H_{25}NO_2$ $(M+H)^+$ 288.1964, found 288.1963.

(*E*)-*N*-(1, 4-Benzodioxine-7-methyl)-*N*-methyl-3-cyclohexylprop-2-en-1-amine Hydrochloride (43).

Yield = 38%. **43** was synthesized by the general procedure of **11** given above as pale yellow oil. ^{1}H NMR (400 MHz, MeOD) δ 6.75 (dd, J = 15.2, 9.5 Hz, 3H), 5.67-5.56

(m, 1H), 5.47 (dt, *J* = 26.9, 9.3 Hz, 1H), 4.21 (s, 4H), 3.37 (s, 2H), 2.95 (d, *J* = 6.7 Hz, 2H), 2.55-2.37 (m, 1H), 2.23-2.08 (s, 3H), 2.09 (s, 1H), 2.03-1.95 (m, 2H), 1.77 (d, *J* = 10.6 Hz, 2H), 1.67 (m, 1H), 1.30 (d, *J* = 14.4 Hz, 2H), 1.18 (dd, *J* = 22.2, 10.8 Hz, 2H); HRMS (ESI) m/z calcd for $C_{19}H_{27}NO_2$ $(M+H)^+$ 302.2120, found 302.2119.

(*E*)-*N*-(1, 4-Benzodioxine-7-methyl)-*N*-methyl-3-(1-furanyl)prop-2-en-1-amine Hydrochloride (44).

Yield = 43%. **44** was synthesized by the general procedure of **11** given above as a white solid, m.p. 136-137 ºC. ^{1}H NMR (400 MHz, MeOD) δ 7.42 (s, 1H), 7.09-6.82 (m, 2H), 6.77 (s, 2H), 6.41 (d, *J* = 15.5 Hz, 1H), 6.28 (d, *J* = 3.2 Hz, 1H), 6.22-6.10 (m, 1H), 4.32-4.14 (m, 4H), 3.84 (m, 2H), 3.68 (d, *J* = 7.0 Hz, 2H), 2.80 (s, 3H); HRMS (ESI) m/z calcd for $C_{17}H_{19}NO_3$ $(M+H)^+$ 286.1443, found 286.1444.

(*E*)-*N*-(1, 4-Benzodioxine-7-methyl)-*N*-methyl-3-(1-thienyl)prop-2-en-1-amine Hydrochloride (45).

Yield = 41%. **45** was synthesized by the general procedure of **11** given above as a white solid, m.p. 152-154 ºC. ^{1}H NMR (400 MHz, MeOD) δ 7.56 (s, 1H), 7.11-6.89 (m, 3H), 6.75 (m, 1H), 6.50-6.32 (m , 2H), 6.16 (dt, *J* = 15.5, 7.6 Hz, 1H), 4.34 (d, *J* = 13.0 Hz, 1H), 4.31 (s, 4H), 4.20-4.10 (m, 1H), 4.07 – 3.93 (m, 1H), 3.76 (dd, *J* = 13.2, 8.2 Hz, 1H), 2.78(s, 3H); HRMS (ESI) m/z calcd for $C_{17}H_{19}NO_2S$ $(M+H)^+$ 302.1215, found 302.1214.

(*E*)-*N*-(1, 4-Benzodioxine-7-methyl)-*N*-methyl-3-(1-naphthyl)prop-2-en-1-amine Hydrochloride (46).

Yield = 39%. **46** was synthesized by the general procedure of **11** given above as a white solid, m.p. 152-154 ºC. ^{1}H NMR (400 MHz, MeOD) δ 7.92-7.84 (m, 2H), 7.78-7.70 (m, 2H), 7.60-7.54 (m, 2H), 7.50 (dd, *J* = 12.4, 4.6 Hz, 2H), 7.07 (s, 1H), 6.95 (d, *J* = 6.7 Hz, 2H), 6.45-6.29 (m, 1H), 4.41 (d, *J* = 12.9 Hz, 1H), 4.26 (s, 4H), 4.20 (d, *J* = 11.6 Hz, 1H), 4.12 (dd, *J* = 11.5, 6.4 Hz, 1H), 4.02-3.92 (m, 1H), 2.84 (s, 3H); HRMS (ESI) m/z calcd for $C_{23}H_{23}NO_2$ $(M+H)^+$ 346.1807, found 346.1807.

(*E*)-*N*-(1, 4-Benzodioxine-7-methyl)-*N*-methyl-7-(4-trifluorophenyl)prop-2,4,6-trien-1-amine Hydrochloride (48).

Yield = 35%. **48** was synthesized by the general procedure of **11** given above as a pale yellow solid, m.p. 162-164 ºC. ^{1}H NMR (400 MHz, MeOD) δ 7.68-7.56 (m, 4H), 7.14-7.04 (m, 1H), 7.02 (d, J = 1.3 Hz, 1H), 6.95 (d, J = 2.1 Hz, 2H), 6.76 (d, J = 15.6 Hz, 1H), 6.69-6.51 (m, 3H), 5.86 (dt, J = 15.1, 7.7 Hz, 1H), 4.31 (d, J = 13.0 Hz, 1H), 4.27 (s, 4H), 4.10 (d, J = 13.0 Hz, 1H), 3.92 (dd, J = 13.4, 7.3 Hz, 1H), 3.75 (dd, J = 13.4, 8.1 Hz, 1H), 2.73 (s, 3H); HRMS (ESI) m/z calcd for $C_{24}H_{24}F_3NO_2$ $(M+H)^+$ 416.1837, found 416.1835.

Biological

Pigment Inhibition Assay.

Newman bacteria were cultured in TSB (4 mL) medium at 37 ºC for 48 h with inhibitor compounds dissolved in DMSO and diluted to a set of concentrations, in duplicate. 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 USA300 LAC, USA400 MW, Mu50 and NRS271 strains were determined in the same way.

Bacterial Growth Assays of *S. aureus* Newman and MRSA Strains

47 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. 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 ºC for 4 h to sufficiently dissolve the 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 ºC overnight, and the OD600 was recorded every half an hour with a Synergy 2 (Biotek) plate reader following the manufacturer's instructions.

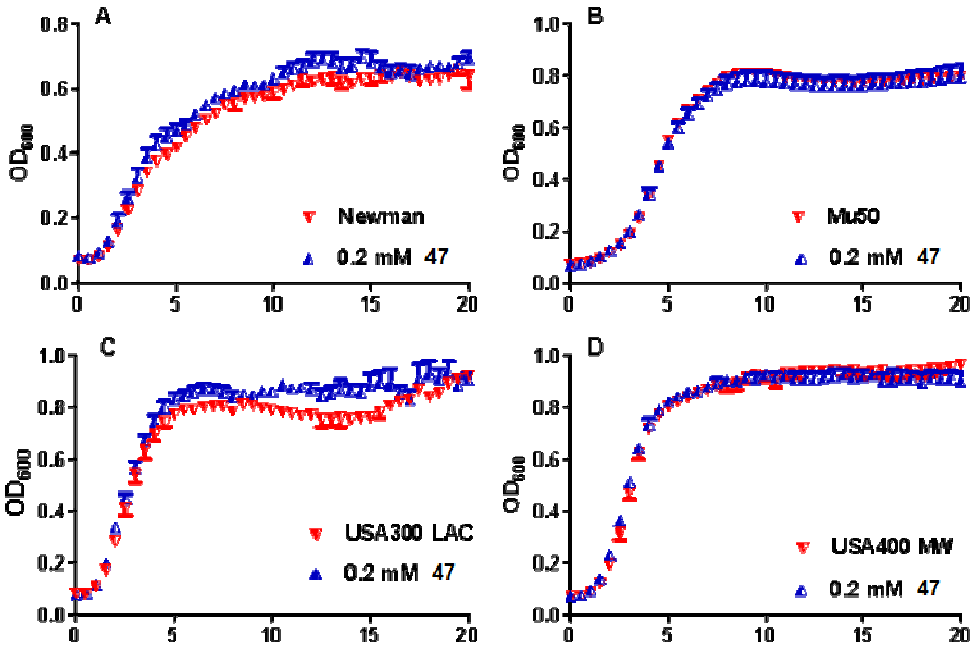


Figure S1. Effect of **47** on the bacterial growth of *S. aureus* Newman (A), USA400 MW2 (B), USA300 LAC (C), and Mu50 (D). Data are presented as means ± SD, n = 3 independent experiments.

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 compounds or mock (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 ºC 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.

Target CrtN Enzyme Determination of 47

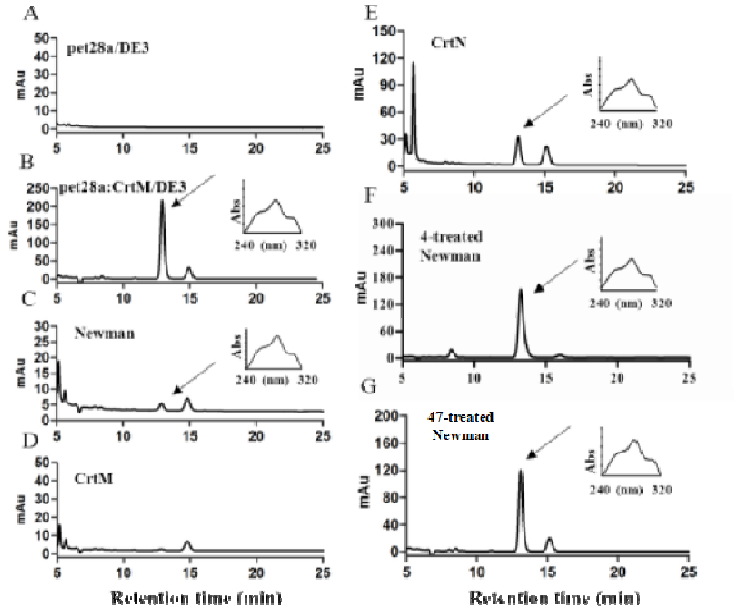


Figure S2. 47 treatment resulted in the inhibition of the *in vivo* function of CrtN. (A-G) HPLC chromatograms (absorption at 286 nm) of the carotenoid extracts from *E. coli* (A), *E. coli* expressing *S. aureus crtM* (B), wild-type *S. aureus* Newman (C), CrtM mutant (D), CrtN mutant (E), 4-treated wild-type *S. aureus* Newman (F), **47**-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.

Since the design and synthesis all revolved around the inhibition of enzyme CrtN, it was cautious to test 4,4'-diapophytoene, the product of CrtM and the substrate of CrtN, by the using of HPLC according to the previous protocol, and to demonstrate whether 1, 4-denzodioxan derivatives have the same target as another lead compound **4**.[20] As shown in *support information* Figure S2, similar HPLC peaks appeared in the expression of CrtM in *E. coli* (Figure S2B), wild-type *S. aureus* Newman (Figure S2C), CrtN mutants (Figure S2E), **4**-treated (Figure S2F) and **47**-treated (Figure S2G) wild-type *S. aureus* Newman, which belonged to 4,4'-diapophytoene with the same profiles of retention time (t=13.7 min) and absorbance (at 286 nm), whereas this peak faded away in the carotenoid extracts of the CrtM mutants (Figure S2D). Synthetically,

these data suggest that CrtN is the target of the denzodioxan derivatives.

Hydrogen Peroxide Killing and Human Whole Blood Killing.

For H_2O_2 killing assay, four strains, including Newman, USA300 LAC, USA400 WM2 and Mu50, were cultured in TSB and grown at 37 ℃ for 24 h with or without 1 μM derivative **47** or *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 1 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 ºC 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 and TSB plates in parallel 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 ºC for 6 h, and then the dilutions were plated on TSA agar and TSB plates in parallel for enumeration of the surviving CFUs.

***S. aureus* Systemic Infection Models.**

6-8-week-old female BALB/c mice (20 g weighed) were obtained from JSJ Lab Animal, Ltd. and housed under specified pathogen-free conditions. five groups (pretreatment), including two groups of **47** with different doses (45 mg/kg and 180 mg/kg), two groups of vancomycin or linezolid with the dose of 180 mg/kg and the mock group, in 12 intervals for 108 hours (4.5 d), which were infected by retro-orbital injection with a suspension of staphylococci 12 hours after the first intraperitoneal injection, while the other two groups (normal treatment), including **47** with two different doses (35 mg/kg or 140 mg/kg), in 12 intervals for 84 hours (3.5 d), were given the first administration to mice 6 hours after challenged with *S.aureus* strains. All the compounds were dissolved in sterile ddH_2O plus 10% castor oil ethoxylated. 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. Animals were sacrificed ~84 hours after infection. Hearts and livers were aseptically removed 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 agar and TSB plates in parallel for the enumeration of CFUs. The statistical significance was determined by the Mann-Whitney Test (two-tailed).

Anti-fungal Assays.

In vitro antifungal activity was determined by measuring the minimal inhibitory concentrations using the broth microdilution recommended by National Committee for Clinical Laboratory Standards (NCCLS). *Microsporum gypseum*, *Trichophyton rubrum* and *Tinea barbae* as common pathogenic fungi were selected as tested fugal strains incubated with serially diluted test compounds in 96-well microtest plates at 28 °C for 7 days. The MIC value was defined as the lowest concentration of test compounds that resulted in a culture with turbidity less than or equal to 80% inhibition when compared with the growth of the control.

Table S2. Antifungal Activity of Analog **47**

compd	antifungal activity MIC (μg/mL)		
	Trichophyton rubrum	*Microsporum gypseum*	*Tinea barbae*
47	8	8	16
ketoconazole	0.25	0.25	16
voriconazole	0.03	0.25	0.125
fluconazole	0.5	8	2

hERG Cardiac Toxicity Assay

Cell Culture and Cell Requirements

A CHO cell line stably transfected with hERG cDNA and expressing hERG channels was applied to this study. The cells were incubated in medium containing the following components: Ham's F12, 10 % (v/v) heat inactivated FBS, 100 μg/ml Hygromycin B and 100 μg/mL Geneticin.

The cells used in QPatch study should satisfy the standard as follows: First, under microscopy examination, single and isolated should be maintained in the majority of cells in suspension; Second, the cells viability should surpass 95 %, with only a few debris and cell clumps (which may block the holes in QPlate during whole-cell clamp recording); Third, before applying to the QPatch stir chamber, cell density should be ranged within 3-8 × 10^6 cells/mL in the final suspension. Fourth, after leaving CO_2 incubator, cells should be maintained in serum-free medium buffered HEPES. To more important, cells in such conditions can only be used for four hours to record after harvesting.

Recording System

Whole-cell recordings were carried out using automated QPatch (Sophion). The cells were voltage clamped at a holding potential of -80 mV. Activated the hERG current by depolarizing at +20 mV for 5 seconds, and then took the current back to -50 mV for 5 seconds to remove the inactivation and observe the deactivating tail current. Subsequently the hERG current amplitude was determined by using the maximum amount of tail current size. The composition of the solutions used for the electrophysiological recordings were shown in Table S3.

Table S3. Composition of Internal and External Solutions Used in hERG QPatch Studies[a].

Reagents	External Solution (mM)	Internal Solution (mM)
$CaCl_2$	2	5.374
$MgCl_2$	1	1.75
KCl	4	120

NaCl	145	-
Glucose	10	-
HEPES	10	10
EGTA	-	5
Na-ATP	-	4
pH	7.4 (adjusted with NaOH), osmolarity ~305 mOsm	7.25 (adjusted with KOH), osmolarity ~290 mOsm

[a] solutions recommended by Sophion.

Automated QPatch Procedures

After achieving break-in (whole-cell) configuration, the cells were recorded for 120 seconds to evaluate current stability. The voltage protocol depicted above was then applied to the cells every 20 seconds throughout the whole procedure. Only stable cells with recording parameters above threshold enabled to enter the drug addiction procedure.

External solution containing 0.2 % DMSO (vehicle) was used for the cells to establish the baseline. After keeping the current stable for 3 minutes, compounds (20 mM stocked in DMSO) was applied after which was initially diluted with DMSO (Final DMSO concentration was 0.2%), and then serial-diluted with external solution to obtain the final μM ranges to applied to the QPatch test. Afterwards, the above solution of compounds (43.3, 14.4, 4.8, 1.6, 0.53, 0.18, 0.059, 0.020, and 0.0066 μM) was added and the cells were kept in the test solution until the compound's effect reached a steady state or for a maximum of 3 minutes. Washout using external solution might be performed until the recovery of the current reached a steady state. Positive control dofetilide (dosing started from 8.6 μM) was applied to the test to detect the same batch of cells used for test compounds to make sure the normal response and the good quality of the cells.

Resistant Level Detection of MRSAs

Mueller-Hinton II Broth(MHB, cation-adjusted) was used to evaluate MIC values of compounds against *Staphylococcus aureus* Mu50 strain , *Staphylococcus aureus* NRS271 strain, *Staphylococcus aureus* USA300 strain, *Staphylococcus aureus* USA400 strain,

Penicillin, Linezolid and vancomycin were dissolved in MHB to 170.67 μg/mL as the initial concentration (150 μL in first well). Further 1:2 serial dilutions were performed by addition of culture broth. 150 μL of each dilution was distributed in 96-well plates, as well as sterility controls and growth controls (containing culture broth, without drugs). Each test and growth control well was inoculated with 50 μL of bacterial suspension (about 1.5×10^5 CFU/well) and the final concentration of compounds would be 128, 64, 32, 16, 8, 4, 2, 1, 0.5, 0.25 μg/mL. The 96-well plates were incubated at 37ºC for 24 h. MIC values of these compounds was defined as the lowest concentration to inhibit the bacterial growth significantly (identify by OD_{600} values read from Synergy 2 Multi-Mode Microplate Reader, Biotek).

Table S4. MIC value of three MRSA vs Penicillin, Linezolid, Vancomycin[a].

	MIC (μg/mL) / Resistance (**S**, **I**, **R**)			
	USA300	USA400	Mu50	NRS271
Penicillin (**S** ≤ 0.12; **R** ≥ 0.25)	8 / **R**	16 / **R**	16 / **R**	8 / **R**
Linezolid (**S** ≤ 4; **R** ≥ 8)	1 / **S**	1 / **S**	0.5 / **S**	16~32 / **R**
vancomycin (**S** ≤ 2; **I**: 4 ~ 8; **R** ≥16)	< 0.25 / **S**	< 0.25/**S**	4 / **I**	<0.25 / **S**

[a]the broth microdilution condition and MIC breakpoints according to: Performance Standards for Antimicrobial Susceptibility Testing (CLSA, 2017, 27th), **S**=susceptible, **R**=resistant, **I**=intermediate.

Cytotoxicity of HEK-293T and HepG2

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 5 000 cells per well and incubated at 37 □ 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 **47** and controls were treated with the cells for 72 h at 37 □ 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.

Liver Microsome Metabolism

The metabolism assay of **47** was tested by using liver microsome, which were obtained from BD Gentest (10722), and NADPH. The incubation and sampling was performed according to the following: make a master-mix containing microsome, phosphate buffer, ultra-pure water (generated from Milli-Q ultra-pure water system) and NADPH (reduced form of nicotinamide-adenine dinucleotide phosphate) solution as demonstrated in Table S5. Then the master solution was pre-warmed in the 37 □ for 2 min. Afterwards the above master solution was added approximate volume of test compounds or control solution (midazolam as positive control and H_2O as negative control) to initiate the reaction according. The final concentration of test compound or midazolam (positive control) in the reaction system was 2 μM.

Table S5. Master-mixture solution preparation.

Buffer	Stock Conc.	Each Reaction (μL)	Final Conc.
NADPH	10 mg/mL	5	1 mg/mL
Microsome	5 mg/mL	5	0.5 mg/ml
Phosphate buffer	0.2M	25	0.1M
Ultra-pure water	-	14.5	-

Sampling

At 0, 5, 15, 30, 45 and 60 minutes for analyte, 50 μL of reaction solution in duplicate was pippeted into a new eppendorff tube, and then 3 fold of volume of cold methanol containing internal standard was added immediately to stop reaction. After centrifugation at 16,000 rpm for 5 minutes to precipitate protein, an aliquot of 100 μL of the supernatant was removed into an autosampler vial for injection.

Sample analysis

Sample analysis was performed by LC-MS/MS using a Waters ACQUITY UPLC chromatography system with a ACQUITY UPLC BEH C18 column (1.7 μm, 50 mm * 2.10 mm). The mobile phase consisted of mixtures of 0.1% formic acid in methanol and 0.1% formic acid in water, which was run in gradient mode at a flow rate of 0.5 mL/min. Mass spectra was detected with an API5500 triple quadruple

equipped with an ESI source. Internal standards were used to track the response of analytes in plasma samples.