Green and Facile Assembly of Diverse Fused N-Heterocycles using
Gold-Catalyzed Cascade Reactions in Water

Supplementary Materials

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Table S1. Survey of the solvents on the yield of product SF1a $^{a}$.

![Reaction scheme]

| Entry | Solvent | Yield (%) $^{b}$ |
|-------|---------|------------------|
| 1     | H₂O     | 91               |
| 2     | Toluene | 86               |
| 3     | Xylene  | 88               |
| 4     | DCE     | 90               |
| 5     | THF     | 75               |
| 6     | CH₃CN   | 63               |
| 7     | DMSO    | 41               |
| 8     | MeOH    | 35               |

$^{a}$ Reaction conditions: 4-pentynoic acid 1a (0.6 mmol), tryptamine 2a (0.5 mmol), AuPPh₃Cl/AgSbF₆ (0.005 mmol), solvent (4.0 ml), 120 °C, 24 h. $^{b}$ Yield refers to isolated yield.
NMR and ESI(+)MS spectrum of SF5a, [D]_n-SF5a, SF5b, [D]_n-SF5b, SF1a, [D]_n-SF1a

**Figure S1** $^1$H NMR spectrum of SF5a in methanol-$d_4$.

**Figure S2** $^{13}$C NMR spectrum of SF5a in methanol-$d_4$. 
Figure S3 HSQC spectrum of SF5a in methanol-\(d_4\).

Figure S4 HMBC spectrum of SF5a in methanol-\(d_4\).
Figure S5 $^1$H-$^1$H COSY spectrum of SF5a in methanol-$d_4$.

Figure S6 ESI(+)MS spectrum of SF5a.
Figure S7 ¹H NMR spectrum of [D]₉-SF₅a in methanol-d₄.
Figure S8 ESI(+)MS spectrum of \([\text{D}]_9\text{SF5a}\).
Figure S9 $^1$H NMR spectrum of SF5b in dimethyl sulfoxide-$d_6$.

Figure S10 $^1$H NMR spectrum of [D]$_6$-SF5b in dimethyl sulfoxide-$d_6$. 
Figure S11 $^1$H NMR spectrum of SF1a in dimethyl sulfoxide-$d_6$.

Figure S12 $^1$H NMR spectrum of [D]$_n$-SF1a in dimethyl sulfoxide-$d_6$. 
**Antibacterial bioassay**

**Bacterial strains, culture and growth conditions, and sample preparation**

_**Staphylococcus aureus** (S. aureus)_ was used in this study and cultured at 37 °C in Mueller-Hinton broth (MH broth). 5 mg compounds were dissolved in 100 μl DMSO, and the resulting solution was used as the sample stock. All the experiments were repeated at least three times.

**Preliminary screening of antibacterial activities**

The preliminary antibacterial activities against _S. aureus_ strain were investigated in 96-well plates, and DMSO was used as the blank control. Briefly, _S. aureus_ strain was seeded into 200 μl MH broth per well to make a density of 1x10^5 CFU/ml. Subsequently, an aliquot of the sample stock was added to make a final compound concentration of 100 μg/ml. After that, the optical density (OD) of the mixture in each well was measured again and recorded as OD_0_. Then the plate was incubated at 37 °C for 24 h, after that, the OD of the mixture in each well was immediately measured again and recorded as OD_24_. ∆OD (∆OD = OD_24_ − OD_0_) was calculated and used to evaluate the antibacterial potency of the compounds. Finally, compounds with ∆OD lower than 0.1 were selected out for further study.

**Minimal inhibitory concentration (MIC) study**

The determination of minimal inhibitory concentration (MIC) of tested compounds was carried out in 96-well plates with DMSO as the blank control. Briefly, _S. aureus_ strain was seeded into 200 μl MH broth per well to make a density of 1x10^5 CFU/ml. Subsequently, an aliquot of the sample stock was added to make 5 final compound concentrations (5 μg/ml, 10 μg/ml, 25 μg/ml, 50 μg/ml, 100 μg/ml and 200 μg/ml). After that, the OD of the mixture in each well at 600 nm wavelength was immediately measured by a spectrometer, and recorded as OD_0_. Then the plates were incubated at 37 °C for 24 h, after that, the OD of the mixture in each well was immediately measured again and recorded as OD_24_. ∆OD (∆OD = OD_24_ − OD_0_) was calculated and used to determine the MIC_90_. MIC_90_ was determined as the lowest concentration that inhibited 90% bacteria growth as compared with DMSO control group.

**Time-Kill Assays**

Time-kill assays were performed in 96-well plates, and DMSO was used as the blank control. Briefly, _S. aureus_ strain was seeded into 200 μl MH broth per well to make a density of 1x10^5 CFU/ml. Subsequently, an aliquot of the sample stock was added to make 5 final compound concentrations (5 μg/ml, 10 μg/ml, 25 μg/ml, 50 μg/ml, 100 μg/ml and 200 μg/ml). After that, the OD of the mixture in each well at 600 nm wavelength was instantly measured by a spectrometer, and recorded as OD_0_. Then the plate was incubated at 37 °C for 2 h, 4 h, 6 h, 8 h, 10 h, 12 h and 24 h, after that, the OD of the mixture in each well was immediately measured again and recorded as OD_k. ∆OD (∆OD = OD_k − OD_0) was calculated and used to draw the time-kill curves.

**Colony-forming units (CFU) study**

At the end of the time-kill assays, the plates were used for CFU study. Concisely, parallel wells were randomly selected and diluted by 10^5 times with MH broth. Then 100 μl diluent was taken and spread on Mueller-Hinton agar. The agar plates were incubated at 37 °C for 24 h. After that, the agar plates were recorded.

**Statistical analysis**: Statistical calculations were processed with Origin Pro 7.5 and Excel 2016.
Antibacterial results and discussion

Preliminary screening results
Preliminary screening disclosed that 21 compounds from the library showed antibacterial activities against the growth of *S. aureus* strain at the concentration of 100 μg/ml (Figure S13), and five of them (compounds SF9d, SF29b, SF33, SF36 and SF41) showed good antibacterial activities, which were selected for further study.

Figure S13 Preliminary screening of antibacterial activities of compounds at 100 μg/ml.

Time-kill assays and colony-forming unit (CFU) studies of compounds SF9d, SF29b, SF33, SF36 and SF41
As shown in Figure S14-S24, time-kill assays and colony-forming units (CFU) studies were also conducted with compounds SF9d, SF29b, SF33, SF36 and SF41. Among them, compound SF36 displayed the most potent antibacterial activity against *S. aureus* strain. Time-kill assay showed that SF36 was bactericidal within 2-24 h at the concentration of 25 μg/ml, preventing bacterial growth of *S. aureus* strain completely (Figure 17). Colony-forming units (CFU) study of SF36 was also carried out (Figure 23). The results showed that the number of clones on the agar plate decreased significantly in a dose-dependent manner, and only few clone was observed at the concentration of 50 μg/ml, indicating the antibacterial potency of this compound intuitively.
Figure S14 Time-kill results of compound SF9d against *S. aureus* strain.

Figure S15 Time-kill results of compound SF29b against *S. aureus* strain.
Figure S16 Time-kill results of compound SF33 against *S. aureus* strain.

Figure S17 Time-kill results of compound SF36 against *S. aureus* strain.
**Figure S18** Time-kill results of compound SF41 against *S. aureus* strain.

**Figure S19** Time-kill results of DMSO against *S. aureus* strain.
Figure S20 CFU results of compound SF9d.

Figure S21 CFU results of compound SF29b.

Figure S22 CFU results of compound SF33.
Figure S23 CFU results of compound SF36.

Figure S24 CFU results of compound SF41.
Copies of $^1$H and $^{13}$C NMR spectra of new compounds

9-fluoro-12b-methyl-1,5,6,12b-tetrahydropyrrolo[2',1':3,4]pyrazino[1,2-a]indol-3(2H)-one (SF9d)
2-hexyl-14b-methyl-1,14b-dihydroindolo[1,2-a]pyrrolo[2,1-c]quinoxalin-3(2H)-one (SF14b)
2-hexyl-8,9-dimethoxy-10b-methyl-1,5,6,10b-tetrahydropyrrolo[2,1-\textit{a}]isoquinolin-3(2\textit{H})-one (SF24b)
2-hexyl-3a-methyl-3,3a-dihydro-1H-benzo[d]pyrrolo[2,1-b][1,3]oxazine-1,5(2H)-dione (SF35b)
11b-methyl-2,3,5,6,11,11b-hexahydro-1H-indolizino[8,7-b]indole (SF47)

Chemical Formula: C_{22}H_{23}N_2
Molecular Weight: 328.32
11c-methyl-2,3,5,6,7,11c-hexahydro-1H-indolizino[7,8-b]indole (SF50)

Chemical Formula: C_{15}H_{18}N_{2}
Molecular Weight: 228.32
14c-methyl-5,6,8,9,10,14c-hexahydroindolo[3',2':3,4]pyrido[2,1-α]isoquinoline (SF51)
15b-methyl-6,8,9,15b-tetrahydro-5H-indolo[2',1':3,4]pyrazino[2,1-a]isoquinoline (SF52)

Chemical Formula: C_{20}H_{19}N_{2}
Molecular Weight: 288.39
11b-methyl-4,5,7,11b-tetrahydro-3H-pyrrolo[3',2':3,4]pyrido[2,1-a]isoindole (SF53)
11b-methyl-4,5,7,11b-tetrahydrothieno[3′,2′:3,4]pyrido[2,1-a]isoindole (SF54)
11b-methyl-4,5,7,11b-tetrahydrothieno[2',3':3,4]pyrido[2,1-a]isoindole (SF55)