

# Bio-inspired synthesis yields a tricyclic indoline that selectively resensitizes methicillin-resistant *Staphylococcus aureus* (MRSA) to $\beta$ -lactam antibiotics

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The continuous emergence of resistant bacteria has become a major worldwide health threat. The current development of new antibacterials has lagged far behind. To discover reagents to fight against resistant bacteria, we initiated a chemical approach by synthesizing and screening a small molecule library, reminiscent of the polycyclic indole alkaloids. Indole alkaloids are a class of structurally diverse natural products, many of which were isolated from plants that have been used as traditional medicine for millennia. Specifically, we adapted an evolutionarily conserved biosynthetic strategy and developed a concise and unified diversity synthesis pathway. Using this pathway, we synthesized 120 polycyclic indolines that contain 26 distinct skeletons and a wide variety of functional groups. A tricyclic indoline, Of1, was discovered to selectively potentiate the activity of  $\beta$ -lactam antibiotics in multidrug-resistant methicillin-resistant *Staphylococcus aureus* (MRSA), but not in methicillin-sensitive *S. aureus*. In addition, we found that Of1 itself does not have antiproliferative activity but can resensitize several MRSA strains to the  $\beta$ -lactam antibiotics that are widely used in the clinic, such as an extended-spectrum  $\beta$ -lactam antibiotic amoxicillin/clavulanic acid and a first-generation cephalosporin cefazolin. These data suggest that Of1 is a unique selective resistance-modifying agent for  $\beta$ -lactam antibiotics, and it may be further developed to fight against resistant bacteria in the clinic.

Antibiotics are one of the most important and widely used medicines. However, the emergence of resistance to the antibiotics has become a global public health threat. Serious infection of multidrug-resistant microorganisms has caused considerable patient mortality and modality (1). For example, more people died from methicillin-resistant *Staphylococcus aureus* (MRSA) infection than those from HIV/AIDS, Parkinson disease, and homicide combined (2). *S. aureus* is the most common Gram-positive bacteria pathogen that can cause skin infection, respiratory disease, and food poisoning. There are two predominant resistance mechanisms in MRSA (3). Foremost, the *mecA* gene (4) encodes penicillin-binding protein 2a (PBP2a) that has low affinity to  $\beta$ -lactam antibiotics such as methicillin, allowing sufficient peptidoglycan cross-linking in the presence of  $\beta$ -lactam antibiotics. The second is the *blaZ* gene (5), which encodes  $\beta$ -lactamases that chemically deactivate  $\beta$ -lactam antibiotics. The pharmaceutical industry has been developing structural analogs of  $\beta$ -lactam antibiotics that have higher affinity to PBP2a and lower activity to  $\beta$ -lactamases. This strategy has kept up with the emergence of new resistant MRSA strains until recently. However, there are not enough analogs in the current antibiotic pipeline to combat current and future resistance emergence (6, 7).

Another successful strategy is to use resistance-modifying agents (RMAs) in combination with antibiotics. RMAs are highly favorable because they can increase the life span of the antibiotics that are currently used in the clinics, which have already been optimized for toxicity and large-scale production. For example, clavulanic acid is a serine-dependent  $\beta$ -lactamase inhibitor from *Streptomyces clavuligerus* (8). Its use in combination with amoxicillin restores the efficacy of amoxicillin against bacteria producing  $\beta$ -lactamases, and this combination has become one of the most prescribed antibiotics

in the United States. Encouraged by the discovery of clavulanic acid, numerous efforts have attempted to discover novel RMAs from natural sources, such as membrane permeabilizing agents and inhibitors of efflux pumps (9). However, the only RMAs that have been proven clinically useful are  $\beta$ -lactamase inhibitors. Recently, there are a number of reports that showed plant extracts from a variety of different species can potentiate the activity of  $\beta$ -lactam antibiotics (10). However, the discovery of the active compounds has been very difficult (11). This challenge is due to the chemical complexity of plant extracts, the lack of standardization, difficulties in access and supply, and the inherent slowness and costs of working with natural products. Only a few plant natural products with RMA activity have been characterized, such as epigallocatechin gallate (i.e., EGCG, a flavonoid from green tea) (12) and reserpine (i.e., a polycyclic indole alkaloid from the root of an Indian medical plant; Fig. 1A) (13). Herein, we describe our recent discovery of a highly selective RMA for  $\beta$ -lactam antibiotics against MRSA from a polycyclic indole alkaloid library.

Natural indole alkaloids represent a large and highly diverse family of nitrogen-containing compounds (14). Most of these are secondary metabolites of medical plants that have been used as traditional medicine for millennia. Many have been used to treat diseases, such as hypertension (reserpine and ajmaline; Fig. 1A) (15, 16) and cancer (vinblastine; Fig. 1A) (17). The broad structural and pharmaceutical properties of indole alkaloids make this family of natural products ideal targets for synthetic efforts. Several synthetic pathways have also been developed for the synthesis of indole alkaloid libraries (18–24). However, systematic and efficient approaches toward highly diverse polycyclic indoline-containing skeletons still remain a main challenge in diversity-oriented synthesis (25, 26).

Inspired by their bioactivity and structural features, we designed a diversity synthesis pathway of a polycyclic indoline alkaloid library, based on a common biosynthetic strategy of natural products (27), such as indole alkaloids (Fig. 1A), polyketides (e.g., erythromycin A) (28), terpenoids (e.g., taxol) (29), and non-ribosomal peptides (e.g., vancomycin) (30). Specifically, as shown in Fig. 1B, we planned to assemble an alkynyl imine **M**, an activating agent Z<sup>1</sup>X (**1**), and an aryl hydrazine (**2**) using a one-pot three-component reaction that we recently developed (31). (Bold terms represent the whole classes of molecules; terms not in bold font are individual compounds.) Based on our previous studies on gold

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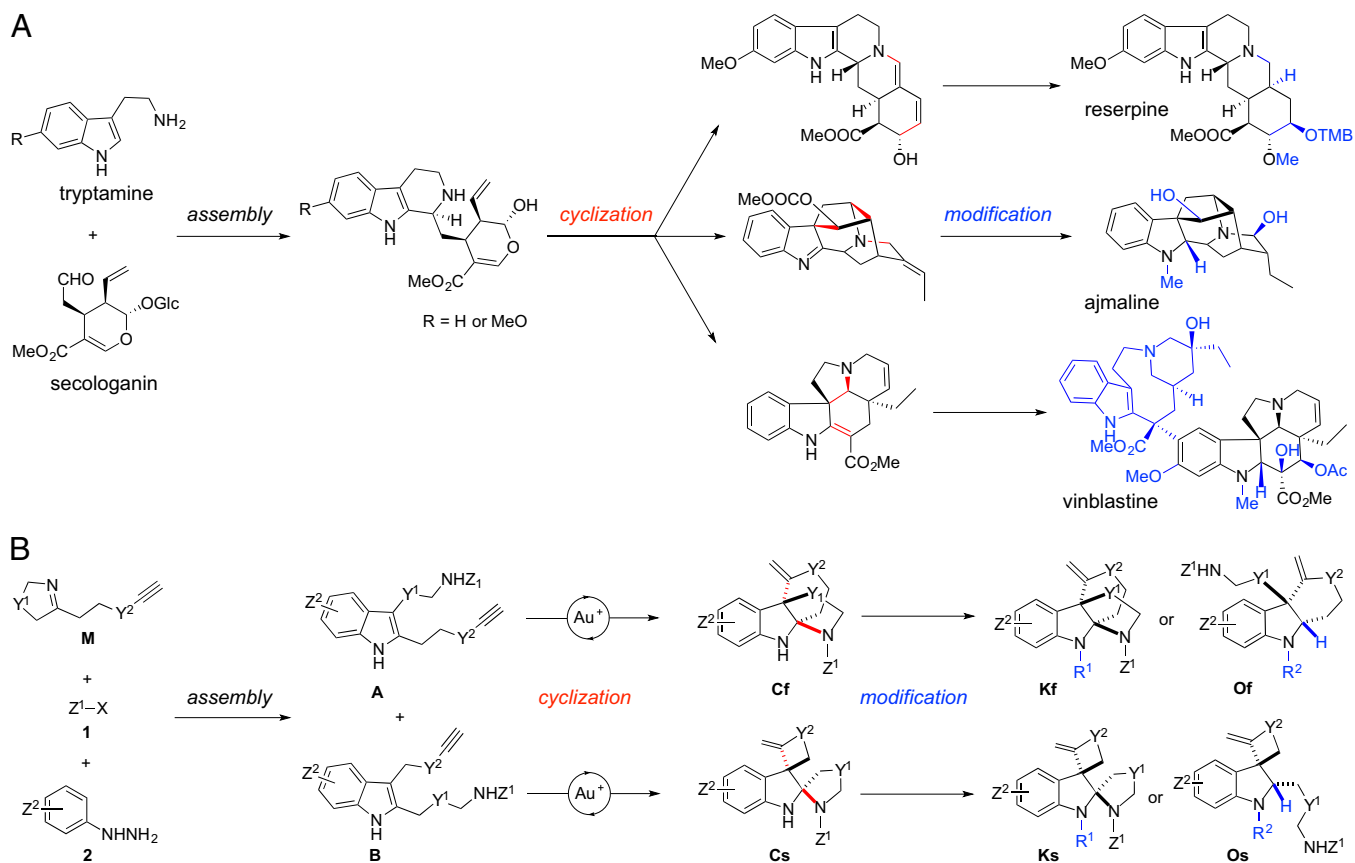
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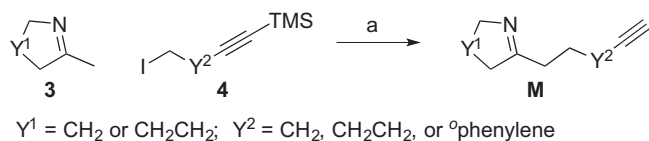


**Fig. 1.** Synthetic strategy for representative polycyclic indole alkaloids and our synthetic library. (A) Biosynthetic strategy for representative indole alkaloids. (B) Synthetic plan for a polycyclic indoline alkaloid library. Bonds in red are formed in the cyclization phase, and functional groups in blue are installed in the modification phase.

catalysis (32, 33), we envisioned that the resulting two regioisomeric indoles (**A** and **B**) could undergo gold(I)-catalyzed tandem cyclization reactions to form a fused (**Cf**) or spiro- (**Cs**) tetracyclic indoline, respectively (34). These indoline products are poised for further modifications, such as alkylations of the aniline nitrogen to afford **Kf** and **Ks**, or tandem ring-opening reduction-reductive aminations to produce **Of** and **Os**. We envisioned that a highly diverse collection of polycyclic indolines may be accessed by varying the linkers  $Y^1$  and  $Y^2$  of alkynyl imines **M**.

## Results and Discussion

**Preparation of Building Blocks M.** The alkynyl imine building blocks **M** were readily synthesized by alkylations of cyclic imine **3** with iodides **4** (Fig. 2) under basic conditions followed by desilylation. Only a single purification using basic alumina is required for each alkynyl imine **M**. Using this general procedure, we obtained six alkynyl imines (**M1**–**6**) in good yields (60–91%), each on multigram scale.

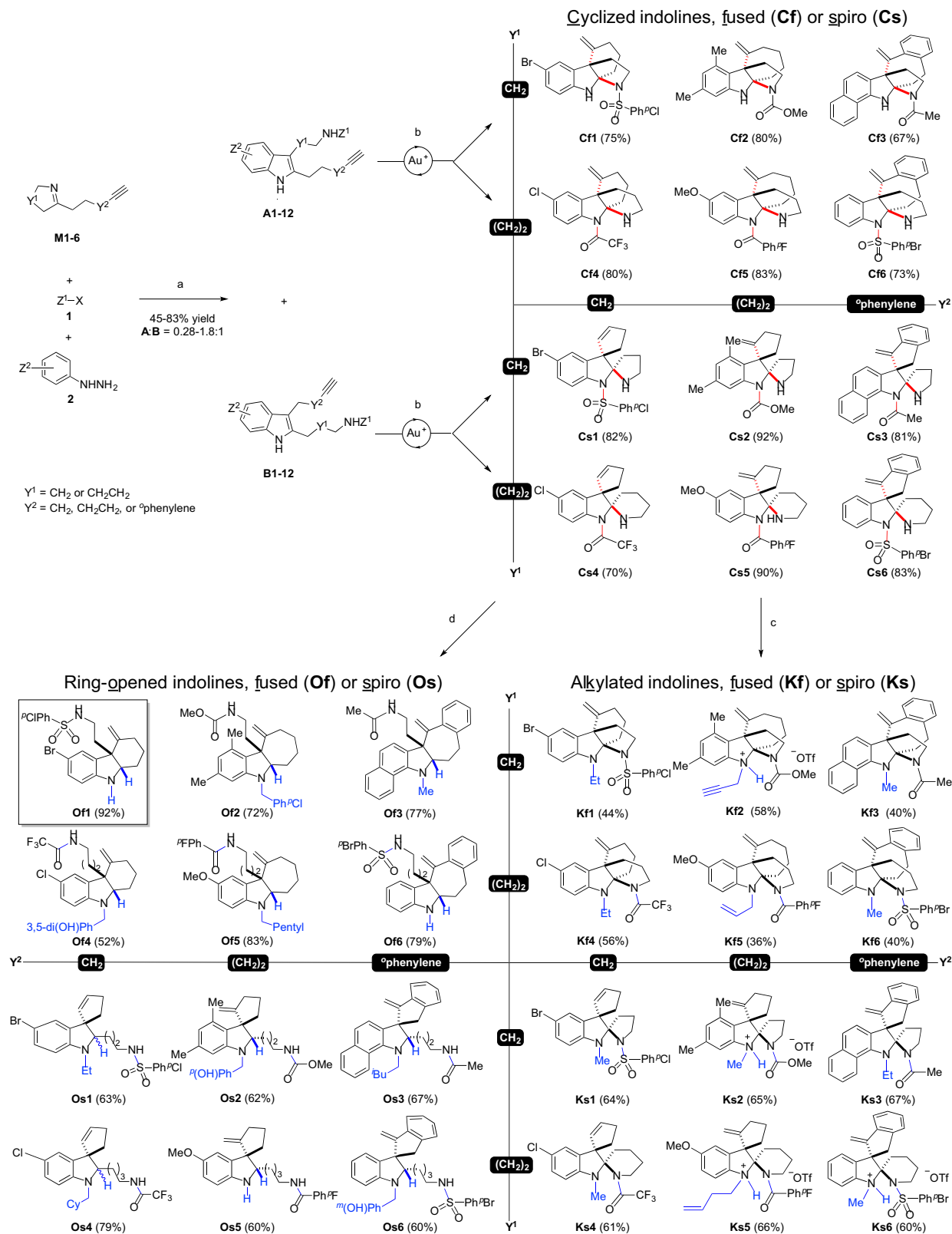


**Fig. 2.** Preparation of alkynyl imine building blocks. Conditions and reagents: (a) (i) LDA, **4**, THF,  $-78^\circ\text{C}$  to  $23^\circ\text{C}$ , 12 h; (ii) TBAF, THF,  $23^\circ\text{C}$ , 10 min, 60–91%. LDA, lithium diisopropylamide; THF, tetrahydrofuran; TBAF, tetrabutylammonium fluoride.

**Assembly of Building Blocks.** With these six alkynyl imines in hand, we next subjected them to the one-pot three-component assembly reactions. Because the resulting two regioisomeric indole products were expected to provide distinct ring skeletons on cyclizations, we slightly modified the reaction conditions and used dimethyl formamide (DMF) as the solvent to obtain moderate selectivity of regioisomers **A** and **B**. Under these modified conditions, all six alkynyl imines were converted to the corresponding indoles in moderate to good yields (45–83%; Table 1). The two regioisomers **A** and **B** from each assembly reaction were separable by silica gel chromatography. This assembly reaction showed excellent compatibility with a wide range of activating

**Table 1.** One-pot synthesis of alkynyl indoles **A** and **B**

Entry	<b>M</b>	$Y^1$	$Y^2$	$Z^1X$	$Z^2$	Yield (%)	<b>A</b> : <b>B</b>
1	<b>M1</b>	$CH_2$	$CH_2$	$^pClPhSO_2Cl$	4-Br	49	0.56:1
2	<b>M2</b>	$CH_2$	$CH_2CH_2$	MeOCOCI	3,5-diMe	55	1.8:1
3	<b>M3</b>	$CH_2$	$\textit{o}$ -phenylene	AcCl	$\textit{o}$ -phenylene	60	1.1:1
4	<b>M4</b>	$CH_2CH_2$	$CH_2$	$(CF_3CO)_2O$	4-Cl	45	0.77:1
5	<b>M5</b>	$CH_2CH_2$	$CH_2CH_2$	$^pFPhCOCl$	4-OMe	55	1.3:1
6	<b>M6</b>	$CH_2CH_2$	$\textit{o}$ -phenylene	$^pBrPhSO_2Cl$	H	54	0.5:1
7	<b>M1</b>	$CH_2$	$CH_2$	$^pClPhCOCl$	2-OMe	60	1:1
8	<b>M2</b>	$CH_2$	$CH_2CH_2$	$^pClPhSO_2Cl$	H	83	0.4:1
9	<b>M3</b>	$CH_2$	$\textit{o}$ -phenylene	MeOCOCI	4-OMe	55	1.8:1
10	<b>M4</b>	$CH_2CH_2$	$CH_2$	$(CF_3CO)_2O$	4-Me	70	1:1
11	<b>M5</b>	$CH_2CH_2$	$CH_2CH_2$	$^pFPhCOCl$	$\textit{o}$ -phenylene	60	1.3:1
12	<b>M6</b>	$CH_2CH_2$	$\textit{o}$ -phenylene	$^pBrPhSO_2Cl$	3,5-diMe	65	0.28:1



**Fig. 3.** Bio-inspired synthesis of polycyclic indolines. Numbers in parentheses are isolated yields. Conditions and reagents: (a) **1**, DMAP, 23 °C, 0.5 h, DMF; **M**, 2–12 h; TsOH·H<sub>2</sub>O, 23–80 °C, 24 h, 45–83%; (b) Ph<sub>3</sub>PAuNTf<sub>2</sub>, 50 °C, toluene, 1–12 h, 67–95%; (c) R<sup>1</sup>OTf, DCM, 23 °C, 2–12 h, 30–92%; (d) AcOH, NaBH<sub>3</sub>CN, MeOH, 0 °C, 0.5 h, and then aldehyde, 0–23 °C, 2–12 h, 35–99%. DMAP, 4-dimethylaminopyridine; DMF, *N,N*-dimethylformamide; TsOH·H<sub>2</sub>O, *p*-toluenesulfonic acid monohydrate; Ph<sub>3</sub>PAuNTf<sub>2</sub>, [bis(trifluoromethanesulfonyl)imidate]-(triphenylphosphine)gold(I); OTf, trifluoromethanesulfonate; DCM, dichloromethane; AcOH, acetic acid; NaBH<sub>3</sub>CN, sodium cyanoborohydride; MeOH, methanol; TMS, trimethylsilyl; Ph, phenyl; <sup>i</sup>Bu, isobutyl; Cy, cyclohexanyl; Me, methyl; Et, ethyl. Full details are in *SI Appendix, SI Methods*.

**Table 2. Of1 selectively potentiates  $\beta$ -lactam antibiotics in multidrug-resistant MRSA**

Compound	MIC ( $\mu\text{g/mL}$ )	MIC (+Of1)* ( $\mu\text{g/mL}$ )	Fold of potentiation	Sensitive range <sup>†</sup> ( $\mu\text{g/mL}$ )
Of1	>128	—	—	—
Methicillin	128	8	16	$\leq 8$
Oxacillin	64	0.5	128	$\leq 2$
Amox/clav	32/16	4/2	8	$\leq 4/2$
Meropenem	4	0.25	16	$\leq 4$
Imipenem	8	1	8	$\leq 4$
Cephalexin	256	16	16	$\leq 8$
Cefazolin	128	4	32	$\leq 8$
Rifampicin	2	1	2	$\leq 1$
Tetracycline	64	64	1	$\leq 4$
Ciprofloxacin	8	8	1	$\leq 1$
Azithromycin	>256	>256	—	$\leq 2$
Erythromycin	>256	>256	—	$\leq 0.5$
Clindamycin	>256	>256	—	$\leq 0.5$
Streptomycin	>256	>256	—	—
Vancomycin	1	1	1	$\leq 2$
Linezolid	2	2	1	$\leq 4$

\*MIC value in the presence of 20  $\mu\text{M}$  Of1.

<sup>†</sup>Values obtained from Clinical Laboratory Standards Institute (CLSI) Performance Standards for Antimicrobial Testing; 17th informational supplement (36).

reagents  $Z^1X$ , such as sulfonyl chlorides, acyl chlorides, anhydrides, and chloroformates. In addition, a variety of aryl hydrazines substituted with either electron-donating groups (e.g., methoxy and methyl) or electron-withdrawing groups (e.g., bromo and chloro) were well tolerated in the reaction conditions. For each alkyne imine, we chose two different combinations of activating groups and hydrazines in the assembly reactions. As such, we ran 12 reactions and prepared 24 highly functionalized alkyne indoles A1–12 and B1–12 (Table 1).

#### Gold(I)-Catalyzed Tandem Cyclizations of Alkyne Indoles to Afford Polycyclic Indolines.

We used a commercially available gold(I) catalyst,  $\text{Ph}_3\text{PAuNTf}_2$ , for the cyclization step to simplify our library production. When subjected to the standard gold catalysis conditions (5 mol%  $\text{Ph}_3\text{PAuNTf}_2$ , toluene, 50  $^\circ\text{C}$ ), each of the 24 alkyne indoles underwent the expected tandem cyclization to produce the corresponding indolines Cf1–12 or Cs1–12 as a single regio- and diastereomer in good to excellent yields (see representative examples in Fig. 3). Nearly all reactions were initiated by the *exo-dig* cyclizations followed by the intramolecular nucleophilic attack of the iminium ion by the nitrogen nucleophiles. The only exceptions were Cs1 and Cs4, which reacted in an *endo-dig* fashion due to the severe ring strain of an *exo-dig* cyclization if they cyclized in the *exo* mode (i.e., 5-*endo-dig* cyclization is favored over 4-*exo-dig* cyclization) (35). Hence, over a dozen distinct polycyclic indoline skeletons were constructed under the same reaction condition in this step. In most cases, the  $Z^1$  groups appeared to migrate to the aniline nitrogens spontaneously after cyclizations, presumably to avoid the severe steric interactions.

**Modifications of the Cyclized Indolines.** To further modify the cyclized indolines, we developed two general methods. When treated with alkyl triflates, the  $Z^1$  groups migrated back to the amine nitrogens and the aniline nitrogens were alkylated to provide **Kf** and **Ks** (Fig. 3). This phenomenon is likely due to the poor accessibility of the amine nitrogens to the alkylating agents. In these transformations, the ring skeletons of the indolines were maintained. However, the migrations of the  $Z^1$  groups caused significant changes of their pharmacological properties (e.g.,  $\text{p}K_a$  values). The alkyl triflates are either commercially available or can be prepared from their corresponding alcohols easily. Alternatively, treatment of the cyclized products **Cf** and **Cs** with

acetic acid and  $\text{NaBH}_3\text{CN}$  at 0  $^\circ\text{C}$  afforded 24 ring-opened indoline products **Of** and **Os**, respectively. The hydride was typically added to the iminium ion intermediates from the opposite face of the *exo*-methylene group (e.g., Of1 and Os5). For the *endo*-cyclization products (e.g., Cs1 and Cs4), the hydride was added nonselectively. Furthermore, subsequent addition of an aldehyde to the above reaction mixture produced the reductive amination product with a ring-opened indoline bearing an additional alkyl group on the aniline nitrogen. We chose two different aldehydes for each cyclized indoline, which provided another 48 ring-opened indoline products.

To summarize, we developed a concise and systematic synthetic pathway that enables rapid access (two to three steps) to highly functionalized and diverse polycyclic indolines. Using this pathway, we synthesized a pilot library of 120 indolines that contain 26 distinct skeletons each bearing three to six rings (*SI Appendix, Fig. S1*). The reactions used in this pathway showed high tolerance of functional groups, which allowed us to choose a wide range of commercially available building blocks, including the activating reagents  $Z^1X$  (e.g., acyl chlorides, sulfonyl chlorides, chloroformates, and anhydrides), aryl hydrazines with both electron-rich (e.g., methyl and methoxy), and electron-deficient (e.g., chloro and bromo) substitutions, and a variety of different aldehydes, both aliphatic and aromatic. The gold(I)-catalyzed tandem cyclizations are particularly interesting, because these reactions, under essentially the same reaction conditions, underwent the expected tandem cyclizations to afford over a dozen different ring skeletons with high yields and diastereoselectivities.

#### Screening of Polycyclic Indolines That Potentiate the Activity of Methicillin in MRSA.

With this highly diverse and functionalized indoline library in hand, we next screened their ability to potentiate

**Table 3. Of1's minimum resensitizing concentrations (MRCs) for MRSAs**

$\beta$ -Lactams	BAA-44	33592	700789	BAA-1683
Oxacillin	2	4	>32	>32
Amox/clav	4	4	4	4
Cefazolin	4	4	—	—

All MRC values are in  $\mu\text{g/mL}$ ; all strain names are ATCC numbers. —, this strain is not resistant to the antibiotic indicated.

the activity of methicillin in MRSA. A multidrug-resistant MRSA strain [American Type Culture Collection (ATCC): BAA-44] was selected for the initial screening. In addition to methicillin, this MRSA strain is also resistant to many other  $\beta$ -lactam antibiotics, as well as antibiotics from other structural classes, such as erythromycin, tetracycline, and ciprofloxacin. We first determined the minimum inhibitory concentration (MIC) of methicillin for this MRSA strain as 128  $\mu\text{g}/\text{mL}$  using the standard Clinical Laboratory Standards Institute (CLSI) broth microdilution method (36). Our initial screen was adapted from this standard microdilution assay; however, the Mueller Hinton Broth (MHB) was supplemented with methicillin at one-quarter of its MIC value (i.e., 32  $\mu\text{g}/\text{mL}$ ). To each well, 20  $\mu\text{M}$  of each individual indoline alkaloid was added, and the plates were incubated at 37  $^{\circ}\text{C}$  for 18 h. Nine indoline alkaloids (8% hit rate) were identified that reduced the MIC of methicillin to at least 32  $\mu\text{g}/\text{mL}$ . We further evaluated the ability of these nine compounds to potentiate methicillin by assessing the MIC of methicillin for MRSA using the standard microdilution method in media supplemented with 20  $\mu\text{M}$  of each alkaloid. Of1 (Fig. 3) was found to be the most active compound and reduced the MIC of methicillin from 128 to 8  $\mu\text{g}/\text{mL}$ . Hence, Of1 resensitizes this multidrug-resistant MRSA strain to methicillin, because *S. aureus* with an MIC  $\leq 8$   $\mu\text{g}/\text{mL}$  is defined as methicillin sensitive.

**Evaluation of the Ability Of1 to Potentiate Other Antibiotics.** To evaluate Of1's ability to potentiate other antibiotics in MRSA, we determined the MIC values of these antibiotics in the presence and absence of 20  $\mu\text{M}$  of Of1. In addition to methicillin, we tested other  $\beta$ -lactam antibiotics, such as oxacillin (i.e., the replacement of methicillin, a narrow spectrum antibiotic), amoxicillin/clavulanic acid (amox/clav, an extended spectrum antibiotic), cephalixin and cefazolin (first-generation cephalosporins), and meropenem and imipenem (carbapenems). Antibiotics from other structural classes tested include streptomycin (aminoglycoside), rifampicin (ansamycin), tetracycline (tetracycline), ciprofloxacin (quinolone), erythromycin and azithromycin (macrolides), clindamycin (lincosamide), vancomycin (glycopeptide), and linezolid (oxazolidinone). As summarized in Table 2, the results showed that Of1 potentiates all  $\beta$ -lactam antibiotics with variable fold of potentiation (8- to 128-fold). Although this MRSA strain is not resistant to carbapenems such as imipenem and meropenem, Of1 still showed 8- and 16-fold potentiation of these, respectively. In addition, this MRSA strain showed intermediate resistance to rifampicin with an MIC of 2  $\mu\text{g}/\text{mL}$ . Of1 showed weak potentiation effect for rifampicin (twofold), but not any other classes of antibiotics tested, such as ciprofloxacin, tetracycline, vancomycin, and linezolid. It should be noted that this MRSA strain is highly resistant to azithromycin, erythromycin, streptomycin, and clindamycin. Of1 (20  $\mu\text{M}$ ) was unable to lower their MICs to 256  $\mu\text{g}/\text{mL}$ , the highest concentration tested.

Furthermore, we evaluated Of1's potentiation effect for  $\beta$ -lactam antibiotics in a methicillin-sensitive *S. aureus* (MSSA) strain (ATCC: 25923). Intriguingly, we found Of1 does not potentiate any  $\beta$ -lactams tested, such as methicillin, amox/clav, and cefazolin (SI Appendix, Table S1). The highly tuned specificity of Of1 for  $\beta$ -lactam antibiotics in MRSA largely rules out the general potentiation mechanisms, such as efflux pump inhibition and enhancement of membrane permeability (37). Instead, Of1 may selectively target  $\beta$ -lactam-specific resistance mechanism, such as  $\beta$ -lactam-induced expression of the *mecA* and/or *blaZ* genes, which encode PBP2a and  $\beta$ -lactamase, respectively (3,38–41). Because Of1 can potentiate the combination of amoxicillin and the  $\beta$ -lactamase inhibitor clavulanic acid, and Of1 does not have the reactive  $\beta$ -lactam functional group, it is highly likely that Of1 modifies the resistance of MRSA via an unknown mechanism.

**Evaluation of the Antiproliferative Activity of Of1 in MRSA and MSSA.** To evaluate the antiproliferative activity of Of1 alone, we performed

the standard microdilution assay for Of1 using both MRSA (ATCC: BAA-44) and MSSA (ATCC: 25923) strains. The MICs of Of1 against both strains were found to be higher than 128  $\mu\text{g}/\text{mL}$ , the highest concentration tested. Considering the effective concentration (20  $\mu\text{M}$  or 10  $\mu\text{g}/\text{mL}$ ) required to resensitize MRSA to methicillin; this result suggests that Of1 has synergistic effect with methicillin, and it does not target any essential genes or gene products.

**Determination of Of1's Minimum Resensitizing Concentrations for MRSA.** Next we determined Of1's minimum resensitizing concentration (MRC) for MRSA with three common  $\beta$ -lactam antibiotics: oxacillin, amox/clav, and cefazolin. A modified broth microdilution assay was used. The modified microdilution assay involves incubating MRSA with Of1 in twofold series dilution in the presence of each individual antibiotic at its highest sensitive concentration (i.e., 2, 4/2, and 8  $\mu\text{g}/\text{mL}$ , respectively). As shown in Table 3, Of1 resensitizes BAA-44 to all three antibiotics, and the MRC of Of1 is dependent on the antibiotic used. We also determined the MRC values for three other MRSA strains. Strain ATCC 33592 behaves similarly to BAA-44, and the MRC for all three antibiotics is 4  $\mu\text{g}/\text{mL}$ . BAA-1683 is not resistant to cefazolin, but resistant to both oxacillin and amox/clav. The MRC for oxacillin is found to be  $>32$   $\mu\text{g}/\text{mL}$  (the highest concentration tested), and the MRC for amox/clav is 4  $\mu\text{g}/\text{mL}$ , similar to the other two strains. Strain ATCC 700789 is particularly interesting, because it is known as a vancomycin intermediate-resistant *S. aureus* (VISA). Our results showed that Of1 is able to resensitize this VISA strain to amox/clav at 4  $\mu\text{g}/\text{mL}$ .

**Mammalian Cytotoxicity of Of1.** To evaluate the cytotoxicity of Of1 in mammalian cells, we treated human liver hepatocellular carcinoma HepG2 cells with Of1 at various concentrations for 24 h. The remaining viable cells were determined using the CellTiter Glo mammalian viability assay (Promega). Of1 showed weak inhibition ( $\sim 10\%$ ) of the growth of HepG2 cells at 4  $\mu\text{g}/\text{mL}$ , which is the MRC value of Of1 required to resensitize all strains to amox/clav or cefazolin. The half growth inhibitory concentration ( $\text{GI}_{50}$ ) of Of1 in HepG2 cells is determined as 8.2  $\mu\text{g}/\text{mL}$ . This was determined by fitting the data using KaleidaGraph (v4.1.1; Synergy Software) (SI Appendix, Fig. S2).

In summary, from the pilot library of 120 highly diverse and functionalized polycyclic indolines, we identified a tricyclic indoline Of1 that selectively potentiates the activity of all  $\beta$ -lactam antibiotics tested (e.g., methicillin, amox/clav, cefazolin, and imipenem) in multidrug-resistant MRSA strains but not in MSSA. In addition, Of1 alone does not have antiproliferative activity in either MRSA or MSSA, and it has low cytotoxicity at the concentration required to resensitize MRSA to amox/clav and cefazolin. These observations suggest that Of1 is a unique resistance-modifying agent that selectively resensitizes MRSA to  $\beta$ -lactam antibiotics through an unknown mechanism. Further structure-activity relationship studies of Of1 and investigation of its mechanism of action are ongoing and will be reported in due course.

## Materials and Methods

Supplementary figures, table, detailed methods, and compound characterization data and NMR spectra can be found in the SI Appendix. *S. aureus* strains ATCC BAA-44 and ATCC 25923 were generously donated to us from Daniel Feldheim and Charles McHenry (Department of Chemistry and Biochemistry, University of Colorado, Boulder, CO), respectively. *S. aureus* strains 33592, 700789, BAA-1683, and HepG2 (ATCC HB-8065) cells were purchased from the ATCC.

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