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Property-Guided Synthesis of Aza-Tricyclic Indolines: Development of Gold Catalysis En Route

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Abstract

Antibiotic resistance is a worldwide public health threat that needs to be addressed by improved antibiotic stewardship and continuing development of new chemical entities to treat resistant bacterial infections. Compounds that work alongside known antibiotics as combination therapies offer an efficient and sustainable approach to counteract antibiotic resistance in bacteria. Guided by property-based analysis, a series of aza-tricyclic indolines (ATIs) were synthesized to optimize their physiochemical properties as novel combination therapies with β -lactams to treat methicillin-resistant *S. aureus* (MRSA) infections. A novel and highly efficient gold-catalyzed tandem cyclization was developed to facilitate the synthesis of these ATIs. One guanidine-containing ATI was discovered to possess both improved anti-MRSA activity and lower mammalian toxicity both in vitro and in vivo. In addition, it also showed significantly enhanced aqueous solubility and metabolic stability. These results indicated that the ATIs are a novel class of anti-MRSA agents suitable for further evaluations as adjuvant therapy in animal model studies.

Keywords

antibiotics; drug design; gold; heterocycles; homogeneous catalysis

Introduction

Antibiotic resistance is not only a worldwide public health concern, but also a huge financial burden to modern society. The discussion of antibiotic resistance at the recent G7 summit in Krün served as a clear reminder to these facts.^[1] According to the recent “Antibiotic Resistance Threats in the United States” by the U.S. Centers of Disease Control and Prevention, at least 23,000 people die as a result of resistant bacterial infections each year in the US alone, and 11,000 of those are caused by methicillin-resistant *S. aureus* (MRSA).^[2] The number of antibiotics entering the clinic has declined continuously since the late 1980s.^[3] While improved resistance surveillance and antibiotic management are critical to

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prevent the aggravation of this crisis,^[4,5] actions must be taken to develop novel therapeutic agents and strategies to address our current need. Increased knowledge on the mechanisms of antibiotic resistance has led to the discovery of new alternatives to antibiotic monotherapy. Small molecules that target virulence factors in pathogenic bacteria have emerged as a promising alternative to antibiotics. However, compounds of this nature have not yet reached the clinic.^[6,7] On the other hand, combination therapy that directly addresses known resistance mechanisms is well established.^[8-12] For example, Augmentin (i.e., the combination of a β -lactam antibiotic amoxicillin and a β -lactamase inhibitor, clavulanic acid) has become one of the most prescribed antibiotics. More recently, several novel β -lactamase inhibitors have been approved for clinical use.^[13] However, MRSA is resistant to essentially all β -lactams by expressing an alternative penicillin-binding protein, PBP2a.^[14, 15]

Our group previously discovered a tricyclic indoline, Of1 (Fig. 1a), from diversity synthesis, that can selectively re-sensitize MRSA to β -lactam antibiotics.^[16, 17] Follow-up structure-activity relationship (SAR) studies of Of1 led to a more potent analogue, **1** (Fig. 1a), by altering the halogenation pattern of Of1.^[18] Although these two compounds showed potent activity in bacterial whole-cell assays, they are not suitable for animal studies, due to their poor physiochemical properties. Recently, several cheminformatic studies concluded that antibacterial agents are biased toward more polar chemical space.^[19-21] The average calculated logarithm of distribution coefficient (octanol/buffer) at pH 7.4 (clogD_{7.4}) of marketed agents targeting Gram-positive bacteria (e.g., *S. aureus*) is -0.2. Most clinical β -lactam antibiotics (e.g., amoxicillin and cefazoline) and β -lactamase inhibitors (e.g., clavulanic acid) contain ionizable groups, which can decrease their permeability and toxicity in mammalian cells while enhancing selectivity for bacteria.

As the clogD_{7.4} of Of1 (i.e., 4.8) is much higher than typical antibacterial agents, we therefore endeavoured to optimize the physiochemical properties of these tricyclic indolines. As piperidine-fused indoline/indole (ATI) is a common motif in many biologically active natural products (e.g., ajmaline and reserpine, Fig. 1b), we decided to investigate the nitrogen-containing ATI as an analogous form of Of1. We hypothesized that introducing ionizable groups to the ATI core may decrease off-target toxicity and maintain anti-MRSA activity by decreasing their permeability to mammalian cells. Herein, we report the property-guided synthesis and evaluation of the anti-MRSA activity and physiochemical properties of the aza-tricyclic indolines (ATIs, **2**, Fig. 1a), as well as a novel and highly efficient gold-catalyzed tandem cyclization that we developed to facilitate the synthesis of ATIs en route.

We planned to synthesize ATIs by adapting the approach we developed for tricyclic indolines. As shown in Figure 1c, the tricyclic indolines were prepared from a gold-catalyzed tandem cyclization of alkynyl indoles **5** followed by a ring-opening reduction of the resulting tetracyclic indolines **6**. We envisioned that a similar gold- or platinum-catalyzed cyclization of a propargyl amine-conjugated tryptamine derivative **8** could be used to form the aza-tetracyclic indolines **9**, which may undergo a ring-opening reduction to produce the desired ATIs **2**.

Results and Discussion

Efficient synthesis of cyclization precursors

To synthesize the cyclization precursor **8**, we first employed our one-pot, three-component reaction using imine **3** (Scheme 1) to give 2-methyl tryptamines,^[22] both nitrogens of which were protected with *tert*-butoxycarbonyl (Boc) groups to afford **7a**.

The methyl group of **7a** was next selectively brominated under radical reaction conditions to produce bromide **10a**, in the presence of another benzylic methylene. Mono-alkylation of propargyl amine with **10a** was achieved by conducting the reaction at $-10\text{ }^{\circ}\text{C}$,^[23] followed by deprotection of both Boc groups using trifluoroacetic acid (TFA). The propargyl amine was then protected by a 4-nitrobenzenesulfonyl (Ns) group to give the cyclization precursor **8a**. The Ns group was chosen because it has been widely utilized in transition metal-catalyzed cyclization of propargyl amine derivatives and it can be removed under mild conditions for further functionalization of the nitrogen atom.^[24-29]

As opposed to the previously developed diversity synthesis strategy, this synthetic route is more efficient and produces only one regioisomer of the alkynyl indole cyclization precursor. The bromination reaction was also found to be highly selective and can tolerate substrates containing a carboxybenzyl group on the tryptamine nitrogen. Thus, a number of cyclization precursors with a variety of different substitutions were synthesized in good yield using this route.

Development of a tandem cyclization of propargylamine-conjugated tryptamine derivatives

With cyclization precursor **8a** in hand, we set out to develop a tandem cyclization reaction to give the desired tetracyclic indoline **9a** (Table 1).

Platinum/gold catalysis has proved well suited for tandem cyclizations of alkynyl indoles;^[30-35] in our previous work, we found triphenylphosphinegold(I) bis(trifluoromethane-sulfonyl)imide ($\text{Ph}_3\text{PAuNTf}_2$) to be an efficient catalyst for the tandem cyclization of alkynyl indoles **5**.^[16, 17, 36] We first evaluated this catalyst in our initial attempt to cyclize **8a**. **8a** appeared to be much less reactive than our previous substrates **5** (Figure 1c) and the desired product **9a** was formed in low yield (entry 1).

The conversion of this reaction was improved by increasing the temperature, but the yield remained sub-optimal (entry 2). We hypothesized that the low reactivity of **8a** was due to its lower electron density and greater steric hindrance of the alkyne, compared to the alkyne in **5**. Therefore, we evaluated catalysts with higher electrophilicity,^[37] as well as platinum catalysts, which have been utilized in efficient carbocyclizations of 1,6-enynes.^[38, 39] However in our system, the majority of the substrate decomposed with all platinum catalysts tested (entries 3-5). Returning to gold catalysis, we found that cationic gold-carbene complex gave small amount of product (entry 7), and its chloride salt was incapable of promoting the reaction. IPr is an *N*-heterocyclic carbene ligand and can stabilize Au-Au bonding. It was also suggested that aurophilic bonded intermediates were involved in gold-catalyzed transformations.^[37,41] These may lead to undesired reaction pathways, resulting in low yield of the desired product.^[40] Hence, we decided to screen additional phosphine

ligands. Based on the observation that ligands like IPr gave poor yields, we hypothesized that increasing the bulk of the phosphine ligand may minimize the formation of di-gold complexes that could lead to side reactions and poor catalyst turnover. Considering the lower electron density of the alkyne in **8a**, we evaluated gold catalyst with electron-rich phosphine ligands. Gratifyingly, we found that catalyst 2-dicyclohexylphosphino-2',4',6'-triisopropylbiphenyl gold(I) bis(trifluoromethanesulfonyl)imide (XPhosAuNTf₂) gave a much improved yield over Ph₃PAuNTf₂ (entry 8). Further survey of additional solvents (e.g., dichloroethane, dioxane, and nitromethane) in this reaction identified 1,4-dioxane as optimal, and a near quantitative yield of the tandem cyclization product **9a** was obtained (entry 9).

We propose a plausible mechanism for the formation of **9a** from **8a** in Scheme 2, based on our previous understanding of this type of gold-catalyzed tandem cyclizations.^[36] The catalyst will first coordinate with the alkyne of substrate **8a** to form intermediate **I**. The 3-position of indole will then nucleophilic attack the activated alkyne in a *6-exo-dig* mode to give iminium ion **II**. A secondary cyclization will afford **III** that can be deprotonated to give intermediate **IV**, which is followed by proto-deauration to yield the desired product **9a**.

Evaluation of the scope and limitation of the optimized tandem cyclization reaction

A variety of other cyclization precursors, **8b-j** (Table 2), were prepared using the aforementioned approach.^[23]

The scope of the new cyclization reaction conditions was then further explored with these substrates. Substrates bearing a variety of functional groups on the propargyl amine nitrogen atom were first investigated under the optimized conditions. Trifluoroacetamide **8b** provided a lower, but acceptable yield of the cyclization product (entry 2). Methanesulfonamide and two other sulfonamides all gave excellent yields (entries 3-5); however, higher catalyst loading was required to consume the 4-chloro-2-nitro-benzensulfonamide **8d** in 12 hours. Additionally, benzyloxycarbonyl (Cbz; entry 6) was also tolerated on the secondary amine with a yield similar to the amide substrate **8f**, probably due to the formation of some undesired oxazole byproduct through a gold-catalyzed cyclodebenzylation process.^[42]

While our previous SAR studies on Of1 showed that halogen substitutions on indoline can significantly impact their biological activity,^[18] we next evaluated substrates with different halogenation patterns on the indole. The results showed that indole with different halogen substitutions in the acyclic precursors were well tolerated and all gave excellent yields.

Removing fluorine from the aromatic system led to a faster reaction (entry 7), probably due to increased nucleophilicity of the indole. As Cbz group is an orthogonal protecting group to Ns, we also investigated substrates bearing a Cbz group on the tryptamine nitrogen atom. Substrates with a Cbz group in this position appeared to be more reactive than their sulfonamide counterparts and gave excellent yields, regardless of the substitutions on the indole (entries 8-10).

Conversion of cyclization products to ATIs

The cyclization products, **9a-c**, were successfully converted to the ATIs under slightly modified reductive ring-opening conditions to furnish **11a-c** (Scheme 3), respectively.

To synthesize additional ATIs with even lower clogD values, we removed the Ns group of **11a** under nucleophilic conditions to afford secondary amine **12**. The amine nitrogen was then converted to a guanidine group **13** using a guanidinylation reagent **15** followed by deprotection with trifluoroacetic acid. A zwitterion analogue **14** was also synthesized by treating **12** with *tert*-butyl bromoacetate followed by treatment with trifluoroacetic acid.

Comparison of the biological activity of Of1 and ATIs

These ATIs, representing a wide range of clogD values (Table 3), were evaluated for their minimum re-sensitizing concentrations (MRCs) for cefazolin (i.e., a first-generation cephalosporin) and amoxicillin/clavulanic acid (i.e., Augmentin, amox/clav) and minimum inhibitory concentrations (MICs) in a multi-drug resistant MRSA strain, ATCC BAA-44.^[23]

To evaluate the mammalian toxicity of ATIs, we also tested their half growth inhibitory concentrations (GI₅₀) in human cervical adenocarcinoma (HeLa) cells. The two sulphonamides, **11a** and **11b**, showed neither antibacterial activity on their own, nor potentiating effects for cefazolin (cef) or amox/clav. Interestingly, the trifluoroacetamide analogue **11c** showed similar activity profile as Of1; it potentiates the activity of β -lactams in BAA-44 without observable antibacterial activity. The more polar analogue, amine **12**, exhibited potent antibacterial activity on its own, albeit with significant mammalian toxicity. More interestingly, the guanidine analogue **13** showed not only improved β -lactam-potentiating activity (MRCs = 2 $\mu\text{g mL}^{-1}$ for both cefazolin and amox/clav) with moderate antibacterial activity (MIC = 8 $\mu\text{g mL}^{-1}$), but also much lower mammalian toxicity with the GI₅₀ of 40 $\mu\text{g mL}^{-1}$ in HeLa cells. Although compound **14** is zwitterionic under physiological conditions and has even lower clogD value, it showed no anti-MRSA activity. Further evaluations of **13** in a variety of MRSA strains, such as community-acquired MRSA strains NRS-100 and NRS-384, and vancomycin-resistant *S. aureus* (VRSA) strain NR-46414, also gave the same results (MRCs = 2 $\mu\text{g mL}^{-1}$).

Evaluations of the physiochemical and pharmacokinetic properties of ATI 13

We next evaluated the physiochemical properties of ATI **13** and found that it had vastly improved physical properties compared to Of1. To begin, we set out to compare their aqueous solubility,^[23] we found the saturation concentration of Of1 to be 0.86 $\mu\text{g mL}^{-1}$, lower than its effective MRC in MRSA. However, the saturated concentration of ATI **13** was much improved at 587 $\mu\text{g mL}^{-1}$, almost 300 fold its MRC in MRSA.^[23] These findings led us to pursue in vivo evaluation of pharmacokinetic (PK) properties of **13**. A single dose (30 mg kg⁻¹) of **13**, administered to mice through intraperitoneal (IP) injection was well tolerated with only minor clinical observations and **13** showed promising in vivo PK properties (Figure 2). The half-life (T_{1/2}) of **13** was 2.5 hr and a maximum concentration (C_{max}) of 8.0 $\mu\text{g mL}^{-1}$ was reached quickly after 30 minutes. The area under the curve (AUC) to the last data point at 24 hr was 18.2 hr $\mu\text{g mL}^{-1}$, which matched the AUC calculated to infinity.

We predicted that, similar to highly polar β -lactam antibiotics, **13** should have low general membrane permeability. To test this, permeability assessments were performed using MDCK cells.^[23] The permeability of **13** was comparable to mannitol, which is a low permeability marker (i.e., $2.11\text{E-}06 \pm 3.53\text{E-}07 \text{ cm s}^{-1}$ for **13**; $1.51\text{E-}06 \pm 2.67\text{E-}07 \text{ cm s}^{-1}$ for mannitol). For comparison, the permeability of metoprolol, a high permeability marker, was measured as $5.35\text{E-}05 \pm 6.05\text{E-}06 \text{ cm s}^{-1}$ in this assay. Despite low permeability *in vitro*, *in vivo* PK studies through IP delivery shows that **13** can reach plasma levels at approximately 4xMRC in MRSA. Overall, ATI **13** exhibited high aqueous solubility, low mammalian membrane permeability and good PK properties *in vivo*.

Conclusion

This report is a clear example of the versatility of gold catalysis that was applied to the property-guided synthesis of biologically active heterocycles. The key cyclization reactions of propargyl amine-conjugated tryptamine derivatives were realized by employing an alternative gold catalyst bearing a more electron-donating phosphine ligand, XPhos. This novel cyclization reaction provided good to excellent yields of the aza-tetracyclic indolines and showed high functional group compatibility.

Overall, an efficient synthetic route was developed to allow the facile synthesis of a series of ATIs, which was designed to possess improved physiochemical properties, compared to the parent compound Of1. A guanidine-containing ATI, **13**, showed not only improved anti-MRSA activity and lower mammalian toxicity, but also significantly improved aqueous solubility and metabolic stability. The improvement of these properties allowed further studies of this novel class of resistance-modifying agents in mice. The *in vivo* pharmacokinetic data of **13** are high promising and reminiscent of those of β -lactam antibiotics, suggesting them as suitable companions for β -lactam antibiotics. Further SAR studies of ATIs and evaluations of their efficacy in animal infection models and are ongoing and will be reported in due course.

This report further demonstrates that gold catalysis is especially well suited to synthesize complex polycyclic heterocycles with applicability in a medicinal chemistry context. This is a new and exciting direction, which builds upon the proven utility of gold catalysis as a highly versatile strategy to develop complex carbocyclic ring systems. More specifically, gold cycloisomerisations of alkynyl indoles provide selective and robust reactions that can be used to develop not only diverse and complex compound collection for the discovery of novel chemical probes, but also drug-like compounds for further evaluation of efficacy in *in vivo* models. In this context, future directions towards asymmetric versions of cycloisomerisations, and recyclable catalysts could blossom the utility of gold catalysis in medicinal chemistry and chemical biology.

Experimental Section

General

All reagents were obtained commercially and used without further purification unless otherwise noted. MRSA strain ATCC BAA-44 was a gift from the laboratory of Daniel

Feldheim. Strains NRS100, NRS384, and NR-46414 were obtained from BEI Resources (<https://www.beiresources.org/>). HeLa cells were purchased from ATCC (www.atcc.org). CellTiter-Glo® luminescent cell viability assay kit was purchased from Promega Corp.

Procedure for gold-catalyzed tandem cyclization to give **9a**

8a (40.0 mg, 0.058 mmol) was dissolved in anhydrous 1,4-dioxane (1.0 mL) in a sealed tube. 5 mol % of the catalyst XPhosAuNTf₂ (2.8 mg, 2.9 μmol) was added as a solid and the reaction was heated at 90 °C for 12 hours under argon atmosphere. The reaction mixture was then concentrated *in vacuo* with diatomaceous earth. The free flowing powder was used directly for column chromatography on silica gel eluting with 80:20 hexanes:ethyl acetate to afford tetracyclic indoline **9a** as a clear oil (97%, 39.0 mg, 0.057 mmol). IR (thin film): 3327, 3108, 2936, 2866, 1730, 1626, 1599, 1532, 1480, 1428, 1402, 1305 cm⁻¹; ¹H NMR (400 MHz, Chloroform-*d*) δ 8.39 (d, *J* = 8.7 Hz, 2H), 8.01 (d, *J* = 8.7 Hz, 2H), 7.81 – 7.71 (m, 2H), 7.47 (d, *J* = 8.5 Hz, 2H), 6.97 (d, *J* = 1.6 Hz, 1H), 6.84 (s, 1H), 5.21 (s, 1H), 5.10 (s, 1H), 4.71 (s, 1H), 4.24 (d, *J* = 14.0 Hz, 1H), 3.97 (d, *J* = 13.1 Hz, 1H), 3.60 (dd, *J* = 22.2, 13.5 Hz, 2H), 3.37 (t, *J* = 8.1 Hz, 1H), 2.99 – 2.89 (m, 1H), 2.40 – 2.28 (m, 1H), 2.22 (dd, *J* = 12.7, 5.8 Hz, 1H) ppm; ¹³C NMR (75 MHz, Chloroform-*d*) δ 150.43, 147.63 (d, *J* = 247.6 Hz), 143.80, 140.24, 140.05, 136.72, 134.69 (d, *J* = 13.0 Hz), 132.99 (d, *J* = 4.7 Hz), 129.61, 129.15, 128.64, 124.56, 122.79 (d, *J* = 3.5 Hz), 119.59 (d, *J* = 20.4 Hz), 116.64, 110.87 (d, *J* = 7.1 Hz), 90.09, 62.26 (d, *J* = 2.0 Hz), 50.61, 48.43, 47.58, 33.50 ppm; HRMS (*m/z*): [M + Li]⁺ calcd. for C₂₆H₂₁BrClFN₄O₆S₂Li, 687.9910; found, 687.9907.

Procedure for reductive ring-opening to give **11a**

A solution of TFA (1.0 M) was prepared in THF (1.0 mL); 2 equivalents of TFA (0.117 mmol, 0.117 mL, 1.0 M in THF) were added to a solution of **9a** (0.040 g, 0.058 mmol, 0.10 M) in a 10:1 mixture of THF:MeOH (0.580 mL) at 0 °C. The reaction was warmed to ambient temperature and stirred for 1 hour. The reaction was concentrated from methanol three times then dissolved in ethyl acetate and the organic layer was washed with a saturated aqueous solution of NaHCO₃. The combined organic layers were dried over anhydrous Na₂SO₄, filtered and concentrated *in vacuo* to give a crude product, which was purified by column chromatography on silica gel with 60:40 hexanes:ethyl acetate to afford **11a** as a yellow oil (92%, 0.037 g, 0.054 mmol). IR (thin film): 3353, 3104, 2026, 2925, 2866, 1532, 1480, 1402, 1350, 1316 cm⁻¹; ¹H NMR (500 MHz, Chloroform-*d*) δ 8.33 (d, *J* = 8.7 Hz, 2H), 7.87 (d, *J* = 8.7 Hz, 2H), 7.70 (d, *J* = 8.6 Hz, 2H), 7.48 (d, *J* = 8.6 Hz, 2H), 6.95 (dd, *J* = 9.4, 1.6 Hz, 1H), 6.66 (d, *J* = 1.6 Hz, 1H), 5.25 (s, 1H), 5.16 (s, 1H), 4.94 (t, *J* = 6.0 Hz, 1H), 3.94 (s, 1H), 3.92 (t, *J* = 3.5 Hz, 1H), 3.83 (d, *J* = 13.1 Hz, 1H), 3.66 (d, *J* = 13.1 Hz, 1H), 3.37 (dd, *J* = 13.8, 3.8 Hz, 1H), 3.27 (dd, *J* = 13.7, 3.5 Hz, 1H), 2.93 – 2.82 (m, 1H), 2.81 – 2.69 (m, 1H), 2.13 – 1.92 (m, 2H) ppm; ¹³C NMR (75 MHz, Chloroform-*d*) δ 150.22, 148.00 (d, *J* = 246.8 Hz), 143.54, 140.72, 139.52, 137.95, 136.49 (d, *J* = 13.0 Hz), 134.91 (d, *J* = 4.8 Hz), 129.63, 128.41 (d, *J* = 9.5 Hz), 124.51, 122.28 (d, *J* = 3.2 Hz), 118.99 (d, *J* = 20.5 Hz), 115.66, 109.99 (d, *J* = 7.1 Hz), 63.90, 52.15, 52.12, 50.18, 47.71, 39.28, 37.31 ppm; HRMS (*m/z*): [M+Li]⁺ calcd. For C₂₆H₂₃BrClFN₄O₆S₂Li, 691.0076; found, 691.0068.

Procedure for removal of 4-nitrobenzenesulfonamide to give **12**

Thiophenol (0.045 mL, 0.50 mmol) and K_2CO_3 (0.057 g, 0.50 mmol) were added to a solution of **11a** (0.057 g, 0.083 mmol, 0.080 M) in anhydrous ACN (1.0 mL). The reaction was refluxed under argon for 3 hours. The reaction was cooled to room temperature and the solvent was removed *in vacuo* to give a residue, which was dissolved in methanol then concentrated onto diatomaceous earth to give a free-flowing solid which was purified by column chromatography on silica gel eluting with 90:8:2 DCM:MeOH:TEA to give **12** as a clear oil (84% yield, 0.035 g, 0.070 mmol). IR (thin film): 3279, 3089, 2936, 2862, 1625, 1592, 1476, 1327 cm^{-1} ; 1H NMR (500 MHz, Chloroform-*d*) δ 7.77 (d, J = 8.5 Hz, 2H), 7.50 (d, J = 8.5 Hz, 2H), 7.06 (dd, J = 9.4, 1.7 Hz, 1H), 6.89 (d, J = 1.7 Hz, 1H), 5.17 (s, 1H), 5.04 (s, 1H), 4.79 (s, 1H), 4.03 (s, 1H), 3.78 (s, 1H), 3.43 (d, J = 14.3 Hz, 1H), 3.36 (d, J = 14.3 Hz, 1H), 3.27 – 3.16 (m, 2H), 3.12 – 3.01 (m, 2H), 3.02 – 2.88 (m, 2H), 2.21 – 2.12 (m, 1H), 2.09 – 1.97 (m, 1H) ppm; ^{13}C NMR (101 MHz, Chloroform-*d*) δ 149.28 (d, J = 246.6 Hz), 143.61, 139.40, 137.91, 137.07 (d, J = 4.8 Hz), 136.31 (d, J = 13.4 Hz), 129.62, 128.68, 122.86, 118.72 (d, J = 21.5 Hz), 110.11 (d, J = 7.2 Hz), 63.34, 51.99, 48.80, 45.40, 39.99, 34.47, 29.85 ppm; HRMS (m/z): $[M+H]^+$ calcd. for $C_{20}H_{21}BrClFN_3O_2S$, 500.0210; found, 500.0212.

Procedure for guanidinylation and deprotection to give **13**

A solution of **12** (0.011 g, 0.022 mmol, 0.070 M) was prepared in DCM (0.314 mL) and cooled to 0°C. To this solution, *N,N*-di-Boc-1H-pyrazole-1-carboxamidine (0.024 mmol, 7.5 mg) was added and the reaction was warmed to room temperature with stirring under argon for 12 hours. The crude reaction was diluted to 1 mL with DCM then washed with 2.0 N NaOH then brine. The organic layer was dried with Na_2SO_4 and concentrated *in vacuo* to give a clear residue which was purified by column chromatography eluting with 70:30 hexanes: ethyl acetate to give diBoc-Guanidine ATI which was used directly in the next step. DiBoc-Guanidine ATI was dissolved in 1 mL 1:1 DCM:TFA at 0°C under argon and the reaction stirred for 4 hours. The reaction was then concentrated *in vacuo* to give **13** as a white solid (90% yield for 2 steps calculated based on **12**, 0.013 g, 0.020 mmol). Melting point: 98.9 – 100.5 °C; IR (thin film): 3357, 3171, 2925, 1659, 1614, 1480, 1432, 1327, 1204 cm^{-1} ; 1H NMR (400 MHz, Methanol-*d*₄) δ 7.70 (d, J = 8.6 Hz, 2H), 7.54 (d, J = 8.6 Hz, 2H), 7.05 (d, J = 9.8 Hz, 1H), 6.76 (s, 1H), 5.33 (s, 1H), 5.28 (s, 1H), 4.02 (s, 1H), 3.91 (d, J = 13.4 Hz, 1H), 3.77 (d, J = 13.2 Hz, 1H), 3.63 (d, J = 14.4 Hz, 1H), 3.25 (d, J = 14.4 Hz, 1H), 3.05 – 2.90 (m, 1H), 2.56 – 2.42 (m, 1H), 2.12 – 2.02 (m, 2H) ppm; ^{13}C NMR (101 MHz, Methanol-*d*₄) δ 158.15, 149.06 (d, J = 244.5 Hz), 142.90, 140.11, 139.85, 138.98 (d, J = 12.8 Hz), 136.70 (d, J = 5.0 Hz), 130.39, 129.66, 123.55 (d, J = 3.03 Hz), 119.66 (d, J = 20.0 Hz), 115.70, 109.53 (d, J = 6.9 Hz) 64.87, 53.41, 51.40, 47.48, 39.64, 38.06 ppm; HRMS (m/z): $[M+H]^+$ calcd. for $C_{21}H_{23}BrClFN_5O_2S$, 542.0428; found, 542.0427.

Procedure for single-dose pharmacokinetic studies

Female CD-1 mice (Charles River Laboratories, CA) at the ages of 6-7 weeks each received a single i.p. dose at 30 mg kg^{-1} of **13**. Blood was collected from the treated mice at 5, 15 and 30 minutes and 1, 1.5, 2, 4, 6, 8 and 24 hr post-dose for processing to plasma. All samples were analyzed by LC-MS/MS for drug levels.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Design and proposed targeted synthesis of aza-tricyclic indolines. **(a)** Structures of tricyclic indolines and aza-tricyclic indolines. **(b)** Structures of aza-tricyclic indoline-containing natural products. **(c)** Previously reported synthetic route to Of1 using a diversity synthesis strategy. **(d)** Planned targeted synthetic route for aza-tricyclic indolines. TMB: 3,4,5-trimethoxybenzoyl.

Figure 2. Single-dose Pharmacodynamics study of **13** in mice. Each data point represents the mean and SD of $n = 3$ mice. The dashed horizontal line is the MRC of **13** in cell culture.

Scheme 1.

Synthesis of the cyclization precursor **8a**. Reagents and conditions: (a) ClsCl, DMAP, **3** then 4-bromo-2-fluorophenylhydrazine·HCl, CH₃SO₃H, CH₃CN, 80°C, 59 %; (b) Boc₂O, DMAP, Et₃N, CH₂Cl₂, 23°C, 86 %; (c) NBS, BPO, CCl₄, 85 °C; (d) H₂NCH₂CCH, CH₃CN, -10 °C 83 % in two steps; (e) TFA, CH₂Cl₂, 0 °C; (f) NsCl, Et₃N, CH₂Cl₂, 89% in two steps. ClsCl, 4-chlorobenzenesulfonyl chloride; DMAP, 4-dimethyl-aminopyridine; Boc, *tert*-butoxycarbonyl; NBS, *N*-bromosuccinimide; BPO, benzoyl peroxide; NsCl, 4-nitrobenzenesulfonyl chloride.

Scheme 2.
Proposed mechanism for tandem cyclization.

Scheme 3.

Synthesis of ATIs. Reagents and conditions: (a) TFA, NaCNBH₃, THF/MeOH, 0 °C; (b) PhSH, K₂CO₃, CH₃CN, 60 °C; (c) i. **15**, CH₂Cl₂, 23 °C; ii. TFA, CH₂Cl₂, 0 °C; (d) i. *Tert*-butyl bromoacetate, Et₃N, CH₂Cl₂, 0 °C; ii. TFA, CH₂Cl₂, 0 °C. Tfa, trifluoroacetamide; TFA, trifluoroacetic acid; Cls, 4-chloro-benzenesulfonyl.

Table 1

Optimization of the tandem cyclization reaction.

Entry	Catalyst	Solvent	<i>T</i> [°C]	Yield [%] ^[a]
1	Ph ₃ PAuNTf ₂	Toluene	60	22
2	Ph ₃ PAuNTf ₂	Toluene	90	43
3	PtCl ₂	Toluene	90	<10
4	PtCl ₄	Toluene	90	0
5	H ₂ PtCl ₆ ·6H ₂ O	Toluene	90	<10
6	IPrAuCl	Toluene	90	0
7	IPrAuCl/AgSbF ₆	Toluene	90	11
8	XPhosAuNTf ₂	Toluene	90	61
9	XPhosAuNTf ₂	1,4-Dioxane	90	97

<p>IPr</p>	<p>XPhos</p>
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^[a]Yields were calculated based on ¹H NMR integration using 4-dimethylaminopyridine as an internal standard.

Table 2Scope of gold-catalyzed tandem cyclization.^[a]

Entry	8	X ¹	X ²	Y	Z	Yield [%] ^[b]
1	8a	Br	F	Cl _s	Ns	97
2	8b	Br	F	Cl _s	Tfa	72
3	8c	Br	F	Cl _s	Ms	90
4	8d	Br	F	Cl _s	CNs	91 ^[c]
5	8e	Br	F	Cl _s	Ts	96
6	8f	Br	F	Cl _s	Cbz	71
7	8g	Br	H	Cl _s	Ns	92 ^[d]
8	8h	Cl	H	Cbz	Ns	96 ^[e]
9	8i	Cl	Cl	Cbz	Ns	95 ^[e]
10	8j	F	H	Cbz	Ns	91 ^[e]

Cl_s, 4-chloro-benzenesulfonyl; Ns, 4-nitro-benzenesulfonyl; Tfa, trifluoroacetyl; Ms, methanesulfonyl; CNs, 4-chloro-2-nitro-benzenesulfonyl; Ts, 4-methyl-benzenesulfonyl; Cbz, benzyloxycarbonyl.

^[a] Standard reaction conditions (5 mol % XPhosAuNTf₂, 1,4-dioxane, 90 °C, 12 hours unless noted otherwise).

^[b] Isolated yield based on complete conversion of substrate.

^[c] 10 mol % catalyst used.

^[d] 2 hour reaction time.

^[e] 2 hour reaction time at 60 °C.

Table 3

Comparison of the biological activity of Of1 and ATIs.

Compound	clogD _{7.4}	MRC Cef ^[a]	MRC amox/clav [a]	MIC [a]	GI ₅₀ [a],[b]
Of1	4.8	4	4	>128	17
11a	4.5	>32	>32	>32	NT
11b	4.1	>32	>32	>32	NT
11c	2.5	4	4	>32	19
12	2.0	4	4	8	6.7
13	0.74	2	2	8	40
14	0.54	>32	>32	>32	NT

Cef-cefazolin; amox/clav-amoxicillin/clavulanic acid; NT- Not tested.

^[a] All MIC, MRC, and GI₅₀ values are in $\mu\text{g mL}^{-1}$;^[b] Determined for HeLa cells.