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Diastereoselective Synthesis and Biological Evaluation of Enantiomerically Pure Tricyclic Indolines

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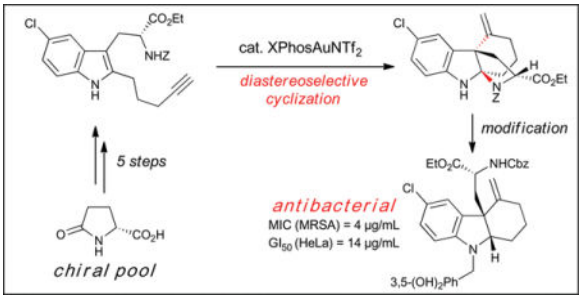
Abstract

Tricyclic indolines are common in both natural products and synthetic chemical probes. In this study we demonstrated that enantiomerically pure tricyclic indolines can be prepared from an inexpensive commmercially available chiral starting material, pyroglutamic acid. The synthesis features a highly diastereoselective gold-catalyzed cyclization of alkyne-tethered indoles and subsequent diastereoselective reductive ring-opening reaction. Using this approach, we synthesized analogs of our previously discovered tricyclic indoline probes that possess antibacterial and resistance-modifying activity. The biological activity against methicillin-resistant *Staphylococcus aureus* (MRSA) of these analogues was evaluated and reported. The synthetic approach reported may be leveraged in the future to prepare diastereopure chemical probes for the determination of biological targets for drug discovery.

TOC Image



Introduction

The development of new antibiotics to stave off the incursion of drug-resistant pathogens has stagnated over the past few decades, with only six first-in-class drugs approved for clinical use between the 1960s and 2012.[1] It has been suggested that, without significant investment in drug discovery, humankind will enter into a second pre-antibiotic era where infection resurges as a major cause of death.[2,3] This prospect is particularly alarming given that 30%

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of deaths in the United States were due to diarrhea and enteritis, pneumonia, and tuberculosis alone at the beginning of the 20th century, before the discovery of penicillin.[4]

Antibiotic development has historically focused on the discovery of new analogues within known structural classes of antibiotics, most of which were discovered from the natural products of soil actinomycetes between the 1940s and 1960s.[5] The risks associated with drug discovery are generally lower when working within a well understood class of antibiotics with more information available about efficacy and toxicology issues.[6] Between 1981 and 2005, 73% of all new antibiotics were cephalosporins, penicillins, quinolones, or macrolides.[7] Unfortunately, new antibiotics that share characteristics with established classes often suffer from shared resistance within those same classes.[1] Though there are highly conserved genes in bacteria accounting for around 300 proteins offering potential targets for new broad spectrum drugs,[8] most current antibiotics work with only a small set of biological targets involved in DNA, RNA, cell wall, or protein synthesis.[1] The need for antibiotics from unexplored sources[9] and with neglected biological targets[10,11] has become clear.

A number of inventive approaches toward the discovery of novel bioactive compounds have been pursued in the past. For example, high-throughput screening was employed to rapidly pick through vast libraries of compounds for activity against cellular targets. Unfortunately, this strategy was largely a failure because even extremely potent compounds rarely had any effect on the growth of live bacteria, probably due to poor membrane penetration in many cases.[5] This pitfall can be circumvented by employing whole-cell screening at the expense of ease and optimization available for target-based screening efforts.[13] Rather than acting on lessons learned from failed drug discovery efforts, many large pharmaceutical companies have practically abandoned antibiotic research in favor of more lucrative alternatives. This paradigm has largely come about due to the inherently unfavorable economics of the antibiotic market compared to other drugs; antibiotics are used in smaller quantities than drugs meant for chronic conditions and their responsible use requires that they be prescribed only cases of serious infections.[12] The rapid emergence of resistance in most cases also makes it likely that the useful lifespans of new drugs will be brief.

In our previous studies, we sought to discover new antibacterials by drawing inspiration from the indole alkaloids, a class of phytochemicals, which had been neglected in modern research yet known to traditional medicine for millennia. Biological screening of small libraries of compounds and follow-up structure-activity relationship studies of indole alkaloid-like molecules led to the discovery of compounds with potent activity against methicillin-resistant *Staphylococcus aureus* (MRSA) in whole-cell assays.[13–15] Two of the most interesting compounds discovered from these studies were **1** and **2** (figure 1). **1** was found to be a resistance-modifying agent (RMA) that re-sensitizes MRSA to β-lactam antibiotics to which the bacteria were previously resistant, while **2** acted as a direct antibacterial agent. The relationship between activity and stereochemistry of these biological probes is unknown, and unfortunately, the synthetic methods used to produce them are not amenable to preparing enantiomerically pure samples for mechanistic studies determining their biological targets. Such studies might include the attachment of a fluorescent or biotin group for pull-down assays as examples.

Our previous methods relied on gold catalysts for the preparation of very complex tetracyclic indoline structures. A chiral catalyst could hypothetically be employed which would prefer one stereochemical outcome in the cyclization step in order to make these methods enantioselective. Unfortunately, gold catalysts coordinate with alkynes in a linear two-coordinate fashion.[16] This, in general, makes gold complexes poor choices as chiral catalysts because the steric bulk of the ligands are far removed from the reactive site and therefore unlikely to have a significant effect on the stereochemical outcome of the reaction. Though the scope of chiral gold catalysts is limited, there has been significant progress in the field of homogenous asymmetric gold-catalysis recently. Such advances include binuclear gold catalysts with atropisomeric bisphosphine ligands, gold(I) catalysts with monodentate phosphoramidite ligands, and gold(I) catalysts with chiral counterions.[17] It was also demonstrated recently that phosphahelicenes can be used as chiral ligands of gold(I) catalysts where the helicity of the ligand imparts high enantioselectivity for enyne cycloisomerization reactions.[18] However, highly stereoselective cyclizations of alkyne-tethered indoles using gold catalysis are rare and often have limited substrate scope.[19]

There are many examples in the literature of studies emphasizing a complementary approach wherein a chiral precursor, such as an alkyne-tethered indole, is used with an achiral gold catalyst.[20] It was hypothesized that a chiral precursor would induce a change in the transition state of the cyclization, ultimately favouring one stereochemical outcome over the others. Computation studies also suggest the stereochemical outcome for these reactions is often under kinetic control.[21] Inexpensive commercially available chiral starting materials could then be used to synthesize a single enantiomer of the cyclization precursor, allowing for the use of methods that were already optimized in previous studies with minimal alterations.

Results and Discussion

Synthesis

Perturbing the structure of a biological probe inherently risks modifying its activity. However, the relationship between functional groups, structure, stereochemistry, and activity can only be determined empirically. Considering this and moving forward with the aim of introducing a new stereocenter into the structures of **1** and **2**, the scaffolds in figure 2 were designed. It was expected that a single diastereomer of the tetracyclic indoline would be favored in the gold-catalyzed cyclization step because of the added bulk of the stereocenter bearing R^1 near the nitrogen nucleophile. This diastereomerically pure tetracyclic indoline could be readily prepared from a corresponding alkynyl indole, which can be derived from pyroglutamic acid. Both enantiomers of this compound are available inexpensively and it is a convenient starting material because of its two reactive sites which can be decorated with various functional groups at the amide nitrogen or the carboxylic acid side chain while preserving the stereocenter.

With a retrosynthetic plan in mind, the forward synthesis (scheme 1) was initiated by the esterification of the carboxylic acid side chain of **3** followed by installation of the carboxybenzyl group to yield compound **4**. The Grignard nucleophile **5** was then leveraged to open the ring and introduce the alkyne moiety, which was subsequently deprotected by

tetrabutylammonium fluoride to afford ketone **6**. The Fischer indole synthesis was subsequently exploited between 4-chlorophenylhydrazine hydrochloride and **6** to generate a mixture of indole regioisomers, which were purified to obtain **7a**. 2,4,6-trichloro-1,3,5-triazine (TCT) was used as an anhydrous acid source to facilitate this reaction under mild conditions.[22] The ratio of **7a** to the undesired regioisomer was determined to be 1 to 2.6 after silica gel chromatography purification. Though the desired isomer was the disfavored product, the yield was high enough to continue. The final preparation of the cyclization precursors was completed by conversion of the carboxybenzyl group of **7a** to a 4-chlorobenzenesulfonyl group yielding analogue **7b**.

The pivotal cyclization step employed the gold catalyst $XPhosAuNTf_2$ to produce the complex tetracyclic structures **8a** and **8b**. The reaction was conducted at low temperature to achieve high diastereoselectivity. The relative stereochemistry of compound **8b** was determined by NOESY NMR experiment. The proton at the ester stereocenter and a proton on the phenyl ring (highlighted in scheme 1) were identified as having a through-space interaction. This observation was used to determine that the ester side chain must be occupying the space towards the six-membered ring, which dictated the relative stereochemistry of the fused ring junction in turn. Although cyclization of **7b** produced **8b** as a single diastereomer, the diastereomeric ratio of **8a** was determined to be 4 to 1 by high-performance liquid chromatography (HPLC) analysis. These results were consistent with computational work we reported in collaboration previously, wherein it was demonstrated that gold-catalyzed cyclizations of alkynylindoles proceed via kinetic control, which dictates the stereochemical outcome of the cyclization steps.[21]

Reductive ring-opening reactions were conducted yielding tricyclic indolines **9a** and **9b**. The product was determined to have a cis ring junction, as the hydride was added to the in situ-generated iminium ion from the convex face of the tricyclic system. Subsequent reductive amination of **9a** in the presence of 3,5-dihydroxybenzaldehyde yielded **10**, an analogue of the antibacterial agent **2**.

Biological Evaluation

The minimal inhibitory concentration (MIC), or the lowest concentration at which *S. aureus* is considered susceptible to an antibacterial, was determined by the standard broth microdilution method detailed in the CLSI handbook.[23] Briefly, 96-well plates were prepared such that each well contained 50μL of total volume with 2-fold serial dilutions of compound in each eight-well column in Mueller Hinton Broth (MHB). A bacterial day culture in the mid log phase of growth (OD_{600} 0.15-0.4) was diluted in MHB to OD_{600} 0.002. Upon addition of 50μL of this culture to each well, the final bacteria concentration was OD_{600} 0.001. Antibiotic controls and synthesized compounds were prepared such that the maximum final concentrations would be 256 and 64 μg/mL respectively. Plates were then sealed and incubated at 37°C with shaking for 18 hours. Results were interpreted by viewing each well for turbidity, indicating overnight growth. The MIC was recorded as the lowest concentration with no visible growth. Each compound was tested in triplicate.

Racemic mixtures of **9b** and **10** were prepared and evaluated against a multi-drug resistant MRSA strain BAA-44.[§] The introduction of the ester stereocenter in **9b** nullified the re-sensitizing activity compared to the initial lead, **1**.[§§] However, we were pleased to discover that **10** was a potent antibacterial agent with an MIC of 4 μg/mL, the same as the lead compound, **2**. The cytotoxicity of **10** in mammalian cells was also evaluated using human cervical adenocarcinoma (HeLa) cells.[§§] The half-growth inhibitory concentration (GI_{50}) of the racemic mixture of **10** was found to be 14 μg/mL, a modest improvement over **2** (GI_{50} = 8 μg/mL).

Pure samples of each enantiomer of **10** were then prepared to determine which were responsible for the observed antibacterial activity. Four MRSA strains were assayed including BAA-44, BAA-1720, ATCC-33592, and NRS-100.[§] Interestingly, both enantiomers of **10** inhibited the growth of all four MRSA strains at 4 μg/mL (table 1), the same MIC as the initial lead compound **2**, suggesting the absolute stereochemistry of the tricyclic indoline is not essential to the antibacterial activity.

Conclusions

The preparation of enantiopure tricyclic indolines was successfully carried out from commercially available chiral starting materials using highly diastereoselective gold-catalyzed cyclization and reductive ring-opening reactions. We demonstrated that the minor perturbation of the cyclization precursor **7** with an added stereocenter was sufficient to induce a change in the pivotal cyclization, yielding an excess of one diastereomer. Although the re-sensitizing activity of **1** was lost in the analogue **9a**, the enantiopure analogues of **2** were found to be active in MRSA with equal potency. The abolition of activity of **9a** was not surprising considering that the addition of a new functional group to a bioactive compound inherently risks altering the desired activity. However, the fact that **10** and **2** had equal potency demonstrates that this strategy is valid as a proof of concept. We hope that this study will pave the way for future research, such as the determination of the biological target of **10** and other compounds like it that have we have previously reported. The relationship between stereochemistry and activity of such compounds may also be elucidated. With the current demand for new antibiotics with unique modes of action, the discovery of structures from previously unexplored chemical space will be of vital importance.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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[§] *BAA-44* was clinically isolated in Portugal with multi-drug resistance across a broad spectrum including methicillin, imipenem, cephalothin, erythromycin, rifampin, ciprofloxacin, doxycycline, and clindamycin. *BAA-1720* was clinically isolated in the United Kingdom with resistance to methicillin (a.k.a. MRSA252). *ATCC-33592* was clinically isolated from human blood in the United States with resistance to methicillin and gentamicin. *NRS-100* was isolated in the United Kingdom as a community-acquired strain with resistance to methicillin (a.k.a. strain COL, a.k.a. NR-45906).

[§§] RMA and HeLa assay protocols are available in the electronic supplementary information (ESI).

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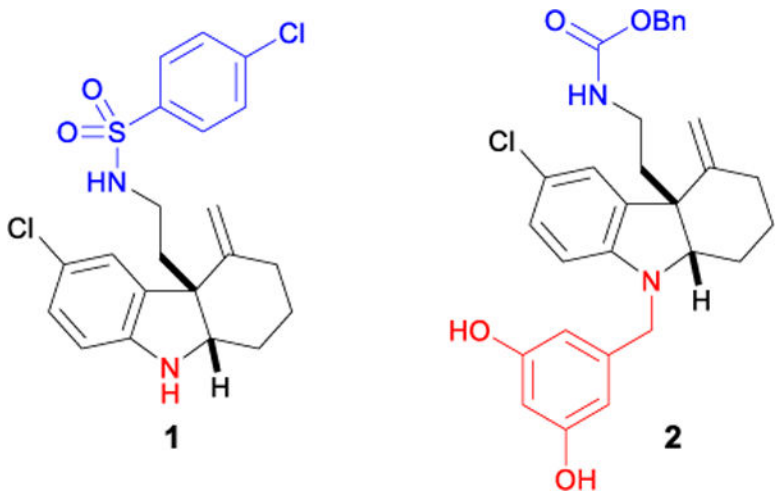


Figure 1.

Previously discovered bioactive compounds. **1** has no antibacterial activity but sensitizes MRSA to β-lactam antibiotics. **2** inhibits MRSA growth directly (MIC = 4 μg/mL).

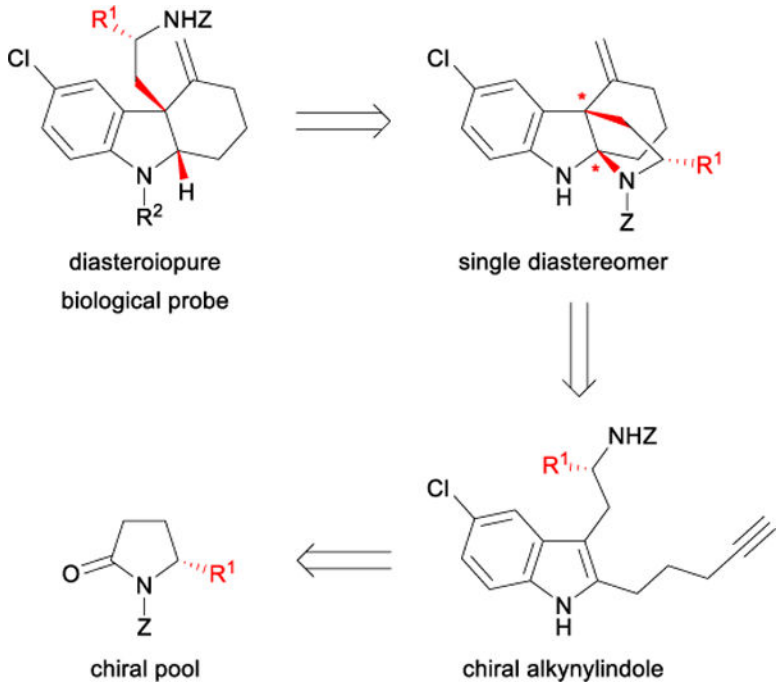
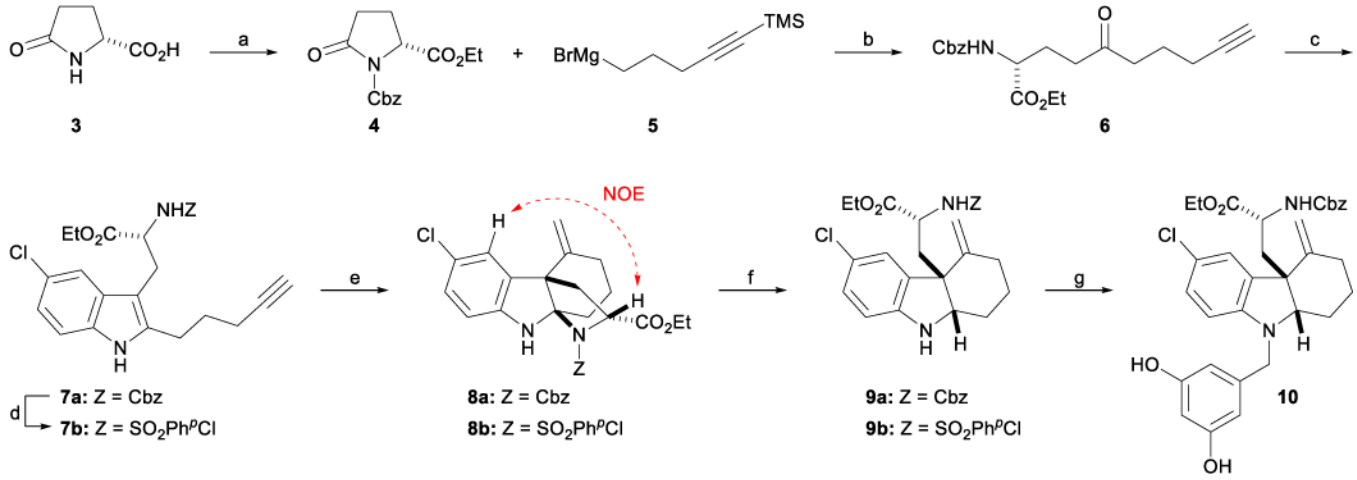


Figure 2.
Retrosynthetic plan



Scheme 1.

Synthesis of **9b** and **10**. *Reagents and conditions:* (a) $SOCl_2$, EtOH then CbzCl, DIPEA, and DMPA in DCM, 51% for two steps; (b) THF, −40°C then acidic workup followed by TBAF in THF, 56% for two steps; (c) 4-chlorophenylhydrazine hydrochloride, 2,4,6-trichlorotriazine, EtOH, 80°C, 23%; (d) $BF_3{\cdot}Et_2O$, Me_2S, DCM then 4-chlorobenzenesulfonyl chloride, NEt_3, DCM, 34% for two steps; (e) $XPhosAuNTf_2$, toluene, −50°C, dr = 4:1, 60%; (f) $NaBH_3CN$, TFA, MeOH; (g) 3,5-dihydroxybenzaldehyde, $NaBH_3CN$, AcOH, MeOH, 57% for steps f and g. *Abbreviations:* Cbz = carboxybenzyl, DIPEA = N,N-diisopropylamine, THF = tetrahydrofuran, TBAF = tetra-n-butylammonium fluoride, DCM = dichloromethane, $XPhosAuNTf_2$ = 2-dicyclohexylphosphino-2′,4′,6′-triisopropylbiphenyl gold(I) bis(trifluoromethanesulfonyl)imide.

Table 1

Biological Evaluation of **10**

Compound	MIC BAA-44	MIC BAA-1720	MIC ATCC-33592	MIC NRS-100	GI_{50} HeLa
2 (racemic)	4	4	4	4	8
10 (racemic)	4	4	4	4	14
10 (L)	4	4	4	4	–
10 (D)	4	4	4	4	–

*All values are reported in μg/mL. **10** (L) and **10** (D) are used to denote the pure enantiomers synthesized from L and D-pyroglutamic acid respectively. Cefazolin and amoxicillin/clavulanic acid were used as antibiotic controls and inhibited the growth of all four bacteria on average at 128 or 256 for cefazolin and 16/8 or 32/16 μg/mL for amoxicillin/clavulanic acid.