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Parallel synthesis of chiral pentaamines and pyrrolidine containing bis-heterocyclic libraries. Multiple scaffolds with multiple building blocks: A double diversity for the identification of new antitubercular compounds

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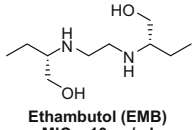
ABSTRACT

Combinatorial chemistry offers a unique opportunity for the synthesis and screening of large numbers of compounds and significantly enhances the prospect of finding new drugs. Collaborative efforts with the Tuberculosis Antimicrobial Acquisition & Coordinating Facility (TAACF), have led to the identification of submicromolar novel antitubercular hits. Chiral pentaamines and bis-heterocyclic compounds with 90–100% inhibition against *Mycobacterium tuberculosis* strain $H_{37}R_v$ were identified. Some of the identified compounds are more active than the existing drug ethambutol.

Tuberculosis (TB) is the biggest reemerging infectious disease in recent history and kills an estimated 3 million people worldwide each year. Although many believe TB to be a scourge of the past, the disease continues to strike people throughout the world at an alarming rate. Almost 2 billion people are infected with *Mycobacterium tuberculosis*, the TB bacterium, and each year, 8 million of them develop active TB.[1–3] Tuberculosis is particularly dangerous to those with weakened immune systems and is the primary cause of death among people infected with HIV.[4] In recent years, drug-resistant strains of TB have emerged, posing a formidable threat to the global population. The discovery and development of new therapeutic anti-TB regimens compounds are urgently needed. Combining the power of combinatorial chemistry and close collaborative interactions with the Tuberculosis Antimicrobial Acquisition & Coordinating Facility (TAACF),[5] a leading laboratory having assays and screening facilities against tuberculosis, a large selection of diversified compounds were tested and led to the identification of potential useful lead compounds. Low molecular weight compounds, especially heterocyclic compounds, offer a high degree of structural diversity and have proven to be broadly and economically useful as therapeutic agents.[6–11] We have proven the inherent strengths of combinatorial chemistry (large numbers of individual compounds and mixture based combinatorial libraries) to accelerate chemical information acquisition for basic research and drug design.[12,13]

Polyamines were reported to have high antitubercular activities.[14] It has been recently reported that active chiral diamines were identified following the screening of a large library of compounds.[14] It has been described that amines which occurred most frequently in active compounds included many with large hydrophobic moieties, suggesting that optimization was perhaps selecting for the isoprenoid binding site of the arabinosyltransferase target of the antitubercular drug ethambutol (EMB) (Fig. 1). Encouraged by the reported antitubercular activities of polyamines, we sent 120 individual chiral pentaamines to TACFF. As



Ethambutol (EMB)
MIC = 10 μg/ml

Figure 1. Chemical structure of ethambutol.

Abbreviations: SPPS, solid phase peptide synthesis; PS-SCL, positional scanning synthetic combinatorial library; TB, tuberculosis; MIC, minimal inhibitory concentration; TAACF, Tuberculosis Antimicrobial Acquisition & Coordinating Facility.

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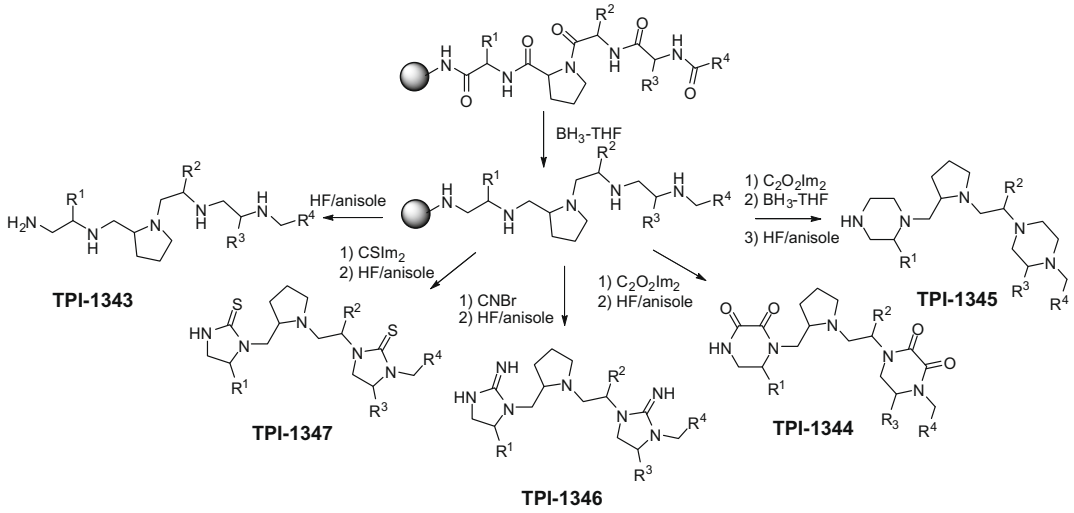

Scheme 1. Parallel synthesis of pentaamine and bis-heterocyclic libraries.

it will be elaborated, different pentaamines, and bis-heterocyclic compounds having hydrophobic groups at R^1, R^2, R^3 and R^4 gave interesting activities (Scheme 1).

Using the tea-bag technology and following the strategy outlined in Scheme 1,[15] we developed an efficient approach for the solid phase synthesis of different pentaamines and pyrrolidine bis-heterocyclic libraries from resin-bound proline-containing acylated tetrapeptides. Starting from resin-bound amino acids (diversity R_1), Boc-proline was coupled using standard solid phase synthesis (SPPS) coupling reagents,[16] followed by Boc deprotection and subsequent coupling of two Boc-amino acids (diversities R_2 and R_3). The N-terminal Boc was cleaved and the generated primary amine was N-acylated with different commercially available carboxylic acids (diversity R_4). The generated resin-bound N-acylated tetrapeptide was exhaustively reduced using borane–THF.[16] Typical reaction conditions for the solid phase reduction of polyamides consists of the treatment of resin-bound peptides with BH_3–THF at 65 °C for 72 h.[17] The generated resin-bound borane-amine complexes are then disproportionate following overnight treatment with neat piperidine at 65 °C. The reduction is free of racemization. Our approach involved the use of proline as a spacer, which, following the exhaustive reduction of the amide groups, yielded resin-bound pentaamine containing two pairs of secondary amines separated by a pyrrolidine ring. One set of compounds were cleaved to afford the corresponding pentaamine library TPI-1343. Applying the concept of 'libraries from libraries',[18] extra sets of tea-bags were prepared, where the resulting pairs of secondary amines were separately treated with different bifunctional reagents such as, thiocarbonyldiimidazole, cyanogen bromide and oxalyldiimidazole to afford following cleavage of the solid support the corresponding libraries, namely pyrrolidine bis-cyclic thiourea TPI-1347, pyrrolidine- bis-cyclic guanidines TPI-1346, and pyrrolidine bis-diketopiperazine TPI-1344, respectively. An extra set of diketopiperazine was prepared and treated prior to cleavage of the solid support with BH_3-THF to afford following reduction of the oxamide and cleavage of the resin the corresponding pyrrolidine bis-piperazine TPI-1345. We used the approach described in Scheme 1, for the preparation of 5 positional scan libraries.[12,19] Twenty six different amino acids were selected for R_1, R_2, and R_3, and 42 carboxylic acids for R_4 (Table 1) to prepare in parallel, a pentaamine library and 4 different mixture based pyrrolidine bis-heterocycles each containing 738,192 individual compounds in positional scanning format.

Along with the synthesis of the mixture based libraries, 120 different individual compounds were synthesized for each library. These individual compounds served as controls to determine whether the individual building blocks used at each of the variable positions could be successfully incorporated into the synthesis of the corresponding libraries. The individual building blocks were varied while the other three positions remained fixed (Scheme 2).

In vitro evaluation of antimycobacterial activity: (description of TAACF assays)

1. Primary screening is conducted at 6.25 μg/mL (or molar equivalent of highest molecular weight compound in a series of congeners) against *M. tuberculosis* $H_{37}Rv$ (ATCC 27294) in BACTEC 12B medium using a broth microdilution assay. The Microplate Alamar Blue Assay (MABA).[20] Compounds exhibiting fluorescence are tested in the BACTEC 460 radiometric system. Compounds affecting <90% inhibition in the primary screen (i.e., MIC >6.25 μg/mL) are not generally evaluated further.
2. Compounds demonstrating at least 90% inhibition in the primary screen are retested at lower concentrations against *M. tuberculosis* $H_{37}Rv$ to determine the actual minimum inhibitory concentration (MIC) using MABA. The MIC is defined as the lowest concentration effecting a reduction in fluorescence of 90% relative to controls.
3. Concurrent with the determination of MICs, compounds are tested for cytotoxicity (IC_{50}) in VERO cells at concentrations $\leqslant$ 62.5 μg/mL or l0 times the MIC for *M. tuberculosis* $H_{37}Rv$ (solubility in media permitting). After 72 h exposure, viability is assessed on the basis of cellular conversion of MTT into a formazan product using the Promega CellTiter 96 non-radioactive cell proliferation assay.

Screening results: Driven by the reported antitubercular activities of polyamines, 120 individual chiral pentaamines (TPI-1343) (Scheme 1) were evaluated in a primary screen against *M. tuberculosis* (Table 2). The individual pentaamines were screened for% inhibition against *M. tuberculosis* strain H37Rv at 6.25 μg/mL. Compounds are considered active with inhibition >90%. Eleven compounds demonstrated higher than 90% inhibition in the primary screen and were tested at lower concentrations to determine the minimum inhibitory concentration (MIC) by serial dilution. As shown in Table 2, pentaamines substituted with different hydrophobic groups provided high inhibition at MIC values ranging from 3 to 6 μg/mL.