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Synthesis and evaluation of (*S,S*)-*N,N′*-bis-[3-(2,2′,6,6′-tetramethylbenzhydryloxy)-2-hydroxy-propyl]-ethylenediamine (S2824) analogs with anti-tuberculosis activity

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ABSTRACT

In order to identify new and potent candidate drugs to treat tuberculosis, a library of compounds was screened, and (*S,S*)-*N,N′*-bis-[3-(2,2′,6,6′-tetramethylbenzhydryloxy)-2-hydroxy-propyl]-ethylenediamine (S2824) was identified as a hit in the screen. This research discusses our efforts to synthesize and test 30 analogs of this hit for activity against *Mycobacterium tuberculosis*. Two compounds with homopiperazine ring possess high in vitro activity against drug sensitive and resistant *M. tuberculosis* with MICs 0.78–3.13 μg/mL (or 1.22–4.88 μM).

Tuberculosis is one of the leading causes of death worldwide due to an infectious agent. The WHO estimates that there are about 9.2 million new cases of active TB disease each year and about 1.7 million deaths.[1] The rising incidence of tuberculosis, especially the multidrug resistant tuberculosis (MDR-TB), has complicated the global fight against TB. Consequently there is an urgent need to call for the development of new drugs with potent antimycobacterial activity.

In order to identify new and potent candidate drug to treat tuberculosis, we used high-throughput screening from our chemical library to discover potential new antituberculosis agents. One compound S2824, (*S,S*)-*N,N′*-bis-[3-(2,2′,6,6′-tetramethylbenzhydryloxy)-2-hydroxy-propyl]-ethylenediamine (**1**) (Fig. 1) was identified as a lead compound, showing interesting activity against *Mycobacterium tuberculosis* with an MIC = 6.25 μg/mL. According to the structure research, we find that it is structurally similar to ethambutol (EMB) shown in Figure 1; EMB is commonly used as one of the first-line drugs for antituberculosis therapy. In addition, SQ109 (*N*-geranyl-*N′*-(2-adamantyl)ethane-1,2-diamine), which has excellent in vitro and vivo activity against *M. tuberculosis*, is synthesized and screened from diamine analogues of EMB.[2–5] Compound S2824 was then selected for further investigation. This Letter describes our efforts to synthesize and test a series of compounds based on the (*S,S*)-*N,N′*-bis-[3-(2,2′,6,6′-tetramethylbenzhydryloxy)-2-hydroxy-propyl]-ethylenediamine for activity against *M. tuberculosis* in vitro. The effects of the most potent hit compounds in drug sensitive and drug resistant strains from clinical isolates of *M. tuberculosis* and cytotoxicity were also investigated.

The modification of compounds S282401–S282430 focused on replacement of benzyl and ethylenediamine in compound 1 by various aromatic moieties (substituted aryl groups, bicycloaryl groups, heteroaryl) and piperazine or homopiperazine group, respectively. Derivatives S282401-S282420 was synthesized by diarylmethanol **2** with (*R*)-2-(chloromethyl) oxirane or (*S*)-2-(chlo-

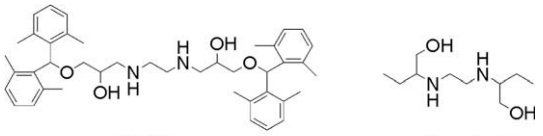

Figure 1. Chemical structures of (*S,S*)-*N,N′*-bis-[3-(2,2′,6,6′-tetramethylbenzhydryloxy)-2-hydroxy-propyl]-ethylenediamine (S2824) and ethambutol (EMB).

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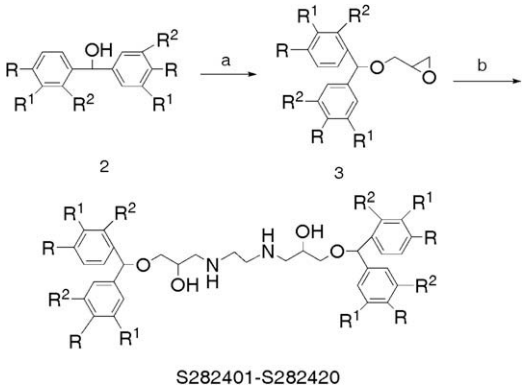
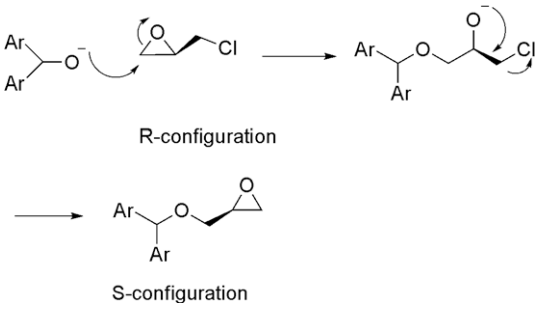
romethyl) oxirane to give intermediate **3**, followed by alkylation of ethylenediamine generated S282401–S282420 (Scheme 1). Moreover, there is an inversion of configuration in the reaction of alkoxides with chloromethyloxirane (Scheme 2). In order to synthesize derivatives S282421–S282424, coupling reaction was carried out with furan or thiophene 4–5 and 2-furaldehyde or 2-thiophenecarboxaldehyde **6**–**7** to obtain difuran-2-ylmethanol or dithiophen-2-ylmethanol **8**–**9**.[6,7] Condensation of **8** or **9** with (*R*)-2-(chloromethyl) oxirane or (*S*)-2-(chloromethyl) oxirane gave intermediate **10**–**13**, followed by alkylation of ethylenediamine which generated S282421–S282424 (Scheme 3). S282425–S282430 were formed by reaction of compound **3** and piperazine or homopiperazine (Scheme 4). All the compounds described in this Letter were purified using silica gel column chromatography, and the structures were confirmed with ^{1}H NMR and HRMS.

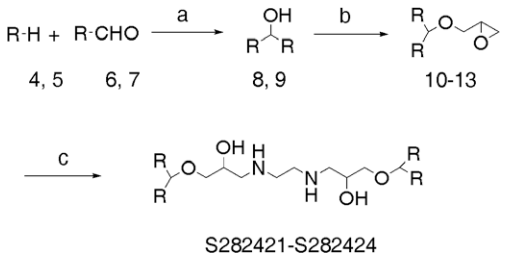
We tested 30 analogs for their in vitro activity against H37Ra at first. In general, all the compounds showed anti-tuberculosis activity. Results showed that the most moderate activity was observed with MICs 25.0 μg/mL. The MICs for (*S*,*S*)-*N*,*N*′-bis-[3-(4,4′-dimethoxybenzhydryloxy)-2-hydroxy-propyl]-ethylenediamine (S282402), (*R*,*R*)-*N*,*N*′-bis-[3-(4,4′-dimethylbenzhydryloxy)-2-hydroxy-propyl]-ethylenediamine (S282405), (*S*,*S*)-*N*,*N*′-bis-[3-(4,4′-dimethylbenzhydryloxy)-2-hydroxy-propyl]-ethylenediamine (S282406) and (*S*,*S*)-*N*,*N*′-bis-[3-(4,4′-dibromobenzhydryloxy)-2-hydroxy-propyl]-ethylenediamine (S282408) were 3.13 μg/mL. The best MICs of 0.78 μg/mL (or 1.22 μM) were found for compounds S282429 and S282430. The data, summarized in Table 1, indicated that small groups such as bromo (S282407 and S282408) or methyl (S282405 and S282406) can further enhance potency. However, larger groups such as tertiary butyl (S282419 and S282420) or oxyisopropyl (S282416 and S282417) caused reduction in potency. When benzene ring was replaced by heteroaromatic, the compounds showed less antimycobacterial activity. Activity could be improved when ethylenediamine was replaced by homopiperazine ring. The data showed in Table 1 indicate that the MIC of S282428 was 1.56 μg/mL (or 2.5 μM) and S282427 displayed relatively moderate activity, although they were only different in configuration.



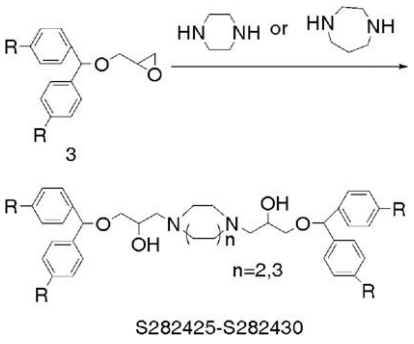
Scheme 1. Reagents and conditions: (a) (*R*)-2-(chloromethyl)oxirane or (*S*)-2-(chloromethyl)oxirane/50% NaOH–H_2O, rt; (b) ethylenediamine/CH_3OH, 60 °C.



Scheme 2. Inversion of configuration in the reaction of alkoxides with chloromethyloxirane.



Scheme 3. Reagents and conditions: (a) *n*-BuLi/THF, −78 °C; (b) (*R*)-2-(chloromethyl) oxirane or (*S*)-2-(chloromethyl) oxirane/50% NaOH–H_2O, rt; (c) ethylenediamine/CH_3OH, 60 °C.



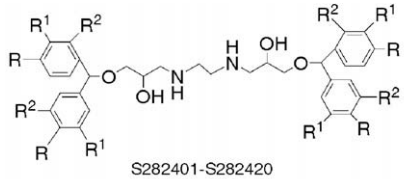
Scheme 4. Reagent and condition: CH_3OH, 60 °C.

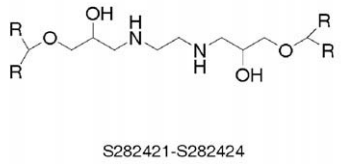
To evaluate whether the anti-TB activity shown by compounds was bacteriostatic or cidal, MBCs and MICs of active compounds with MICs equivalent to and lower than 3.13 μg/mL against H37Ra strain were determined for strain H37Rv. A compound is generally considered to be bactericidal if the ratio of MIC to MBC is ⩽4.[8] As shown in Table 2, S282405, S282406 and S282430 could be considered to be bactericidal due to the low ratios obtained. On the other hand, S282428 and S282429 induced moderate bactericidal activity at concentrations equivalent to their MIC. However, S282402 and S282408 appeared to be primarily bacteriostatic.

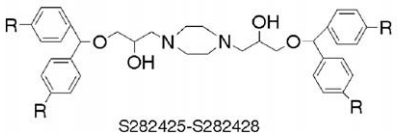
Three of the most potent hit compounds with good antimycobacterial activity were subjected to the determination of MICs against drug-sensitive and MDR clinical isolates of *M. tuberculosis* by BACTEC 460 system.[9] Data in Table 3 showed that S282429 and S282430 were highly active against all the isolates tested and were equally active against the drug-sensitive and MDR-TB, with MICs ranging from 0.78 to 3.13 μg/ml (or 1.22–4.88 μM).

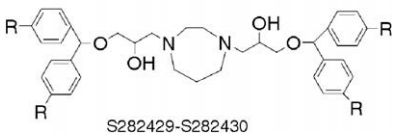
Compared with the activity of EMB, the hit compounds were more active against drug-resistant strains of *M. tuberculosis*. Moreover, the MICs range of S282429 and S282430 do not change for MDR strains compared to drug-sensitive strains of *M. tuberculosis*. The susceptibilities of MDR strains can be indicated by the radios of MICs against resistance and sensitive strains,[8] which were generally ∼2 for S282429 and S282430. This indicated that there is a little cross-resistance with current anti-TB drugs thereby supporting a novel mechanism of action. Four strains tested were resistant to isoniazid, rifampicin, streptomycin and ethambutol, indicating that S282429 and S282430 will maintain activity on MDR strains. However, S282428 showed moderate activity against drug-resistant strains compared with S282429 and S282430 for the activity on the drug sensitive stains and the drug-resistant strains varied 2–4-fold.

Table 1
Structures and in vitro activity against *M. tuberculosis* H37Ra









Compound	R	R^1	R^2	Iso	MIC[a] μg/mL (or μM)
S282401	OCH_3	H	H	4*R*,11*R*	6.25 (9.46)
S282402	OCH_3	H	H	4*S*,11*S*	3.13 (4.73)
S282403	CH_3OCH_2O	H	H	4*R*,11*R*	6.25 (8.0)
S282404	CH_3OCH_2O	H	H	4*S*,11*S*	6.25 (8.0)
S282405	CH_3	H	H	4*R*,11*R*	3.13 (5.24)
S282406	CH_3	H	H	4*S*,11*S*	3.13 (5.24)
S282407	Br	H	H	4*R*,11*R*	6.25 (7.34)
S282408	Br	H	H	4*S*,11*S*	3.13 (3.67)
S282409	OCH_3	OCH_3	H	4*R*,11*R*	12.5 (16.0)
S282410	OCH_3	OCH_3	H	4*S*,11*S*	12.5 (16.0)
S282411	OCH_2CH_2O	H	4*R*,11*R*	12.5	12.5 (16.17)
S282412	OC_2H_5	H	H	4*R*,11*R*	6.25 (8.72)
S282413	OC_2H_5	H	H	4*S*,11*S*	6.25 (8.72)
S282414	OCH_2O	H	4*R*,11*R*	12.5	12.5 (17.44)
S282415	OCH_2O		H	4*S*,11*S*	6.25 (8.72)
S282416	OC_3H_7-*i*	H	H	4*R*,11*R*	6.25 (8.09)
S282417	OC_3H_7-*i*	H	H	4*S*,11*S*	12.5 (16.18)
S282418	OCH_3	OCH_3	OCH_3	4*R*,11*R*	12.5 (13.87)
S282419	*t*-C_4H_9	H	H	4*R*,11*R*	25.0 (32.67)
S282420	*t*-C_4H_9	H	H	4*S*,11*S*	12.5 (16.34)
S282421		—	—	4*R*,11*R*	25.0 (49.95)
S282422		—	—	4*S*,11*S*	12.5 (24.97)
S282423		—	—	4*R*,11*R*	12.5 (22.13)
S282424		—	—	4*S*,11*S*	12.5 (22.13)
S282425	Br	—	—	4*S*,11*S*	25.0 (28.33)
S282426	Br	—	—	4*R*,11*R*	25.0 (28.33)
S282427	CH_3	—	—	4*S*,11*S*	25.0 (40.14)
S282428	CH_3	—	—	4*R*,11*R*	1.56 (2.50)
S282429	CH_3	—	—	4*R*,11*R*	0.78 (1.22)
S282430	CH_3	—	—	4*S*,11*S*	0.78 (1.22)

[a] MICs were determined for H37Ra as described in Supplementary data. MIC of ethambutol (EMB) was 1.56 μg/mL or 7.64 μM.

According to the research, anti-TB activity was improved when ethylenediamine was replaced by homopiperazine ring, which suggest a synthetic direction for further improvement of antituberculosis activity. Furthermore, active compounds including S282428, S282429 and S282430 were also assessed for cytotoxicity using an in vitro assay with the THP-1 cell line. In generally, the selectivity indices (SI) obtained by dividing the cytotoxic IC_{50} values by the broth MICs were higher than 10, which suggests that the compounds may be considered suitable for further screening. The compounds S282428, S282429 and S282430 with SIs were 12, 26.92, and 30.77, respectively, suggesting that there is a larger therapeutic window for the selective killing of *M. tuberculosis* with these three compounds.

In summary, high-throughput screening of a chemical library identified a hit compound with anti-tuberculosis activity and an unknown mechanism of action. Based on this hit compound, a series of compounds were synthesized and the in vitro antituberculosis activity investigated. S282429 and S282430 with homopiperazine ring displayed better efficacies for *M. tuberculosis* in vitro including activity for MDR-TB clinical isolates (MIC = 0.78–3.13 μg/mL) and higher SI values (>10). Further development of these compounds includes modification of S282429 and S282430,

Table 2
MICs and MBCs against *M. tuberculosis* H37Rv

Compound	H37Rv		
	MIC[a] (μg/mL)	MBC[b] (μg/mL)	MBC/MIC
S282402	3.13	25	8
S282405	3.13	6.25	2
S282406	3.13	6.25	2
S282408	3.13	25	8
S282428	1.56	6.25	4
S282429	0.78	3.13	4
S282430	0.78	1.56	2
EMB	1.56	3.13	2

[a] MICs were determined for H37Rv as described in Supplementary data.
[b] The MBC was determined for H37Rv strain by sub-culturing onto drug-free solid medium and enumeration of CFU.

Table 3
MICs of compounds for drug sensitive and resistant clinical isolates of *M. tuberculosis*

Isolate resistance profile[a]	MIC (μg/mL)			
	S282428	S282429	S282430	EMB
Sensitive	3.13	0.78	0.78	3.13
Sensitive	3.13	1.56	1.56	3.13
Sensitive	3.13	1.56	1.56	3.13
INH,RIF	6.25	1.56	3.13	6.25
INH,RIF	12.5	3.13	1.56	3.13
INH,RIF	6.25	3.13	1.56	3.13
INH,RIF,EMB,STR	6.25	1.56	1.56	12.5
INH,RIF,EMB,STR	12.5	3.13	3.13	>12.5
INH,RIF,EMB,STR	6.25	3.13	0.78	>12.5
INH,RIF,EMB,STR	6.25	3.13	1.56	>12.5

[a] Susceptibility of drug-resistant isolates to the following compounds were tested at Shanghai Pulmonary Hospital where the isolates were collected: The resistance cutoffs for the following drugs were: isoniazid (INH) at 0.2 μg/mL, rifampin (RIF) at 2.0 μg/mL, ethambutol (EMB) at 7.5 μg/mL, streptomycin (STR) at 4.0 μg/

evaluation of the most active compounds in the mouse model of tuberculosis infection. The data presented in this paper provide a clear indication that the promising activities of these compounds may be important for development of much needed drugs for improved treatment of MDR/XDR-TB.[10,11]

Acknowledgements

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Supplementary data

Supplementary data (the detailed synthetic and microbiological procedures, as well as full spectral characterization of the new compounds) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.09.035.

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