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Discovery, synthesis, and biological evaluation of piperidinol analogs with anti-tuberculosis activity

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ABSTRACT

Direct anti-tuberculosis screening of commercially available compound libraries identified a novel piperidinol with interesting anti-tuberculosis activity and drug like characteristics. To generate a structure activity relationship about this hit a 22 member optimization library was generated using parallel synthesis. Products of this library 1-((*R*)-3-(4-chlorophenoxy)-2-hydroxypropyl)-4-(4-chloro-3-(trifluoromethyl) phenyl)piperidin-4-ol and 1-((*S*)-3-(4-(trifluoromethyl) phenoxy)-2-hydroxypropyl)-4-(4-chloro-3-(trifluoromethyl) phenyl) piperidin-4-ol demonstrated good anti-tuberculosis activity. Unfortunately, side effects were observed upon in vivo anti-tuberculosis testing of these compounds precluding their further advancement, which may be in part due to the secondary pharmacology associated with the aryl piperidinol core.

1. Introduction

Tuberculosis continues to pose a serious world health threat for which there is an urgent need to develop new therapeutic options.[1] In our ongoing effort to discover novel anti-tuberculosis therapeutics we have been performing primary anti-tuberculosis screening of commercially available libraries. This effort produced a piperidinol hit (**1** in Fig. 1). The anti-tuberculosis activity of **1** was confirmed by resynthesis and biological reevaluation showing good anti-tuberculosis activity with an MIC 1.5 μg/mL. Compound **1** was then selected for further investigation because: (i) it is structurally similar to existing orally bioavailable drugs haloperidol and penfluridol[2,3] shown in Figure 1; (ii) there has been reported bioactivity of a number of compounds bearing 4-chloro-3-trifluoromethylphenyl-4-piperidinol scaffold as antivirals,[4] antibacterials,[5] mu receptor agonists,[6] and nociceptin (N/OFQ) receptor ligands[7] suggesting that it is a biologically useful scaffold for a variety of targets; (iii) there are no reports of similar compounds with anti-tuberculosis activity and thus **1** represents a potentially novel scaffold or pharmacophore for anti-tuberculosis activity, which is unlikely to be cross resistant to existing tuberculosis therapies. To this end, we became interested in the synthesis and evaluation of a hit optimization library about compound **1** in an effort to obtain a preliminary structure-activity relationship (SAR). In this manuscript, the design and synthesis of a series of analogs related to **1** is reported and the resulting anti-tuberculosis SAR is discussed.

2. Chemistry

The chemistry to make racemic and chiral aryl piperidinol analogs is shown in Schemes 1 and 2, respectively.[8,9] The synthesis of racemic products **1** and **2** starts from commercially available piperidinol and racemic epoxide starting materials. Epoxide ring opening by piperidine afforded diol product **1** and **2** in 75% and 79% yield, respectively (Scheme 1). The synthesis of the chiral piperidinol compounds **4a**–**p** was first performed by reacting optically active epoxide intermediate (*S*)-(+)-epichlorohydrin or (*R*)-(−)-epichlorohydrin with a substituted phenol or thiophenol in acetonitrile under reflux in the presence of cesium carbonate to afford chiral epoxide derivative **3**.[8] The yield of this reaction was limited by a competing epoxide ring opening with the phenoxide nucleophile.[10,11] However, the bis-substituted byproducts[12] (scheme 2) were most easily removed by column chromatography purification in the next step, therefore the crude product intermediate **3** was used directly without further purification. The crude preparation of **3** was reacted with 4-[4-chloro-3-(trifluoromethyl)-phenyl]-4-piperidinol in ethanol under reflux to afford alcohol diols **4a**–**p** in 19–50% two step overall yield after purification by silica gel column chromatography (Scheme 2).[9] Based on the biological data of the first series, a second set of compounds was designed and synthesized to evaluate changes to the 4-Cl, 3-CF_3 phenyl piperidinol core of **4b** in an effort to gain further understanding of the A

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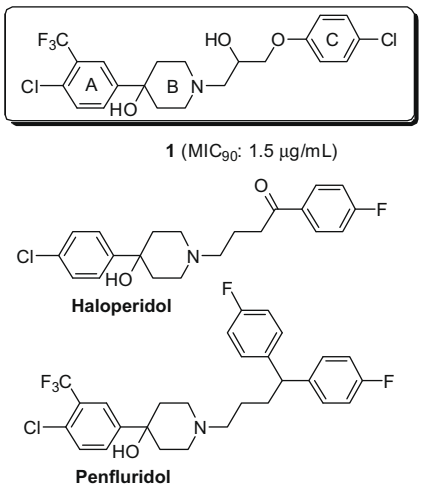
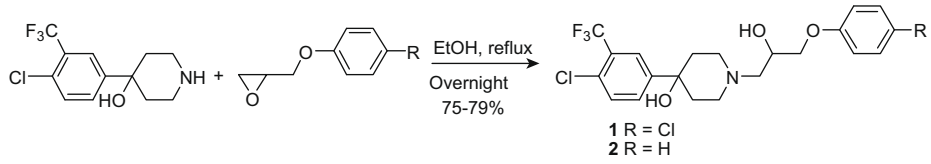


Figure 1. Chemical structures of piperidinol library hit **1**, haloperidol, and penfluridol.

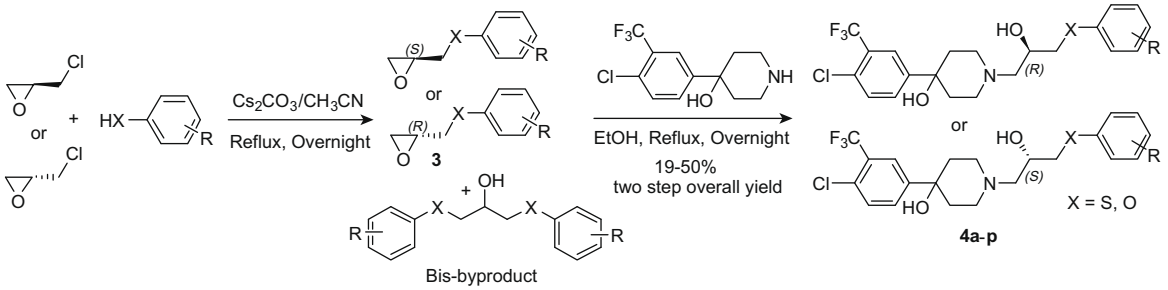
and B-ring SAR, compound **4b** was selected for further optimization because it is the most active from the first series and has the highest therapeutic index. As outlined in Scheme 3, the same synthetic strategy was employed as in the synthesis of **4a**-**p** except that starting materials bearing different piperidine scaffolds were used. Optically active epoxide (*S*)-(+)-epichlorohydrin was firstly reacted with 4-chlorophenol in the presence of cesium carbonate to give the chiral epoxide derivative, which was subsequently treated with a panel of 4-piperidines in ethanol under reflux to afford alcohol **5a**–**e**, in overall yield 37–57% after purification by silica gel chromatography. (Scheme 3).

3. Biological activity

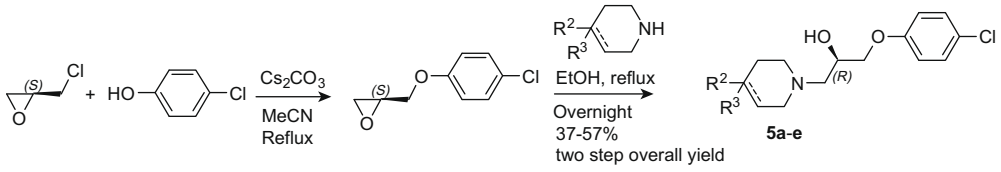
All derivatives were evaluated for anti-tuberculosis activity and the results are described in Tables 1 and 2. In general, compounds **1**, **2**, and **4a**–**p** demonstrated anti-tuberculosis activity with minimum inhibitory concentrations (MICs) ranging from 1.4 to 18.8 μg/mL (Table 1). The most active compounds are: **4b** (MIC 1.4 μg/mL) with *R* stereochemistry at the central secondary hydroxyl group and chloro substitution at para position to the aryl C-ring; and **4m** (MIC 1.7 μg/mL) with *S* stereochemistry at the central secondary hydroxyl group and trifluoromethyl group at para position of the aryl C-ring. In addition, among eight pairs of enantiomers synthesized, the *R* isomers **4b**, **4j**, and **4l** of three pairs of enantiomers showed more potent anti-tuberculosis activity than their corresponding *S* isomers, whilst in the remaining five pairs of enantiomers, the *S* isomers demonstrated approximately equal (**4c** and **4o**) or more than one fold active (**4e**, **4g**, and **4m**) anti-tuberculosis activity compared with their corresponding *R* isomers. Based on these data, there appears to be no correlation or preference between stereochemistry at the central secondary hydroxyl group and anti-tuberculosis activity. The SAR with respect to the phenoxy C ring was clearer with 4-chloro (**4a,b**) and 4-trifluoromethyl (**4m**,**n**) analogs being the most active and unsubstituted phenyl (**4c**, **d** and **4e**, **f**) and the 2,5-dimethyl substituted (**4o,p**) analogs being the least active. The anti-tuberculosis activities of the second set of compounds are shown in Table 2. Compared with parent compound **4b**, compounds **5a**–**c** had significant decrease of anti-tuberculosis activity. This demonstrated the importance of 4-chloro and 3-CF_3 groups. Removal of either of these groups is detrimental to anti-tuberculosis activity by comparing compound **5b**, **5c**, and **4b,** removal of both groups in compound **5a** led to the significant loss of activity. The tertiary hydroxyl group also has a significant effect on activity as well. Replacement of it with a cyano



Scheme 1. Synthesis of piperidinol racemate analogs **1** and **2**.

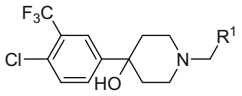


Scheme 2. Synthesis of optically active piperidinol analogs **4a**–**p**.



Scheme 3. Synthesis of optically active piperidinol analogs **5a**–**e**.

Table 1
In vitro anti-tuberculosis activity, cytotoxic LD_{50}, and therapeutic indices of piperidinol analogs **1**, **2**, and **4** optimized at R^1 position



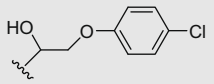
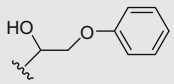
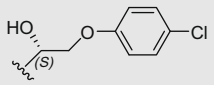
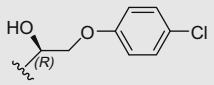
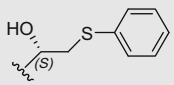
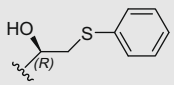
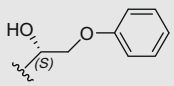
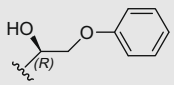
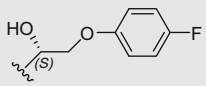
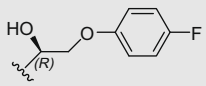
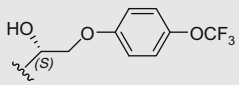
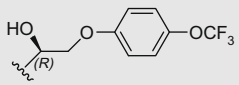
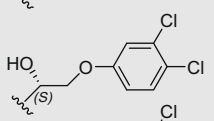
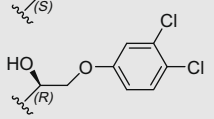
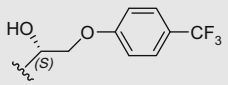
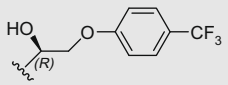
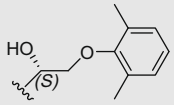
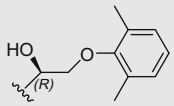
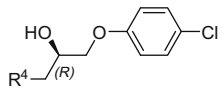
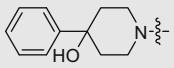
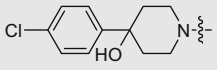
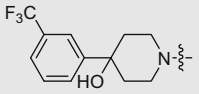
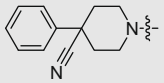
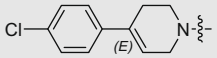
Compd	R^1	MIC (μg/mL)	Cytotoxic LD_{50} (μg/mL)	Therapeutic index
1		1.5	17.9	11.9
2		10.4	19.5	1.9
4a		3.8	17.9	4.7
4b		1.4	18.2	13.3
4c		5.2	35.7	6.8
4d		8.3	37.1	4.4
4e		8.3	34.9	4.2
4f		18.8	27.7	1.5
4g		3.9	25.8	6.6
4h		10.4	20.8	2.0
4i		7.3	17.6	2.4
4j		3.6	17.7	4.9
4k		7.3	18.7	2.6
4l		3.1	18.0	5.8
4m		1.7	18.7	11.0
4n		5.2	18.3	3.5
4o		4.7	19.0	4.1
4p		5.9	19.3	3.3

Table 2
In vitro anti-tuberculosis activity of piperidinol analogs **5** optimized at R^4 position



Compd	R^4	MIC (μg/mL)
5a		100
5b		25
5c		25
5d		25
5e		>200

group resulted in a more active compound **5d** and replacement with a dehydration product **5e** results led to a complete loss of anti-tuberculosis activity (Table 2).

The cytotoxicities of the compounds were next evaluated against eukaryotic Vero cells (Table 1) to determine their suitability for further advancement. In general cytotoxicities were observed producing narrow therapeutic indices that ranged from 1.5 for **4f** to 13.3 for **4b**. However, the selectivity of **4b** and **4m** was sufficient to advance for testing in vivo (>10). In subsequent experiments for anti-tuberculosis efficacy of **4b** in mice,[13] severe adverse effects were observed resulting in death at doses higher than 50–100 mg/kg. These symptoms precluded further testing of these compounds.

4. Conclusions

In summary, high-throughput screening of a commercial library identified a piperidinol hit with anti-tuberculosis activities and an unknown mechanism of action. Based on this hit compound, two expanded sets of compounds **4a–p** and **5a–e** were subsequently designed, synthesized, and evaluated toward anti-tuberculosis activity. Two compounds **4b** and **4m** were identified with good activity (MIC: 1.4–1.7 μg/mL) and acceptable therapeutic indices (>10). We have showed that improvements in the therapeutic index can be made (Table 1), unfortunately these compounds produced significant side effects when tested in vivo for anti-tuberculosis activity in mice likely due to secondary pharmacology associated with 4-[4-chloro-3-(trifluoromethyl)-phenyl]-4-piperidinol core. This study may highlight some of the problems likely to be encountered as more investigators switch to direct anti-tuberculosis screening rather than single enzyme screening to discover novel anti-tuberculosis compounds from compound libraries. As such commercial libraries are rarely truly random and are most often designed around pharmacologically active scaffolds utilized in discovery of human non-infectious diseases, which could compromise their use for infectious disease particularly those requiring long-term treatment such as tuberculosis. As observed in our study, this increases the risk of unwanted side effects from residual secondary pharmacology associated with the chemical scaffold that may preclude further advancement of the series.

5. Experimental

All solvents were purchased from Sigma-Aldrich and Fisher Scientific and used as received, and remaining chemicals were from Sigma–Aldrich. Thin layer chromatography (TLC) analysis was performed on Merck Silica Gel $60F_{254}$ plates and the spots were visualized under a UV lamp. Melting point (mp) was determined using OptiMelt Automated Melting Point System (Stanford Research Systems) and is uncorrected. ^{1}H NMR spectra were recorded at 500 MHz on a Varian Inova NMR instrument. Mass spectra were recorded on a Bruker Esquire LC/MS using ESI. Analytical RP-HPLC was conducted on a Shimadzu HPLC system with a Phenomenex C18 column (100 Å, 3 μm, 4.6 × 50 mm), flow rate 1.0 mL/min and a gradient of solvent A (water with 0.1% TFA) and solvent B (acetonitrile): 0–2.00 min 100% A; 2.00–7.00 min 0–100% B (linear gradient). UV detection at 254 and 218 nm.

5.1. General procedure for the synthesis of the piperidinols 1, 2, 4a–p, and 5a–e

Acetonitrile (15 mL) was added to (*S*)-(+)-epichlorohydrin or (*R*)-(−)-epichlorohydrin (0.188 mL, 2.4 mmol, 1.2 equiv), substituted phenol (2 mmol, 1 equiv), followed by the addition of Cesium carbonate (1.3 g, 4 mmol, 2 equiv). The reaction mixture was stirred under reflux overnight. The reaction was complete based on TLC monitoring. The reaction mixture was filtered and washed with acetonitrile. The filtrate was evaporated in vacuo to give crude product **3**, which was used directly for the next step without further purification.

The crude product obtained above was dissolved in ethanol (15 mL), followed by the addition of 4-[4-chloro-3-(trifluoromethyl)-phenyl]-4-piperidinol or the other 4-piperidines (2 mmol). The reaction was stirred under reflux overnight and the reaction was complete based on HPLC analysis. The resulting solution was evaporated in vacuo and the residue was purified by column chromatography on silica gel (hexane–50% ethyl acetate in hexane in a volume of 255 mL, followed by 100% ethyl acetate in a linear gradient in a volume of 204 mL). Evaporation of appropriate fractions gave final product as white powder.

5.1.1. 1-(3-(4-Chlorophenoxy)-2-hydroxypropyl)-4-(4-chloro-3-(trifluoromethyl)phenyl)piperidin-4-ol, 1 (racemate)

0.35 g (1 mmol scale), 75.4% yield. Mp: 147–149 °C. ^{1}H NMR, 500 MHz (DMSO-d_6): δ 7.92 (d, J = 2.0 Hz, 1H), 7.76 (dd, J = 1.7 and 8.5 Hz, 1H), 7.66 (d, J = 8.3 Hz, 1H), 7.32 (dd, J = 3.3 and 8.8 Hz, 2H), 6.98 (dd, J = 3.3 and 8.8 Hz, 2H), 5.15 (s, 1H, O*H*), 4.85 (d, J = 4.2 Hz, 1H, O*H*), 4.01 (dd, J = 3.5 and 9.5 Hz, 1H, C*H*^{a}O), 3.96 (m, 1H, C*H*OH), 3.88 (dd, J = 5.8 and 9.5 Hz, 1H, C*H*$^{a'}$O), 2.72 (m, 2H), 2.50 (m, 3H, overlapped with DMSO peak), 2.42 (dd, J = 6.5 and 12.6 Hz, 1H, NC*H*^{b}), 1.95 (m, 2H), 1.57 (m, 2H). Mass spectrum (ESI) m/z $(MH)^+$ 464.1, $(M-H)^-$ 461.9. HPLC purity: 100% (254 nm), t_R = 5.52 min.

5.1.2. 4-(4-Chloro-3-(trifluoromethyl)phenyl)-1-(2-hydroxy-3-phenoxypropyl)piperidin-4-ol, 2 (racemate)

0.68 g (2 mmol scale), 79.1% yield. Mp: 127–130 °C. ^{1}H NMR, 500 MHz (DMSO-d_6): δ 7.93 (d, J = 2.0 Hz, 1H), 7.77 (dd, J = 2.0 and 8.5 Hz, 1H), 7.66 (d, J = 8.5 Hz, 1H), 7.29 (m, 2H), 6.95 (m, 3H), 5.15 (s, 1H, O*H*), 4.82 (br s, 1H, O*H*), 3.99 (m, 2H, C*H*^{a}O and C*H*OH), 3.88 (dd, J = 5.4 and 9.0 Hz, 1H, C*H*$^{a'}$O), 2.73 (m, 2H), 2.50 (m, 3H, overlapped with DMSO peak), 2.43 (dd, J = 6.4 and 12.5 Hz, 1H, NC*H*^{b}), 1.95 (m, 2H), 1.57 (m, 2H). Mass spectrum (ESI) m/z $(MH)^+$ 430.1, $(M-H)^-$ 428.0. HPLC purity: 100% (254 nm), t_R = 5.38 min.

5.1.3. 1-((*S*)-3-(4-Chlorophenoxy)-2-hydroxypropyl)-4-(4-chloro-3-(trifluoromethyl)phenyl)piperidin-4-ol, 4a

0.39 g (2 mmol scale), 42.0% 2-step overall yield. Mp: 146–149 °C. ^{1}H NMR, 500 MHz (DMSO-d_6): δ 7.92 (d, J = 2.0 Hz, 1H), 7.76 (dd, J = 1.7 and 8.5 Hz, 1H), 7.66 (d, J = 8.5 Hz, 1H), 7.32 (dd, J = 3.5 and 8.9 Hz, 2H), 6.98 (dd, J = 3.4 and 8.9 Hz, 2H), 5.15 (s, 1H, O*H*), 4.84 (d, J = 4.6 Hz, 1H, O*H*), 4.01 (dd, J = 3.5 and 9.5 Hz, 1H, C*H*^{a}O), 3.96 (m, 1H, C*H*OH), 3.88 (dd, J = 5.9 and 9.5 Hz, 1H, C*H*$^{a'}$O), 2.72 (m, 2H), 2.50 (m, 3H, overlapped with DMSO peak), 2.41 (dd, J = 6.5 and 12.6 Hz, 1H, NC*H*^{b}), 1.95 (m, 2H), 1.56 (m, 2H). Mass spectrum (ESI) m/z (MH)$^+$ 464.1, (M−H)$^-$ 461.9. HPLC purity: 99.1% (218 nm), t_R = 5.59 min.

5.1.4. 1-((*R*)-3-(4-Chlorophenoxy)-2-hydroxypropyl)-4-(4-chloro-3-(trifluoromethyl)phenyl)piperidin-4-ol, 4b

0.30 g (2 mmol scale), 32.3% 2-step overall yield. Mp: 144–146 °C. ^{1}H NMR, 500 MHz (DMSO-d_6): δ 7.93 (d, J = 2.0 Hz, 1H), 7.76 (dd, J = 2.0 and 8.5 Hz, 1H), 7.66 (d, J = 8.3 Hz, 1H), 7.32 (dd, J = 3.5 and 9.0 Hz, 2H), 6.98 (dd, J = 3.4 and 9.0 Hz, 2H), 5.14 (s, 1H, O*H*), 4.84 (d, J = 4.4 Hz, 1H, O*H*), 4.01 (dd, J = 3.7 and 9.8 Hz, 1H, C*H*^{a}O), 3.96 (m, 1H, C*H*OH), 3.88 (dd, J = 5.9 and 9.5 Hz, 1H, C*H*$^{a'}$O), 2.72 (m, 2H), 2.50 (m, 3H, overlapped with DMSO peak), 2.41 (dd, J = 6.4 and 12.5 Hz, 1H, NC*H*^{b}), 1.95 (m, 2H), 1.56 (m, 2H). Mass spectrum (ESI) m/z (MH)$^+$ 464.1, (M−H)$^-$ 461.9. HPLC purity: 100% (254 nm), t_R = 5.58 min; 98.8% (218 nm), t_R = 5.59 min.

5.1.5. 4-(4-Chloro-3-(trifluoromethyl)phenyl)-1-((*S*)-2-hydroxy-3-(phenylthio)propyl)piperidin-4-ol, 4c

0.17 g (2 mmol scale), 19.1% 2-step overall yield. Mp: 117–119 °C. ^{1}H NMR, 500 MHz ($CDCl_3$): δ 7.84 (s, 1H), 7.56 (d, J = 8.1 Hz, 1H), 7.45 (d, J = 8.3 Hz, 1H), 7.37 (d, J = 7.8 Hz, 2H), 7.28 (t, J = 7.8 Hz, 2H), 7.18 (t, J = 7.3 Hz, 1H), 3.89 (m, 1H, C*H*OH), 3.58 (br s, 2H, O*H* and O*H*), 3.03 (m, 2H, CH_2S), 2.81 (d, J = 11.0 Hz, 1H, NC*H*$^{b'}$), 2.71 (m, 2H), 2.58 (dd, J = 2.9 and 12.5 Hz, 1H, NC*H*^{b}), 2.45 (t, J = 10.3 Hz, 2H), 2.09 (m, 1H), 2.01 (m, 2H), 1.70 (m, 2H). Mass spectrum (ESI) m/z (MH)$^+$ 446.1, (M−H)$^-$ 443.9. HPLC purity: 99.4% (254 nm), t_R = 5.48 min; 99.5% (218 nm), t_R = 5.48 min.

5.1.6. 4-(4-Chloro-3-(trifluoromethyl)phenyl)-1-((*R*)-2-hydroxy-3-(phenylthio)propyl)piperidin-4-ol, 4d

0.22 g (2 mmol scale), 24.7% 2-step overall yield. Mp: 116–119 °C. ^{1}H NMR, 500 MHz (DMSO-d_6): δ 7.93 (d, J = 2.0 Hz, 1H), 7.77 (dd, J = 2.0 and 8.3 Hz, 1H), 7.68 (d, J = 8.3 Hz, 1H), 7.37 (d, J = 7.6 Hz, 2H), 7.31 (t, J = 7.8 Hz, 2H), 7.17 (t, J = 7.3 Hz, 1H), 5.14 (s, 1H, O*H*), 4.88 (d, J = 4.6 Hz, 1H, O*H*), 3.80 (m, 1H, C*H*OH), 3.19 (dd, J = 4.6 and 13.2 Hz, 1H, C*H*^{a}S), 2.96 (dd, J = 6.7 and 13.2 Hz, 1H, C*H*$^{a''}$S), 2.65 (m, 2H), 2.46 (m, 3H), 2.40 (dd, J = 6.4 and 12.7 Hz, 1H, NC*H*^{b}), 1.94 (m, 2H), 1.55 (m, 2H). Mass spectrum (ESI) m/z (MH)$^+$ 446.1, (M−H)$^-$ 443.9. HPLC purity: 99.3% (254 nm), t_R = 5.48 min; 99.3% (218 nm), t_R = 5.48 min.

5.1.7. 4-(4-Chloro-3-(trifluoromethyl)phenyl)-1-((*S*)-2-hydroxy-3-phenoxypropyl)piperidin-4-ol, 4e

0.40 g (2 mmol scale), 46.5% 2-step overall yield. Mp: 124–126 °C. ^{1}H NMR, 500 MHz (DMSO-d_6): δ 7.93 (d, J = 1.7 Hz, 1H), 7.77 (dd, J = 1.7 and 8.3 Hz, 1H), 7.67 (d, J = 8.3 Hz, 1H), 7.29 (m, 2H), 6.94 (m, 3H), 5.15 (s, 1H, O*H*), 4.81 (d, J = 4.4 Hz, 1H, O*H*), 3.99 (m, 2H, C*H*OH and C*H*^{a}O), 3.89 (dd, J = 5.5 and 9.2 Hz, 1H, C*H*$^{a'}$O), 2.74 (m, 2H), 2.51 (m, 3H), 2.43 (dd, J = 6.4 and 12.7 Hz, 1H, NC*H*^{b}), 1.96 (m, 2H), 1.57 (m, 2H). Mass spectrum (ESI) m/z (MH)$^+$ 430.1, (MNa)$^+$ 452.1, (M−H)$^-$ 427.9. HPLC purity: 100% (254 nm), t_R = 5.42 min; 100% (218 nm), t_R = 5.43 min.

5.1.8. 4-(4-Chloro-3-(trifluoromethyl)phenyl)-1-((*R*)-2-hydroxy-3-phenoxypropyl)piperidin-4-ol, 4f

0.43 g (2 mmol scale), 50.0% 2-step overall yield. Mp: 124–126 °C. ^{1}H NMR, 500 MHz (DMSO-d_6): δ 7.93 (d, J = 1.2 Hz, 1H), 7.77 (dd, J = 1.2 and 8.5 Hz, 1H), 7.67 (d, J = 8.5 Hz, 1H), 7.30 (t, J = 7.6 Hz, 2H), 6.94 (m, 3H), 5.15 (s, 1H, O*H*), 4.81 (d, J = 4.4 Hz, 1H, O*H*), 3.99 (m, 2H, C*H*OH and C*H*^{a}O), 3.89 (dd, J = 5.6 and 9.3 Hz, 1H, C*H*$^{a'}$O), 2.74 (m, 2H), 2.51 (m, 3H), 2.43 (dd, J = 6.4 and 12.5 Hz, 1H, NC*H*^{b}), 1.96 (m, 2H), 1.57 (m, 2H). Mass spectrum (ESI) m/z (MH)$^+$ 430.1, (MNa)$^+$ 452.1, (M−H)$^-$ 427.9. HPLC purity: 100% (254 nm), t_R = 5.41 min; 99.4% (218 nm), t_R = 5.42 min.

5.1.9. 1-((*S*)-3-(4-Fluorophenoxy)-2-hydroxypropyl)-4-(4-chloro-3-(trifluoromethyl)phenyl)piperidin-4-ol, 4g

0.35 g (2 mmol scale), 39.1% 2-step overall yield. Mp: 114–115 °C. ^{1}H NMR, 500 MHz (DMSO-d_6): δ 7.93 (d, J = 2.0 Hz, 1H), 7.77 (dd, J = 1.7 and 8.5 Hz, 1H), 7.67 (d, J = 8.5 Hz, 1H), 7.12 (m, 2H), 6.97 (m, 2H), 5.15 (s, 1H, O*H*), 4.81 (d, J = 4.2 Hz, 1H, O*H*), 3.98 (m, 2H, C*H*OH and C*H*^{a}O), 3.87 (dd, J = 5.4 and 9.0 Hz, 1H, C*H*$^{a'}$O), 2.72 (m, 2H), 2.50 (m, 3H, overlapped with DMSO peak), 2.42 (dd, J = 6.4 and 12.7 Hz, 1H, NC*H*^{b}), 1.95 (m, 2H), 1.56 (m, 2H). Mass spectrum (ESI) m/z (MH)$^+$ 448.1, (MNa)$^+$ 470.1, (M−H)$^-$ 445.9. HPLC purity: 99.5% (254 nm), t_R = 5.44 min; 99.5% (218 nm), t_R = 5.45 min.

5.1.10. 1-((*R*)-3-(4-Fluorophenoxy)-2-hydroxypropyl)-4-(4-chloro-3-(trifluoromethyl)phenyl)piperidin-4-ol, 4h

0.38 g (2 mmol scale), 42.4% 2-step overall yield. Mp: 114–116 °C. ^{1}H NMR, 500 MHz (DMSO-d_6): δ 7.93 (d, J = 1.7 Hz, 1H), 7.77 (dd, J = 1.7 and 8.5 Hz, 1H), 7.67 (d, J = 8.5 Hz, 1H), 7.12 (m, 2H), 6.98 (m, 2H), 5.15 (s, 1H, O*H*), 4.81 (d, J = 4.4 Hz, 1H, O*H*), 3.98 (m, 2H, C*H*OH and C*H*^{a}O), 3.87 (dd, J = 5.4 and 9.0 Hz, 1H, C*H*$^{a'}$O), 2.72 (m, 2H), 2.50 (m, 3H, overlapped with DMSO peak), 2.42 (dd, J = 6.4 and 12.5 Hz, 1H, NC*H*^{b}), 1.96 (m, 2H), 1.57 (m, 2H). Mass spectrum (ESI) m/z (MH)$^+$ 448.1, (M−H)$^-$ 445.9. HPLC purity: 99.2% (254 nm), t_R = 5.43 min; 98.2% (218 nm), t_R = 5.44 min.

5.1.11. 1-((*S*)-3-(4-(Trifluoromethoxy)phenoxy)-2-hydroxypropyl)-4-(4-chloro-3-(trifluoromethyl)phenyl)piperidin-4-ol, 4i

0.43 g (2 mmol scale), 41.8% 2-step overall yield. Mp: 123–126 °C. ^{1}H NMR, 500 MHz (DMSO-d_6): δ 7.93 (d, J = 1.7 Hz, 1H), 7.77 (dd, J = 1.7 and 8.5 Hz, 1H), 7.67 (d, J = 8.5 Hz, 1H), 7.29 (d, J = 9.0 Hz, 2H), 7.06 (d, J = 9.0 Hz, 2H), 5.15 (s, 1H, O*H*), 4.85 (d, J = 4.6 Hz, 1H, O*H*), 4.04 (dd, J = 3.4 and 9.5 Hz, 1H, C*H*^{a}O), 3.98 (m, 1H, C*H*OH), 3.92 (dd, J = 5.9 and 9.5 Hz, 1H, C*H*$^{a'}$O), 2.72 (m, 2H), 2.50 (m, 3H, overlapped with DMSO peak), 2.42 (dd, J = 6.4 and 12.7 Hz, 1H, NC*H*^{b}), 1.95 (m, 2H), 1.57 (m, 2H). Mass spectrum (ESI) m/z (MH)$^+$ 514.2, (M−H)$^-$ 511.9. HPLC purity: 99.0% (254 nm), t_R = 5.70 min; 98.3% (218 nm), t_R = 5.71 min.

5.1.12. 1-((*R*)-3-(4-(Trifluoromethoxy)phenoxy)-2-hydroxypropyl)-4-(4-chloro-3-(trifluoromethyl)phenyl)piperidin-4-ol, 4j

0.43 g (2 mmol scale), 41.8% 2-step overall yield. Mp: 123–125 °C. ^{1}H NMR, 500 MHz (DMSO-d_6): δ 7.93 (d, J = 2.0 Hz, 1H), 7.77 (dd, J = 2.0 and 8.5 Hz, 1H), 7.67 (d, J = 8.5 Hz, 1H), 7.29 (d, J = 8.8 Hz, 2H), 7.06 (d, J = 9.0 Hz, 2H), 5.15 (s, 1H, O*H*), 4.85 (d, J = 4.6 Hz, 1H, O*H*), 4.04 (dd, J = 3.4 and 9.8 Hz, 1H, C*H*^{a}O), 3.98 (m, 1H, C*H*OH), 3.92 (dd, J = 6.1 and 9.8 Hz, 1H, C*H*$^{a'}$O), 2.72 (m, 2H), 2.50 (m, 3H, overlapped with DMSO peak), 2.42 (dd, J = 6.4 and 12.7 Hz, 1H, NC*H*^{b}), 1.95 (m, 2H), 1.57 (m, 2H). Mass spectrum (ESI) m/z (MH)$^+$ 514.2, (M−H)$^-$ 511.9. HPLC purity: 98.9% (254 nm), t_R = 5.71 min; 97.5% (218 nm), t_R = 5.72 min.

5.1.13. 1-((*S*)-3-(3,4-Dichlorophenoxy)-2-hydroxypropyl)-4-(4-chloro-3-(trifluoromethyl)phenyl)piperidin-4-ol, 4k

0.41 g (2 mmol scale), 41.1% 2-step overall yield. Mp: 121–124 °C. ^{1}H NMR, 500 MHz (DMSO-d_6): δ 7.93 (d, J = 2.0 Hz, 1H), 7.77 (dd, J = 2.0 and 8.5 Hz, 1H), 7.67 (d, J = 8.5 Hz, 1H), 7.52 (d, J = 8.8 Hz, 1H), 7.27 (d, J = 2.9 Hz, 1H), 7.00 (dd, J = 2.9 and 9.0 Hz,

1H), 5.15 (s, 1H, O*H*), 4.87 (d, *J* = 4.2 Hz, 1H, O*H*), 4.08 (m, 1H), 3.95 (m, 2H), 2.72 (m, 2H), 2.50 (m, 3H, overlapped with DMSO peak), 2.41 (dd, *J* = 5.9 and 12.5 Hz, 1H, NC*H*^{b}), 1.96 (m, 2H), 1.57 (m, 2H). Mass spectrum (ESI) *m*/*z* $(MH)^+$ 500.0. HPLC purity: 96.1% (254 nm), t_R = 5.69 min; 95.8% (215 nm), t_R = 5.69 min.

5.1.14. 1-((*R*)-3-(3,4-Dichlorophenoxy)-2-hydroxypropyl)-4-(4-chloro-3-(trifluoromethyl)phenyl)piperidin-4-ol, 4l

0.41 g (2 mmol scale), 41.1% 2-step overall yield. Mp: 136–138 °C. ^{1}H NMR, 500 MHz (DMSO-d_6): δ 7.93 (d, *J* = 1.7 Hz, 1H), 7.77 (dd, *J* = 2.0 and 8.5 Hz, 1H), 7.67 (d, *J* = 8.5 Hz, 1H), 7.52 (d, *J* = 9.0 Hz, 1H), 7.27 (d, *J* = 2.9 Hz, 1H), 7.00 (dd, *J* = 2.9 and 9.0 Hz, 1H), 5.15 (s, 1H, O*H*), 4.87 (d, *J* = 3.9 Hz, 1H, O*H*), 4.08 (m, 1H), 3.95 (m, 2H), 2.71 (m, 2H), 2.50 (m, 3H, overlapped with DMSO peak), 2.41 (dd, *J* = 5.9 and 12.5 Hz, 1H, NC*H*^{b}), 1.96 (m, 2H), 1.56 (m, 2H). Mass spectrum (ESI) *m*/*z* $(MH)^+$ 500.0. HPLC purity: 95.8% (254 nm), t_R = 5.68 min; 95.2% (218 nm), t_R = 5.69 min.

5.1.15. 1-((*S*)-3-(4-(Trifluoromethyl)phenoxy)-2-hydroxypropyl)-4-(4-chloro-3-(trifluoromethyl)phenyl)piperidin-4-ol, 4m

0.35 g (2 mmol scale), 35.2% 2-step overall yield. Mp: 135–137 °C. ^{1}H NMR, 500 MHz (DMSO-d_6): δ 7.93 (d, *J* = 2.0 Hz, 1H), 7.77 (dd, *J* = 2.0 and 8.5 Hz, 1H), 7.67 (d, *J* = 8.5 Hz, 1H), 7.65 (d, *J* = 8.5 Hz, 2H), 7.15 (d, *J* = 8.8 Hz, 2H), 5.15 (s, 1H, O*H*), 4.89 (d, *J* = 4.2 Hz, 1H, O*H*), 4.12 (m, 1H), 4.00 (m, 2H), 2.73 (m, 2H), 2.50 (m, 3H, overlapped with DMSO peak), 2.44 (dd, *J* = 5.9 and 12.5 Hz, 1H, NC*H*^{b}), 1.95 (m, 2H), 1.57 (m, 2H). Mass spectrum (ESI) *m*/*z* $(MH)^+$ 498.1. HPLC purity: 98.4% (218 nm), t_R = 5.68 min.

5.1.16. 1-((*R*)-3-(4-(Trifluoromethyl)phenoxy)-2-hydroxypropyl)-4-(4-chloro-3-(trifluoromethyl)phenyl)piperidin-4-ol, 4n

0.21 g (2 mmol scale), 21.1% 2-step overall yield. Mp: 138–141 °C. ^{1}H NMR, 500 MHz (DMSO-d_6): δ 7.92 (d, *J* = 1.7 Hz, 1H), 7.77 (dd, *J* = 1.5 and 8.3 Hz, 1H), 7.67 (d, *J* = 8.3 Hz, 1H), 7.65 (d, *J* = 8.5 Hz, 2H), 7.15 (d, *J* = 8.5 Hz, 2H), 5.15 (s, 1H, O*H*), 4.89 (d, *J* = 4.2 Hz, 1H, O*H*), 4.12 (m, 1H), 4.00 (m, 2H), 2.73 (m, 2H), 2.50 (m, 3H, overlapped with DMSO peak), 2.44 (dd, *J* = 5.9 and 12.5 Hz, 1H, NC*H*^{b}), 1.95 (m, 2H), 1.57 (m, 2H). Mass spectrum (ESI) *m*/*z* $(MH)^+$ 498.1. HPLC purity: 96.8% (218 nm), t_R = 5.69 min.

5.1.17. 1-((*S*)-3-(2,6-Dimethylphenoxy)-2-hydroxypropyl)-4-(4-chloro-3-(trifluoromethyl)phenyl)piperidin-4-ol, 4o

0.37 g (2 mmol scale), 40.4% 2-step overall yield. Mp: 138–141 °C. ^{1}H NMR, 500 MHz (DMSO-d_6): δ 7.93 (d, *J* = 2.0 Hz, 1H), 7.76 (dd, *J* = 1.7 and 8.3 Hz, 1H), 7.68 (d, *J* = 8.5 Hz, 1H), 7.01 (d, *J* = 7.3 Hz, 2H), 6.91 (t, *J* = 7.3 Hz, 1H), 5.16 (s, 1H, O*H*), 4.80 (d, *J* = 4.6 Hz, 1H, O*H*), 3.99 (m, 1H, C*H*OH), 3.76 (dd, *J* = 3.7 and 9.3 Hz, 1H, C*H*^{a}O), 3.70 (dd, *J* = 5.6 and 9.3 Hz, 1H, C*H*$^{a'}$O), 2.75 (m, 2H), 2.57 (dd, *J* = 5.9 and 12.5 Hz, 1H, NC*H*$^{b'}$), 2.50 (m, 2H, overlapped with DMSO peak), 2.44 (dd, *J* = 6.6 and 12.5 Hz, 1H, NC*H*^{b}), 2.26 (s, 6H, CH_3 and CH_3), 1.95 (m, 2H), 1.59 (m, 2H). Mass spectrum (ESI) *m*/*z* $(MH)^+$ 458.1, $(MNa)^+$ 480.1, $(M-H)^-$ 455.9. HPLC purity: 100% (254 nm), t_R = 5.58 min; 100% (218 nm), t_R = 5.58 min.

5.1.18. 1-((*R*)-3-(2,6-Dimethylphenoxy)-2-hydroxypropyl)-4-(4-chloro-3-(trifluoromethyl)phenyl)piperidin-4-ol, 4p

0.43 g (2 mmol scale), 47.0% 2-step overall yield. Mp: 138–141 °C. ^{1}H NMR, 500 MHz (DMSO-d_6): δ 7.94 (d, *J* = 1.7 Hz, 1H), 7.76 (dd, *J* = 1.5 and 8.3 Hz, 1H), 7.67 (d, *J* = 8.5 Hz, 1H), 7.01 (d, *J* = 7.6 Hz, 2H), 6.91 (t, *J* = 7.6 Hz, 1H), 5.16 (s, 1H, O*H*), 4.80 (d, *J* = 4.4 Hz, 1H, O*H*), 3.99 (m, 1H, C*H*OH), 3.76 (dd, *J* = 3.9 and 9.3 Hz, 1H, C*H*^{a}O), 3.70 (dd, *J* = 5.6 and 9.3 Hz, 1H, C*H*$^{a'}$O), 2.75 (m, 2H), 2.57 (dd, *J* = 6.1 and 12.5 Hz, 1H, NC*H*$^{b'}$), 2.50 (m, 2H, overlapped with DMSO peak), 2.44 (dd, *J* = 6.6 and 12.5 Hz, 1H, NC*H*^{b}), 2.26 (s, 6H, CH_3 and CH_3), 1.95 (m, 2H), 1.59 (m, 2H). Mass spectrum (ESI) *m*/*z* $(MH)^+$ 458.1, $(MNa)^+$ 480.1, $(M-H)^-$ 455.9. HPLC purity: 100% (254 nm), t_R = 5.58 min; 100% (218 nm), t_R = 5.58 min.

5.1.19. 1-((*R*)-3-(4-Chlorophenoxy)-2-hydroxypropyl)-4-phenylpiperidin-4-ol, 5a

0.41 g (2 mmol scale), 56.7% 2-step overall yield. Mp: 108–111 °C. ^{1}H NMR, 500 MHz (DMSO-d_6): δ 7.48 (d, *J* = 7.6 Hz, 2H), 7.32 (m, 4H), 7.20 (t, *J* = 7.3 Hz, 1H), 6.99 (d, *J* = 9.0 Hz, 2H), 4.84 (d, *J* = 4.6 Hz, 1H, O*H*), 4.77 (s, 1H, O*H*), 4.02 (dd, *J* = 3.4 and 9.5 Hz, 1H, C*H*^{a}O), 3.97 (m, 1H, C*H*OH), 3.88 (dd, *J* = 6.1 and 9.5 Hz, 1H, C*H*$^{a'}$O), 2.69 (m, 2H), 2.50 (m, 3H, overlapped with DMSO peak), 2.41 (dd, *J* = 6.6 and 12.7 Hz, 1H, NC*H*^{b}), 1.93 (m, 2H), 1.57 (m, 2H). Mass spectrum (ESI) *m*/*z* $(MH)^+$ 362.3, $(MNa)^+$ 384.1. HPLC purity: 100% (254 nm), t_R = 5.16 min; 100% (215 nm), t_R = 5.16 min.

5.1.20. 1-((*R*)-3-(4-Chlorophenoxy)-2-hydroxypropyl)-4-(4-chlorophenyl)piperidin-4-ol, 5b

0.35 g (2 mmol scale), 44.2% 2-step overall yield. Mp: 146–148 °C. ^{1}H NMR, 500 MHz (DMSO-d_6): δ 7.50 (d, *J* = 8.5 Hz, 2H), 7.36 (d, *J* = 8.3 Hz, 2H), 7.33 (d, *J* = 8.8 Hz, 2H), 6.99 (d, *J* = 8.8 Hz, 2H), 4.89 (s, 1H, O*H*), 4.84 (d, *J* = 4.4 Hz, 1H, O*H*), 4.01 (dd, *J* = 3.7 and 9.8 Hz, 1H, C*H*^{a}O), 3.97 (m, 1H, C*H*OH), 3.88 (dd, *J* = 6.1 and 9.8 Hz, 1H, C*H*$^{a'}$O), 2.69 (m, 2H), 2.50 (m, 3H, overlapped with DMSO peak), 2.41 (dd, *J* = 6.4 and 12.5 Hz, 1H, NC*H*^{b}), 1.90 (m, 2H), 1.56 (m, 2H). Mass spectrum (ESI) *m*/*z* $(MH)^+$ 396.2, $(MNa)^+$ 418.1, $(M-H)^-$ 393.9. HPLC purity: 100% (254 nm), t_R = 5.33 min; 99.3% (215 nm), t_R = 5.33 min.

5.1.21. 1-((*R*)-3-(4-Chlorophenoxy)-2-hydroxypropyl)-4-(3-(trifluoromethyl)phenyl)piperidin-4-ol, 5c

0.32 g (2 mmol scale), 37.2% 2-step overall yield. Mp: 101–104 °C. ^{1}H NMR, 500 MHz (DMSO-d_6): δ 7.83 (s, 1H), 7.77 (d, *J* = 7.3 Hz, 1H), 7.57 (m, 2H), 7.33 (dd, *J* = 3.4 and 8.2 Hz, 2H), 6.99 (dd, *J* = 3.4 and 8.8 Hz, 2H), 5.06 (s, 1H, O*H*), 4.85 (d, *J* = 4.4 Hz, 1H, O*H*), 4.02 (dd, *J* = 3.7 and 9.8 Hz, 1H, C*H*^{a}O), 3.97 (m, 1H, C*H*OH), 3.89 (dd, *J* = 5.9 and 9.5 Hz, 1H, C*H*$^{a'}$O), 2.72 (m, 2H), 2.50 (m, 3H, overlapped with DMSO peak), 2.42 (dd, *J* = 6.6 and 12.7 Hz, 1H, NC*H*^{b}), 1.97 (m, 2H), 1.57 (m, 2H). Mass spectrum (ESI) *m*/*z* $(MH)^+$ 430.2, $(MNa)^+$ 452.1, $(M-H)^-$ 427.9. HPLC purity: 98.9% (254 nm), t_R = 5.45 min; 98.7% (215 nm), t_R = 5.45 min.

5.1.22. 1-((*R*)-3-(4-Chlorophenoxy)-2-hydroxypropyl)-4-phenylpiperidine-4-carbonitrile, 5d

0.30 g (2 mmol scale), 40.4% 2-step overall yield. Mp: 74–76 °C. ^{1}H NMR, 500 MHz (DMSO-d_6): δ 7.54 (d, *J* = 7.6 Hz, 2H), 7.45 (t, *J* = 7.3 Hz, 2H), 7.37 (t, *J* = 7.3 Hz, 1H), 7.33 (d, *J* = 8.8 Hz, 2H), 6.99 (d, *J* = 8.8 Hz, 2H), 4.94 (d, *J* = 3.4 Hz, 1H, O*H*), 3.99 (m, 2H, C*H*^{a}O and C*H*OH), 3.89 (dd, *J* = 7.1 and 10.7 Hz, 1H, C*H*$^{a'}$O), 3.05 (m, 2H), 2.50 (m, 3H, overlapped with DMSO peak), 2.42 (m, 1H), 2.04 (m, 4H). Mass spectrum (ESI) *m*/*z* $(MH)^+$ 371.2, $(MNa)^+$ 393.1. HPLC purity: 100% (254 nm), t_R = 5.38 min; 98.5% (215 nm), t_R = 5.38 min.

5.1.23. (*R*)-1-(4-Chlorophenoxy)-3-(4-(4-chlorophenyl)-5,6-dihydropyridin-1(2H)-yl)propan-2-ol, 5e

0.28 g (2 mmol scale), 37.0% 2-step overall yield. Mp: 124–126 °C. ^{1}H NMR, 500 MHz (DMSO-d_6): δ 7.46 (d, *J* = 8.5 Hz, 2H), 7.38 (d, *J* = 8.5 Hz, 2H), 7.32 (d, *J* = 9.0 Hz, 2H), 6.98 (d, *J* = 8.8 Hz, 2H), 6.20 (br t, 1H, =C*H*), 4.94 (d, *J* = 4.2 Hz, 1H, O*H*), 4.00 (m, 2H, C*H*^{a}O and C*H*OH), 3.89 (dd, *J* = 6.8 and 10.7 Hz, 1H, C*H*$^{a'}$O), 3.17 (m, 2H), 2.71 (m, 2H), 2.58 (dd, *J* = 5.6 and

12.5 Hz, 1H, NC$H^{b'}$), 2.50 (m, 1H, overlapped with DMSO peak, NCH^{b}), 2.46 (m, 2H). Mass spectrum (ESI) m/z $(MH)^+$ 378.1, $(MNa)^+$ 400.1. HPLC purity: 99.7% (254 nm), t_R = 5.57 min; 99.7% (215 nm), t_R = 5.56 min.

5.1.24. MIC determination

MIC values were determined against *Mycobacterium tuberculosis* (H37Rv) by the microbroth dilution method.[14] A broth culture of *M. tuberculosis* was grown in Middlebrook 7H9 medium with 10% OADC supplement to an OD_{600} of 0.4–0.6 and aliquots were frozen. The CFU/mL of these aliquots were determined. The tested compounds were serially diluted in DMSO and 4 μL added to microtier plate wells containing 100 μL of 7H9 medium. Subsequently, 2×10^4 TB bacteria in 100 μL of 7H9 medium were added to each well. The plates were incubated at 37 °C for 9–11 days. The MIC_{90} was determined by visual inspection and defined as the concentration that inhibited 90% of growth. For each experiment, controls of INH were run; the MIC of INH under these conditions was between 0.032 and 0.064 μg/mL.

5.1.25. Cytotoxicity study

The LD_{50} for each drug was determined using a microplate dilution method.[15] African green monkey kidney cells (Vero cells) were grown in L15 medium without phenol (Invitrogen). The media was supplemented with glutamine, 10 mL/L penicillin-streptomycin solution (10,000 IU/10,000 μg/mL), and 10% bovine calf serum at 37 °C. The microtiter plates were sealed and testing was conducted for 72 h at 37 °C, alamarBlue® was added to each well, and plates were incubated overnight at 37 °C. Plates were read at 570 and 600 nm using a spectrophotometric plate reader, and the absorbance readings were used to calculate the 50% lethal concentration.

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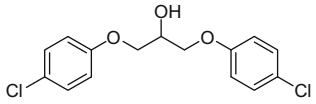
Supplementary data

Supplementary data (^{1}H NMR spectra and HPLC profiles of all target molecules) associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2009.04.005.

References and notes

1. Sacchettini, J. C.; Rubin, E. J.; Freundlich, J. S. *Nat. Rev. Microbiol.* **2008**, *6*, 41.
2. Williams, D. A.; Lemke, T. L. *Foye's Principles of Medicinal Chemistry*, 5th ed.; Lippincott Williams & Wilkins, 2002.
3. Gallant, D. M.; Mielke, D. H.; Spirtes, M. A.; Swanson, W. C.; Bost, R. *Am. J. Psychiat.* **1974**, *131*, 699.
4. Manikowski, A.; Verri, A.; Lossani, A.; Gebhardt, B. M.; Gambino, J.; Focher, F.; Spadari, S.; Wright, G. E. *J. Med. Chem.* **2005**, *48*, 3919.
5. Zhi, C.; Long, Z. Y.; Gambino, J.; Xu, W. C.; Brown, N. C.; Barnes, M.; Butler, M.; LaMarr, W.; Wright, G. E. *J. Med. Chem.* **2003**, *46*, 2731.
6. Chen, Z.; Davies, E.; Miller, W. S.; Shan, S.; Valenzano, K. J.; Kyle, D. J. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 5275.
7. Chen, Z.; Miller, W. S.; Shan, S.; Valenzano, K. J. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3247.
8. Yang, W.; Wang, Y.; Roberge, J. Y.; Ma, Z.; Liu, Y.; Michael Lawrence, R.; Rotella, D. P.; Seethala, R.; Feyen, J. H.; Dickson, J. K., Jr. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 1225.
9. Shagufta; Srivastava, A. K.; Sharma, R.; Mishra, R.; Balapure, A. K.; Murthy, P. S.; Panda, G. *Bioorg. Med. Chem.* **2006**, *14*, 1497.
10. Surendra, K.; Krishnaveni, N. S.; Nageswar, Y. V.; Rao, K. R. *J. Org. Chem.* **2003**, *68*, 4994.
11. Das, B.; Krishnaiah, M.; Thirupathi, P.; Laxminarayana, K. *Tetrahedron Lett.* **2007**, *48*, 4263.
12. Representative characterization of Bis-byproduct (R = 4-Cl, X = O):



^{1}H NMR, 500 MHz (DMSO-d_6): 7.33 (d, J = 8.8 Hz, 4H), 6.99 (d, J = 9.0 Hz, 4H), 5.44 (d, J = 4.6 Hz, 1H, OH), 4.14 (m, 1H), 4.07 (dd, J = 4.6 and 9.8 Hz, 2H), 4.01 (dd, J = 5.9 and 10.0 Hz, 2H). Mass spectrum (ESI) m/z $(MH)^+$ 335.1. HPLC purity: 100% (254 nm and 215 nm), t_R = 7.00 min.

13. Lenaerts, A. J. M.; Veronica, G.; Brooks, J. V.; Orme, I. M. *Antimicrob. Agents Chemother.* **2003**, *47*, 783.
14. Hurdle, J. G.; Lee, R. B.; Budha, N. R.; Carson, E. I.; Qi, J.; Scherman, M. S.; Cho, S. H.; McNeil, M. R.; Lenaerts, A. J.; Franzblau, S. G.; Meibohm, B.; Lee, R. E. *J. Antimicrob. Chemother.* **2008**, *62*, 1037.
15. Collins, L.; Franzblau, S. G. *Antimicrob. Agents Chemother.* **1997**, *41*, 1004.