Putting Tuberculosis (TB) To Rest: Transformation of the Sleep Aid, Ambien, and “Anagrams” Generated Potent Antituberculosis Agents

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Putting Tuberculosis (TB) To Rest: Transformation of the Sleep Aid, Ambien, and "Anagrams" Generated Potent Antituberculosis Agents

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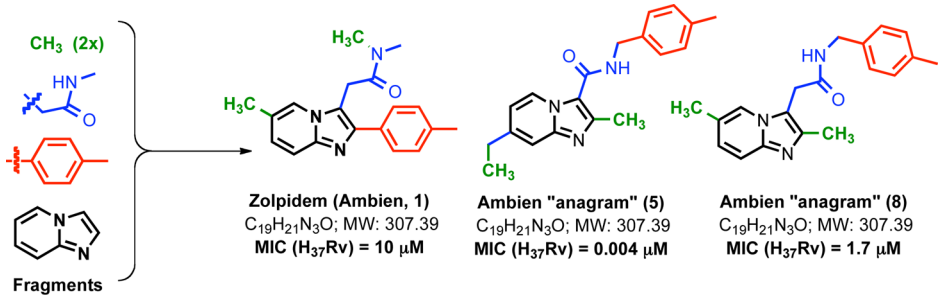


ABSTRACT:

Zolpidem (Ambien, 1) is an imidazo[1,2-a]pyridine-3-acetamide and an approved drug for the treatment of insomnia. As medicinal chemists enamored by how structure imparts biological function, we found it to have strikingly similar structure to the antitubercular imidazo[1,2-a]pyridine-3-carboxyamides. Zolpidem was found to have antituberculosis activity (MIC of 10−50 μM) when screened against replicating Mycobacterium tuberculosis (Mtb) H $_{37}$Rv. Manipulation of the Zolpidem structure, notably, to structural isomers ("anagrams"), attains remarkably improved potency (5, MIC of 0.004 μM) and impressive potency against clinically relevant drug-sensitive, multi- and extensively drug-resistant Mtb strains (MIC < 0.03 μM). Zolpidem anagrams and analogues were synthesized and evaluated for their antitubercular potency, toxicity, and spectrum of activity against nontubercular mycobacteria and Gram-positive and Gram-negative bacteria. These efforts toward the rational design of isomeric anagrams of a well-known sleep aid underscore the possibility that further optimization of the imidazo[1,2-a] pyridine core may well "put TB to rest".

KEYWORDS: *tuberculosis, zolpidem, imidazopyridine analogues, anti-TB, Ambien*

Prior to Robert Koch's identification of Mycobacterium tuberculosis (Mtb) as the causative agent of tuberculosis in 1882, tuberculosis was romanticized within literature.[1,2] John Keats, Edgar Allan Poe, Charlotte Brontë, Fyodor Dostoyevsky, and Victor Hugo (among other prominent writers) all had firsthand experience with tuberculosis, yet their writing failed to convey the true suffering caused by an active Mtb infection. Rather, these authors portrayed this terrible disease as a romantic interlude preceding a peaceful death.[1−4] In Brontë's Jane Eyre, one of Jane's classmates says "I am very happy, Jane; and when you hear that I am dead, you must be sure and not grieve; there is nothing to grieve about. We all must die one day, and the illness which is removing me is not painful; it is gentle and gradual; my mind is at rest."[5]

Tuberculosis (TB) is today understood to be a sinister global health problem that claims more lives than any other disease.[6] Although TB is most prominent among the weak and impoverished, the reality is that social economic status does not make one immune.[7] In 2011 alone, 70000 children died and millions more were orphaned as a result of TB.[8] Indeed, the challenge today is greater as multidrug-resistant strains of Mtb (which do not respond to current chemotherapy) are on the

rise.[8] The reality for the millions who suffer from active TB is a death every 20 s.

As medicinal chemists, we endeavor to find the patterns hidden in structures that impart biological function. We look for them in patents and in literature, and sometimes we find them within approved drugs. Zolpidem (**1**, *N*,*N*-dimethyl-2-(6-methyl-2-*p*-tolyl-imidazo[1,2-*a*]pyridin-3-yl)acetamide; Ambien) is one of the best known sleep aids and ironically bears strong structural similarity to the imidazo[1,2-*a*]pyridine-3-carboxamide antitubercular agents (**2**, **3**, **4**) we discovered to have outstanding in vitro potency against both replicating *Mtb* $H_{37}Rv$ and multi- and extensively drug-resistant (XDR) *Mtb* strains (Figure 1a).[9−12]

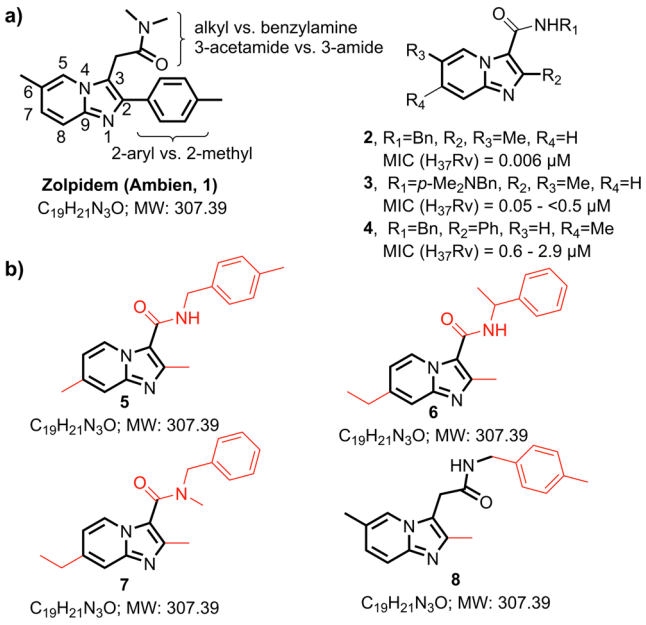


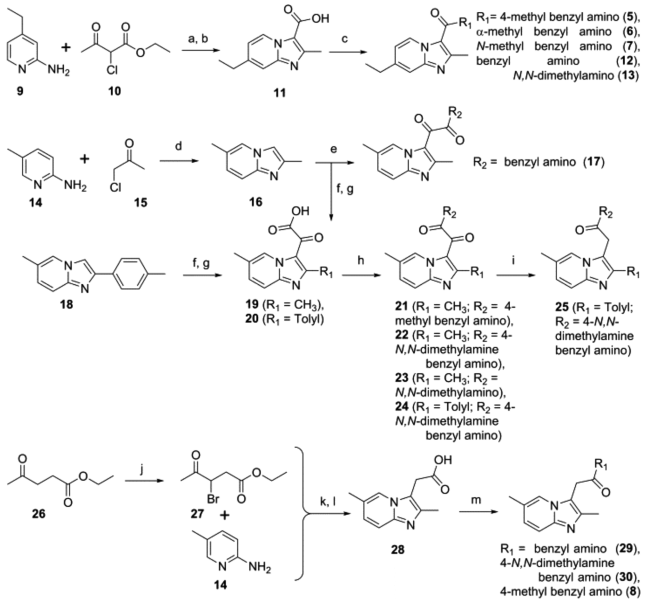
Figure 1. (a) Probes of the SAR around the zolpidem scaffold in comparison to previously published antitubercular imidazo[1,2-*a*]-pyridines (**2**−**4**). (b) Zolpidem "anagram" (isomeric) scaffold analogues explored for their antitubercular activity through specific site modifications. The conserved zolpidem structure remains in black, whereas anagram structure alterations are shown in red.

Zolpidem interacts with the GABA-benzodiazepine receptor complex with selectivity for the type 1 (omega-1) benzodiazepine receptor subtype[13,14] to produce its sedative effects in vivo.[15] Zolpidem is rapidly transformed in vivo to three inactive metabolites,[16] thus achieving a desirable short duration of action.[17] Conversely, the mechanism of our imidazo[1,2-*a*]pyridine-3-carboxamides appears to be related to ATP synthesis[9,18] through the cytochrome *bc1* complex[19] and demonstrated herein by screening of seven resistant mutant strains as well as a hypersensitive strain. When given to mice, they do not produce any noticeable sedative effects in vivo even at 10 times the effective dose for zolpidem (300−500 mg/kg compared to 3−30 mg/kg).[19,20] They also have lower clearance and, in pharmacology studies, longer half-lives than does zolpidem itself.[9,12] Imidazo[1,2-*a*]pyridine-3-carboxamides reduce the burden of replicating *M. tuberculosis* in the lungs and spleens of mice, thus demonstrating proof of the in vivo efficacy necessary for clinical development.[19,21,22]

Our curiosity as to whether zolpidem would have antitubercular activity itself prompted a literature search. The Collaborative Drug Discovery (CDD) database indicated that it was screened by the Southern Research Institute and was not active against *Mtb* (0% inhibition at 10 μM).[23] Nonetheless, we rescreened zolpidem (using the high-throughput microplate alamar blue assay (MABA) at the Institute for Tuberculosis Research, University of Illinois in Chicago), and we observed an MIC of 49−53 μM using two different (GAS[24] and 7H12) growth media.[25] Further screening (at the Infectious Disease Research Institute, Seattle, WA, USA) confirmed activity against *Mtb* (MIC of 10−11 μM in 7H9-Tw-OADC media).[11] The different MIC value suggested that the activity of zolpidem is sensitive to the carbon source of the media,[26] to the degree of aeration,[27] and/or to the presence of bovine serum albumin,[28] as is seen for other exploratory antitubercular drugs. Its potency was still orders of magnitude less active than our previously reported imidazo[1,2-*a*]pyridine-3-carboxamides (micromolar for **1** versus nanomolar for **2**). Knowing that there was weak antitubercular potency within the zolpidem structure and potent activity expressed by our imidazo[1,2-*a*]pyridine-3-carboxamides, we conceptualized alteration of the *N*,*N*-dimethyl-3-acetamide moiety of zolpidem to attain significantly improved antitubercular potency (Figure 1a). We did not anticipate that the 2-tolyl (compared to 2-methyl), or even placement of the methyl at the 6- or 7-position of the imidazopyridine core, would substantially affect potency. Therefore, beyond just probing the structure−activity relationship (SAR) around these positions, we wondered whether the design of specific isomers or "anagrams" of Ambien (that is, compounds with the same empirical formula but rearranged structural moieties) would result in improved biological activity (**5**−**8**, Figure 1b). Herein, we report that rationally designed zolpidem anagrams, as well as additional analogues, have substantial activity against replicating, multi- and extensively drug-resistant *Mtb* clinical strains.

The syntheses of these 4 zolpidem anagrams (Figure 1b) and 10 additional analogues (Scheme 1) can all be reduced to amide bond formation between the corresponding imidazo[1,2-*a*]pyridine-3-carboxylic acid (**11**), imidazo[1,2-*a*]pyridine-3-oxoacetic acids (**19** and **20**), and imidazo[1,2-*a*]pyridine-3-acetic acid (**28**) with the appropriate amines. The syntheses of acids **11** and **28** required only slight modification of our published procedure for the synthesis of compound **2**,[12] namely, reaction of 2-amino-4-ethylpyridine (**9**) with **10** followed by saponification to prepare carboxylic acid intermediate (**11**); in a similar way, reaction of 2-amino-5-methylpyridine (**14**) with ethyl 3-bromo-4-oxopentanoate (**27**)[29] followed by saponification gave acetic acid intermediate (**28**). Oxoacetic acids **19** and **20** were prepared from modified procedures related to the synthesis of zolpidem.[30,31] In brief, that involved reaction of 2,6-dimethylimidazo[1,2-*a*]pyridine (**16**) or 6-methyl-2-(4-methylphenyl)imidazo[1,2-*a*]pyridine (**18**) with oxalyl chloride and then quenching the resulting acid chloride with sodium hydroxide followed by acidifying to collect the α-carbonyl acid intermediates of **19** and **20** (Scheme 1).[30,31] These imidazo[1,2-*a*]pyridine-3-oxoacetic acids (**19** and **20**) were coupled with the desired amines to give a set of unique biologically active imidazo[1,2-*a*]pyridine-3-oxoacetamides (**21**−**24**). We also explored the direct synthesis of the 3-oxoacetamides from **16** and found that, after formation of the acid chloride, subsequent amine addition gave the desired product as demonstrated by the synthesis of **17**, albeit in a low nonoptimized yield (29%). Reduction of these α-carbonyl compounds with zinc produced the imidazo[1,2-*a*]pyridine-3-

Scheme 1. Synthesis of Zolpidem "Anagrams" (5−8), Imidazo[1,2-*a*]pyridine-3-carboxamides (11, 12), Imidazo[1,2-*a*]pyridine-3-oxoacetamides (17, 21−24), and Imidazo[1,2-*a*]pyridine-3-acetamides (25, 29, 30)[a]



[a]Reagents: (a) $NaHCO_3$, DME, reflux, 32 h; (b) 1, LiOH, EtOH, 56 h; 2, HCl; (c) EDC, DMAP, R_1, CH_3CN, 16 h; (d) $NaHCO_3$, DME, reflux, 32 h; (e) 1, oxalyl chloride, 4 h; 2, R_2, Et_3N, DCE, 35 °C, 16 h; (f) 1, oxalyl chloride, DCE, 1 h; 2, Et_3N, 3 h; (g) 1, NaOH aq solution, 0 °C, 2 h; 2, HCl, 16 h; (h) EDC, DMAP, R_2, CH_3CN, 16 h; (i) zinc powder, pyridine, acetic anhydride, acetic acid, 55 °C, 16 h; (j) NBS, CH_2Cl_2, Et_3N, 12 h; (k) DME, reflux, 16 h; (l) 1. LiOH, EtOH, 56 h; 2, HCl; (m) EDC, DMAP, R_1, CH_3CN, 16 h.

acetamide architecture as demonstrated by the reduction of **24** to give **25**. However, it was again much more efficient to couple the imidazo[1,2-*a*]pyridine-3-acetic acid (**28**) to the appropriate amines with EDC to give the desired imidazo[1,2-*a*]pyridine-3-acetamides (**8**, **29**, **30**).

Antituberculosis assays revealed that steric bulk at the 2-position did not significantly affect potency in the 3-carboxyamide series (see Table S2 in the Supporting Information). This trend continued with the 3-acetamide series as **30** had similar potency to **25** (both had MICs from 1 to 3 μM in MABA) but not in the α-dicarbonyl series as **22** (MICs of 5−14 μM in MABA) was more active than **24** (>50 μM in MABA) (Table 1). When screening slow-growing mycobacteria such as *M. tuberculosis*, a 2−4-fold change in MICs can be rationalized due to differences in growth media, growth density, carbon source, amount of serum present, and other assay factors.[33] Therefore, definitive SAR determination by whole cell screening can be challenging but not insurmountable as trends do clearly appear. Next, comparing the activity of 3-carboxylates to that of the 3-oxoacetamides and 3-acetamides, it was apparent that the 3-carboxylates were much more potent as demonstrated by compounds **2**, **3**, **5**, and **12** with MICs ranging from 0.003 to 0.05 μM, which were 2−3 orders of magnitude more active than homologous 3-oxoacetamides (**17**, **21**, **22**) and 3-acetamides (**29**, **8**, **30**). Differentiation of the potency of the 3-oxoacetamides (**17**, **21**, **22**) and 3-acetamides (**29**, **8**, **30**) was not as definitive and needed to be viewed on a case by case basis as some compounds such as **21** and **8** had similar levels of activity (MICs of ∼2−4 μM), whereas other compounds such as **24** (MIC of >20 μM) and **25** (MIC of 1−3 μM) were very different. Finally, we found that benzyl amides were more effective than *N*,*N*-dimethyl amides in all three series (3-carboxylates, 3-oxoacetamides, and 3-acetamides) as clearly demonstrated by comparing compounds **12** and **13** (lowest MICs of 0.02−12 μM), **17** and **23** (lowest MICs of 4−38 μM), or **25** to zolpidem (**1**) (lowest MICs of 3−10 μM).

Table 1. In Vitro Evaluation of Compounds 1−8 and 12−30 against Replicating *Mtb* H_{37}Rv in Various Media

		MIC (μM) *Mtb* H_{37}Rv in assay and media		
compd ID	calcd ClogP[a]	MABA GAS	MABA 7H12	7H9-Tw-OADC
Ambien, **1**	3.02	49	53	10
2	3.60	0.11	0.05	0.006
3	3.76	0.1	0.18	0.05
4	5.30	2.9	0.6	nd
5	4.62	<0.195	<0.195	0.004
6	4.43	2.4	2.9	2.5
7	3.70	36.8	16.4	>20
8	3.20	2.9	4.3	1.7
12	4.12	<0.195	<0.195	0.02
13	1.51	12.1	43.7	66
17	2.74	3.8	20.7	8.5
21	3.24	2.2	4.3	4.5
22	2.90	4.9	13.9	3.1
23	0.64	38	>128	>20
24	5.23	>50	>50	>20
25	4.94	1.2	2.8	nd
29	2.70	19.0	19.3	>20
30	2.87	1.5	2.6	0.7

[a]ClogP calculated by ChemDraw version 12.0. Minimum inhibitory concentration (MIC) was determined against *Mtb* grown in various media, glycerol−alanine−salts (GAS), Middlebrook 7H12, and Middlebrook 7H9, and by three different readouts of growth (MABA, optical density, and fluorescence). nd, not determined. An expanded table with standard deviation calculated and positive controls can be found in the Supporting Information (Table S1).

Of special interest were our rationally designed zolpidem anagrams **5**−**8**, which all have the same chemical formula and, thus, the same exact mass with only specific structural rearrangements made in an attempt to enhance antituberculosis activity (Figure 1b). As we had anticipated, most of these isomers did have improved activity compared to zolpidem (Table 1). Compound **5** was the most potent compound with an MIC of 0.004 μM. It was more potent than most standard TB drugs (Table S1) and nearly as potent as rifampicin (a first-line TB treatment)[34] and BTZ043 (a very potent clinical candidate)[35] with MICs of 0.1 and 0.003 μM, respectively. The least potent was **7** (MICs of 16−37 μM), which bears an *N*-methyl benzyl amide and lacks a hydrogen bond donor. Other secondary amide compounds lacking a hydrogen bond donor were evaluated, and all had weak antituberculosis activity (see Table S3 in the Supporting Information). Compound **6**, however, has an α-methyl benzyl amide and retained good potency despite being racemic (MICs of 2−3 μM). Curious as to which stereochemistry would be the most potent, we prepared both enantiomers and determined that the (*R*)-enantiomer was >3 times more active than the (*S*)-enantiomer (see Table S4 in the Supporting Information). Finally, compound **8** relocated the 2-tolyl moiety found in zolpidem (**1**) to the 3-acetamide region, and potency was increased by >5 times (MIC of 2 μM for **8** compared to 10 μM for **1**).

Ambien anagrams **5** and **8** were screened against a panel of drug-sensitive, multi- and extremely drug resistant *Mtb* clinical strains with PA-824[32] as a positive control (Figure S2 in the Supporting Information). The results were encouraging as **5** had an impressive MIC of <0.03 μM (<0.01 μg/mL) against most of the clinical strains. These compounds were also evaluated for their in vitro toxicity to VERO cells[36] and in three human cell lines (HeLa, PC-3, and MCF-7) (Table S1). None of the compounds tested showed any observable toxicity to the VERO or PC-3 cell lines, but four of the tested compounds (**3**, **8**, **24**, and **30**) did show some toxicity in the HeLa cell line (with IC_{50} values of 13, 8, 17, and 10 μM, respectively).

Broad screening of 14 compounds (**2**, **3**, **5**−**8**, **12**, **13**, **17**, **21**, **23**−**25**, and **30**) against a panel of Gram-positive and Gram-negative organisms using an agar diffusion assay[37] revealed that these compounds are highly selective for *M. tuberculosis* and *Mycobacterium vaccae* (Table S5 in the Supporting Information). Compounds **2** and **30** showed the largest zones of inhibition (72 and 37 mm, respectively) against *M. vaccae* when screened at 2 mM but showed little to no inhibition of *Bacillus subtilis*, *Micrococcus luteus*, *Pseudomonas aeruginosa*, and *Escherichia coli* (Table S5). Compound **25** displayed the broadest spectrum of activity with some inhibition of *Staphylococcus aureus*, *B. subtilis*, *M. luteus*, and *M. vaccae* with moderate zones of inhibition of 15, 15, 17 (partial), and 26 mm, respectively when screened at 2 mM (Table S4 in the Supporting Information). Next, the MICs of 10 compounds (**1**−**3**, **5**, **8**, **17**, **21**, **23**, **25**, and **30**) were determined against a panel of nontubercular mycobacteria (*Mycobacterium smegmatis*, *Mycobacterium abscessus*, *Mycobacterium chelonae*, *Mycobcaterium marinum*, *Mycobacterium avium*, *Mycobacterium bovis* BCG, and *Mycobacterium kansasii*) as well as *E. coli*, *S. aureus*, and *Candida albicans* for compounds **2**, **3**, **17**, **22**, and **23** (Table S6 in the Supporting Information). Compounds **2**, **3**, **5**, and **17** were the most active against the nontuberculosis mycobacteria, showing growth inhibition of *M. avium* (MICs of 16, 16, 46, and 2 μM, respectively), and *M. kansasii* (MICs of 4, 2, 6, and 63 μM, respectively) as well as against other *Mtb* complex bacteria such as *M. bovis* BCG (MICs of 1, 0.3, 0.5, and 29 μM, respectively). Compounds **21**, **25**, and **30** were weaker inhibitors of *M. bovis* BCG (MICs of 15, 24, and 10 μM, respectively) and *M. kansasii* (MICs of 54, 48, and 50 μM, respectively). None of the compounds tested were active against *M. smegmatis*, *M. abscessus*, *M. marinum*, *M. chelonae*, *E. coli*, *S. aureus*, or *C. albicans* at the highest concentration tested (either 50 or 128 μM) with the exception of compound **2**, which inhibited *M. chelonae* (MIC of 94 μM). Zolpidem (**1**) showed growth inhibition of only *M. bovis* BCG (MIC of 49 μM) despite having *Mtb* activity (MIC of 10−50 μM).

Because the targets of these classes are of great interest, compounds **5**, **8**, and **21** were screened in a panel of resistant mutants[38] known to target the bc_1 complex along with Q203, a known inhibitor of the bc_1 complex.[21] All three scaffolds were inhibitors of the bc_1 complex as demonstrated by their loss of potency to the seven *qcrB* mutant strains and the laboratory-adapted H37Rv strain (Table S7 in the Supporting Information). Similarly, the cytochrome *bd* oxidase mutant (cydKO) was found to be susceptible to these imidazo[1,2-*a*]pyridines, and compounds **5**, **8**, **21** and Q203 inhibited resazurin dye reduction to give good MICs even in the laboratory-adapted H37Rv strain (Table S7 in the Supporting Information), a phenotype observed with other QcrB inhibitors.[38]

In conclusion, the rational redesign of the structural moieties found in zolpidem provides access to very potent antituberculosis compounds (MICs as low as 0.004 μM) and thus demonstrates the inherent in vitro potency, selectivity, and low toxicity of imidazo[1,2-*a*]pyridines. We believe that further manipulation of this antitubercular scaffold has the potential to "put TB to rest".

■ ASSOCIATED CONTENT

Supporting Information

The following file is available free of charge on the ACS Publications website at DOI: 10.1021/id500008t.

Experimental procedures, additional SAR, syntheses, and ^{1}H and ^{13}C NMR of all new compounds; details of all microbiological and antibacterial (*Mtb*, NTM, MDR- and XDR-*Mtb*, broad spectrum) studies (PDF)

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Wilsey, A. M. (2012) Half in love with easeful death: tuberculosis in literature. *Humanities Capstone Projects*, Paper 11; http://commons.pacificu.edu/cashu/11.

(2) Martinez, G. G. (2013) Romanticizing tuberculosis: poetry, prose, opera, and society of the romantic era; https://digital.library.txstate.edu/handle/10877/4661.

(3) Latimer, D. (1990) Erotic susceptibility and tuberculosis: literary images of a pathology. *MLN 105*, 1016−1031 http://www.jstor.org/stable/2905165.

(4) Byrne, K. (2011) *Tuberculosis and the Victorian Literary Imagination*; Cambridge University Press: Cambridge, UK.

(5) Brontë, C. (2005) *Jane Eyre*; Barnes & Noble Classics: New York.

(6) Toth, A., Fackelmann, J., Pigott, W., and Tolomeo, O. (2004) Tuberculosis prevention and treatment. *Can. Nurse 100*, 27−30.

(7) World Health Organization (WHO). (2012) Global tuberculosis report; http://www.who.int/tb/publications/global_report/gtbr12_main.pdf.

(8) Stop TB Partnership. (2012) *Combating tuberculosis in children*, Stop TB Partnership, Geneva, Switzerland; http://www.who.int/tb/challenges/childtb_factsheet.pdf.

(9) Moraski, G. C., Markley, L. D., Hipskind, P. A., Boshoff, H., Cho, S., Franzblau, S. G., and Miller, M. J. (2011) Advent of imidazo[1,2-*a*]pyridine-3-carboxamides with potent multi- and extended drug resistant antituberculosis activity. *ACS Med. Chem. Lett. 2*, 466−470 DOI: 10.1021/ml200036r.

(10) Moraski, G. C., Markley, L. D., Chang, M., Cho, S., Franzblau, S. G., Hwang, C. H., Boshoff, H., and Miller, M. J. (2012) Generation and exploration of new classes of antitubercular agents: the optimization of oxazolines, oxazoles, thiazolines, thiazoles to imidazo-[1,2-*a*]pyridines and isomeric 5,6-fused scaffolds. *Bioorg. Med. Chem. 7*, 2214−2220 DOI: 10.1016/j.bmc.2012.02.025.

(11) Ollinger, J., Bailey, M.-A., Moraski, G. C., Casey, A., Florio, S., Alling, T., Miller, M. J., and Parish, T. (2013) A dual read-out assay to evaluate the potency of compounds active against *Mycobacterium tuberculosis*. *PLoS One 8*, No. e60531, DOI: 10.1371/journal.-pone.0060531.

(12) Moraski, G. C., Markley, L. D., Cramer, J., Hipskind, P. A., Boshoff, H., Bailey, M.-A., Alling, T., Ollinger, J., Parish, T., and Miller, M. J. (2013) Advancement of imidazo[1,2-*a*]pyridines with improved pharmacokinetics and nanomolar activity against *Mycobacterium tuberculosis*. *ACS Med. Chem. Lett. 4*, 675−679 DOI: 10.1021/ml400088y.

(13) Sanger, D. J., Benavides, J., Perrault, G., Morel, E., Cohen, C., Joly, D., and Zivkovic, B. (1994) Recent developments in the behavioral pharmacology of benzodiazepine (ω) receptors: evidence for the functional significance of receptor subtypes. *Neurosci. Biobehav. Rev. 18*, 355−372 DOI: 10.1016/0149-7634(94)90049-3.

(14) Byrnes, J. J., Greenblatt, D. J., and Miller, L. G. (1992) Benzodiazepine receptor binding of non-benzodiazepines *in vivo*: alpidem, zolpidem, and zopiclone. *Brain Res. Bull. 29*, 905−908.

(15) Hoehns, J. D., and Perry, P. J. (1993) Zolpidem: a nonbenzodiazepine hypnotic for treatment of insomnia. *Clin. Pharm. 12*, 814−828.

(16) Von Moltke, L. L., Greenblatt, D. J., Granda, B. W., Duan, S. X., Grassi, J. M., Venkatakrishnan, K., Harmatz, J. S., and Shader, R. I. (1999) Zolpidem metabolism *in vitro*: responsible cytochromes, chemical inhibitors, and *in vivo* correlations. *Br. J. Clin. Pharmacol. 48*, 89−97 DOI: 10.1046/j.1365-2125.1999.00953.x.

(17) Wagner, J., and Wagner, M. L. (2000) Non-benzodiazepines for the treatment of insomnia. *Sleep Med. Rev. 4*, 551−581 DOI: 10.1053/smrv.2000.0126.

(18) Mak, P. M., Rao, S. P. S., Tan, M.-P., Lin, X., Chyba, J., Tay, J., Ng, S.-H., Tan, B.-H., Cherian, J., Duraiswamy, J., Bifani, P., Vim, V., Lee, B.-H., Ma, N.-L., Beer, D., Thayalan, P., Kuhen, K., Chatterjee, A., Supek, F., Glynne, R., Zheng, J., Boshoff, H. I., Barr, C. E., Dick, T., Pethe, K., and Camacho, L. R. (2012) A high-throughput screen to identify inhibitors of ATP homeostasis in non-replicating *Mycobacterium tuberculosis*. *ACS Chem. Bio. 7*, 1190−1197 DOI: 10.1021/cb2004884.

(19) Abrahams, K. A., Cox, J. A. G., Spivey, V. L., Loman, N. J., Patten, M. J., Constantinidoou, C., Fernandex, R., Alemparte, C., Remuinan, M. J., Barros, D., Ballell, L., and Besra, G. S. (2012) Identification of novel imidazo[1,2-*a*]pyridine inhibitors targeting *M. tuberculosis* QcrB. *PLoS One 7*, No. e52951, DOI: 10.1371/journal.-pone.0052951.

(20) Cope, D. W., Wulff, P., Oberto, A., Aller, M. I., Capogna, M., Ferraguti, F., Halbsgurth, C., Hoeger, H., Jolin, H. E., Jones, A., Mckenzie, A. N. J., Ogris, W., Poeltl, A., Sinkkonen, S. T., Vekovischeva, O. Y., Korpi, E. R., Sieghart, W., Sigel, E., Somogyi, P., and Wisden, W. (2004) Abolition of zolpidem sensitivity in mice with a point mutation in the GABAA receptor gamma2 subunit. *Neuropharmacology 47*, 17−34 DOI: 10.1016/j.neuro-pharm.2004.03.007.

(21) Pethe, K., Bifani, P., Jang, J., Kang, S., Park, S., Ahn, S., Jiricek, J., Jung, J., Jeon, H. K., Cechetto, J., Lee, H., Kempf, M., Jackson, M., Lanaerts, A. J., Pham, H., Jones, V., Seo, M. J., Kim, Y. M., Seo, M., Seo, J. J., Park, D., Ko, Y., Choi, I., Kim, R., Kim, S. Y., Lim, S.-B., Yim, S.-A., Nam, J., Kang, H., Kwon, H., Oh, C.-T., Cho, Y., Jang, Y., Kim, J., Chua, A., Tan, B. H., Nanjundappa, M. B., Rao, S. P. S., Barnes, W. S., Wintjens, R., Walker, J. R., Alonso, S., Lee, S., Kim, J., Oh, S., Oh, T., Nehrbass, U., Han, S.-J., No, Z., Lee, J., Brodin, P., Cho, S.-N., Nam, K., and Kim, J. (2013) Discovery of Q203, a potent clinical candidate for the treatment of tuberculosis. *Nat. Med. 19*, 1157−1160 DOI: 10.1038/nm.3262.

(22) Cheng, Y., Moraski, G. C., Cramer, J., Miller, M. J., and Schorey, J. S. (2014) Bactericidal activity of an imidazo[1,2-*a*]pyridine using a mouse *M. tuberculosis* infection model. *PLoS One 9*, No. e87483, DOI: 10.1371/journal.pone.0087483.

(23) The screening data were found in the collaborative drug discovery (CDD) portal as a public molecule SMR0000469145 (or CDD-730); https://app.collaborativedrug.com/. For more information on CDD, see: Ekins, S., Kaneko, T., Lipinski, C. A., Bradford, J., Dole, K., Spektor, A., Gregory, K., Blondeau, D., Ernst, S., Yang, J., Goncharoff, N., Hohmana, M. M., and Bunin, B. A. (2010) Analysis and hit filtering of a very large library of compounds screened against *Mycobacterium tuberculosis Mol. BioSyst. 6*, 2316−2324, DOI: 10.1039/c0mb00104j.

(24) De Voss, J. J., Rutter, K., Schroeder, B. G., Su, H., Zhu, Y., and Barry, C. E. (2000) The salicylate-derived mycobactin siderophores of *Mycobacterium tuberculosis* are essential for growth in macrophages. *Proc. Natl. Acad. Sci. U.S.A. 97*, 1252−1257 DOI: 10.1073/pnas.97.3.1252.

(25) Franzblau, S. G., DeGroote, M. A., Cho, S. H., Andries, K., Nuermberger, E., Orme, I. M., Mdluli, K., Angulo-Barturen, I., Dick, T., Dartois, V., and Lenaerts, A. J. (2012) Comprehensive analysis of methods used for the evaluation of compounds against *Mycobacterium tuberculosis*. *Tuberculosis 92*, 453−488 DOI: 10.1016/j.tube.2012.07.003.

(26) Pethe, K., Sequeira, P. C., Agarwalla, S., Rhee, K., Kuhen, K., Phong, W. Y., Patel, V., Beer, D., Walker, J. R., Duraiswamy, J., Jiricek, J., Keller, T. H., Chatterjee, A., Tan, M. P., Ujjini, M., Roa, S. P. S., Camacho, L., Bifani, P., Mak, A. P., Ma, I., and Barnes, S. W. (2010) A chemical genetic screen in *Mycobacterium tuberculosis* identifies carbon-source-dependent growth inhibitors devoid of *in vivo* efficacy. *Nat. Commun. 57*, 1−8 DOI: 10.1038/ncomms1060.

(27) Solovev, V. N., and Zueva, V. S. (1965) Significance of the degree of aeration of media for the antibacterial action of antitubercular agents. *Biull. Eksp. Biol. Med.*, 63−65.

(28) Aoyagi, A. (1977) Clinical significance of antitubercular agents and protein binding. *Kekkaku 52*, 101−103.

(29) Abignente, E., de Caprariis, P., De Martino, G., and Patscot, R. (1987) Research on heterocyclic compounds. XXI. Synthesis of imidazo[2,1-*b*]benzothiazole and imidazo[2,1-*b*]thiazole derivatives. *J. Heterocycl. Chem. 24*, 155−158.

(30) Rey, M., Rossler, A., Derungs, G., and Pak, J. K. (2002) Process for the preparation of imidazopyridines. WO 2002014306 A1 (Cilag Ag).

(31) Alok, S., Sakhardande, R., Santosh, C., Chaudhari, H., and Jadhav, M. (2007) Process for the preparation of imidazopyridines. WO 2007023504 A1 (Saxena Alok).

(32) Stover, C. K., Warrener, P., VanDevanter, D. R., Sherman, D. R., Arain, T. M., Langhorne, M. H., Anderson, S. W., Towell, J. A., Yuan, Y., McMurray, D. N., Kreiswirth, B. N., Barry, C. E., and Baker, W. R. (2000) A small-molecule nitroimidazopyran drug candidate for the treatment of tuberculosis. *Nature 405*, 962−966 DOI: 10.1038/35016103.

(33) Ogawa, T., and Otani, N. (1961) Studies on inactivation of antitubercular agents in media. III. Examination of the media with effects of inactivating antitubercular agents. *Kekkaku*, 138−141.

(34) Cohn, D. L., Catlin, B. J., Peterson, K. L., Judson, F. N., and Sbarbaro, J. A. (1990) A 62-dose, 6-month therapy for pulmonary and extrapulmonary tuberculosis. A twice-weekly, directly observed, and cost-effective regimen. *Ann. Int. Med. 112*, 407−415 DOI: 10.7326/0003-4819-76-3-112-6-407.

(35) Lechartier, B., Hartkoorn, R. C., and Cole, S. T. (2012) *In vitro* combination studies of benzothiazinone lead compound BTZ043 against *Mycobacterium tuberculosis*. *Antimicrob. Agents Chemother. 56*, 5790−5793 DOI: 10.1128/AAC.01476-12.

(36) Lilienkampf, A., Mao, J., Wan, B., Wang, Y., Franzblau, S. G., and Kozikowski, A. P. (2009) Structure−activity relationships for a series of quinoline-based compounds active against replicating and nonreplicating *Mycobacterium tuberculosis*. *J. Med. Chem. 52*, 2109−2118 DOI: 10.1021/jm900003c.

(37) Espinel-Ingroff, A. (2006) Comparison of three commercial assays and a modified disk diffusion assay with two broth microdilution reference assays for testing zygomycetes, *Aspergillus* spp., *Candida* spp., and *Cryptococcus neoformans* with posaconazole and amphotericin B. *J. Clin. Microbiol. 44*, 3616−3622 DOI: 10.1128/JCM.01187-06.

(38) Arora, K., Ochoa-Montano, B., Tsang, P. S., Blundell, T. L., Dawes, S. S., Mizrahi, V., Bayliss, T., Mackenzie, C. J., Cleghorn, L. A. T., Ray, P. C., Wyatt, P. G., Uh, E., Lee, J., Barry, C. E., and Boshoff, H. I. (2014) Respiratory flexibility in response to inhibition of cytochrome *c* oxidase in *Mycobacterium tuberculosis*. *Antimicrob. Agents Chemother. 58*, 6962−6965 DOI: 10.1128/AAC.03486-14.