Article

TBA-354: A new drug for the treatment of persistent tuberculosis

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Keywords: *nitroimidazoles*, *pretomanid*, *TBA-354*, *tuberculosis drugs*, *drug development*

Introduction

Tuberculosis (TB), resulting from infection with the bacterium *Mycobacterium tuberculosis* (*M. tb*), is a resurgent and major worldwide health problem. TB was the principal cause of early death in Europe in the 17th and 18th centuries, and was still a greatly feared disease in the 19th and early part of the 20th century (the "white plague"), when there was no active treatment available. Yet by the middle of the 20th century it had essentially disappeared as a health issue in the developed world. This was primarily due to the introduction of an effective combination of the drugs isoniazid, ethambutol (bacterial cell wall synthesis inhibitors), pyrazinamide (energy metabolism inhibitor) and rifampin (bacterial RNA synthesis inhibitor); collectively known as "Rifafour" (Fig. 1).

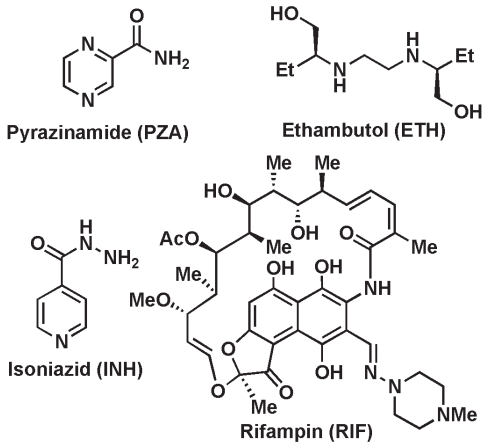

Fig. 1. Structures of the first-line drugs for TB

TB has always been a major disease in the less-developed world, and a major potential global issue, with best estimates suggesting that about 1 person in 3 worldwide carries a dormant infection. The large majority of these, with active immune systems, never develop the active disease. A suggested major factor in the recent resurgence of TB is the spread of HIV, which by weakening the immune system allows reactivation of latent disease. The current TB epidemic peaked in 1990, with an estimated 8 million new cases and 2.9 million deaths from the disease, but remains a major global health problem, with 8.6 million cases and 1.3 million deaths in 2012 (25% of the deaths being among HIV-positive people).[1]

However, a drawback to current treatment is the complex regimen and long duration needed (often more than 12 months), due to the limited activity of the front-line drugs against the "persistent" form of the disease, encapsulated in low-oxygen environments by macrophages.[2] Such treatment times result in levels of incomplete compliance which aids the development of drug resistance. This has given rise to a steep increase in cases of multi-drug resistant TB (MDR-TB; defined as resistance to isoniazid and rifampicin, the two most powerful first-line drugs), which comprised 5.2% of new TB cases in 2012 but nearly 14% of all TB deaths. Worse, in 2012 more than 90 countries reported cases of extensively drug-resistant TB (XDR-TB; resistant to all first-line and to many second-line drugs).[1]

The spectacular success of TB drugs in the 1950s meant that research efforts on improved drugs were not seriously undertaken until quite recently; the "Rifafour" combination remains front-line treatment after more than 50 years. Only recently, sparked off largely by global charities such as the Global Alliance for TB (GATB) and the Gates Foundation, have new drugs for TB started to be approved. Among these have been the nitroimidazole-based bioreductive compounds aimed at the "persistent" form of the disease, focussing on shortening and simplifying treatment, improving compliance and hopefully thus limiting the transition to MDR/XDR.

The nitroimidazole class of TB drugs

Nitroimidazoles such as CGI17341 have long been known to have anti-tubercular activity, but were too mutagenic to use (Fig. 2). The first of the clinically-useful compounds was the nitroimidazooxazine pretomanid (PA-824), initially discovered by the small company Pathogenesis[3] and developed to clinical trial by the GATB. Pretomanid showed substantial *in vitro* and *in vivo* activity against both replicating and non-replicating (low-oxygen) cultures of *M. tb*, while lacking the mutagenic profile of previous simpler and more hydrophilic nitroimidazoles. In a Phase IIA early bactericidal activity (EBA) trial (where a new drug replaces a standard drug in a combination for 14 days), a combination of pretomanid, pyrazinamide and the fluoroquinoline moxifloxacin was particularly effective.[4] It showed the most rapid onset of activity and was clearly superior to the standard Rifafour treatment. This pretomanid combination is currently in Phase III clinical trial.[5] A related compound, the nitroimidazooxazole delamanid, developed by Otsuka Pharmaceutical, has recently gained registration approval from the US Food and Drug Administration for use in TB treatment.[6]

Development of TBA-354

Introduction

Concomitant with their drug pretomanid beginning initial clinical trials in 2005, the GATB considered the development of a second generation analogue, looking for a combination of higher potency, better solubility, longer

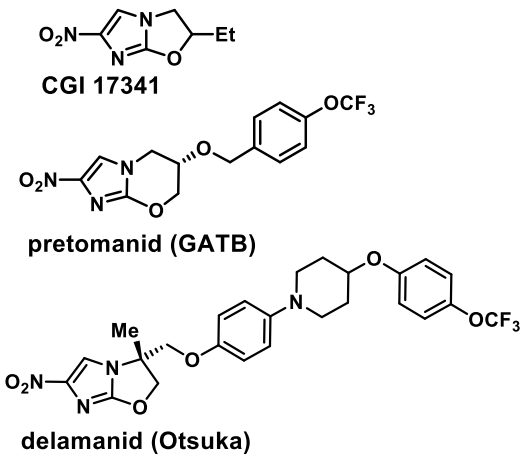

Fig. 2. Structures of nitroimidazole-based drugs for TB

half-life and, ideally, a broader spectrum of action. The Auckland Cancer Society Research Centre at the University of Auckland was selected for this work, due largely to our experience in nitroimidazole chemistry, gained previously in the development of hypoxia-activated bioreductive prodrugs for cancer therapy.

Variation of the nitroimidooxazine chromophore

When this work began, the molecular target of pretomanid was unknown, so we first focused on the nitroimidazooxazine chromophore. From the presence of the nitro group, and earlier work[2] that showed polar metabolites were produced when pretomanid was incubated with *M. tb*, it seemed likely that bioreduction was involved, so we first looked at varying reduction potential by changing the chromophore unit (Fig. 3; chromophore variations). The results showed that very limited alterations to the chromophore were permitted, and no correlation between reduction potential and activity was seen.[7] Furthermore, pulse radiolysis studies indicated that the active compounds (including pretomanid) showed unusual reduction of the imidazole ring in preference to the nitro group.[8]

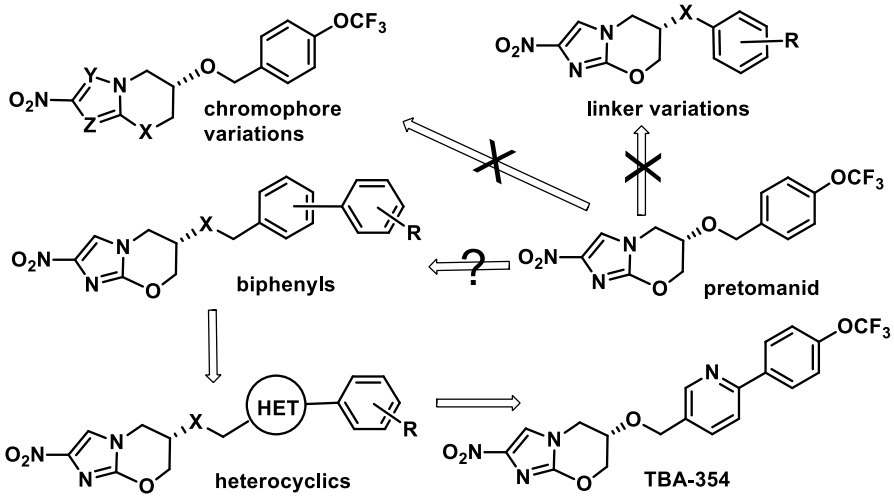

Fig. 3. The development pathway from pretomanid to TBA-354

At about the same time, a US group showed that the major stable metabolite from incubation of pretomanid with its putative *M. tb* target (now known as deazaflavin dinucleotide reductase; Ddn) was the des-nitro compound, which they surmised to be generated by initial reduction of the C2-C3 imidazole bond and hydride transfer to the 3-position to give the unstable intermediate M3, followed by release of nitric oxide (Fig. 4).[9] We later isolated M3 and confirmed its structure.[10]

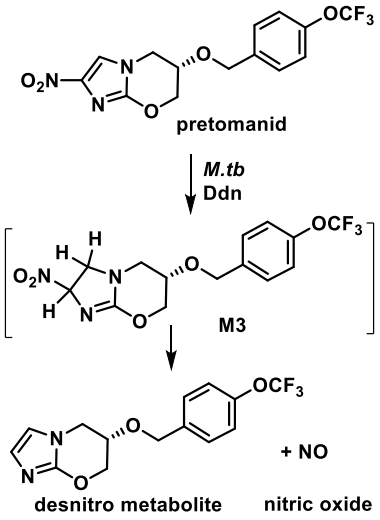

Fig. 4. Bioreductive metabolism of pretomanid

In an extensive study[11] of more lipophilic biphenyl analogues of pretomanid (Fig. 3; biphenyls) we showed that para-linked compounds were the most active, suggesting a reasonably linear and restricted lipophilic binding pocket in the target enzyme. The activity of the compounds (as the minimum inhibitory concentration for 90% inhibition of *M. tb* growth; MIC) correlated positively with overall lipophilicity (clogP) and the electron-donating ability of the R substituents.

Many of the biphenyl compounds also showed good efficacy in a mouse model of acute *M. tb* infection, using a once daily oral dose of 100 mg/kg for 5 days a week for 3 weeks.[11] Activity was measured as the ratio of the fold decrease in colony forming units (CFUs) recovered from the lungs of compound-treated mice compared to the corresponding fold CFU decrease achieved by treatment with pretomanid as internal control. By this measure, many compounds were >200-fold more effective than pretomanid. But with poor aqueous solubility becoming a limitation with the biphenyls, two ways of improving aqueous solubility were explored.

Variation of the side-chain: seeking solubility

One way studied to improve solubility was to replace the OCH_2 linker group with a wide range of more polar and/or more flexible groups, in both the pretomanid and biphenyl series (Fig. 3; linker variations). Previous studies by others on analogues where the benzyl ether linker was replaced by more hydrophilic (but less flexible) urea, carbamate and amide linkers showed these retained good *in vitro* potency,[3] albeit against *M. bovis*, not *M. tb*. We thus looked at

a significant number of new analogues with a wide range of both rigid[12] and flexible[13] linkers, both in the pretomanid and biphenyl series, but while many had excellent *in vitro* potency none approached the high efficacy of the biphenyls *in vivo*.

A second approach to improve solubility was to replace the first phenyl ring of the biphenyls with different 5-membered ring heterocycles, ranging in lipophilicity from thiophenes to tetrazoles (Fig. 3; heterocycles). Several of these series, most notably N-methylimidazoles and1,3,4-oxadiazoles, were substantially more soluble than the biphenyls, and many had comparable MIC values comparable to pretomanid against *M. tb* in culture, but none showed the extensive improvement in efficacy over pretomanid in the *in vivo* mouse assay demonstrated by many of the biphenyls.[14]

Success finally came by replacing one of the phenyl rings with a pyridyl unit.[15] With two rings, as well as the nature of the link between them to study, there were a large number of possible combinations, but it finally emerged that substitution in the first ring, particularly in the 3´-position, gave compounds that were significantly (>100-fold) more effective than pretomanid in reducing lung CFUs in the mouse model, among then TBA-354. The ionisable nitrogen also provided much more soluble compounds. Analogues with a pyridyl terminal ring were less successful and less soluble, the latter because an electron-withdrawing group was needed on this ring, thus lowering the pyridine pKa.

After this work was completed, the crystal structure of the truncated core protein of the target enzyme, *M.tb* Ddn, with the F420 cofactor bound, was published.[16] From this, the authors constructed a model of the putative binding site of pretomanid, locating it near the F420 cofactor binding site, with the nitro group H-bonded to several Ddn residues, which were shown to be important by point mutation studies. This model orients the side chain of pretomanid towards the N-terminus of the enzyme, where there are numerous aromatic residues potentially able to form van der Waal and charge-transfer binding interactions with the drug phenyl or biphenyl rings. This was supported by mutation of one or more of these residues to non-aromatic ones, which resulted in mutant enzymes of lower activity. It is also consistent with the observed structure-activity relationships of this class of drugs, where potency (due to more efficient metabolism by the enzyme?) is enhanced by lipophilic and electron-deficient biphenyl side chains. From this study it is possible to suggest a binding model for TBA-354 where the drug has opportunities for further binding contacts (Fig. 5).

TB-354 emerged as the preferred candidate from a small number of analogues that were extensively evaluated for activity in mouse models of chronic ('persistent") TB, and for pharmacokinetic, genetic and safety profiling. A comparison of TB-354 with pretomanid (Table 1) shows that the former has superior *in vitro* and *in vivo* activity against *M. tb* and appropriate pharmacokinetics, including a 4-fold longer half-life. These properties should have a favourable impact on the cost of goods - an important

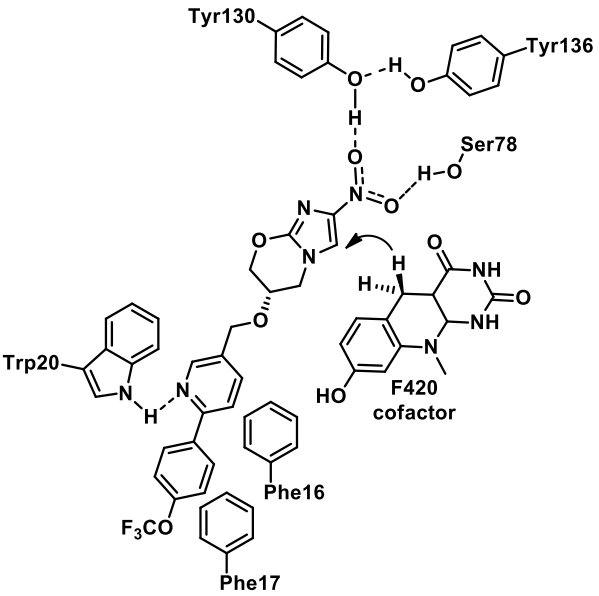

Fig. 5. Suggested binding mode of TBA-354 to Ddn (after Cellitti *et al*.[16])

issue for a drug which, if successful, will primarily be used in less developed countries. Finally, while the primary goal for drugs such as pretomanid and TBA-354 is to shorten initial treatment regimens to minimise non-compliance and the concomitant risk of drug-resistant TB, it is pleasing that both drugs retain activity against clinical isolates of human MDR and XDR TB (with TBA-354 retaining its potency edge) as compared with the front-line drugs rifampin and isoniazid (Table 2).[17]

Table 1. Comparative properties of pretomanid and TBA-354

Property	pretomanid	TBA-354
MW	359.3	436.4
clogP[a] (ACD v12)	2.79	3.49
tPSA[a]	95	107
Sol (μM) pH=2	30	104
Papp A->B[b]	24	24
aerobic MIC (μM)[c]	0.05	0.006
hypoxic MIC (μM)[d]	2.64	0.27
Fold redn CFUs[e]	1.00	>90
Microsome (%)[f]	82	83
HPP binding (%)[g]	81	97
$T_{1/2}$ (h) rat	4.2	24
AUC rat (h*ng/mL)[h]	7159	81266
F (%) rat[i]	81	62
Ames test	negative	negative
hERG (μM)[j]	20	8.2

[a]Calculated with ACD programme (v.12.02); [b]Permeability in a confluent monolayer of Caco-2 human colorectal carcinoma cells (x10^{-6} cm/sec); [c]Aerobic (replicating) *M. tb* assay; [d]Non-replicating *M. tb* assay; [e]Ratio of reduction in *M. tb* colony-forming units compared to pretomanid in a head-to-head study in mice, following drug dosing at 100 mg/kg daily for 15 days. [f]Percent drug remaining after 1 h exposure to human microsomes; [g]Percent binding to human plasma proteins. [h]Under fasting conditions. [i]Oral bioavailability. [j]Inhibition of the hERG potassium ion channel (cause of long QT syndrome)

Table 2. Potency of TBA-354, pretomanid, rifampicin and isoniazid against clinical isolates of human wild-type (WT), multidrug-resistant (MDR) and extensively drug resistant (XDR) *M. tb*

class	MIC (mg/ml)[a]			
	TBA-354	**preto**	**RMP**	**INH**
WT	<0.0063	0.042	0.033	0.047
MDR	0.016	0.25	10	4.77
XDR	<0.0075	0.14	>20	>1.25
XDR	0.015	0.25	>20	>20
XDR	0.013	0.21	>20	>20
XDR	0.015	0.21	>20	>20

[a]Data from ref. 17.

Summary

This research programme arose from a rather speculative offer to the GATB from the ACSRC to help with their PA-824 second generation development programme because of our expertise in nitroimidazole chemistry. A small part in the programme quickly morphed into our undertaking the entire drug design and synthesis role. Initial work on the chromophore showed that it was already optimal, and that activity depended on the initial site of reduction, contributing to an understanding of the mechanism of action. Increased potency (and insolubility) was seen with biphenyl analogues, and the rest of the programme really focused around solving the solubility problem. A number of approaches were explored, with the successful one being the introduction of an appropriate pyridyl unit in the side chain. Despite the lack of information at the start of the work about either a molecular target or a mechanism, the programme was able to recommend a clinical candidate in four years, although it took a few more years for the validation, safety and regulatory work to be completed (Table 3). We are extremely pleased that TBA-354 has recently been approved by the US Federal Drug Administration for clinical trial.

Acknowledgements

To all the staff and students in the ACSRC and the Dept of Molecular Medicine & Pathology who contributed to this work; chemistry leader Associate Professor Brian Palmer, chemists Associate Professor Bob Anderson, Drs Adrian Blaser, Iveta Kmentova, Sujata Shinde, Hamish Sutherland, Andrew Thompson, pharmacologist Associate Professor Nuala Helsby, and students Mridula Dogra and Andreij Maroz. Primary screening against *M. tb* in culture was carried out in Professor Scott Franzblau's laboratory in the Institute for Tuberculosis Research, University of Illinois at Chicago. Funding for the programme was provided by the Global Alliance for Tuberculosis Drug Development, New York.

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Table 3. Timeline for the development of TBA-354

Date	Event
mid-2005	First clinical trial of pretomanid (PA-824) by GATB. ACSRC wins one of the contracts to develop a second generation analogue
2006	ACSRC takes over the entire second generation medchem programme
2008	Independent papers from ACSRC and the US National Institute of Allergy and Infectious Diseases (refs 8 and 9) help define the mechanism of pretomanid
2009	Selection of SN 31354 as the preferred second generation clinical candidate; renamed TBA-354
Oct 2011	TBA-354 endorsed by international committee to proceed to IND filing
Sept 2012	First public disclosure of TBA-354 (symposium at the 52nd Interscience Conference on Antimicrobial Agents & Chemotherapy (ICAAC) in San Francisco
2014	Phase III trial of pretomanid/moxifloxacin/pyrazinamide combination begins
Sept 2014	US FDA approves the IND for TBA-354 to proceed to clinical trial
Late 2014	First clinical trial of TBA-354 expected to begin

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