TDR/PRD/TB/03.1W

The current anti-TB drug research and development pipeline

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Special Programme for Research and
Training in Tropical Diseases (TDR)

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SUMMARY

This report details compounds with potential for the treatment of tuberculosis (TB) and categorizes them into the discovery, preclinical or clinical phases of development. It was prepared following discussion of a draft document submitted by Dr Toshiko Imamura (WHO; financially assisted by the Rockefeller Foundation) to delegates at the meeting in Geneva on The Current Anti-TB Drug Candidates, December 5-6, 2000. This meeting was jointly convened and sponsored by the UNDP/World Bank/WHO Special Programme for Research and Training In Tropical Diseases (TDR) and the Global Alliance for TB Drug Research and Development (GATB), with the specific objective of identifying a short list of promising candidates for development as drugs for tuberculosis.

As a result of the review, it is recommended that the compounds listed below are worthy of further pursuit by the global R & D community:

Discovery research:

- **thiolactomycin** analogues
- **ethambutol** analogues
- **mefloquine/halofantrine** analogues
- **diterpenoids**
- **9-benzylpurines**
- **benzoxazines**
- **imidazo(4,5-c)pyridines**
- **deazapteridines**.

Preclinical development:

- **calanolides A and B**
- **nitroimidazopyrans PA 824 and PA 1343**
- **quinolones PD 161148 and CS-940**
- **poloxamer 315**.

Clinical development:

- **moxifloxacin**
- **sitafloxacin**
- **gemifloxacin**
- **T-3811ME**.

It was also recommended that the families of antifungal **azoles**, antibacterial **nitroimidazoles**, **quinolones** and **oxazolidinones** be further studied in the discovery research phase in an attempt to identify molecules with greater anti-TB potential than current candidates which have been discussed specifically in this report. The use of inhibitors of bacterial **drug efflux** mechanisms and small molecule **immunomodulators** were also felt to be areas worth investigating further in order to identify agents as possible adjuncts to existing TB drug regimens.

INTRODUCTION

Tuberculosis (TB) is a disease of antiquity which is thought to have evolved sometime between the seventh and sixth millennia BC.[1] Current estimates suggest that one third of the world's population are infected resulting in some 2 million deaths per year.[2] The introduction of the first drugs for TB treatment some 50 years ago - streptomycin, para-aminosalicylic acid, isoniazid - led to optimism that the disease could be controlled if not eradicated.[3] These medicaments, coupled with generally increasing standards of health care, caused a rapid decline of tuberculosis in many industrialized countries which produced a climate of indifference to the need for fresh drugs. As a result of this apathy and the perception by the pharmaceutical industry that such agents would be unlikely to generate a suitable return on investment, few new drugs have been introduced in the last 30 years.[4] However, since the 1980's the disease has been undergoing a resurgence driven by a variety of changes in social, medical and economic factors. Thus, a dramatic increase in immuno-suppressed individuals due mainly to AIDS (but also to cancer chemotherapy and organ-transplant practices), coupled with increasing urbanization and poverty in developing countries, has compromised primary heath care structures and led to large increases in TB incidence.[5] Concomitant with the resurgence of TB has been the occurrence of multidrug-resistant disease which has exposed the frailties of the current drug armamentarium.[6]

There is now recognition that new drugs to treat TB are urgently required, specifically for use in shorter treatment regimens than are possible with the current agents and which can be employed to treat multidrug-resistant and latent disease. A variety of new initiatives have been created to tackle these objectives, the most recent of which is the establishment of the so-called Global Alliance for TB Drug Development.[7] The Alliance, a public/private partnership in which WHO is a partner, is a not-for-profit venture that will accelerate the discovery and development of new drugs to fight TB using a virtual operating model to outsource projects. As a first step to identifying suitable candidate molecules, information in the public domain on anti-TB compounds was reviewed at a meeting held in Geneva, 5-6 December 2000, under the joint auspices of the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (TDR) and the Global Alliance for TB Drug Development (GATB).

The information in this report was initially presented at the meeting in a draft document prepared by Dr Toshiko Imamura (WHO; financially assisted by the Rockefeller Foundation). This contained data extracted mainly from the public domain via Medline, R&D Focus, Pharma-Transfer, World Drug Alert (Accord), Investigational Drugs weekly highlights, a Boston Consulting Group presentation to the Global Alliance for TB Drug Development (working session, 11 May 2000), and various pharmaceutical company web-sites. In some instances non-confidential information was also obtained direct from representatives of companies involved in the development of several of the compounds cited. As a result of the review, additional material was added by the delegates and used to produce the ensuing report.

COMPOUNDS WITH ANTI-TB ACTIVITY

As in the original draft document, this report presents chemical, biological, and in some cases, clinical data for each of the candidate molecules and uses this to conduct a SWOT (Strength, Weakness, Opportunity, Threat) analysis. The compounds are grouped according to their perceived state of development for the TB indication - discovery research, preclinical development and clinical development. It should be noted that for many of the compounds, particularly those in the discovery and preclinical phases, there were insufficient data to enable accurate assessments of their anti-TB potential to be made. Nevertheless, despite this paucity of information, it was possible to conclude which molecules should be progressed further to generate the required data to facilitate go/no go recommendations.

Discovery

In assessing the compounds discussed below, particular attention was paid to the following properties:

Drug like:

- molecular structure compatible with ADME (absorption, distribution, metabolism, excretion) requirements
- avoid metabolically fragile molecules, high molecular weight, very polar, and highly lipophilic compounds
- avoid compounds containing known toxocophores and chemically reactive groups.

Novelty:

- at target site, avoid cross resistance with existing drugs
- structure makes patent protection feasible and may also avoid cross resistance with existing drugs even if directed to the same target protein.

In vitro data:

- activity v *M. tuberculosis* strains
- activity v mammalian cells
- bacteriostatic v bactericidal activity.

In vivo data:

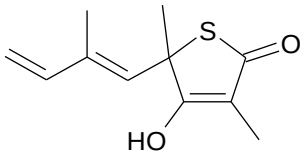
- efficacy
- ADME
- toxicity.

Potential cost of goods:

- ease of synthesis; achiral preferred to chiral molecules
- availability of starting materials
- lack of hazardous intermediates.

Many of the above parameters were not known with certainty, but sufficient details were usually available to permit a judgment to be made.

1. Thiolactomycin and analogues

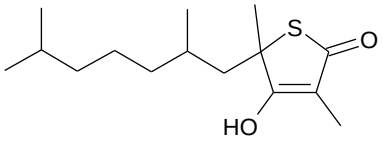


Thiolactomycin

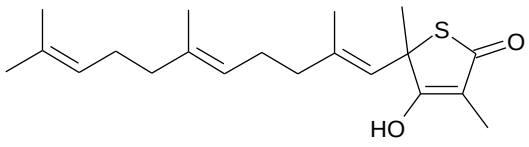
Thiolactomycin is an antibiotic of considerable interest because of its selective activity in disrupting essential fatty acid synthesis in bacteria, plants and some protozoa, but not in eukaryotes. This has lead to expectations that inhibitors of the thiolactomycin target enzyme, FAS-II, are of potential value in the treatment of malaria,[8] African trypanosomiasis (sleeping sickness)[9] and various bacterial indications including TB.[10] Currently, various organizations are active in this area of research e.g. Glaxo SmithKline, US National Institutes of Health (NIH), University of Newcastle, UK.

Thiolactomycin selectively inhibits the mycobacterial acyl carrier protein-dependent type II fatty acid synthase (FAS-II) but not the multifunctional type I fatty acid synthase (FAS-I) present in mammals.[10,11] It also has been shown to block long-chain mycolate synthesis in a dose-dependent manner in purified, cell wall-containing extracts of *Mycobacterium smegmatis*.[11] The primary mode of action of isoniazid is also disruption of mycolic acid synthesis, but there is no cross-resistance between the two molecules. This is because isoniazid requires activation by a katG catalase-peroxidase enzyme and in many resistant strains of M. tuberculosis this enzyme is mutated and unable to convert the drug to its active species.[12] Consequently, thiolactomycin is active in vitro against a wide range of strains of *M. tuberculosis*, including those resistant to isoniazid, albeit at somewhat high concentrations. For example, complete inhibition of growth on solid media of the virulent strain *M. tuberculosis* Erdmman is seen at 25mcg/ml.[11] In rodents, thiolactomycin is well absorbed orally[13] with an LD50 of 1.689g/kg.[14] It has activity in mice against various models of bacterial infection, by both the oral and sub-cutaneous routes, but does not appear to be especially potent, with ED50's >70mg/kg.[13] No reports appear to have been published on its efficacy towards *M. tuberculosis* in any animal models.

Although the above activity is interesting, it is insufficient to warrant further progression of thiolactomycin itself as an anti-TB agent. However, synthetic routes are available which allow access to analogues as both enantiomerically pure molecules[15] and racemic mixtures,[10] e.g. compounds (1) and (2), which are reported to have greater activity than the parent in inhibiting *M. tuberculosis* H37Rv in vitro.[10] Undoubtedly, as more structural details of the target protein(s) emerge, they will provide an added stimulus to aid design of more potent analogues.



(1)



(2)

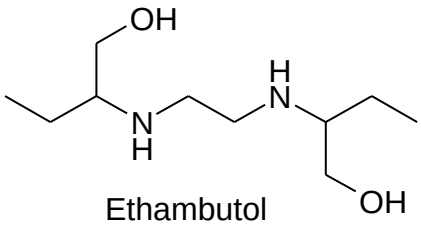
SWOT analysis

Strengths	Weaknesses
• Synthetic routes to thiolactomycin and analogues have been developed • The mechanism of action is known • Active against isoniazid-resistant strains • Well absorbed orally	• No reports of in vivo activity v *M. tuberculosis* • Parent molecule is chiral - cost implications if analogues also chiral
Opportunities	**Threats**
• Both pharmas and academia are pursuing inhibitors of FAS-II and progress might be accelerated by collaborating with GATB/TDR	• Unknown at this stage

Recommendation

Pursue further thiolactomycin analogues and other inhibitors of FAS-II.

2. Ethambutol analogues



Ethambutol

Ethambutol is one of the main drugs used in TB-treatment regimens and in most countries it has now replaced streptomycin and thiacetazone. Although the mode of action is not known with certainty, ethambutol interferes with construction of the arabinogalactan layer of the mycobacterial cell wall.[16] In general, it is well tolerated but has been reported to induce ocular toxicity as a result of depletion of copper and zinc levels.[17] The structure of ethambutol is favourable to the preparation of analogues by combinatorial chemical techniques, and a 100 000 strong library has been constructed at the NIH using solid phase synthesis.[18] Biological screening of these analogues has resulted in the identification of 150 compounds worthy of further evaluation. Of these, five have been tested in vivo for TB activity and at least one, NIH 241, has been shown to have good oral activity at 10mg/kg – comparable with ethambutol efficacy at 100mg/kg.[18] Cross resistance with the parent drug is unlikely to be a serious consideration as, where this does occur clinically, it is not at a high level. The analogues tested to date, like the parent molecule, all appear to have a static rather than a cidal action and are not active against non-mycobacterial microbes. However, unlike ethambutol, they are poor metal ion chelators and consequently are not expected to induce the same ocular toxicities.

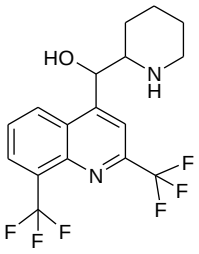
SWOT analysis

Strengths	Weaknesses
• More potent in vivo than ethambutol • Likely to have less toxicity than ethambutol	• Cytostatic action • Parent molecule is chiral - cost implications if analogues also chiral
Opportunities	**Threats**
• Progress might be accelerated by collaborating with GATB/TDR	• Unknown at this stage

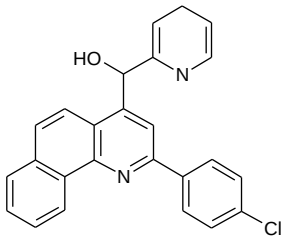
Recommendation

Pursue further.

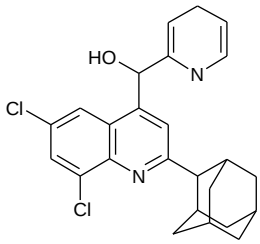
3. Mefloquine and analogues



Mefloquine



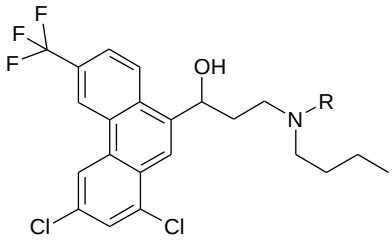
WR-3016



WR-3017

The antimalarial drug mefloquine (a 4-aminoquinoline methanol), and several analogues, have been reported to have activity against a variety of bacteria including *Mycobacterium*.[19] From a series of quinolinemethanols obtained from WRAIR, two compounds, WR-3016 and WR-3017, showed potent inhibitory activity in vitro in the *M. avium* complex-1 (MAC) assay with MIC50 values of 1 and 2 mcg/ml respectively, compared to 16 mcg/ml for mefloquine.[20] However, these two compounds were not as active as the parent drug in an in vivo MAC assay. NIH is engaged in a programme of screening other mefloquine analogues from the WRAIR.[21] Ideally this should include the two enantiomers of mefloquine[22] and might be worth extending to test a few representative 4-aminoquinoline antimalarials such as chloroquine. Other relevant samples might be obtainable from other groups pursuing these types of antimalarial compounds such as those at Liverpool[23] and Tulane[24] Universities.

There is also interest in the anti-TB properties of the mefloquine analogue desbutylhalofantrine. This compound is in development for its antimalarial properties with the apparent advantage over the parent drug halofantrine of lower cardiotoxicity. In Phase I clinical trials, sufficient blood levels were reached to suggest that it would be effective in TB patients[25] and a patent application has been filed for this indication.[26]



R = n-butyl, halofantrine
R = H, desbutlyhalofantrine

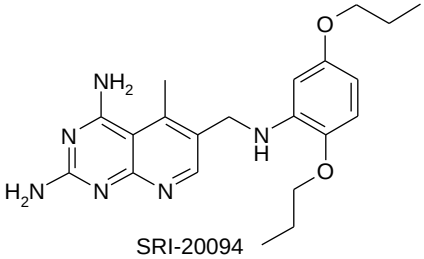
SWOT analysis

Strengths	**Weaknesses**
• Mefloquine has good in vivo activity • Desbutylhalofantrine, in clinical development for malaria, has antimycobacterial activity • Many analogues available	• None apparent but possibility of strong protein-binding should be checked
Opportunities	**Threats**
• Progress might be accelerated by collaborating with GATB/TDR	• Unknown at this stage

Recommendation

Pursue mefloquine and desbutylhalofantrine further and encourage testing of more analogues.

4. Deazapteridines



SRI-20094

From a series of 2,4-diamino-5-deazapteridine derivatives synthesized at the Southern Research Institute, SRI-20094 displayed potent inhibition of MM6 cells infected with *M. avium* complex strain NJ3440 with an MIC of at most 0.13 mcg/ml. SRI also showed excellent inhibition of dihydrofolate reductase (DHFR) of the *M. avium* complex, with an IC50 value of 1.0 nM as compared to 4100, 1.0 and 1.4 nM for the known agents trimethoprim, trimetrexate and piritrexim respectively. It displayed limited inhibition for human DHFR having an IC50 value of 7300 nM. SRI-20094 is claimed to be of potential value for the treatment of *M. avium* infections and, in particular, for persons co-infected with HIV.[27] Other close analogues of this compound have previously been reported to be highly active against *M. tuberculosis* with MICs of ~0.1mg/l.[28]

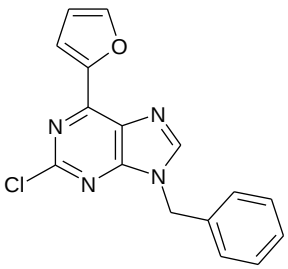
SWOT analysis

Strengths	Weaknesses
• 20094 is a very potent and selective inhibitor of mycobacterial DHFR • Knowledge of the tertiary structure of *M. tuberculosis* DHFR will allow a rational search for other inhibitors • Many other DHFR analogues available	• In vivo information lacking on lead compound
Opportunities	**Threats**
• Progress of deazapteridine series might be accelerated by collaborating with GATB/TDR	• Unknown at this stage

Recommendation

Pursue SRI compounds further and encourage testing of the many types of DHFR inhibitors produced by both industry and academia. The tertiary structure of the *M. tuberculosis* enzyme could profitably be used to aid in the identification of TB-specific inhibitors.[29] In addition, the recently developed yeast-based system incorporating the DHFR allele is also an attractive possibility for use in high throughput screening for novel enzyme inhibitors.[30]

5. 9-Benzylpurines



2-Chloro-6(2-furanyl)-9-benzylpurine

From a series of 9-benzylpurines synthesized at the University of Oslo, 2-chloro-4(2-furanyl)-9-benzylpurine was shown to potently inhibit *M. tuberculosis* H37Rv in vitro with a MIC value of 0.78 mcg/ml.[31] It also exhibited low cytotoxicity towards VERO cells (IC50 value - 8.1 mcg/ml) - selectivity index (MIC/IC50) of 10.4. No in vivo data are yet available.

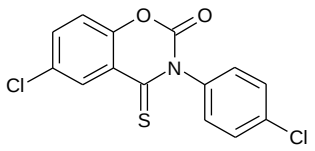
SWOT analysis

Strengths	Weaknesses
• Synthesis of some 40 analogues to date • Good activity in vitro with low cytotoxicity • Structurally dissimilar to existing TB drugs and hence cross-resistance unlikely	• No in vivo data • No information on mode of action
Opportunities	**Threats**
• Funding opportunity for GATB/TDR to aid further progress	• If no funds become available then research will cease

Recommendation

Pursue further and encourage selection of a limited number of analogues (two or three) for in vivo evaluation.

6. Benzoxazines



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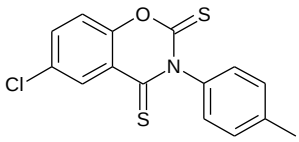


(4)

Evaluation of a series of 6-chloro-3-phenyl-4-thioxo-2H-1,3-benzoxazine-2(3H)-ones and 6-chloro-3-phenyl-2H-1,3-benzoxazine-2,4(3H)-dithiones, revealed compounds (3) and (4) to have potent antimycobacterial activity against *M. tuberculosis* (MIC values 0.5 mcmol/l), *M. avium* (16 and 16 mcmol/l), *M. kansasii* 235/80 (2 and 2 mcmol/l), and *M. kansaii* 6509/96 (1 and 0.5 mcmol/l), compared with MIC values of 4, 500, 8 and 500 mcmol/l for isoniazid after 14 days.[32] No in vivo data were reported or information as to mode of action. However, the presence of the thioamide moiety is suggestive of some structural similarities to existing TB drugs such as thiacetazone and may result in cross-resistance.

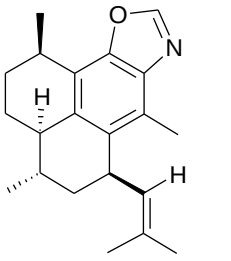
SWOT analysis

Strengths	Weaknesses
• Range of analogues available • Lead compounds have good in vitro activity v spectrum of mycobacteria species	• No in vivo data • No information as to mode of action
Opportunities	**Threats**
• Progress might be accelerated by collaborating with GATB/TDR	• Unknown at this stage

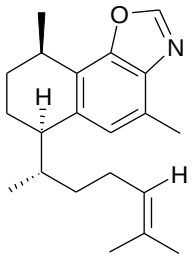
Recommendation

Pursue further and encourage evaluation of lead compounds in vivo and v thiacetazone-resistant strains of TB.

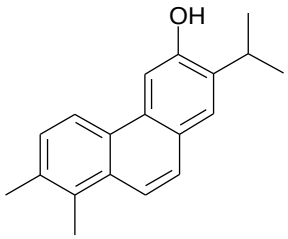
7. Diterpenoids



Pseudopteroxazole



Seco-pseudopteroxazole



(5)

Screening marine natural products from the West Indian gorgonian coral *Pseudopterogorgia elisabethae* for anti-tuberculosis activity resulted in the isolation and identification of two active diterpenoid alkaloids, pseudopteroxazole and seco-pseudopteroxazole. Both compounds are novel diterpenoids containing the uncommon benzoxazole moiety.[33] Against *M. tuberculosis* H37Rv pseudopteroxazole was claimed to be a potent inhibitor giving 97% growth inhibition at 12.5mcg/ml whilst seco-pseudopteroxazole was somewhat less active. No in vivo data on the compounds have been reported and no attempts appear to have been made to obtain the compounds synthetically. However, other workers have been successful in cultivating corals artificially and if it became necessary to obtain these diterpenoids in greater quantity then such a process might be an attractive alternative to *de novo* synthesis or large scale extraction of the coral from its natural habitat.

Post-meeting it was noted that various plant-derived diterpenoids have also been reported to have tuberculosis activity.[34,35] Several of these, e.g. (5), are considerably more active than the marine diterpenoids - MIC of (5) v *M. tuberculosis* H37Rv = 0.46mcg/ml. Such compounds, although still novel, seem structurally more amenable to analogue synthesis.

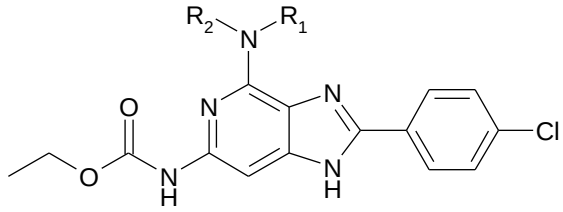
SWOT analysis

Strengths	Weaknesses
• Novel structures suggest cross-resistance with existing drugs is unlikely • In vitro activity of some diterpenes is comparable to drug standards	• No in vivo data • Mode of action unknown
Opportunities	**Threats**
• GATB/TDR should encourage further testing on various diterpenoids	• Not known at this stage

Recommendation

Pursue diterpenes further and in particular, encourage in vivo assessment of compounds such as (5).

8. Imidazo(4,5-c)pyridines



R_1 and R_2 are various alkyl groups

From a series of imidazo(4,5-c)pyridines synthesized at the Southern Research Institute (SRI), one compound - see above for general formula (R1, R2 undisclosed) - inhibited *M. tuberculosis* H37Rv and other strains with MICs in the range 0.256-2.56mcg/ml.[36] Imidazo(4,5-c)pyridines were originally prepared as antimitotic agents for cancer chemotherapy but in the current work, less cytotoxic agents were selected and found to have anti-TB activity.[37]

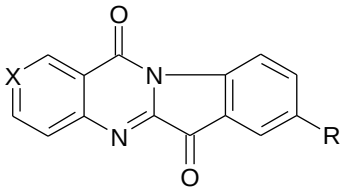
SWOT analysis

Strengths	Weaknesses
• Range of analogues synthetically accessible • Structurally dissimilar to existing TB drugs hence cross-resistance unlikely	• No in vivo data • Mode of action unknown
Opportunities	**Threats**
• Progress might be accelerated by collaborating with GATB/TDR	• If SRI cannot attract funds, the development of the compounds may stall

Recommendation

Pursue further and encourage in vivo testing on the lead compounds.

9. Tryptanthrin and analogues



Tryptanthrin, R = H, X = CH
PA 505, R = $CH(Me)(CH_2)_5Me$, X = N

Tryptanthrin is a structurally novel indoloquinazolinone alkaloid, first isolated by Chinese scientists, which has been tested against various strains of *M. tuberculosis*.[38] Against a drug-sensitive strain, H37Rv, the MIC of tryptanthrin was 1.0 mcg/ml compared to 0.03 mcg/ml for isoniazid. When tested against a panel of multidrug-resistant strains of *M. tuberculosis*, whilst tryptanthrin maintained its potency (MICs of 0.5-1mcg/ml), isoniazid had decreased activity with MIC's 4-16mcg/ml. Many analogues of this lead structure have been synthesized and evaluated for their potential in tuberculosis chemotherapy. However, to date it is not been possible to identify a compound sufficiently efficacious in animal models to warrant further progression. For example the PathoGenesis Corporation compound, PA-505, whilst having potent in vitro activity towards *M. tuberculosis* H37Rv - MIC 0.015mcg/ml - had only modest effects in decreasing *M. tuberculosis* in the spleen of infected mice when given at 50mg/kg/day orally for ten days.[38] Peak plasma concentrations of 2-3.5 mcg/ml were reached after two hours, falling to half this concentration by 7.5 hours, and it was concluded that, for most of the rest of the 24-hour period, there would be insufficient drug concentration to exert a bactericidal effect. Finally, although the mode of action of the tryptanthrins is not known, from structural considerations they may well be DNA intercalators which, if correct, would raise toxicological issues.

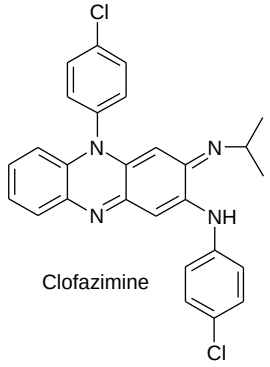
SWOT analysis

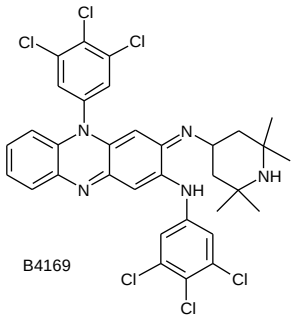
Strengths	**Weaknesses**
• Synthetic routes to tryptanthrin and analogues have been developed • Active against drug-resistant strains • Some moderate activity seen in vivo	• lack of toxicity data • Poor in vivo activity
Opportunity	**Threat**
• Collaboration with GATB/TDR might induce pharmas to re-investigate the area	• Not known at this stage

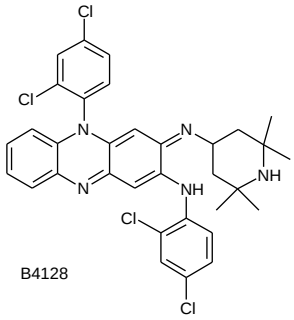
Recommendation

Until compounds emerge with the appropriate in vivo profile, do not pursue further

10. Tetramethylpiperidino (TMP) phenazines







The anti-TB activity of a series of novel tetramethylpiperidinophenazines closely related to the anti-leprosy drug clofazimine has been reported - see also page 26. The intra- and extra-cellular activities of these compounds were compared to clofazimine and rifampicin against *M. tuberculosis* H37Rv (ATCC 27294).[39] One of the phenazines, B4169, potently inhibited the bacterium with an MIC value of 0.015 mcg/ml; the corresponding value for clofazimine was 0.06 mcg/ml. The compounds were also more active than clofazimine against a range of clinical *M. tuberculosis* isolates including multidrug-resistant strains. Additionally several of the phenazines, e.g. B4128, showed significant intracellular activity (~60% inhibition of growth) at 0.001 mcg/ml against *M. tuberculosis* -infected monocyte-derived macrophages and were superior to both clofazimine and rifampicin. No in vivo data were reported and the compounds have a static rather than cidal action. There is no information as to mode of action although they might be expected to participate in a variety of redox reactions. Additionally, since they have close structural similarities to clofazimine, most likely they will suffer from the undesirable property of the latter of imparting marked coloration to the skin of patients.

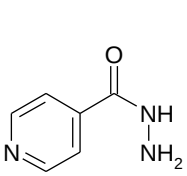
SWOT analysis

Strengths	**Weaknesses**
• Potent activity v multidrug-resistant strains • Active intra- and extra-cellularly	• In vivo data lacking • Probably highly coloured • No data on mode of action
Opportunity	**Threat**
• None perceived	• Not known at this stage

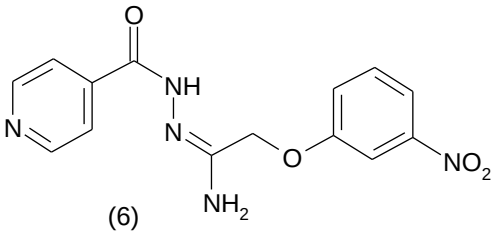
Recommendation

In the absence of in vivo data to refute the above shortcomings, do not pursue further.

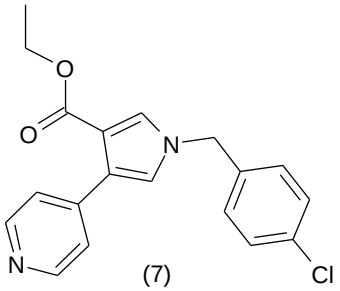
11. Isoniazid derivatives and analogues



Isoniazid



(6)



(7)

Various analogues and derivatives of isoniazid continue to be synthesized, mainly by scientists in academia, and their potential for TB treatment explored, e.g. (6),[40] (7).[41] However, these compounds are likely to be ineffectual against isoniazid-resistant strains of TB either because their activity results from regeneration of this drug or because of close structural similarities.

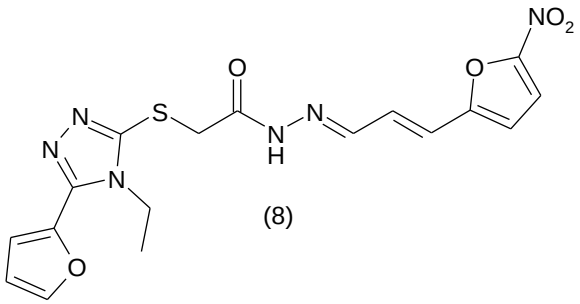
SWOT analysis

Strengths	**Weaknesses**
• Ease of synthesis • Known mode of action	• Cross-resistance with isoniazid
Opportunity	**Threat**
• None perceived induce pharmas to re-investigate the area	• Little of interest to attract pharma

Recommendation

Do not pursue further unless data emerge to suggest some major advantage over isoniazid.

12. 1,2,4 –Triazoles



(8)

Various 1,2,4-triazoles have been synthesized and evaluated against *M. tuberculosis* H37Rv. Compound (8), at 6.25 mcg/ml, gave 61% inhibition.[42] Other close analogues were inactive and no in vivo data were reported.

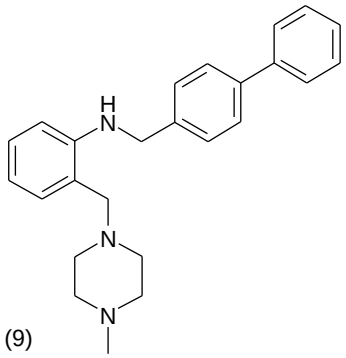
SWOT analysis

Strengths	Weaknesses
• Variety of analogues synthetically accessible	• No in vivo data • From structural considerations, the molecules do not appear to be too metabolically robust
Opportunity	**Threat**
• None apparent	• Little of interest to attract pharma

Recommendation

Do not pursue further unless data emerge to show activity in vivo.

13. Toluidine derivatives



(9)

A number of simple derivatives of toluidines have shown interesting in vitro activity against *M. tuber - culosis* 103471,[43] with the best compounds, exemplified by (9), having MICs of 4 mcg/ml - cf MICs of isoniazid, 0.25 mcg/ml, and streptomycin, 0.5 mcg/ml. However, no in vivo data are reported and there are concerns that these aromatic amines will undergo rapid metabolic degradation, possibly to toxic metabolites.

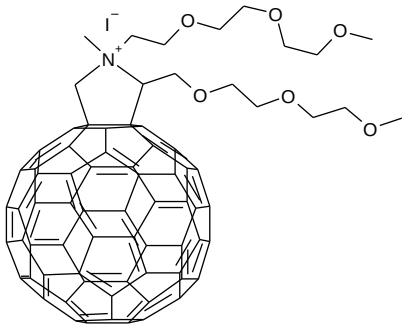
SWOT analysis

Strengths	Weaknesses
• Ease of synthesis	• No in vivo data
Opportunity	**Threat**
• None perceived	• Little of interest to attract pharma

Recommendation

Do not pursue until in vivo data obtained.

14. Fulleropyrrolidines



(10)

From a series of fullerene derivatives, compound (10) displayed anti-mycobacterial activity. It inhibited the growth of *M. tuberculosis* strain H6/99, a human clinical isolate, with a MIC value of 5 mcg/ml, and strain H37Rv with a MIC value of 50 mcg/ml.[44] Some fullerene derivatives have also shown in vitro activity against the HIV protease[45] offering the tantalising possibility of joint efficacy towards both AIDS and TB. However, the presence of the quartenary nitrogen atom in (10) suggests that toxicity issues might be a problem. In addition, the large size of these molecules probably precludes oral absorption.

SWOT analysis

Strengths	**Weaknesses**
• Offers possibility of efficacy v TB and AIDS • Novel structures suggest cross-resistance with existing TB drugs is unlikely	• High molecular weight will preclude oral absorption • Not particularly active in vitro • Toxicity concerns • Lack of in vivo data • Potential cost of synthesis
Opportunity	**Threat**
• GATB/TDR support might permit critical areas of concern to be rapidly addressed	• In the absence of further support, research may cease into TB indication

Recommendation

Do not pursue.

15. Granulysin

Chemical structure - not applicable

Granulysin is a novel antimicrobial protein produced by human cytotoxic T-lymphocytes and natural killer cells.[46] The protein is of interest as it has been found to reduce the viability of a broad spectrum of pathogenic bacteria, fungi, and parasites in vitro. Furthermore, synthetic 22- and 29-residue peptides of granulysin directly kill extracellular *M. tuberculosis*, altering the membrane integrity of the bacillus.[47] However, formidable barriers exist to producing a therapeutic agent from this area of work, especially cost and lack of oral bioavailability of peptides of this size.

SWOT analysis

Strengths	**Weaknesses**
• Novel action • Little likelihood of cross-resistance	• No in vivo data • Cost of peptides • Potential lack of oral bioavailability • Immunogenicity
Opportunity	**Threat**
• GATB/TDR support might permit critical areas of concern to be rapidly addressed	• In the absence of further support, research may cease into TB indication

Recommendation

Do not pursue.

16. Stazyme

Chemical structure - not applicable

Stazyme is a preparation from *Staphylococcus clavelios* containing several different-sized proteins which cause significant mycobacterial growth inhibition.[48] At concentrations of 50 and 200 mcg/ml of total protein, stazyme was highly bactericidal against *M. smegmatis*, and bacteriostatic against *M. tubercu - losis* and *M. avium*. It was able to break the permeability barrier of *M. avium* isolates, significantly enhancing the activity of other anti-TB drugs. Identification of the mycobacteriolytic determinant in stazyme may be helpful as a tool to define novel drug targets in mycobacteria. However, the current work is unlikely to yield any chemotherapeutic agent suitable for progression into the clinic in the foreseeable future.

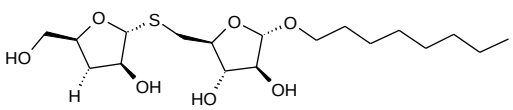
SWOT analysis

Strengths	Weaknesses
• Novel action • Enhances the in vitro potency of other anti-TB	• No in vivo data • Heterogeneous preparation • Potential cost • Potential lack of oral bioavailability • Immunogenicity
Opportunity	**Threat**
• None perceived at this stage	• Lack of commercial potential

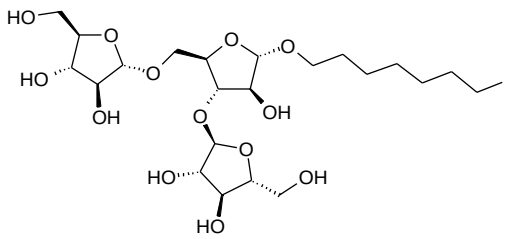
Recommendation

Do not pursue.

17. Saccharides



SR-9581



(11)

The arabinose disaccharide SR-9581 is active in vitro against *M. tuberculosis*, with a MIC value of 4 mcg/ml.[49] It decreased the viability of *M. tuberculosis* by 76.1%, 97.8% and 99.9% at 8, 16 and 32 mcg/ml respectively within 3 days. In a separate report,[50] another saccharide, an arabinofuranoside oligosaccharide (11), is claimed to be a substrate for mycobacterial arabinosyltransferases - no data are presented in the Derwent abstract. Both compounds can be expected to disrupt mycobacterial cell wall biosynthesis.

SWOT analysis

Strengths	Weaknesses
• In vitro activity against defined biochemical target	• No in vivo data • Saccharides might be costly to synthetize and have poor ADME characteristics
Opportunity	**Threat**
• None perceived at this stage	• None perceived at this stage

Recommendation

Do not consider further until more promising and detailed information becomes available.

Other areas of interest

A. In addition to the above, several compounds were considered specifically under the 'preclinical' or 'clinical' section of this report but it was felt that it would be beneficial to re-investigate a range of their analogues at the earlier discovery research phase. The series recommended for further study were the quinolones, oxazolidinones, miconazole analogues and the nitroimidazoles. The key issues/advantages were perceived as follows:

18. Quinolones (see pages 21, 22, 29-33)

The current molecules in this series will have mainly been selected for further development/marketing on the basis of broad-spectrum antimicrobial efficacy in short-term treatment regimens. However, for TB chemotherapy it would be beneficial to progress representatives of the series which have a more selective action on *mycobacteria* at the expense of other microbes. This would help avoid the undesirable effect of severely depleting the beneficial gut fauna. Another approach to identifying a more appropriate anti-TB quinolone would be to select those members of the series concentrating in lung tissues. However, this is more difficult and far more time consuming and expensive than determining activity in vitro against a panel of bacteria. It would be highly desirable to compare all potential candidates for further progression (including those belonging to other series discussed in the preclinical section of this report) in a standard series of tests - e.g. efficacy, ADME, toxicity studies in mice, in vitro tests v quinolone-sensitive and -resistant strains of *M. tuberculosis*.

The side-effects of the quinolone antibacterials - particularly the newer fluoroquinolones[51] - are well documented and include, for example, phototoxicity, neurotoxicity, and drug-drug interactions (possibly caused by the affinity of these compounds for cytochrome P450). Less frequently observed problems include renal, hepatic and cardiac toxicity. Since the treatment regimens for TB therapy are likely to be longer than for most other bacterial diseases, it is a cause of some concern that these toxicities might occur more frequently and blight a drug marketed for both TB and more commercially attractive diseases. Consequently, the selection of a mycobacterial-disease specific drug is attractive despite the additional costs of developing this as a new chemical entity.

19. Oxazolidinones (see page 25)

Similar remarks apply to this series as to the quinolones. However, far less information is in the public domain as to the likely toxicities of these agents following long-term administration since they are newcomers to the antimicrobial market.

20. Miconazole analogues (see pages 26, 27)

It remains to be established whether the in vitro effects of miconazole result from the presence of a pharmacophore shared by the rest of the anti-fungal azoles or whether they are unconnected and the antimicrobial properties result from other structural features. This can readily be ascertained by screening a selection of the anti-fungal drugs.

21. Nitroimidazoles (see pages 23-24)

Commercial interest in this series of compounds is influenced by both Novartis and th PathoGenesis Corporation abandoning their lead compounds CGI 17341 and PA824/PA1343. The target enzyme for the compounds has been identified at the NIH and, although the protein is not yet disclosed in the public domain, it is known to be involved in cell wall synthesis.[52] The compounds are also known to require reductive activation. Further collaboration is to be encouraged between NIH and Pathogenesis to try and identify more efficacious molecules. However, two major areas of concern also need to be addressed - possible mutagenicity resulting from the presence of a nitro group, and the opportunity for

the ready development of drug resistance. The latter is prompted by the fact that the nitroimidazoles induce a high rate of mutation.[53] Furthermore, the obligate activating (reducing) enzyme, using a flavin co-factor, is not essential for the survival of mycobacteria,[54] leading to suspicions that this might cause the ready emergence of drug-resistant bacteria. Since the drugs will inevitably be used in combination therapy, these issues, whilst still needing consideration, should not be viewed in too pessimistic a light.

B. The above reviews have focused on compounds having direct action on *mycobacteria*. However, strategies could be explored which might enhance the effectiveness of existing TB drugs. Specifically immunomodulators and efflux pump inhibitors were deemed worthy of further interest:

22. Immunomodulators

Some limited data are available to suggest that immunomodulators might be useful in TB treatment. Gamma-interferon has been administered by aerosol to MDR TB patients and shown to have some bacteriological effects,[55] and the outcome of formal trials is now awaited. Small molecular weight modulators, which could be expected to be significantly cheaper and suitable for oral administration, have also shown preliminary promise. Thus compounds such as thalidomide, which block TNFα production, have been reported to have beneficial effects on weight gain in both HIV-positive and -negative TB patients.[56] Other small molecular weight immunomodulators such as tucaresol (GlaxoSmithKline) might also be useful,[57] and encouragement should be provided to these pharmas to evaluate the potential of the compounds for TB therapy.

23. Efflux pump inhibitors

Inhibition of proteins responsible for functioning as bacterial efflux pumps is a strategy worth exploring as a possible means of negating MDR disease. Preliminary data on *M. smegmatis* show that efflux proteins of the proton antiporter family can confer low-level resistance to fluoroquinolones.[58] There have also been reports of a series of bacterial efflux inhibitors that have been identified by Microcide working in collaboration with Daiichi.[59] Data are not yet in the public domain and a watching brief should be kept on this potentially interesting area.

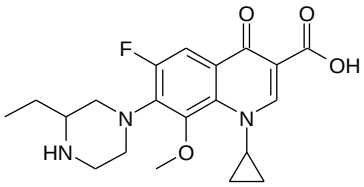
C. The meeting did not seek to address novel biochemical targets except to note the current interest in isocitrate lyase as a potential TB drug target (see below). It was suggested that, at some stage, a meeting be convened to discuss this and other novel targets.

24. Isocitrate lyase

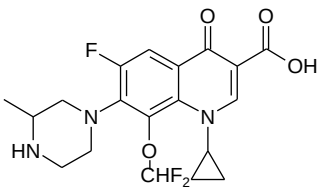
A recent report indicates that the persistence of *M. tuberculosis* in mice is facilitated by isocitrate lyase, an obligate enzyme for the metabolism of fatty acids.[60, 61] Biochemical studies suggest that, in chronically infected lung tissues, fatty acids may be a major source of carbon and energy in *M. tuberculosis* metabolism. The data show that, whilst isocitrate lyase is important for survival of *M. tuberculosis* in the lung during the persistent phase of infection, it is not essential for growth in the acute stage.[61] Since conventional antimicrobials target processes required for bacterial cell wall growth and division, such as cell wall biosynthesis and chromosome replication, combination therapy of existing TB drugs with an isocitrate lyase inhibitor might be expected to expedite eradication of tuberculosis infections. Further work is now required to conclusively validate this enzyme as a target for drug discovery. The three-dimensional structure of *M. tuberculosis* isocitrate lyase, in association with the simple prototype inhibitors 3-bromopyruvate and 3-nitropyruvate,[62] has been solved, and the search is now underway for more drug-like molecules. Since the dimensions of the active site are somewhat constrained, this is unlikely to be an easy task.

Preclinical

25. Quinolones PD 161148 and CS-940



PD 161148

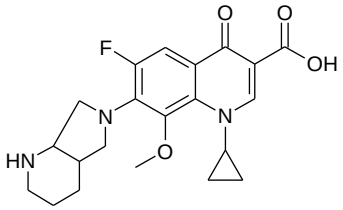


CS-940

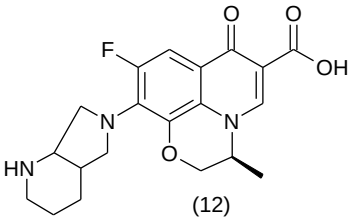
The title compounds, despite obvious structural similarities, were synthesized by different pharmas - PD 161148 [63]originated at Parke-Davis (now Warner-Lambert) and CS-940 [64] at Sankyo. Both have been selected on the basis of their potent broad-spectrum activity against gram-negative, -positive and anaerobic bacteria, and have been tested against a variety of mycobacteria in comparison to other quinolones. Unfortunately, since these studies were conducted by two different groups, no comparative data between the two compounds are available. CS-940 was screened against 100 clinical isolates of *M. tuberculosis* and, along with sparfloxacin, was found to be have an average IC50 of 0.25-0.5mcg/ml and to be more potent than ofloxacin, ciprofloxacin and balofloxacin (IC50s of 0.5 to 2.0mcg/ml), with norfloxacin (IC50s of 8 to 16 mcg/ml) being the least active. PD 161148 was also tested against various clinical isolates of *M. tuberculosis* where it was compared to the desmethoxy analogue and ciprofloxacin and found to be some 3-4-fold more active. Against ciprofloxacin-resistant strains, the presence of the C-8 methoxy group in PD 161148 enhanced lethality. Both PD 161148 and CS-940 are amongst the most active of all third-generation quinolones but unfortunately there are no in vivo tuberculosis data in the public domain which would allow the two compounds to be further separated. Structural considerations do however suggest that, of the two, PD 161148, which contains only one fluorine atom, might be less expensive to manufacture and have a lower propensity to induce phototoxicity.

SWOT analysis

Strengths	**Weaknesses**
• Potent bactericidal activity • Active against both quinolone -sensitive and -resistant *M. tuberculosis*	• No in vivo TB efficacy, ADME or toxicity data available • From structural considerations, the molecules do not appear to be too metabolically robust
Opportunity	**Threat**
• Collaboration with GATB/WHO might induce pharmas to progress the compounds rapidly for TB	• Numerous other quinolones are in development



moxifloxacin

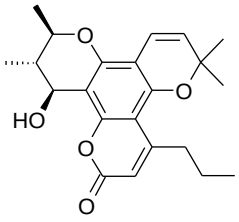


(12)

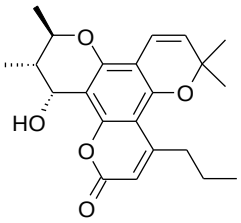
Recommendation

Pursue further and encourage a side-by-side comparison with other quinolones such as moxifloxacin and the levofloxacin analogues exemplified by (12). Data on the latter quinolones65 emerged after the review, but again only in vitro mycobacterial data are available.

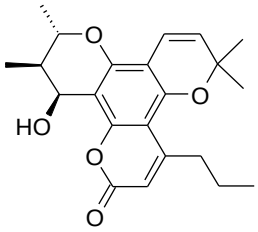
26. Calanolides



Calanolide A



Calanolide B



(13)

Calanolide A is a naturally occurring pyranocoumarin[66] of considerable interest because of its dual activity against TB and HIV infections.[67,68] The compound, an inhibitor of HIV-1 reverse transcriptase,[69] is being progressed by Sarawak MediChem Pharmaceuticals and is in Phase I/II development stage for the HIV indication. It also displays good in vitro activity towards *M. tuberculosis*. In a preliminary assessment of its activity, calanolide A was comparable to the positive control isoniazid and remained effective against rifampin- and streptomycin-resistant TB strains.[67] Sarawak has developed a process for the large-scale synthesis of calanolide A thus reducing the dependency upon obtaining the material from scarce natural resources.[67] Other analogues have been obtained either from plant extracts or by synthesis[70] and some, e.g.(13), have been patented for their antimycobacterial properties.[71] In addition, calanolide B, which unlike calanolide A, is readily available in substantial quantities from renewable natural sources, e.g. from *Calophyllum* seed oil,[72] is claimed to have a similar spectrum of activity to calanolide A against *mycobacteria* and may be a more cost-effective treatment.[73]

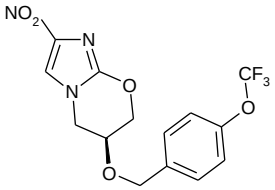
SWOT analysis

Strengths	**Weaknesses**
• Possible dual role in treating TB and AIDS • Synthetic route developed to calanolide A and analogues; calanolide B readily obtainable from renewable natural sources • No cross-resistance with rifampin or streptomycin	• For the TB indication, no in vivo efficacy, ADME or toxicity data in the public domain
Opportunity	**Threat**
• Collaboration with GATB/WHO could induce Sarawak to proceed with the TB indication	• Unless funds become available, Sarawak will concentrate on the AIDS indication

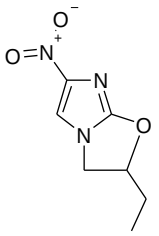
Recommendation

Pursue further and encourage Sarawak to produce sufficient data to aid assessment of the TB indication.

27. Nitroimidazoles



PA 824



CGI 17341

The Ciba-Geigy 5-nitroimidazole derivative CGI 17341 showed considerable potential for the treatment of tuberculosis in preclinical studies. In vitro, at 0.04 to 0.3 mcg/ml, the compound inhibited both drug-susceptible and multidrug-resistant strains of *M. tuberculosis* and showed no cross-resistance with isoniazid, rifampicin, streptomycin or ethambutol. Against *M. tuberculosis* in vitro, its activity was comparable to that of isoniazid and rifampicin and superior to streptomycin, ciprofloxacin, norfloxacin and the oxazolidinone DuP 721. In *M. tuberculosis*-infected mice, oral treatment with CGI 17341 on days 11 and 12 post infection resulted in an ED50 of 7.7 mg/kg and a significant dose-dependent increase in survival time.[74] Unfortunately despite the initial promise of CGI 17341, its development was terminated when the company was absorbed into Novartis[75] most likely as a result of perceived lack of commercial potential and mutagenicity.[74]

In a revival of interest into the 5-nitroimidazoles, a promising series of nitroimidazopyrans has been recently identified by the PathoGenesis Corporation for the treatment of TB and related mycobacterial diseases.[76,54] These compounds were not mutagenic and showed potent bactericidal activity against replicating and static *M. tuberculosis*, including multidrug-resistant strains. One compound, PA 824, displayed MIC values ranging from 0.015 to 0.25 mcg/ml against cultured replicating *M. tuberculosis* pan-sensitive and rifampin mono-resistant clinical isolates. Although this was not the most active member of the series, it showed impressive efficacy when administered orally to both *M. tuberculosis*-infected mice (25mg/kg) and guinea pigs (40mg/kg), and was comparable to isoniazid at 25 mg/kg.[54] In addition, it displayed low levels of toxicity with an acute toxic threshold value of 1000 mg/kg single dose, and a chronic threshold value of >500 mg/kg daily for 28 days. A related compound, PA 1343 (structure not disclosed), is claimed to be even more active than PA 824, although the MIC value quoted, 0.015 mcg/ml, against *M. tuberculosis* in vitro,[77] falls within the range of those for PA 824. Furthermore, when PA1343 was administered by oral gavage to mice infected with *M. tuberculosis*, treatment at 25 mg/kg was effective and comparable to PA 824 and isoniazid. Oral bioavailability of PA 1343 in mice was 74% with a half life of 4.2 hours. In contrast, PA 824 has poor oral bioavailability - 2% - which is attributed to its low aqueous solubility of 0.02mg/ml.[77] This can be markedly improved using a lipid coated cyclodextrin formulation,[77] but for TB treatment this is probably not a feasible option due to cost consequences.

Despite the promising data on PA 824 and PA 1343 and generic claims that mutagenicity is not a problem, the presence of the nitro moiety in these compounds raises concerns about the possibility of genotoxicity and this issue remains to be addressed.

SWOT analysis

Strengths	Weaknesses
• Potent cidal activity against drug-sensitive and -resistant strains of *M. tuberculosis* • Active orally in vivo	• Concerns about the toxic potential of the nitro group
Opportunity	**Threat**
• Collaboration with GATB/WHO would encourage PathoGenesis to proceed with further development	• In the absence of third party support, the project will be cancelled

Recommendation

Pursue further; in particular encourage genotoxicity studies.

28. Poloxamer 315 (CRL-1072)

Chemical structure - not applicable

Poloxamer 315 is a methyloxirane surfactant polymer from the CytRx Corporation that appears to disrupt the cell membranes of microbes or their intracellular components. The highly purified polymer has been shown to be active against both *M. tuberculosis*[78] and *M. avium*.[79] In vitro studies against *M. tuberculosis* in broth culture show MIC values of 3.1-6.2 mcg/ml whilst, in a macrophage assay, these drop to 0.92 to 1.25 mcg/ml.[80] The compound was active against strains of *M. tuberculosis* resistant to isoniazid, streptomycin and rifampin. In vivo, 2 mg/kg/day of 315 administered intravenously three times a week for three weeks allowed survival of *M. tuberculosis*-infected mice and reduced CFU (colony forming unit) counts in lungs and spleens by one to two log units.[80] In an acute toxicity study with 315 in mice, the maximum tolerated intravenous dose was 125mg/kg.[79] Pharmacokinetic analysis revealed very little of the drug in blood with high concentrations being found in liver, kidney and spleen. In subchronic toxicology studies, poloxamer 315 was non-toxic following oral administration at doses up to 100 mg/kg/day for 28 days.[78] An IND (investigational new drug) has been obtained for this compound in the USA but as yet no plans have been made to progress the compound clinically.[78] In order to evaluate its true potential, oral activity should be determined in various animal models and it would be instructive to compare efficacy against other surfactants first reported to have anti-tuberculosis activity some 50 years ago.[81]

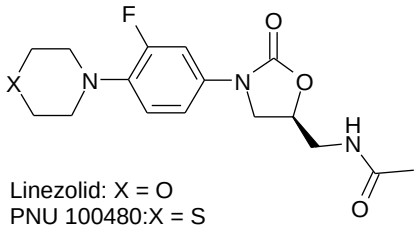
SWOT analysis

Strengths	Weaknesses
• Acts on microbes extra- & intra-cellularly • Synergises with other anti-TB drugs • IND obtained	• Non-specific mechanism of action • Accumulation in tissues may be problematic with long treatment regimens
Opportunity	**Threat**
• Collaboration with GATB/WHO may encourage clinical progression for TB indication	• None perceived

Recommendation

Pursue further with a view to encouraging further efficacy and toxicity studies.

29. Oxazolidinones PNU 100480 and AZD 2563



Linezolid: X = O
PNU 100480:X = S

The oxazolidinones are a promising new class of synthetic antimicrobial agents with a unique mechanism of action in inhibiting protein synthesis.[82] In general they display bacteriostatic activity against many of the important human pathogens including drug-resistant microbes.[82] One compound, linezolid,[83] has already reached the market place and other members of this class are in varying stages of development. The oxazolidinones have activity against *M. tuberculosis* with linezolid (U-100766) inhibiting multidrug-resistant isolates in vitro at 2 mcg/ml.[84] Oxazolidinones containing a thiomorpholine moiety in place of the morpholine unit present in linezolid have been reported to be particularly active against *M. tuberculosis* with MICs of 0.125 mcg/ml.[85] One member of this series, the Pharmacia Upjohn compound PNU-100480, was tested in a murine model against ten viable strains of *M. tuberculosis* in comparison to linezolid and isoniazid. When treatment was started one-day post infection and the compounds given by gavage for four weeks, PNU-100480 proved comparable to isoniazid and more active than linezolid.[86] Further comparisons are warranted with other oxazolidinones, such as the AstraZeneca compound AZD 2563[87] (structure not available) which is being progressed clinically for the treatment of MDR bacterial infections - no information is yet available as to its activity against mycobacteria species.

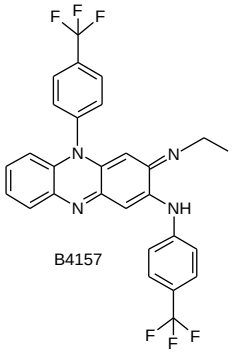
SWOT analysis

Strengths	**Weaknesses**
• Novel mode of action - not cross-resistant with other inhibitors of protein synthesis • PNU 100480 active in vivo v *M. tuberculosis*	• No ADME or toxicity data for PNU 100480 • No TB data for AZT 2563 • Potential cost
Opportunity	**Threat**
• Collaboration with GATB/WHO might encourage pharmas to generate definitive TB data	• Availability of other oxazolidinones

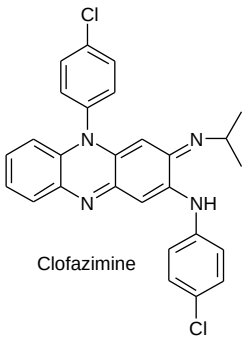
Recommendation

The data are too incomplete to allow a careful evaluation of PNU 100480. The compound should be assessed in further tests in comparison with other oxazolidinones in order to select a candidate for tuberculosis treatment rather than as a broad-spectrum antimicrobial.

30. Phenazine B4157



B4157



Clofazimine

B4157[88] is a phenazinamine derivative, closely related to the antileprosy drug clofazimine, which has been investigated at the University of Illinois as a potential treatment for tuberculosis.[89] In vitro, clofazimine and B4157 were tested against 20 strains of *M. tuberculosis*, including 16 drug-resistant strains, and all were found to be susceptible to B4157 including one which showed moderate resistance to clofazimine. The MICs of B4157 and clofazimine at which 90% of strains were inhibited were 0.12 and 1.0 mcg/ml, respectively.[88] However, against *M. tuberculosis* in C57BL/6 mice at 20 mg/kg, clofazimine was slightly superior to B4157. Both compounds prevented mortality and caused significant reduction of CFUs in the lungs and spleens. The animals treated with B4157 showed less pigmentation than those receiving clofazimine.[88]

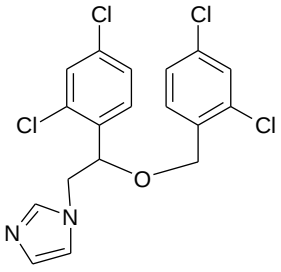
SWOT analysis

Strengths	**Weaknesses**
• More active than clofazimine in vitro clofazimine • Doesn't colour mice as strongly as clofozimine	• Lack of definitive toxicity and ADME data • Concentration in and colouration of skin likely to be problematic
Opportunity	**Threat**
• None perceived	• None perceived

Recommendation

Problems with concentration in tissues and colouration are likely to blight progression of any phenazine and, unless more compelling evidence to progress B4157 becomes available, it should not be pursued.

31. Azoles



Miconazole

Miconazole is a well-established antifungal agent which has been reported to have anti-TB activity in vitro (MIC 2 mcg/ml against *M. tuberculosis* H37Ra). The strength of the compound is that, as well as inhibiting replicating bacteria, it also has some effect on stationary phase bacilli.[90] Unfortunately, miconazole is not orally active and hence is of little further interest for progressing further for a TB indication. However, there are many 2nd and 3rd generation anti-fungal azoles which are clinically efficacious by the oral route, including the anti-fungal fluconazole which is much used in AIDS patients.[91]

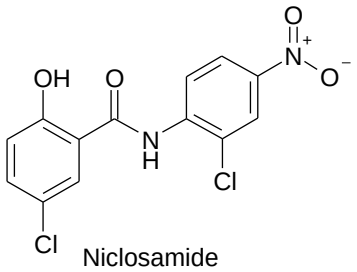
SWOT analysis

Strengths	**Weaknesses**
• Miconazole is active v growing and static *M. tuberculosis* • Many anti-fungal azoles available	• No structure-activity relationships (SAR) to suggest any connection between anti-fungal and TB activity
Opportunity	**Threat**
• GATB/WHO should promote the testing of a wide variety of anti-fungal azoles	• Not known at this stage

Recommendation

Pursue azoles further via screening a wide range of anti-fungal azoles to develop structure-activity relationships.

32. Niclosamide



Niclosamide

The anthelmintic drug niclosamide was found to have anti-TB activity in vitro (MIC 0.5-1 mcg/ml) against *M. tuberculosis* H37Ra. As well as being active against growing cells, it has the interesting property of acting against stationary phase non-replicating bacterial cells.[90] However, although niclosamide has been extremely useful for the treatment of human tapeworm infections, it is not absorbed to any significant extent from the intestine. This explains why no systemic pharmacological effects are observed although the compound has been reported to be mutagenic in vitro and to have effects on sperm morphology in animals.[92] This pharmacokinetic profile, along with its mutagenic capability, considerably blights the potential of this compound for TB chemotherapy.

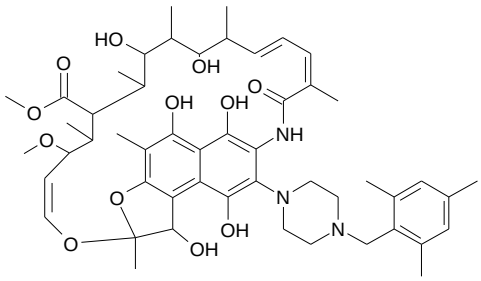
SWOT analysis

Strengths	Weaknesses
• Cheap • Active against MDR-TB strains • Bactericidal	• The compound is mutagenic and has reproductive toxicity • No in vivo TB data
Opportunity	**Threat**
• None perceived	• None perceived

Recommendation

Do not pursue.

33. Rifamycin derivatives



CGP 7040

A number of rifamycin derivatives, exemplified by CGP 7040, have been compared in vitro to rifampicin against rifampicin-sensitive and rifampicin-resistant strains of *M. tuberculosis* and *M. avium/intracellu - lare/scrofulaceum* (MAIS) complex. The compounds had MICs 4 to 8 times lower than those of rifampicin against sensitive *M. tuberculosis* strains, but of the 35 rifampicin-resistant strains of *M. tuberculosis*, only 3-11% were sensitive - cf rifabutin, 31%.93 Overall CGP7040 was more active than rifabutin and rifampicin against *M. avium* and was superior to rifampicin towards *M. tuberculosis*. In addition it was found to be considerably more stable than rifampicin.[94]

However, preclinical studies in collaboration with the University of Colorado were terminated and the compound is not available for licensing.[95]

SWOT analysis

Strengths	Weaknesses
• More active than rifampicin in vitro	• Cross resistant to rifampicin • No in vivo data in public domain
Opportunity	**Threat**
• None perceived	• None perceived

Recommendation

Do not pursue.

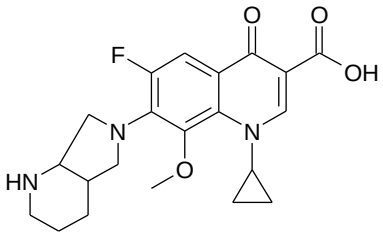
Clinical

Quinolones

Since the initial breakthrough discovery of nalidixic acid, a steady stream of analogues has followed this compound to the market place, each new drug ever-widening the quinolone antibacterial profile. The narrow antibacterial spectrum of nalidixic acid (limited to *Enterobacteriaceae*) was first expanded through drugs such as norfloxacin, pefloxacin, ofloxacin and ciprofloxacin to improve the utility for treating gram-negative bacterial infections. These were followed by drugs e.g. temafloxacin, sparfloxacin, grepafloxacin and gatifloxacin, which, whilst maintaining gram-negative activity, had additional effects on gram-positive microbes. More recently the bacterial profile of the quinolones has been enlarged again by such drugs as clinafloxacin, trovafloxacin, gemifloxacin and moxifloxacin to encompass anaerobic bacteria. In general these drugs are well tolerated[51,96] but, in some cases, side-effects have been sufficiently severe to cause withdrawal from the market (grepafloxacin - cardiotoxicity; trovafloxacin - hepatotoxicity).

In line with the expanding indications for the quinolones, a number of these compounds have shown promise for the treatment of mycobacterial infections, and ciprofloxacin[97] and ofloxacin[98] are currently finding utility as second-line or alternative TB drugs. However, resistance to these drugs develops quickly when used as single agent treatments or as add-ons to other drugs which are already failing.[99] Consequently, in seeking to evaluate the potential of the latest generation of quinolones as TB treatments, the issue of cross resistance to the existing drugs needs to be addressed - possibly by pursuing compounds inherently more bactericidal than ofloxacin and ciprofloxacin. A further advance would be to select quinolones with increased half lives to facilitate once daily dosing.

34. Moxifloxacin



Moxifloxacin

The Bayer quinolone moxifloxacin (BAY12-8039)[100] is the newest member of this 4th generation class of antibiotic to progress through the clinical pipeline. It has recently been launched in Germany (1999) for the treatment of respiratory tract infections,[101] and wider registration is now being sought. In November 1999, a US Food and Drug Administration (FDA) advisory panel recommended moxifloxacin for approval for skin and soft tissue infections, acute sinusitis, community-acquired pneumonia and acute exacerbation and chronic bronchitis. Because other quinolones have been associated with cardiac QT prolongation or arrhythmia, the final approval will be based on assessment of safety considerations. However, in the period September 1999 to April 2000, 1.2 million patients in Germany were treated with the drug and no ventricular arrhythmia attributable to QT prolongation was observed.[101]

The drug has been shown to be active against *M. tuberculosis* in vitro and in vivo in various test systems.[102,103] Against *M. tuberculosis* CSU93, a highly virulent, recently isolated clinical strain, the MIC of moxifloxacin was 0.25 mcg/ml. Oral administration of drug to mice at 100mg/kg produced peak serum concentrations of 7.8 mcg/ml within 0.25 hour of dosing. On this basis, mice were infected with a sublethal inoculum of *M. tuberculosis* CSU93 and then treated with moxifloxacin at 100 mg/kg per day for 8 weeks. This resulted in a significant decrease in the log 10 CFU counts in the organs of treated, compared to untreated, mice - 0.6 ± 0.2 versus 5.65 ± 0.3 in the lungs and 1.5 ± 0.7 versus

4.9 ± 0.5 in the spleens, respectively (p< 0.001 in both organs).[103] In other studies in *M. tuberculo - sis* H37Rv-infected mice, orally administered moxidectin at 100mg/kg/d given six times weekly was as bactericidal as isoniazid at 25mg/kg over a similar dosing schedule.[102] It was also demonstrated that 8 weeks of treatment with moxifloxacin (100 mg/kg/d) or with moxifloxacin plus isoniazid (100 mg/kg and 25 mg/kg, respectively, per day) sterilized the lungs in seven of eight and in eight of eight mice, respectively.[103] Interestingly, surviving bacilli isolated from animals infected with a high-titre inoculum and treated for 7 weeks with low-dose moxifloxacin (20 mg/kg per day) did not show any marked resistance to the drug.

The above data suggest that moxifloxacin should be effective in the treatment of TB. Furthermore, the elimination half life of the drug in man, mean value 12 hours[104] (compare isoniazid – 1 to 2 hours) supports the possibility of once-a-day treatment.

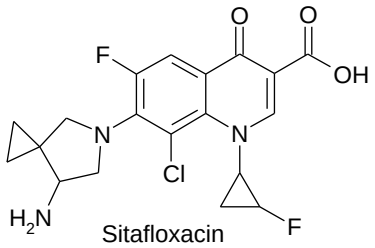
SWOT analysis

Strengths	**Weaknesses**
• Safe and well tolerated • Excellent oral bioavailability & long T1/2 • Already registered in Germany - other approvals expected soon	• No clinical data for TB indication
Opportunity	**Threat**
• Collaboration with GATB/WHO might encourage Bayer to generate clinical TB data	• Many other quinolones either in development or marketed already

Recommendation

Pursue further and encourage Bayer to conduct Phase II/III trials for TB indication.

35. Sitafloxacin



Sitafloxacin

The Daiichi quinolone Sitafloxacin (DU-6859a)[105] is in Phase III trials in both Japan and the USA.[106] The compound has outstanding activity against a broad range of bacteria. When compared to a number of other quinolones - ciprofloxacin, trovafloxacin, clinafloxacin, levofloxacin, gatifloxacin and moxifloxacin - sitafloxacin was the most active against 3344 gram-positive cocci and 406 anaerobes, and against 5046 gram-negative bacteria it either equalled or bettered clinafloxacin.[107] The mechanistic basis for this potency is believed to reside with sitafloxacin's ability to equally inhibit both DNA gyrase and topoisomerase IV, and its IC50s against these enzymes were amongst the lowest of the quinolones.[108] Sitafloxacin was equipotent with gatifloxacin and sparfloxacin, and more active than levofloxacin and ofloxacin, when tested against *M. tuberculosis* - MICs at which 90% of strains of *M. tuberculosis* inhibited (MIC90s) were ~ 0.2mcg/ml.[109,110] No data appear to have been published for the activity of the compound against *M. tuberculosis* in vivo.

In clinical studies of sitafloxacin in healthy volunteers oral bioavailability was at least 70%; it was well tolerated with no serious adverse effects and the elimination half life was 4.4 to 5 hours.[105] The latter parameter casts some doubt upon a once per day dosing schedule for this drug. However, a study in which the postantibiotic effect was determined for sitafloxacin (and several other quinolones) against various bacteria concluded that this factor would permit dosing on a once every 24 hours basis.[111]

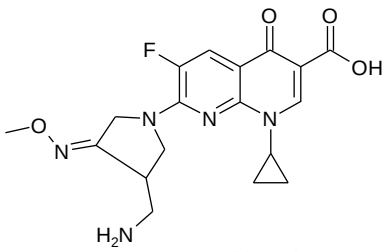
SWOT analysis

Strengths	Weaknesses
• Potent, 4th generation fluoroquinolone • Well absorbed and tolerated in man	• No in vivo TB data
Opportunity	**Threat**
• Collaboration with GATB/WHO might encourage Daiichi to generate clinical TB data	• The company has indicated a lack of enthusiasm for developing the drug for a TB indication[106]

Recommendation

Pursue further and initiate discussions with manufacturer as to how best to proceed.

36. Gemifloxacin



Gemifloxacin

Gemifloxacin is another quinolone in the late stages of development and it is being progressed by SmithKline Beecham on license from LG Chem. Following various Phase III trials, it has now been submitted for approval in the USA for the treatment of respiratory infections.[112,113] In healthy volunteers, oral bioavailability is approximately 70%, it is well tolerated and has a mean elimination half life of 7.4 hours. These characteristics, coupled with its potent antibacterial activity, suggest its suitability for a once-daily dosing regimen.[114]

Data on the anti-TB potential of gemifloxacin appear to be limited to a report comparing its activity to five other quinolones against 250 clinical isolates of *M. tuberculosis* susceptible or resistant to first-line antituberculosis drugs. In these assays, gemifloxacin showed a MIC90 value of 8mcg/ml, compared with 1mcg/ml for levofloxacin, trovafloxacin and grepafloxacin.[115] Extrapolating these data to serum concentrations seen in healthy volunteers suggests that, even at the highest non-toxic dose tested (800mg), gemifloxacin would not be effective for TB treatment.

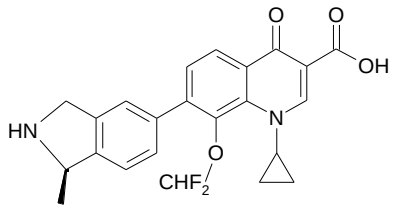
SWOT analysis

Strengths	Weaknesses
• Potent, 4th generation fluoroquinolone • Well absorbed and tolerated in man	• No in vivo TB data • Data from healthy volunteers suggest difficulty in reaching a therapeutic dose for TB
Opportunity	**Threat**
• Collaboration with GATB/WHO might encourage SKB or LG Chemicals to generate more definitive TB data	• Many other quinolones either in development or marketed already

Recommendation

Despite high MIC value, pursue further by encouraging generation of TB-relevant data.

37. T-3811ME



T-3811ME

T-3811ME is unique amongst the other broad-spectrum quinolones featured in this report in that it lacks the presence of a fluorine atom at the 6-position of the ring. Since the synthesis of norfloxacin, virtually all of the quinolones of interest have contained this structural feature. However, the Toyama compound shows similar broad spectrum and potent activity against bacteria as the best of the fluoroquinolones.[116] Against ten strains of *M. tuberculosis*, T-3811ME has an MIC90 value of 0.0625mcg/ml - comparable with ciprofloxacin and levofloxacin, and more active than trovafloxacin. No other TB-relevant data appear to have been published but the compound is being advanced for other bacterial indications. It is being progressed in the USA in collaboration with BristolMeyers Squib, with Phase II/III trials shortly to commence.[117]

SWOT analysis

Strengths	Weaknesses
• Potent, broad-spectrum quinolone lacking 6-F atom present in all other 3rd/4th generation fluoroquinolones • Progressing into Phase III trials so presumably well absorbed and tolerated in man	• No in vivo TB data • No ADME data in public domain
Opportunity	**Threat**
• Collaboration with GATB/WHO might encourage Toyoma or BMS Chemicals to generate more TB data	• The company has indicated a lack of enthusiasm for developing the drug for a TB indication[117]

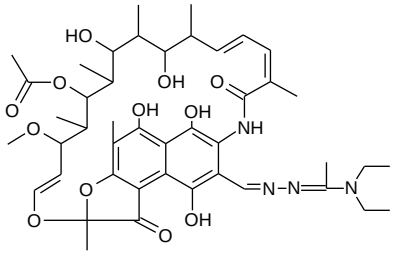
Recommendation

Pursue further and initiate discussions with manufacturers as to how best to proceed.

Rifamycins

Rifampicin has been used for front-line TB therapy for over 30 years. More recently in 1998, a longer-acting rifamycin, rifapentine[118] (T1/2 10-15 hours v 2-3 hours for rifampicin), received approval by the FDA in the USA as an anti-TB drug. This drug can be given twice weekly initially and then once weekly during the continuation phase of treatment.[119] However, in comparative studies with rifampicin, it appears to be slightly less effective and there is also a significant interaction with the AIDS drug indinavir.[118] Continuing interest in new rifamycins is focused on longer-acting compounds which can be given just once weekly to simplify short-course chemotherapy regimens. Furthermore, unlike rifapentine, the ideal new drug would not be cross resistant to the other rifamycins, have no drug-drug interactions (particularly with AIDS treatments), and would not require special storage or packaging.

38. Rifametane



Rifametane

Rifametane (SPA-S-565) is a new semi-synthetic rifamycin being progressed by Societa Prodotti Antibiotici (SPA), Milan, Italy. It has a bactericidal spectrum and potency similar to that of rifampicin, but with much better pharmacokinetic properties.[120] This is reflected in the fact that, although the MIC90 values of the two compounds were the same against 20 strains of *M. tuberculosis*, in TB-infected mice rifametane proved to be the more effective orally.[121] In healthy male volunteers, the pharmacokinetics and safety of a 300 mg single oral dose of rifametane were compared to a 300 mg dose of rifampicin.[122] The data clearly showed the pharmacokinetic profile of rifametane to be significantly more favourable than that of rifampicin. Thus, the elimination half-life for rifametane was 10.58 hours compared with 1.89 hours for rifampicin, and the mean residence time was 18.05 hours for rifametane and 3.93 hours for rifampicin. The drug was well tolerated and no changes clinically or statistically in laboratory parameters were found. In another Phase 1 trial carried out in collaboration with Glaxo India, a single oral dose of 150mg was administered and the half-life and area under curve (AUC) were some 6 or 7-fold that of rifampicin. Encouragingly, serum drug levels above the MIC for *M. tuberculosis* were maintained for up to 48 hours after drug administration.[123] Currently SPA are collaborating with Glaxo India in preparing to advance rifametane into Phase II trials.[124]

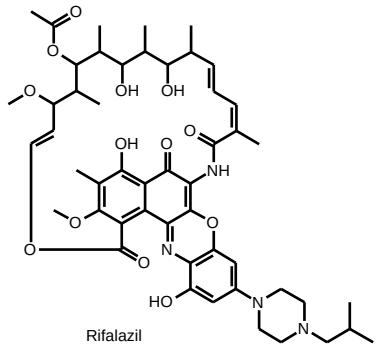
SWOT analysis

Strengths	Weaknesses
• Equipotent to rifampicin v *M. tuberculosis* but better pharmacokinetic profile	• Cross resistance with rifampicin
Opportunity	**Threat**
• Collaboration with GATB/WHO might encourage SPA to generate definitive TB data	• Competition with rifapentine

Recommendation

Maintain a watching brief on progress with the compound, particularly with reference to key issues such as likely product cost, stability of bulk chemical and formulated drug, interaction with protease inhibitors used in AIDS patients.

39. Rifalazil



Rifalazil

Rifalazil (KRM 1648) was synthesized at the Kaneka Corporation and shown to have superior activity to rifampicin against *M. tuberculosis* in vitro and in vivo.[125] The compound was progressed clinically in association with PathoGenesis through Phase I trials in the USA, and into a Phase II trial in Brazil using pulmonary TB patients.[126,127] However, due to severe side-effects in the four-day Phase II trial, the development of rifalazil has been terminated.[128]

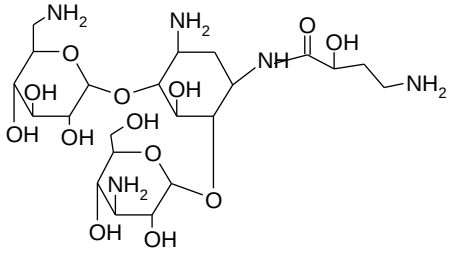
SWOT analysis

Strengths	**Weaknesses**
• More potent than rifampicin	• Toxicity in Phase II studies caused abandonment of project
Opportunity	**Threat**
• None apparent	• None apparent

Recommendation

Do not pursue.

40. MiKasome



Amikacin

Amikacin is an aminoglycoside used as a second-line anti-TB drug.[129] The anti-mycobacterial activity of liposome-encapsulated drug, MiKasome (Gilliad Sciences), has been found to be effective against *Mycobacterium avium* infections in vitro and in animal models.[130] In preclinical studies,[131] 48 hr after delivery to the lungs via liposome, over half of the antibiotic remained in this tissue. In animals, pharmacokinetic data showed that MiKasome produced 7-fold higher peak plasma levels compared to free drug (amikacin) administered intravenously (iv). Additionally, the AUC was 150-fold higher with the liposomal material and a single dose of liposomal amikacin produced therapeutic levels of antibiotic for more than 72 hr.[131] In a preliminary clinical study, a patient with tuberculosis treated with MiKasome for 49 days exhibited negative culture.[132] Once weekly dosing maintained constant amikacin dose levels. Pilot Phase II studies showed that MiKasome was able to resolve *M. tuberculosis* infections in adults and children who had failed conventional therapies.[131]

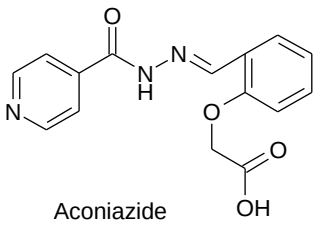
SWOT analysis

Strengths	**Weaknesses**
• MiKasome was effective as an anti-TB drug in limited Phase II clinical studies	• Administration is either by iv route or by aerosol • Liposomal preparation is expensive and stability is a concern
Opportunity	**Threat**
• If widely used, would generate significant revenue	• If proved highly effective, socio-economic problems might result due to cost considerations

Recommendation

Do not pursue.

41. Aconiazide



Aconiazide

Aconiazide is a pro-drug of isoniazid which was designed to be less toxic than the parent drug. The latter is metabolized to hydrazine and acetylhydrazine, which have both been implicated in the toxicity of isoniazid. Because aconiazide is converted to isoniazid and 2-formylphenoxyacetic acid, it was expected that the acid would bind to the isoniazid metabolites and so lower toxicity.[133] This proved to be the case and aconiazide is indeed less toxic than the parent drug and lacks carcinogenicity.[133] In healthy patients it was found to produce proportionately lower levels of isoniazid in serum than the parent molecule itself.[134] For some time, further progression of the compound was delayed due to problems with its commercial synthesis. However, additional toxicology data are now being submitted to the FDA by Lincoln Diagnostics in order to conduct clinical trials in tuberculosis patients, and the compound has been granted Orphan Drug status in the US.[135]

SWOT analysis

Strengths	Weaknesses
• Lower toxicity than isoniazid in animals	• Cost will be higher than isoniazid • Cross resistance with isoniazid
Opportunity	**Threat**
• None apparent	• None apparent

Recommendation

Do not pursue.

42. SRL 172

Chemical structure - not applicable

SRL 172 is a preparation containing heat-killed *Mycobacterium vaccae* and comprises Th1 adjuvant with bacterial antigens to induce host protective immunity. Extensive clinical studies on this immunomodulator are being conducted by SR Pharma for various indications including tuberculosis. In one study in Argentina it was added to routine chemotherapy in the treatment of pulmonary tuberculosis.[136] Patients receiving SRL 172 (plus drugs) were found to have reduced sputum smear positivity of AFB, increased weight gain and shortened time to becoming apyrexial, compared to drug treatment alone. It was concluded that the results indicated a switch to Th1 immunological status and improved clinical status. Another study in 100 subjects with relapsed or chronic tuberculosis showed SRL 172 to keep 97% of patients disease-free six months after treatment had finished, compared with 70% who received placebo. In other trials in tuberculosis patients, SRL 72 showed little difference, if any, to those receiving placebo.[137]

SWOT analysis

Strengths	Weaknesses
• Safe and well tolerated • Probably cost effective	• Unimpressive clinical data • Treatment will be by iv not the oral route
Opportunity	**Threat**
• None perceived	• None apparent

Recommendation

Do not pursue.

OVERALL CONCLUSIONS

It is recommended that the compounds listed below are worthy of further pursuit by the global R & D community.

Discovery research:

- **thiolactomycin** analogues
- **ethambutol** analogues
- **mefloquine/halofantrine** analogues
- **diterpenoids**
- **9-benzylpurines**
- **benzoxazines**
- **imidazo(4,5-c)pyridines**
- **deazapteridines**.

Preclinical development:

- **calanolides A and B**
- **nitroimidazopyrans PA 824 and PA 1343**
- **quinolones PD 161148 and CS-940**
- **poloxamer 315**.

Clinical development:

- **moxifloxacin**
- **sitafloxacin**
- **gemifloxacin**
- **T-3811ME**.

It was also recommended that the families of antifungal **azoles**, antibacterial **nitroimidazoles**, **quinolones** and **oxazolidinones** be further studied in the discovery research phase in an attempt to identify molecules with greater anti-TB potential than current candidates which have been discussed specifically in this report. The use of inhibitors of bacterial **drug efflux** mechanisms and small molecule **immunomodulators** were also felt to be areas worth investigating further in order to identify agents as possible adjuncts to existing TB drug regimens.

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