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MINIREVIEW

New Small-Molecule Synthetic Antimycobacterials

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Centre for Carbohydrate Chemistry, School of Chemical Sciences and Pharmacy, University of East Anglia, Norwich NR4 7TJ, United Kingdom,[1] and GlaxoSmithKline, Gunnels Wood Road, Stevenage, Hertfordshire SG1 2NY, United Kingdom[2]

Drugs for treating tuberculosis (TB) have been available for over half a century, and yet the incidence of disease worldwide continues to rise year by year. In 2002, the last year for which statistics are available, it is estimated that 24,000 people developed active disease and close to 5,000 people died from TB every day (110). Coinfection with human immunodeficiency virus is driving the increase in incidence (68, 105), and the cause of death in 31% of AIDS cases can be attributed to TB in the African region (25, 87). When coupled with the emergence of multidrug-resistant strains of *Mycobacterium tuberculosis* (MDR-TB) (108), the scale of the problem becomes clear, as it will inevitably become even more difficult to treat TB in the future. It is now more than a decade since the World Health Organization declared TB "a global health emergency" (109).

The reasons for these problems are numerous (27, 39). Compliance with even the best available regimen is poor, and treatment failure is all too common. This regimen comprises daily isoniazid (INH), rifampin (RIF), pyrazinamide (PZA), and ethambutol (EMB) treatment for 2 months followed by 4 months of daily doses of INH and RIF (structures shown in Fig. 1). To overcome this, the World Health Organization is encouraging widespread implementation of its DOTS (directly observed therapy, short course) strategy (107). A study in China has shown that cure rates as high as 95% can be achieved through DOTS implementation (107), but treatment is labor intensive, making it difficult to deliver unless substantial infrastructure is in place. Detecting drug resistance is problematic—the process can take up to 12 weeks—and the delay means patients can be exposed to suboptimal therapy, and this leads to MDR-TB.

The need for new drugs to extend the range of TB treatment options is acute. New chemical entities with novel mechanisms of action will most likely possess activity against MDR-TB (15). However, these alone will not provide the breakthrough that is needed. The key to improving therapy is to develop new agents with potent sterilizing activity that will lead to a shortening of the duration of chemotherapy (36).

NEW LEADS IN TUBERCULOSIS DRUG DISCOVERY

The number of TB research publications with a drug discovery focus increased in the mid-1990s. In particular, research aimed at understating both the pathogen and the disease process led to the identification of key biochemical processes that are potential targets for drug therapy (35). For example, the determination of the complete genome sequence of *M. tuberculosis* revealed a detailed picture of the organism's metabolic processes (23). Likewise, numerous new molecules have been disclosed as potential leads for TB drug discovery. These have been identified in two complementary screening strategies to secure active entities, which are based on either whole-cell evaluation or profiling against specific biochemical targets. As the treatment of TB infections typically necessitates extended oral dosing regimens, an agent is needed that is both economical to produce and preferably highly specific for mycobacteria to minimize unwanted side effects associated with disturbance of the normal gut flora (70).

While other small-molecule reviews on therapeutically relevant areas exist (26), no such work exists for TB. This review surveys new synthetic molecules with antimycobacterial activity disclosed between 1998 and 2004 and excludes natural products (reviewed recently in references 24 and 72) and direct analogues of current antitubercular agents. When available, information about their modes of action is also detailed.

Hits, leads, and tools. The terms "hit" and "lead" are widely used in drug discovery, but there is little generality applied to the criteria used to define either term, or sometimes, even to those that differentiate them (44). In this review, we have used the term *hit* to describe individual or small numbers of structurally related molecules that have established antitubercular activity regardless of other important drug discovery considerations. *Leads* are defined by molecules within a series that display a more substantial structure-activity relationship (SAR) around a given hit, coupled with other important factors such as evidence of selectivity and perhaps pharmacokinetic and/or in vivo data. In an effort to try and quantify the attractiveness of a given hit or lead, we have used calculated physicochemical parameters as a means of predicting the likelihood of a compound possessing a desirable pharmacokinetic profile (Tables 1 and 2). A number of the agents described herein fall short of satisfying the various criteria to be classified as either a hit or a lead and appear to have little chance of delivering appropriate pharmacokinetic profiles without substantial chemical manipulation. Nonetheless, such molecules may prove useful as tools for validating a biochemical process as a target for therapy or for supporting crystallographic studies that might underpin structure-based drug design.

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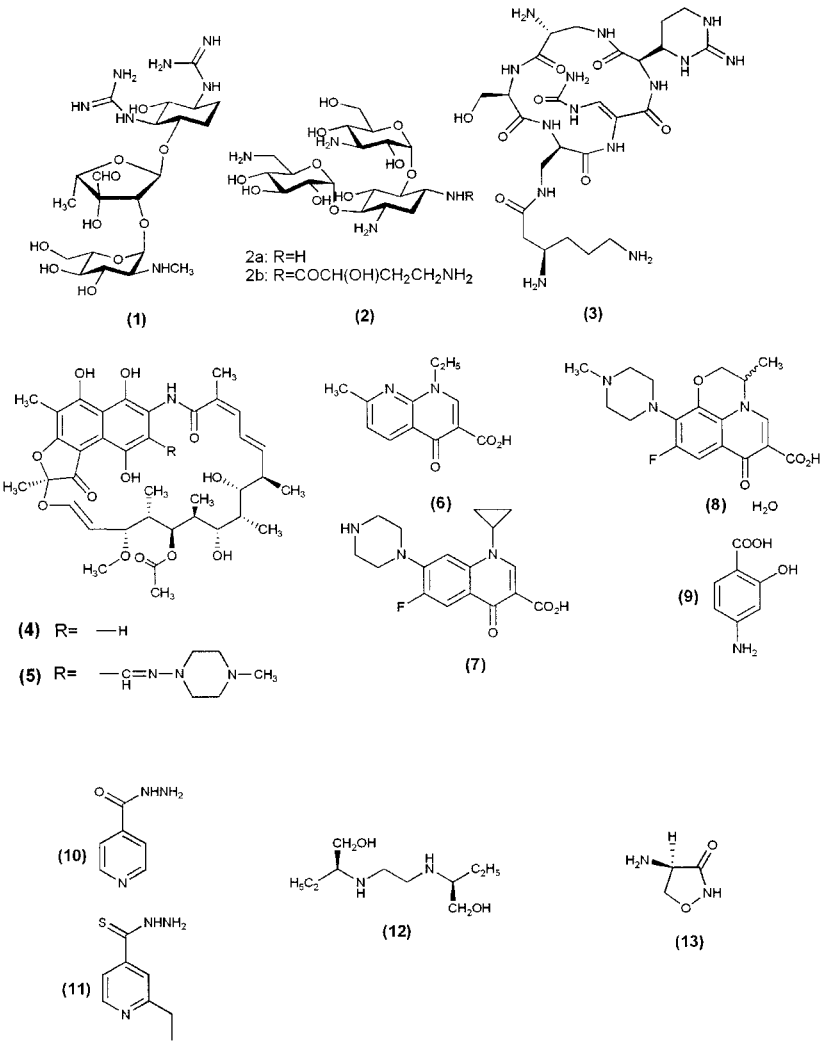


FIG. 1. Structures of streptomycin (1), kanamycin (2a), amikamycin (2b), capreomycin (3), rifamycin (4), rifampicin (5), nalidixic acid (6), ciprofloxacin (7), ofloxacin (8), *p*-aminosalicyclic acid (9), isoniazid (10), ethionamide (11), ethambutol (12), and cycloserine (13).

PARAMETERS CONSIDERED IN ASSESSING "QUALITY" OF MOLECULES

The following parameters have been used to assess new families of chemical entities: best MIC (MIC for *M. tuberculosis* strain $H_{37}Rv$ or $H_{37}Ra$ [most examples included have at least one compound with an MIC <6.25 μg/ml]); whether the biochemical target is known; physicochemical parameters as a method of quantifying drug likeness (61, 64, 97); the "Lipinski rule of 5" (64), which provides a method for assessing the likelihood that a given molecule could be orally bioavailable based on a series of physicochemical requirements, no more than one of which should be violated (the data presented are those listed by SciFinder, calculated using ACD in software), including (i) no more than 5 H-bond donors (guideline 1), (ii) no more than 10 H-bond acceptors (guideline 2), (iii) molecular weight no higher than 500 (guideline 3), and (iv) calculated octanol/water partition (clogP) no higher than 5 (guideline 4); flexibility (it was recently suggested that to secure good oral bioavailability, the number of rotatable bonds in a given molecule should be kept below 10 [97]; many molecules reported display long chains in their structure); favorable toxicological profile as assessed by selectivity index (SI) expressed as the 50% inhibitory concentration/MIC and/or data from animal models; and the numbers of synthetic steps and total compounds reported which give insight into the ease of synthesis and how much SAR has been delineated.

ANTIMYCOBACTERIAL LEADS WITH KNOWN MODE OF ACTION

Mycobacterial cell wall biosynthesis inhibitors. A key target for antimycobacterial chemotherapy is cell wall biosynthesis. The complex lipoglycan calyx on the mycobacterial cell surface provides a significant physical barrier to intracellular-acting drugs; lack of penetration is thought to be a reason why many antibiotics show no activity against *M. tuberculosis* (41). Inhibition of synthesis is known to be lethal to the bacterium as evidenced by the action of isoniazid and ethambutol, and the

TABLE 1. Antimycobacterial drug leads with known or suspected modes of action

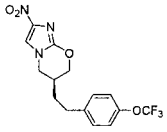
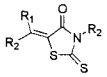
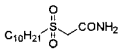
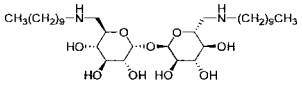
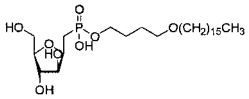
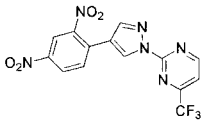
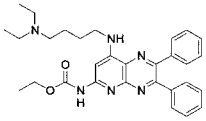
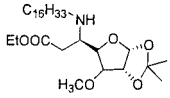
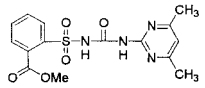
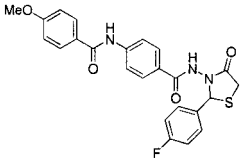
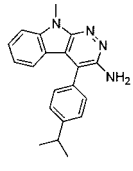
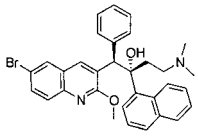
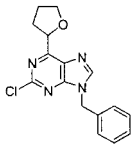
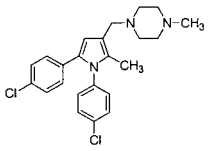
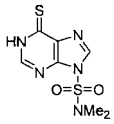
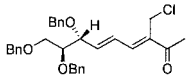
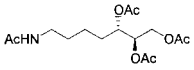
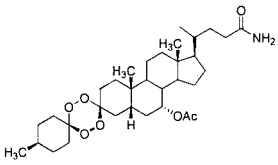
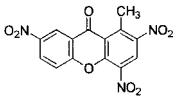
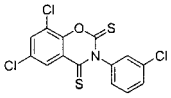
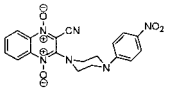
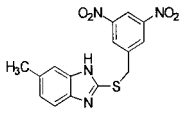
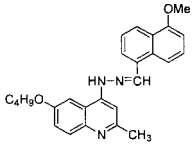
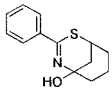
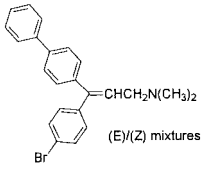
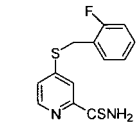
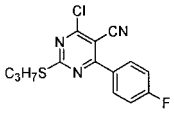
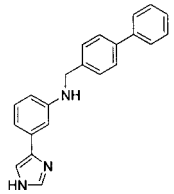
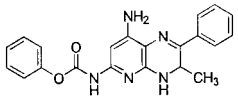
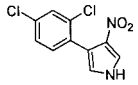
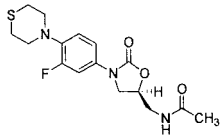
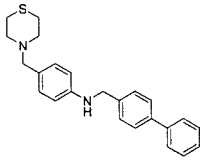
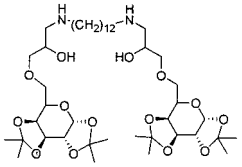
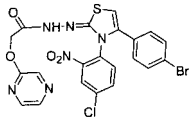
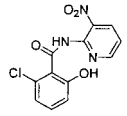
Compound	Structure	MIC (μg/ml)	Target	Mol wt	H acceptors + donors	SI/toxicity	clogP	Lipinski alert?	Synthesis, no. of compounds assayed/SAR?	Status (reference[s])
14 (PA-824)		0.13	Cell wall mycolate biosynthesis?	357.3	7 + 0	Toxic threshold at 1 g/kg in mice	3.74	No	No details 328/ SAR	Candidate (10, 89)
15 (5372)		16	dTDP-rhamnose biosynthesis						No details 8,000/ SAR	Hit (66)
16		0.75	β-Ketoacyl synthase?	363.4	5 + 1		2.60	No	3 steps/30/SAR	Tool (49)
17		1.3	Mycolyl transferase?	479.6	5 + 8		0.10	No	6 steps/19/SAR	Tool (80)
18		3.13	Araf transferase	523.6	8 + 4		6.06	Yes	5 steps/6/no SAR	Tool (21)
19		2.5	Mycolic acid biosynthesis	380.2	10 + 0		2.10	No	1 step/?/SAR	Hit (60)
20 (SRI-3072)		0.15	FtsZ (ID_{50}, 52 μM)	540.7	8 + 2	42	7.67	Yes	No details/200	Hit (104)
21		3.12	D-Alanine racemase and/or D-alanylalanine synthase	513.7	7 + 1		9.81	Yes	5 steps/22/no SAR	Tool (95)
22		0.3–1.8	Acetolactate synthase	364.4	9 + 2		1.67	No	3 steps/?/no SAR	Lead (45)
23		6.25	Peptidoglycan biosynthesis	465.5	7 + 2		2.42	No	4 steps/18/SAR	Hit (2)
24		1.42	MAO	316.4	3 + 1		4.8	No	2 steps/40/SAR	Hit (98)
25		0.03–0.12	ATP synthase proton pump	551.5	4 + 1	No toxicity in humans compared to placebo	7.7	Yes	5 steps/>200/ SAR	Candidate (3)

TABLE 2. Antimycobacterial drug leads with unknown modes of action

Compound	Structure	MIC (μg/ml)	Mol wt	H acceptors + donors	SI/toxicity	clogP	Lipinski alert?	Synthesis/no. of compounds assayed/ SAR?	Status (reference[s])
26		0.78	314.2	5 + 0	10.6	1.96	No	2 steps/22/SAR	Lead (46, 74)
27 (BM 212)		0.7	414.4	3 + 0	5.6	5.32	No	4 steps/50/SAR	Lead (28)
28		0.78	259.3	7 + 1	513	−0.65	No	1 step/11/SAR	Hit (86)
29		3.1	505.4	4 + 0		7.55	Yes	5 steps/12/no SAR	Hit (76)
30		≤6.25	331.3	8 + 1		−0.63	No	6 steps/19/no SAR	Hit (75)
31		3.12	575.4	8 + 1	1.1	7.41	Yes	4 steps/23/SAR	Hit (88)
32		4	345.2	11 + 1		4.11	No	3 steps/61/SAR	Hit (83)
33		4	374.7	2 + 0		5.72	No	?/10/SAR	Hit (100)
34		0.1	392.4	10 + 0	>125	1.12	No	3 steps/20/SAR	Hit (73, 113)
35		1.3	330.3	8 + 1		4.11	No	2 steps/40/SAR	Hit (50)
36		0.78	413.5	3 + 1	6.67	7.90	No	2 steps/66/SAR	Hit (82)
37		≤6.25	233.3	2 + 1		1.53	No	1 step/8/no SAR	Hit (55)
38		1.6	392.3	1 + 0	2.5	6.58	No	2 steps/15/no SAR	Hit (29)

Continued on following page

TABLE 2—*Continued*

Compound	Structure	MIC (μg/ml)	Mol wt	H acceptors + donors	SI/toxicity	clogP	Lipinski alert?	Synthesis/no. of compounds assayed/ SAR?	Status (reference[s])
39		1.2	278.4	2 + 2		2.12	No	2 steps/40/SAR	Hit (51)
40		0.78	307.8	3 + 0		4.95	No	3 steps/17/SAR	Hit (1)
41		4	325.4	2 + 1	16	5.04	No	3 steps/36/SAR	Hit (14)
42		1.3	373.4	4 + 4		4.73	No	4 steps/25/SAR	Hit (90)
43		1	257.1	4 + 1	1	3.11	No	1 step/27/SAR	Hit (31)
44 (U-100480)		≤0.125	353.4	6 + 1	50 mg/kg well tolrated in rats	−0.36	No	3 steps/?/SAR	Lead (9)
45		0.5	374.5	2 + 1	16	4.75	No	4 steps/4/no SAR	Hit (6)
46		1.56	833.1	14 + 4	50 mg/kg per day	4.4	Yes	3 steps/12/no SAR	Hit (94)
47		0.3	561.8	12 + 1	LD_{50} >500 μg/ml	7.3	Yes	4 steps/60/SAR	Hit (16)
48		<1	293.6	6 + 2		2.1	No	1 step/100/SAR	Hit (100)

recent deconvolution of biochemical events leading to cell wall formation has exposed a rich supply of targets that may be further exploited (60, 62, 63, 65, 69, 91).

One of the most significant new antitubercular drug candidates disclosed recently is PA-824 (Table 1, compound 14) (10, 89). This nitroimidazopyran was identified from a 328-compound library. PA-824 possesses excellent potency against both $H_{37}H_v$ and drug-resistant strains of *M. tuberculosis* and demonstrates interesting activity against oxygen-starved cultures. Its biological target has been proposed to be an enzyme involved in mycolate biosynthesis, since treatment of *M. tuberculosis* with the compound was found to give rise to an accumulation of hydroxymycolic acid (a known precursor to cell wall ketomycolate) (112) with a parallel reduction in ketomy-

colate. The compound is effective in murine and guinea pig infection models after oral administration, with reduction of bacillary load similar to that of isoniazid-treated controls. Toxicity thresholds were 5- to 10-fold above the doses necessary to emulate the effect of isoniazid in infection models (89).

The rhodamine derivative 5372 (Table 1, compound 15) (66) is an example of target-based drug design. This structural motif is very similar to that used in the 4-thiazolidinones, putative diphosphate surrogates that affect sugar nucleotide biosynthesis during peptidoglycan formation (2). While having relatively poor antimycobacterial activity (MIC, 16 μg/ml), encouraging activity against enzymes involved in mycobacterial dTDP-rhamnose biosynthesis was observed (81% inhibition in a coupled RmlB, RmlC, and RmlD assay at 10 μM).

Another example of target-based drug design is the series of alkyl-sulfinyl amides, such as compound 16 (Table 1) (49), that inhibit β-ketoacyl synthase (KAS). KAS is one of the accessory fatty acid synthases peculiar to mycobacteria. The sulfonyl amides are thought to mimic the putative tetrahedral transition state formed during KAS catalysis. These compounds show good MICs (0.75 μg/ml), but their selectivity toward mycobacteria is not known. The compounds are easily synthesized (three steps) from inexpensive precursors, but their length and flexibility are undesirable properties in drug leads.

A related target in mycolic acid biosynthesis is the mycolyl transferase activity exhibited by proteins of the antigen 85 complex (57). These proteins recognize and transfer mycolate from trehalose mono- and dimycolates. A family of 6,6′-diamino-6,6′-dideoxytrehalose-based derivatives with different alkylamines or alkysulfonamide functionality was synthesized and assayed for whole-cell activity against *M. tuberculosis* $H_{37}R_a$ (80). A potent new antimycobacterial with an MIC of 1.3 μg/ml was found in compound 17 (Table 1). Further work is necessary to evaluate the potential of such compounds, which have an unattractive physicochemical make up for lead optimization, but they could be useful tools.

Arabinofuranosyl transferases have also been proposed as potential targets for drug development (106). Arabinofuranose units are not present in humans; hence, a drug based on the inhibition of such transferases removes the likelihood of mechanism-based toxicity. Decaprenylphosphoarabinose is the probable donor molecule for the arabinose unit (84). Some *C*-phosphonate analogs of the putative donor have been synthesized, and compound 18 (Table 1) was found to be active (MIC, 3.13 μg/ml) (21). This is a good example of a likely biochemical tool, given the inherent structural features.

The enoyl acyl carrier protein reductase enzyme InhA has been validated as an antimycobacterial target (7). High-throughput screening of a structurally diverse library of compounds showed that indole-5-amides, 4-aryl-substituted piperazines, and various pyrazole derivatives provided useful core templates that display good InhA inhibition (60). A second more focused library yielded Genz-8575 (Table 1, compound 19), a potent InhA inhibitor (91% inhibition at 40 μM). Crystal structures of InhA with bound inhibitors were obtained. This enzyme inhibition profile transferred to potent activity (MIC 2.5 μg/ml) against $H_{37}R_v$. Good activities versus *M. tuberculosis* and *Plasmodium falciparum* were observed, with no effect against six other common infectious agents, making this an attractive lead series.

Other targets. SRI-3072 (Table 1, compound 20), originally synthesized as a tubulin polymerase inhibitor (92, 93), was found to affect *M. tuberculosis* growth with a potent MIC (0.15 μg/ml) and good selectivity index (SI = 42) (104). Moreover, it was found to be an inhibitor of FtsZ (a bacterial tubulin polymerase homologue). Activity of a number of compounds in the 200-compound library assayed was found to be linked to FtsZ inhibition. This provides an attractive hit, with clear scope for further diversification that may help identify analogues with more favorable physicochemical profiles.

D-Alanine racemase is a cytoplasmic enzyme responsible for the conversion of L-alanine to D-alanine, a key building block in peptidoglycan biosynthesis. Inhibitors of this enzyme, such as the marketed agents D-cycloserine and fludalanine, have been found to possess potent antitubercular activity. To target this enzyme, some 5-amino-furanoside derivatives were synthesized (Table 1, compound 21) (95). The most active compound was found to possess reasonable antimycobacterial activity (MIC, 3.12 μg/ml), but no specific information about D-alanine racemase inhibition was provided.

Sulfometuron methyl (Table 1, compound 22) is a commercially available herbicide with inhibitory activity against acetolactate synthase, an enzyme which catalyses a key step in branched-chain amino acid biosynthesis (111). Compound 21 showed potent *M. tuberculosis* activity (MIC, 0.3 to 1.8 μg/ml) and displayed good activity in a murine model (45), with no overt toxicity at 500 mg/kg. The encouraging physicochemical parameters and ease of synthesis make this an attractive lead.

The 4-thiazolidinone derivative, compound 23 (Table 1) (2), showed good inhibitory activity against the bacterial MurB enzyme which converts UDP-GlcNAc into UDP-MurNAc, an intermediate in the assembly of the MurNAc-pentapeptide for cell wall peptidoglycan biosynthesis. The compound was found to possess moderate antimycobacterial activity (6.25 μg/ml) as well as bacteriostatic and bactericidal activity against a wide range of fungi and bacteria including *Escherichia coli* and *Staphylococcus aureus* (2).

The correlation between monoamine oxidase (MAO) inhibition and antitubercular activity was first reported in the 1960s (77), a concept that was revisited in the recent paper exploring the SAR of pyradazinoindoles against these targets (98). Compounds with modest (micromolar) levels of MAO inhibition were shown to have potent antitubercular activity (MIC, 1.42 μg/ml for compound 24 [Table 1]). The favorable physicochemical profile thus confirms this as an attractive hit.

Very recently, R207910 (Table 1, compound 25) (3) has generated great excitement. The compound is extremely potent against a variety of mycobacterial species (MIC, 0.03 to 0.12 μg/ml). In addition, no cross-resistance was found against a panel of different drug-resistant isolates. In fact, this notion was further supported by the identification, through genetic analysis of resistant mutants, of the molecule's target as the proton pump for *M. tuberculosis* ATP synthase. Moreover, in mouse studies using oral treatment, the bactericidal activity obtained after 2 months of therapy by a combined treatment of RIF plus INH plus PZA was matched by the combinations of R207910 plus INH plus PZA and R207910 plus RIF plus PZA after just 1 month of therapy. This novel mode of action together with a very favorable pharmacokinetic profile (allowing for a less frequent dosage regime) and the lack of serious

adverse effects in humans make R207910 possibly the most promising antimycobacterial drug prospect in the last 40 years. It is important to mention how the diarylquinolone challenges our objective criteria; it would be flagged in a Lipinski analysis, with a molecular weight of 555.5 (influenced by the bromine) and a high clogP (7.72). However, hydrogen bond donors/acceptors fall well within the guidelines (1 and 4, respectively), and the molecule has eight rotatable bonds. The Lipinski filter is perceived to be a useful flag for potentially insoluble compounds; thus, it is likely that dimethylaminoethyl substituent is present to enhance the aqueous solubility of the molecule.

ANTIMYCOBACTERIAL LEADS WITH UNKNOWN MODES OF ACTION

Several 9-benzylpurines have been found to exhibit very good inhibitory activity against *M. tuberculosis* (Table 2, compound 26) (46, 74). The MIC of the leading compound was 0.78 μg/ml, with moderate toxicity levels against Vero cell lines (SI = 10.6). Potent antimycobacterial activity was maintained against strains resistant to INH, RIF, and EMB. Experiments with infected bone marrow macrophages also suggested that the compound was capable of attacking *M. tuberculosis* inside these cells. The ease of synthesis and the possibility of considerable further structural variation make this an attractive lead. No information regarding potential molecular targets was provided.

BM 212 (Table 2, compound 27) (11) belongs to a series of pyrrole derivatives with very good MICs (0.7 μg/ml) and moderate toxicity levels against Vero cells (SI = 5.6). More recent work (28) has improved these values to an MIC of 0.4 μg/ml and a selectivity index of 20. The leading compound in this pyrrole derivative showed no significant cross-resistance with INH, RIF, EMB, and streptomycin, and it was found to possess intracellular antimycobacterial activity in macrophage assays. More recently, work has been patented regarding this family of compounds (58). The patented molecules show enhanced activity as well as reduced levels of toxicity in mice. Their activity profile seems to suggest a new mode of action that could be specially useful against multidrug-resistant strains of the disease.

9-Sulfonylated or sulfenylated 6-mercaptopurines (86) (Table 2, compound 28) show potent antimycobacterial activity (from 0.39 to 3.39 μg/ml) combined with very good selectivity indexes (45 to >200). These molecules are derived from 6-mercaptopurine, a drug commonly used for the treatment of leukemia, other cancerous tumors (19), and inflammatory bowel disease (18). The lead structures show moderate levels of antitubercular activity against drug-resistant strains. The oxidation state of the sulfur atom has an impact on the MICs for resistant strains, since a sulfenylated derivative showed significantly better MICs than the sulfonylated compounds (37). As is often the case (22), poor correlation was found between the antimycobacterial properties of the compounds against *M. tuberculosis* and *M. avium*. These are attractive hits, with calculated physicochemical parameters within a desirable range.

A range of α,β-unsaturated acyclic sugar ketones (75, 76) (Table 2, compound 29) has been evaluated. Compound 29 possesses good antimycobacterial activity (MIC, 3.1 μg/ml), but no information regarding toxicity was provided. This hit is an alkylating agent; any optimization program would therefore need to address the role of this functionality at an early stage. A series of alditol derivatives (Table 2, compound 30) with some structural similarity to the previously described series showed good antimycobacterial activity (MIC, 6.25 μg/ml) without including an alkylating center, although no further data were disclosed.

Some 1,2,4,5-tetraoxacycloalkanes (Table 2, compound 31), previously explored as antimalarial agents (32), have also been found to possess notable antimycobacterial activity (MIC, 3.12 μg/ml) (88). The poor selectivity (SI = 1.1) and unknown mode of action of this hit suggest that more work is required before such molecules can be regarded as genuine leads.

Over the past 25 years, a number of compounds based on the xanthone template have been reported to exhibit antitubercular activity (43). Several series of compounds have been synthesized, and SAR correlations have been established (48, 78, 79). Compound 32 (82) (Table 2) is a representative example. Its antimycobacterial activity is moderate (MIC, 4 μg/ml) with a low clogP (2.62) and low molecular weight and is therefore an attractive hit. Even though no cytotoxicity data were provided for the given example, other molecules belonging to the same family have been reported to show low toxicity against HT 29 cell lines (67).

Several 3-phenyl-6,8-dichloro-2*H*-1,3-benzoxazine-2,4(3*H*)-dithiones have been synthesized (e.g., compound 33 [Table 2]) and shown to have antitubercular activity (100, 100A). The best compound of the series showed moderate activity against mycobacteria (MIC, 4 μg/ml). More recently, a theoretical QSAR model has been proposed that could rationalize the activities found (38). The compound has a relatively high clogP value (5.72), and no data were provided regarding cytotoxicity.

The antibacterial properties of quinoxaline 1-oxides have been known now for more than 20 years (30). More recently, their antimycobacterial profile has been described (81), in addition to their growth-promoting activity (85) and evidence of genotoxicity versus both *E. coli* and *Salmonella enterica* (71). Their widespread activity seems to emanate from enzymatic, single-electron reduction of quinoxaline 1,4-dioxides under hypoxic conditions leading to DNA damage (40). Compound 34 (Table 2) is representative of this group of molecules (20, 73). The leading compound showed very good antitubercular activity (MIC, 0.1 μg/ml) and selectivity towards mycobacteria (SI > 125) in addition to reasonable activity in an *M. tuberculosis*-infected macrophage model (113). Favorable physicochemical profiles and ease of synthesis make these attractive hits, although their genotoxicity is a concern, especially if this proves to be a mechanism-based property.

Waisser et al. reported the antimycobacterial activity associated with a series of compounds containing an alkyl-mercaptan group attached to an electron-deficient carbon atom (102). Following this observation, significant antitubercular activity was observed in different series of molecules incorporating this functionality (52, 53, 101). The most active compound in this class, compound 35 (Table 2), serves as an example (50). The alkyl-mercaptan functionality is attached to a benzimidazole ring, a heterocycle that, along with structurally related benzothiazole, is often found in molecules with antimycobacterial activity (42, 99). The chosen example showed moderate to

good MIC levels (1.3 μg/ml) and falls well within the Lipinski parameters. These data are supported by the low cytotoxicity of this class of compounds (54), confirming their potential for further lead development (SI > 72).

4-Quinolylhydrazones, structural hybrids of isoniazid and quinolones (96, 96A), are another interesting group of molecules that show marked antitubercular activity (82). The selected representative, compound 36 (Table 2), showed a good MIC (0.78 μg/ml) but poor selectivity for mycobacteria (SI = 6.67). This activity translated well across the series into an *M. tuberculosis*-infected macrophage model, with some compounds showing up to a 10-fold increase in activity relative to the initial hit. A major concern with this series of compounds is the high clogP (7.9 for compound 36).

The leading compound in a series of 1,3-thiazine derivatives, compound 37 (Table 2), displays good antimycobacterial activity (55, 56); no toxicity data were reported. These compounds have relatively low molecular weights and three potential points of diversity, making them attractive templates for high-throughput analogue synthesis.

Another new class of moderately potent antimycobacterials is represented by compound 38 (Table 2), a derivative of 3-[4′-Y-(1,1′-biphenyl)-4-yl]-*N*,*N*-dimethyl-3-(4-X-phenyl)-2-propen-1-amine (29). These molecules were found to display good antitubercular activity against both $H_{37}R_a$ and $H37R_v$ isolates (MIC as low as 1.6 μg/ml) and other drug-resistant clinical isolates of *M. tuberculosis*. However, high toxicity against V79 mammalian cells (SI < 2) and high lipophilicity are issues that need to be addressed with respect to this hit.

Another example of the successful use of an electron-withdrawing group attached to sulfur as a template for antimycobacterial activity is the series of fluorobenzyl derivatives represented by compound 39 (Table 2) (51), with MICs for the series down to 1.2 μg/ml. Evidence of the antitubercular activity of such compounds was accompanied by a QSAR study of the effects of different substituents on the benzyl group. From that study, activity in the series was clearly related to the electron-withdrawing ability of the substituent(s) on the benzyl ring. This mirrors the effects observed with compound 35 (Table 2). Even though cytotoxicity has not been evaluated, this class of molecules could form a promising core for lead generation, as they have a low molecular weight and satisfy the Lipinski requirements.

The alkylmercapto group was used again in a set of chloropyrimidine derivatives represented by compound 40 (Table 2) (1). These molecules exhibit good antimycobacterial activity, with an MIC of 0.78 μg/ml for several compounds in the series, most notably those whose heterocyclic ring is attached to a mercaptopropyl chain. This represents an attractive hit based on low molecular weight, low clogP, and ease of synthesis. Additionally, these compounds were shown to be highly specific for *M. tuberculosis*, with only weak activity observed against other bacteria and fungi (1). No toxicity data have been reported.

The antimicrobial activity of many toluidine derivatives has been described previously (12, 13). One such compound also displayed moderate antimycobacterial activity, thereby prompting its investigation within a more focused antitubercular program (14). Toluidine derivative 41 (Table 2) is moderately active against mycobacteria (MIC, 4 μg/ml) and is also moderately selective versus mammalian Vero cells (SI = 16). No correlation was found between the activities of the compounds against *M. tuberculosis* and *M. smegmatis*, but comparable activity against *M. avium* and *M. gordonae* was found. No activity was found against a range of gram-positive and gram-negative bacteria, perhaps suggesting a specific mycobacterial target. These compounds are easily synthesized from inexpensive precursors, and further work should address metabolism and complex pharmacology issues associated with such charged, lipophilic molecules.

Some deazapteridine derivatives, originally synthesized as potential anticancer agents (92, 103), were found to exhibit moderate activity against *M. tuberculosis* (90). Even though the cytotoxic properties of the compounds had previously been correlated with their tubulin binding capacity, the active antitubercular molecules were found not to inhibit the polymerization of mycobacterial FtsZ. Compound 42 (Table 2) is a representative example of the library of deazapteridines reported. This compound, among other members of the series, was found to display moderate antimycobacterial activity against both *M. tuberculosis* and *M. avium.* The compounds showed marked inhibition of proliferation in cultured L1210 cells (mouse DBA/2 lymphocytic leukemia) (17), which, coupled with their favorable physicochemical make-up, makes them potentially attractive hits.

The naturally occurring antibiotic pyrrolnitrin, isolated from *Pseudomonas pyrrocinia* (4), is used as a topical antifungal agent (5). The compound was also found to exhibit moderate antimycobacterial activity (MIC, 8 μg/ml). The antifungal activity is due to inhibition of protein kinase III (kinases have already been shown to be potential drug targets [33]), which is involved in the osmosensing signal transduction pathway. The synthesis of a series of analogues was undertaken (31), including the representative nitropyrrole, compound 43 (Table 2). The best MIC observed for the series was 1 μg/ml, but this was accompanied across all derivatives by pronounced levels of cytotoxicity (SI < 1). Should the cytotoxicity problem be solvable, the ease of synthesis, low molecular weight, and low clogP values make this an attractive hit for further evaluation.

The oxazolidinone family of molecules probably represents the most significant recent development in the field of novel antimicrobials; the U.S. Food and Drug Administration granted approval to the antibiotic linezolid in 2000 (8). Some oxazolidinones, such as compound 44 (Table 2) and U-100480 (9), have been identified as potential antimycobacterials. Several analogues were found to possess MICs <0.125 μg/ml. This potent activity in vitro was complemented by good antitubercular activity against multiple strains of *M. tuberculosis* (9). Most significantly, activity comparable to that of isoniazid was observed in a mouse model of *M. tuberculosis* infection. No toxic effects were observed when rats were administered up to 50 mg/kg of the compound for 29 days (well above the therapeutically active dose), and good pharmacokinetics have been secured across this synthetically accessible series (47, 59).

Compound 45 is representative of a class of putative non-oxazolidinone analogues of U-100480 (Table 2). Arima et al. reported on the replacement of the acetamidomethyloxazolidinone moiety with a biphenylmethyl group, with the retention of notable activity against *M. tuberculosis* (5) (MIC, 0.5 μg/ml) and with a moderate selectivity index (SI = 16). The similarity

of this compound to compound 41 should be noted, which indicates likely shortcomings.

A series of galactopyranosyl amino alcohols exemplified by compound 46 in Table 2 was described as an interesting slant towards inhibition of mycobacterial cell wall biosynthesis (94). This compound, a dimeric hybrid of a galactofuranosyl ethambutol analogue, displayed potent in vitro activity (MIC for *M. tuberculosis* in vitro of 1.56 μg/ml). However, on progression into a murine model, toxicity was observed at dosage levels (50 mg/kg per day) that offered no significant protection against *M. tuberculosis* infection. The high molecular weight, hydrogen bond donor count, and flexibility of this molecule contribute to its classification as a hit.

An additional series of compounds based on the thiazoline template was synthesized and evaluated by Bonde and Gaikwad (16). The most potent compound (compound 47 [Table 2]) showed good MICs (0.3 μg/ml) combined with low toxicity using a hemolytic assay (50% lethal dose values, >500 μg/ml). Some SAR was delineated; for example, replacing the thiazoline with a thiazolidinone substantially reduced activity. However, the loss of activity of some examples with time raises likely stability issues, with further concerns raised by cross-reactivity against other gram-positive and -negative bacteria. These molecules are large and lipophilic, which contribute to a poor physicochemical profile.

The antimycobacterial properties of various salicylanilides and related benzoxazine-diones (100, 100A) have been described, exemplified by compound 48 (Table 2). This compound showed good levels of antimycobacterial activity (MIC, <1 μg/ml) comparable to that of INH for some strains. No data, beyond an interesting QSAR analysis of the benzoxazine-diones, have been disclosed that might establish these compounds as strong hits, although they do have an attractive physicochemical profile.

CONCLUDING REMARKS

A large number of new small synthetic molecules with potential as antimycobacterial drug leads have been described. From these, the diarylquinoline drug candidates R207910 and PA-824 represent the most advanced tuberculosis-specific agents. PA-824 was expected to enter phase I clinical trials by the end of 2004 (34). Nevertheless, many of the hits and leads described herein merit further investigation from both a chemical and biological perspective. Defining the mode of action is a logical next step, as this information would permit the full range of biochemical, enzymological, and structural tools to be deployed, greatly increasing the chances that a drug candidate can be identified. An intensive medicinal chemistry effort is called for to improve the SAR and assess the true potential of each series. Unfortunately, most of the compounds described are interesting only because of their activity against growing *M. tuberculosis*. Further effort must be made to identify compounds acting on key targets that are essential for persistence of *M. tuberculosis* if a real breakthrough in therapy is to be made. Further work on the molecules described here and others emerging from both screening and focused medicinal chemistry programs should lead to new clinical agents becoming a reality in the coming years.

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