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The identification of potential new anti-tubercular chemotherapeutics is paramount due to the recent emergence of extensively drug-resistant strains of Mycobacterium tuberculosis (XDR-TB). Libraries of NAS-21 and NAS-91 analogues were synthesized and evaluated for their whole-cell activity against Mycobacterium bovis BCG. NAS-21 analogues 1 and 2 demonstrated enhanced whole-cell activity in comparison to the parental compound, and an M. bovis BCG strain overexpressing the dehydratase enzyme Rv0636 was resistant to these analogues. NAS-91 analogues with ortho-modifications gave enhanced whole-cell activity. However, extension with biphenyl modifications compromised the whole-cell activities of both NAS-21 and NAS-91 analogues. Interestingly, both libraries demonstrated in vitro activity against fatty acid synthase II (FAS-II) but not FAS-I in cell-free extracts. In in vitro assays of FAS-II inhibition, NAS-21 analogues 4 and 5 had IC₅₀ values of 28 and 19 µg ml⁻¹, respectively, for the control M. bovis strain, and the M. bovis BCG strain overexpressing Rv0636 showed a marked increase in resistance. In contrast, NAS-91 analogues demonstrated moderate in vitro activity, although increased resistance was again observed in FAS-II activity assays with the Rv0636-overexpressing strain. Fatty acid methyl ester (FAME) and mycolic acid methyl ester (MAME) were synthesized and evaluated for their whole-cell activity against fatty acid synthase II (in vitro assays of FAS-II inhibition, NAS-21 analogues showed increased whole-cell activity against fatty acid synthase II (FAS-II). However, NAS-91 analogues showed moderate in vitro activity, although increased resistance was observed in FAS-II activity assays with the Rv0636-overexpressing strain.

INTRODUCTION

Fatty acids. FAS-II, however, is incapable of de novo fatty acid synthesis and accepts short-chain (C₁₆) acyl-CoA primers from FAS-I via a condensation reaction carried out by β-ketoacyl-ACP synthase III (mtFabH) (Brown et al., 2005; Choi et al., 2000). This newly formed β-ketoacyl-ACP is reduced by a β-ketoacyl-ACP reductase (MabA) (Banerjee et al., 1998) to form a β-hydroxyacyl-ACP intermediate. The product is then dehydrated by β-hydroxyacyl-ACP dehydratase (designated FabZ in Escherichia coli), followed by further reduction with the enoyl-ACP reductase, InhA, to complete the FAS-II cycle (Banerjee et al., 1994; Kikuchi & Kusaka, 1984). Subsequent FAS-II cycles are initiated by the acyl-ACP primed β-ketoacyl-ACP synthases KasA and KasB, respectively (Kremer et al., 2000; Mdluli et al., 1998; Schaeffer et al., 2001), to afford a meromycolic acid (C₅₆), which is then condensed with a C₁₆ fatty acid (Gande et al., 2004; Portevin et al., 2005; Takayama et al., 2005). The oxymycolic acid intermediate is then reduced to form the mature mycolic acid (Lea-Smith et al., 2007).

Abbreviations: ACP, acyl carrier protein; FAMES/MAMEs, fatty/mycolic acid methyl esters; FAS, fatty acid synthase; MeOH, methanol; MTBE, methyl tert-butyl ether; NaOMe, sodium methoxide; TB, tuberculosis.
The dehydratase enzymes FabZ and FabA have been extensively studied in both *E. coli* and *Plasmodium falciparum* (Lee song et al., 1996; Sharma et al., 2003). Both FabZ and FabA catalyse the dehydration of the ϒ-hydroxylacyl-ACP to a *trans*-2-enoyl ACP in the third step of fatty acid elongation. In addition to performing the dehydration step, FabA has the ability to isomerize *trans*-2- to *cis*-3-decanoyl-ACP (Fig. 1), as an essential step in the formation of unsaturated fatty acids in *E. coli* (Kass & Bloch, 1967; Kass et al., 1967). The pivotal role played by FabZ and FabA makes them good potential drug targets against *M. tuberculosis*. The identification of the key FAS-II dehydratase step in mycobacteria has remained an enigma until very recently (Sacco et al., 2007).

In an attempt to establish whether Rv0636 represented the potential dehydratase candidate, overexpression studies were performed in *M. bovis* BCG against a series of flavonoid inhibitors known to target FabZ (Brown et al., 2007b). Of the five flavonoids tested, four were found to be active against *M. bovis* BCG with MICs ranging from 150 to 220 μM, the most potent being butein. The activity of the flavonoids against the hypothesized gene product Rv0636 indicated that the overexpression in *M. bovis* BCG conferred resistance to butein and isoliquiritigenin (Brown et al., 2007b). The data suggested that the flavonoids are inhibitors of mycobacterial FAS-II and in particular Rv0636, reiterating the potential candidacy of this gene product as the dehydratase enzyme of the FAS-II in *M. tuberculosis*.

Sacco et al. (2007) had independently demonstrated that the Rv0635–Rv0637 operon encoded dehydratase activity. The recombinant expression of the candidate protein cluster, Rv0635-Rv0636-Rv0637, led to the formation of two heterodimers, Rv0635-Rv0636 (HadAB) and Rv0636-Rv0637 (HadBC), which were shown to also occur in *Mycobacterium smegmatis* (Sacco et al., 2007). Both heterodimers exhibited the enzymic properties expected for mycobacterial FAS-II dehydratases, including a marked specificity for both long-chain (>C12) and ACP-linked substrates (Sacco et al., 2007). Furthermore, the authors of this study were able to show the function of Rv0636 or HadAB/ HadBC as a ϒ-hydroxyacyl dehydratase when coupled with MabA and InhA enzymes from *M. tuberculosis* FAS-II.

Further research into potential dehydratase inhibitors has yielded the identification of NAS-21 and NAS-91, which have been shown to target ϒ-hydroxyacyl-ACP dehydratase FabZ of *P. falciparum* (Sharma et al., 2003). A decrease in the rate of enzyme activity was observed in the presence of both NAS-21 and NAS-91 using spectrometric and HPLC methods. The authors of that study also showed that the incorporation of [2,1-^14^C]malonyl-CoA into fatty acids in cell-free extracts of *P. falciparum* was inhibited to different extents by NAS-21 and NAS-91. The incorporation of [1,2-^14^C]acetic acid into fatty acids was reduced by 26 and 46 %, respectively, in the presence of 10 μM NAS-21 and NAS-91. To investigate the potential anti-mycobacterial therapeutic activity of NAS-21 and NAS-91, we synthesized a library of these FabZ inhibitors. Using a similar strategy to that previously presented (Brown et al., 2007b), we evaluated the analogues for their whole-cell activity against *M. bovis* BCG and an Rv0636-overexpressing *M. bovis* BCG strain, and and their *in vitro* activity against FAS-I and FAS-II in cell-free assays using *M. smegmatis* extracts.

**METHODS**

**Synthesis of NAS-21 analogues.** A series of NAS-21 analogues were developed using a previously described method (Sharma et al., 2003) (Scheme 1). In brief, acetoephene derivatives were condensed with ethyl trifluoroacetate in the presence of 25 % NaOMe (in MeOH) and methyl-tert-butyl ether. Diversity was introduced into these reactions using a variety of commercially available acetoephene derivatives, yielding analogues 1–6 (Table 1). Biphenyl analogues 7–9 were developed via Suzuki coupling of 4-iodoacetoephene with aryl boronic acid derivatives. The product was then subjected to treatment with ethyl trifluoroacetate, 25 % NaOMe in MeOH and MTBE (Scheme 1) to yield the desired analogues 7–9. An example of the Suzuki coupling reaction for analogue 7 is as follows. 4-Iodoacetoephene (100 mg, 0.292 mmol, 1 eq.), ethylene glycol dimethyl ether (3 ml), aqueous Na2CO3 (0.5 ml, 1 M) and 4-fluorophenylboronic acid (48.97 mg, 0.37 mmol, 1.2 eq.) in a round-bottom flask was degassed for 10 min. Bis[triphenylphosphine] palladium chloride (8 mg, 7×10^-3), 5 mol% was then added and the mixture was heated under reflux for 6 h. The mixture was partitioned between water (10 ml) and ethyl acetate (10 ml) and separated. The aqueous layer was acidified to pH 2 with dilute hydrochloric acid (2 M) and the product was extracted with ethyl acetate (2× 10 ml). The organic layers were combined, washed with saturated brine (3×10 ml), dried and reduced to give the crude product. Purification was achieved via two separate silica gel columns. The first column used chloroform in methanol (95:5, v/v) as eluant and the second column used ethyl acetate in petroleum ether (35:65). The title analogue gave a white solid in 78 % yield (62 mg). ^1H NMR (CDCl3, 300 MHz) δ: 2.60 (s, 3H, CH3, H-14), 7.05 (d, 2H, H-5, H-7, J = 8.5 Hz), 7.50 (d, 2H, H-4, H-8, J = 8.5 Hz), 7.60 (d, 2H, H-3, H-9, J = 8.0 Hz), 7.95 (d, 2H, H-2, H-10, J = 8.0 Hz). ^13C NMR (CDCl3, 75 MHz) δ: 23.8 (C-14), 116.0, 127.4, 129.8 (C-2, 3, 4, 5, 7, 8, 9, 10), 132.0 (C-12), 136.7 (C-1), 142.0 (C-11), 168.4 (C-6), 195.6 (C-13); m/z (EI) 214.2 [M+] (100 %); HRMS calculated for C14H11FO [M+] 214.2319 found 214.2327.

![Fig. 1. Dehydration and isomerization of 3-hydroxydecanoyl-ACP by *E. coli* FabA.](http://mic.sgmjournals.org)
Synthesis of NAS-91 analogues. NAS-91 was synthesized as described by Sharma et al. (2003). The reaction involves the coupling of 2-bromo-4-chlorophenol with 5-chloro-8-hydroxyquinolone, using caesium carbonate, copper (I) chloride (0.5 eq.) and N-methylpyrrolidinone as the solvent (Scheme 2) (Ullmann & Sponagel, 1905). An alternative method was developed to synthesize the remaining NAS-91 analogues in Table 2. A linker arm was introduced into 5-chloro-8-hydroxyquinolone by reacting it with benzyl bromide derivatives. This method was employed to generate a library of seven novel NAS-91 analogues (Franzblau et al., 1999) (Scheme 3). Diversity was introduced into this library by utilizing a variety of commercially available benzyl bromide derivatives. This method was employed to generate a library of seven novel NAS-91 analogues (10-16) (Table 2), which contain a methylene linker arm connected to the oxygen of 5-chloro-8-hydroxyquinolone. As an example, analogue 10 was synthesized as follows. 5-Chloro-8-hydroxyquinolone (500 mg, 2.78 mmol, 1 eq.) was dissolved in 5 ml dimethylformamide. To this was added caesium carbonate (452 mg, 1.39 mmol, 0.5 eq.). After 20 min of mixing at room temperature, benzyl bromide (0.37 ml, 2.78 mmol, 1 eq.) was dissolved in 5 ml dimethylformamide. To this reaction mixture was quenched at room temperature overnight. The reaction mixture was quenched with water. The organic layer was extracted with ethyl acetate, washed at room temperature overnight. The reaction mixture was quenched with water, and the supernatant was added dropwise and the reaction was stirred for 85 % yield (635 mg).1H NMR (CDCl3, 300 MHz) δ: 5.35 (s, 2H, CH2, H-11), 6.85 (d, 1H, H-7, J=8.44 Hz), 7.15–7.45 (m, 4H, H-12, H-14, H-15, H-16), 7.48–7.50 (m, 3H, H-3, H-6, H-13), 8.40 (d, 1H, H-4, J=8.54 Hz), 8.90 (d, 1H, H-2, J=4.17 Hz). 13C NMR (CDCl3, 75 MHz) δ: 68.9 (C-11), 107.8 (C-7), 120.4 (C-3), 124.3 (C-5), 124.5 (C-6), 125.2 (C-13, C-17), 126.1, 126.8, 127.2 (C-15, C-16, C-17), 130.8 (C-4), 134.5 (C-12), 147.2 (C-9), 153.2 (C-8); 130.9 (m), 132.1 (m), 141.4 (s), 162.5 (s); m/z 369.06 (M+) (30 %), 91.00 [C6H4(CH2)3] (100 %). HRMS calculated for C16H13ClNO [M+] 269.0600 found 269.0602.

Bacterial strains, growth conditions and MIC<sub>90</sub> determination. All reagents were of assay grade and purchased from Sigma-Aldrich. Overexpression of pVV16-Rv0636 (Brown et al., 2007b) was conducted in M. bovis BCG on Middlebrook 7H10 agar supplemented with oleic-albumin-dextrose-catalase (OADC) enrichment (BD and Company) and containing 25 µg kanamycin ml<sup>−1</sup> and 50 µg hygromycin ml<sup>−1</sup> (Kremer et al., 1995). Liquid cultures of M. bovis BCG were grown at 37 °C in Sauton’s medium containing 25 µg kanamycin ml<sup>−1</sup> and 50 µg hygromycin ml<sup>−1</sup>. MIC<sub>90</sub> values of NAS analogues against M. bovis BCG/pVV16 and M. bovis BCG/pVV16-Rv0636 were determined by Alamar Blue as described previously (Kremer et al., 2002a). The final extract containing the FAS-I and FAS-II activities was dissolved in 5 ml 50 mM MOPS pH 7.9, 5 mM β-mercaptoethanol, 10 mM MgCl<sub>2</sub>. Protein concentrations were determined using the BCA protein assay reagent kit (Pierce). FAS-I and FAS-II assays were conducted as previously described using the 40–80 % ammonium sulfate fraction (Kremer et al., 2002b; Slayden et al., 1996).

RESULTS AND DISCUSSION

Biological evaluation of NAS-21 analogues

NAS-21 has been shown to target the β-hydroxylacyl-ACP dehydratase FabZ of P. falciparum (Sharma et al., 2003). The mycobacterial FabZ (Rv0635–637) is a potentially attractive target for such an inhibitor; therefore we sought to synthesize and evaluate NAS-21 analogues against M. bovis BCG, which possesses a similar drug profile to M. tuberculosis in terms of sensitivity (Larsen et al., 2002; Vilcheze et al., 2005). The results of the whole-cell analysis of NAS-21 analogues against M. bovis BCG pVV16 and M. bovis BCG/pVV16-Rv0636, which overexpresses Rv0636, are shown in Table 1. It is clearly evident that the...
COCH₂COCF₃ group plays a central role in the activity of NAS-21 analogues, because the simple conversion of this group to the COCH₃ functionality resulted in the inactivation of the compounds (MIC₉₉ > 250 μg ml⁻¹) (data not shown). Two possible explanations for this observed decrease in activity are (i) the di-keto nature of the analogue may mimic the β-keto substrate utilized in FAS-II and may act as a competitive inhibitor or (ii) the presence of the CF₃ group may stabilize the formation of the keto–enol tautomer of these analogues, which may increase the interaction of the drug with the enzyme's active site. Extension in the para-position of the aromatic ring in NAS-21 resulted in analogues with a significant reduction in activity (Table 1). This is demonstrated by the

<table>
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<th>Label</th>
<th>Structure</th>
<th>Whole-cell M. bovis BCG activity MIC₉₉ (μg ml⁻¹)</th>
<th>In vitro M. smegmatis FAS-II activity IC₅₀ (μg ml⁻¹)</th>
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effects of (i) the simple modification of the methyl group in 4 to an ethyl group in 5 and (ii) the introduction of a


Table 2. Structures of NAS-91 analogues, whole-cell inhibitory activity against M. bovis BCG and in vitro inhibition of M. smegmatis FAS-II activity

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<th>Whole-cell M. bovis BCG activity MIC&lt;sub&gt;90&lt;/sub&gt; (µg ml&lt;sup&gt;-1&lt;/sup&gt;)</th>
<th>In vitro M. smegmatis FAS-II activity IC&lt;sub&gt;50&lt;/sub&gt; (µg ml&lt;sup&gt;-1&lt;/sup&gt;)</th>
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biphenyl group (7–9), thus indicating that modifications extending in this position are not well tolerated. Comparable whole-cell activity of the parent drug NAS-21 and analogues 1, 2 and 4 was demonstrated against M. bovis BCG/pVV16. A significant decrease in whole-cell FAS-I or FAS-II, respectively. In both cases fatty acyl-CoAs, either acetyl-CoA or palmitoyl-CoA, for utilized by using priming units in the form of two different radiolabel into extractable lipids. Specific assays were performed on extracts isolated from both analogues strain was also observed, suggesting that the product of this gene may represent the cellular target for NAS-21 and analogues 1, 2 and 4.

Effects of NAS-21 analogues on activity of FAS-I and FAS-II in cell-free extracts

To further evaluate the biological properties of NAS-21 analogues and to investigate their potential target, Rv0636, a series of in vitro FAS-I and FAS-II assays were performed on crude cell-free extracts of M. smegmatis as previously described by Slayden et al. (1996). The analysis was performed on extracts isolated from both M. smegmatis/ pVV16 and M. smegmatis/pVV16-Rv0636. The activity of each analogue was measured by the incorporation of each analogue was demonstrated against whole cells of M. bovis BCG/pVV16 and M. bovis BCG/pVV16-Rv0636, did not give the same marked response compared to 4 and 5 in relation to FAS-II inhibition with the same strains (Table 1). Analogues 7–9 were inactive against whole cells; however, moderate activity was observed in FAS-II assays (Table 1), implying that cell permeability may be a contributing factor towards the lack of whole-cell activity of these biphenyl-containing analogues.

Biological evaluation of NAS-91 analogues

NAS-91 showed poor whole-cell activity against both M. bovis BCG/pVV16 and M. bovis BCG/pVV16-Rv0636 and no inhibition was observed even at high concentrations (>250 μg ml⁻¹) (Table 2). The observed poor inhibition of M. bovis BCG growth was surprising since Gratraud et al. (2008) recently reported an MIC value of 25 μg ml⁻¹ against M. bovis BCG for NAS-91, although the MIC values reported for NAS-21 (50 μg ml⁻¹) by Gratraud et al. (2008) are similar to the values (63 μg ml⁻¹) reported in this study. A key feature of note in the studies by Gratraud et al. (2008) was that the MIC values were determined on Middlebrook 7H11 agar plates by visualizing plaques following serial dilution. In contrast, in this present study, MIC values for NAS-91 (as well as NAS-21) were determined using the more established and sensitive Alamar Blue method (Franzblau et al., 1998) in Sauton’s liquid medium. It is clear that MIC values for NAS-91 in particular are different on liquid and solid media. This is not totally surprising since similar observations have been reported for drug inhibition of mycobacterial strains. For instance, M. smegmatis is sensitive to econazole and clotrimazole on LB solid agar plates, with MIC values of 2 and 0.5 μg ml⁻¹, respectively (Burguiere et al., 2005). However, when M. smegmatis is cultured in Sauton’s liquid medium, the MIC values are higher than those determined on agar plates, with econazole at 20 μg ml⁻¹ (10-fold higher) and clotrimazole at 15 μg ml⁻¹ (30-fold higher), respectively. Interestingly, it is clear that a concentration of 100 μg ml⁻¹ of NAS-91 in liquid media is only partially inhibiting mycolate synthesis (50%) in the Gratraud et al. (2008) study, which is at four times the MIC value on solid media. This is further evidence for the MIC value for NAS-91 being different on solid and liquid media.
Analogues 13–16 demonstrated significantly improved whole-cell activity in comparison to NAS-91. The simple introduction of a methyl modification in analogue 15 resulted in the most improved whole-cell activity, with an MIC<sub>99</sub> value of 18 μg ml<sup>−1</sup> against <i>M. bovis</i> BCG/pVV16. Encouragingly, resistance was shown against analogue 15 when <i>M. bovis</i> BCG/pVV16-Rv0636 was used, with an increase in MIC<sub>99</sub> to 100 μg ml<sup>−1</sup>. Structurally, analogues 13 and 14 indicate that there is more scope to extend the modification in the ortho-position by two or more carbons. Analogues 10 and 11 were primarily developed to assess the feasibility of introducing a linker arm into the analogues whilst changing the functionalities on the aromatic ring. As indicated in Table 2, the low biological activity of these analogues was comparable to that of NAS-91. Initially it was felt that the linker arm might have compromised activity by reorientating the analogue within the active site, thus affecting its interactions with the target. However, as observed with analogues 13–16, modifications in the ortho-position of the aromatic ring greatly increase the potency of this analogue, suggesting it is the nature of the modification on 10 and 11 which has compromised their whole-cell activity. From the activities observed with analogues 13–16 it is evident that the hydroxyl group of the secondary aromatic functionality does not play an important part in the protein–drug interaction, as activity was still observed in these analogues. Finally, the introduction of a second aromatic group in the para-position (12) compromised the whole-cell activity against both <i>M. bovis</i> BCG/pVV16 and <i>M. bovis</i> BCG/pVV16-Rv0636. This initial study suggests that there is limited scope to further extend in the para-position with a second aromatic ring; however, this requires verification by formulating a more comprehensive library.

**Effects of NAS-91 analogues on activity of FAS-I and FAS-II in cell-free extracts**

To further evaluate the activities of the NAS-91 analogues, a series of <i>in vitro</i> FAS-I and FAS-II assays were performed on crude cell-free extracts of <i>M. smegmatis</i>. As with the NAS-21 analogues, the crude <i>M. smegmatis</i> cell-free FAS-I assays revealed that none of the analogues inhibited FAS-I (data not shown). Analogues 10 and 12 demonstrated similar effects to NAS-91 against FAS-II activity in <i>M. smegmatis</i> cell-free extracts (Table 2). Encouragingly, analogues 11 and 13–15 gave a marked increase in <i>in vitro</i> activity against FAS-II, and extracts from <i>M. smegmatis</i>...
pVV16-Rv0636 FAS-II extract showed resistance to these analogues. Analogues 13–15 also showed good whole-cell activity against whole-cell *M. bovis* BCG/pVV16, providing further evidence that these analogues would form a good basis to generate a secondary library of NAS-91 analogues.

**Effects of NAS-21 and NAS-91 analogues on FAME and MAME synthesis**

*M. bovis* BCG/pVV16 was grown in the presence of the NAS analogues at various concentrations, followed by [1,2-14C]acetate labelling and analysis by TLC separation of FAMEs and MAMEs. An example of the results, for analogues 1 and 15, is shown in Fig. 2. There was a decrease in the incorporation of radioactivity into FAMEs and MAMEs in the presence of NAS-21, analogues 1 and 15. Since analogues 1 and 15 were shown not to inhibit FAS-I (data not shown), the experiment was repeated; equal counts were loaded and the TLC profiles of FAMEs and MAMEs reanalysed (Fig. 3A, D). It is clear from this analysis that analogues 1 and 15 only inhibit the synthesis of α- and keto-MAMEs and not that of FAMEs (Fig. 3), consistent with the earlier in vitro data (Tables 1 and 2). As an additional control the synthesis of cell envelope lipids was also examined (Fig. 3). Analogues 1 and 15 again do not inhibit general fatty acid synthesis as the synthesis of PGL (Fig. 3B, E) and phospholipids (Fig. 3C, F) remains unaffected. Resistance was also observed upon the overexpression of pVV16-Rv0636, supporting the earlier MIC99 and in vitro studies and thereby strengthening the evidence that these analogues target Rv0636 (Fig. 2). Similar results were observed with the other active analogues (2, 4, 13, 14 and 16).

**Concluding remarks**

In conclusion, no activity was observed against FAS-I for either NAS-21 or NAS-91. In general, all the analogues showed in vitro activity against FAS-II extracts, and the Rv0636-overexpressing strain carrying pVV16-Rv0636 showed a marked increase in resistance. Whole-cell FAME and MAME analysis for most analogues demonstrated a decrease in both mycolic acid and fatty acid biosynthesis. Interestingly, this effect of the analogues was also reduced in *M. bovis* BCG/pVV16-Rv0636, thus further implicating Rv0636 as the target for these FabZ dehydratase inhibitors. The present study extends the initial findings of Gratraud et al. (2008), who did not perform FAS-I and FAS-II in vitro enzyme studies, using NAS-21 and NAS-91 to examine mycolate inhibition directly. Although the study of Gratraud et al. (2008) demonstrated that NAS-21 and NAS-91 also inhibited oleate biosynthesis it is clear that this represents a secondary target since it is non-essential, in contrast to Rv0636, which has been shown to

![Fig. 3. TLC-autoradiography of *M. bovis* BCG lipids after NAS-21 and NAS-91 analogue treatment. (A, D) Analysis of FAMEs and MAMEs following treatment with NAS-21 analogue 1 (20 µg ml⁻¹) and NAS-91 analogue 15 (15 µg ml⁻¹) and resolved by TLC using equal counts (50 000 c.p.m.) as described in Methods. Lipid extractions were performed as described by Dobson et al. (1985) and a 50 000 c.p.m. aliquot analysed using silica gel plates (5735 silica gel 60F254; Merck). (B, E) Phenolic glycolipids (PGL) were identified by 2D TLC [direction 1, chloroform/methanol (94:4; v/v); direction 2, toluene/acetone (80:20, v/v)]. (C, F) Phospholipids (P), phosphatidylinositol (PI), acyl-phosphatidylinositol dimannoside (Ac1PIM2) and diacyl-phosphatidylinositol dimannoside (Ac2PIM2) were identified by 2D TLC [direction 1, chloroform/methanol/water (60:30:6, by vol.); direction 2, chloroform/acetic acid/methanol/water (40:25:3:6, by vol.)]. Autoradiograms were produced by overnight exposure to Kodak X-Omat AR film to reveal 14C-labelled FAMEs, MAMEs and lipids.](http://mic.sgmjournals.org)
be essential (Brown et al., 2007a). In comparison to the FAS-II flavonoid inhibitors (Brown et al., 2007b), our NAS-21 and NAS-91 analogues demonstrated a marked enhancement in activity; in some cases an eightfold increase is observed. Therefore NAS-21 and NAS-91 analogues represent good candidates for further development of drugs targeting the mycobacterial FAS-II dehydratase. However, to fully establish the potential therapeutic properties of NAS-21 and NAS-91, their inhibition against the heterodimers Rv0635-Rv0636 (HadAB) and Rv0636-Rv0637 (HadBC) must be also evaluated. The recent development of an in vitro assay for the FAS-II dehydratase activity (Sacco et al., 2007) will help us to better understand the inhibitory activity of these compounds.

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