European Journal of Medicinal Chemistry 45 (2010) 5910–5918

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Design, synthesis and inhibitory activity against *Mycobacterium tuberculosis* thymidine monophosphate kinase of acyclic nucleoside analogues with a distal imidazoquinolinone

Olga Familiar [a], Hélène Munier-Lehmann [b,c], José Antonio Aínsa [d], María-José Camarasa [a], María-Jesús Pérez-Pérez [a,*]

[a] Instituto de Química Médica (CSIC), Juan de la Cierva 3, E-28006 Madrid, Spain
[b] Institut Pasteur, Unité de Chimie et Biocatalyse, 28 Rue du Dr. Roux, 75724 Paris Cedex, France
[c] CNRS URA2128, France
[d] Departamento de Microbiología, Medicina Preventiva y Salud Publica, Universidad de Zaragoza, Domingo Miral s/n, 50009 Zaragoza and CIBER Enfermedades Respiratorias, Spain

ARTICLE INFO

Article history:
Received 5 July 2010
Received in revised form
23 September 2010
Accepted 27 September 2010
Available online 15 October 2010

Keywords:
Thymine
Imidazoquinolinone
Enzymatic inhibitors
M. tuberculosis
Kinase
Bioisoster

ABSTRACT

Thymidine monophosphate kinase from *Mycobacterium tuberculosis* (TMPKmt) has been proposed as an attractive target in the search of new agents to fight against tuberculosis. We recently reported that thymine derivatives carrying a naphtholactam or naphthosultam moiety at position 4 of a (*Z*)-butenyl chain inhibit TMPKmt in the subμM range. Here we describe the replacement of the planar naphtholactam and naphthosultam rings in our identified hits by 5,6-dihydro-1*H*-imidazo[4,5,1-*ij*]quinolinones and a 5,6-dihydro-1*H*,4*H*-1,2,5-thiadiazolo[4,3,2-*ij*]quinoline-2,2-dioxide where the planarity has been broken. Interestingly, these non-planar compounds were similarly potent against the target enzyme than their aromatic analogues, suggesting a bioisosteric behavior that may also be applied to other biologically active compounds. The synthesis of the different targeted imidazoquinolinones has been successfully performed via a hypervalent iodide mediated oxidative cyclization of *N*-methoxyureas catalized by bis(trifluoroacetoxy)iodobenzene (PIFA) expanding the reported use of this reagent for the synthesis of differently substituted imidazoquinolinones.

1. Introduction

Infection with *Mycobacterium tuberculosis*, the causative agent of tuberculosis, is one of main global health problems. More than 9 million people develop tuberculosis annually [1]. Besides this high incidence, the armamentarium of drugs to fight this global epidemic is inadequate. Moreover, the increasing impact of multidrug-resistant (MDR) and extensively drug-resistant (XDR) tuberculosis demands new efforts in this field. In the fight against this increasing global health problem, the search of novel compounds and novel targets is one of the active areas of research [2,3].

M. tuberculosis thymidine monophosphate kinase (TMPKmt) has been proposed as an attractive candidate to interfere with the replication of the pathogen [4,5]. TMPK is crucial for maintaining the thymidine triphosphate (dTTP) pools that are required for DNA synthesis in replicating organisms [5–7]. Since *M. tuberculosis* lacks the salvage pyrimidine pathway, TMPKmt has been proposed as a key target for the replication of the bacteria. Very recently, and to further stress the importance of this enzyme, TMPKmt detection assays have been proposed to reduce the time-lines for positive identification and drug resistance profile among *M. tuberculosis* cultures [8].

Several research groups have been working in the identification of TMPKmt inhibitors with a nucleoside structure [9–13] or based on benzylthymines [14,15]. Moreover, computational studies have been performed to establish structure–activity relationships and/or to identify the pharmacophore sites on the inhibitors [16–18]. Recently we reported on acyclic nucleoside analogues that potently and selectively inhibited TMPKmt [19]. Our most potent compounds were (*Z*)-butenyl thymines carrying a naphtholactam (**1**) or naphthosultam (**2**) moiety at position 4 (Fig. 1), with K_i values in the submicromolar range, and are among the most potent TMPKmt inhibitors described.

Docking studies performed with these compounds and their target enzyme TMPKmt revealed that the thymine base and the (*Z*)-butenyl unit established favorable interactions in the active site of the enzyme, while the CO or SO_2 group at the distal substituent established a key interaction with the guanidinium group of Arg95

* Corresponding author. Tel.: +34 91 258 75 79; fax: +34 91 5644853.
E-mail address: mjperez@iqm.csic.es (M.-J. Pérez-Pérez).

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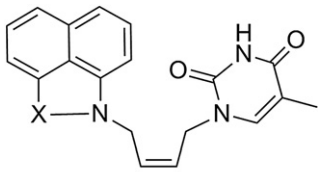
doi:10.1016/j.ejmech.2010.09.056



1 X=CO K_i= 0.42 μM

2 X=SO_2 K_i= 0.27 μM

Fig. 1. Chemical formulae and *Ki* values against TMPKmt of previously described acyclic nucleoside inhibitors.

(Fig. 2) [19]. Despite their significant inhibitory activity against TMPKmt, these compounds were highly insoluble. It is plausible that the high degree of planarity of the thymine base and the naphtholactam or naphthosultam substituents favored stacking interactions that prevented the accessibility of the solvent. Therefore it was desirable to break the planarity of these substituents. Since the thymine base is crucial for the right interaction with the enzyme active site, we focused our efforts at the naphtholactam and naphthosultam site. Still the substituent at the distal site should keep a CO or SO_2 group able to favorably interact with Arg95, in a similar way to the lead compounds **1** and **2**.

Therefore the new derivatives that fulfill our requirements can be represented by the general formula represented in Fig. 3 where the naphtholactam and naphthosultam have been replaced by a 5,6-dihydro-1*H*-imidazo[4,5,1-*ij*]quinolinone (X = CO) and a 5,6-dihydro-1*H*,4*H*-1,2,5-thiadiazolo[4,3,2-*ij*]quinoline-2,2-dioxide (X = SO_2), respectively, and in this way the planarity at the distal site has been broken. Moreover an amino group has been incorporated at positions 5 or 8 of the heterocyclo-quinolinones with the aim to improve the solubility and to facilitate further functionalization. It is interesting to mention that breaking the planarity and increasing the sp^3 character have recently been proposed as a general strategy in medicinal chemistry to progress from an initial hit toward a bioactive compound [20]. Another interesting example in this direction is the transformation of insoluble dye indirrubin into highly active CDK2 inhibitors by breaking the planarity and replacing sp^2 by sp^3 carbon centers [21].

The most convenient synthesis of the 5,6-dihydro-1*H*-imidazo[4,5,1-*ij*]quinolin-2(4*H*)-one was described by Romero et al. [22],

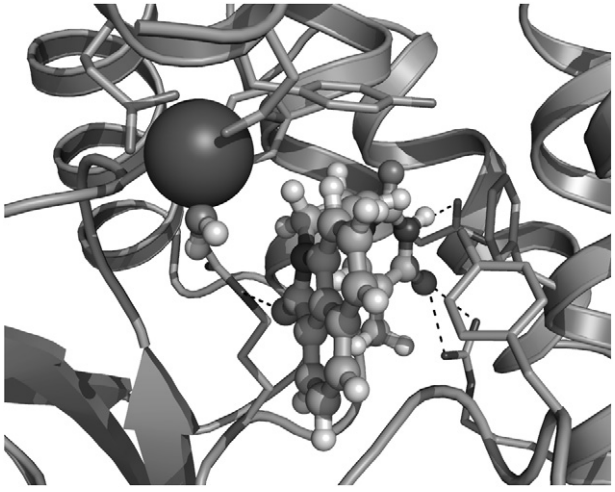
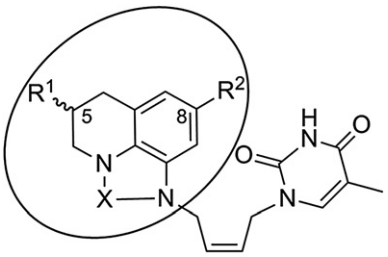


Fig. 2. Detail of the substrate binding site in the complex of compound **1** with TMPKmt based on docking studies. Only the side chains of relevant residues are shown as sticks. The black dashes represent the proposed hydrogen bonds involved in binding of the inhibitor. The magnesium ion is represented as a sphere.



X = CO, SO_2

R^1 = H, NH_2

R^2 = H, NH_2

Fig. 3. Chemical formulae of the proposed inhibitors.

applying the methodology reported by Kawase [23]. This strategy implies, as the key reaction, the oxidative cyclization of *N*-methoxyureas with the powerful oxidant bis(trifluoroacetoxy) iodobenzene (abbreviated as PIFA or BTI) followed by removal of the methoxy group. The advantages of PIFA, as recently reviewed [24], include its low toxicity, commercial availability and ease of handling. Based on the work published by Romero et al. [22,25], we hypothesized that this PIFA-mediated reaction could also be applied to the synthesis of the required imidazoquinolinones with an amino group at position 5 or 8, that either had not been described or had been previously obtained by a more lengthy synthetic procedure, as in the case of the amino group at position 5 [26]. Once obtained, the heterocycles were alkylated with (*Z*)-1,4-dichlorobut-2-ene followed by incorporation of the thymine base to afford the target compounds.

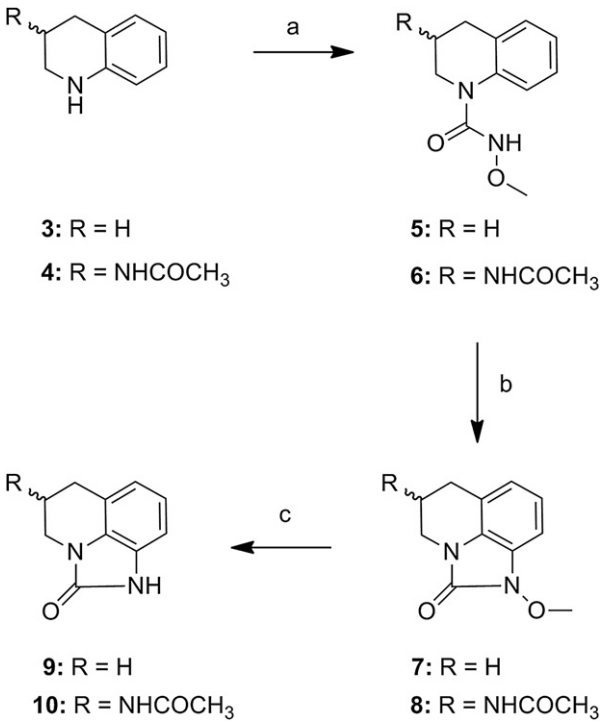
2. Results and discussion

2.1. Chemistry

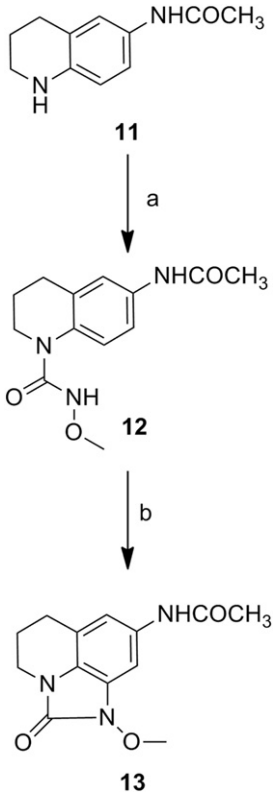
As mentioned, our synthetic approach started with the synthesis of the imidazoquinolinones at the distal site. For the non-substituted compound (Fig. 3, X = CO, $R^1 = R^2 = H$), the starting material was the commercially available 1,2,3,4-tetrahydroquinoline (**3,** Scheme 1). For the synthesis of the 5-amino derivative (Fig. 3, X = CO; $R^1 = NH_2$; $R^2 = H$), the synthetic pathway began from the 3-acetamido-1,2,3,4-tetrahydroquinoline (**4**) obtained from 3-aminoquinoline by described procedures [27,28]. Reaction of **3** with triphosgene in the presence of Et_3N in THF at 0 °C followed by addition of methoxyamine hydrochloride afforded the methoxyurea **5** [22] in 66% yield (Scheme 1).

Similarly, the 3-acetamidotetrahydroquinoline (**4**) reacted under identical conditions to afford the urea **6** in 91% yield. Next, treatment of **5** with PIFA in CH_2Cl_2 in the presence of TFA as initially described by Romero [22] afforded the desired cyclized compound **7** but in a modest yield (34%). When the same reaction was performed in $CHCl_3$ [23], the yield of **7** was increased to 78%. The application of these reaction conditions to **6**, that is oxidation with PIFA in $CHCl_3$ at 0 °C, afforded after 4 h the cyclic derivative **8** in 90% yield. Finally, the methoxy group in **7** and **8** was removed by catalytic hydrogenation in EtOH in the presence of $Pd(OH)_2/C$ (20%) to provide the NH derivatives **9** [22] and **10** (83 and 65% yield, respectively).

Next we envisioned the synthesis of 5,6-dihydro-1*H*-imidazo[4,5,1-*ij*]quinolin-2(4*H*)-one with an amino group at position 8. Initially, we applied an approach similar to that described for the synthesis of **10**. In this case the starting material was the 6-acetamido-1,2,3,4-tetrahydroquinoline **11** (Scheme 2), obtained from



Scheme 1. (a) Et_3N, triphosgene, THF, 0 °C, 1 h, then addition of methoxyamine hydrochloride, rt, 72 h; (b) PIFA, $CHCl_3$, rt, 4 h; (c) H_2, $Pd(OH)_2/C$ (20%), EtOH, 30 °C, 24 h.



Scheme 2. (a) Et_3N, triphosgene, THF, 0 °C, 1 h, then addition of methoxyamine hydrochloride, rt, 72 h; (b) PIFA, $CHCl_3$, rt, 4 h.

6-aminoquinoline following reported procedures [29]. Compound **11** was transformed into the methoxyurea (**12**) in 57% yield and then submitted to aromatic electrocyclization with PIFA. However, this step afforded the target compound **13** in a very low yield (15%). This low yield could be due to the presence of an additional arylic NH in **12** that is susceptible to be oxidized under the experimental conditions leading to complex mixtures, as reported in other examples [22].

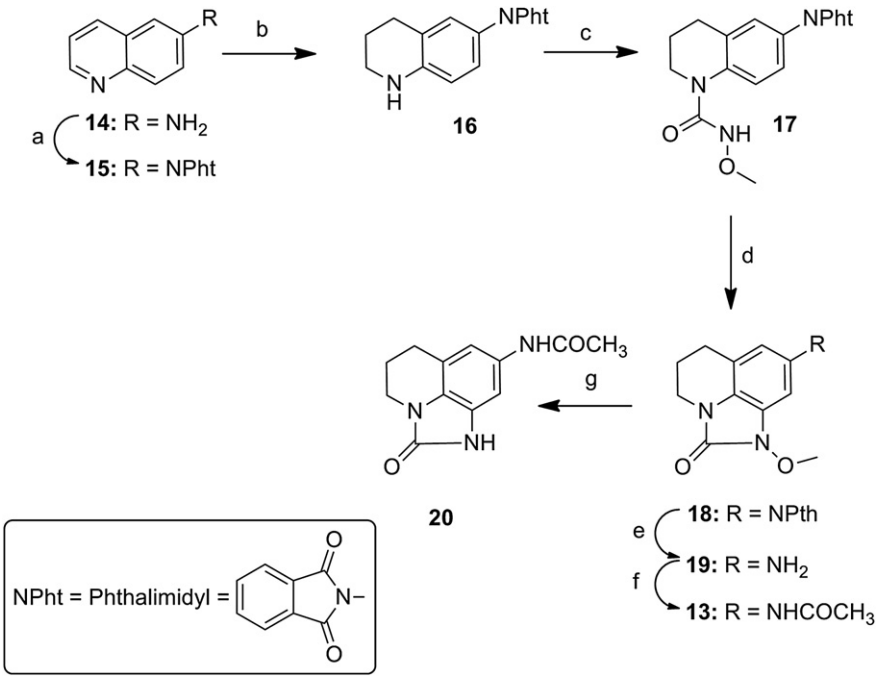
Thus, the amino group of the starting 6-aminoquinoline was protected as a phthalimido in order to avoid the presence of an alternate activated N–H. Reaction of 6-aminoquinoline with phthalic anhydride afforded the 6-protected derivative **15** in 98% yield (Scheme 3). Selective reduction of the pyridine ring in **15** was accomplished by treatment with sodium cyanoborohydride to provide **16** in 81% yield. Reaction of **16** with triphosgene followed by treatment with methoxyamine hydrochloride yielded the urea **17** (74%). In this case the aromatic electrocyclization with PIFA effectively afforded the cyclic compound **18** (73% yield). However, all attempts to remove the *N*-methoxy group in **18** were unsuccessful. Therefore, we decided to replace the phthalimido group in **18** by another protecting group, i.e., an acetyl group. Treatment of **18** with hydrazine hydrate quantitatively afforded the amino derivative **19** that was further protected by acetylation to afford compound **13** (73% yield). Catalytic hydrogenation of **13** employing $Pd(OH)_2/C$ (20%) in EtOH allowed the successful removal of the methoxy group to provide the NH free compound (**20,** 84% yield).

Treatment of compounds **9**, **10** and **20** (Scheme 4) with NaH or MeONa in dry DMF followed by "in situ" reaction with (*Z*)-1,4-dichlorobut-2-ene afforded the monochlorides **21**–**23** in good yields (67%, 86% and 65% yield, respectively). Reaction of **21**–**23** with silylated thymine in the presence of NaI in CH_3CN successfully afforded the *N*1-substituted thymine derivatives **24**, **25** and **27** in good to high yields. Removal of the acetyl group in compounds **25** and **27** was accomplished by treatment with HCl (1.6 M) in dioxane at reflux, to yield compounds **26** and **28**.

As mentioned in the introduction, the naphthosultam derivative **2** was also shown to be a potent inhibitor of the target enzyme TMPKmt. Therefore, it was considered of interest to synthesize a similar analogue where the naphthosultam ring was replaced by the corresponding 5,6-dihydro-1*H*,4*H*-1,2,5-thiadiazolo[4,3,2-*ij*]quinoline-2,2-dioxide (**29**, Scheme 5). Compound **29** was synthesized following a described procedure [30] by reaction of 8-amino-1,2,3,4-tetrahydroquinoline [31] with sulfamide in diglyme. Reaction of **29** with (*Z*)-1,4-dichlorobut-2-ene in the presence of NaH in dry DMF afforded the monosubstituted compound **30** in 46% yield (Scheme 5). Reaction of **30** with silylated thymine in refluxing CH_3CN and in the presence of NaI gave the thymine derivative **31** in 79% yield. Alternatively, compound **30** reacted with silylated 5-bromouracil to afford the *N*1-substituted compound **32** in 49% yield.

2.2. Enzymatic assays

The synthesized nucleoside analogues **24**–**28, 31** and **32** were evaluated against TMPKmt using a spectrophotometric assay [32]. This assay is routinely used for TMPK activity determination since it is fast and easily carried out. The results are shown in Table 1. The natural substrate thymidine-5′-phosphate (dTMP) and the previously described inhibitors **1** and **2** are included for comparative purposes. Replacement of the naphtholactam in compound **1** by the non-substituted heterocyclo-quinoline as in compound **24** reduced only 3-fold the inhibitory activity against TMPKmt. However introduction of an acetamido or an amino group at position 5 in the imidazoquinolinone has a more significant impact in the inhibitory activity, with K_i values of 5.8 and 7.1 μM as shown for compounds **25** and **26**, respectively. Interestingly, for compounds **27** and **28** where



Scheme 3. (a) Phthalic anhydride, glacial AcOH, 110 °C, 2 h; (b) $NaCNBH_3$, glacial AcOH, rt, 24 h; (c) Et_3N, triphosgene, THF, 0 °C, 1 h, then addition of methoxyamine hydrochloride, rt, 72 h; (d) PIFA, $CHCl_3$, rt, 4 h; (e) NH_2–$NH_2 \cdot H_2O$, EtOH, rt, 24 h; (f) Ac_2O, CH_2Cl_2, rt, 30 min; (g) H_2, $Pd(OH)_2/C$ (20%), EtOH, 30 °C, 2 d.

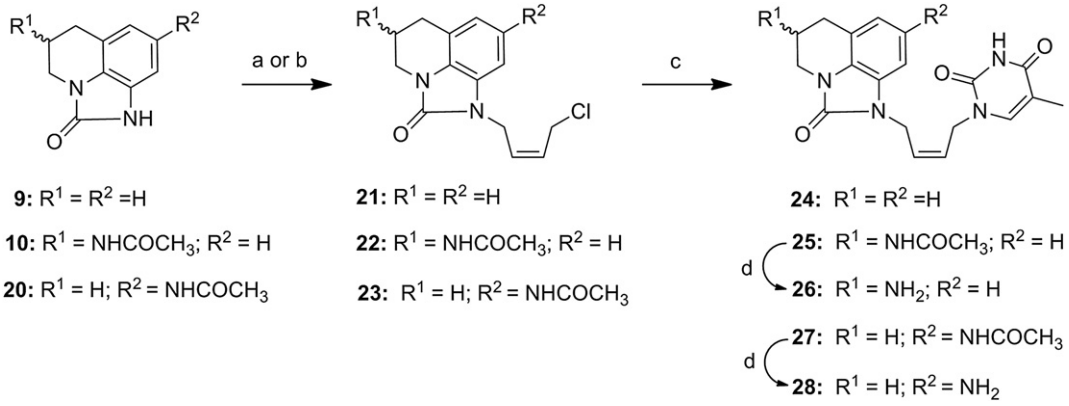
the acetamido and amino groups are incorporated at position 8 of the heterocycle, the K_i remains around 0.9 and 2.5 μM respectively. It is also noticeable that compound **31** with a 5,6-dihydro-1*H*,4*H*-1,2,5-thiadiazolo[4,3,2-*ij*]quinoline-2,2-dioxide as the distal substituent is 2-fold more active than the analogous compound in the carbonyl series (compound **24**). Finally, introduction of 5-bromouracil as the base instead of thymine as shown for compound **32** further improves the potency against TMPKmt 2-fold, providing a K_i value of 0.30 μM. Thus, compound **32** is the most inhibitory against TMPKmt of the here described compounds with a K_i value very similar to those reported for our lead compounds **1** and **2**, and 15-fold lower than the K_m for the natural substrate dTMP.

2.3. Water solubility studies

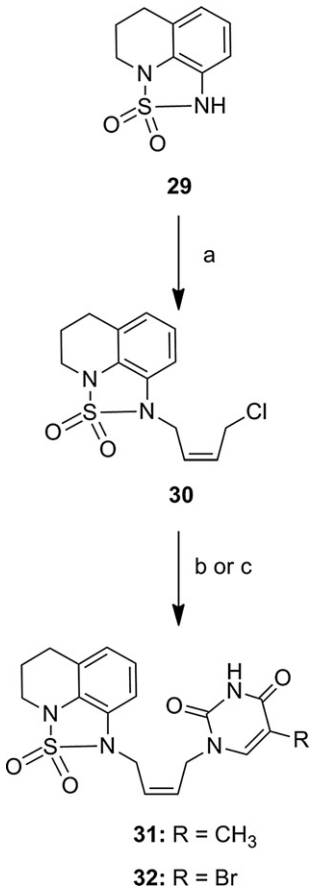
In order to determine whether breaking the planarity at the distal substituent has an impact on water solubility, as hypothesized, three of the compounds under study were incubated in PBS and analyzed by HPLC. The naphtolactam **2**, reference compound from our previous series, was hardly soluble in PBS with an estimated solubility of 0.003 mg/mL. The unsubstituted imidazoquinolinone **24** was 3-fold more soluble while the imidazoquinolinone with an amino group at position 8, compound **28**, was 200-fold more soluble than the reference compound **1**, with a solubility value of 0.64 ± 0.04 mg/mL. Thus, in our case, the incorporation of an amino group has a much more pronounced impact on solubility than breaking the planarity of the heterocycle.

2.4. Antimycobacterial evaluation

The synthesized nucleoside analogues (**24**–**28**, **31**, **32**) were evaluated for their growth inhibitory activity against *Mycobacterium bovis* BCG-Pasteur and *Mycobacterium smegmatis* mc^2155 following the colorimetric resazurin microtiter plate assay (REMA) [33]. Unfortunately no significant inhibitory activity was detected at the highest concentration tested (160 μg/mL).



Scheme 4. (a) NaH, DMF, 0 °C, 30 min, then addition of (*Z*)-1,4-dichlorobut-2-ene, 80 °C, 3h, (conditions for **9**); (b) NaOMe, DMF, rt, 15 min, then addition of (*Z*)-1,4-dichlorobut-2-ene, 80 °C, 1 h, (conditions for **10** and **20**); (c) Thymine, *N*,*O*-bistrimethylsilylacetamide, CH_3CN, 80 °C till solubilization of thymine, then addition of **21**–**23**, NaI, CH_3CN, 80 °C, 10h; (d) HCl (1.6 M), dioxane, Δ, 7 h.



Scheme 5. (a) NaH, DMF, 0 °C, 30 min, then addition of (*Z*)-1,4-dichlorobut-2-ene, 80 °C, 3 h; (b) Thymine, *N*,*O*-bistrimethylsilylacetimide, CH_3CN, 80 °C till solubilization of thymine, then addition of **30**; (c) 5-bromouracil, HMDS, $(NH_4)_2SO_4$ (cat), 150 °C, overnight, evaporation under vacuum, then addition of **30**, NaI, CH_3CN, 80 °C, 16 h; (e) HCl (1.6 M), dioxane, Δ, 7 h.

The lack of mycobacterial inhibition might be ascribed, among other factors, to an inefficient crossing of the bacterial wall so that the compounds cannot reach the target enzyme or, if they do so, the concentrations attained are not sufficient to effectively inhibit the mycobacterial growth. It should be mentioned that the complex nature of the mycobacterial wall seriously hampers the delivery of compounds inside the pathogen, and this is particularly important for compounds that have been designed to interact with an intracellular target, as here is the case. Indeed this has been and still is a major concern in the search for novel amtimycobacterial agents [34].

Table 1
Evaluation of the synthesized compounds against TMPKmt.

Compound	K_i
dTMP	4.5[a]
1	0.42 ± 0.01
2	0.27 ± 0.01
24	1.16 ± 0.08
25	5.80 ± 0.43
26	7.07 ± 0.65
27	0.93 ± 0.15
28	2.46 ± 0.64
31	0.57 ± 0.06
32	0.30 ± 0.06

[a] Km value.

3. Conclusions

New acyclic nucleoside analogues structurally related to our lead compounds **1** and **2** have been synthesized and tested against TMPKmt. The synthesis of the desired imidazoquinolinones was accomplished by a PIFA-mediated electrocyclization of *N*-methoxyureas followed by elimination of the methoxy groups by catalytic hydrogenation. This approach has allowed the synthesis of imidazoquinolinones with an acetamido group at positions 5 or 8, compounds **10** and **20**, so far unknown. These compounds can be transformed into their corresponding amino analogues by treatment with refluxing HCl. The results obtained from the evaluation against the target enzyme TMPKmt revealed that these partially saturated analogues, in particular those compounds with an unsubstituted heterocycle at the distal site (compounds **24**, **31** and **32**), behave quite similar to their aromatic analogues **1** and **2** against TMPKmt. Thus, in our hands, the 5,6-dihydro-1*H*-imidazo[4,5,1-*ij*]quinolinone and a 5,6-dihydro-1*H*,4*H*-1,2,5-thiadiazolo[4,3,2-*ij*]quinoline-2,2-dioxide behave as bioisosteres of the naphtolactam and naphthosultam rings and therefore may be useful in other areas of research where these heterocycles are present in biologically active compounds.

4. Experimental

Melting points were obtained on a Reichert–Jung Kofler apparatus and are uncorrected. Microanalyses were obtained with a Heraeus CHN-O-RAPID instrument. Electrospray mass spectra were measured on a quadrupole mass spectrometer equipped with an electrospray source (Hewlett–Packard, LC/MS HP 1100). ^1H and ^{13}C NMR spectra were recorded on a Varian INNOVA 300 operating at 299 MHz (^1H) and 75 MHz (^{13}C), and on a Varian MERCURY 400 operating at 399 MHz (^1H) and 100 MHz (^{13}C).

Analytical TLC was performed on silica gel 60 F254 (Merck) precoated plates (0.2 mm). Spots were detected under UV light (254 nm) and/or by charring with phosphomolybdic acid or ninhydrin. Separations on silica gel were performed by preparative centrifugal circular thin layer chromatography (CCTLC) on a Chromatotron (Kieselgel 60 PF254 gipshaltig (Merck)); layer thickness: 1 or 2 mm; flow rate: 4 or 8 mL min^{-1}, respectively. Flash chromatography was performed in a Horizon HPFG system (Biotage) with Flash 25 or 40 silica gel cartridges. All experiments involving water-sensitive compounds were conducted under scrupulously dry conditions.

4.1. Synthesis of differently substituted imidazoquinolinones

4.1.1. N-Methoxy-3,4-dihydroquinoline-1(2H)-carboxamide (*5*) [22]

A solution 1,2,3,4-tetrahydroquinoline (**3**) (500 mg. 3.75 mmol) and Et_3N (875 μl, 11.36 mmol) in dry THF (10 mL) was added to a solution of triphosgene (393 mg, 1.32 mmol) in dry THF (14 mL) at 0 °C. The mixture was stirred at 0 °C for 1 h. Then, methoxyamine hydrochloride (626 mg, 7.50 mmol) and Et_3N (0.87 mL, 11.36 mmol) were added. The reaction was allowed to reach room temperature and was further stirred for 4 d. The reaction was quenched by addition of H_2O and extracted with Et_2O (2 × 40 mL). The combined organic extracts were washed with brine (30 mL), dried over $MgSO_4$ and filtered. Volatiles were removed under vacuum and the residue was purified by flash column chromatography (CH_2Cl_2/Et_2O, 10:1) to yield 510 mg (66%) of **5** as brown oil. MS (ES): *m*/*z* 206 $[M]^+$; ^1H NMR ($CDCl_3$) δ: 1.92 (m, 2H, CH_2), 2.71 (t, 2H, J = 6.6 Hz, CH_2), 3.72 (t, 2H, J = 6.0 Hz, CH_2), 3.75 (s, 3H, CH_3), 7.06 (d, 1H, J = 7.1 Hz, Ar), 7.15 (m, 1H, Ar), 7.33 (d, 1H, J = 8.1 Hz, Ar), 7.79 (br s, 1H, NH).

*4.1.2. (+/−)-3-Acetamido-3,4-dihydro-N-methoxyquinoline-1 (2H)-carboxamide (**6**)*

Compound **4** (644 mg, 3.39 mmol) reacted with triphosgene (353 mg, 1.19 mmol) and methoxyamine hydrochloride (564 mg, 6.76 mmol) as described for the synthesis of **5**. The final residue was purified by flash column chromatography (CH_2Cl_2/MeOH, 35:1) to yield 813 mg (91%) of **6** as brown oil. MS (ES): *m/z* 264 $[M + 1]^+$; ^1H NMR ($CDCl_3$) δ: 1.95 (s, 3H, CH_3), 2.71 (dd, 1H, $J = 16.5$, 5.4 Hz, CH_2), 3.14 (dd, 1H, $J = 16.5$, 6.1 Hz, CH_2), 3.64 (dd, 1H, $J = 13.2$, 4.4 Hz, CH_2), 3.76 (s, 3H, OCH_3), 3.87 (dd, 1H, $J = 13.9$, 4.8 Hz, CH_2), 4.42 (m, 1H, CH), 5.70 (br s, 1H, NHAc), 7.18 (m, 3H, Ar), 7.43 (d, 1H, $J = 7.6$ Hz, Ar), 7.78 (br s, 1H, NH); ^{13}C NMR ($CDCl_3$) δ: 23.0 (CH_3), 32.9, 45.1 (CH_2), 45.5 (CH), 64.2 (CH_3), 122.9, 125.2, 126.8, 128.4, 129.6, 137.4 (Ar), 157.8, 169.9 (CO).

*4.1.3. 1-Methoxy-5,6-dihydro-1H-imidazo[4,5,1-ij]quinolin-2(4H)-one (**7**) [22]*

Bis(trifluoroacetoxy)iodobenzene (PIFA) (1.3 g, 2.94 mmol) was added to a solution of **5** (1.0 g, 5.37 mmol) in $CHCl_3$ (19 mL) at 0 °C, in small portions over 20 min. The reaction was allowed to reach room temperature and was further stirred for 1.5 h. The mixture was washed with an aqueous solution of $NaHCO_3$ (40 mL) and back-extracting the aqueous phase with diethyl ether (3 × 40 mL). The combined organic extracts were dried over $MgSO_4$, filtered and volatiles removed under vacuum. The residue was purified by flash column chromatography HPFC (CH_2Cl_2/MeOH, 20:1) to yield 390 mg (78%) of **7** as brown oil. MS (ES): *m/z* 205 $[M + 1]^+$; ^1H NMR ($CDCl_3$) δ: 2.12 (m, 2H, CH_2), 2.85 (t, 2H, $J = 6.1$ Hz, CH_2), 3.85 (t, 2H, $J = 5.9$ Hz, CH_2), 4.04 (s, 3H, CH_3), 6.86–7.04 (m, 3H, Ar).

*4.1.4. (+/−)-5-Acetamido-1-methoxy-5,6-dihydro-1H-imidazo [4,5,1-ij]quinolin-2(4H)one (**8**)*

Compound **6** (815 mg, 3.1 mmol) reacted with PIFA (1.6 g, 3.7 mmol) as described for the synthesis of **7**. The final residue was purified by flash column chromatography (CH_2Cl_2/MeOH, 10:1) to yield 729 mg (90%) of **8** as colourless oil. MS (ES): *m/z* 262 $[M + 1]^+$; ^1H NMR ($CDCl_3$) δ: 1.93 (s, 3H, CH_3), 2.91 (dd, 1H, $J = 16.6$, 4.5 Hz, CH_2), 3.11 (dd, 1H, $J = 16.5$, 4.3 Hz, CH_2), 3.80 (dd, 1H, $J = 12.7$, 3.3 Hz, CH_2), 4.00 (dd, 1H, $J = 12.6$, 4.0 Hz, CH_2), 4.11 (s, 3H, OCH_3), 4.80 (m, 1H, CH), 5.49 (br s, 1H, NHAc), 6.89–7.10 (m, 3H, Ar); ^{13}C RMN ($CDCl_3$) δ: 23.6 (CH_3), 30.1, 42.4 (CH_2), 43.6 (CH), 66.1 (OCH_3), 105.5, 116.3, 121.2, 122.4, 125.0, 125.5, 125.8 (Ar), 146.5, 169.9 (CO).

*4.1.5. 5,6-Dihydro-1H-imidazo[4,5,1-ij]quinolin-2(4H)-one (**9**) [22]*

A solution of **7** (187 mg, 0.92 mmol) in EtOH (9 mL) was placed in a Parr hydrogenation bottle and 20% $Pd(OH)_2/C$ (62 mg) was added. The mixture was hydrogenated at 40 psi at 30 °C for 1 day. The reaction mixture was filtered through Celite and the filtrate evaporated. The residue was purified by flash column chromatography HPFC (CH_2Cl_2/MeOH, 40:1) to yield 132 mg (83%) of **9** as a white solid. Mp: 212–213 °C (CH_2Cl_2:MeOH); Mp: 213–214 °C (lit) [35]; MS (ES): *m/z* 175 $[M + 1]^+$; ^1H NMR ($CDCl_3$) δ: 2.11 (m, 2H, CH_2), 2.83 (t, 2H, $J = 6.0$ Hz, CH_2), 3.96 (t, 2H, $J = 5.5$ Hz, CH_2), 6.81–6.96 (m, 3H, Ar), 10.82 (br s, 1H, NH).

*4.1.6. (+/−)-5-Acetamido-5,6-dihydro-1H-imidazo[4,5,1-ij] quinolin-2(4H)-one (**10**)*

A solution of **8** (610 mg, 2.34 mmol) in EtOH (22 mL) was placed in a Parr hydrogenation bottle and 20% $Pd(OH)_2/C$ (221 mg) was added. The mixture was hydrogenated at 40 psi at 30 °C for 1 day. The reaction mixture was filtered through Celite and the filtrate evaporated. The residue was purified by flash column chromatography HPFC (CH_2Cl_2/MeOH, 30:1) to yield 351 mg (65%) of **10** as colourless oil. MS (ES): *m/z* 232 $[M + 1]^+$; ^1H NMR (DMSO − d_6) δ: 2.07 (s, 3H, CH_3), 2.76 (dd, 1H, $J = 16.2$, 3.7 Hz, CH_2), 2.95 (dd, 1H, $J = 16.2$, 4.4 Hz, CH_2), 3.51 (dd, 1H, $J = 12.1$, 6.3 Hz, CH_2), 3.78 (dd, 1H, $J = 12.2$, 4.0 Hz, CH_2), 4.25 (m, 1H, CH), 6.76–6.89 (m, 3H, Ar), 8.05 (br s, 1H, NHAc), 10.70 (br s, 1H, NH); ^{13}C NMR (DMSO − d_6) δ: 22.6 (CH_3), 29.2, 42.1 (CH_2), 42.8 (CH), 106.5, 116.5, 119.2, 120.6, 126.5, 127.0 (Ar), 153.7, 169.3 (CO).

*4.1.7. 6-Acetamido-3,4-dihydro-N-methoxyquinoline-1(2H)-carboxamide (**12**)*

Compound **11** [36] (203 mg, 1.07 mmol) reacted with triphosgene (112 mg, 0.38 mmol) and methoxyamine hydrochloride (179 mg, 2.14 mmol) as described for the synthesis of **5**. The final residue was purified by flash column chromatography (CH_2Cl_2/MeOH, 30:1) to yield 160 mg (57%) of **12** as a white solid. Mp: 127–129 °C (hexane/CH_2Cl_2). MS (ES): *m/z* 264 $[M + 1]^+$; ^1H NMR ($CDCl_3$) δ: 1.92 (m, 2H, CH_2) 2.17 (s, 3H, CH_3), 2.70 (t, 2H, $J = 6.6$ Hz, CH_2), 3.71 (t, 2H, $J = 6.3$ Hz, CH_2), 3.76 (s, 3H, OCH_3), 7.19 (dd, 1H, $J = 8.7$, 2.2 Hz, Ar), 7.30 (d, 1H, $J = 8.7$ Hz, Ar), 7.33 (br s, 1H, NH), 7.39 (d, 1H, $J = 1.9$ Hz, Ar), 7.79 (br s, 1H, NH); ^{13}C NMR ($CDCl_3$) δ: 22.9 (CH_3), 24.0, 26.9 41.9 (CH_2), 64.7 (OCH_3), 114.2, 120.1, 121.7, 122.6, 127.7 (Ar), 141.8, 168.5 (CO). Anal. Calc. for $C_{13}H_{17}N_3O_3$: C: 59.30; H: 6.51; N: 15.96; found: C: 59.45; H: 6.38; N: 15.83.

*4.1.8. 8-Acetamido-5,6-dihydro-1-methoxy-1H-imidazo[4,5,1-ij] quinolin-2(4H)-one (**13**)*

Compound **12** (160 mg, 0.61 mmol) reacted with PIFA (313 mg, 0.73 mmol) as described for the synthesis of **7**. The final residue was purified by flash column chromatography (CH_2Cl_2/MeOH, 10:1) to yield 25 mg (15%) of **13** as a white solid. Mp: 151–153 °C (hexane/CH_2Cl_2). MS (ES): *m/z* 262 $[M + 1]^+$; ^1H NMR ($CDCl_3$) δ: 2.10 (m, 2H, CH_2), 2.18 (s, 3H, CH_3), 2.81 (t, 2H, $J = 6.0$ Hz, CH_2), 3.83 (m, 2H, CH_2), 4.06 (s, 3H, OCH_3), 6.89 (s, 1H, Ar), 7.21 (br s, 1H, NH), 7.27 (d, 1H, $J = 1.6$ Hz, Ar); ^{13}C NMR ($CDCl_3$) δ: 21.7 (CH_3), 23.6, 24.3, 38.8 (CH_2), 64.7 (OCH_3), 98.4, 113.0, 119.6, 119.8, 124.5, 132.4 (Ar), 150.2, 168.6 (CO). Anal. Calc. for $C_{13}H_{15}N_3O_3$: C: 59.76; H: 5.79; N: 16.08; found: C: 59.60; H: 5.88; N: 16.01.

*4.1.9. 2-(Quinolin-6-yl)isoindolin-1,3-dione (**15**) [37]*

A suspension of phthalic anhydride (955 mg, 6.44 mmol) and 6-aminoquinoline (**14**) (1.0 g, 5.37 mmol) in AcOH (80 mL) was stirred at 120 °C for 2 h in presence of 4 Å activated molecular sieves. Volatiles were removed and the residue was purified by flash column chromatography HPFC (hexane/ethyl acetate, 3:1) to yield 1.7 g (98%) of **15** as a white solid Mp: 210–211 °C (hexane/ethyl acetate); Mp: 213–214 °C (lit[31]); MS (ES): *m/z* 275 $[M + 1]^+$; ^1H NMR (DMSO − d_6) δ: 7.62 (dd, 1H, $J = 8.3$, 4.2 Hz, Ar), 7.86 (dd, 1H, $J = 8.9$, 2.2 Hz, Ar), 7.97 (m, 4H, Ar), 8.10 (d, 1H, $J = 2.1$ Hz, Ar), 8.16 (d, 1H, $J = 8.9$ Hz, Ar), 8.46 (d, 1H, $J = 7.9$ Hz, Ar), 8.99 (dd, 1H, $J = 4.0$, 1.4 Hz, Ar); ^{13}C NMR (DMSO − d_6) δ: 122.1, 123.5, 126.2, 127.6, 129.0, 129.3, 129.9, 131.5, 134.9, 136.6, 146.2, 151.1 (Ar), 167.0 (CO).

*4.1.10. 2-(1,2,3,4-Tetrahydroquinolin-6-yl)isoindolin-1,3-dione (**16**)*

Sodium cyanoborohydride (691 mg, 11.01 mmol) was added to a stirred solution of **15** (722 mg, 2.62 mmol) in acetic acid (21 mL) at 0 °C in small portions over a 5 min period. The reaction was allowed to reach room temperature and was further stirred for 1 day. Then, $Na_2SO_4 \cdot 10H_2O$ (300 mg) was added and the reaction mixture was stirred at room temperature for 1 h. The mixture was filtered through Celite and the filtrate evaporated. The residue was diluted with CH_2Cl_2 (40 mL), washed with a saturated solution of $NaHCO_3$ (20 mL), and the aqueous layer was further extracted with CH_2Cl_2 (30 mL). The organic layers were combined, dried over $MgSO_4$, filtered, and evaporated. The residue was purified by flash column chromatography HPFC (hexane/ethyl acetate, 4:1) to yield 589 mg (81%) of **16** as an orange solid. Mp: 142–143 °C (hexane/ethyl acetate); MS (ES): *m/z* 279 $[M + 1]^+$; ^1H NMR ($CDCl_3$) δ: 1.93 (m, 2H,

CH_2), 2.79 (t, 2H, $J = 6.3$ Hz, CH_2), 3.31 (t, 2H, $J = 5.4$ Hz, CH_2), 4.05 (br s, 1H, NH), 6.52 (d, 1H, $J = 9.1$ Hz, Ar), 6.93 (m, 2H, Ar), 7.78 (m, 2H, Ar), 7.91 (m, 2H, Ar); ^{13}C NMR ($CDCl_3$) δ: 21.5, 26.9, 41.7 (CH_2), 114.0, 119.8, 121.5, 123.4, 125.4, 127.8, 131.8, 134.0, 144.9 (Ar), 168.0 (CO). Anal. Calc. for $C_{17}H_{14}N_2O_2$: C: 73.37; H: 5.07; N: 10.07; found: C: 73.12; H: 5.25; N: 10.01.

4.1.11. 6-(1,3-Dioxoisoindolin-2-yl)-N-methoxy-3,4-dihydroquinoline-1(2H)-carboxamide (**17**)

Compound **16** (308 mg, 1.11 mmol) reacted with triphosgene (116 mg, 0.39 mmol) and methoxyamine hydrochloride (185 mg, 2.22 mmol) as described for the synthesis of **5**. The final residue was purified by flash column chromatography HPFC (hexane/ethyl acetate, 2:1) to yield 289 mg (74%) of **17** as a white solid. Mp: 176–177 °C (hexane/ethyl acetate); MS (ES): m/z 351 $[M]^+$; ^{1}H NMR ($CDCl_3$) δ: 1.98 (m, 2H, CH_2), 2.81 (t, 2H, $J = 6.6$ Hz, CH_2), 3.78 (t, 2H, $J = 6.5$ Hz, CH_2), 3.80 (s, 3H, OCH_3), 7.24 (d, 2H, $J = 7.9$ Hz, Ar), 7.53 (d, 1H, $J = 8.1$ Hz, Ar), 7.76 (br s, 1H, NH), 7.81 (m, 2H, Ar), 7.96 (m, 2H, Ar); ^{13}C NMR ($CDCl_3$) δ: 23.3, 27.0, 43.7 (CH_2), 64.6 (CH_3), 123.7, 123.8, 124.9, 127.4, 127.9, 131.7, 132.9, 134.5, 137.7 (Ar), 157.5, 167.3 (CO). Anal. Calc. for $C_{19}H_{17}N_3O_4$: C: 64.95; H: 4.88; N: 11.96; found: C: 64.81; H: 4.95; N: 11.83.

4.1.12. 8-(1,3-Dioxoisoindolin-2-yl)-5,6-dihydro-1-methoxy-1H-imidazo[4,5,1-ij]quinolin-2(4H)-one (**18**)

Compound **17** (266 mg, 0.75 mmol) reacted with PIFA (390 mg, 0.91 mmol) as described for the synthesis of **7**. The final residue was purified by flash column chromatography (hexane/ethyl acetate, 2:1) to yield 190 mg (73%) of **18** as a yellow solid. Mp: 215–216 °C (hexane/ethyl acetate); MS (ES): m/z 350 $[M + 1]^+$; ^{1}H NMR ($CDCl_3$) δ: 2.16 (m, 2H, CH_2), 2.90 (t, 2H, $J = 5.9$ Hz, CH_2), 3.88 (t, 2H, $J = 5.7$ Hz, CH_2), 4.09 (s, 3H, OCH_3), 6.90 (s, 1H, Ar), 6.99 (s, 1H, Ar), 7.80 (m, 2H, Ar), 7.96 (m, 2H, Ar); ^{13}C NMR ($CDCl_3$) δ: 23.3, 27.0, 43.7 (CH_2), 64.6 (CH_3), 123.7, 123.8, 124.9, 127.4, 127.9, 131.7, 132.9, 134.5, 137.7 (Ar), 157.5, 167.3 (CO). Anal. Calc. for $C_{19}H_{15}N_3O_4$: C: 65.32; H: 4.33; N: 12.03; found: C: 65.38; H: 4.21; N: 11.91.

4.1.13. 8-Amino-5,6-dihydro-1-methoxy-1H-imidazo[4,5,1-ij]quinolin-2(4H)-one (**19**)

A suspension containing **18** (294 mg, 0.84 mmol) in EtOH (28 mL) was treated with NH_2–$NH_2 \cdot H_2O$ (81 μl, 0.70 mmol) at room temperature and stirred for 1 day. The mixture was filtered and the filtrate evaporated. The residue was purified by an SCX cartridge washing with CH_2Cl_2 and then eluting with 2M NH_3 in MeOH, to yield 182 mg (99%) of **19** as a brown solid that was used as such for the next step. MS (ES): m/z 220 $[M + 1]^+$; ^{1}H NMR ($CDCl_3$) δ: 2.05 (m, 2H, CH_2), 2.71 (t, 2H, $J = 6.0$ Hz, CH_2), 3.60 (br s, 2H, NH_2), 3.77 (t, 2H, $J = 5.8$ Hz, CH_2), 4.01 (s, 3H, CH_3), 6.24 (s, 1H, Ar), 6.33 (s, 1H, Ar).

4.1.14. Acetylation of **19**. Synthesis of **13**

A solution of **19** (1.0 g, 6.93 mmol) in dry CH_2Cl_2 (2 mL) and acetic anhydride (105 μL, 1.12 mmol) was stirred at room temperature for 30 min. The reaction was quenched by addition of water and volatiles were removed. The residue was purified by flash column chromatography (hexane/Ethyl acetate, 2:1) to yield 179 mg (73%) of **13** as a white solid. (See analytical and spectroscopic data described in this paper for compound **13**).

4.1.15. 8-Acetamido-5,6-dihydro-1H-imidazo[4,5,1-ij]quinolin-2(4H)-one (**20**)

A suspension containing **13** (176 mg, 0.67 mmol) and 20% Pd $(OH)_2$/C (117 mg) in MeOH (10 mL) was placed in a Parr hydrogenation bottle. The mixture was hydrogenated at 40 psi at 30 °C for 1 day. The reaction mixture was filtered through Celite and the filtrate evaporated. The residue was purified by flash column chromatography (CH_2Cl_2/MeOH, 30:1) to yield 130 mg (84%) of **20** as a white solid. Mp: 285–287 °C (CH_2Cl_2/MeOH); MS (ES): m/z 232 $[M + 1]^+$; ^{1}H NMR ($CDCl_3$) δ: 1.96 (m, 2H, CH_2) 1.99 (s, 3H, CH_3), 2.70 (t, 2H, $J = 5.9$ Hz, CH_2), 3.65 (t, 2H, $J = 5.9$ Hz, CH_2), 6.89 (s, 1H, Ar), 7.29 (s, 1H, Ar), 9.71 (br s, 1H, NH), 10.51 (br s, 1H, NH); ^{13}C NMR ($CDCl_3$) δ: 23.9 (CH_3), 21.6, 23.1, 38.0 (CH_2), 98.7, 110.2, 118.5, 123.5, 126.2, 133.0 (Ar), 153.6, 167.6 (CO). Anal. Calc. for $C_{12}H_{13}N_3O_2$: C: 62.33; H: 5.67; N: 18.17; found: C: 62.20; H: 5.92; N: 18.03.

4.1.16. (Z)-1-(4-Chlorobut-2-enyl)-5,6-dihydro-1H-imidazo[4,5,1-ij]quinolin-2(4H)-one (**21**)

To a solution of 5,6-dihydro-1*H*-imidazo[4,5,1-*ij*]quinoline-2(4*H*)-one (**9**) (100 mg, 0.57 mmol) in dry DMF (0.6 mL) at 0 °C, NaH (60% oil suspension) (34 mg, 0.85 mmol) was added. The mixture was stirred at 0 °C for 30 min. Then, the (*Z*)-1,4-dichloro-2-butene (102 μl, 0.97 mmol) was added and the mixture was heated at 80 °C for 5 h. After reaching rt, the reaction was quenched by addition of a NH_4Cl solution and evaporated to dryness. The final residue was purified by flash column chromatography (hexane/ethyl acetate, 3:1) to yield 100 mg (67%) of **21** as yellow oil. MS (ES): m/z 263 $[M + 1]^+$,with a Cl isotopic pattern; ^{1}H NMR ($CDCl_3$) δ: 2.11 (m, 2H, CH_2), 2.85 (t, 2H, $J = 6.1$ Hz, CH_2), 3.86 (t, 2H, $J = 5.6$ Hz, CH_2), 4.29 (d, 2H, $J = 8.0$ Hz, CH_2), 4.58 (d, 2H, $J = 6.8$ Hz, CH_2), 5.71 (m, 1H, CH=CH), 5.84 (m, 1H, CH=CH), 6.82–7.00 (m, 3H, Ar); ^{13}C NMR ($CDCl_3$) δ: 21.8, 23.8, 37.6, 39.0, 42.1 (CH_2), 127.0, 128.4 (CH=CH), 104.8, 117.6, 119.5, 120.7, 126.1, 126.8 (Ar), 153.0 (CO).

4.1.17. (+/−)(Z)-5-Acetamido-1-(4-chlorobut-2-enyl)-5,6-dihydro-1H-imidazo[4,5,1-ij]quinolin-2(4H)-one (**22**)

To a solution of **10** (330 mg, 1.43 mmol) in dry DMF (2 mL), NaOMe (77 mg, 1.43 mmol) was added and the mixture was stirred at rt for 15 min. Then, the (*Z*)-1,4-dichloro-2-butene (525 μl, 4.99 mmol) was added and the mixture was stirred at rt for 1 h. The reaction was quenched by addition of a NH_4Cl solution and evaporated to dryness. The residue was purified by flash chromatography HPFC (CH_2Cl_2/MeOH, 30:1) to yield 392 mg (86%) of **22** as a yellow oil. MS (ES): m/z 320 $[M + 1]^+$, with a Cl isotopic pattern; ^{1}H NMR ($CDCl_3$) δ: 1.78 (s, 3H, CH_3), 2.78 (dd, 1H, $J = 16.3$, 6.7 Hz, CH_2), 2.97 (dd, 1H, $J = 16.3$, 3.8 Hz, CH_2), 3.59 (dd, 1H, $J = 12.2$, 6.1 Hz, CH_2), 3.84 (dd, 1H, $J = 12.1$, 3.8 Hz, CH_2), 4.28 (m, 1H, CH), 4.47 (d, 2H, $J = 7.9$ Hz, CH_2), 4.55 (d, 2H, $J = 6.2$ Hz, CH_2), 5.64 (m, 1H, CH=CH), 5.81 (m, 1H, CH=CH), 6.85–6.96 (m, 3H, Ar), 8.05 (br s, 1H, NHAc); ^{13}C NMR ($CDCl_3$) δ: 23.7 (CH_3), 30.4, 38.2, 38.9, 44.0 (CH_2), 42.7 (CH), 128.9, 129.6 (CH=CH), 106.8, 116.3, 121.1, 122.2, 126.5, 127.0 (Ar), 153.7, 169.3 (CO).

4.1.18. (Z)-8-Acetamido-1-(4-chlorobut-2-enyl)-5,6-dihydro-1H-imidazo[4,5,1-ij]quinolin-2(4H)-one (**23**)

Compound **20** (115 mg, 0.54 mmol) reacted with (*Z*)-1,4-dichloro-2-butene (199 μL, 1.89 mmol) as described for the synthesis of **22**. The final residue was purified by flash chromatography HPFC (CH_2Cl_2/MeOH, 30:1) to yield 112 mg (65%) of **23** as colourless oil. MS (ES): m/z 319 $[M]^+$, with a Cl isotopic pattern; ^{1}H NMR ($CDCl_3$) δ: 2.10 (m, 2H, CH_2), 2.17 (s, 3H, CH_3), 2.81 (t, 2H, $J = 6.0$ Hz, CH_2), 3.84 (t, 2H, $J = 5.8$ Hz, CH_2), 4.30 (d, 2H, $J = 7.9$ Hz, CH_2), 4.55 (d, 2H, $J = 6.8$ Hz, CH_2), 5.70 (m, 1H, CH=CH), 5.87 (m, 1H, CH=CH), 6.87 (s, 1H, Ar), 7.16 (br s, 1H, NH), 7.44 (s, 1H, Ar); ^{13}C NMR ($CDCl_3$) δ: 23.8 (CH_3), 21.9, 24.5, 38.6, 39.0 (CH_2), 128.5, 129.1 (CH=CH), 99.7, 112.9, 119.5, 127.3, 127.4, 131.7 (Ar), 153.5, 168.3 (CO).

4.2. General procedure for the reaction of compounds **21**–**23** with thymine

To a suspension of thymine (214 mg, 1.70 mmol) in dry CH_3CN (6 mL), *N*,*O*-bis(trimethylsilyl)acetamide (0.6 mL) was added and

the mixture was heated at 80 °C until total solubilization of thymine. Then, NaI (73 mg, 0.5 mmol) and a solution of the corresponding halide **21**–**23** (1.0 mmol) in CH_3CN (4 mL) were added. The reaction was kept at 80 °C for 8 h. The mixture was filtered and the isolated solid contained the target compound. The filtrate was treated with a solution of sodium bisulphite (10 mL) and extracted with ethyl acetate (25 mL). The organic layer was dried over $MgSO_4$, filtered, and evaporated. The residue obtained was purified by column chromatography. The yields given include the amount obtained from the isolated solid together with that obtained after column chromatography.

4.2.1. (Z)-1-(4-(Thymin-1-yl)but-2-enyl)-5,6-dihydro-1H-imidazo[4,5,1-ij]quinolin-2(4H)-one (**24**)

According to the general procedure, thymine (91 mg, 0.72 mmol) reacted with the chloride **21** (95 mg, 0.36 mmol). After purification by flash column chromatography HPFC (CH_2Cl_2/MeOH, 40:1), 110 mg (87%) of **24** were obtained as a white solid. Mp: 211–212 °C (CH_2Cl_2/MeOH); MS (APCI): *m*/*z* 353 $[M + 1]^+$; ^1H NMR ($CDCl_3$) δ: 1.92 (s, 3H, 5-CH_3), 2.13 (m, 2H, CH_2), 2.87 (t, 2H, $J = 6.1$ Hz, CH_2), 3.87 (t, 2H, $J = 5.9$ Hz, CH_2), 4.60 (m, 4H, CH_2), 5.67 (m, 1H, CH=CH), 5.81 (m, 1H, CH=CH), 6.81 (m, 3H, Ar), 7.36 (s, 1H, H-6), 8.26 (br s, 1H, NH). ^{13}C NMR ($CDCl_3$) δ: 12.3 (5-CH_3), 21.8, 23.8, 37.6, 39.0, 44.2 (CH_2), 110.0 (C5), 127.3, 128.4 (CH=CH), 105.2, 119.6, 119.8, 120.9, 126.4, 127.1 (Ar), 140.4 (C6), 150.9 (C2), 153.1 (CO), 164.2 (C4). Anal. Calc. for $C_{19}H_{20}N_4O_3$: C: 64.76; H: 5.72; N: 15.90; found: C: 64.71; H: 6.10; N: 15.72.

4.2.2. (+/−)(Z)-5-Acetamido-1-(4-(thymin-1-yl)but-2-enyl)-5,6-dihydro-1H-imidazo[4,5,1-ij]quinolin-2(4H)-one (**25**)

According to the general procedure, thymine (154 mg, 1.22 mmol) reacted with the chloride **22** (229 mg, 0.72 mmol). After purification by column chromatography flash HPFC (CH_2Cl_2/MeOH, 10:1) 241 mg (82%) of **25** were obtained as a yellow amorphous solid. MS (ES): *m*/*z* 410 $[M + 1]^+$; ^1H NMR (DMSO − d_6) δ: 1.75 (s, 3H, 5-CH_3), 1.78 (s, 3H, CH_3), 2.79 (dd, 1H, $J = 16.1$, 6.4 Hz, CH_2), 2.97 (dd, 1H, $J = 16.8$, 4.3 Hz, CH_2), 3.60 (dd, 1H, $J = 12.2$, 6.3 Hz, CH_2), 3.85 (dd, 1H, $J = 12.2$, 3.9 Hz, CH_2), 4.29 (m, 1H, CH), 4.49 (d, 2H, $J = 5.2$ Hz, CH_2), 4.61 (d, 2H, $J = 4.9$ Hz, CH_2), 5.61 (m, 2H, CH=CH), 6.85 (d, 1H, $J = 7.5$ Hz, Ar), 6.93 (t, 1H, $J = 7.5$ Hz, Ar), 7.02 (d, 1H, $J = 7.4$ Hz, Ar), 7.59 (s, 1H, H-6), 8.06 (d, 1H, $J = 6.9$ Hz, NHAc), 11.30 (br s, 1H, NH); ^{13}C NMR (DMSO − d_6) δ: 12.5 (5-CH_3), 22.8 (CH_3), 29.5, 38.1, 43.1, 44.5 (CH_2), 42.8 (CH), 127.8, 128.9 (CH=CH), 109.5 (C5), 106.6, 117.4, 120.5, 121.6, 125.8, 127.4 (Ar), 141.6 (C6), 151.3, 153.1 (C2, CO), 164.9 (C4), 170.6 ($COCH_3$). Anal. Calc. for $C_{21}H_{23}N_5O_4 \cdot H_2O$: C: 59.01; H: 5.90; N: 16.38; found: C: 58.72; H: 6.25; N: 16.06.

4.2.3. (+/−)(Z)-5-Amino-1-(4-(thymin-1-yl)but-2-enyl)-5,6-dihydro-1H-imidazo[4,5,1-ij]quinolin-2(4H)-one (**26**)

A solution of **25** (40 mg, 0.12 mmol) in a mixture of dioxane (4 mL) and 1.6 M of HCl (1 mL) was refluxed for 7 h. After cooling, the mixture was filtered and the filtrated was treated with a solution of 30% NH_3 (5 mL) and CH_2Cl_2 (30 mL). The organic phase was dried over $MgSO_4$, filtered, and evaporated. The residue obtained was purified by flash column chromatography HPFC (CH_2Cl_2/MeOH, 10:1) to yield 37 mg (83%) of **26** as a white amorphous solid. MS (ES): *m*/*z* 377 $[M + 1]^+$; ^1H NMR (DMSO − d_6) δ: 1.75 (s, 3H, 5-CH_3), 2.90 (dd, 1H, $J = 16.8$, 5.8 Hz, CH_2), 2.97 (dd, 1H, $J = 16.8$, 4.3 Hz, CH_2), 3.96 (m, 2H, CH_2), 4.00 (m, 1H, CH_2), 4.49 (d, 2H, $J = 5.2$ Hz, CH_2), 4.62 (d, 2H, $J = 4.9$ Hz, CH_2), 5.60 (m, 2H, CH=CH), 6.89 (d, 1H, $J = 7.5$ Hz, Ar), 6.97 (t, 1H, $J = 7.6$ Hz, Ar), 7.05 (d, 1H, $J = 7.6$ Hz, Ar), 7.60 (s, 1H, H-6), 11.30 (br s, 1H, NH); ^{13}C NMR (DMSO − d_6) δ: 11.9 (5-CH_3), 28.9, 37.7, 41.9, 44.0 (CH_2), 43.9 (CH), 108.9 (C5), 127.6, 128.5 (CH=CH), 106.3, 115.3, 119.7, 121.1, 125.4, 127.2 (Ar), 141.0 (C6), 150.8, 152.4 (C2, CO), 164.2 (C4). Anal. Calc. for $C_{19}H_{20}N_5O_3 \cdot 2H_2O$: C: 56.57; H: 6.25; N: 17.36.; found: C: 56.43; H: 6.40; N: 17.10.

4.2.4. (Z)-8-Acetamido-1-(4-(thymin-1-yl)but-2-enyl)-5,6-dihydro-1H-imidazo[4,5,1-ij]quinolin-2(4H)-one (**27**)

According to the general procedure, thymine (46 mg, 0.36 mmol) reacted with **23** (77 mg, 0.24 mmol). The reaction was allowed to reach rt and filtered to afford 95 mg (96%) of **27** as a yellow solid. Mp: 288–289 °C (CH_3CN); MS (ES): *m*/*z* 410 $[M + 1]^+$; ^1H NMR ($CDCl_3$) δ: 1.76 (s, 3H, 5-CH_3), 2.00 (s, 3H, CH_3), 2.05 (m, 2H, CH_2), 2.74 (t, 2H, $J = 5.9$ Hz, CH_2), 3.72 (t, 2H, $J = 5.5$ Hz, CH_2), 4.50 (t, 2H, $J = 4.8$ Hz, CH_2), 4.56 (t, 2H, $J = 4.5$ Hz, CH_2), 5.61 (m, 2H, CH=CH), 6.96 (s, 1H, H-6), 7.34 (s, 1H, Ar), 7.58 (s, 1H, Ar), 9.80 (br s, 1H, NH), 11.30 (br s, 1H, NH); ^{13}C NMR ($CDCl_3$) δ: 11.8 (5-CH_3), 23.8 (CH_3), 21.5, 23.1, 37.4, 43.7 (CH_2), 108.8(C5),128.3, 131.2 (CH=CH), 98.1, 110.0, 118.9, 122.1, 126.6, 127.7 (Ar), 150.8 (C2), 152.4 (CO), 164.2 (C4), 167.6 (CO). Anal. Calc. for $C_{21}H_{23}N_5O_4$: C: 61.60; H: 5.66; N: 17.10; found: C: 61.54; H: 5.60; N: 17.08.

4.2.5. (Z)-8-Amino-1-(4-(thymin-1-yl)but-2-enyl)-5,6-dihydro-1H-imidazo[4,5,1-ij]quinolin-2(4H)-one (**28**)

A solution of **27** (90 mg, 0.22 mmol) in a mixture of dioxane (9 mL) and 1.6 M of HCl (2 mL) was refluxed for 7 h. After cooling, the mixture was filtered and the filtrated was treated with a solution 30% NH_3 (5 mL) and extracted with CH_2Cl_2 (30 mL). The organic phase was dried over $MgSO_4$, filtered, and evaporated. The residue obtained was purified by CCTLC in the Chromatotron (CH_2Cl_2/Acetone, 1:2) to yield 71 mg (88%) of **28** as a light brown solid. Mp: 247–249 °C. MS (ES): *m*/*z* 368 $[M + 1]^+$; ^1H NMR (DMSO − d_6) δ: 1.76 (s, 3H, 5-CH_3), 2.01 (t, 2H, $J = 5.0$ Hz, CH_2), 2.80 (t, 2H, $J = 5.0$ Hz, CH_2), 3.75 (t, 2H, $J = 5.0$ Hz, CH_2), 4.51 (d, 2H, $J = 4.7$ Hz, CH_2), 4.64 (d, 2H, $J = 4.5$ Hz, CH_2), 5.62 (m, 2H, CH=CH), 6.72 (s, 1H, H-6), 6.84 (s, 1H, Ar), 7.61 (s, 1H, Ar), 9.99 (br s, 2H, NH_2), 11.28 (br s, 1H, NH); ^{13}C NMR (DMSO − d_6) δ: 12.6 (5-CH_3), 21.8, 21.9, 23.7, 38.4, 44.6 (CH_2), 109.6 (C-5), 101.3, 101.4, 114.3, 120.7, 128.0, 128.7, 128.8 (Ar, CH=CH), 141.6 (C-6), 151.5 (C-2), 153.1 (CO), 164.9 (C-4), 167.6 (CO). Anal. Calc. for $C_{19}H_{21}N_5O_3$: C: 62.11; H: 5.76; N: 19.06; found: C: 62.03; H: 5.69; N: 18.98.

4.3. Synthesis of 5,6-dihydro-1H,4H-1,2,5-thiadiazolo[4,3,2-ij]quinolin-2,2-dioxide derivatives

4.3.1. 1-[(Z)-4-Chlorobut-2-enyl]-5,6-dihydro-1H,4H-1,2,5-thiadiazolo[4,3,2-ij]quinolin-2,2-dioxide (**30**)

Compound **29** [26] (179 mg, 0.85 mmol) reacted with (*Z*)-1,4-dichloro-2-butene (0.15 mL, 1.45 mmol) for 4 h, as described for the synthesis of **21**. The final residue was purified by flash column chromatography (hexane/ethyl acetate, 2:1) to yield 116 mg (46%) of **30** as colourless oil. MS (ES, positive mode): *m*/*z* 229 $[M + 1]^+$, with a Cl isotopic pattern; ^1H NMR ($CDCl_3$) δ: 2.18 (m, 2H, CH_2), 2.76 (t, 2H, $J = 6.2$ Hz, CH_2), 3.68 (t, 2H, $J = 5.7$ Hz, CH_2), 4.22 (d, 2H, $J = 7.7$ Hz, CH_2), 4.39 (d, 2H, $J = 6.5$ Hz, CH_2), 5.81 (m, 1H, CH=CH), 5.91 (m, 1H, CH=CH), 6.59–6.89 (m, 3H, Ar); ^{13}C NMR ($CDCl_3$) δ: 21.7, 23.8, 38.7, 40.0, 41.2 (CH_2), 128.9, 130.2 (CH=CH), 106.9, 120.4, 121.6, 121.9, 126.6, 128.4 (Ar).

4.3.2. 1-[(Z)-(4-(Thymin-1-yl)but-2-enyl)]-5,6-dihydro-1H,4H-1,2,5-thiadiazolo[4,3,2-ij]quinolin-2,2-dioxide (**31**)

The halide **30** (110 mg, 0.36 mmol) was made to react with thymine (79 mg, 0.63 mmol) as already described in the general procedure. The final residue was purified by flash column chromatography HPFC (CH_2Cl_2/MeOH, 40:1) to yield 110 mg (79%) of **31** as a yellow solid. Mp: 289–290 °C (CH_2Cl_2/MeOH); MS (ES): *m*/*z* 389 $[M + 1]^+$; ^1H NMR (DMSO − d_6) δ: 1.74 (s, 3H, 5-CH_3), 2.05 (m, 2H, CH_2), 2.71 (t, 2H, $J = 6.1$ Hz, CH_2), 3.58 (t, 2H, $J = 5.4$ Hz, CH_2),

4.43 (d, 2H, $J = 4.3$ Hz, CH_2), 4.55 (d, 2H, $J = 3.9$ Hz, CH_2), 5.66 (m, 2H, CH=CH), 6.77–6.88 (m, 3H, Ar), 7.51 (s, 1H, H-6), 11.30 (br s, 1H, NH); ^{13}C NMR (DMSO – d_6) δ: 12.5 (5-CH_3), 21.4, 23.3, 38.7, 40.3, 44.6 (CH_2), 109.5 (C5), 107.6, 121.1, 122.0, 126.3, 127.0, 128.4 (Ar), 128.6, 129.3 (CH=CH), 141.8 (C6), 151.5 (C2), 165.1 (C4). Anal. Calc. for $C_{18}H_{20}N_4O_4S$: C: 55.66; H: 5.19; N: 14.42; S: 8.25; found: C: 55.37; H: 5.14; N: 14.35; S: 8.07.

4.3.3. 1-[(2Z)-(4-(5-Bromouracil-1-yl)but-2-enyl)]-5,6-dihydro-1H,4H-1,2,5-thiadiazolo[4,3,2-ij]quinolin-2,2-dioxide (**32**)

A suspension of 5-bromouracil (503 mg, 2.64 mmol) in hexamethyldisilazane (HMDS) (5 mL) was heated at 150 °C in the presence of ammonium sulfate (10 mg) overnight till it became a clear solution. The excess of HMDS was removed under reduced pressure. Then, the halide **30** (394 mg, 1.32 mmol) and NaI (97 mg, 0.7 mmol) in dry CH_3CN (6 mL) were added to the silylated base. The mixture was heated at 80 °C overnight. The reaction was allowed to reach room temperature, diluted with ethyl acetate (20 mL) and washed with a cooled $NaHCO_3$ solution (15 mL). The organic phase was decanted, dried over $MgSO_4$, filtered, and evaporated. The residue was purified by flash column chromatography HPFC (CH_2Cl_2/MeOH, 40:1) to yield 295 mg (49%) of **32** as a pale yellow solid. Mp: 240–241 °C (CH_2Cl_2/MeOH); MS (ES): m/z 453 $[M + 1]^+$, with a Br isotopic pattern; ^{1}H NMR (DMSO – d_6) δ: 2.05 (m, 2H, CH_2), 2.71 (t, 2H, $J = 6.1$ Hz, CH_2), 3.59 (t, 2H, $J = 5.4$ Hz, CH_2), 4.47 (d, 2H, $J = 5.3$ Hz, CH_2), 4.55 (d, 2H, $J = 4.4$ Hz, CH_2), 5.68 (m, 2H, CH=CH), 6.78–6.89 (m, 3H, Ar, H-6), 8.20 (s, 1H, Ar), 11.82 ppm (br s, 1H, NH); ^{13}C NMR (DMSO – d_6) δ: 20.8, 22.6, 44.68, (CH_2), 94.5 (C5), 106.8, 120.2, 121.1, 121.3, 125.6, 127.6 (Ar), 128.0, 128.2 (CH=CH), 144.8 (C6), 150.2 (C2), 159.6 (C4). Anal. Calc. for $C_{17}H_{17}BrN_4O_4S$: C: 45.04; H: 3.78; N: 12.36; S: 7.07; Br: 17.63; found: C: 45.33; H: 4.01; N: 12.18; S: 6.98; Br: 17.90.

4.4. Enzymatic assays

In vitro tests were performed on recombinant TMPKmt. TMPKmt production and purification has been described elsewhere [5]. TMPK activity was determined using the coupled spectrophotometric assay according to Blondin et al. [32] at $\lambda = 334$ nm in an Eppendorf ECOM 6122 spectrophotometer. The reaction medium (0.5 mL final volume) contained 50 mM Tris–HCl pH 7.4, 50 mM KCl, 2 mM $MgCl_2$, 0.2 mM NADH, 1 mM phosphoenol pyruvate and 2 units each of lactate dehydrogenase, pyruvate kinase, and nucleoside diphosphate kinase. The concentrations of ATP and dTMP were kept constant at 0.5 and 0.05 mM, respectively, whereas the concentrations of analogues varied between 0.005 and 0.2 mM. The K_i values were calculated using the classical competitive inhibition model (for more details see Ref. [4]).

4.5. Water solubility

Excess amount of the compound under study was suspended in PBS, sonicated for 10 min at room temperature, and then equilibrated 24 h at room temperature. The samples were centrifuged at 14 000 rpm in an Eppendorf microcentrifuge for 1.5 min at room temperature. An aliquot of the clear supernatant was removed, diluted and analyzed by HPLC by comparison with a five-point standard curve with known concentrations.

4.6. Antimycobacterial evaluation against M. bovis (BCG) and M. smegmatis

The different compounds were assayed for their inhibitory potency toward *M. bovis* BCG-Pasteur and *M. smegmatis* mc^{2}155 growth *in vitro* using a micromethod as previously described [33].

Acknowledgements

This work has been supported by grants from the Spanish MICINN (SAF2009-13914-C02-01 to M.-J.C and M.J.P.P.; BIO-2009-09405 to J.-A.A.) and the Comunidad de Madrid (BIPEDD-CM, S-BIO/0214/2006). H.M.-L. gratefully acknowledges the Institut Pasteur (GPH Tuberculose, DARRI), CNRS and INSERM.

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