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Substituted benzyl-pyrimidines targeting thymidine monophosphate kinase of *Mycobacterium tuberculosis*: Synthesis and in vitro anti-mycobacterial activity

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Abstract—A series of N^1-(4-substituted-benzyl)-pyrimidines were synthesized as potential inhibitors of thymidine monophosphate kinase of *Mycobacterium tuberculosis* (TMPKmt). Key SAR parameters included the chain length substitution in para position of the benzyl ring, the functional group terminating the alkyl chain, and the substituent on the C-5 pyrimidine ring. Synthesized molecules were assayed against both recombinant enzyme and mycobacteria cultures. The most potent compounds have K_i values in the micromolar range and an MIC_{50} of 50 μg/mL against *Mycobacterium bovis*. These results will guide the design of a new generation of lead compounds.

1. Introduction

Tuberculosis (TB) is the major cause of death from a single infectious agent among adults in developing countries, after HIV.[1] The emergence of multi-drug resistant strains of *Mycobacterium tuberculosis* and revival of TB in the industrialized world due to HIV infections have rendered the quest for new drugs against TB a priority. Since the late 1990s, numerous synthetic molecules with anti-mycobacterial activity have been reported with known or suspected modes of action.[2,3] Most of them were identified on the basis of their activity against whole cells. Several compounds are currently under clinical evaluation,[3,4] the diarylquinolone R207910 developed by Johnson and Johnson[5] and the nitroimidazopyran PA-824 by the TB Alliance[6] being the most promising ones so far. Determination in 1998 of the complete genome sequence of *M. tuberculosis* strain H37Rv[7] and the use of mycobacterial genetic tools[8] helped to identify new potential drug targets. Regulatory proteins, enzymes involved in the biosynthesis of essential amino acids and cofactors, enzymes for cell wall biosynthesis, and DNA metabolism are among the key targets studied for anti-mycobacterial chemotherapy.[9] Taking advantage of available high-resolution structures of proteins, structure-based inhibitor design is now underway with the prospect of yielding new classes of drugs.[10]

We are currently studying thymidine monophosphate kinase of *M. tuberculosis* (TMPKmt) as a promising target for the development of new anti-tuberculous agents. TMPK is a member of the nucleoside monophosphate kinase family (NMPK) and catalyzes the phosphorylation of dTMP into dTDP using ATP as a phosphoryl donor. NMPKs are essential for growth in numerous organisms, including mycobacteria.[11] TMPK is the last specific enzyme for the synthesis of dTTP and represents a key enzyme in *M. tuberculosis* metabolism. Biochemical and structural characterization of TMPKmt has revealed subtle differences compared to the corresponding human enzyme (22% of sequence

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identity),[12] which were exploited for the development of selective inhibitors. Single or multiple chemical modifications have been introduced into the pyrimidine moiety and the sugar part of thymidylate, leading to compounds with K_i values in the micromolar range and with a high selectivity index for TMPKmt.[13–15] A TMPKmt inhibitor derived from a 2′,3′-bicyclic thymidine analogue, which showed some activity against mycobacteria cultures, was recently identified.[16]

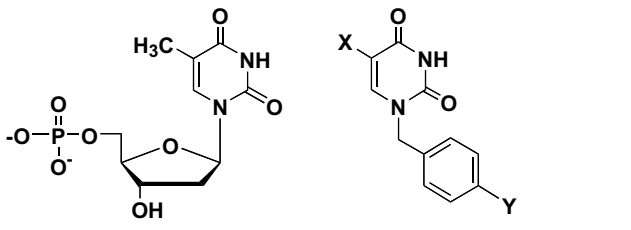
As part of our ongoing program to discover potent and selective inhibitors of TMPKmt, we applied a fragment-based de novo drug design program (LEA3D) to the dTMP binding site of TMPKmt[17] with the aim of generating new ligand families.[18,19] Molecule **1**, 3-[4-(thymin-1-ylmethyl)phenyl] propionamide (Fig. 1), was identified as a potential non-nucleosidic ligand. A limited set of substituted benzyl-thymines were first synthesized and evaluated for their inhibitory potency (K_i) of recombinant TMPKmt.[18,19] The benzyl-thymine derivatives reported so far showed notable inhibition of TMPKmt that led to the possibility of replacing the ribose moiety with a benzyl group.[18] With the aim to improve the inhibitory potency of these thymidylate surrogates and identify potent anti-mycobacterial compounds, we decided to further study this new class of molecules.[20]

In the present work, we describe the convenient synthetic access to a series of *para*-substituted benzyl-pyrimidine derivatives. Four structural variations based on the benzyl-pyrimidine substructure (see Fig. 1) were explored: the chain arm length in para position of the benzyl ring, the saturation of the alkyl chain, the functional group ending the chain, and the substituent at position 5 of the nucleobase. The inhibitory potency of synthesized molecules was determined on recombinant TMPKmt and compared to that on human TMPK (TMPKh). Furthermore, these benzyl-pyrimidines were evaluated for their anti-mycobacterial activity against *Mycobacterium bovis* bacteria.

2. Results and discussion

2.1. Chemistry

The synthesis of the benzyl-pyrimidine derivatives started with the N^1-benzylation of thymine or uracil



dTMP 1 X = CH_3, Y = $(CH_2)_2CONH_2$

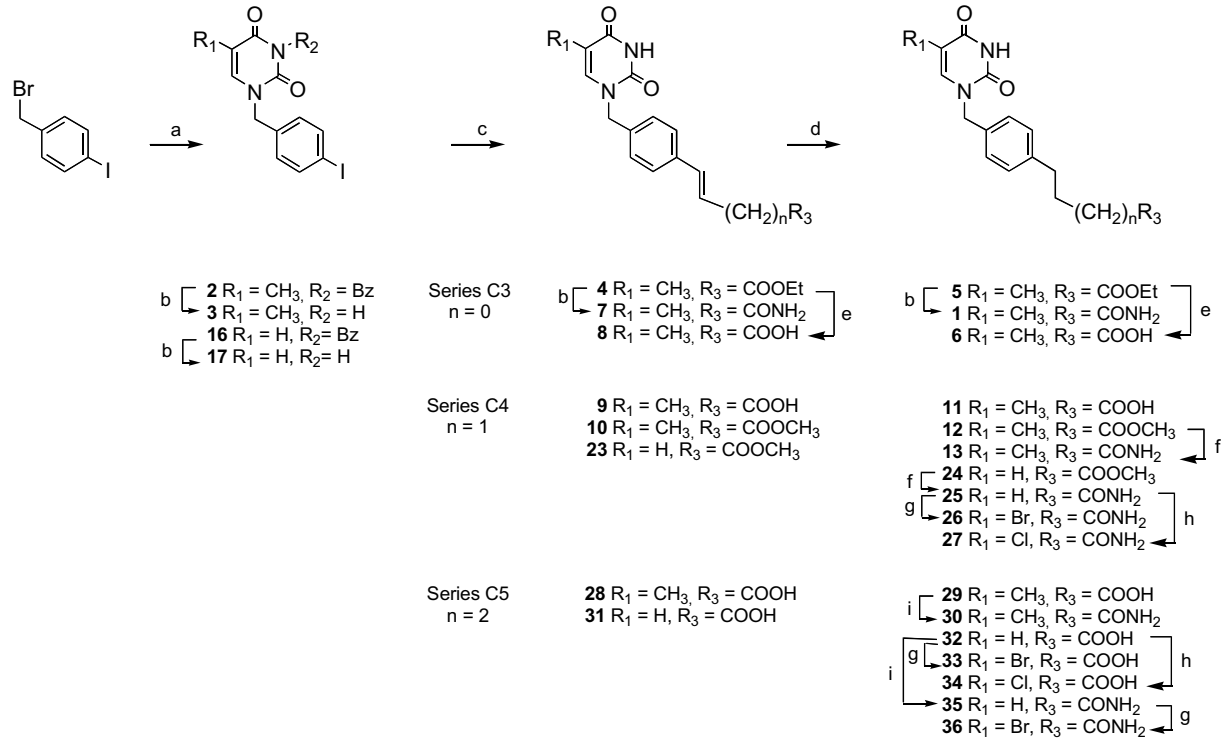
Figure 1. Structures of dTMP and 3-(4-(thymin-1-ylmethyl)phenyl)propionamide (**1**).

moiety (Scheme 1). For regio-selectivity purposes, the pyrimidine moiety was suitably N^3-protected by a benzoyl group.[21] Various alkenyl or alkynyl carboxylic acids, esters or alcohols (according to the selected chain length) were then introduced by the Heck[22] or the Sonogashira[23] palladium-catalyzed coupling reaction (Schemes 1 and 2, respectively). The resulting coupling products were further hydrogenated into saturated derivatives.

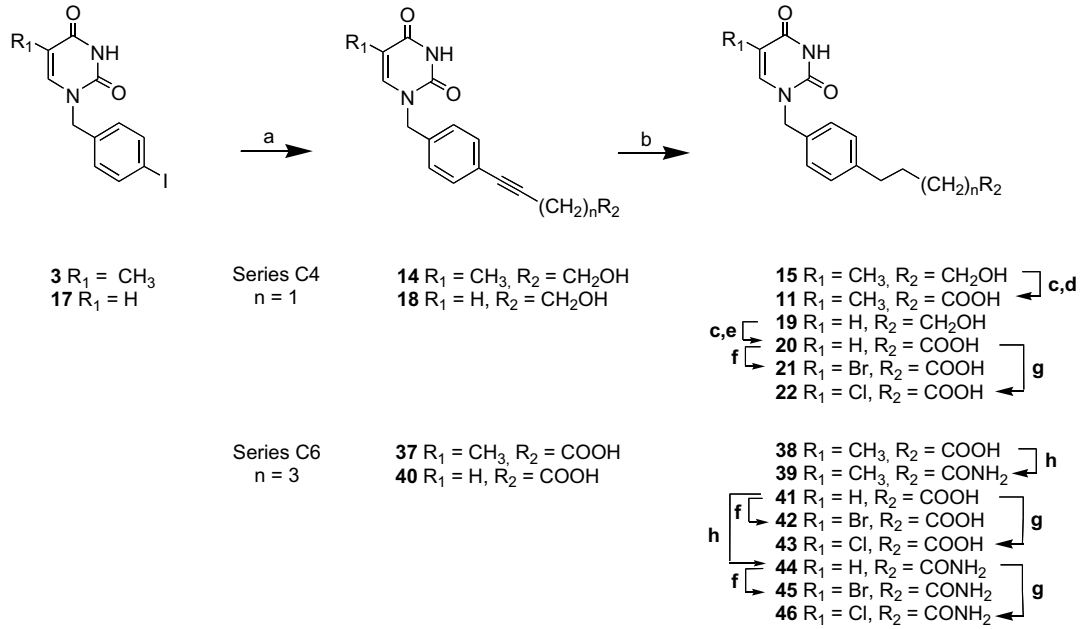
Series C3. Benzyl-thymine derivatives substituted by a C3 chain in *para* position were obtained following the Heck palladium-catalyzed coupling reaction. Starting from the commercially available 4-iodo-benzyl bromide (Scheme 1), N^3-benzoylthymine was first alkylated to give **2** and then debenzoylated into iodide **3** with an overall yield of 85% (compared to 70% for direct N^1-alkylation). Reaction of **3** and ethyl acrylate in a mixture of anhydrous acetonitrile and triethylamine in the presence of $Pd(OAc)_2$and tri-*o*-toluylphosphine at 90 °C resulted in the *trans*-olefin **4** with a 97% yield. Hydrogenation on Pd black of the acrylic ethyl ester **4** produced the propionic ethyl ester **5**. Treatment of **5** with aqueous ammonia gave propionamide **1**. Reaction of **5** in 1 N NaOH yielded the propionic acid **6**. Similarly, ammonolysis of **4** gave the propenamide **7**, while saponification of **4** gave the acrylic acid derivative **8**.

Series C4. Two approaches were investigated to synthesize C4 acid **11** depending on the precursor chosen for introducing the terminal function (carboxylic acid derivatives or alcohol) as depicted in Schemes 1 and 2. As a first approach, the Heck reaction between iodide **3** and 3-butenoic acid at 60 °C gave one stereoisomer (*E*-3) **9** with a 28% yield and 27% of unreacted **3** (Scheme 1). Subsequent hydrogenation of **9** gave saturated C4 acid **11**. When **3** was reacted with methyl 3-butenoate at 90 °C, the coupling yield was increased and ester **10** was isolated as a mixture of *E*-3/*E*-2 isomers (9/1) with a 60% yield. Catalytic hydrogenation into butanoic ester **12**, followed by saponification produced **11**, while treatment of **12** with ammonia in MeOH gave C4 amide **13**. Following the second approach, the aryl iodide **3** and a commercially available C4 alkyne were reacted according to the Sonogashira coupling reaction (Scheme 2). Thus, **3** and 3-butyn-1-ol in the presence of tetrakis(triphenylphosphine)palladium and copper(I) iodide was refluxed in a mixture of anhydrous dichloromethane and triethylamine. Compound **14** was isolated as the major product with a 43% yield, as well as 27% of unreacted iodide **3**. Alkynol **14** was then reduced to give alcohol **15**. Oxidation of **15** with pyridinium dichromate (PDC) in the presence of *tert*-butanol,[24] followed by acid hydrolysis of the intermediate *tert*-butyl ester produced C4 acid **11** from **15** (46% yield). While both approaches gave rise to the target acid **11**, the second one allowed the preparation of alcohol derivatives that could be of interest for biological evaluation.

Our previous work outlined that the introduction of a bromine (or chlorine) atom at 5-position of the pyrimidine moiety improves the affinity of nucleoside analogues.[12,13] The synthesis of the 5-bromo and 5-chloro-



Scheme 1. Reagents and conditions: (a) N^3-benzoylthymine or N^3-benzoyluracil/K_2CO_3/DMF/rt; (b) 36% aq NH_4OH/MeOH/rt; (c) ethyl acrylate (for **4**), 3-butenoic acid (for **9**), methyl 3-butenoate (for **10** and **23**) or 4-pentenoic acid (for **28** and **31**)/$Pd(OAc)_2$/P(*o*-Tol)$_3$/Et_3N/CH_3CN/60, 80 or 90 °C; (d) H_2/Pd black/ MeOH/rt and 5 bars H_2/10% Pd on C/MeOH/rt for **29**; (e) 1N NaOH/MeOH/rt; (f) sat. NH_3/MeOH/rt or 65 °C; (g) Br_2/CCl_4/pyridine/rt; (h) *N*-chlorosuccinimide/Ac_2O/AcOH/60 °C; (i) MeOH/DowexH$^+$/reflux; then satd NH_3/MeOH.



Scheme 2. Reagents and conditions: (a) 3-butyn-1-ol for **14** and **18**, or 5-hexynoic acid for **37** and **40**/$Pd(PPh_3)_4$/CuI/Et_3N/CH_2Cl_2 or CH_3CN/rt, 50 or 90 °C; (b) H_2/Pd black/MeOH/rt; (c) PDC/*t*-BuOH/Ac_2O/CH_2Cl_2/rt; (d) TFA/CH_2Cl_2/rt; (e) 2N NaOH/MeOH/rt; (f) Br_2/CCl_4/pyridine/rt; (g) *N*-chlorosuccinimide/Ac_2O/AcOH/60 °C; (h) MeOH/Dowex H$^+$/reflux, then satd NH_3/MeOH.

uracil benzyl analogues was undertaken using both coupling methods starting from iodide **17**. Heck coupling reaction of iodide **17** and methyl 3-butenoate at 60 °C produced **23** as one stereoisomer (*E*-3) in 44% yield (Scheme 1). When the reaction was performed at 90 °C, **23** was isolated with a 75% yield as a mixture of *E*-3/*E*-2 isomers in a 9/1 ratio. Catalytic hydrogenation of **23** into saturated ester **24**, conversion into amide

25, followed by bromination or chlorination[25] gave 5-bromo or 5-chloro-uracil derivative **26** (82% yield) and **27** (26% yield), respectively. On the other hand, Sonogashira coupling of iodide **17** and 3-butyn-1-ol produced alkyne **18** at a 64% yield (Scheme 2). Catalytic hydrogenation into alcohol **19** (88% yield), PDC oxidation into *tert*-butyl ester (70% yield) followed by saponification gave C4 uracil acid derivative **20** (80% yield). Bromination and chlorination of **20** gave the corresponding 5-Br and 5-Cl acid derivatives, **21** (70% yield) and **22** (49% yield), respectively.

Series C5. The C5 derivatives were synthesized as illustrated in Scheme 1. Heck reaction between iodide **3** and 4-pentenoic acid gave the coupling product **28** (82%) as a mixture of three stereoisomers, *E*-4, *E*-3, and *E*-2 in a ratio of 7/7/3 according to ^{1}H NMR analysis. Attempts to separate these stereoisomers by reverse phase HPLC were unsuccessful. Hydrogenation of olefins **28** yielded the saturated C5 acid **29**. Acid **29** was converted into amide **30** via the methyl ester. Similarly, benzyl-uracil derivative was synthesized by Heck coupling of uracil iodide **17** and 4-pentenoic acid (69% yield), followed by catalytic hydrogenation of olefins **31**–**32** (95% yield). Bromination and chlorination of **32** afforded 5-bromo and 5-chloro derivatives **33** (64% yield) and **34** (56% yield), respectively. Conversion of acid **32** into amide **35**, followed by bromination gave **36** (35% yield).

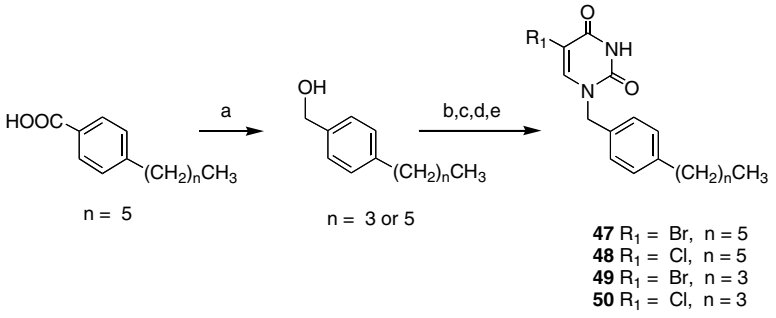
Series C6. Sonogashira coupling of **3** with 5-hexynoic acid gave alkyne **37** in 50% yield. Catalytic hydrogenation afforded saturated C6 acid **38** (89% yield). Acid **38** was converted into C6 amide **39** via the corresponding methyl ester (42% yield in two steps). Similarly, reaction of 5-hexynoic acid and uracil iodide **17** afforded alkyne **40** in 72% yield. Catalytic hydrogenation afforded C6 acid **41** (83%), which was brominated and chlorinated to give 5-bromo and 5-chloro-uracil derivatives **42** and **43**, respectively (92% and 49% yields, respectively). Acid **41** was converted into amide **44**, which was brominated and chlorinated into compounds **45** and **46** (87% and 72% yields, respectively).

Furthermore, benzyl-pyrimidines substituted by a linear alkyl chain were synthesized in order to examine the influence of the functional group ending the chain (Scheme 3). C6 derivatives **47** and **48** were obtained from 4-hexyl benzoic acid by reduction of the carboxylic acid (58%), followed by chlorination of the primary alcohol (91%) and then reaction with N^3-benzoyl uracil (59% yield). After treatment with ammonia (83%), bromination (94%), or chlorination (76%) at the 5-position of uracil afforded the corresponding C6 derivatives **47** and **48**, respectively. Similarly, benzyl-pyrimidines substituted by a butyl chain were prepared starting from 4-butylbenzyl alcohol to yield brominated **49** and chlorinated **50** derivatives.

2.2. Inhibitory activity on TMPKmt

The ability of the synthesized benzyl-pyrimidines to inhibit TMPKmt in the presence of ATP and dTMP was examined with recombinant enzyme using an enzymatic assay, as previously described.[12] Most of the molecules exhibited inhibitory potency with K_i values ranging between 6.5 and 202 μM (Table 1). The most potent compounds correspond to benzyl-pyrimidines (thymine, 5-bromo-uracil or 5-chloro-uracil) substituted by a saturated C4 carboxylic acid (**11**, K_i = 13 μM; **21**, K_i = 10 μM **22**, K_i = 6.5 μM). The inhibitory potency of **11** and **22** is notable when compared to their nucleosidic homologues, dT (27 μM) and 5-chloro-dU (10 μM),[15] and to N^1-benzyl-thymidine (75 μM)[18] and 4-bromobenzyl-thymine (**53**) (38 μM, Table 1). Indeed, the lowest K_i within the thymine series is observed for carboxylic acid with a chain length of 4 carbons (**11**: K_i = 13 μM) compared to 3 carbons (**6**, K_i = 55 μM), 5 carbons (**29**, K_i = 58 μM) and 6 carbons (**38**, K_i = 47 μM). Compounds in the thymine series terminating with a carboxamide group are generally less active than the acid derivatives. The highest inhibitory potency is observed for a molecule with a 6 carbon chain (**39**, K_i = 26.5 μM) compared to 3 carbons (**1**, K_i = 89 μM), 4 carbons (**13**, K_i = 112 μM), and 5 carbons (**30**, K_i = 55 μM). Benzyl-thymine substituted with unsaturated alkyl chains (double or triple bond) show reduced affinity compared to their saturated homologues (compared **8** and **6**, **9** and **11**, **14** and **15**, **37** and **38**).

As previously observed in the deoxyribonucleoside series,[12,13] the introduction of a bromine atom (or a chlorine) at position 5 on the pyrimidine improves the



Scheme 3. Reagents and conditions: (a) 1 M BH_3-THF complex; (b) $SOCl_2/CH_3CN$/90 °C; (c) N^3-benzoyluracil/K_2CO_3/DMF/rt; (d) 36% aq NH_4OH/MeOH/rt; (e) Br_2/pyridine/CCl_4/rt (**47** and **49**) or *N*-chlorosuccinimide/Ac_2O/AcOH/60 °C (**48** and **50**).

Table 1. Evaluation of synthesized molecules on *M. tuberculosis* and human TMPKs, and on *M. bovis* (BCG) bacteria

Compound	X	Y	TMPKmt K_i (μM)	TMPKh K_i (μM)	SI	MIC_{50} (μg/mL)	Compound	X	Y	TMPKmt K_i (μM)	TMPKh K_i (μM)	SI	MIC_{50} (μg/mL)
dT			27[15]	180[15]	7	NI[e]	**5BrdU**			5[15]	214[15]	43	NI[e]
1	CH_3	$CH_2CH_2CONH_2$	89			NI[e]	**33**	Br	$(CH_2)_4COOH$	38			100
6	CH_3	CH_2CH_2COOH	55			NI[e]	**34**	Cl	$(CH_2)_4COOH$	16	122	7.6	120
7	CH_3	$CH{=}CHCONH_2$	195			NI[d]	**36**	Br	$(CH_2)_4CONH_2$	35			NI[e]
8	CH_3	$CH{=}CHCOOH$	NI[a]			NI[e]	**37**	CH_3	$C{\equiv}C(CH_2)_3COOH$	96			150
9	CH_3	$CH{=}CHCH_2COOH$	39			NI[e]	**38**	CH_3	$(CH_2)_5COOH$	47			195
11	CH_3	$(CH_2)_3COOH$	13	252	19	NI[e]	**39**	CH_3	$(CH_2)_5CONH_2$	26.5			230
13	CH_3	$(CH_2)_3CONH_2$	112			NI[e]	**42**	Br	$(CH_2)_5COOH$	34	92	3	80
14	CH_3	$C{\equiv}CCH_2CH_2OH$	70			NI[e]	**43**	Cl	$(CH_2)_5COOH$	23			125
15	CH_3	$(CH_2)_4OH$	51			365	**45**	Br	$(CH_2)_5CONH_2$	19.5			NI[e]
20	H	$(CH_2)_3COOH$	202			225	**46**	Cl	$(CH_2)_5CONH_2$	26			288
21	Br	$(CH_2)_3COOH$	10	293	29	50	**47**	Br	$(CH_2)_5CH_3$	NI[b]			NI[d]
22	Cl	$(CH_2)_3COOH$	6.5	790	120	45	**48**	Cl	$(CH_2)_5CH_3$	NI[b]			NI[d]
25	H	$(CH_2)_3CONH_2$	NI[a]			270	**49**	Br	$(CH_2)_3CH_3$	NI[b]			NI[d]
26	Br	$(CH_2)_3CONH_2$	39	NI[a]		55	**50**	Cl	$(CH_2)_3CH_3$	NI[b]			NI[d]
27	Cl	$(CH_2)_3CONH_2$	39	NI[a]		51	**51**	Br	$(CH_2)_3COOCH_3$	52			180
29	CH_3	$(CH_2)_4COOH$	58			120	**52**	Cl	$(CH_2)_3COOCH_3$	59			260
30	CH_3	$(CH_2)_4CONH_2$	55			NI[e]	**53**	CH_3	Br	38			NI[d]

For the general structure, see Figure 1. The K_i values are means of three independent assays. SI = (K_i TMPKh)/(K_i TMPKmt). The MIC_{50} values are means of two independent assays performed in duplicate. NI, no inhibition (a) until 2 mM, (b) until 0.2 mM, (c) until 400 μg/mL, (d) until 40 μg/mL, (e) at 10 μg/mL.

affinity of benzyl derivatives, whatever the arm length and the functional group ending the alkyl chain. Thus, 5-bromo-uracil derivatives **21** (C4 acid), **33** (C5 acid), and **42** (C6 acid) are more potent than their respective thymine homologues, **11**, **29**, and **38**. When the C4 chain terminates with methyl ester (**51** and **52**, obtained by 5-halogenation from **24**), carboxamide (**13**, **26**, and **27**) or primary alcohol (**14**, **15**) affinity is reduced (4- to 10-fold) compared to the acid homologues **11**, **21**, and **22**. Moreover, substitution of the benzyl ring with hydrophobic alkyl chains led to inactive compounds (**47**, **48**, **49**, and **50**).

2.3. Anti-mycobacterial activity and cytotoxicity

The benzyl-pyrimidine derivatives were evaluated for inhibitory activity against *M. bovis* (BCG) bacteria by the microplate assay.[26] The genome of *M. bovis* (BCG) being more than 98% homologous to *M. tuberculosis*, the screening for potential drug sensitivity may be performed with little risk of infection using the BCG vaccine strain. Due to the poor solubility of some derivatives, all inhibitors were tested in the presence of DMSO. The percentage of DMSO (ranging from 0.125% to 4%) that is tolerated by cells was first controlled. As the presence of 1.5% DMSO had no detectable effect on bacterial growth, compounds were tested at concentrations of 1–400 μg/mL and a final concentration of 0.8% DMSO. The minimum inhibitory concentration (MIC) inhibiting 50% of growth (MIC_{50}) is given in Table 1. Isoniazid (INH) was used as a reference standard at concentrations ranging from 0.01 to 5 μg/mL (MIC_{50} of 0.05 μg/mL).

Within the thymine series, compound **29** (C5 acid) showed the highest activity (MIC_{50} = 120 μg/mL) among the acid and amide derivatives. The benzyl-thymines with a C6 arm (acids **37**, **38**, and amide **39**) exhibited weaker activity (MIC_{50} = 150–230 μg/mL) than **29**, while those with a chain shorter than 5 carbons (**1**, **6**, **11**, and **13**) were devoid of activity against *M. bovis* up to a concentration of 400 μg/mL. When the nucleobase is a 5-bromo or 5-chloro-uracil, the benzyl derivatives substituted with C4 chain (acids **21**, **22**, and amides **26**, **27**) showed significant activity (MIC_{50} = 45–55 μg/mL) compared to poorly active uracil (**20**, **25**) (MIC_{50} = 225–270 μg/mL) or inactive thymine homologues (**11**, **13**). The activity was slightly decreased for acid derivatives having a chain longer than 4 carbons (MIC_{50} = 80–125 μg/mL for **33**, **34**, **42**, and **43**). The removal of the polar terminal group (amide or acid) from the chain had a detrimental impact on activity. Thus, the absence of the carboxylic acid or amide group led to inactive compounds (**47**–**50**). The conversion of the carboxylic acids (**21**, **22**) into methyl esters (**51**, **52**) led to significantly less active molecules (5- to 9-fold).

For compounds displaying $K_i \leqslant 16$ μM or MIC_{50} lower than 100 μg/mL, selectivity index (SI) versus TMPKh was measured (Table 1). Molecules having a saturated C4 chain ending with carboxylic acid or amide (**11**, **21**, **22, 26**, and**27**) showed low affinity for the TMPKh (SI > 19), whatever the substituent at the 5-position.

Compounds having a chain length of 5 or 6 carbons (**34** and **42**) appeared less selective (SI from 3 to 8).

The cytotoxicity of selected compounds was examined on monkey kidney cell lines (Vero cells). Molecules tested were devoid of cytotoxicity at 400 μg/mL, with the exception of molecules **21** and **27**, which exhibited a weak toxicity (respectively, 14% and 10% inhibition at 100 μg/mL).

3. Conclusion

In the search for new anti-tuberculosis agents that work by mechanisms of action other than current anti-TB drugs, we studied the inhibitory potency of a series of substituted benzyl-pyrimidines on TMPKmt. The synthesis of this new family of molecules was easily achieved in good yields by introducing different functionalized alkyl chains using Heck and Sonogashira coupling chemistry. By varying the arm length and the nature of the terminal function, we identified potent and selective inhibitors of recombinant TMPKmt. The highest K_i (in the micromolar range) and selectivity index values (20–120) correspond to benzyl-pyrimidines substituted by a chain length of 4 carbons and a terminal carboxylic acid function (**11**, **21**, and **22**). Other functional groups tested led to significantly less potent compounds. When the chain was longer, the affinity and selectivity of the acid carboxylic derivatives were reduced and were similar to the amide derivatives. Molecular modeling suggested possible orientations of the ligands.[18,20] Docking of molecule **11** in TMPKmt showed that, in addition to hydrogen network of thymine, the acid function can provide favorable interactions with Arg95 (Fig. 2). Indeed Arg95 participates in key interactions with the substrates, in particular with the 5′-*O*-phosphate of dTMP.[18] It can thus be considered as a key factor to afford inhibition. For benzyl-thymine ending with an amide (**1**, **13**, **30**, and **39**), the binding mode of **39** corresponds to an optimal interaction with Glu166 and the carboxyl backbone of Ala161 (Fig. 2).

Finally, among the strongest inhibitors of this series, four compounds (**21**, **22**, **26**, and **27**) showed moderate inhibitory potency (MIC_{50} of 50 μg/mL) on the growth of *M. bovis* (BCG) bacteria.

The obtained results confirm that TMPKmt represents a valuable target for designing anti-mycobacterial drugs. We are currently working on further modifications and optimizations of this new class of molecules for obtaining more efficient agents. Recently, a set of 5′-thiourea-substituted α-thymidine derivatives has been synthesized as potent and selective inhibitors of TMPKmt, which resulted in the identification of a molecule with significant inhibitory activity against *M. bovis* and *M. tuberculosis*.[27] Thus, two structurally different molecules, α-nucleoside analogue and non-nucleosidic ligand, both acting as TMPKmt inhibitors, proved to be capable of inhibiting bacterial growth of *M. bovis*.

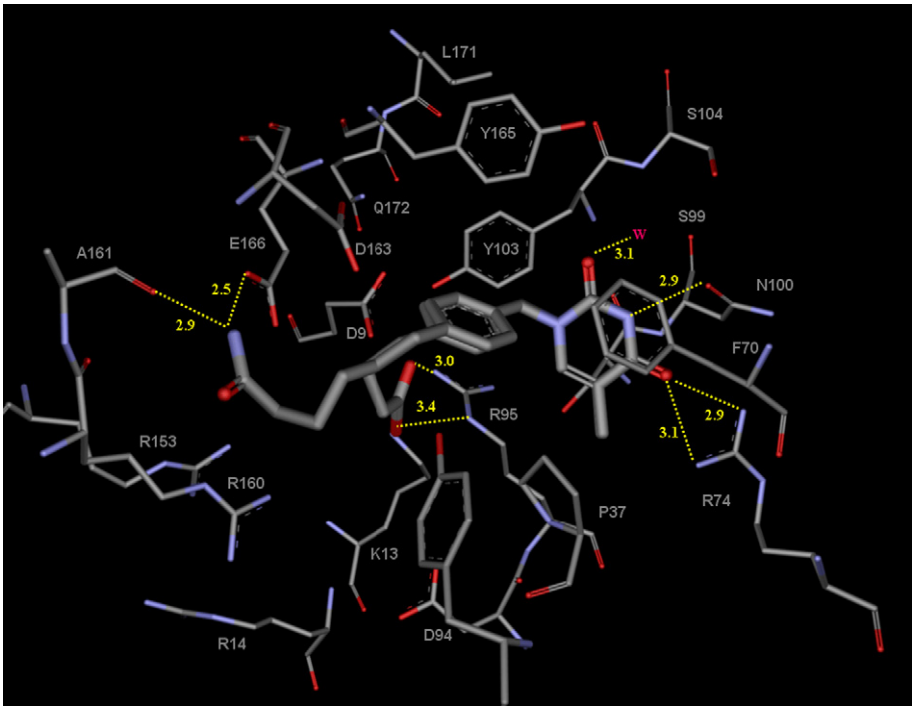


Figure 2. Active site of TMPKmt. Superimposition of the predicted binding modes of molecules **11** and **39** by FlexX.[18] The hydrogen bond network is depicted in yellow. The thymine base is able to form a significant π-stack with Phe70 and four hydrogen bonds with Arg74, Asn100, and a water molecule (W in magenta). The carboxylate group of **11** is able to interact with Arg95, whereas the amide function of **39** interacts with Glu166 and the backbone carboxyl of Ala161.

4. Experimental

4.1. Synthesis: general information

Anhydrous solvents and reagents were purchased from Sigma–Aldrich and used without purification (except for anhydrous Et_3N). Reactions were monitored by thin-layer chromatography (TLC) on precoated Merck silica gel plates (60 F_{254}/0.2 mm thickness) and visualised by UV light, then revealed by sulfuric acid–anisaldehyde spray followed by heating. Column chromatography was performed with Merck silica gel 60 (230–400 mesh). Preparative HPLCs were carried out on a Perkin Elmer system (200 Pump) with a C18 reverse phase column (Kromasil, 5μ–100 Å, 250 × 10 mm) using a flow rate of 5.5 mL/min and a linear gradient of CH_3CN in 10 mM triethylammonium acetate buffer at pH 7.5 over 20 min. Eluted products were visualized using a diode array detector. Purity of all compounds evaluated on TMPKmt was verified by analytical HPLC (Kromasil, 5μ–100 Å, 150 × 4.6 mm) using a flow rate of 1 mL/min and a linear gradient of CH_3CN in 20 mM triethylammonium acetate in 20 min and was found up to 96% (diode array detector). ^1H and ^{13}C NMR spectra were recorded on a Bruker Avance 400 spectrometer, operating at 400.13 MHz and 100.62 MHz, respectively. Chemical shifts are given in ppm (δ) relative to residual solvent peak for ^1H and ^{13}C, coupling constants (J) are reported in Hertz, and the normal abbreviations are used. ESI-TOF mass spectra were recorded by the mass spectroscopy laboratory (CNRS-ICSN, Gif-sur-Yvette).

4.2. General procedures

4.2.1. Saponification. The appropriate methyl or ethyl ester dissolved in MeOH was treated with 1 N NaOH (1.2 equiv). After completion of the reaction (TLC), the solution was acidified to pH 3–4 by addition of a cationic resin (Dowex 50W-H^+), filtered. The filtrate was evaporated under vacuum and purified as specified. For biological assays, acid was neutralized by addition of dilute NaOH until pH 7.

4.2.2. Catalytic hydrogenation. Pd black (10% w/w) was added to the appropriate alkene or alkyne in MeOH. Hydrogen was applied for 5–9 h. The reaction mixture was filtered on Celite, rinsed with MeOH, and the filtrates were evaporated under vacuum. The resulting product was then purified by silica gel column chromatography or reverse phase HPLC as specified.

4.2.3. 4-[4-(5-Methyl-2,4-dioxo-3,4-dihydro-2*H*-pyrimidin-1-yl-methyl)-phenyl]-buten-2-oic acid (9). A stirred mixture of **3** (0.36 g, 1.06 mmol), 3-butenoic acid (0.11 g, 1.27 mmol), $Pd(OAc)_2$ (5 mg, 0.022 mmol) and P(*o*-Tol)$_3$ (13 mg, 0.043 mmol) in CH_3CN (10 mL) with Et_3N (10 mL) was heated at 60 °C under argon for 72 h. The reaction mixture was filtered on Celite, rinsed with CH_2Cl_2, and the filtrates were concentrated to dryness. The crude residue was dissolved in CH_2Cl_2 and washed with 10% HCl, the organic layer was dried, concentrated under vacuum. The residue was purified by column chromatography on silica gel (0–10% gradient of methanol in dichloromethane) to give iodide **3** (100 mg, 27%), then **9** as *E*-3 stereoisomer (85 mg, 28%). ^1H NMR (DMSO-d_6) δ 1.68 (d, 3H, CH_3, J = 1.2), 3.09 (d, 2H, CH_2, J = 7.0), 4.73 (s, 1H, $PhCH_2$), 6.24 (dt, 1H, CH, J = 15.9, J = 7.0), 6.39 (d, 1H, CH, J = 15.9), 7.17 (d, 2H, H ortho), 7.32 (d, 2H, H meta), 7.54 (d, 1H, H6, J = 1.1), 11.24 (br s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ 12.78 (CH_3), 34.02 (CH_2), 50.65 ($PhCH_2$), 109.87 (C5), 124.73 (CH), 127.10 (2×CH meta), 128.66 (2×CH ortho), 132.33 (CH), 136.87 (C arom.), 137.12 (C arom.), 142.10 (C6), 151.85 (C2), 165.08 (C4), 173.68 (COOH). HRMS (ESI-TOF) *m/z* calcd for $C_{16}H_{16}N_2O_4$+Na 323.1008; found 323.1044.

4.2.4. 4-[4-(5-Methyl-2,4-dioxo-3,4-dihydro-2*H*-pyrimidin-1-yl-methyl)-phenyl]-buten-2-oic acid methyl ester (10). As for **9**, reaction of **3** (0.24 g, 0.70 mmol) and methyl 3-butenoate (0.09 g, 0.84 mmol) in CH_3CN (7 mL) with Et_3N (0.12 mL) at 90° for 72 h afforded after purification by column chromatography (0–8% MeOH in CH_2Cl_2) compound **10** (0.18 g, 60%). ^1H NMR ($CDCl_3$) δ 1.85 (d, 3H, CH_3, J = 1.1), 3.25 (dd, 2H, CH_2, J = 1.2, J = 7.1), 3.70 (s, 3H, OCH_3), 4.86 (s, 2H, $PhCH_2$), 6.29 (m, 1H, =CH), 6.46 (d, 1H, H, J = 15.9), 6.99 (d, 1H, H6, J = 1.2), 7.22 (d, 2H, H ortho), 7.34 (d, 2H, H meta), 10.11 (br s, 1H, NH). ^{13}C NMR ($CDCl_3$) δ 12.75 (CH_3), 38.51 (CH_2), 51.00 ($PhCH_2$), 52.36 (OCH_3), 111.62 (C5), 122.93 (=CH), 127.25 (CH meta), 128.51 (CH ortho), 133.09 (=CH), 135.20 (C arom.), 137.37 (C arom.), 140.21 (C6), 151.88 (C2), 165.00 (C4), 172.30 ($COOCH_3$).

4.2.5. 4-[4-(5-Methyl-2,4-dioxo-3,4-dihydro-2*H*-pyrimidin-1-yl-methyl)-phenyl]-butanoic acid (11). *Method A:* Hydrogenation of **9** (80 mg, 0.27 mmol) in MeOH gave **11** (77 mg, 95%). MS (ESI-TOF) *m/z* 325.1 (100%, M+Na)$^+$. HRMS (ESI-TOF) *m/z* calcd for $C_{16}H_{18}N_2O_4$+Na 325.1178; found 325.0357. *Method B:* Hydrogenation of **14** (68 mg, 0.23 mmol) in MeOH (5 mL) gave **15** (97%). To a stirred solution of **15** (120 mg, 0.42 mmol) in dry CH_2Cl_2 (4 mL) were added *tert*-butanol (0.79 mL, 8.4 mmol), acetic anhydride (0.40 mL, 4.2 mmol) and pyridinium dichromate (0.32 g, 0.84 mmol). After stirring for 6 h at room temperature, **15** was consumed as judged by TLC. The crude mixture was loaded on a silica gel column chromatography conditioned in ethyl acetate, let on the silica gel for 15 min, then eluted with ethyl acetate. The fractions containing the expected ester were pooled, concentrated under vacuum, and purified on silica gel column (2% MeOH in CH_2Cl_2) to give *tert*-butyl ester (70 mg). Treatment with 80% aq solution of TFA (20 mL) at room temperature gave **11** (58 mg, 46% in two steps). ^1H NMR (DMSO-d_6) δ 1.75 (d, 3H, CH_3, J = 1.2), 1.78 (m, 2H, CH_2), 2.20 (t, 2H, CH_2, J = 7.4), 2.56 (t, 2H, CH_2, J = 7.7), 4.80 (s, 2H, $PhCH_2$), 7.19 (m, 4H, H arom.), 7.61 (d, 1H, H6, J = 1.2), 11.30 (br s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ 12.78 (CH_3), 27.12 (CH_2), 33.97 (CH_2), 34.90 (CH_2), 50.61 ($PhCH_2$), 109.83 (C5), 128.37 (CH ortho), 129.44 (CH meta), 135.37 (C arom.), 141.92 (C arom.), 142.12 (C6), 151.86 (C2), 165.08 (C4), 175.09 (COOH).HRMS

(ESI-TOF) *m/z* calcd for $C_{16}H_{18}N_2O_4$+Na 325.1164; found 325.1162.

4.2.6. 4-[4-(5-Methyl-2,4-dioxo-3,4-dihydro-2*H*-pyrimidin-1-yl-methyl)-phenyl]-butanoic acid methyl ester (12). Hydrogenation of **10** afforded **12** (95%) after silica gel column chromatography. ^{1}H NMR (DMSO-d_6) δ 1.80 (d, 3H, CH_3), 1.86 (m, 2H, CH_2), 2.36 (t, 2H, CH_2, J = 7.4), 2.62 (t, 2H, CH_2, J = 7.5), 3.63 (m, 3H, OCH_3), 4.85 (s, 1H, $PhCH_2$), 7.23 (d, 2H, H meta), 7.27 (d, 2H, H ortho), 7.67 (d, 1H, H6, J = 1.1), 11.32 (br s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ 12.77 (CH_3), 27.01 (CH_2), 33.55 (CH_2), 34.78 (CH_2), 50.61 ($PhCH_2$), 52.07 (OCH_3), 109.83 (C5), 128.39 (CH ortho), 129.46 (CH meta), 135.43 (C arom.), 141.71 (C arom.), 142.11 (C6), 151.85 (C2), 165.07 (C4), 173.95 ($COOCH_3$). MS (ESI-TOF) *m/z* 339.1 (100%, $M+Na)^+$.

4.2.7. 4-[4-(5-Methyl-2,4-dioxo-3,4-dihydro-2*H*-pyrimidin-1-yl-methyl)-phenyl]-butanamide (13). Compound **12** (40 mg, 0.13 mmol) was treated with a saturated solution of ammonia in MeOH (4 mL) at 65 °C overnight to give after purification on silica gel (5% MeOH in CH_2Cl_2) unreacted ester **12** (8 mg, 20%) and amide **13** (20 mg, 47%). ^{1}H NMR (DMSO-d_6) δ 1.76 (m, 5H, CH_2 and CH_3), 2.05 (t, 2H, CH_2, J = 7.5), 2.54 (t, 2H, CH_2), 4.80 (s, 1H, $PhCH_2$), 6.73 (br s, 1H, $CONH_2$), 7.17 (d, 2H, H ortho), 7.21 (d, 2H, H meta), 7.62 (d, 1H, H6, J = 1.2), 7.25 (br s, 1H, $CONH_2$), 11.31 (br s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ 12.78 (CH_3), 27.64 (CH_2), 35.17 and 35.36 (2×CH_2), 50.63 ($PhCH_2$), 109.83 (C5), 128.35 (CH ortho), 129.44 (CH meta), 135.28 (C arom.), 142.13 (C6), 142.17 (C arom.), 151.86 (C2), 165.09 (C4), 174.82 ($CONH_2$). HRMS (ESI-TOF) *m/z* calcd for $C_{16}H_{19}N_3O_4$+Na 324.1324; found 324.1342.

4.2.8. 1-[4-(4-Hydroxy-butyn-1-yl)-benzyl]-5-methyl-1*H*-pyrimidine-2,4-dione (14). To a solution of **3** (0.51 g, 1.50 mmol) in dry CH_2Cl_2 (15 mL) were added under argon freshly distilled Et_3N (0.31 mL), 3-butyn-1-ol (0.13 g, 1.95 mmol), tetrakis(triphenylphosphine)palladium (0.06 g, 0.05 mmol) and cuprous iodide (26 mg, 0.02 mmol). After heating at 90 °C under argon overnight, the reaction mixture was concentrated to dryness. To the residue was added CH_2Cl_2 (100 mL) and the resulting solution was washed with 10% HCl, and water. The organic layer was dried over Na_2SO_4, then evaporated in vacuo. Purification by silica gel column chromatography (0–3% MeOH in CH_2Cl_2) afforded recovered iodide **3** (0.52 g, 27%) and **14** as a white powder (0.18 g, 43%). ^{1}H NMR (DMSO-d_6) δ 1.75 (d, 3H, CH_3, J = 1.2), 2.54 (m, 2H, CH_2), 3.58 (m, 2H, CH_2), 4.83 (s, 2H, $PhCH_2$), 4.92 (t, 1H, OH), 7.25 (d, 2H, H ortho, J = 8.0), 7.37 (d, 2H, H meta, J = 8.0), 7.61 (d, 1H, H6, J = 1.2), 11.33 (br s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ 12.77 (CH_3), 24.09 (CH_2), 50.72 ($PhCH_2$), 60.57 (CH_2), 81.54 (C), 89.68 (C), 109.98 (C5), 123.32 (C arom.), 128.40 (CH ortho), 132.38 (CH meta), 137.63 (C arom.), 142.14 (C6), 151.84 (C2), 165.13 (C4). HRMS (ESI-TOF) *m/z* calcd for $C_{16}H_{17}N_2O_3$+Na 307.1059; found 307.1048.

4.2.9. 1-(4-Hydroxy-butyn-1-yl-benzyl)-1*H*-pyrimidine-2,4-dione (18). As for **14**, reaction of **17** (0.52 g, 1.87 mmol) and 3-butyn-1-ol (0.17 g, 2.40 mmol) in dry CH_2Cl_2 (28 mL) with Et_3N (32 mL), at 80 °C for 48 h afforded after column chromatography **18** (0.33 g, 64%). ^{1}H NMR (DMSO-d_6) δ 2.54 (t, 2H, CH_2, J = 6.8), 3.57 (q, 2H, CH_2), 4.87 (s, 2H, $PhCH_2$), 4.89 (t, 1H, OH), 5.60 (dd, 1H, H5, J = 7.8, J = 2.1), 7.25 (d, 2H, H arom., J = 8.3), 7.38 (dd, 2H, H arom., J = 6.5, J = 2.7), 7.76 (d, 1H, H6), 11.33 (br s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ 24.12 (CH_2), 50.88 ($PhCH_2$), 60.56 (CH_2), 81.52 (C), 89.72 (C), 102.26 (C5), 123.36 (CH ortho), 128.41 (CH meta), 132.38 (C arom.), 137.50 (C arom.), 146.38 (C6), 151.85 (C2), 164.50 (C4). MS (ESI-TOF) *m/z* 293.1 (100%, $M+Na)^+$, 333.2 (5%, $M+Na+K)^+$.

4.2.10. 1-[4-(4-Hydroxy-butyl)-benzyl]-1*H*-pyrimidine-2,4-dione (19). Hydrogenation of **18** (0.19 g, 0.71 mmol) gave **19** (0.17 g, 88%). ^{1}H NMR (DMSO-d_6) δ 1.41 (m, 2H, CH_2), 1.57 (m, 2H, CH_2), 2.55 (t, 2H, CH_2, J = 7.6), 3.39 (m, 2H, CH_2), 4.35 (t, 1H, OH), 4.83 (s, 2H, $PhCH_2$), 5.58 (dd, 1H, H5, J = 7.8, J = 2.3), 7.19 (m, 4H, H arom.), 7.74 (d, 1H, H6, J = 7.8), 11.30 (br s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ 28.26 (CH_2), 32.93 (CH_2), 35.46 (CH_2), 50.84 ($PhCH_2$), 61.34 (CH_2), 102.14 (C5), 128.40 (CH ortho), 129.42 (CH meta), 134.95 (C arom.), 142.74 (C arom.), 146.45 (C6), 151.86 (C2), 164.50 (C4). MS (ESI-TOF) *m/z* 297.1 (100%, $M+Na)^+$, 431.4 (40%).

4.2.11. 4-[4-(2,4-Dioxo-3,4-dihydro-2*H*-pyrimidin-1-yl-methyl)-phenyl]-butanoic acid (20). Chromic oxidation of **19** (135 mg, 0.5 mmol) as for **11** gave the corresponding *tert*-butyl ester (125 mg, 70%). Saponification of the ester (105 mg, 0.29 mmol) afforded **20** as a white powder (68 mg as sodium salt, 80%). ^{1}H NMR (DMSO-d_6) δ 1.77 (m, 2H, CH_2), 2.21 (t, 2H, CH_2, J = 7.4), 2.57 (t, 2H, CH_2, J = 7.7), 4.83 (s, 2H, $PhCH_2$), 5.58 (dd, 1H, H5, J = 2.2, J = 7.8), 7.20 (m, 4H, H arom.), 7.74 (d, 1H, H6, J = 8.8), 11.30 (br s, 1H, NH), 12.05 (br s, 1H, COOH). ^{13}C NMR (DMSO-d_6) δ 27.08 (CH_2), 33.91 (CH_2), 34.89 (CH_2), 50.83 ($PhCH_2$), 102.15 (C5), 128.40 (CH ortho), 129.46 (CH meta), 135.19 (C arom.), 141.97 (C arom.), 146.44 (C6), 151.86 (C2), 164.50 (C4), 175.04 (COOH). HRMS (ESI-TOF) *m/z* calcd for $C_{15}H_{16}N_2O_4$+Na 311.1008; found 311.1022.

4.2.12. 4-(5-Bromo-2,4-dioxo-3,4-dihydro-2*H*-pyrimidin-1-ylmethyl)-butanoic acid (21). A 1 M solution of bromine in CCl_4 (1.3 equiv) was added to uracil derivative **20** (50 mg, 0.17 mmol) in pyridine (20 mL/mmol). After stirring for 90 min at room temperature, the reaction was complete (TLC) and the solution was concentrated under vacuum. The residue was purified by silica gel column chromatography (0–20% MeOH in CH_2Cl_2) to afford compound **21** as a pale yellow powder (45 mg, 70%). ^{1}H NMR (DMSO-d_6) δ 1.77 (m, 2H, CH_2), 2.21 (t, 2H, CH_2, J = 7.3), 2.57 (t, 2H, CH_2, J = 7.6), 4.84 (s, 2H, $PhCH_2$), 7.18 (d, 2H, H meta, J = 8.1), 7.25 (d, 2H, H ortho), 8.35 (d, 1H, H6), 11.83 (br s, 1H, NH), 12.05 (br s, 1H, COOH). ^{13}C NMR (DMSO-d_6) δ 27.08 (CH_2), 33.92 (CH_2), 34.90 (CH_2), 51.32 ($PhCH_2$),

95.92 (C5), 128.51 (C arom.), 129.45 (CH ortho), 134.84 (CH meta), 142.09 (C arom.), 146.02 (C6), 151.22 (C2), 160.44 (C4), 175.04 (COOH). MS (ESI-TOF) *m/z* 389.0 (100%, $M+Na)^+$, 391.0 (86%, $M+Na)^+$. HRMS (ESI-TOF) *m/z* calcd for $C_{15}H_{15}N_2O_4{}^{79}Br+Na$ 389.0113 and $C_{15}H_{15}N_2O_4{}^{81}Br+Na$ 391.0092; found 389.0140 (100%) and 391.0136 (87%).

4.2.13. 5-[5-Chloro-4-(2,4-dioxo-3,4-dihydro-2*H*-pyrimidin-1-yl-methyl)-phenyl]-butanoic acid (22). A solution of uracil derivative **20** (62 mg, 0.21 mmol) in a 19/1 mixture of acetic acid and acetic anhydride (30 mL/mmol) was stirred at 60 °C. *N*-Chlorosuccinimide (1.5 equiv) was added little by little over 5 h. When the reaction was judged complete (TLC), the solution was concentrated under vacuum. The residue was purified by silica gel column chromatography, followed by reverse phase HPLC to afford compound **22** as a pale yellow powder (34 mg, 49%). ^{1}H NMR (DMSO-d_6) δ 1.77 (m, 2H, CH_2), 2.19 (t, 2H, CH_2, $J = 7.4$), 2.57 (t, 2H, CH_2, $J = 7.7$), 4.84 (s, 2H, $PhCH_2$), 7.18 (d, 2H, H meta, $J = 8.1$), 7.25 (d, 2H, H ortho, $J = 8.1$), 8.29 (s, 1H, H6), 11.89 (br s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ 27.22 (CH_2), 34.18 (CH_2), 34.96 (CH_2), 51.36 ($PhCH_2$), 107.42 (C5), 128.50 (CH ortho), 129.45 (CH meta), 134.77 (C arom.), 142.18 (C arom.), 143.65 (C6), 151.03 (C2), 160.32 (C4), 175.19 (COOH). HRMS (ESI-TOF) *m/z* calcd for $C_{15}H_{15}N_2O_4{}^{35}Cl+Na$ 345.0618; found 345.0637.

4.2.14. 4-[4-(2,4-dioxo-3,4-dihydro-2*H*-pyrimidin-1-yl-methyl)-phenyl]-buten-3-oic acid methyl ester (23). As for **10**, coupling of **17** (0.26 g, 0.80 mmol) and methyl 3-butenoate (0.10 g, 1.04 mmol) in CH_3CN (8 mL) with Et_3N (0.14 mL) at 60 °C for 24 h afforded after silica gel chromatography compound **23** as major *E*-3 stereoisomer (0.18 g, 75%). ^{1}H NMR ($CDCl_3$) δ 3.28 (dd, 2H, CH_2, $J = 1.0$, $J = 7.1$), 3.74 (s, 3H, OCH_3), 4.91 (s, 2H, $PhCH_2$), 5.71 (d, 1H, H5, $J = 7.8$), 6.34 (m, 1H, CH), 6.50 (d, 1H, CH, $J = 15.9$), 7.16 (d, 1H, H6, $J = 7.8$), 7.25 (d, 2H, H ortho, $J = 8.3$), 7.40 (m, 2H, H meta, $J = 8.3$), 8.98 (br s, 1H, NH).^{13}C NMR ($CDCl_3$) δ 38.53 (CH_2), 51.27 ($PhCH_2$), 52.38 (OCH_3), 103.09 (C5), 123.21 (CH), 127.50 (CH meta), 128.78 (CH ortho), 132.99 (CH), 130.46 (CH) 134.54 (C arom.), 137.69 (C arom.), 144.11 (C6), 151.33 (C2), 163.65 (C4), 172.23 ($COOCH_3$).

4.2.15. 4-(2,4-Dioxo-3,4-dihydro-2*H*-pyrimidin-1-ylmethyl)-butanamide (25). Hydrogenation of **23** (0.26 g, 0.87 mmol), followed by treatment of crude **24** with ammonia in MeOH gave after purification on silica gel **25** (0.19 g, 84%). ^{1}H NMR (DMSO-d_6) δ 1.76 (m, 2H, CH_2), 2.05 (t, 2H, CH_2, $J = 7.4$), 2.54 (t, 2H, CH_2, $J = 7.5$), 3.34 (s, 2H, $PhCH_2$), 5.59 (d, 1H, H5, $J = 7.9$), 6.72 (br s, 1H, $CONH_2$), 7.20 (m, 4H, H arom.), 7.24 (br s, 1H, $CONH_2$), 7.75 (d, 1H, H6, $J = 7.9$), 11.31 (br s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ 27.62 (CH_2), 35.17 (CH_2), 35.35 (CH_2), 50.84 ($PhCH_2$), 102.15 (C5), 128.37 (CH ortho), 129.46 (CH meta), 135.10 (C arom.), 133.07 (C arom. Ph), 142.24 (C arom. Ph), 146.46 (C6), 151.87 (C2), 164.50 (C4), 174.79 ($CONH_2$). HRMS (ESI-TOF) *m/z* calcd for $C_{15}H_{17}N_3O_3+Na$ 310.1168; found 310.1145.

4.2.16. 4-(5-Bromo-2,4-dioxo-3,4-dihydro-2*H*-pyrimidin-1-ylmethyl)-butanamide (26). Bromination of **25** (105 mg, 0.36 mmol) as for **21** afforded after purification on silica gel **26** as a pale yellow powder (110 mg, 82%). ^{1}H NMR (DMSO-d_6) δ 1.76 (m, 2H, CH_2), 2.05 (t, 2H, CH_2, $J = 7.5$), 2.54 (t, 2H, CH_2, $J = 7.8$), 4.84 (s, 2H, $PhCH_2$), 6.71 (br s, 1H, $CONH_2$), 7.18 (d, 2H, H meta, $J = 8.1$), 7.24 (br s, 1H, $CONH_2$), 7.25 (d, 2H, H ortho), 8.36 (s, 1H, H6), 11.83 (br s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ 27.63 (CH_2), 35.18 (CH_2), 35.36 (CH_2), 51.32 ($PhCH_2$), 95.93 (C5), 128.49 (CH ortho), 129.46 (CH meta), 134.76 (C arom.), 142.37 (C arom.), 146.01 (C6), 151.24 (C2), 160.47 (C4), 174.79 ($CONH_2$). HRMS (ESI-TOF) *m/z* calcd for $C_{15}H_{16}N_3O_3{}^{79}Br+Na$ 389.0113 and $C_{15}H_{16}N_3O_3{}^{81}Br+Na$ 390.0252; found 388.0299 (100%) and 390.0282 (83%).

4.2.17. 4-(5-Chloro-2,4-dioxo-3,4-dihydro-2*H*-pyrimidin-1-ylmethyl)-butanamide (27). Chlorination of **25** (100 mg, 0.35 mmol) as for **22** afforded after purification on silica gel **27** as a powder (30 mg, 26%). ^{1}H NMR (DMSO-d_6) δ1.76 (m, 2H, CH_2), 2.05 (t, 2H, CH_2, $J = 7.4$), 2.54 (t, 2H, CH_2, $J = 7.7$), 4.84 (s, 2H, $PhCH_2$), 6.71 (br s, 1H, $CONH_2$), 7.18 (d, 2H, H meta, $J = 8.1$), 7.24 (br s, 1H, $CONH_2$), 7.25 (d, 2H, H ortho), 8.29 (s, 1H, H6), 11.86 (br s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ 27.63 (CH_2), 35.18 (CH_2), 35.36 (CH_2), 51.37 ($PhCH_2$), 107.41 (C5), 128.49 (CH ortho), 129.46 (CH meta), 134.69 (C arom.), 142.37 (C arom.), 143.67 (C6), 151.00 (C2), 160.28 (C4), 174.79 ($CONH_2$). HRMS (ESI-TOF) *m/z* calcd for $C_{15}H_{15}N_2O_4{}^{35}Cl+Na$ 344.0778 and $C_{15}H_{15}N_2O_4{}^{37}Cl+Na$ 346.0748; found 344.0798 (100%) and 346.0798 (4%).

4.2.18. 5-Bromo-1-(4-hexyl-benzyl)-1*H*-pyrimidine-2,4-dione (47) and 5-chloro-1-(4-hexyl-benzyl)-1*H*-pyrimidine-2,4-dione (48). To a solution of 4-hexyl benzoic acid (1 g, 4.85 mmol) in anhydrous THF (7 mL) at 0 °C, a 1 M borane solution in THF (9.7 mL, 9.7 mmol) was added dropwise. The reaction mixture was stirred for 8 h at room temperature, then water (5 mL) was added. After 1 h, the reaction mixture was concentrated under vacuum, taken up in water (60 mL) and extracted with ethyl acetate (60 mL× 3). The organic layers were dried over Na_2SO_4, concentrated under vacuum and the residue purified by silica gel chromatography (CH_2Cl_2/MeOH) to give 4-hexyl alcohol (0.54 g, 58%) and unreacted acid (20%). To a solution of 4-hexyl alcohol (0.53 g, 2.66 mmol) in anhydrous CH_3CN (7 mL) was added $SOCl_2$ (0.24 mL, 3.3 mmol). After heating at 90 °C for 1 h, the reaction mixture was concentrated, taken up in CH_2Cl_2 and washed twice with water. The organic layer was dried, concentrated under vacuum, and purified by silica gel chromatography (CH_2Cl_2) to give 4-hexylbenzyl chloride (0.53 g, 90%). To N^3-benzoyl-uracil (0.48 g, 2.23 mmol), K_2CO_3 (0.31 g, 2.23 mmol) in DMF (5 mL) was added 4-hexylbenzyl chloride (0.52 g, 2.45 mmol). After stirring overnight at room temperature and usual work-up (general procedure A), the benzoylated benzyl-uracil derivative was isolated after silica gel chromatography (CH_2Cl_2) (0.51 g, 58%). Treatment with 33% aq ammonia (room temperature overnight) gave after silica gel chromatography (0–3%

MeOH in CH_2Cl_2) 1-(4-hexyl-benzyl)-1*H*-pyrimidine-2,4-dione (0.30 g, 83%). Compounds **47** (0.17 g, 94%) and **48** (0.12 g, 76%) were obtained by bromination or chlorination of 1-(4-hexyl-benzyl)-1*H*-pyrimidine-2,4-dione. Compound **47**: ^{1}H NMR (DMSO-d_6) δ 0.85 (t, 3H, CH_3), 1.27 (m, 6H, 3×CH_2), 1.53 (m, 2H, CH_2), 2.55 (m, 2H, CH_2), 4.84 (s, 2H, $PhCH_2$), 7.17 (d, 2H, H arom.), 7.23 (d, 2H, H arom.), 8.33 (s, 1H, H6), 11.80 (br s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ 14.77 (CH_3), 22.88 (CH_2), 29.17 (CH_2), 31.74(CH_2), 31.93 (CH_2), 35.65 (CH_2), 51.33 ($PhCH_2$), 95.90 (C5), 128.45 and 129.39 (CH arom.), 134.58 (C arom.), 142.88 (C arom.), 145.98 (C6), 151.21 (C2), 160.42 (C4). HRMS (ESI-TOF) *m*/*z* calcd for $C_{17}H_{20}N_2O_2{}^{79}Br$ 363.0708; found 363.0705; calcd for $C_{17}H_{20}N_2O_2{}^{81}Br$ 365.0688; found 365.0696. Compound**48**: ^{1}H NMR (DMSO-d_6) δ 0.85 (t, 3H, CH_3), 1.27 (m, 6H, 3×CH_2), 1.54 (m, 2H, CH_2), 2.54 (m, 2H, CH_2), 4.83 (s, 2H, $PhCH_2$), 7.17 (d, 2H, H arom.), 7.23 (d, 2H, H arom.), 8.27 (s, 1H, H6), 11.84 (br s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ 14.77 (CH_3), 22.88 (CH_2), 29.17 (CH_2), 31.74 (CH_2), 31.93 (CH_2), 35.65 (CH_2), 51.37 ($PhCH_2$), 107.42 (C5), 128.45 and 129.39 (CH arom.), 134.52 (C arom.), 142.88 (C arom.), 143.62 (C6), 150.98 (C2), 160.25 (C4). HRMS (ESI-TOF) *m*/*z* calcd for $C_{17}H_{20}N_2O_2{}^{35}Cl$ 319.1213; found 319.1206; calcd for $C_{17}H_{20}N_2O_2{}^{37}Cl$ 321.1184; found 321.1206.

4.2.19. 5-Bromo-1-(4-butyl-benzyl)-1*H*-pyrimidine-2,4-dione (49) and 5-chloro-1-(4-butyl-benzyl)-1*H*-pyrimidine-2,4-dione (50). To a solution of 4-butylbenzyl alcohol (1.0 g, 6.1 mmol) in anhydrous CH_3CN (16 mL) was added $SOCl_2$ (0.53 mL, 7.3 mmol). After heating at 90 °C for 1 h, the reaction mixture was concentrated, taken up in CH_2Cl_2 and washed twice with water. The organic layer was dried, concentrated under vacuum, and purified by silica gel chromatography to give 4-butylbenzyl chloride (1.05 g, 95%). To N^3-benzoyl-uracil (1.08 g, 5 mmol), K_2CO_3 (0.69 g, 5 mmol) in DMF (10 mL) was added 4-butylbenzyl chloride (1.0 g, 5.5 mmol). After stirring overnight at room temperature and usual work-up (general procedure A), the benzoylated benzyl-uracil derivative was isolated after silica gel chromatography (CH_2Cl_2) (1.14 g, 63%). Treatment with 33% aq ammonia (room temperature overnight) gave after silica gel chromatography (0–3% MeOH in CH_2Cl_2) 1-(4-butyl-benzyl)-1*H*-pyrimidine-2,4-dione (0.72 g, 89%). Compounds **49** (0.16 g, 82%) and **50** (0.15 g, 85%) have been synthesized by bromination or chlorination of 1-(4-butyl-benzyl)-1*H*-pyrimidine-2,4-dione (0.15 g, 0.6 mmol). Compound **49**: ^{1}H NMR (DMSO-d_6) δ 0.89 (t, 3H, CH_3), 1.29 (m, 2H, CH_2), 1.53 (m, 2H, CH_2), 2.55 (m, 2H, CH_2), 4.84 (s, 2H, $PhCH_2$), 7.18 (d, 2H, H arom.), 7.23 (d, 2H, H arom.), 8.33 (s, 1H, H6), 11.80 (br s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ 14.58 (CH_3), 22.57 (CH_2), 33.91 (CH_2), 35.31 (CH_2), 51.33 ($PhCH_2$), 95.90 (C5), 128.45 and 129.40 (CH arom.), 134.57 (C arom.), 142.85 (C arom.), 145.97 (C6), 151.21 (C2), 160.41 (C4). HRMS (ESI-TOF) *m*/*z* calcd for $C_{15}H_{17}N_2O_2{}^{79}Br$+Na 359.0371; found 359.0387; calcd for $C_{15}H_{17}N_2O_2{}^{81}Br$+Na 361.0351; found 361.0356. Compound **50**: ^{1}H NMR (DMSO-d_6) δ 0.89 (t, 3H, CH_3), 1.30 (m, 2H, CH_2), 1.53 (m, 2H, CH_2), 2.56 (m, 2H, CH_2), 4.84 (s, 2H, $PhCH_2$), 7.18 (d, 2H, H arom.), 7.24 (d, 2H, H arom.), 8.27 (s, 1H, H6), 11.83 (br s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ 14.58 (CH_3), 22.57 (CH_2), 33.93 (CH_2), 35.31 (CH_2), 51.37 ($PhCH_2$), 107.42 (C5), 128.45 and 129.40 (CH arom.), 134.53 (C arom.), 142.85 (C arom.), 143.63 (C6), 150.99 (C2), 160.26 (C4). HRMS (ESI-TOF) *m*/*z* calcd for $C_{15}H_{16}N_2O_2{}^{35}Cl$ 291.0900; found 291.0887; calcd for $C_{15}H_{16}N_2O_2{}^{37}Cl$ 293.0871; found 293.0939.

4.3. Inhibition assays

Tested compounds were dissolved in DMSO. The concentrations of stock solutions were determined using the molar extinction coefficient of thymidine (ε 9700 at 267 nm). For compounds **1**, **6**, **7**, **8**, **11**, **21** and **36**, the discrepancies between the K_i values previously reported[18] and those given in Table 1 are due to the concentration calculation (molar extinction coefficient of thymine versus thymidine). The K_i determinations TMPK were performed as previously described.[18] The data are the mean of three experiments. The standard deviation ranges from 3% to 11%.

4.4. Biological assays on *M. bovis* (BCG)

A micro-method of culture was performed in 7H9 Middlebrook broth medium containing 0.2% glycerol, 0.5% Tween 80 and supplemented with oleic acid, albumin, dextrose and catalase (Becton-Dickinson). Dilutions of each compound were prepared from DMSO stock solutions (1–5 mg/mL), then deposited in 96-well plates at final concentrations ranging from 1 to 400 μg/mL. Control wells were treated with an equivalent amount of DMSO. Each condition was run in triplicate, using as a positive control cells incubated without compound. The bacterial inoculum was prepared at a concentration in the order of 10^7 bacteria (*M. bovis* BCG 1173P2) in 7H9 medium and stored at −80 °C until used. The bacteria, adjusted to 10^5 per mL, were delivered in a volume of 100 μL per well. The covered plates were sealed with Parafilm and incubated at 37 °C in plastic boxes in a humidified normal atmosphere. On day 8 of incubation, 30 μL of a resazurin (Sigma) solution at 0.01% (wt/vol) in water were added to each well. After overnight incubation at 37 °C, plates were assessed for color development using optical density differences at 570 and 630 nm on a ELISA reader. A change from blue to pink indicated reduction of resazurin and therefore bacterial growth. MIC_{50} values were determined using the dose–response curves [DO = f(Log C)].

4.5. Cytotoxicity assay (against VERO cells lines)

Cytotoxicity evaluation was based on the colorimetric MTT assay (Roche Applied Science). VERO cells were maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum, 50 mg/mL penicillin and 100 μg/mL streptomycin. Proliferating cells seeded in 96-well plates (6 × 103 cells/mL) were incubated with the compounds at different concentrations

(ranging from 5 to 400 μg/mL) in a final volume of 100 μL at 37 °C in a humidified atmosphere with 5% CO_2. After 72 h, 20 μL of MTT labeling reagent at 7.5 mg/mL in PBS was added to each well and cells were further incubated for 4 h under the same conditions. The purple formazan crystals formed were dissolved by addition to each well of a solution of 10% SDS and 0.01 M HCl. After complete solubilization of formazan, optical density at 550/570 nm was measured with an Elisa reader. Each condition was run in duplicate using as a positive control cells incubated in absence of compound.

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Supplementary data

Experimental details and analytical data for compounds **3**, **17**, and for compounds from series C3, C5 and C6 (Schemes 1 and 2) can be found in the online version. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2008.04.045.

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