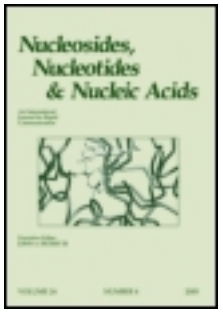

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A NEW FAMILY OF INHIBITORS OF *MYCOBACTERIUM TUBERCULOSIS* THYMIDINE MONOPHOSPHATE KINASE

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Tuberculosis (TB) ranks among the leading causes of death worldwide from a single infectious agent, particularly in developing countries. The emergence of multidrug resistant strains of *Mycobacterium tuberculosis* and the unfortunate revival of TB due to HIV infection have rendered the quest for new antimycobacterial drugs against TB a priority.

We currently are studying thymidine monophosphate kinase of *M. tuberculosis* (TMPKmt) as a potential target for developing new antituberculosis agents.[1] TMPK catalyzes the transfer of γ-phosphate from ATP to dTMP. TMPK is the last specific enzyme for the synthesis of dTTP and it represents a key enzyme in its metabolism. Biochemical and structural characterization of *M. tuberculosis* TMPK (TMPKmt) revealed distinct features when compared to its counterpart from yeast, *E. coli* and human (TMPKh).[1–3]

As a part of our ongoing program to design inhibitors of TMPKmt.[4–7] we applied a new fragment-based algorithm called LEA3D to the dTMP binding site of TMPKmt with the aim to generate new ligand families.[8] Molecule (**1**), thus, was identified as a potential inhibitor of TMPKmt, the benzyl ring acting as a substitute for the sugar part of dTMP (Figure 1). The target amide **1**, as well as the carboxylic acid derivative **2**, were synthesized. The evaluation of their inhibitory potency (*Ki*) on recombinant TMPKmt

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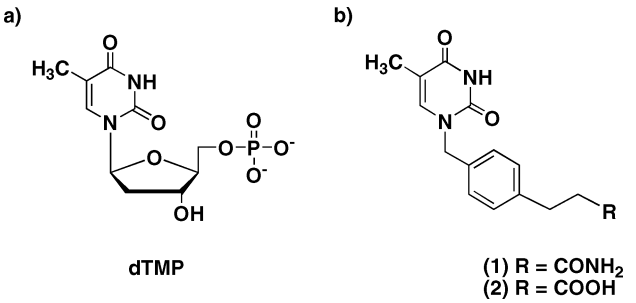


FIGURE 1 Structures of dTMP and 3-(4-thymidylmethyl-phenyl)-propionamide (**1**).

concluded to the possible replacement of the sugar part by a benzyl group (Table 1).

We decided to further explore this family of benzyl-thymine derivatives by varying the arm length in para position of the benzyl ring, the nature of the terminal function, as well as the substituent at C-5 position on the pyrimidine ring (CH_3, Br, or Cl). We report here the results obtained in the thymine series.

CHEMISTRY

The benzyl-thymine derivatives were synthesized according to palladium-catalyzed coupling reactions between an aryl halide and a suitable alkene or alkyne. Typically, the first step is the N^1-arylation of

TABLE 1 Evaluation of benzyl thymine derivatives on *M. tuberculosis* TMPK and on *M. bovis* (BCG) bacteria

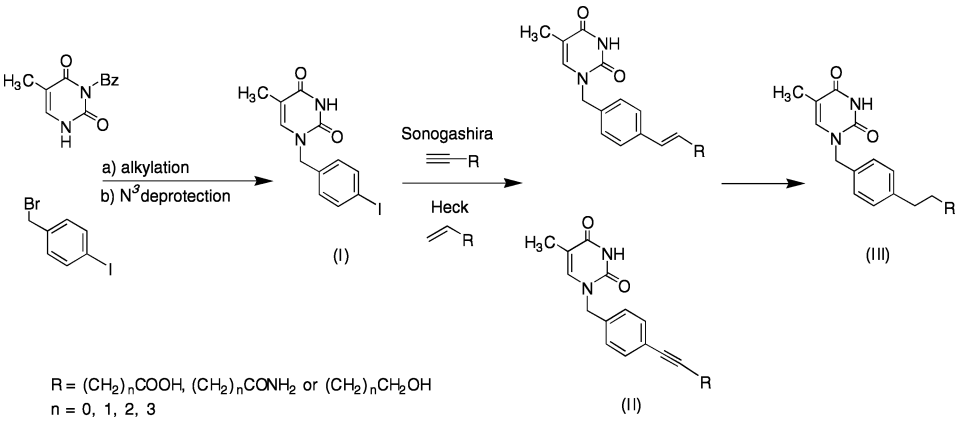
Cpd	R	TMPKmt *Ki* (μM)	FlexX score	MIC_{50} (μg/mL)
1	$(CH_2)_2CONH_2$	89	−32	N.I.[b]
2	$(CH_2)_2COOH$	55	−30	N.I.[b]
3	CH=CHCOOH	N.I.[a]		N.D.
4	$CH{=}CHCONH_2$	195		N.D.
5	$(CH_2)_3COOH$	13	−36	N.I.[b]
6	$CH{=}CHCH_2COOH$	39	−28	N.I.[b]
7	$C{=}CCH_2CH_2OH$	70	−29	N.I.[b]
8	$(CH_2)_3CH_2OH$	51	−33	N.D.
6	$(CH_2)_3CONH_2$	112	−32	N.I.[b]
7	$(CH_2)_4COOH$	58	−41	120
8	$(CH_2)_4CONH_2$	55		N.D.
9	$(CH_2)_5COOH$	47	−48	195
10	$(CH_2)_5CONH_2$	27	−36	230

N.D.: not determined.

[a]N.I.: no inhibition until 2 mM.

[b]N.I.: no inhibition until 400 μg/mL.

The MIC_{50} values are the means of two independent assays performed in duplicate.



SCHEME 1 General synthetic scheme.

thymine (Scheme 1). For selectivity purpose, the pyrimidine moiety was suitably N^3 protected by a benzoyl group. Starting from this key halide intermediate (I), various commercially available carboxylic acids, esters or alcohols (according to the selected chain length) were introduced by Heck or Sonogashira coupling reaction. The resulting coupling products (II) were further hydrogenated into saturated derivatives (III).

INHIBITORY ACTIVITY ON TMPKmt

The ability of synthesized compounds to inhibit in vitro TMPKmt in the presence of ATP and dTMP was examined on recombinant enzyme using an enzymatic assay as previously described.[1] Selectivity of the most active compounds was measured against TMPKh. All molecules tested exhibited inhibitory potency with *Ki* values ranged between 13 and 195 μM (except for compound **3** that is devoid of activity) (Table 1). The highest inhibitor corresponds to molecule **5** having a chain length of 4 carbons with a terminal carboxylic acid function.

STRUCTURAL ANALYSIS

In an attempt to rationalize these results, the inhibitors were docked into the active site of TMPKmt. The crystal structure of TMPKmt in complex with dTMP (PDB structure 1G3U) was selected for the docking using the program FlexX as in our previous studies (Table 1).[8] Molecular modelling suggested possible orientations of the ligands: due to the arm flexibility, two binding modes are predicted, the alkyl chain adopting either an elbowed or extended conformation (Figure 2). An interaction between Arg95 and the terminal COOH of molecule **5** can explain that the optimal length within the carboxylic acid series corresponds to an alkyl chain

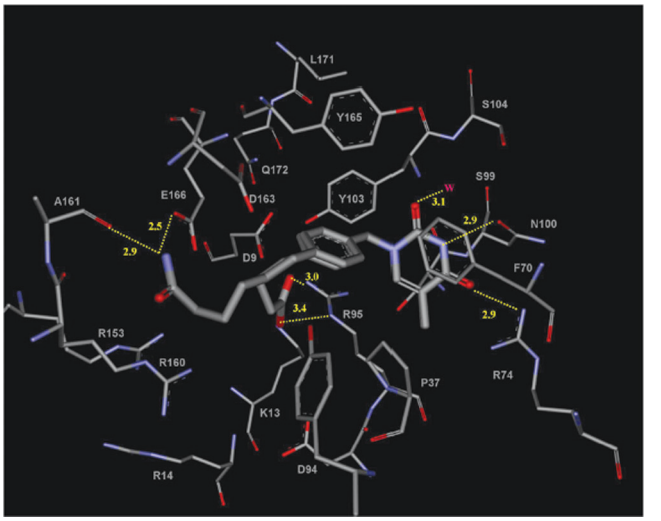


FIGURE 2 The predicted binding mode of molecules **5** and **10**. The hydrogen bond network is depicted in yellow. The thymine base is able to form a significant π-stack with Phe70 and three hydrogen bonds with Arg74, Asn100 and a water molecule (W in magenta). The carboxylate group of **5** is able to interact with Arg 95, the nitrogen of the amide function of **10** is able to interact with Glu 166 and the backbone carboxyl of Ala 161.

of 4 carbons. Within the carboxamide series, the strongest inhibitors have a 6 carbons chain, that corresponds to an optimal interaction between the nitrogen of the amide function with Glu166 and the backbone carboxyl of Ala161 (Figure 2, molecule **10**).

ANTIMYCOBACTERIAL ACTIVITY AND CYTOTOXICITY

These thymine derivatives were evaluated for inhibitory activity against whole-cell *M. bovis* (BCG strain) (Table 1). Compounds **7**, **9**, and **10** having a chain length of 5 or 6 carbons exhibited moderate activity (MIC_{50} up to 120 μg/ml) without any cytotoxicity at concentrations up to 400 μg/ml (Vero cells).

In conclusion, among the molecules investigated we identified a new family of benzyl-thymines as potent and selective inhibitors of recombinant TMPKmt. Some members of these series showed moderate inhibitory potency on the growth of *M. bovis* (BCG) bacteria while no cytotoxiciy was detected. We are currently working on further modification and optimization of this new class of molecules.

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