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Research Article

Design, Synthesis, and Antimycobacterial Activity of Novel Theophylline-7-Acetic Acid Derivatives With Amino Acid Moieties

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The theophylline-7-acetic acid (7-TAA) scaffold is a promising novel lead compound for antimycobacterial activity. Here, we derive a model for antitubercular activity prediction based on 14 7-TAA derivatives with amino acid moieties and their methyl esters. The model is applied to a combinatorial library, consisting of 40 amino acid and methyl ester derivatives of 7-TAA. The best three predicted compounds are synthesized and tested against *Mycobacterium tuberculosis* H37Rv. All of them are stable, non-toxic against human cells and show antimycobacterial activity in the nanomolar range being 60 times more active than ethambutol.

Key words: antimycobacterial activity, discriminant analysis, drug design, theophylline-7-acetic acid derivatives, tuberculosis

Abbreviations: 2D and 3D QSAR, two-dimensional and three-dimensional quantitative structure – activity relationship; 7-TAA, theophylline-7-acetic acid; anti-TB, antitubercular; BCG, *M. bovis* bacilli Calmette–Guerin; MIC, minimum concentration of compound required to inhibit bacterial growth completely; TB, tuberculosis.

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Despite the availability of effective treatment, tuberculosis (TB) is still one of the three main infectious disease killers worldwide. Among HIV-infected patients, TB is the leading cause of mortality.a TB is caused by *Mycobacterium tuberculosis* – a bacterium with unique cell wall structure (1). More than 60% of the mycobacterial cell wall is lipids. Lipids form a thick shell around the micro-organism and prevent attacks by lysozyme and oxygen radicals inside of macrophages. This lipid barrier explains the resistance of *M. tuberculosis* against stains, many antibiotics, acids and alkaline compounds. The current anti-TB treatment consists of multi-drug attack (between 2 and 4 drugs of different groups) for a long period of time (between 6 and 18 months) (2,3). Such treatment is associated with lower rates of failure, relapses and acquired drug resistance (4) but also with high toxicity, poor tolerance, high cost and less efficacy on the drug-resistant forms of *M. tuberculosis*. The need of more effective short-course chemotherapeutics is urgent and critical.

The advances in anti-TB drug and vaccine research during the last decades are encouraging. More than 20 new chemical entities in preclinical research and nine compounds belonging to five chemical classes (flouroquinolones, nitroimidazoles, diarylquinolines, oxazolidinones, and ethylenediamines) are in clinical trials (3,4). Several new vaccines are also under development, including recombinant BCG, inactivated whole-cells, adjuvanted subunits, and viral vector-based.b Aside from these advances, many other compounds from different chemical classes are currently tested against various strains of *M. tuberculosis*. Ligand-based methods as pharmacophore search, 2D and 3D QSAR studies, fingerprints, and similarity search are involved actively (5–9), but structure-based methods targeting specific *M. tuberculosis* enzymes also take place (10–14).

Recently, we developed 14 theophylline-7-acetic acid (7-TAA) derivatives with amino acid moiety and tested them for antimycobacterial activity (15). The tests showed that some of the compounds are between 11 and 28 times more active than ethambutol against *M. tuberculosis* H37Rv. The compounds were non-toxic on human embryonal kidney cell line HEK-293T. Here, we used the structures and activities of these 14 derivatives and develop a model for anti-TB activity prediction. We applied this model on a combinatorial library, consisting of 20 naturally occurring amino acid derivatives of 7-TAA and their methyl esters. The best predicted compounds were synthesized and tested against *M. tuberculosis* H37Rv. All of them

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showed antimycobacterial activity 60 times higher than that of ethambutol.

Methods and Materials

Discriminant analysis

The structures of the 14 synthesized and tested 7-TAA derivatives were described by 178 molecular descriptors using MDL QSAR v.2.2.c The descriptors were grouped into five types: molecular connectivity χ (chi) indices, which represent molecular structure by encoding significant topological features of whole molecule; κ shape indices – a family of graph-based structure descriptors that represent shape; electrotopological state (*E*-state) indices, which represent the electron density at each atom and the ability of those electrons to participate in intermolecular interactions; molecular properties – weight, *logP*, *log D7.4*, number of rings, number of hydrogen bond donors and acceptors, etc.; and 3D molecular properties such as polarizability, surface area, volume, etc. Compounds with MIC values in the range 0.260–0.647 μM were classified as *Class 1*, whereas compounds with MIC values between 5.477 and 5.964 μM were classified as *Class 0*.

The descriptors relevant to antimycobacterial activity were selected by genetic algorithm (GA) (16), as implemented in the MDL QSAR v.2.2. GA allows one to select a subset of the most significant predictors using two evolutionary operations: random mutation and genetic recombination (crossover). The algorithm was used in the study with default values for the size of initial population (*32*), choice of parents (tournament selection), types of crossover (uniform crossover) and mutation (one-point mutation), and fitness function (Friedman's lack-of-fit scoring function with two parameters) (17). The selected variables were used in a stepwise linear regression, as implemented in the MDL QSAR v.2.2. It was used in a forward mode with default value for F-to-enter (2.00) and F-to-remove (1.99).

The derived models were assessed in terms of *sensitivity* (true *Class 1*/all *Class 1* compounds), *specificity* (true *Class 0*/all *Class 0* compounds), and *accuracy* (true *Class 1* and *0*/all compounds). The best performing model was validated by leave-one-out cross-validation (LOO-CV) at cutoff 0.5. Values above 0.5 were considered as *Class 1*; below 0.5 – as *Class 0*.

Synthesis

Reagents were of commercial grade and used without further purification. Thin layer chromatography (TLC) was performed on aluminum sheets precoated with Merck Kieselgel 60 F_{254} 0.25 mm (Merck, Kenilworth, NJ, USA). Flash column chromatography was carried out using Silica Gel 60 230–400 mesh (Merck). Commercially available solvents for reactions, TLC and column chromatography were used after distillation. Melting points of the compounds were determined using "Electrothermal" MEL-TEMP apparatus (uncorrected). The NMR spectra were recorded on a Bruker Avance II+ 600 spectrometer (600 for ^{1}H MHz, 150 MHz for ^{13}C NMR) with TMS as internal standards for chemical shifts (δ, ppm). ^{1}H and ^{13}C NMR data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, br = broad, m = multiplet), coupling constants (Hz), integration. LC–MS analyses were performed using a Q Exactive Plus Orbitrap Mass spectrometer (Thermo Fisher Scientific, Bremen, Germany), equipped with an electrospray (ESI) probe. The spectra were recorded on a positive mode using a MS Full Scan mode. ^{1}H, ^{13}C NMR, and LC-MS data are given in Supporting Information.

Synthesis of (S)-N-(2-(theophylline-7-yl)acetyl) phenylalanine methyl ester

To a stirred suspension of theophyline-7-acetic acid (0.500 g; 2.1 mmol) and L-phenylalanine methyl ester.HCl (0.455 g; 2.1 mmol) in CH_2Cl_2 (50 mL) was added N-ethyldiisopropylamine (0.36 mL; 2.1 mmol). After 10 min, the reaction became a clear solution and EDC (0.443 g, 2.31 mmol), and HOBt (0.313 g; 2.31 mmol) were added. The mixture was stirred overnight at r.t., quenched with water and extracted with CH_2Cl_2 (2 × 10 mL). The combined organic extracts were washed with water (3 × 50 mL) and sat. aq. $NaHCO_3$ (1 × 50 mL), dried above Na_2SO_4 and concentrated under reduced pressure to give the desired product. Yield: 99%; white crystals; m.p. 174–176 °C.

Synthesis of (S)-N-(2-(theophylline-7-yl)acetyl) tyrosine methyl ester

To a stirred suspension of theophyline-7-acetic acid (0.500 g; 2.1 mmol) and L-tyrosine methyl ester.HCl (0.486 g; 2.1 mmol) in CH_2Cl_2 (50 mL) was added N-ethyldiisopropylamine (0.36 mL; 2.1 mmol). After 10 min the reaction became a clear solution and EDC (0.443 g, 2.31 mmol), and HOBt (0.313 g; 2.31 mmol) were added. The mixture was stirred overnight at r.t., quenched with water (50 mL) and extracted with CH_2Cl_2/MeOH = 10:1 (3 × 20 mL). The combined organic extracts were dried above Na_2SO_4 and concentrated under reduced pressure. The product was purified by flash column chromatography (silica, CH_2Cl_2/MeOH = 20:1). Yield: 92%; white crystals; m.p. 112–116 °C.

Synthesis of (S)-N-(2-(theophylline-7-yl)acetyl) histidine methyl ester

To a stirred suspension of theophyline-7-acetic acid (0.500 g; 2.1 mmol) and L-histidine methyl ester.HCl (0.486 g; 2.1 mmol) in CH_2Cl_2 (50 mL) were added N-ethyldiisopropylamine (0.36 mL; 2.1 mmol), EDC (0.443 g, 2.31 mmol), and HOBt (0.313 g; 2.31 mmol). The mixture

was stirred 48 h at r.t. A white precipitate, the highly hygroscopic product, was formed over time. Filtration afforded the crude product, which was additionally purified by recrystallization from MeOH. Yield: 48%; white crystals; m.p. 102–105 °C.

Antimycobacterial activity

The antimycobacterial activity was determined through the proportional method of Canetti toward reference strain *M. tuberculosis* H37Rv (18). A sterile suspension/solution of each tested compound was added to Löwenstein–Jensen egg-based medium before its coagulation (30 min at 85 °C). Each compound was tested at four concentrations – 2, 0.2, 0.1, and 0.05 mg/mL (in DMSO). Tubes with Löwenstein–Jensen medium (5 mL) containing tested compounds and those without them (controls) were inoculated with a suspension of *M. tuberculosis* H37Rv (105 cells/mL) and incubated for 45 days at 37 °C. The ratio between the number of colonies of *M. tuberculosis* grown in medium containing compounds and the number of colonies in control medium were calculated and expressed as percentage of inhibition. The MIC is defined as the minimum concentration of compound required to inhibit bacterial growth completely (0% growth). The MIC values are calculated and given as μM.

Cytotoxicity

The human hepatocellular crcinoma cell line HEP-G2 was obtained from the German Collection of Microorganisms and Cell Cultures. Cells were kept in controlled environment RPMI-1640 medium, supplemented with 10% heat-inactivated fetal calf serum and 2 mM L-glutamine, at 37 °C in an incubator with 5% CO_2 humidified atmosphere.

The cytotoxicity of the newly synthesized compounds was assessed using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide]-dye reduction assay as described by Mossman with some modifications (19,20). In brief, exponentially growing cells were seeded in 96-well microplates (100 μL/well) at a density of 3.5×10^5 cell/mL and allowed to grow for 24 h before the exposure to the studied compounds. Stock solutions of the tested compounds were freshly prepared in DMSO and thereafter were subset to serial dilutions with growth medium to obtain the desired final concentrations. At the final dilutions the solvent concentration never exceeded 0.5%. Cells were exposed to the tested agents for 72 h, whereby for each concentration a set of at least eight separate wells was used. After the exposure period MTT solution (10 mg/mL in phosphate-buffered saline) aliquots (10 μL/well) were added to each well. The plates were further incubated for 4 h at 37 °C and the MTT-formazan crystals formed were dissolved through addition of 110 mL of 5% HCOOH in 2-propanol. The MTT-formazan absorption of the samples was measured by a multimode microplate reader DTX 880 (Beckman Coulter, Indianapolis, IN, USA) at 580 nm. Cell survival fractions were calculated as percentage of the untreated control. The experimental data were fitted to sigmoidal concentration–response curves and the corresponding IC_{50} values (concentrations causing 50% reduction in cellular survival versus the untreated control) were estimated via non-linear regression (GraphPad Software, Inc., La Jolla, CA, USA).

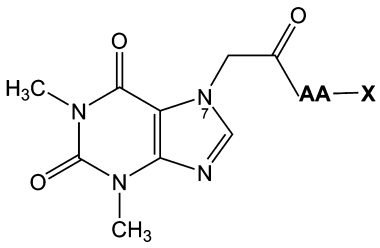
Results

Discriminant analysis of 7-TAA derivatives with antimycobacterial activity

A combinatorial library of 40 7-TAA derivatives with amino acid moiety was created: 20 naturally occurring amino acid derivatives and 20 amino acid methyl esters (Figure 1). The molecules were modeled, energy minimized by MM+ (21), and the electron density in molecules was calculated by AM1 (22).

Fourteen compounds of the library were synthesized and tested previously for antimycobacterial activity. Results are given in Table 1. Based on their MIC values, the compounds were divided in two classes. Half of the compounds had MIC values in the range 0.260–0.647 μM, whereas the MIC values of the other half were between 5.477 and 5.964 μM. The first class was assigned as *Class 1*, the second – as *Class 0*. A discrimininant analysis was applied to derive a model for anti-TB activity prediction. The chemical structures were described by 178 molecular descriptors computed using MDL QSAR version 2.2.c The descriptors relevant to the activity were selected by GA and stepwise regression as described in the Methods and materials section. The best performing model in terms of *sensitivity* and *specificity* at cutoff 0.5 is given below:

$$\begin{aligned} Class1 = {} & 1.541(\pm 0496) G_{\min} + 0.005(\pm 0.002) f_w \\ & - 0.344(\pm 0.111) SsCH3\_acnt \\ & - 0.508(\pm 0.109) SssCH2\_acnt \\ & + 0.508(\pm 0.230) SssS + 2.445 \end{aligned}$$



AA: Ala, Arg, Asn, Asp-CH_3, Cys, Gln, Glu-CH_3, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, Val
X: H, CH_3

Figure 1: Structure of 7-TAA derivatives with amino acid substituents.

Table 1: Combinatorial library of 7-TAA derivatives with amino acid moiety. Antimycobacterial activity (MIC), descriptors, relevant to the anti-TB activity, experimental and predicted class affiliation are given

ID[a]	MIC, μM[b]	Class exp[c]	G_{min}	f_w	*SsCH3_acnt*	*SssCH2_acnt*	*SssS*	Class pred[d]
7-Ala (3b)	0.647	1	−1.165	309.282	3	1	0.000	**0.417**
7-Arg			−1.188	395.398	2	4	0.000	−0.204
7-Asn			−1.889	338.280	2	1	0.000	−0.035
7-Asp-CH_3			−1.875	353.291	3	1	0.000	−0.286
7-Cys			−1.206	341.348	2	2	0.000	0.524
7-Gln			−1.312	366.334	2	3	0.000	−0.026
7-Glu-CH_3			−1.305	381.345	3	3	0.000	−0.287
7-Gly (3a)	6.774	0	−1.169	295.255	2	2	0.000	**0.607**
7-His			−1.222	374.336	2	2	0.000	0.659
7-Ile			−1.114	351.362	4	2	0.000	0.025
7-Leu (3d)	5.923	0	−1.115	351.362	4	2	0.000	**0.032**
7-Lys			−1.115	367.385	2	5	0.000	−0.734
7-Met			−1.115	369.401	3	3	1.482	0.700
7-Phe			−1.161	385.379	2	2	0.000	0.806
7-Pro (3h)	5.964	0	−1.033	335.319	2	4	0.000	**−0.481**
7-Ser			−1.440	325.281	2	2	0.000	0.086
7-Thr			−1.473	339.308	3	1	0.000	0.268
7-Trp (3e)	0.471	1	−1.174	424.416	2	2	0.000	**0.941**
7-Tyr			−1.222	401.379	2	2	0.000	0.789
7-Val (3c)	5.923	0	−1.132	337.335	4	1	0.000	**0.577**
7-Ala-CH_3 (2b)	0.619	1	−0.811	323.308	4	1	0.000	**0.824**
7-Arg-CH_3			−0.879	409.425	3	4	0.000	−0.005
7-Asn-CH_3			−1.653	352.307	3	1	0.000	0.052
7-Asp-$(CH_3)_2$			−1.639	367.318	4	1	0.000	−0.199
7-Cys-CH_3			−0.896	355.374	3	2	0.000	0.726
7-Gln-CH_3			−1.058	380.360	3	3	0.000	0.087
7-Glu-$(CH_3)_2$ (2f)	>5.060	0	−1.057	395.372	4	3	0.000	**−0.302**
7-Gly-CH_3 (2a)	0.647	1	−0.581	309.282	3	2	0.000	**0.976**
7-His-CH_3	***0.128***	***1***	−0.962	388.363	3	2	0.000	0.783
7-Ile-CH_3			−0.778	365.389	5	2	0.000	0.267
7-Leu-CH_3 (2d)	5.477	0	−0.769	365.389	5	2	0.000	**0.407**
7-Lys-CH_3			−0.768	381.412	3	5	0.000	−0.476
7-Met-CH_3 (2g)	0.260	1	−0.762	383.428	4	3	1.548	**0.214**
7-Phe-CH_3	***0.125***	***1***	−0.878	399.406	3	2	0.000	0.965
7-Pro-CH_3 (2h)	5.725	0	−0.598	349.346	3	4	0.000	**0.282**
7-Ser-CH_3			−1.204	339.308	3	2	0.000	0.173
7-Thr-CH_3			−1.237	353.335	4	1	0.000	0.355
7-Trp-CH_3 (2e)	0.456	1	−0.923	438.443	3	2	0.000	**1.171**
7-Tyr-CH_3	***0.120***	***1***	−0.964	415.406	3	2	0.000	0.910
7-Val-CH_3 (2c)	0.570	1	−0.792	351.362	5	1	0.000	**0.545**
EMB.2HCl[e]	7.220							

The experimental MIC values and the class affiliation of the best predicted and synthesized new derivatives are given in bold italic.
[a]The number of compounds synthesized and tested previously are given in bold (21).
[b]Minimum concentration of compound required to inhibit bacterial growth completely. The MIC of the best predicted compounds are given in bold.
[c]Class affiliation according to experimental MIC.
[d]Class affiliation predicted by the discriminant model. The class affiliations predicted by LOO-CV are given in bold.
[e]Ethambutol dihydrochloride (reference compound).

$n = 14$ *sensitivity* $= 1.000$ *specificity* $= 1.000$ *accuracy* $= 1.000$ at cutoff $= 0.5$

LOO-CV *sensitivity* $= 0.714$ *specificity* $= 0.714$ *accuracy* $= 0.714$ at cutoff $= 0.5$

where G_{min} represents the minimum *E*-state value in the molecule; f_w gives the molecular weight; *SsCH3_acnt* and *SssCH2_acnt* count the CH_3 and CH_2 groups in molecule, respectively; *SssS* is the sum of all bisubstituted sulfur *E*-state values in molecule. No intercorrelation between the descriptors in the model was observed ($r < |0.7|$). The descriptors relevant to the anti-TB activity and the predicted by leave-one-out cross-validation (LOO-CV) class affiliations are given in Table 1.

The LOO-CV recognized correctly five of seven compounds of *Class 1* and the same number of *Class 0*. The compounds **7-Ala** and **7-Met-CH_3** were underestimated

(predicted to be *Class 0* but they are *Class 1*), whereas the compounds **7-Gly** and **7-Val** were overestimated (predicted to be *Class 1* but they are *Class 0*).

Anti-TB activity prediction of the combinatorial library of 7-TAA derivatives

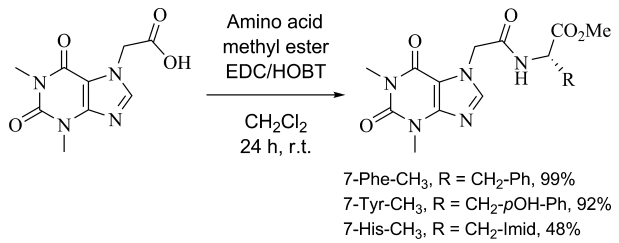
The model derived on the set of 14 7-TAA derivatives was used to predict the anti-TB activities of the non-synthesized compounds from the combinatorial library. The class affiliations are given in Table 1 as *Class* pred (last column). At cutoff 0.5, the values above 0.5 are considered as predicted *Class 1*, otherwise – as predicted *Class 0*. Among the 26 non-synthesized 7-TAA derivatives, only nine were predicted to belong to *Class 1*. The best three predicted from the methyl ester subset were **7-Phe-CH_3**, **7-Tyr-CH_3**, and **7-His-CH_3**. They were synthesized and tested for antimycobacterial activity.

Synthesis and antimycobacterial activity of the best predicted compounds

The synthesis of the three target structures was accomplished in one step by coupling theophylline-7-acetic acid with an appropriate amino acid methyl ester. The reactions were performed using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide and hydroxybenzotriazole (EDC/HOBt) as coupling reagents in dichloromethane (Scheme 1). The phenylalanine and the tyrosine analogues were isolated in quantitative yields. The histidine derivative appeared to be very hygroscopic, and was isolated without aqueous work up, by direct filtration of the reaction mixture and recrystallization of the collected solid from MeOH.

The antimycobacterial activity of the newly synthesized 7-TAA derivatives was tested as described in Methods and materials section. The MIC values and the class affiliation are given in Table 1 (the bold italic values in columns 2 and 3). They range from 0.128 to 0.120 μM and are the highest activities in the set of the 7-TAA derivatives.

The newly synthesized compounds displayed chemical stability under the conditions of antimycobacterial determination. No changes were detected on their ^{1}H NMR spectra in dimethyl sulfoxide-d6 after 5, 12, and 45 days heating at 37 °C of the NMR tubes with the initially prepared solutions.



Scheme 1: Synthesis of theophylline acetamides.

In addition, the cytotoxic (possible hepatotoxic) activity of the compounds was assessed against the human transformed liver HEP-G2 cells after 72 h exposure. The substances at the maximal applied concentration of 400 μM did not reach 50% inhibition of cellular viability, hence they were not cytotoxic against human cells (IC_{50} > 400 μM).

Discussion

In this study, 14 theophilline-7-acetic acid derivatives with amino acid substituents were classified as highly active (*Class 1,* MIC < 1 μM) and less active (*Class 0*, MIC > 5 μM) antimycobacterials according to MIC values derived previously. A set of 178 descriptors was used to derive a model discriminating between highly active and less active compounds. The relevant descriptors were selected by GA and stepwise regression. The best performed model showed 71% accuracy in the LOO-CV. It contained five descriptors relevant for the anti-TB activity of the studied compounds: G_{min}, f_w, *SsCH3_acnt*, *SssCH2_acnt,* and *SssS.*

The descriptor G_{min} represents the minimum *E*-state value in the molecule. It is related to the most electrophilic atom and takes negative values. Its coefficient in the model is positive which means that less polar compounds will belong to *Class 1*. Indeed, five of the compounds in *Class 1* are amino acid methyl esters which are less polar than the corresponding amino acid derivatives.

The descriptor f_w gives the molecular weight of the compounds. Its positive coefficient in the model points that the weightier molecules will belong to *Class 1*. The amino acid methyl esters are weightier than the corresponding amino acid derivatives and potentially are more active. The descriptor f_w correlates well with the *logP* values of the tested compounds (r = 0.743). The substitution of f_w with *logP* does not change sensitivity, specificity, and accuracy of both fitted and LOO-CV models (data not shown). Thus, f_w could be considered as a lipophilicity measure in this particular case. The more lipophilic 7TAA derivatives are more active against *M. tuberculosis* H37Rv. As the mycobacterial cell wall is formed mainly by lipids, the more lipophilic molecules will be able to cross the wall and enter the cell.

The descriptors *SsCH3_acnt* and *SssCH2_acnt* count the CH_3 and CH_2 groups in molecule, respectively. Both have negative coefficients in the model which means that compounds with less number of CH_3 and CH_2 groups will be more active. The methyl esters have one more CH_3 group than the corresponding amino acid derivatives but the

same number of CH_2 groups. The negative contribution of CH_3 count seems to be controversial to the contributions of G_{min} and f_w. Actually, this descriptor captures the three exceptions to the methyl ester subset – the esters of Glu, Leu, and Pro – which are expected to belong to *Class 1* according to the first two descriptors in the model but they belong to *Class 0*, and the two exceptions to the amino acid subset – the Ala and Trp derivatives – which are expected to belong to *Class 0* but they belong to *Class 1*.

The descriptor *SssS* represents the sum of all bisubstituted sulfur *E*-state values in molecule. As only Met has such atom, this descriptor takes a value only for the Met analogs. In the tested set there is only one Met analog – compound **7-Met-CH_3**. It belongs to *Class 1* and brings a positive coefficient for the descriptor *SssS*. As a unique structure in the set, this compound was mispredicted in the LOO-CV.

The derived model was used to predict the antimycobacterial activity of 26 novel non-synthesized 7TAA derivatives. The three best predicted compounds were synthesized and tested. All of them showed activities higher than the activities in the initial set and 60 times more active than the classical anti-TB drug ethambutol.

Recently, some of these 7TAA compounds were screened for antiproliferative activity against two human leukemic cell lines, namely acute myeloid leukemia (HL-60) and chronic myeloid leukemia (K-562) (23). The IC_{50} values were in the range 330.4–1051.9 μM. The amino acid esters demonstrated higher activity than the corresponding acidic derivatives. Higher chemosensitivity was observed against HL-60. The compounds were non-toxic on the non-malignant cell line HEK-293T. No correlation exists between the cytotoxic IC_{50} values and the antimycobacterial MIC values found in this study ($r \approx 0$). Hence, the antimycobacterial activity of 7TAA compounds is not connected with their antiproliferative effects.

In this study was found also that the methyl esters of the tested 7TAA derivatives are more active than the corresponding amino acids. The aromatic residues, like His, Phe, Trp and Tyr, are preferred for antimycobacterial activity. Some of the aliphatic residues, as Ala, Met, and Val, are also well accepted. Surprisingly, the Leu derivatives in both subsets are less active. One of the Gly derivatives, the methyl ester **7-Gly-CH_3**, shows good activity, whereas the other one is a weak inhibitor of *M. tuberculosis* H37Rv. The favorable effect of aromatic substituents on the antimycobacterial activity has been observed in different purine-related and non-purine scaffolds (24–28). X-ray data (29,30) and docking studies (31,32) with the target enzyme InhA, an enzyme essential for mycolic acid biosynthesis in *M. tuberculosis* show that two aromatic moieties connected by a short spacer is a suitable pharmacophore for enzyme inhibition.

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Conflict of interest

All authors declare that they have no conflict of interest.

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Notes

[a]World Health Organization, Global tuberculosis report 2014, http://www.who.int/tb/publications/global_report/en/.
[b]Frick M. (2014) The tuberculosis vaccine pipeline. Pipeline report (http:www.pipelinereport.org/2014/tb-vaccine).
[c]MDL QSAR version 2.2, MDL Information Systems, Inc., San Leandro, USA.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. 1H, 13C NMR and LC-MC data of the synthesized compounds.