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Synthesis and antimycobacterial activity of 5-formylaminopyrimidines; analogs of antibacterial purines

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ABSTRACT

Pyrimidine analogs of antimycobacterial purines have been synthesized and their biological activities evaluated. Several 5-formamidopyrimidines exhibited profound activity against *Mycobacterium tuberculosis* in vitro ($IC_{90} \leqslant 1.5$ μg/mL), and they were essentially inactive against other bacteria.

Tuberculosis (TB) still claims ca. 2 mill. deaths pr year worldwide and resistance to existing drugs is a growing problem.[1] We have previously studied 6-aryl-9-benzylpurines as antimycobacterial agents.[2] Structures of some purines with profound antimycobacterial activity as well as a summary of SAR knowledge are shown in Figure 1. These compounds display several properties which make them highly interesting as potential drugs against tuberculosis such as high selectivity towards *Mycobacterium tuberculosis* (*Mtb*) compared to other microorganisms, activity against several drug resistant strains of *Mtb*, generally low toxicity towards mammalian cells, and ability to affect *Mtb* inside macrophages. After exploring SAR of intact purines,[2] we decided also to study non-purine analogs[3] of the compounds described above in order identify the real pharmacophore. We herein report synthesis and antimycobacterial activity for pyrimidine analogs with the general structure shown in Figure 1.

We have previously found that 2-nitropurines are valuable synthetic intermediates,[2f,4] and the 2-nitropurine **1a** can be converted to the 2-oxopurine **3** (Scheme 1).[2f] A careful study of this reaction revealed that the ring-opening product **2a** was formed initially, and after 1 h the pyrimidine **2a** could be isolated in 70% yield. After a prolonged reaction time, compound **2a** disappeared and the only product present was compound **3**[4] (Scheme 1). The oxopurine **3** may have been formed via an elusive unstable species **4**, arbitrary drawn as one possible tautomer. Alternatively, there is an equilibrium between purine **1a** and **2a** and a slow irreversible reaction of compound **1a** to oxopurine **3**.

Since the formamidopyrimidine **2a** was readily available by a ring-opening of the corresponding purine **1a** (Scheme 1), we subjected a series of purines **1** to the same set of reaction conditions (Table 1).[5] Compounds **1** chosen as starting materials, generally exhibit profound antimycobacterial activity,[2] and the products **2** are thus 5-formamidopyrimidine analogs of antimycobacterial purines. Compounds **1a** and **1c–1e**, carrying electron withdrawing substituents in the purine 2-position, participated readily in the ring-opening reaction and >90% conversion was generally seen after 1 h. In case of the 2-nitropurine **1a** and the 2-fluoropurine **1c**, minor amounts of the 2-oxopurine **3** were also formed, due to facile substitution in the 2-position. Without an activating group at C-2, the reaction was much slower and for the purines **1b** and **1f–1i** an 18 h reaction time was required in order to get the desired ring-opening products **2** in reasonable yields. The least reactive purine was compound **1i** carrying a powerful electron donor (NMe_2) in the 2-position. In the case of purine **1i**, prolonged reaction time (66 h) did not improve the yield of **2i**.

Due to restricted rotation around the amide bond in the 5-formylaminopyrimidines **2**, two rotamers were generally observed in the NMR spectra, with the s-*cis* rotamer as the major form in DMSO-d_6. NH-CHO coupling constants were in the area of 11–12 Hz for the s-*trans* rotamer and ca. 1 Hz, when observed, for the s-*cis* rotamers. These values are in good agreement with coupling constants found for other 5-formylaminopyrimidines.[6] At ambient temperature the s-*cis*: s-*trans* ratios were ca. 8:2 with only minor variations in the ratio depending on the pyrimidine 2-substituent.

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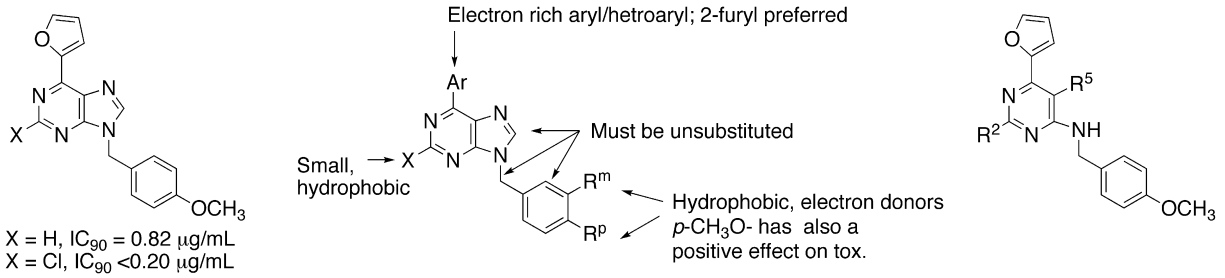
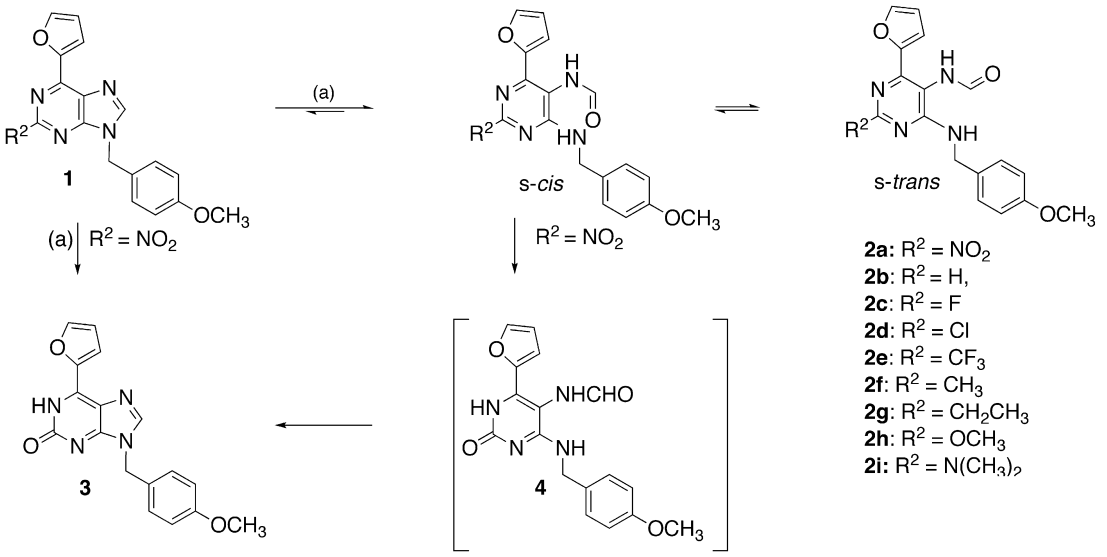


Figure 1. Structures of some potent antimycobacterial purines, summary of SAR knowledge, and general structure of pyrimidines described herein.

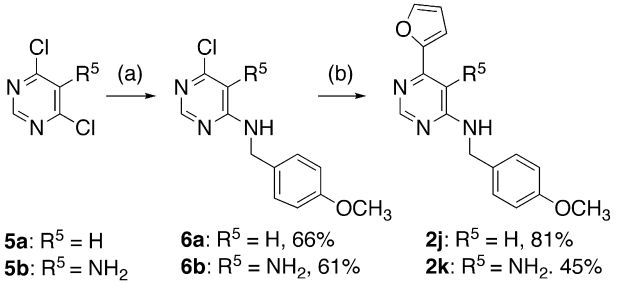


Scheme 1. Reagents: (a) Bu_4NOH, H_2O, THF.

Table 1
Synthesis of 5-formamidopyrimidines **2** by ring-opening of purines **1**

R^2	Time (h)	Yield **2**[a] (%)	Recovered **1**[a] (%)
NO_2	1	70, **2a**	0, **1a**[b]
H	18	66, **2b**	3, **1b**
F	1	72, **2c**	0, **1c**[c]
Cl	1	81, **2d**	5, **1d**
CF_3	1	59, **2e**	7, **1e**
CH_3	18	44, **2f**	26, **1f**
CH_2CH_3	18	32, **2g**	53, **1g**
OCH_3	18	65, **2h**	12, **1h**
$N(CH_3)_2$	18	14, **2i**	85, **1i**

[a] Yield of isolated compound.
[b] 11% of the 2-oxopurine **6** was present in the crude product according to ^1H NMR.
[c] 7% of the 2-oxopurine **6** was present in the crude product according to ^1H NMR.



Scheme 2. Reagents and conditions: (a) p-CH_3O–C_6H_4–CH_2NH_2, Et_3N, n-BuOH, Δ; (b) (2-Furyl)$SnBu_3$, $(Ph_3P)_2PdCl_2$, DMF, 90 °C.

In order to evaluate the importance of the formamido group for biological activity, we also chose to synthesize pyrimidines **2j** and **2k** by reaction of the dichloropyrimidines **5a**–**b** with *p*-methoxybenzylamine followed by (2-furyl)tributyltin under Stille conditions (Scheme 2).

The furylpyrimidines **2** were screened for antibacterial activity against *M. tuberculosis* $H_{37}Rv$ in vitro and the results are presented in Table 2.[7] Except for the dimethylaminopyrimidine **2i**, profound activities were found for all formamidopyrimidines, whereas the pyrimidines **2j** and **2k**, lacking the formamide functionality, were essentially inactive. IC_{90} values for the purines **1b** and **1d**, corresponding to the formamidopyrimidines **2b** and **2d**, are included in Figure 1. The values found for the pyrimidines, are comparable to those found earlier for the corresponding purines, and both in the purine[2f] and pyrimidine series small and lipophilic substituents (i.e., F, Cl, CH_3) in the 2-position are benificial for the inhibition of *Mtb* growth.

Even though formamidopyrimidines **2a**–**2k** are synthetically available from the corresponding purines **1**, one cannot exclude that the ring-opening process may be reversed in the bioassay used to determine antimycobacterial activity. Hence, any bioactivity observed from formamidopyrimidines may actually (in part) be caused by the parent purine **1**. However, NMR studies indicate that most formamidopyrimidines were not prone to cyclization. Especially pyrimidines with electron withdrawing substituents in the 2-position were highly stable and these compounds were also the most active antimycobacterials.

Table 2
Antibacterial and cytotoxic data for pyrimidines **2**[a]

Compd	R^2	R^5	IC_{90} *M. tuberculosis* $H_{37}Rv$ (μg/mL)[b]	IC_{50} *M. tuberculosis* $H_{37}Rv$ (μg/mL)[b]	MIC *S. aureus* (μg/mL)[c]	MIC *E. coli* (μg/mL)[d]
2a	NO_2	NHCHO	1.1	0.59	>64	>64
2b	H	NHCHO	0.56	0.22	>64	>64
2c	F	NHCHO	<0.20	<0.20	>64	>64
2d	Cl	NHCHO	0.20	<0.20	>64	>64
2e	CF_3	NHCHO	0.58	0.26	n.d.	n.d.
2f	CH_3	NHCHO	0.33	<0.20	n.d.	n.d.
2g	CH_2CH_3	NHCHO	0.53	0.26	n.d.	n.d.
2h	OCH_3	NHCHO	1.5	0.53	n.d.	n.d.
2i	$N(CH_3)_2$	NHCHO	26	11	n.d.	n.d.
2j	H	H	n.d.[e]	n.d.	n.d.	n.d.
2k	H	NH_2	n.d.[e]	n.d.	n.d.	n.d.

[a] General structure of pyrimidines **2** is shown in Figure 1.
[b] IC_{90} amicain 0.13 and IC_{50} amicain 0.07 μg/mL.
[c] MIC gentamycin 0.1 μg/mL.
[d] MIC gentamycin 0.5 μg/mL.
[e] 0% inhibition of *Mtb* at 6.25 μg/mL.

In accordance with previous findings on the structurally related purines,[2] the pyrimidines exhibit a selective antimycobacterial activity. Compounds **2a**–**2d** were essentially inactive against *Staphylococcus aureus* and *Escherichia coli* (MIC > 64 μg/mL, Table 2).[8] The nitropyrimidine **2a** was also examined for cytotoxicity against VERO cells,[9] and the EC_{50} value was found to be >40 μg/mL (62% cell viabiliy at 40 μg/mL).

Except for some similarities with antimycobacterial purines previously described by us,[2] the potent pyrimidines discussed herein has no structural resemblance with any other compounds studies as (potential) antimycobacterial drugs. This may indicate that the pyrimidines do not act by the same mechanism as any existing TB-drug, and such drug leads are preferred since there is an increased possibility that resistance will not be developed within a reasonable time frame. Furthermore, the pyrimidines are easily synthesized and they comply with the Lipinski rule. The latter is a good indication that they will display acceptable oral availability.

Acknowledgments

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Supplementary data

Supplementary data (analytical data for novel compounds) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.04.082.

References and notes

1. See for instance: Bhowruth, V.; Dover, L. G.; Besra, G. S. *Prog. Med. Chem* **2007**, *45*, 169. and references therein.
2. (a) Bakkestuen, A. K.; Gundersen, L.-L.; Langli, G.; Liu, F.; Nolsøe, J. M. J. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 1207; (b) Gundersen, L.-L.; Nissen-Meyer, J.; Spilsberg, B. *J. Med. Chem.* **2002**, *45*, 1383; (c) Andresen, G.; Gundersen, L.-L.; Nissen-Meyer, J.; Rise, F.; Spilsberg, B. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 567; (d) Bakkestuen, A. K.; Gundersen, L.-L.; Utenova, B. T. *J. Med. Chem.* **2005**, *48*, 2710; (e) Brændvang, M.; Gundersen, L.-L. *Bioorg. Med. Chem.* **2005**, *13*, 6360; (f) Brændvang, M.; Gundersen, L.-L. *Bioorg. Med. Chem.* **2007**, *15*, 7144.
3. Brændvang, M.; Gundersen, L.-L. *Tetrahedron Lett.* **2007**, *48*, 3057.
4. Brændvang, M.; Gundersen, L.-L. *Synthesis* **2006**, 2993.
5. *General procedure for the synthesis of pyrimidines* **2a**–**2i**: Compound **1** was dissolved, by heating if necessary, in THF (8 mL). The mixture was stirred at ambient temperature and tetrabutylammonium hydroxide (0.67 mL, 1.0 mmol, 1.5 M sol. in water) was added over 2 min. The reaction mixture was stirred for the time given in Table 1. In case of compounds **2b** and **2f**–**2i**, a small amount of silica gel was added, the mixture evaporated and the product isolated by flash chromatography on silica gel. In case of compounds **2a** and **2c**–**2e**, the reaction mixture was poured into satd aq NH_4Cl (30 mL). The resulting mixture was extracted with EtOAc (2 × 30 mL), the combined organic layers were washed with water (2 × 30 mL) and brine (30 mL), dried ($MgSO_4$) and evaporated. The products were purified by flash chromatography on silica gel.
6. (a) Raoul, S.; Bardet, M.; Cadet, J. *Chem. Res. Toxicol.* **1995**, *8*, 924; (b) Fenn, M. D.; Lister, J. H. *J. Chem. Soc., Perkin Trans. 2* **1976**, 29.
7. a The pyrimidines were screened for antimycobacterial activities essentially as described in Ref. 1 Compounds were tested in ten twofold dilutions, from 100 μg/mL to 0.19 μg/mL, against *Mycobacterium tuberculosis* H_{37} Rv (ATCC 27294) in BACTEC 12B medium using the Microplate Alamar Blue Assay (MABA). The IC_{90} and IC_{50} values are determined from the dose–response curve as the IC_{90} using the curve fitting program XLFIT, formula 205.; (b) Collins, L. A.; Franzblau, S. G. *Antimicrob. Agents Chemother.* **1997**, *41*, 1004.
8. The antimicrobial data (*S. aureus* and *E. coli*) were obtained as described before: (a) Vik, A.; Hedner, E.; Charnock, C.; Samuelsen, Ø.; Larsson, R.; Gundersen, L.-L.; Bohlin, L. *J. Nat. Prod.* **2006**, *69*, 381; (b) Vik, A.; Hedner, E.; Charnock, C.; Tangen, L. W.; Samuelsen, Ø.; Larsson, R.; Bohlin, L.; Gundersen, L.-L. *Bioorg. Med. Chem.* **2007**, *15*, 4016.
9. The pyrimidines were screened for mammalian cell cytotoxicity to VERO cells essentially as described in Ref. 1: After 72 h exposure, viability is assessed using the CellTiter 96® Non-Radioactive Cell Proliferation Assay (MTT) reagent from Promega. Cytotoxicity is determined from the dose–response curve as the EC_{50} using the curve fitting program XLFIT, formula 205.