Synthesis and Antimycobacterial Activity of 6-Arylpurines: The Requirements for the *N*-9 Substituent in Active Antimycobacterial Purines

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6-Arylpurines carrying a variety of substituents in the 9-position were prepared by Stille coupling between appropriately substituted 6-chloropurines and aryl(tributyl)tin, and the compounds were screened for antibacterial activity against *Mycobacterium tuberculosis* H_{37}-Rv. The lowest minimum inhibitory concentration value, 0.78 μg/mL, was found for 9-benzyl-2-chloro-6-(2-furyl)purine. This compound exhibited relatively low cytotoxicity, and it was active against several singly drug-resistant strains of *M. tuberculosis*.

Introduction

Tuberculosis (TB) is the major cause of death from a single infectious agent among adults in developing countries, and there has been an unfortunate revival of TB in the industrialized world. Human immunodeficiency virus (HIV) infections have further increased TB morbidity and mortality. Multidrug-resistant tuberculosis, defined as resistance to the two most important drugs, isoniazid and rifampin, is a growing problem among HIV-infected patients. About 1/3 of the world population is infected with *Mycobacterium tuberculosis* even though most of them carry a dormant infection. There are ca. 8 million new cases of TB each year, and it has been estimated that ca. 30 million people will die from tuberculosis in about 10 years.[1] The World Health Organization (WHO) declared TB a global emergency in 1993. There is an urgent need for new antimycobacterial agents, but no new chemotherapeutic agents directed specifically against TB have been introduced the last 25−30 years. TB is not an attractive target condition for the pharmaceutical industry because the majority of patients live in countries with inadequate health care budgets.[2]

We have found that several 9-benzylpurines exhibit inhibitory activity against *M. tuberculosis*.[3] In addition to our work, we are only aware of two other papers on antimycobacterial purine derivatives; both were published very recently. The natural product Agelasine F[4] and some synthetic 9-sulfonyl-6-mercaptopurines[5] also exhibit minimum inhibitory concentrations (MICs) against *M. tuberculosis* in the same range as the more active compounds described herein.

Chemistry

The aim of the present study was to determine the requirements for the *N*-9 substituent in active antimycobacterial purines. Based on our preliminary results, we chose 6-arylpurines as our target compounds because 6-(2-furyl)-, 6-(2-thienyl)-, and to a certain extent 6-phenyl-9-benzylpurines have shown high antimycobacterial activity.[3] Aryl substituents can easily be introduced in the purine 6-position by palladium catalyzed cross coupling between 6-halopurines and organometallic reagents, mainly organotin,[6] organozinc,[6] and organoboron compounds.[7] The syntheses of target compounds **2a**, **2e**, **2f**, **2g**, **2h**, **2j**, **2m**, **4a**, and **5a** (Scheme 1) have been reported before. The novel 6-arylpurines **2** and **5** were prepared by Stille coupling between the appropriately substituted 6-chloropurine **1** or **3** and aryl(tributyl)tin compounds. As we have previously reported,[8] the free NH function in 6-chloropurine **1a** does not interfere with the Stille coupling, and 6-(2-furyl)-purine **2a** was formed by the bis(triphenylphosphine)-palladium(II) chloride catalyzed reaction between 6-chloropurine **1a** and 2-furyl(tributyl)tin in DMF at 90 °C. Employing these conditions, the target 6-aryl-9-alkylpurines were also easily available in high yields, but regioselective coupling between 2,6-dichloropurines and organotin reagents[6a] required milder conditions. Switching to the more reactive catalyst tetrakis[tri(2-furyl)phosphine]palladium(0)[9] and lowering the reaction temperature to 50 °C allowed coupling in the more reactive purine 6-position to give the 2-chloro-6-arylpurines **2o**, **2q**, and **5d** exclusively.

The nucleosides **5** were also formed by Stille couplings, but here protection of the riboside hydroxy groups was required for reasonable yields. We used both acyl and TBS (*tert*-butyldimethylsilyl) protecting groups, and in both instances the protecting groups in the initial coupling products **4** were removed when the crude product was stirred with a saturated solution of potassium fluoride in methanol. This treatment also converts the coproduct tributyltin chloride to the corresponding fluoride which is far more easily separable from the desired purine products by flash chromatography.

Antimycobacterial Activity

The 6-arylpurines **2**, **4a**, and **5** were screened for antibacterial activity against *M. tuberculosis* H_{37}Rv at 6.25 μg/mL concentration in BACTEC 12B medium using the microplate alamar blue assay.[10,11] The results are presented in Table 2. The 9-unsubstituted purines **2a** and **2b** as well as the compounds carrying rather

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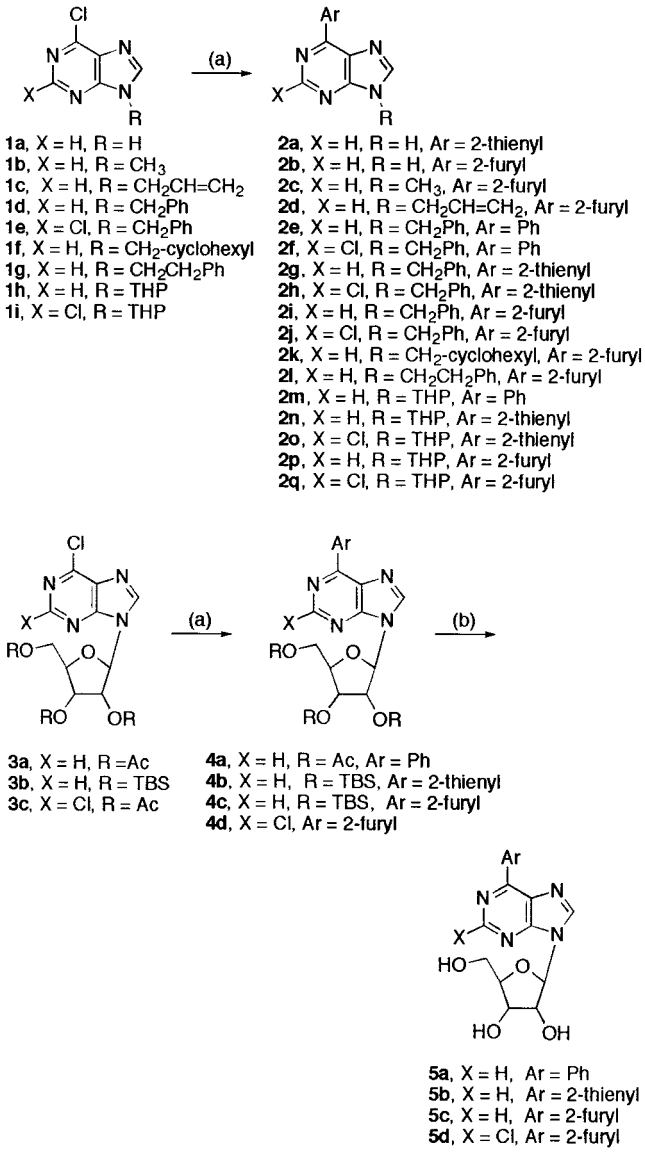
Scheme 1[a]



[a] (a) $ArSnBu_3$, $(Ph_3)_2PdCl_2$ or $[(2\text{-furyl})_3P]_4Pd$, DMF; (b) KF, CH_3OH.

small alkyl substituents such as methyl (**2c**) and allyl (**2d**) were basically inactive at this concentration. The 9-benzylpurines **2e**−**2j**, on the other hand, exhibited significant antimycobacterial activity. For compounds displaying at least 90% inhibition of bacterial growth in the initial screening, MICs against *M. tuberculosis* were determined (Table 1). MIC is defined as the minimum concentration of compound required to give 90% inhibition of bacterial growth. The results show that among the aryl groups tested, the 2-furyl group (**2i** and **2j**) is superior for desired antibacterial effect and that the 2-thienyl group (**2g** and **2h**) is better than the phenyl substituent (**2e** and **2f**). Furthermore, it is clear that a chlorine atom situated in the purine 2-position (compounds **2f**, **2h**, and **2j**) enhances activity compared to purines without substituent in the 2-position. The lowest MIC value, 0.78 μg/mL, was found for the 2-chloro-6-(2-furyl)purine **2j**. MIC for the tuberculosis first-line drug rifampin in the same assay was 0.25 μg/mL.

When the phenyl group in the 9-substituent in 9-benzyl-6-(2-furyl)purine **2i**, was replaced by the totally saturated cyclohexyl ring to give compound **2k**, the antimycobacterial activity dropped considerably, and the same situation occurred when the bridge between the purine and the phenyl group was increased from a methylene to an ethylene bridge in compound **2l**. Purines **2m**−**2q**, carrying a 9-tetrahydropyranyl (THP) protecting group, were essentially without any activity. A similar lack of activity was seen with the 6-arylpurine ribosides **4a** and **5**. The results described herein show that the requirements regarding the 9-substituent in antimycobacterial 6-arylpurines are quite restricted, and among the compounds tested, the benzyl group is definitely the most suitable.

Table 1. Antimycobacterial Activity against *M. tuberculosis* and Cytotoxic Activity of Purines **2** and **5**

compd	% inhib. *M. tub.* 6.25 μg/mL.	MIC against *M. tub.* $H_{37}Rv$	IC_{50} VERO cells	selectivity index (SI = IC_{50}:MIC)	inhib. [^{3}H]-thymidine incorporation (IC_{50})
2a	7	n.d.	n.d.	n.d.	<10
2b	6	n.d.	n.d.	n.d.	n.d.
2c	0	n.d.	n.d.	n.d.	<10
2d	7	n.d.	n.d.	n.d.	11
2e	69[a]	n.d.	n.d.	n.d.	<10[b]
2f	>90[a]	12.5	*c*	*c*	76
2g	>90	6.25	>10	>1.6	<10[b]
2h	>90	1.56	>10	>6.4	n.d.
2i	>90	3.13	8.6	2.7	59
2j	>90	0.78	8.1	10.6	27
2k	22	n.d.	n.d.	n.d.	60
2l	26	n.d.	n.d.	n.d.	53
2m	3	n.d.	n.d.	n.d.	<10
2n	0	n.d.	n.d.	n.d.	23
2o	7	n.d.	n.d.	n.d.	61
2p	0	n.d.	n.d.	n.d.	15
2q	6	n.d.	n.d.	n.d.	36
4a	4	n.d.	n.d.	n.d.	n.d.
5a	14	n.d.	n.d.	n.d.	99 (0.4)
5b	6	n.d.	n.d.	n.d.	n.d.
5c	0	n.d.	n.d.	n.d.	n.d.
5d	7	n.d.	n.d.	n.d.	100 (3.4)

[a] Determined at 12.5 μg/mL. [b] Determined at 1.00 μg/mL due to low solubility. [c] Solubility too low to determine IC_{50}. [d] Ac_3Rib = 2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl.

Table 2. Minimum Innibitory Concentration of Compound **2j** against Drug-Resistant Strains of *M. tuberculosis*, *M. tuberculosis* Erdman, *M. avium*, and *L. casei*

MIC[a] *M. tub.* $H_{37}RV$	MIC *M. tub.* Erdman	MIC INH[b]	MIC RMP[b]	MIC EMB[b]	MIC KM[b]	MIC CIP[b]	MIC *M. avium*	MIC *L. casei*
0.78	12.5	3.13	1.56	<0.39	0.78	<0.39	25	>10

[a] All concentrations are given in μg/mL. [b] MIC against *M. tuberculosis* strains resistant to INH (isoniazid), RMP (rifampin), EMB (ethambutol), KM (kanamycin), and CIP (ciprofloxacin).

Having identified several active antimycobacterial purines, the next step was to examine the toxicity of the drug candidates. Compounds exhibiting reasonably low MICs were tested for cytotoxicity (IC_{50}) in VERO cells, and a selectivity index (SI), defined as IC_{50}:MIC, was calculated. The IC_{50} and SI values are shown in Table 2. The thienylpurines **2g** and **2h** were somewhat less toxic than the furylpurines **2i** and **2j**, and a chloro substituent in the purine 2-position resulted in a minor increase in toxicity. Generally, compounds with an MIC $\leq$ 6.25 μg/mL and an SI $\geq$ 10 are interesting compounds, and an MIC $\leq$ 1 μg/mL in a novel compound class is considered an excellent lead,[11] which makes the 2-chloro-6-furyl-9-benzylpurine **2j** a very promising an-

timycobacterial compound. In addition to the assessment of toxicity to VERO cells, most compounds described herein were screened for cytostatic activity against chronic myelogenous leukemia cells (K-562) based on their ability to inhibit [^{3}H]thymidine incorporation. The percent inhibition of incorporation at 10 μg/mL as well as EC_{50} values for the most toxic compounds are given in Table 2. The compounds described herein exhibited only a low cytostatic effect, except for the nucleosides **5a** and **5d**. This finding was in good agreement with previous reports on cytotoxic 6-arylpurine nucleosides.[7,12] However, the cytotoxic nucleosides were essentially inactive as antimycobacterials.

It has been estimated that up to 50 million people are infected with drug-resistant forms of TB. The development of drug resistance in the population has increased concern that TB may once again become an incurable disease. Of particular concern is the development of multidrug-resistant forms of the disease (MDR-TB), defined as forms resistant to two or more of the front line anti-TB agents.[13] These forms of the disease are more often fatal and are difficult and expensive to treat.[14] The most active compound **2j** was tested against various drug-resistant strains as well as the *M. tuberculosis* strain Erdman, the *Mycobacterium avium* complex, which causes opportunistic infections in AIDS patients, and against the Gram positive bacteria *Lactobacillus casei* (Table 2). An extremely attractive feature with the furylpurine **2j** is that this compound displayed only a very minor cross resistance with isoniazid (INH) and rifampin (RMP) and absolutely no cross resistance with ethambutol (EMB), kanamycin (KM), and ciprofloxacin (CIP). This is in sharp contrast to the 9-sulfonyl and sulfenylmercaptopurines which exhibit a much more profound cross resistance with INH, RMP, EMB, and KM.[5] Compound **2j** was not especially active against the *M. avium* complex or the Gram positive bacteria *L. casei*. Compounds **2** and **5** had MICs against *L. casei* higher than 10 μg/mL (data not shown). This finding may suggest that the compounds described herein will not affect the Lactobaccilli present as part of the normal flora in the intestinal system.

The mechanism by which the purines described herein exhibit their antimycobacterial activity is not known. However, the lack of cross-resistance with many known antimycobacterial drugs suggests that these compounds do not share entirely the same antibacterial mode of action as rifampin, kanamycin, and ciprofloxacin, isoniazid, and ethambutol. These known antimycobacterials are inhibitors of RNA synthesis, DNA-replication, protein synthesis, and synthesis of the cell wall component mycolic acid and arabinogalactan, respectively. The ineffectiveness of the purine nucleosides **5** also makes inhibition of DNA/RNA synthesis less likely. Moreover the complete inactivity against the Gram positive bacteria *L. casei* found for all target compounds **2** and **5** points toward a specific mycobacterial target for the drug action.

Compound **2j** was tested for killing of *M. tuberculosis* Erdman in monolayers of mouse bone marrow macrophages.[15] The concentration of the purine **2j** effecting 99% reduction of bacterial growth (EC_{99}) after 7 days was 8.46 μg/mL and the concentration effecting a 90% reduction of growth (EC_{90}) was 0.038 μg/mL. These results demonstrate that **2j** is able to attack *M. tuberculosis* inside macrophages. The EC_{90}:MIC (*M. tuberculosis* H_{37}Rv) is 0.049 while compounds with EC_{90} up to 16 × MIC are considered to exhibit some activity.[11] In vivo studies of compound **2j** as well as synthesis of analogues of this lead compound are currently in progress.

Experimental Section

The ^{1}H NMR spectra were recorded at 300 MHz with a Bruker Avance DPX 300 instrument or at 200 MHz with a Varian Gemini 200 instrument. The ^{1}H decoupled ^{13}C NMR spectra were recorded at 75 or 50 MHz using the above-mentioned spectrometers. Mass spectra under electron impact conditions (EI) were recorded at 70 eV ionizing voltage with a VG Prospec instrument and are presented as *m*/*z* (% rel int.). Elemental analyses were performed by Ilse Beetz Mikroanalytisches Laboratorium, Kronach, Germany. Melting points were determined with a C. Reichert melting point apparatus and are uncorrected. Silica gel for flash chromatography was purchased from Merck, Darmstadt, Germany (Merck No. 9385). Analytical thin-layer chromatography was performed with E. Merck silica gel $60F_{254}$ 0.25 mm plates (Merck No. 1.05554). DMF was distilled from BaO and stored over 4 Å molsieve. All 6-chloropurines **1** and **3** used in the syntheses of compounds **2**, **4**, and **5** were available by literature methods. The following compounds **2**, **4**, and **5** were synthesized as previously reported: 6-(2-thienyl)-1*H*-purine **2a**,[7] 9-benzyl-6-phenyl-9*H*-purine **2e**,[16] 9-benzyl-2-chloro-6-phenyl-9*H*-purine **2f**,[6a] 9-benzyl-6-(2-thienyl)-9*H*-purine **2g**,[16] 9-benzyl-2-chloro-6-(2-thienyl)-9*H*-purine **2h**,[6a] 9-benzyl-2-chloro-6-(2-furyl)-9*H*-purine **2j**,[6a] 6-phenyl-9-(tetrahydro-2*H*-pyran-2-yl)-9*H*-purine **2m**,[8] 9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-6-phenyl-9*H*-purine **4a**,[8] 6-phenyl-9-(β-D-ribofuranosyl)-9*H*-purine **5a**.[8] All other reagents were commercially available and used as received. Antibacterial effect against *L. casei* (NCDO 2713)[17] and cytotoxicity against chronic myelogenous leukemia cells (cell line K-562)[18] were measured as described previously.

General Procedure for Coupling between 6-Chloropurines and Arylstannanes. A mixture of chloropurine (0.5 mmol) and bis(triphenylphosphine)palladium(II) chloride (0.025 mmol) and organostannane (0.6 mmol) in dry DMF (2 mL) was heated under N_2 at 90−95 for ca. 18 h and evaporated in vacuo. A saturated solution of potassium fluoride in methanol (20 mL) was added to the residue, and the resulting mixture was stirred at ambient temperature overnight and evaporated in vacuo together with a small amount of silica gel. The residue was added on top of a silica gel column and the product isolated by flash chromatography. Couplings on purines **1e**, **1i**, and **3c** were carried out as described above except that tetrakis[tri-(2-furyl)phosphine]palladium(0) was used as catalyst and the reaction temperature was 50 °C.

6-(2-Furyl)-1*H*-purine (2b). Off-white microcrystalline solid, yield 53%, mp ca. 280 °C (subl.).

6-(2-Furyl)-9-methyl-9*H*-purine (2c). Off-white small needles, yield 88%, mp 191−193 °C.

6-(2-Furyl)-9-(2-propen-1-yl)-9*H*-purine (2d). Yellow needles, yield 68%, mp 126−128 °C.

9-Benzyl-6-(2-furyl)-9*H*-purine (2i). Colorless crystals, yield 93%, mp 223−225 °C.

9-(Cyclohexylmethyl)-6-(2-furyl)-9*H*-purine (2k). Off-white microcrystalline solid, yield 89%, mp 198−200 °C.

6-(2-Furyl)-9-(2-phenylethyl)-9*H*-purine (2l). Off-white microcrystalline solid, yield 88%, mp 119−120 °C.

9-(Tetrahydro-2*H*-pyran-2-yl)-6-(2-thienyl)-9*H*-purine (2n). Off-white crystals, yield 93%, mp 139−140 °C.

2-Chloro-9-(tetrahydro-2*H*-pyran-2-yl)-6-(2-thienyl)-9*H*-purine (2o). Yellow crystals, yield 58%, mp 143−144 °C.

6-(2-Furyl)-9-(tetrahydro-2*H*-pyran-2-yl)-9*H*-purine (2p). EtOAc−hexane (1:1) was used for flash chromatography; colorless crystals, yield 90%, mp 133−134 °C.

2-Chloro-6-(2-furyl)-9-(tetrahydro-2*H*-pyran-2-yl)-9*H*-purine (2q). Off-white crystals, yield 86%, mp 138−140 °C.

9-(β-D-Ribofuranosyl)-6-(2-thienyl)-9*H*-purine (5b). Colorless microcrystalline solid, yield 87%, mp 128−130 °C (lit.[12] 126−129 °C).

6-(2-Furyl)-9-(β-D-ribofuranosyl)-9*H*-purine (5c). Colorless microcrystalline solid, yield 92%, mp 171−173 °C (lit.[12] 165−168 °C).

2-Chloro-6-(2-furyl)-9-(β-D-ribofuranosyl)-9*H*-purine (5d). Yellow crystals, yield 49%, mp 151−153 °C.

Activity against Mycobacteria. The primary screening was conducted at 6.25 μg/mL against *M. tuberculosis* $H_{37}Rv$ (ATCC 27294) in BACTEC 12B medium using the microplate alamar blue assay (MABA).[10] Compounds exhibiting fluorescence were tested in the BACTEC 460-radiometric system,[10] and compounds demonstrating at least 90% inhibition in the primary screen were re-tested at lower concentrations against *M. tuberculosis* $H_{37}Rv$ to determine the actual MIC in the MABA. MIC for rifampin was 0.25 μg/mL. For 2-chloro-6-(2-furyl)-9-(phenylmethyl)-9*H*-purine **2j**, MICs were also determined in the MABA for drug-resistant strains of *M. tuberculosis*, [strains resistant to isoniazid (ATCC 35822), rifampin (ATCC 35838), ethambutol, kanamycin, and ciprofloxacin] as well as the drug-sensitive strain *Mycobacterium Erdman* and also *M. avium*. (ATCC 25291).

Activity against *M. tuberculosis* Erdman in Monolayers of Mouse Bone Marrow Macrophages. 2-Chloro-6-(2-furyl)-9-(phenylmethyl)-9*H*-purine **2j** was tested for killing of *M. tuberculosis* Erdman in monolayers of mouse bone marrow macrophages at 4-fold concentrations equivalent to 0.25, 1, 4, and 16 times the MIC.[15] EC_{99} and EC_{90} were defined as the lowest concentration effecting a 90% and 99% reduction in colony forming units at 7 days compared to drug-free controls.

Cytotoxicity against VERO Cell Lines. Compounds were tested for cytotoxicity (IC_{50}) in VERO cells at concentrations less than or equal to 62.5 μg/mL. After 72 h exposure, viability was assessed on the basis of cellular conversion of MTT into a formazan product using the Promega CellTiter 96 nonradioactive cell proliferation assay.

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Supporting Information Available: List of the references for synthesis of the starting materials and detailed information on synthetic procedures and spectroscopic and analytical data. This material is available free of charge via the Internet at http://pubs.acs.org.

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