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9-Benzylpurines with Inhibitory Activity Against *Mycobacterium tuberculosis*

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Abstract—9-Benzylpurines with a variety of substituents in the 2-, 6- and/or 8-position have been prepared and screened for antimycobacterial effects. High inhibitory activity against *Mycobacterium tuberculosis* was found for 9-benzylpurines carrying a phenylethynyl-, *trans*-styryl or aryl substituents in the 6-position and generally chlorine in the 2-position tends to increase activity.

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 industrialised world. Human immunodeficiency virus (HIV) infections has further increased TB morbidity and mortality. Multidrug-resistant tuberculosis (MDR-TB) defined as resistance to the two most important drugs, isoniazid (INH) and rifampicin (RMP), is a growing problem among HIV-infected patients. It has been estimated that ca. 30 million people will die from tuberculosis in about 10 years, and there is an urgent need for new antimycobacterial agents.[1] We herein report that several 9-benzylpurines exhibit inhibitory activity against *Mycobacterium tuberculosis*.

A variety of 6-substituted or 2,6-disubstituted 9-benzylpurines **2**, **3** and **4** were prepared by one or two Stille couplings on the parent (di)halopurines **1**, **2** or **4** as outlined in Scheme 1.[2–4] Reaction conditions for the preparation of compounds not previously reported are summarised in Table 1.

The coupling products **2**, **3** and **4** (Scheme 1) as well as 9-benzylpurine **5** (Table 2, entry 1), the methylketones 6 and **7** (Table 2, entries 9 and 16), the nitrile **8** (Table 2, entry 17), the halopurines **1d**, **1e** and **1f** (Scheme 1) and the aminopurine **9** (Table 2, entry 41) were screened for inhibitory effect against *M. tuberculosis* at 12.5 μg/mL concn using the Microplate Alamar Blue Assay (MABA).[5] For compounds exhibiting 90% inhibition or more in the initial testing, minimum inhibitory concn against *M. tuberculosis* were also determined. The results are given in Tables 2 and 3. 9-Benzylpurine (**5**) itself exhibited no inhibitory effect (Table 2, entry 1), but highly active compounds were found among 9-benzylpurines carrying substituents in the 6-position such as phenylethynyl- (Table 3, entry 1), *trans*-styryl (Table 3, entries 2–5), and aryl (Table 3, entries 7–12). Generally chlorine in the 2-position tends to increase activity, whereas compounds with a 2-amino substituent in most instances exhibit lower activity than their C-2 unsubstituted analogues. Among the purines monosubstituted in the 2-position (Table 2, entries 36–41), we discovered no highly active compounds.

Also cytotoxicity (IC_{50}) in VERO cells as well as selectivity index (SI), defined as IC_{50}/MIC, were found for several of the active compounds described in Table 3. However, in some instances the solubility of the compound in the tissue culture medium was too low for IC_{50} to be determined. The 6-furylpurines **2o** and **2p** (Figure 1; Table 3, entries 11–12) exhibited the highest activities among the compounds examined. Especially compound **2p** with a MIC close to that of RMP as well as a SI at ca. 10, should be considered as an excellent lead compound.

The position of the *N*-benzyl substituent appears to be crucial for antimycobacterial activity. The 7-benzylpurines **10**[2,6] (Fig. 2) are regioisomers of the highly active 9-benzylpurines **2i**, **2k**, **2m** and **2n**, but none of the compounds **10** showed any activity against *M. tuberculosis* at 12.5 μg/mL concn.

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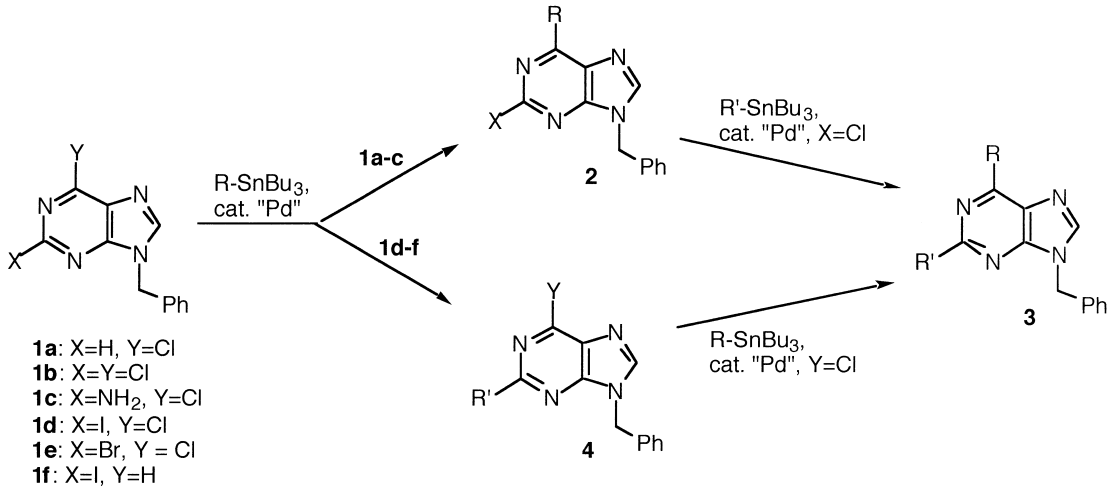


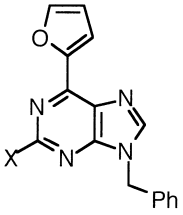
Scheme 1.

Also some 8-substituted and 6,8-disubstituted 9-benzylpurines **11**, prepared by Stille couplings on the parent (di)halopurines **12** as reported before,[9,10] were screened for inhibitory effect against *M. tuberculosis* at 12.5 μg/mL concn. A general structure for compounds **11** and **12** is shown in Figure 3 and the biological test results are summarised in Table 4. None of the compounds **11** or **12**

Table 1. Pd-catalyzed coupling between purines **1**,**2** and **4** or organotin reagents[a]

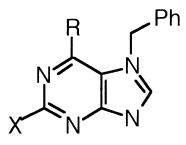
Starting material	Subst. in the purine 2 position (X/R′−)	Subst. in the purine 6 position (R−/Y)	Temperature (°C)	Time (h)	Yield (%) **2**, **3** of **4**
1a	H−	Ph−C≡C−	75	2.5	77,**2a**
1c	NH_2−	Ph−C≡C−	80	21	64,**2c**
1c	NH_2−	(*E*)−PhCH=CH−	100	21	77,**2g**
1c	NH_2−	Ph−	110	24	30,**21**
1a	H−	2−Furyl−	95	17	93,**2g**
4e	Ph−	Ph−C≡C−	75	19	66,**3a**
1b	2-Thienyl−	2-Thienyl−	70	40	72,**3f**
1f	Ph−C≡C−	H−	60	18	85,**4h**
1f	2-furyl−	H−	60	20	90,**4j**
1f	2-thienyl−	H−	60	24	96,**4k**

[a]The reactions were performed in DMF using 1.5 equiv $RSnBu_3$ (2.5 equiv for compound **3f**) and 5 mol% of $(Ph_3P)_2PdCl_2$.



2o: X = H-,
2p: X = Cl-

Figure 1.



10a: X = H-, R = 2-thienyl-
10b: X = Cl-, R = 2-thienyl-
10c: X = Cl-, R = Ph-
10d: X = Cl-, R = CH_2=C(OEt)-

Figure 2.

Table 2. Initial screening of inhibitory activity of 2-, 6-, and 2,6-substituted 9-benzylpurines against *M. tuberculosis*

Entry	Compound[a]	Substituent in the 2-position	Substituent in the 6-position	% Inhibition of *M. tuberculosis* at 12.5 μg/ml[b]
1	**5**[7]	H−	H−	0
2	**2a**	H−	Ph−C≡C−	29
3	**3a**	Ph−	Ph−C≡C−	99
4	**3b**[3]	Cl−	Ph−C≡C−	36
5	**2c**	NH_2−	Ph−C≡C−	60
6	**2d**[2]	H−	CH_2=CH−	13
7	**2e**[2]	H−	(*E*)−PhCH=CH−	67
8	**3b**[3]	CH_2=C(OEt)−	(*E*)−PhCH=CH−	99
9	**6**	CH_3CO−	(*E*)−PhCH=CH−	99
10	**3c**[3]	Ph−	(*E*)−PhCH=CH−	99
11	**2f**[3]	Cl	(*E*)−PhCH=CH−	98
12	**2g**	NH_2−	(*E*)−PhCH=CH−	9
13	**2h**[2]	H−	CH_2=C(OEt)−	56
14	**3d**[3]	(*E*)−PhCH=CH−	CH_2=C(OEt)−	33
15	**2i**[3]	Cl−	CH_2=C(OEt)−	94
16	**7**[2]	H−	CH_3CO−	0
17	**8**[8]	H−	NC−	57
18	**2j**[2]	H−	Ph−	69
19	**3e**[3]	(*E*)−PhCH=CH−	Ph−	22
20	**2k**[3]	Cl−	Ph−	98
21	**2l**	NH_2−	Ph−	44
22	**2m**[2]	H−	2-Thienyl	92
23	**3f**	2-Thienyl−	2-Thienyl−	90
24	**2n**[3]	Cl−	2-Thienyl−	98
25	**2o**	H−	2-Furyl−	95
26	**2p**[3]	Cl−	2-Furyl−	98
27	**4a**[3]	Ph-C≡C−	Cl−	22
28	**4b**[3]	CH_2=CH−	Cl−	0
29	**4c**[3]	(*E*)−PhCH=CH−	Cl−	76
30	**4d**[3]	CH_2=C(OEt)−	Cl−	5
31	**4e**[3]	Ph−	Cl−	67
32	**4f**[3]	2-furyl−	Cl−	100
33	**4g**[3]	2-thienyl−	Cl−	44
34	**1e**[3]	Br−	Cl−	0
35	**1d**[3]	I−	Cl−	76
36	**4h**	Ph−C≡C−	H−	0
37	**4i**[9]	CH_2=CH−	H−	14
38	**4j**	2-Furyl−	H−	0
39	**4k**	2-Thienyl−	H−	44
40	**1f**[9]	I−	H−	0
41	**9**[9]	NH_2−	H−	1

[a]Reference to synthesis of the compounds.
[b]The compounds were tested against *M. tuberculosis* H37Rv in BACTEC 12B medium using the Microplate Alamar Blue Assay (MABA). Compounds exhibiting fluorescence are tested in the BACTEC 460-radiometric system.[5]

Table 3. Minimum inhibitory activity of 2-, 6-, and 2,6-substituted 9-benzylpurines against *M. tuberculosis*. cytoxicity to VERO cells and selectivity index

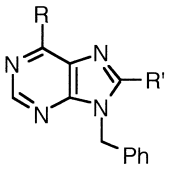
Entry	Compound	Substituent in the 2-position	Substituent in the 6-position	MIC for *M. tuberculosis*[a,b] (μg/mL)	Cytotoxicity (IC_{50}) in VERO cells[c] (μg/mL)	Selectivity index (SI)[d]
1	**3a**	Ph–	Ph–C≡C–	6.25[e]	–[f]	–
2	**3b**	CH2=C(OEt)–	(*E*)–PhCH=CH–	>12.5	n.d.	–
3	**6**	CH_3CO–	(*E*)–PhCH=CH–	6.25	>10	>1.6
4	**3c**	Ph–	(*E*)–PhCH=CH–	12.5	–[f]	–
5	**2f**	Cl–	(*E*)–PhCH=CH–	>6.25[e]	8.3	<1.3
6	**2i**	Cl–	CH_2=C(OEt)–	>6.25[e]	–[f]	–
7	**2k**	Cl–	Ph–	12.5	–[f]	–
8	**2m**	H–	2-Thienyl–	6.25[g]	n.d.	–
9	**3f**	2-Thienyl–	2-Thienyl–	>12.5	n.d.	–
10	**2n**	Cl–	2-Thienyl	1.56	>10	>6.4
11	**2o**	H–	2-Furyl–	3.13[e]	8.6	2.7
12	**2p**	Cl–	2-Furyl–	0.78[e]	8.1	10.4
13	**4f**	2-Furyl–	Cl–	>12.5[g]	n.d.	–

[a]MIC (minimum inhibitory concentration) against *M. tuberculosis* H37Rv in BACTEC 12B medium using the Microplate Alamar Blue Assay (MABA). Compounds exhibiting fluorescence are tested in the BACTEC 460-radiometric system.[5] MIC was defined as the lowest concn effecting a reduction in fluorescence of 90% relative to controls.
[b]The min inhibitory concn (MIC) found for RMP under these conditions was 0.125 μg/mL.
[c]Cytotoxcity (IC_{50}) in VERO cells at concentrations less than or equal to 62.5 μg/mL or 10 times the MIC for *M. tuberculosis* H37Rv. After 72 h exposure, viability as assessed on the basis of cellular conversion of Mtt into formazan product using the Promega Cell Titer 96 non-radioactive cell proliferation assay.
[d]SI = IC_{50}/MIC.
[e]The MIC for RMP was 0.25 μg/mL.
[f]Insoluble in tissue culture media.
[g]The MIC for RMP was 0.03 μg/mL.

Table 4. Inhibitory activity of 8- and 6,8-substituted 9-benzylpurines against *M. tuberculosis*

Entry	Compound[a]	Substituent in the 6-position (R)	Substituent in the 8-position (R′)	%Inhibition of *M. tuberculosis* at 12.5 μg/mL[b,c]
1	**11a**[10]	CH_2=C(OEt)	Cl–	29[d]
2	**11b**[10]	Ph–	Cl–	52[d]
3	**11c**[10]	2-Thienyl–	Cl–	3[d]
4	**11d**[10]	Cl–	Ph–C≡C–	0
5	**11e**[10]	Cl–	CH_2=CH–	31
6	**11f**[10]	Cl–	CH_2=C(OEt)–	0[d]
7	**11g**[10]	Cl–	Ph–	31
8	**12a**[10]	Cl–	I–	0
9	**11h**[9]	H–	CH_2=CH–	40
10	**12b**[9]	H–	I–	0

[a]Reference to synthesis of the compound.
[b]The compounds were tested against *M. tuberculosis* H37Rv in BACTEC 12B medium using the Microplate Alamar Blue Assay (MABA). Compounds exhibiting fluorescence are tested in the BACTEC 460-radiometric system.[5]
[c]The MIC found for RMP under these conditions was 0.125 μg/mL.
[d]% Inhibition of *M. tuberculosis* at 6.25 μg/mL.



11, 12

Figure 3.

exhibited 90% inhibition or more in the initial testing and their minimum inhibitory concn were not determined.

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4. The compounds not previously reported were prepared using the coupling procedures described in refs 2 and 3 as well as in Table 1. **2a**: Yield 77%. ^{1}H NMR ($CDCI_3$, 300 MHz): 5.48 (s, 2 H), 7.3–7.4 (m, 8H), 7.7–7.8 (m, 2H), 8.13 (s, 1H), 9.01 (s, 1H). MS (EI): 310 (100, M^+). **2c**: Yield 64%. ^{1}H NMR ($CDCI_3$, 300 MHz): 5.10 (br s, 2H), 5.30 (s, 2H), 7.2–7.3 (m, 2H), 7.4 (m, H), 7.7 (m, 2H), 7.79 (s, 1H). MS (El): 325 (100, M^+). **2g**: Yield 77%. ^{1}H NMR ($CDC1_3$, 200 MHz): δ5.25 (br s, 2H), 5.30 (s, 2H), 7.3–7.5 (m, 8H). 7.67 (d, *J* 16.1 Hz, 1H), 7.6–7.7 (m, 2H), 7.76 (s, 1H), 8.33 (d, *J* 16.1 Hz, 1H). MS (EI): 327 (100, M^+). **2I**: Yield 30%. ^{1}H NMR ($CDCl_3$, 200 MHz): δ5.06 (br s, 2H), 5.36 (s, 2H), 7.3–7.4 (m, 5H), 7.5–7.6 (m, 3H), 7.82 (s, 1H), 8.7 (m, 2H). MS (EI): 301 (1, M^+), 224 (100). **2o**: Yield 93%. ^{1}H NMR ($CDCl_3$, 300 MHz): 5.50 (s, 2H), 6.7 (m, 1H), 7.3–7.4 (m, 5H). 7.8 (br s, IH), 7.86 (d, *J* 3.3 Hz, 1H), 8.10 (s, 1H), 9.01 (s, 1H). MS (EI): 276(100, M^+). **3a**: Yield 72%. ^{1}H NMR ($CDCI_3$, 300 MHz): δ5.54 (s, 2H), 7.4–7.5 (m, 11H), 7.8 (m, 2H), 8.09 (s, 1H), 8.6 (m, 2H). MS (EI): 386 (100, M^+). **3f**: Yield 72%. ^{1}H NMR ($CDC1_3$, 200 MHz): δ5.51 (s, 2H), 7.21 (dd, *J* 5.0 and 3.7 Hz, 1H), 7.3–7.4 (m, 7H), 7.50 (dd, *J* 5.O and 1.2 Hz, H), 7.66 (dd, *J* 5.0 and 1.1 Hz, 1H), 8.03 (s, IH), 8.l5 (dd, *J* 3.6 and 1.2 Hz, IH), 8.69 (dd, *J* 3.7 and 1.1 Hz, 1H). MS (EI): 374 (100, M^+). **4h**: Yield 85%. ^{1}H NMR ($CDCI_3$, 300 MHz): 5.45 (s, 2H), 7.34 (m, S H), 7.68 (m, 2H), 8.04 (s, 1H), 9A2 (s, 1H). MS(El): 310 (100,M^+). **4j**: Yield 90%. ^{1}H NMR ($CDCI_3$, 300 MHz): 65.45 (s, 2H), δ6.56 (m, IH), 7.3–7.4 (m, 6H), 7.62 (m, 1H), 7.97 (s, IH, H-8), 9.12 (s, 1H, H-6). MS (EI): 276 (100, M^+). **4k**: Yield 96%. ^{1}H NMR ($CDCI_3$, 200 MHz): δ5.43 (s, 2H), 7.14 (m, 1H), 7.3–7.4 (m, 5H), 7.44 (m, 1H), 7.97 (s, 1H), 8.03 (m, 1H), 9.07 (s, 1H). MS (EI): 292 (100, M^+). This compound was prepared by acidic hydrolysis of compound **3b** essentially as described in ref. 2. ^{1}H NMR ($CDCI_3$, 200 MHz): δ2.96 (s, 3H), 5.58 (s, 2H), 7.3–7.4 (m, 8H), 7.7–7.8 (m, 3H), 8.20 (s, 1H), 8.59 (d, *J* 16.2 Hz, 1H). MS (EI): 354 (92, M^+), 91 (100).

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