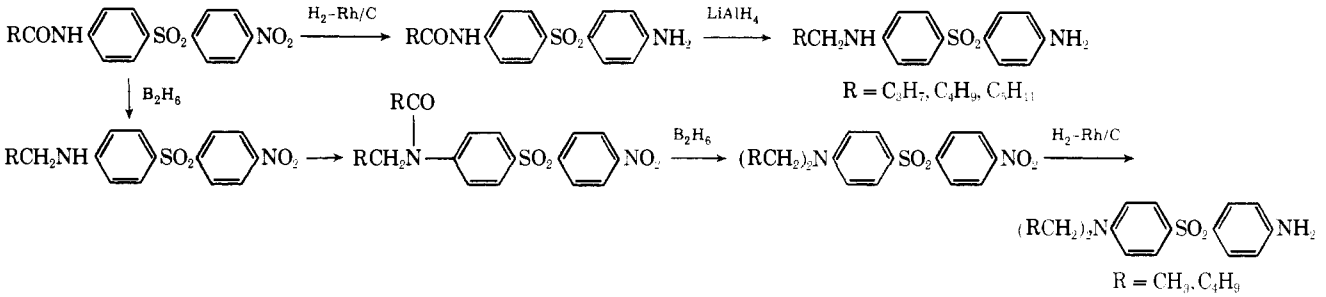
Scheme I



16 hr. After removal of most of the HOAc *in vacuo*, the residue was diluted with H_2O (50 ml) and the white precipitate collected to yield 1.91 g (89%). Recrystallization from C_6H_6 gave mp 163–165°. *Anal.* ($C_{12}H_8FNO_4S$).

4-Amino-4′-fluorodiphenyl Sulfone. A mixture of the nitro fluoro sulfone (0.50 g), 25 mg of 5% Rh/C, and 20 ml of MeOH was stirred under H_2 (1 atm) for 5 hr (theoretical uptake). The catalyst was removed and the solution concentrated to 10 ml to afford white crystals (0.29 g, 64%), mp 203–208° (lit.[9] mp 200–201°).

4-Butyramido-4′-nitrodiphenyl Sulfone. To an ice-cold, stirred suspension of 4-amino-4′-nitrodiphenyl sulfone (2.20 g, 8 mmol) in 2,6-lutidine (20 ml) was slowly added 1.70 g (16 mmol) of butyryl chloride. After 10 min the mixture was heated for 15 min at 95–100° and poured into 450 ml of ice H_2O. The precipitate was collected and recrystallized from EtOH–H_2O to afford 1.92 g (35%), mp 185–189°. *Anal.* ($C_{16}H_{16}N_2O_5S$).

Similarly prepared were the *N*-valeryl [63%, mp 170–171° ($C_{17}H_{18}N_2O_5S$)] and *N*-hexanoyl compounds [51%, mp 164–168° ($C_{18}H_{20}N_2O_5S$)].

4-Amino-4′-butyramidodiphenyl Sulfone. A mixture of the nitro sulfone (1.80 g), 5% Rh/C (300 mg), and MeOH (100 ml) was stirred under H_2 (1 atm) for 4 hr (theoretical uptake). After removal of catalyst and solvent the crude product was crystallized (C_6H_6–*i*-PrOH): 0.80 g (50%); mp 185–189°. *Anal.* ($C_{16}H_{18}N_2O_3S$).

The *N*-valeryl [66%, mp 163–167° ($C_{17}H_{20}N_2O_3S$)] and *N*-hexanoyl compounds [87%, mp 158–160° ($C_{18}H_{22}N_2O_3S$)] were similarly obtained.

4-Amino-4′-butylaminodiphenyl Sulfone. A mixture of 4-amino-4′-butyramidodiphenyl sulfone (0.80 g, 2.5 mmol), $LiAlH_4$ (0.28 g, 7.5 mmol), and THF (20 ml) was stirred at reflux for 15 hr. After decomposition of excess hydride with MeOH and H_2O, the THF was evaporated and the residue extracted with Et_2O. The extract was dried ($MgSO_4$) and evaporated to dryness. The residue was crystallized (EtOH–H_2O) to yield 0.37 g (51%), mp 195–196° (lit.[10] mp 193–199°); *N*-amyl compound (50%), mp 151–153° (lit.[10] 150–151°); *N*-hexyl (50%), mp 154–156° (lit.[10] 152–153°).

4-Ethylamino-4′-nitrodiphenyl Sulfone. To an ice-cold stirred solution of 1 *M* borane (6.5 ml, 6.5 mmol) in THF was slowly added 1.00 g (3.1 mmol) of 4-acetamido-4′-nitrodiphenyl sulfone in 50 ml of THF. The solution was refluxed for 8 hr, cooled to 0–5°, treated with 2 *N* HCl (3 ml), and warmed for 15 min. The THF was removed *in vacuo* and the residue treated with 20% NaOH. The orange solid was collected, washed with H_2O, and dried: yield 0.90 g (94%). Recrystallization (95% EtOH) gave mp 215–227° (lit.[10] mp 223°). *Anal.* ($C_{14}H_{14}N_2O_4S$).

Similar reduction of 4-valeramido-4′-nitrodiphenyl sulfone afforded the 4-amylamino-4′-nitrodiphenyl sulfone (70%): mp 136–140° (lit.[10] 142–143°).

4-*N*-Acetyl-*N*-ethylamino-4′-nitrodiphenyl Sulfone. A mixture of 4-ethylamino-4′-nitrodiphenyl sulfone (0.80 g), HOAc (3 ml), and Ac_2O (0.36 ml) was refluxed for 2 hr, cooled, and poured over ice. The precipitate was collected, washed with H_2O, and dried (0.80 g, 88%). Recrystallization (*i*-PrOH) gave mp 136–140°. *Anal.* ($C_{16}H_{16}N_2O_5S$).

4-*N*-Valeryl-*N*-amylamino-4′-nitrodiphenyl Sulfone. A mixture of 4-amylamino-4′-nitrodiphenyl sulfone (4.36 g, 12.5 mmol), NaH (55% in oil, 0.82 g, 18.8 mmol), DMF (5.8 ml), and C_6H_6 (150 ml) was stirred at reflux under N_2 for 2 hr, cooled, and treated with valeryl chloride (2.96 ml, 25 mmol), Reflux was continued for 15 hr. After washing with H_2O, the organic phase was dried ($MgSO_4$) and evaporated *in vacuo*, and the residue was crystallized from cyclohexane: yield 4.79 g (91%). Recrystallization (95% EtOH) gave mp 85–87.5°. *Anal.* ($C_{22}H_{28}N_2O_5S$).

4-Diethylamino-4′-nitrodiphenyl Sulfone. Reduction of the *N*-ethyl-*N*-acetyl nitro sulfone with BH_3 in a manner similar to that described above for 4-acetamido-4′-nitrodiphenyl sulfone afforded the *N,N*-diethyl nitro sulfone (74%); recrystallization (*i*-PrOH) gave mp 139–142°. Chromatography on silica gel (elution with Et_2O–C_6H_6, 3:7) followed by recrystallization (EtOH) gave the analytical sample, mp 148–155°. *Anal.* ($C_{16}H_{18}N_2O_4S$).

4-Diamylamino-4′-nitrodiphenyl Sulfone. Analogous reduction of the *N*-amyl-*N*-valeryl nitro sulfone gave the *N,N*-diamyl nitro sulfone (90%); recrystallization (95% EtOH) gave mp 126–129°. *Anal.* ($C_{22}H_{30}N_2O_4S$).

4-Amino-4′-diethylaminodiphenyl Sulfone. Hydrogenation of the diethylamino nitro sulfone over 5% Rh/C in MeOH afforded the amino sulfone: yield (48%); mp 192–222° after recrystallization from EtOH. *Anal.* ($C_{16}H_{20}N_2O_2S$).

4-Amino-4′-diamylaminodiphenyl Sulfone. Similar hydrogenation of the diamylamino nitro sulfone yielded the amino diamylamino sulfone (50%), mp 136–140° (95% EtOH). *Anal.* ($C_{22}H_{32}N_2O_2S$).

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Potential Antileprotic Agents. 2. Inhibition of Mycobacterial Dihydrofolic Reductase

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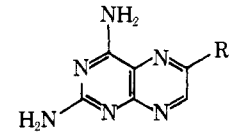
As part of a program for developing new antileprotic agents we have focused attention upon disruption of folate

†This note is dedicated to Alfred Burger in recognition of his many significant contributions to medicinal chemistry.

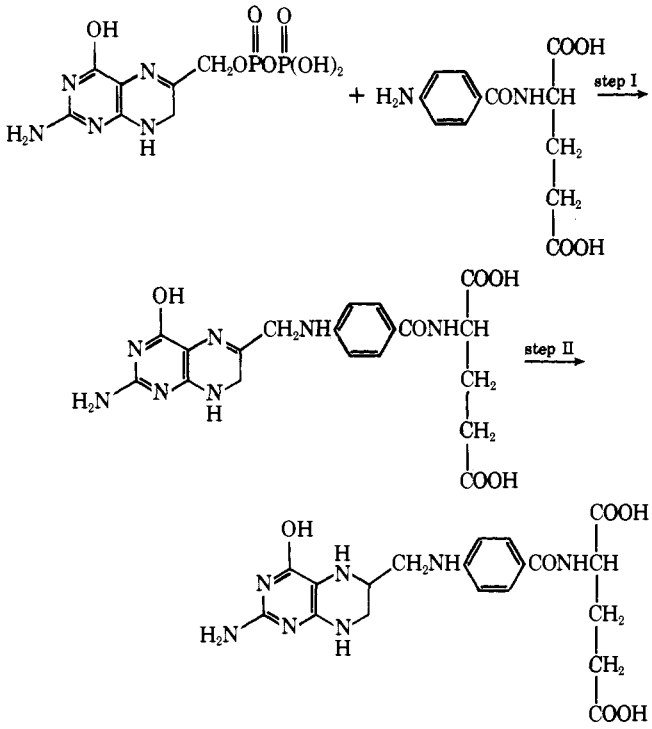
Table I. Physical Properties and Biological Data in *M.* sp. 607 for 2,4-Diamino-6-substituted Pteridines



Compd	R	Yield, %	Mp, °C	Formula[a]	Inhibn of mycobacterial dihydrofolic reductase K_i	K_m[d]/K_i	Mycobacterial growth inhibn, MIC, nmol/ml
6a	CH_3[b]				5.1×10^{-6}	0.8	>568
6b	n-C_4H_9	23	277–285	$C_{10}H_{14}N_6$	1.0×10^{-9}	4,100	917
6c	$(CH_3)_2CHCH_2$	34	296–299	$C_{10}H_{14}N_6$	9×10^{-10}	4,560	688
6d	n-C_5H_{11}	70	280–284	$C_{11}H_{16}N_6$	1.2×10^{-9}	3,415	862
6e	$(CH_3)_2CH(CH_2)_2$	76	297–304	$C_{11}H_{16}N_6$	1.0×10^{-9}	4,100	775
6f	$C_6H_5CH_2$	66	287–299	$C_{13}H_{12}N_6$	9×10^{-10}	4,560	>794
6g	3,4,5-$(OCH_3)_3C_6H_2CH_2$	51	264–268	$C_{16}H_{18}N_6O_3 \cdot 0.25H_2O$	5×10^{-9}	820	347
6h	3,4,5-$(OCH_3)_3C_6H_2(CH_2)_2$[c]				1.8×10^{-8}	230	>281
6i	3,4-$(Cl_2)C_6H_3CH_2$	31	>300	$C_{13}H_{10}N_6Cl_2$[e]	2×10^{-9}	2,050	>311
Trimethoprim					7.0×10^{-8}	58.5	34
Pyrimethamine					7.5×10^{-8}	54.6	101
Aminopterin					1.8×10^{-10}	23,111	742
Amethopterin (Methotrexate)					3.5×10^{-10}	11,714	2200

[a] All compounds were analyzed for C, H, and N. [b] Cyclo Chemical Corp. [c] See ref 4. [d] K_m = Michaelis constant for dihydrofolic acid at pH 6.0 and 25° = 4.1×10^{-6}. [e] N: calcd, 26.2; found, 25.7.

biosynthesis in mycobacterial organisms. In paper 1 of this series[1] a rationale was presented for sequential blockade of the de novo folate pathway in a model system, *Mycobacterium* species 607. Several compounds of the diphenyl sulfone class were examined for their ability to suppress growth of the organism, presumably *via* inhibition of step I, the condensation of *p*-aminobenzoylglutamate with 2-amino-4-hydroxy-7,8-dihydro-6-pteridylmethylpyrophosphate to form dihydrofolate. In this second communication we report some initial studies on compounds designed to inhibit step II, reduction of dihydrofolate to tetrahydrofolate in *M.* sp. 607.



The dihydrofolic reductase inhibitors currently in use as antimicrobial agents in combination therapy with "sulfa" compounds are trimethoprim and pyrimethamine. However, it was observed by Shepard‡ that trimethoprim alone was without effect against *Mycobacterium leprae* and did not potentiate the effect of diaminodiphenyl sulfone. Our measurements (Table I) show that trimethoprim and pyrimethamine were poor inhibitors of the dihydrofolic reductase isolated from *M.* sp. 607. Whole cell data showed trimethoprim to be a moderate growth inhibitor, while pyrimethamine was a poor one. Aminopterin and methotrexate were found to be powerful inhibitors of the mycobacterial enzyme but inactive as growth inhibitors.

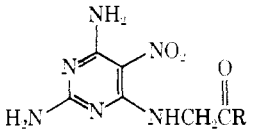
Since the mycobacteria do not require exogenous folate, it is likely that they do not have active transport provision for passing such charged molecules of the aminopterin type through the cell wall. However, in a previous communication[3] we reported the growth inhibition of *Streptococcus faecium* (a folate-dependent organism) by some 2,4-diamino-6-aralkylpteridines. It seemed likely to us that such compounds could acquire passive entry into the mycobacteria. Accordingly, a series of 2,4-diamino-6-substituted pteridines was prepared and growth inhibition and enzyme inhibition data for *M.* sp. 607 were obtained. These data are presented in Table I.

The data indicate that moderately strong binding of inhibitor to enzyme takes place for some of the compounds ($K_m/K_i \sim 4000$). The weak binding of the 6-methyl compound **6a** suggests the presence of a lipophilic region on the enzyme with a requirement for the 6 substituent to be greater than CH_3 to obtain substantial binding.[4] There was not a significant spread of inhibitory potencies for the other alkyl pteridines **6b**–e and the benzyl analog **6f.** It is interesting that **6f** was reasonably inhibitory, whereas substitution of the phenyl ring with electron-donating or -withdrawing groups caused pronounced decreases in inhibition. The high minimum inhibitory concentrations (MIC) obtained for these compounds suggest that penetration of the cell wall is poor in this mycobacterial system. However, the isobutyl analog **6c** was selected as a representative compound for this series and is currently being tested for activity against *M. leprae* in the mouse footpad assay.[5]

The synthesis of the unknown pteridines was carried out by the method previously described[3] as shown in Scheme I. Addition of an appropriate acid chloride to excess CH_2N_2 and subsequent treatment with dry HCl afforded the chloromethyl ketone **1.** Displacement with

‡ C. C. Shepard, unpublished data cited in a review; see ref 2.

Table II. 2,4-Diamino-5-nitro-6-pyrimidinylaminomethyl Ketones

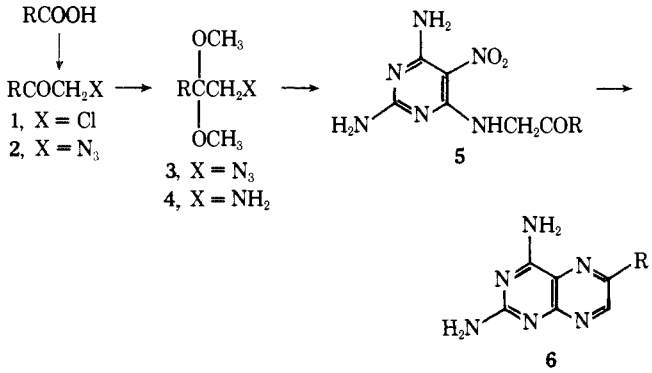


Compd	R	Yield, %	Mp, °C	Formula[a]
5b	n-C_4H_9[b]			
5c	$(CH_3)_2CHCH_2$	47	186–189	$C_{10}H_{16}N_6O_3$
5d	n-C_5H_{11}	25	184–188	$C_{11}H_{18}N_6O_3$
5e	$(CH_3)_2CH(CH_2)_2$	18	190.5–192	$C_{11}H_{18}N_6O_3 \cdot 0.5H_2O$
5f	$C_5H_5CH_2$	79	207–210	$C_{13}H_{14}N_6O_3$
5g	3,4,5-$(OCH_3)_3C_6H_2CH_2$[b]	85		
5i	3,4-$(Cl)_2C_6H_3CH_2$	87	190–193	$C_{13}H_{12}Cl_2N_6O_3$

[a] Analyzed for C, H, and N. [b] Crude material used directly in next step.

NaN_3 gave the azido ketone **2** which was blocked as the ketal **3** prior to reduction with $NaBH_4$ in 2-PrOH. The amino ketals **4** so obtained were subjected to short path distillation *in vacuo* as the only significant purification required in the above sequence. Coupling of the amino ketals **4** with 2,4-diamino-5-nitro-6-chloropyrimidine followed by acid hydrolysis (90% CF_3COOH) yielded the pyrimidinyl ketones **5**. Reduction of the 5-nitro group with Zn–HOAc and *in situ* oxidation (dilute H_2O_2) of the resulting 7,8-dihydropteridines afforded the 2,4-diamino-6-substituted pteridines **6**.

Scheme I



b, R = n-C_4H_9
c, R = $CH_2CH(CH_3)_2$
d, R = n-C_5H_{11}
e, R = $CH_2CH_2CH(CH_3)_2$
f, R = $CH_2C_6H_5$
g, R = 3,4,5-$(OCH_3)_3C_6H_2CH_2$
i, R = 3,4-$(Cl)_2C_6H_3CH_2$

Experimental Section§

Chloromethyl Ketones 1. A mixture of 20.3 g (0.09 mol) of 3,4,5-trimethoxyphenylacetic acid, 8.4 ml (0.1 mol) of $SOCl_2$, and 100 ml of C_6H_6 was refluxed for 5 hr. The solvent was removed *in vacuo* and the residual acid chloride taken up in Et_2O (60 ml). The solution was added dropwise to CH_2N_2 (0.27 mol in 300 ml of Et_2O) at 0–5°. After standing for 2 hr in the cold, the mixture was gassed with dry HCl for 1 hr and filtered. The filtrate was evaporated under reduced pressure to leave **1g** as a white solid, 14.5 g (63%), mp 75–76.5° (lit.[6] mp 75°). The other chloromethyl ketones were similarly obtained as syrups.

Azidomethyl Ketones 2. A mixture of 10 g of **1**, 2 equiv of NaN_3, and 90 ml of 80% MeOH was stirred 4 hr at room temperature. The MeOH was removed *in vacuo*, and the residue was partitioned between Et_2O and H_2O. The Et_2O extract was dried ($MgSO_4$) and evaporated under reduced pressure to give the azidomethyl ketone in 38–91% yield as a syrup possessing a strong 4.75-μ band in the infrared.

§ Compounds followed by empirical formulas were analyzed for C, H, and N with values ±0.4% of theoretical values.

Azidomethyl Ketals 3. A mixture of 20 g of **2**, 50 ml of trimethyl orthoformate, 50 ml of MeOH, and 2 g of *p*-TsOH was refluxed for 15 hr. The solvents were removed *in vacuo*, and the residue was partitioned between Et_2O and saturated $NaHCO_3$. The organic layer was dried ($MgSO_4$) and the Et_2O removed to leave the ketal as a syrup. The ir spectra showed N_3 at 4.75 μ, ketal C–O at 9.5, and loss of C=O at 5.8.

Aminomethyl Ketals 4. A mixture of 1 equiv of **3**, 2.3 equiv of $NaBH_4$, and 20 vol of 2-PrOH was stirred at reflux for 24 hr. The solvent was removed *in vacuo*, and the residue was partitioned between Et_2O and H_2O. The organic layer was dried ($MgSO_4$) and evaporated. The crude aminomethyl ketals were vacuum distilled through a short path column to afford colorless liquid products.

2,4-Diamino-5-nitro-6-pyrimidinylaminomethyl Ketones 5 (Table II). A mixture of equimolar amounts of **4**, 2,4-diamino-5-nitro-6-chloropyrimidine,[7] *s*-collidine, and 80 vol of DMF was stirred at 90° under N_2 for 45 min. The cooled mixture was filtered and the filtrate poured into 200 vol of ice H_2O. The crude, solid ketals were obtained in 25–55% yield and used without further purification.

The intermediate ketal **2g** was stirred with 50 ml of 90% CF_3COOH at room temperature for 15 hr. The solvent was removed *in vacuo*, and the residue was diluted with H_2O and taken to pH 8 with saturated K_2CO_3. The solid ketone was collected by filtration and washed with H_2O and Et_2O. Purification was accomplished by recrystallization from MeOH or a 2-$MeOC_2H_4OH$–H_2O mixture.

2,4-Diamino-6-substituted Pteridines 6 (Table I). A mixture of 1.0 g of **5** and 100 ml of glacial HOAc was heated on a steam bath under N_2. To this hot mixture was added an equal weight of zinc dust over a 30-min period. After cooling to room temperature, the mixture was filtered. The filtrate was treated dropwise with 30% H_2O_2 (3 equiv) and allowed to stand at ambient temperature for 2 hr. The solution was cooled to 0° and slowly alkalized to pH 8 with concentrated NH_4OH. The yellow solid was collected by filtration and washed with H_2O. The pteridines were recrystallized from MeOH, except **6f** and **6i**, which required 2-$MeOC_2H_4OH$. The uv spectra (pH 1) of the pteridines showed absorption maxima at 245 and 340 nm.

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