Selective Inhibitors of *Candida albicans* Dihydrofolate Reductase: Activity and Selectivity of 5-(Arylthio)-2,4-diaminoquinazolines

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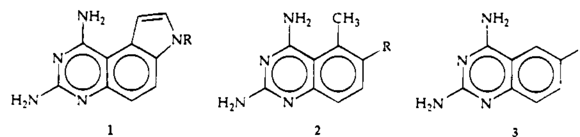
The recent increase in fungal infections, especially among AIDS patients, has resulted in the need for more effective antifungal agents. In our search for such agents, we focused on developing compounds which inhibit fungal dihydrofolate reductase (DHFR). A series of 25 5-(arylthio)-2,4-diaminoquinazolines were synthesized as potentially selective inhibitors of *Candida albicans* DHFR. The majority of the compounds were potent inhibitors of *C. albicans* DHFR and much less active against human DHFR. High selectivity, as defined by the ratio of the I_{50} values for human and *C. albicans* DHFR, was achieved by compounds with bulky and rigid 4-substituents in the phenylthio moiety. For example, 5-[(4-morpholinophenyl)thio]-2,4-diaminoquinazoline displayed a selectivity ratio of 540 and was the most selective inhibitor synthesized to date. Substitution in the 2- or 3-position of the 5-phenylthio group provided only marginal selectivity. 6-Substituted-5-[(4-*tert*-butylphenyl)thio]-2,4-diaminoquinazolines showed potent activity against the *C. albicans* enzyme but were equally active against human DHFR. Most of the selective compounds were also good inhibitors of *C. albicans* cell growth, with minimum inhibitory concentration values as low as 0.05 μg/mL.

Introduction

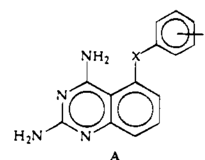
The increasing occurrence of systemic fungal infections, particularly among AIDS patients, has stimulated the search for better antifungal agents.[1] In a program aimed at identifying novel antifungal agents, we focused on developing compounds that would selectively inhibit the enzyme dihydrofolate reductase (DHFR) from fungal species. Inhibition of DHFR is a well-established mechanism of drug action.[2] DHFR catalyzes the reduction of dihydrofolic acid to tetrahydrofolic acid (FH_4). Cofactors derived from FH_4 are crucial to a number of metabolic processes including the biosynthesis of DNA.[3] The inability to synthesize DNA as a result of the inhibition of DHFR can ultimately lead to cell death.

The choice of DHFR as the molecular target for the development of antifungal agents is complicated by the fact that the enzyme is ubiquitous and is found in both microorganisms and humans. Thus, to minimize DHFR-related toxicities to the human host, the ideal antifungal agent should selectively inhibit the fungal enzyme. Such species-selective inhibition of DHFR has proven clinically useful in other therapeutic applications.[2,4] For example, the therapeutic value of the antibacterial agent trimethoprim lies in its ability to selectively inhibit the bacterial enzyme.

Certain pyrrolo-2,4-diaminoquinazolines **1**,[5] which are potent but nonselective inhibitors of DHFR, have been reported to have *in vitro* antifungal activity.[5a] Other DHFR inhibitors reported to have *in vitro* antifungal activity include 5-methyl-6-alkyl-2,4-diaminoquinazolines **2**[6] and 6-substituted-2,4-diaminoquinazolines **3**.[7] One analogue of **3**, 2,4-diamino-6-[2-(3,4-dichlorophenyl)acetamido]quinazoline, was reported to have *in vivo* activity in mice infected with *Cryptococcus neoformans*.[8]



In this paper, we report the synthesis and biological activity of a series of 5-(arylthio)-2,4-diaminoquinazolines (A, X = S). The design of these compounds was based on a three-dimensional molecular model of *Candida albicans* DHFR which was constructed using the X-ray crystal structure of L1210 DHFR[9] and the amino acid sequence of the *C. albicans* enzyme.[10] The molecular modeling details are not reported in this paper because subsequent X-ray crystallographic studies[11] of *C. albicans* DHFR showed that its structure was different from the homology-based model in features important to the binding of the inhibitors described here. Nevertheless, the majority of the compounds reported herein were potent and selective inhibitors of *C. albicans* DHFR.



Chemistry

The procedure for the synthesis of compounds **8a–e**, **i**, **k–p**, **s**, **u–y** was analogous to a previously reported route[12] shown in Scheme 1. Reacting 2,6-dinitrobenzonitrile (**4**) with aryl thiolates at 0 °C resulted in displacement of a nitro group to give 2-(arylthio)-6-nitrobenzontriles **5a–e**, **g–r** (Table 1). A trace amount of a byproduct identified as the corresponding bis(aryl sulfide) **6** was obtained. Compound **6** was the predomi-

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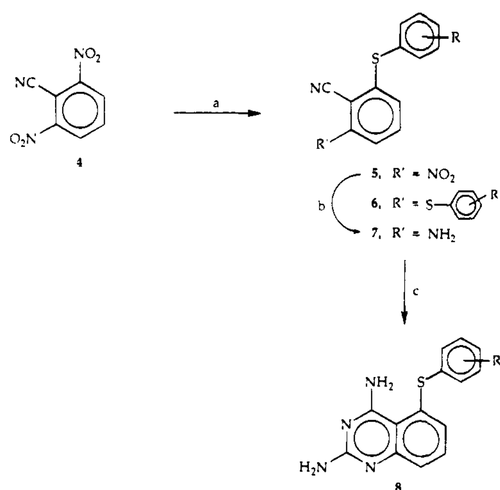
Scheme 1[a]



[a] (a) ArSH, K_2CO_3, DMF; (b) $SnCl_2{\cdot}2H_2O$, HCl, diglyme; (c) $HNC(Cl)NH_2{\cdot}HCl$, fused at 180−190 °C.

nant product if the reaction was run at room temperature. 2-Nitro-6-[[4-(*N,N*-diethylamino)phenyl]thio]benzonitrile (**5f**) was prepared from 2-nitro-6-[(4-aminophenyl)thio]benzonitrile (**5l**) using acetaldehyde and sodium cyanoborohydride.

Intermediates **5a−r** were reduced with stannous chloride under acidic conditions,[12] resulting in 6-(arylthio)-2-aminobenzonitriles **7a−k, m−r** (Table 2). Compound **5k** was selectively reduced to 2-amino-6-[(4-nitrophenyl)thio]benzonitrile (**7k**), albeit in a 43% yield. The electron-withdrawing cyano group might have influenced this selective reduction. Compound **7l** was obtained by the treatment of **7h** with cuprous cyanide.[13] Fusing **7a−r** with chloroformamidine hydrochloride[14,15] (Scheme 1) at 180−190 °C gave compounds **8a−e, i, k−p, s, u−y** listed in Table 3.

A second approach, shown in Scheme 2, was a modification of a published procedure in which a fluoro group of commercially available 2,6-difluorobenzonitrile (**9**) was readily displaced by a series of alkyl alcohols, alkylamines, and alkylthiols.[16] In a similar fashion, aryl thiolates, formed using potassium *tert*-butoxide in dimethyl sulfoxide, displaced one of the fluoro groups in **9** to give 2-(arylthio)-6-fluorobenzonitriles **10a−e, g−h** (Table 4). A trace amount of the bis(aryl sulfide) **6** was formed when the reaction was run at 5−10 °C and was the predominant product if the reaction was run at room temperature. Compound **10f** was prepared by the reaction of 3,4-dihydro-2*H*-pyran with **10e** catalyzed by pyridinium *p*-toluenesulfonate.[17] Cyclization of **10a−d, f−h** with guanidine carbonate in *N,N*-dimethylacetamide[16] gave the desired **8f−h,j,l,q,t** listed in Table 3. Compound **8r** was obtained by the deprotection of **8h** catalyzed by hydrochloric acid.

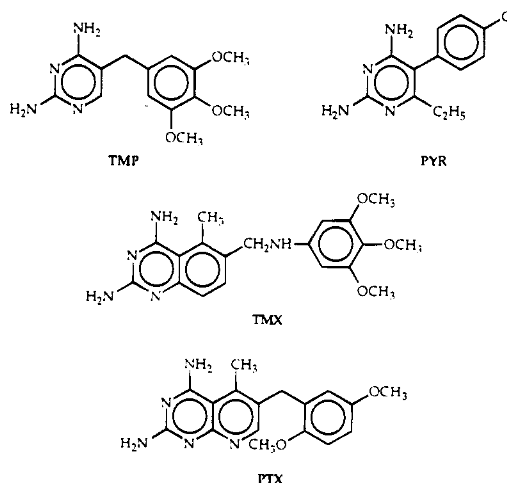
Four 6-substituted analogues of the 5-(4-*tert*-butylphenyl)thio analogue **8d**, compounds **13a−d**, were synthesized and are listed in Table 5. As shown in Scheme 3, the synthesis of **13a** began with displacement of the chloro group in **11**[18] by the anion of 4-*tert*-butylthiophenol to give **12**, which was then hydrogenated with palladium on carbon as the catalyst to yield **13a**. Reduction of the nitro group in **11** with stannous chloride under acidic conditions resulted in compound **14**.[18] Diazotization of **14**, followed by treatment with potassium cyanide,[18] gave compound **15**,[18] which was reacted with the anion of 4-*tert*-butylthiophenol to give **13b**.

The synthesis of **13c,d**, as shown in Scheme 4, began with 2,3-dichlorophenol (**16**) which was first alkylated with either ethyl iodide or isobutyl bromide to give **17a,b**, respectively. Nitration of **17a,b** gave mixtures of **18a** and **19a**, and **18b** and **19b**, respectively. After chromatographic separation,[19] **19a,b** were reacted with cuprous cyanide to give **20a,b**,[20] respectively. Displacement of the chloro group in **20a,b** by the anion of 4-*tert*-butylthiophenol resulted in **21a,b**, which were reduced with stannous chloride/hydrochloric acid to give **22a,b**. Fusion of **22a,b** with chloroformamidine hydrochloride at 185 °C resulted in **13c,d**. Nitration of **16** before alkylation led to complicated product mixtures.

Results and Discussion

Although our goal was to develop broad-spectrum antifungal agents, *C. albicans* was chosen as a primary target because of its role as the leading cause of fungal infections in the clinic.[1,21] *C. albicans* currently is the fifth leading cause of infections arising in a hospital setting.[22] Hospital-acquired infections due to *C. albicans* are nearly as common as those due to bacteria such as *Escherichia coli*.[22]

Initially, we attempted to identify lead compounds by testing a diverse group of existing DHFR inhibitors including trimethoprim (TMP), pyrimethamine (PYR), trimetrexate (TMX), and piritrexim (PTX). As shown in Table 6, TMP was a weak inhibitor of *C. albicans* DHFR, and it showed a selectivity index of only 10. PYR, TMX, and PTX were much more active than TMP against *C. albicans* DHFR, but these compounds showed selectivity indices of less than 1. However, none of these compounds showed *in vitro* activity against *C. albicans* cell growth, as measured by their minimum inhibitory concentration (MIC) values. A large number of TMP, PYR, TMX, and PTX analogues from Burroughs Wellcome Co. files were also tested as enzyme inhibitors, and none was found to be both highly active and selective.[23]



Since screening known DHFR inhibitors failed to identify a useful antifungal lead compound, we concentrated on a synthetic effort which was aided by molecular modeling. A model of the active site of *C. albicans* DHFR was built using the three-dimensional structure of L1210 DHFR[9] as the template. Although crystal structures for bacterial DHFR are known,[24] we chose the crystal structure of a mammalian DHFR because inhibitor profiles suggested that *C. albicans* DHFR was more similar to the mammalian than to the bacterial

Table 1. Physical Constants for 2-(Arylthio)-6-nitrobenzonitriles **5a–r**[a]

compd	R	yield (%)	mp (°C)	empirical formula	elemental anal.
5a	H	73	106–107	$C_{13}H_8N_2O_2S$	C,H,N,S
5b	4-CH_3	89	106–107	$C_{14}H_{10}N_2O_2S$	C,H,N,S
5c	4-$CH(CH_3)_2$	94	116–118	$C_{16}H_{14}N_2O_2S$	C,H,N,S
5d	4-$C(CH_3)_3$	99	134–136	$C_{17}H_{16}N_2O_2S$	C,H,N,S
5e	4-$C(CH_3)_2CH_2CH_3$	69[b]	76–78	$C_{18}H_{18}N_2O_2S$	C,H,N,S
5f	4-$N(CH_2CH_3)_2$	83	143–144	$C_{17}H_{17}N_3O_2S$	C,H,N,S
5g	4-Cl	90[c]	148–149	$C_{13}H_7N_2ClO_2S$	C,H,N,Cl,S
5h	4-Br	71	154–156	$C_{13}H_7N_2BrO_2S$	C,H,N,Br,S
5i	4-F	95	155–156	$C_{13}H_7N_2FO_2S$	C,H,N,S
5j	4-CF_3	58[d]	124–126	$C_{14}H_7N_2F_3O_2S$	C,H,N,S
5k	4-NO_2	87[e]	214–215	$C_{13}H_7N_3O_4S$	C,H,N,S
5l	4-NH_2	63[e]	168–170	$C_{13}H_9N_3O_2S$	C,H,N,S
5m	4-OCH_3	93	139–140	$C_{14}H_{10}N_2O_3S$	C,H,N,S
5n	3,4,5-$(OCH_3)_3$	86	200–201.5	$C_{16}H_{14}N_2O_5S$	C,H,N,S
5o	3-OCH_3	81	144–147	$C_{14}H_{10}N_2O_3S$	C,H,N,S
5p	2-OCH_3	47[f]	142–143	$C_{14}H_{10}N_2O_3S$	C,H,N,S
5q	3-Cl	99[b]	161–164	$C_{13}H_7N_2ClO_2S$	C,H,N,Cl,S
5r	2-Cl	67[g]	151–153	$C_{13}H_7N_2ClO_2S$	C,H,N,Cl,S

[a] Compounds were prepared according to method A (see the Experimental Section, **5b**) except **5f**, which was prepared by the reductive amination of **5l** with acetaldehyde (see the Experimental Section). [b] Purified by flash column chromatography[19] on silica gel with EtOAc–hexane (1:4). [c] Recrystallized from EtOH–H_2O. [d] Purified by flash column chromatography on silica gel with EtOAc–hexane (3:7). [e] Recrystallized from EtOH. [f] Purified by flash column chromatography on silica gel with EtOAc–hexane (1:1). [g] Purified by flash column chromatography on silica gel with EtOAc–hexane (3:2).

Table 2. Physical Constants for 6-(Arylthio)-2-aminobenzonitriles **7a–r**[a]

compd	R	yield (%)	mp (°C)	empirical formula	elemental anal.
7a	H	94	73–74	$C_{13}H_{10}N_2S$	C,H,N,S
7b	4-CH_3	86	114–116	$C_{14}H_{12}N_2S$	C,H,N,S
7c	4-$CH(CH_3)_2$	77[b]	80–82	$C_{16}H_{16}N_2S$	C,H,N,S
7d	4-$C(CH_3)_3$	73[b]	117–120	$C_{17}H_{18}N_2S$	C,H,N,S
7e	4-$C(CH_3)_2CH_2CH_3$	80[c]	122–124	$C_{18}H_{20}N_2S$	C,H,N,S
7f	4-$N(CH_2CH_3)_2$	58[d]	135–137	$C_{17}H_{19}N_3S$	C,H,N,S
7g	4-Cl	91	137–138.5	$C_{13}H_9N_2ClS$	C,H,N,Cl,S
7h	4-Br	99[b]	120–123	$C_{13}H_9N_2BrS$	C,H,N,Br,S
7i	4-F	88[b]	122–124	$C_{13}H_9N_2FS$	C,H,N,S
7j	4-CF_3	81	118–120	$C_{14}H_9N_2F_3S$	C,H,N,S
7k	4-NO_2	43[b]	212–213	$C_{13}H_9N_3O_2S$	C,H,N,S
7l	4-CN	41	176–177	$C_{14}H_9N_3S$	C,H,N,S
7m	4-OCH_3	55	95–98	$C_{14}H_{12}N_2S$	C,H,N,S
7n	3,4,5-$(OCH_3)_3$	94	108–110	$C_{16}H_{16}N_2O_5S$	C,H,N,S
7o	3-OCH_3	51	98–100	$C_{14}H_{12}N_2OS$	C,H,N,S
7p	2-OCH_3	70[e]	145–147	$C_{14}H_{14}N_2OS$	C,H,N,S
7q	3-Cl	58[f]	88–90	$C_{13}H_9N_2ClS$	C,H,N,Cl,S
7r	2-Cl	62[e]	113–115	$C_{13}H_9N_2ClS$	C,H,N,Cl,S

[a] Compounds were prepared according to method B (see the Experimental Section, **7b**) except **7l**, which was prepared by the displacement of the bromo group in **7h** by CuCN[13] (see the Experimental Section). [b] Purified by flash column chromatography on silica gel with CH_2Cl_2. [c] Purified by flash column chromatography on silica gel with EtOAc–hexane (1:1). [d] Purified by filtering a solution of **7f** in CH_2Cl_2 through a silica gel pad. [e] Purified by flash column chromatography on silica gel with EtOAc–hexane (2:3). [f] Recrystallized from EtOAc–hexane.

enzyme. For example, benzylpyrimidines such as TMP which are potent inhibitors of bacterial DHFR and poor inhibitors of vertebrate DHFR showed poor affinity for the *Candida* enzyme. Additionally, *C. albicans* DHFR consists of 192 residues, compared to 187 residues in L1210 DHFR and 159 residues in *E. coli* DHFR. The active site of human DHFR[25] was similarly constructed based on the 88% homology between L1210 and human DHFR.

In general, the residues in the active site of the constructed human and *C. albicans* DHFR were similar, but some subtle differences were apparent. A region of the active site near the helix formed by residues 55–62 of the fungal enzyme was of particular interest because phenylalanine-66 in the *C. albicans* enzyme corresponded to asparagine-64 in human DHFR. Our strategy focused on exploiting this difference. Inhibitors with a hydrophobic moiety projecting toward this part of the active site were envisaged to interact more favorably with phenylalanine-66 than with asparagine-64. On the basis of simple molecular graphics modeling, we chose compounds of the general structure A, where para R groups appeared to be able to interact with those two residues. In structure A, atom X potentially could be any divalent atom or a methylene group. In our initial work, we chose to study a series of 5-(arylthio)-2,4-diaminoquinazolines (A, X = S).

X-ray crystallographic studies of several members bound to *C. albicans* DHFR subsequently established that the high degree of selectivity of these 5-(arylthio)-2,4-diaminoquinazolines was not due to residue differences mentioned above.[11] Thus our initial postulates regarding selectivity were invalid.

Compounds **8a–y** listed in Table 3 were prepared and tested for their activities against *C. albicans* and human DHFR as well as against *C. albicans* cell growth. The results of the *in vitro* assays are listed in Table 7 together with selectivity indices. Substituents in the 5-arylthio moiety were chosen to explore electronic and steric properties. For comparison, a few compounds were synthesized with substituents in the 2- or 3-position of the phenylthio moiety.

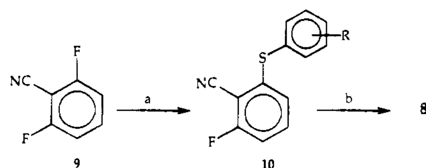
As shown in Table 7, substituent variations appeared to be well-tolerated, and the compounds exhibited good

Table 3. Physical Constants for 5-(Arylthio)-2,4-diaminoquinazoliness **8a–y**[a]

compd	R	method	yield (%)	mp (°C)	empirical formula	elemental anal.
8a	H	C	80	240–241	$C_{14}H_{12}N_4S{\cdot}0.2H_2O$	C,H,N,S
8b	4-CH_3	C	73	203–204.5	$C_{15}H_{14}N_4S$	C,H,N,S
8c	4-$CH(CH_3)_2$	C	56	193–195	$C_{17}H_{18}N_4S$	C,H,N,S
8d	4-$C(CH_3)_3$	C	22	192 dec	$C_{18}H_{20}N_4S{\cdot}0.7H_2O$	C,H,N,S
8e	4-$C(CH_3)_2CH_2CH_3$	C	60[b]	207–208	$C_{19}H_{22}N_4S$	C,H,N,S
8f	4-*n*-hexyl	E	64[b]	170–172	$C_{20}H_{24}N_4S$	C,H,N,S
8g	4-cyclohexyl	E	63[b]	213–214	$C_{20}H_{24}N_4S{\cdot}0.1H_2O$	C,H,N,S
8h	4-OTHP[c]	E	42[b]	195–197	$C_{19}H_{20}N_4O_2S$	C,H,N,S
8i	4-$N(CH_2CH_3)_2$	C	31[d]	193–195	$C_{18}H_{21}N_5S$	C,H,N,S
8j	4-morpholino	E	57	260–262	$C_{18}H_{19}N_5OS{\cdot}0.2H_2O$	C,H,N,S
8k	4-Cl	C	75	223–224	$C_{14}H_{11}N_4ClS$	C,H,N,Cl,S
8l	4-Br	C	50	225–226	$C_{14}H_{11}N_4BrS$	C,H,N,Br,S
8l	4-Br	E	68	227–228	$C_{14}H_{11}N_4BrS$	C,H,N,Br,S
8m	4-F	C	25	210–212	$C_{14}H_{11}N_4FS$	C,H,N,S
8n	4-CF_3	C	50[b]	226–228	$C_{15}H_{11}N_4F_3S$	C,H,N,S
8o	4-NO_2	C	39	206 dec	$C_{14}H_{11}N_5O_2S{\cdot}0.8H_2O$	C,H,N,S
8p	4-CN	C	71[b]	236–237	$C_{15}H_{11}N_5O_2S{\cdot}0.2H_2O$	C,H,N,S
8q	4-NH_2	E	17[b]	227 dec	$C_{14}H_{13}N_5OS{\cdot}0.4H_2O$	C,H,N,S
8r	4-OH		44	232–235	$C_{14}H_{12}N_4OS{\cdot}0.5H_2O$	C,H,N,S
8s	4-OCH_3	C	93	222–224	$C_{15}H_{14}N_4OS{\cdot}0.2H_2O$	C,H,N,S
8t	3,4-$(OCH_3)_2$	E	92	243–245	$C_{16}H_{16}N_4O_2S$	C,H,N,S
8u	3,4,5-$(OCH_3)_3$	C	27	178–180	$C_{17}H_{18}N_4O_3S$	C,H,N,S
8v	3-OCH_3	C	51	178–180	$C_{15}H_{14}N_4OS$	C,H,N,S
8w	2-OCH_3	C	59[b]	218–220	$C_{15}H_{14}N_4OS$	C,H,N,S
8x	3-Cl	C	80	215–217	$C_{14}H_{11}N_4ClS$	C,H,N,Cl,S
8y	2-Cl	C	62[b]	272 dec	$C_{14}H_{19}N_4ClS$	C,H,N,Cl,S

[a] Compounds were prepared according to methods C and E (see the Experimental Section, **8b,t**, respectively) except **8r**, which was prepared by the deprotection of **8h** with HCl (see the Experimental Section). [b] Purified by flash column chromatography on silica gel with $MeOH-CH_2Cl_2$ (1:9). [c] THP–tetrahydropyran. [d] Purified by filtering through a silica gel pad with MeOH–EtOAc (1:4).

Scheme 2[a]



[a] (a) ArSH, *t*-BuOK, DMSO; (b) $(H_2NC(NH)NH_2)_2{\cdot}H_2CO_3$, DMA, Δ.

Table 4. Physical Constants for 2-(Arylthio)-6-fluorobenzonitriles **10a–g**[a]

compd	R	yield (%)	mp (°C)	empirical formula	elemental analysis
10a	4-morpholino	87	189–190	$C_{17}H_{15}N_2FOS$	C,H,N,S
10b	4-cyclohexyl	76[b]	80–81	$C_{19}H_{18}NFS$	C,H,N,S
10c	4-Br	68[c]	227–228	$C_{13}H_7NBrFS$	C,H,N,Br,S
10d	4-NH_2	30[b]	112–113	$C_{13}H_9N_2FS$	C,H,N,S
10e	4-OH	53	110–111	$C_{13}H_8NFOS$	C,H,N,S
10f	4-OTHP[d]	72	91–95	$C_{18}H_{16}NFO_2S$	C,H,N,S
10g	3,4-$(OCH_3)_2$	74	137–138	$C_{15}H_{12}NFO_2S$	C,H,N,S
10h	4-*n*-hexyl	64		$C_{19}H_{20}NFS$	C,H,N,S

[a] Compounds were prepared according to method D (see the Experimental Section, **10g**) except **10f**, which was prepared by the reaction of **10e** with dihydropyran[17] (see the Experimental Section). [b] Purified by flash column chromatography on silica gel with EtOAc–hexane (1:4). [c] Purified by flash column chromatography on silica gel with EtOAc–hexane (1:9). [d] THF–tetrahydropyran.

inhibitory activity against *C. albicans* DHFR with I_{50} values generally equal to or less than 0.5 μM. The *tert*-butyl-substituted analogue **8d** was the most active inhibitor in the series with an I_{50} value of 8 nM.

Eight of the compounds had selectivity indices equal to or greater than 100. Variations in the electronic properties of the 4-substituents in the phenylthio moiety did not appear to affect the selectivity of these compounds for the *C. albicans* enzyme. However, the bulkiness (as measured by molar refractivity[26]) of substituents appeared to influence selectivity. Thus, **8j**, with the bulkiest para substituent, had the highest selectivity index of 540. This was followed by the cyclohexyl-substituted **8g** and the *tert*-butyl-substituted **8d**. Adding a methyl group to **8d**, as in **8e**, significantly reduced activity against *C. albicans* DHFR and therefore lowered selectivity for the enzyme. Flexible substituents such as those found in **8f,i** resulted in marginal selectivity for *C. albicans* DHFR.

Substituents at the 4-position of the phenylthio moiety appeared to be required for optimum selectivity. For example, 3,4-dimethoxy-substituted **8t** and 3,4,5-trimethoxy-substituted **8u** displayed good selectivity, but the 3-methoxy- and 2-methoxy-substituted analogues **8v,w** were much less selective. Similarly, the selectivity for *C. albicans* DHFR of the 3-chloro derivative **8x** and the 2-chloro-substituted **8y** was minimal compared to that of the 4-chloro analogue **8k**.

In an attempt to further optimize the potency of **8d**, the most potent analogue in Table 7, four 6-substituted analogues of **8d** were synthesized. Substitution at the 6-position appeared to be compatible with the active site geometry of the *C. albicans* enzyme model and was expected to enhance affinity for the enzyme. However, as shown in Table 8, only one compound, the isobutoxy-substituted **13d**, showed higher affinity than **8d** for *C. albicans* DHFR. Unfortunately, **13d** was also more potent against the human enzyme. The selectivity index of compound **13d** was 25-fold lower than that of **8d**.

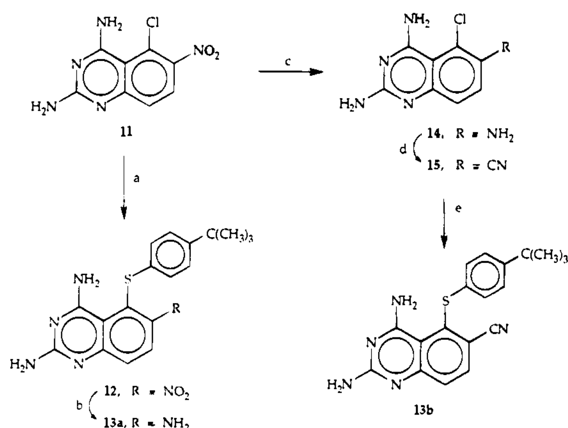
Ten of the compounds in Table 7 had *C. albicans* MIC values equal to or less than 0.5 μg/mL. These values compared favorably with those reported for the nonselective pyrrolo-2,4-diaminoquinazolines (>0.64 μg/mL)[5a] and those shown by TMX and PTX (>10 μg/mL).

Three compounds, **8a,d,l**, with *C. albicans* MIC values ranging from 0.05 to 0.25 μg/mL, were chosen for *in vivo* assays in mouse models of *Candida* nephritis. None of the compounds showed significant activity when administered in concentrations ranging from 20 to 200 mg/kg using intraperitoneal, subcutaneous, and oral routes. For example, the oral efficacy of **8l** was investigated at

Table 5. Physical Constants for C6-Substituted-5-[(4-*tert*-butylphenyl)thio]-2,4-diaminoquinazolines **13a–d**

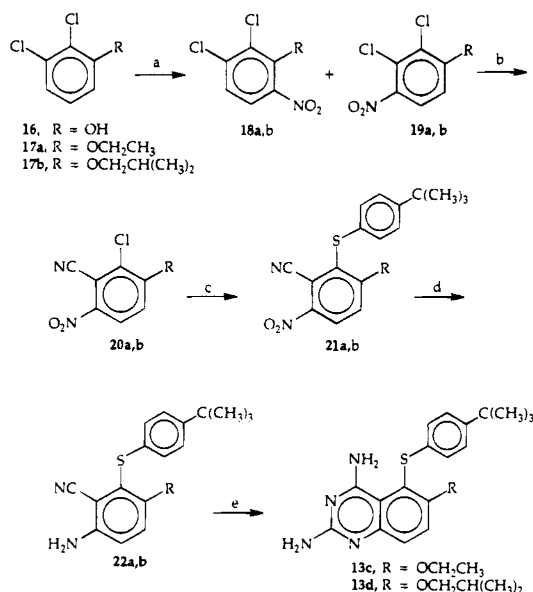
compd	R	yield (%)	mp (°C)	empirical formula	elemental analysis
13a	NH_2	8	220–221	$C_{18}H_{21}N_5S$	C,H,N,S
13b	CN	53	277–279 dec	$C_{19}H_{19}N_5S$	C,H,N,S
13c	OCH_2CH_3	72	200–201	$C_{20}H_{24}N_4OS$	C,H,N,Br,S
13d	$OCH_2CH(CH_3)_2$	44	173–174	$C_{22}H_{28}N_4OS{\cdot}H_2O$	C,H,N,S

Scheme 3[a]



[a] (a) p-$C(CH_3)_3C_6H_4SH$, NaH, DMF; (b) H_2, Pd/C, DMF; (c) $SnCl_2{\cdot}2H_2O$, HCl, diglyme; (d) $NaNO_2$, HCl, KCN; (e) p-$C(CH_3)_3$-C_6H_4SH, NaH, DMF.

Scheme 4[a]



[a] (a) HNO_3; (b) CuCN, N-methylpyrrolidinone; (c) p-$C(CH_3)_3$-C_6H_4SH, t-BuOK, DMSO; (d) $SnCl_2{\cdot}2H_2O$, HCl, diglyme; (e) $HNC(Cl)NH_2{\cdot}HCl$, fused at 185 °C.

Table 6. Inhibition of Dihydrofolate Reductase and *in Vitro* Antifungal Activity of Known Inhibitors

	DHFR I_{50} (μM)		selectivity index (human/*C. albicans* DHFR I_{50})	*C. albicans* MIC (μg/mL)
compd	*C. albicans*	human		
TMP	50	490	10	>50
PYR	5.0	2.6	0.5	>50
TMX	0.04	<0.001	<0.03	>50
PTX	0.04	0.002	0.05	>10

100 and 200 mg/kg, both with coadministration of 200 mg/kg sulfamethoxazole. No efficacy was observed at 100 mg/kg, and at 200 mg/kg, toxicities began to be discerned. Sulfamethoxazole prevents the incorporation of p-aminobenzoic acid in the biosynthesis of folic acid and thus synergizes the activity of DHFR inhibitors.[27] When used alone, sulfamethoxazole showed no *in vivo* activity.

Preliminary pharmacokinetic studies of **8l** in mice indicated that the compound was able to penetrate the brain, kidneys, and lungs after oral administration. At an oral dose of 200 mg/kg in mice, the kidney level of **8l** was 34 μg/g of tissue when measured at a 2 h time point. This level was about 140-fold higher than the *C. albicans* MIC value. The reason for the lack of *in vivo* efficacy for these compounds is not clear to us at present.

In summary, this is the first report on the preferential inhibition of *C. albicans* DHFR versus the corresponding human enzyme. Although the series of 5-(arylthio)-2,4-diaminoquinazolines showed a lack of *in vivo* activity, this report demonstrated that inhibition of *C. albicans* DHFR could potentially be a viable means for the development of antifungal agents.

Experimental Section

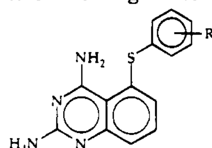
Melting points were determined with a Thomas Hoover or a Mel-Temp apparatus and are uncorrected. ^{1}H-NMR spectra were recorded on Varian XL-200 spectrometers with tetramethylsilane as the internal standard. Chemical ionization (CI) mass spectra were recorded by Oneida Research Service, Whitesboro, NY, with a Finnigan MAT TSQ mass spectrometer. Elemental analyses were carried out by Atlantic Microlabs, Inc., Atlanta, GA. 3,4,5-Trimethoxythiophenol, 4-morpholinylthiophenol, 4-cyclohexylthiophenol, and 4-(trifluoromethyl)thiophenol were prepared according to the procedure of Newman and Angier.[28] All other thiols were purchased from Aldrich: thiophenol, 4-thiocresol, and 4-chloro-, 4-bromo-, 4-fluoro-, 4-nitro-, 4-amino-, 4-methoxy-, 4-hydroxy-, and 4-chlorothiophenols; from Alfa, 3-methoxy-, 2-methoxy, and 3-chlorothiophenols; from Fairfield Chemical Co., 4-isopropyl-, 4-*tert*-amyl-, and 4-*tert*-butylthiophenols; from Maybridge Chemical Co. Inc., U.K., 3,4-dimethoxythiophenol; from Columbia Organic Chemical Co., 4-n-hexylthiophenol. 2,6-Dinitrobenzonitrile was purchased from Fairfield Chemical Co., and 2,6-difluorobenzonitrile was purchased from Aldrich. All purchased starting materials were used without further purification. All solvents used were reagent grade. Dimethyl sulfoxide (DMSO), N,N-dimethylacetamide (DMAC), and N,N-dimethylformamide (DMF) were dried over 4 Å sieves.

The following methods, A–E, are representative of procedures used to prepare compound series **5**, **7**, **8**, and **10** as indicated in Tables 1–4.

Method A. 2-Nitro-6-(4-tolylthio)benzonitrile (5b). An ice water bath-cooled mixture of 10 g (0.052 mol) of 2,6-dinitrobenzonitrile, 6.43 g (0.052 mol) of 4-thiocresol, and 7.19 g (0.052 mol) of anhydrous K_2CO_3 in 70 mL of DMF was stirred for 0.5 h. Approximately 50 mL of pyridine and 300 mL of H_2O were added to the reaction mixture. The yellow precipitate was collected by filtration, washed with 1 N NaOH and water, and dried to give 12.5 g (89%) of **5b** as a yellow solid: mp 106–107 °C; NMR (Me_2SO-d_6): δ 2.37 (s, 3H, CH_3), 7.22 (dd, 1H, aromatic), 7.35 (d, 2H, aromatic), 7.5 (d, 2H, aromatic), 7.75 (t, 1H, aromatic), 8.12 (dd, 1H, aromatic); MS (CI) m/e 271 (M^+ + 1, 100). Anal. ($C_{14}H_{10}N_2O_2S$) C, H, N, S.

Method B. 2-Amino-6-(4-tolylthio)benzonitrile (7b). To a water bath-cooled solution of 5 g (0.018 mol) of **5b** in 85 mL of diglyme was added dropwise, with stirring, 12.66 g (0.056 mol) of $SnCl_2{\cdot}2H_2O$ in 35 mL of concentrated HCl. The water bath was removed, and the reaction mixture was stirred at room temperature for 0.5 h. This reaction mixture was poured into a vigorously stirring mixture of 100 mL of 50% NaOH and 300 g of crushed ice. The precipitate was collected by filtration and washed with 1 N NaOH and water. Recrystallization from $MeOH/H_2O$ gave 1.92 g of **7b** as a yellow solid.

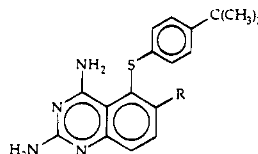
Table 7. Inhibition of Dihydrofolate Reductase and *in Vitro* Antifungal Activity of 5-(Arylthio)-2,4-diaminoquinazolines **8a–y**



compd	R	DHFR I_{50} (μM) *C. albicans*	DHFR I_{50} (μM) human	selectivity index (human/*C. albicans* DHFR I_{50})	*C. albicans* MIC (μg/mL)
8a[a]	H	0.034	0.62	18	0.050
8b	4-CH_3	0.023	0.94	41	0.25
8c	4-$CH(CH_3)_2$	0.077	2.4	31	0.25
8d	4-$C(CH_3)_3$	0.008	2.0	250	0.10
8e	4-$C(CH_3)_2CH_2CH_3$	0.30	3.9	10	10
8f	4-*n*-hexyl	0.04	0.55	14	50
8g	4-cyclohexyl	0.05	>10	>200	1.0
8h	4-OTHP[b]	0.026	0.20	8.0	13
8i	4-$N(CH_2CH_3)_2$	0.20	4.3	22	2.5
8j	4-morpholino	0.13	70	540	>50
8k	4-Cl	0.03	2.1	70	0.50
8l	4-Br	0.03	3.1	100	0.25
8m	4-F	0.05	2.0	40	0.25
8n	4-CF_3	0.48	2.6	5.0	25
8o	4-NO_2	0.23	24	100	50
8p	4-CN	0.32	33	100	>50
8q	4-NH_2	0.06	0.32	5.0	2.5
8r	4-OH	0.034	0.18	5.0	10.
8s	4-OCH_3	0.02	3.5	175	0.25
8t	3,4-$(OCH_3)_2$	0.05	4.1	79	>50
8u	3,4,5-$(OCH_3)_3$	0.04	4.4	110	5.0
8v	3-OCH_3	0.05	1.3	26	0.25
8w	2-OCH_3	0.10	0.40	4.0	10
8x	3-Cl	0.042	0.27	6.0	0.50
8y	2-Cl	0.06	0.092	2.0	1.0

[a] See: Harris, N. V.; Smith, C.; Bowden, K. Antifolate and Antibacterial Activities of 5-Substituted 2,4-Diaminoquinazolines. *J. Med. Chem.* **1990**, *33*, 434–444, for activity of **5a** against DHFR from other species. [b] THP–tetrahydropyran.

Table 8. Inhibition of Dihydrofolate Reductase and *in Vitro* Antifungal Activity of C6-Substituted-5-[(4-*tert*-butylphenyl)-thio]-2,4-diaminoquinazolines **13a–d**



compd	R	DHFR I_{50} (μM) *C. albicans*	DHFR I_{50} (μM) human	selectivity index (human/ *C. albicans* DHFT I50)	*C. albicans* MIC (mg/mL)
13a	NH_2	0.25	4.3	17	5.0
13b	CN	0.14	9.1	65	>5.0
13c	OCH_2CH_3	0.057	0.82	14	1.0
13d	$OCH_2CH(CH_3)_2$	0.0030	0.030	10	0.80

The mother liquor was concentrated to give an additional 1.8 g of **7b**, resulting in a total of 3.72 g (86%) of **7b**: mp 114–116 °C; NMR (Me_2SO-d_6): δ 2.3 (s, 3H, CH_3), 6.13 (br s, 2H, NH_2), 6.15 (d, 1H, aromatic), 6.6 (d, 1H, aromatic), 7.15 (t, 1H, aromatic), 7.2 (d, 1H, aromatic), 7.3 (d, 1H, aromatic); MS (CI) *m/e* 241 (M^+ + 1, 100). Anal. ($C_{14}H_{12}N_2S$) C, H, N, S.

Method C. 5-(4-Tolylthio)-2,4-diaminoquinazoline (8b). A mixture of 0.95 g (0.004 mol) of **7b** and 1.36 g (0.012 mol) of chloroformamidine hydrochloride[14,15] was heated in a test tube to 180–190 °C for 0.5 h. The resultant product was dissolved in MeOH and basified with concentrated NH_4OH. The precipitate was collected by filtration and recrystallized from EtOH to give 0.52 g (73%) of **8b** as an off-white solid: mp 203–204.5 °C; NMR (Me_2SO-d_6): δ 2.2 (s, 3H, CH_3), 6.06 (br s, 2H, NH_2), 7.02 (d, 2H, aromatic), 7.1 (d, 2H, aromatic), 7.11 (dd, 1H, aromatic), 7.23 (dd, 1H, aromatic), 7.4 (t with fine splittings, 1H, aromatic), 7.64 (br s, 2H, NH_2); MS (CI) *m/e* 283 (M^+ + 1, 100). Anal. ($C_{15}H_{14}N_4S$) C, H, N, S.

Method D. 2-[(3,4-Dimethoxyphenyl)thio]-6-fluorobenzonitrile (10g). To a suspension of 0.88 g (0.008 mol) of *t*-BuOK in 10 mL of DMSO was added 1.33 g (0.008 mol) of 3,4-dimethoxythiophenol. The reaction mixture was cooled in an ice/water bath, and 1.03 g (0.008 mol) of 2,6-difluorobenzonitrile in 1 mL of DMSO was added. The resultant mixture was stirred for 10 min in the ice/water bath and then at room temperature for 20 min. This mixture was poured into ice/water and basified with 1 N NaOH. The white precipitate was collected by filtration, washed with water, and dried in an oven. Purification by flash column chromatography[19] on silica gel with 30% EtOAc in hexane gave 1.57 g (74%) of **10g** as a white powder: mp 137–138 °C; NMR (Me_2SO-d_6): δ 3.75 (s, 3H, OCH_3), 3.8 (s, 3H, OCH_3), 6.7 (d, 1H, aromatic), 7.1 (d, 1H, aromatic), 7.15–7.25 (m, 2H, aromatic), 7.3 (d, 1H, aromatic), 7.6 (q with fine splittings, 1H, aromatic); MS (CI) *m/e* 290 (M^+ + 1, 100). Anal. ($C_{15}H_{12}NFO_2S$) C, H, N, S.

Method E. 5-[(3,4-Dimethoxyphenyl)thio]-2,4-diaminoquinazoline (8t). A mixture of 1.5 g (0.005 mol) of **10g** and 1.4 g (0.008 mol) of guanidine carbonate was heated in 16 mL of DMAC at 145 °C for 3.5 h. The reaction mixture was cooled to room temperature, and CH_2Cl_2 was added. The resulting white precipitate was collected by filtration. The solid was washed with warm water and EtOH and dried in an oven overnight to give 1.57 g (92%) of **8t** as a white powder: mp 243–245 °C; NMR (Me_2SO-d_6): δ 3.7 (s, 3H, OCH_3), 3.72 (s, 3H, OCH_3), 6.1 (br s, 2H, NH_2), 6.72 (dd, 1H, aromatic), 6.95 (d, 1H, aromatic), 6.97 (d, 1H, aromatic), 7.05

(d, 1H, aromatic), 7.2 (d, 1H, aromatic), 7.4 (t, 1H, aromatic), 7.62 (br s, 2H, NH_2); MS (CI) *m/e* 329 (M^+ + 1, 100). Anal. ($C_{16}H_{16}N_4O_2S$) C, H, N, S.

2-[[(4-*N,N*-Diethylamino)phenyl]thio]-6-nitrobenzonitrile (5f). To a stirred ice/water-cooled mixture of 1.5 g (0.006 mol) of **5l** and 3 mL (0.006 mol) of acetaldehyde in 10 mL of CH_3CN was added 1.04 g (0.017 mol) of $NaCNBH_3$. This was followed by the dropwise addition of 0.6 mL (0.01 mol) of glacial AcOH. The resultant mixture was gradually warmed to room temperature. After stirring for 3–4 h, an additional 2 mL of acetaldehyde and 0.6 mL of glacial AcOH were added. The reaction mixture was stirred overnight. The mixture was diluted with EtOAc, washed with 1 N NaOH and brine, and dried over $MgSO_4$. The volatiles were removed by spin evaporation to give a red oil. The oil was crystallized from EtOH to give 1.49 g (83%) of **5f** as red crystals: NMR (Me_2SO-d_6): δ 1.1 (t, 6H, NCH_2CH_3), 3.35 (q, 4H, NCH_2CH_3), 6.8 (d, 2H, aromatic), 7.1 (d, 1H, aromatic), 7.4 (d, 2H, aromatic), 7.75 (t, 1H, aromatic), 8.05 (d, 1H, aromatic); MS (CI) *m/e* 328 (M^+ + 1, 100). Anal. ($C_{17}H_{17}N_3O_2S$) C, H, N, S.

2-Amino-6-[(4-cyanophenyl)thio]benzonitrile (7l). A stirred mixture of 0.4 g (0.005 mol) of CuCN and 1.2 g (0.004 mol) of **7h** in 2 mL of DMF was refluxed for 5 h. The resulting dark material was poured into a solution of 3 mL of 2 N HCl containing 1.55 g (0.006 mol) of $FeCl_3 \cdot 6H_2O$. The resultant mixture was stirred at 60–70 °C for ca. 20 min. This mixture was extracted with hot toluene (40 mL × 5). The toluene extracts were combined, washed with water and 1 N NaOH, and dried over $MgSO_4$. Solvent was removed, and the crude product was purified by flash column chromatography on silica gel with 30% EtOAc in hexane as the eluent. This yielded 0.41 g (41%) of **7l** as a yellow powder: mp 176–177 °C; NMR (Me_2SO-d_6): δ 6.35 (br s, 2H, NH_2), 6.75 (d, 1H, aromatic), 6.88 (d, 1H, aromatic), 7.25 (d, 2H, aromatic), 7.35 (t, 1H, aromatic), 7.75 (d, 2H, aromatic); MS (CI) *m/e* 252 (M^+ + 1, 100). Anal. ($C_{14}H_9N_3S$) C, H, N, S.

2-Fluoro-6-[[4-[(tetrahydro-2*H*-pyran-2-yl)oxy]phenyl]thio]benzonitrile (10f). To a solution of 1.04 g (0.004 mol) of **10e** in 20 mL of CH_2Cl_2 was added 0.54 g (0.006 mol) of dihydropyran and 0.11 g (0.0004 mol) of pyridinium *p*-toluenesulfonate. The resultant reaction mixture was stirred at room temperature overnight. Approximately 25 mL of brine was added to the reaction mixture. The organic layer was separated, dried over Na_2SO_4, and concentrated. Purification by flash column chromatography on silica gel with 40% EtOAc in hexane gave 1 g (72%) of **10f** as a white powder: mp 91–95 °C; NMR (Me_2SO-d_6): δ 1.3–1.7 (m, 3H, tetrahydropyran), 1.7–1.9 (m, 3H, tetrahydropyran), 3.4–3.62 (m, 1H, OCH_2), 3.62–3.8 (m, 1H, OCH_2), 5.5 (br s, 1H, OCH), 6.7 (d, 1H, aromatic), 7.1 (d, 2H, aromatic), 7.25 (t, 1H, aromatic), 7.5 (d, 2H, aromatic), 7.55 (dd, 1H, aromatic); MS (CI) *m/e* 330 (M^+ + 1, 100). Anal. ($C_{18}H_{16}NFO_2S$) C, H, N, S.

5-[(4-Hydroxyphenyl)thio]-2,4-diaminoquinazoline (8r). To 0.2 g (0.0005 mol) of **8h** in a round-bottomed flask was added 10 mL of 2 N HCl. The resultant mixture was stirred at room temperature for 1.5 h. The precipitate was collected by filtration, washed with water, and dried. Purification by flash column chromatography on silica gel with 10% MeOH in CH_2Cl_2 gave 0.07 g (44%) of **8r** as an off-white powder: mp 232–235 °C; NMR (Me_2SO-d_6): δ 6.2 (br s, 2H, NH_2), 6.75 (d, 2H, aromatic), 6.9 (d, 1H, aromatic), 7.2 (d, 3H, aromatic), 7.36 (t, 1H, aromatic), 7.8 (br s, 2H, NH_2), 9.8 (br s, 1H, OH); MS (CI) *m/e* 285 (M^+ + 1, 100). Anal. ($C_{14}H_{12}N_4OS \cdot 0.5H_2O$) C, H, N, S.

6-Nitro-5-[(4-*tert*-butylphenyl)thio]-2,4-diaminoquinazoline (12). To a stirred suspension of 0.72 g (0.015 mol) of 50% oil-dispersed NaH in 60 mL of dry DMF under an N_2 atmosphere was added 2.51 g (0.015 mol) of 4-*tert*-butylthiophenol, and the mixture was stirred for 5 min. This was followed by the dropwise addition of a solution of 3.23 g (0.014 mol) of 5-chloro-6-nitro-2,4-diaminoquinazoline (11)[18] in 160 mL of dry DMF. The resultant mixture was heated to 80–90 °C (oil bath temperature) for 22 h. The reaction mixture was poured into an Erlenmeyer flask containing approximately 1.1 L of ice/water. The yellow precipitate was collected by filtration, washed repeatedly with water, and dried. This yielded 4.27 g (83%) of **12** as a yellow solid. An analytical sample was prepared by recrystallization from MeOH: mp 270–272 °C; NMR (Me_2SO-d_6): δ 1.2 (s, 9H, $C(CH_3)_3$), 6.55 (br s, 2H, NH_2), 7.0 (d, 2H, aromatic), 7.3 (d, 2H, aromatic), 7.35 (d, 1H, aromatic), 7.9 (br s, 2H, NH_2), 7.95 (d, 2H, aromatic); MS (CI) *m/e* 370 (M^+ + 1, 100). Anal. ($C_{18}H_{19}N_5O_2S$) C, H, N, S.

6-Amino-5-[(4-*tert*-butylphenyl)thio]-2,4-diaminoquinazoline (13a). A warm solution of 4 g (0.011 mol) of **12** and 11 g of 5% Pd/C in 130 mL of DMF was shaken in the presence of hydrogen at 2–3 atm for 5 h. The resultant reaction mixture was filtered through a Celite pad and concentrated. The crude product was purified by flash column chromatography on silica gel with 10% MeOH in CH_2Cl_2 as the eluent to give 2.48 g (68%) of **13a** as a yellow powder: mp 220-221 °C; NMR (Me_2SO-d_6): δ 1.2 (s, 9H, $C(CH_3)_3$), 5.5 (br s, 2H, NH_2), 5.6 (br s, 2H, NH_2), 6.95 (d, 2H, aromatic), 7.2 (s, 2H, aromatic), 7.25 (d, 2H, aromatic), 7.8–8.1 (br s, 2H, NH_2); MS (CI) *m/e* 340 (M^+ + 1, 100). Anal. ($C_{18}H_{21}N_5S$) C, H, N, S.

6-Cyano-5-[(4-*tert*-butylphenyl)thio]-2,4-diaminoquinazoline (13b). To a mixture of 0.06 g (0.0015 mol) of 60% NaH and 0.25 g (0.0015 mol) of 4-*tert*-butylthiophenol in 4 mL of dry DMF was added 0.31 g (0.0014 mol) of **15**.[18] The resultant mixture was heated at 90 °C for 4 h. This mixture was cooled to room temperature and poured into a beaker containing ca. 65 mL of ice/water. The precipitate was filtered, washed with water, and dried in an oven at 60 °C. Purification by flash column chromatography on silica gel with 3% MeOH in CH_2Cl_2 resulted in 0.27 g (55%) of **13b** as an off-white powder: mp 276 °C dec; NMR (Me_2SO-d_6): δ 1.2 (s, 9H, $C(CH_3)_3$), 6.6 (br s, 2H, NH_2), 7.0 (d, 2H, aromatic), 7.2–7.4 (m, 2H, aromatic), 7.8 (d, 2H, aromatic), 8.2 (br s, 2H, NH_2); MS (CI) *m/e* 350 (M^+ + 1, 100). Anal. ($C_{19}H_{19}N_5S$) C, H, N, S.

2,3-Dichloro-1-ethoxybenzene (17a). To a stirred mixture of 5 g (0.031 mol) of 2,3-dichlorophenol and 4.23 g (0.031 mol) of K_2CO_3 in 50 mL of dry acetone was added dropwise 7.18 g (0.046 mol) of ethyl iodide in 10 mL of dry acetone. The resultant mixture was refluxed for 7 h. After cooling to room temperature, the precipitate was removed by filtration, and the filtrate was concentrated *in vacuo*. This concentrate was partitioned between ether and water. The ether layer was collected, and the water layer was re-extracted with ether. The ether extracts were combined, washed with 0.1 N NaOH, and dried over Na_2SO_4. Solvent was removed, and the crude product was purified by flash column chromatography to yield 5.55 g (95%) of **17a** as a colorless oil: NMR (Me_2SO-d_6): δ 1.35 (t, 3H, OCH_2CH_3), 4.15 (q, 2H, OCH_2CH_3), 7.1 (d, 1H, aromatic), 7.2 (d, 1H, aromatic), 7.3 (t, 1H, aromatic); MS (CI) *m/e* 191 (M^+ + 1, 100). Anal. ($C_8H_8Cl_2O$) C, H, Cl.

2,3-Dichloro-1-isobutoxybenzene (17b). This compound was prepared in a manner analogous to that described for **17a** using isobutyl bromide to give a 33% yield of **17b** as a colorless oil: NMR (Me_2SO-d_6): δ 1.0 (d, 6H, $OCH_2CH(CH_3)_2$), 2.0 (m, 1H, $OCH_2CH(CH_3)_2$), 3.85 (d, 2H, $OCH_2CH(CH_3)_2$), 7.0–7.4 (m, 3H, aromatic); MS (CI) *m/e* 219 (M^+ + 1, 100). Anal. ($C_{10}H_{12}Cl_2O$) C, H, Cl.

2,3-Dichloro-6-nitro-1-ethoxybenzene (18a) and 2,3-Dichloro-4-nitro-1-ethoxybenzene (19a). HNO_3 (70%, 50 mL) was cautiously added to 5 g (0.026 mol) of **17a** in a 250 mL round-bottomed flask. The resultant mixture became homogeneous after warming to 40 °C for 20 min. After stirring at room temperature overnight, the mixture was poured into a flask containing 300 g of ice. The precipitate was collected by filtration and washed repeatedly with water. Purification by flash column chromatography on silica gel with 10% EtOAc in hexane as the eluent gave 2.03 g (33%) of **18a** as an off-white powder: mp 56–57 °C; NMR (Me_2SO-d_6): δ 1.35 (t, 3H, OCH_2CH_3), 4.15 (q, 2H, OCH_2CH_3), 7.65 (d, 1H, aromatic), 8.0 (d, 1H, aromatic); MS (CI) *m/e* 236 (M^+ + 1, 100). Anal. ($C_8H_7NCl_2O_3$) C, H, N, Cl.

Further elution gave 2.58 g (42%) of **19a** as an off-white powder: mp 61–63 °C; NMR (Me_2SO-d_6): δ 1.35 (t, 3H, OCH_2CH_3), 4.30 (q, 2H, OCH_2CH_3), 7.32 (d, 1H, aromatic), 8.12 (d, 1H, aromatic); MS (CI) *m/e* 236 (M^+ + 1, 100). Anal. ($C_8H_7NCl_2O_3$) C, H, N, Cl.

2,3-Dichloro-6-nitro-1-isobutoxybenzene (18b) and 2,3-Dichloro-4-nitro-1-isobutoxybenzene (19b). These com-

pounds were prepared in a manner analogous to that described for **18a** and **19a** above to give a 38% yield of **18b** as a yellow oil: NMR (Me_2SO-d_6): δ 1.0 (d, 6H, $OCH_2CH(CH_3)_2$), 2.0 (m, 1H, $OCH_2CH(CH_3)_2$), 3.85 (d, 2H, $OCH_2CH(CH_3)_2$), 7.65 (d, 1H, aromatic), 7.95 (d, 1H, aromatic); MS (CI) *m/e* 264 (M^+ + 1, 28). Anal. ($C_{10}H_{11}NCl_2O_3$) C, H, N, Cl.

Further elution gave a 37% yield of **19b** as a solid: mp 43–44 °C; NMR (Me_2SO-d_6): δ 1.0 (d, 6H, $OCH_2CH(CH_3)_2$), 2.0 (m, 1H, $OCH_2CH(CH_3)_2$), 4.0 (d, 2H, $OCH_2CH(CH_3)_2$), 7.35 (d, 1H, aromatic), 8.1 (d, 1H, aromatic); MS (CI) *m/e* 264 (M^+ + 1, 100). Anal. ($C_{10}H_{11}NCl_2O_3$) C, H, N, Cl.

2-Chloro-3-ethoxy-6-nitrobenzonitrile (20a). A stirred mixture of 2.36 g (0.01 mol) of **19a** and 0.98 g (0.011 mol) of CuCN in 25 mL of 1-methyl-2-pyrrolidinone was heated to 150 °C overnight in an oil bath. The reaction mixture was poured into a flask containing 100 g of ice. The precipitate was collected by filtration. Flash column chromatography on silica gel with 40% EtOAc in hexane as the eluent provided 1.41 g (62%) of **20a** as an off-white solid: mp 145–146 °C; NMR (Me_2SO-d_6): δ 1.42 (t, 3H, OCH_2CH_3), 4.35 (q, 2H, OCH_2CH_3), 7.65 (d, 1H, aromatic), 8.40 (d, 1H, aromatic); MS (CI) *m/e* 227 (M^+ + 1, 100). Anal. ($C_9H_7N_2ClO_3$) C, H, N, Cl.

2-Chloro-3-isobutoxy-6-nitrobenzonitrile (20b). This compound was prepared in a manner analogous to that described for **20a** above to give a 62% yield of **20b** as an off-white powder: mp 110–112 °C; NMR (Me_2SO-d_6): δ 1.0 (d, 6H, $OCH_2CH(CH_3)_2$), 2.1 (m, 1H, $OCH_2CH(CH_3)_2$), 4.1 (d, 2H, $OCH_2CH(CH_3)_2$), 7.65 (d, 1H, aromatic), 8.35 (d, 1H, aromatic); MS (CI) *m/e* 225 (M^+ + 1, 100). Anal. ($C_{11}H_{11}N_2ClO_3$) C, H, N, Cl.

3-Ethoxy-6-nitro-2-[(4-*tert*-butylphenyl)thio]benzonitrile (21a). To a stirred suspension of 0.68 g (0.006 mol) of *t*-BuOK in 5 mL of dry DMSO was added 1.01 g (0.006 mol) of 4-*tert*-butylthiophenol. The reaction mixture was cooled in an ice/water bath. This was followed by the dropwise addition of 1.3 g (0.006 mol) of **20a** in 8 mL of DMSO. The mixture was stirred for 2 h and then poured into a flask containing 80 g of ice. The precipitate was collected by filtration, washed with 1 N NaOH and water, and dried to furnish 2.0 g (99%) of **21a** as a yellow powder. Purification of 0.2 g of this powder by flash column chromatography on silica gel with 30% EtOAc in hexane as the eluent gave 0.17 g of analytically pure **21a**: mp 160–162 °C; NMR (Me_2SO-d_6): δ 1.02 (t, 3H, OCH_2CH_3), 1.21 (s, 9H, $C(CH_3)_3$), 4.12 (q, 2H, OCH_2CH_3), 7.2 (d, 1H, aromatic), 7.3 (d, 1H, aromatic), 7.5 (d, 1H, aromatic), 8.4 (d, 1H, aromatic); MS (CI) *m/e* 357 (M^+ + 1, 100). Anal. ($C_{19}H_{20}N_2O_3S$) C, H, N, S.

3-Isobutoxy-6-nitro-2-[(4-*tert*-butylphenyl)thio]benzonitrile (21b). This compound was prepared in a manner analogous to that described for **21a** above to give a 98% yield of **21b** as a yellow powder: mp 110–112 °C; NMR (Me_2SO-d_6): δ 0.7 (d, 6H, $OCH_2CH(CH_3)_2$), 1.2 (s, 9H, $C(CH_3)_3$), 1.7 (m, 1H, $OCH_2CH(CH_3)_2$), 3.9 (d, 2H, $OCH_2CH(CH_3)_2$), 7.1 (d, 2H, aromatic), 7.3 (d, 2H, aromatic), 7.5 (d, 1H, aromatic), 8.45 (d, 1H, aromatic); MS (CI) *m/e* 385 (M^+ + 1, 100). Anal. ($C_{21}H_{24}N_2O_3S$) C, H, N, S.

6-Amino-3-ethoxy-2-[(4-*tert*-butylphenyl)thio]benzonitrile (22a). To an ice bath-cooled and stirred suspension of 1.8 g (0.005 mol) of **21a** in 25 mL of diglyme was added a solution of $SnCl_2 \cdot 2H_2O$ in 10 mL of concentrated HCl. The resultant reaction mixture was gradually brought to room temperature and stirred for 2 h. This mixture was poured into a flask containing a solution of 14 g of NaOH in 14 mL of H_2O and 100 g of ice. The solution was extracted with EtOAc (3×). The EtOAc extracts were washed with 1 N NaOH and water and dried over Na_2SO_4. The solvent was removed by spin evaporation, and the resultant crude product was purified by flash column chromatography on silica gel with 30% EtOAc in hexane as the eluent to give 1.13 g (69%) of **22a** as a beige powder: mp 80–81 °C; NMR (Me_2SO-d_6): δ 1.02 (t, 3H, OCH_2CH_3), 1.3 (s, 9H, $C(CH_3)_3$), 3.95 (q, 2H, OCH_2CH_3), 5.8 (s, 2H, NH_2), 6.9 (d, 1H, aromatic), 7.1 (d, 1H, aromatic), 7.3 (d, 1H, aromatic), 7.35 (d, 1H, aromatic); MS (CI) *m/e* 327 (M^+ + 1, 88). Anal. ($C_{19}H_{22}N_2OS$) C, H, N, S.

6-Amino-3-isobutoxy-2-[(4-*tert*-butylphenyl)thio]benzonitrile (22b). This compound was prepared in a manner analogous to that described for **22a** above to give a 69% yield of **22b** as a beige powder: mp 100–101 °C; NMR (Me_2SO-d_6): δ 0.75 (d, 6H, $OCH_2CH(CH_3)_2$), 1.2 (s, 9H, $C(CH_3)_3$), 1.7 (m, 1H, $OCH_2CH(CH_3)_2$), 3.6 (d, 2H, $OCH_2CH(CH_3)_2$), 5.7 (br s, 2H, NH_2), 6.85 (d, 2H, aromatic), 7.0 (d, 2H, aromatic), 7.2 (d, 1H, aromatic), 7.3 (d, 1H, aromatic); MS (CI) *m/e* 355 (M^+ + 1, 100). Anal. ($C_{21}H_{26}N_2OS$) C, H, N, S.

5-[(4-*tert*-Butylphenyl)thio]-6-ethoxy-2,4-diaminoquinazoline (13c). A mixture of 1.05 g (0.003 mol) of **22a** and 1.11 g (0.01 mol) of chloroformamidine hydrochloride in a test tube was fused at 185 °C for 30 min. The mixture was cooled to room temperature and suspended in MeOH. To this suspension was added concentrated NH_4OH until it became basic. The precipitate was collected and washed with H_2O. Purification by flash column chromatography on silica gel with 10% MeOH in CH_2Cl_2 gave 0.86 g (72%) of **13c** as a yellow powder: mp 200–201 °C; NMR (Me_2SO-d_6): δ 1.12 (t, 3H, OCH_2CH_3), 1.22 (s, 9H, $C(CH_3)_3$), 4.0 (q, 2H, OCH_2CH_3), 5.9 (br s, 2H, NH_2), 7.0 (d, 2H, aromatic), 7.3 (d, 2H, aromatic), 7.4 (d, 1H, aromatic), 7.5 (d, 1H, aromatic), 8.5 (br s, 2H, NH_2); MS (CI) *m/e* 369 (M^+ + 1, 100). Anal. ($C_{20}H_{24}N_4OS$) C, H, N, S.

5-[(4-*tert*-Butylphenyl)thio]-6-isobutoxy-2,4-diaminoquinazoline (13d). This compound was prepared in a manner analogous to that described for **13c** above to give a 44% yield of **13d** as a yellow powder: mp 173–174 °C; NMR (Me_2SO-d_6): δ 0.8 (d, 6H, $OCH_2CH(CH_3)_2$), 1.2 (s, 9H, $C(CH_3)_3$), 1.8 (m, 1H, $OCH_2CH(CH_3)_2$), 3.75 (d, 2H, $OCH_2CH(CH_3)_2$), 5.9 (br s, 2H, NH_2), 6.95 (d, 2H, aromatic), 7.25 (d, 2H, aromatic), 7.4 (d, 1H, aromatic), 7.5 (d, 1H, aromatic), 8.5 (br s, 2H, NH_2); MS (CI) *m/e* 397 (M^+ + 1, 100). Anal. ($C_{22}H_{28}N_4OS$) C, H, N, S.

Enzyme Assays. *C. albicans* DHFR was expressed in *E. coli* BL21 (DE3, p1869) and purified to homogeneity as previously described.[29] The standard enzyme assay was performed in 0.1 M imidazole chloride buffer, pH 6.4, with 12 mM mercaptoethanol, 60 μM NADPH, and 45 μM dihydrofolic acid in a final volume of 1 mL at 30 °C. I_{50} is the concentration of inhibitor that decreases the velocity of the standard assay by 50%. The enzyme (0.2 nM), NADPH, and varying concentrations of inhibitor were preincubated for 2 min at 30 °C, and the reaction was initiated by the addition of dihydrofolic acid. Steady state velocities were measured, and I_{50} values were calculated from a linear regression plot of the percentage inhibition vs the logarithm of inhibitor concentration. The precision of the I_{50} determination is generally about ±30%.

Recombinant human DHFR, prepared as described by Prendergast et al.,[30] was obtained from Dr. J. Freisheim, Medical College of Ohio. The enzyme (2 nM) was assayed in 50 mM Sorenson's phosphate buffer, pH 7.0, as described above for *C. albicans* DHFR.

***C. albicans* MIC Test.** The minimum inhibitory concentration (MIC) is defined as the lowest concentration of compound that prevents visible overnight growth of *C. albicans* in liquid culture. The defined culture medium contained NH_4Cl (2 g), K_2HPO_4 (0.35 g), $MgSO_4$ (0.24 g), sodium citrate (0.3 g), piperazine-*N,N'*-bis[2-ethanesulfonic acid] (3.4 g), biotin (40 mg), and glucose (10 g) in 1 L of water at a final pH of 7.1. Stock cultures were prepared from *C. albicans* strain P712 that had been harvested during logarithmic growth and stored frozen in 10% glycerol at −70 °C. For the MIC test, 16 × 125 mm tubes were prepared containing culture medium and 4500 colony-forming units (CFU) of *C. albicans* without and with drug in a final volume of 3 mL. Final concentrations of drug in each tube were 50–0.01 μg/mL. The tubes were placed in a gyrotory water bath (28 °C, 160 rpm) and incubated for 20–24 h. During this time the control tubes became turbid (light absorbance at 530 nm approximately equal to 1 absorbance unit), and the MIC was determined by inspection ($A_{530} < 0.03$). Alternatively, the assay was performed in a 96-well microtiter plate with a final volume of 0.05 mL and serial 4-fold dilutions of drug from 50 to 0.01 μg/mL. The precision of the MIC assay is ±1 tube or well.

Pharmacokinetics. Mouse brains, kidneys, and lungs were extracted with acidic methanol, centrifuged, and then purified as plasma by extraction from C2 Bond-Elut columns (Analytichem International). Compound levels were determined by reverse phase HPLC (Waters μBondapak C18),

utilizing a linear 0–48% acetonitrile gradient. Standard curves of plasma or tissues spiked with authentic compound were used for quantification.

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References

(1) (a) Walsh, T. J.; Jarosinski, P. F.; Fromtling, R. A. Increasing Usage of Systemic Antifungal Agents. *Diagn. Microbiol. Infect. Dis.* **1990**, *13*, 37–40. (b) Walsh, T. J.; Pizzo, A. Treatment of Systemic Fungal Infections: Recent Progress and Current Problems. *Eur. J. Clin. Microbiol. Infect. Dis.* **1988**, *7*, 460–475. (c) Graybill, J. R. New Antifungal Agents. *Eur. J. Clin. Microbiol. Infect. Dis.* **1989**, *8*, 402–412. (d) *Recent Trends in the Discovery, Development and Evaluation of Antifungal Agents*; Fromtling, R. A., Ed.; J. R. Prous: Barcelona, Spain, 1987; pp 81–92. (e) *Antifungal Drugs (Annals of the New York Academy of Sciences)*; St. Georgiev, V., Ed.; The New York Academy of Sciences: New York, 1988; Vol. 544. (f) Koltin, Y. Targets for Antifungal Drug Discovery. In *Annual Reports in Medicinal Chemistry*; Bristol, J. A., Ed.; Academic Press, Inc.: San Diego, CA, 1990; Vol. 25, pp 141–148. (g) Sternberg, S. The Emerging Fungal Threat. *Science* **1994**, *266*, 1632–1634.

(2) (a) Roth, B.; Falco, E. A.; Hitchings, G. H.; Bushby, S. R. M. 5-Benzyl-2,4-diaminopyrimidines as Antibacterial Agents. I. Synthesis and Antibacterial Activity *in vitro*. *J. Med. Pharm. Chem.* **1962**, *5*, 1103–1123. (b) Burchall, J. J.; Hitchings, G. H. Inhibitor Binding Analysis of Dihydrofolate Reductases from Various Species. *Mol. Pharmacol.* **1965**, *1*, 126–136. (c) Schweitzer, B. I.; Dicker, A. P.; Bertino, J. R. Dihydrofolate Reductase as a Therapeutic Target. *FASEB J.* **1990**, *4*, 2441–2452.

(3) Blakley, R. L. *The Biochemistry of Folic Acid and Related Pteridines*; N. Holland: Amsterdam, The Netherlands, 1969.

(4) Roth, B.; Cheng, C. C. Recent Progress in the Medicinal Chemistry of 2,4-Diaminopyrimidines. *Prog. Med. Chem.* **1982**, *19*, 270–331.

(5) (a) Castaldo, R. A.; Gump, D. W.; McCormack, J. J. Activity of 2,4-Diaminoquinazoline Compounds against *Candida* species. *Antimicrob. Agents Chemother.* **1979**, *15*, 81–86. (b) McCormack, J. J.; Allen, B. A.; Ledig, K. W.; Hillcoat, B. L. Inhibition of Dihydrofolate Reductases by derivatives of 2,4-diaminopyrroloquinazoline. *Biochem. Pharmacol.* **1979**, *28*, 3227–3229. (c) Ledig, K. W. 7-(Substituted)-7H-pyrrolo[3,2-f]quinazoline-1,3-diaminines. U.S. Patent 4,118,561, 1978.

(6) Colwell, W. T.; Degraw, J. I.; Ryan, K. J.; Lawson, J. A.; Cheng, A. Antifungal Activity of Some 2,4-Diamino-5-methyl-6-alkyl Quinazolines. In *Chemistry and Biology of Pteridines 1989, Pteridines and Folic Acid Derivatives*; Curtius, H.-Ch., Ghisla, S., Blau, N., Eds.; Walter de Gruyter: Berlin, Germany, 1990; pp 1052–1055.

(7) Hynes, J. B.; Hough, L. V.; Smith, A. B.; Gale, G. R. Activity of Selected 2,4-Diaminoquinazolines against *Candida albicans in Vitro* (39516). *Proc. Soc. Exp. Biol. Med.* **1976**, *153*, 230–232.

(8) Hariri, A. R.; Larsh, H. W. *In Vitro* and *In Vivo* Activity of 2,4-Diamino-6-[2-(3,4-dichlorophenyl)acetamido]quinazoline against *Cryptococcus neoformans*. *Proc. Soc. Exp. Biol. Med.* **1976**, *151*, 173–176.

(9) Stammers, D. K.; Champness, J. N.; Beddell, C. R.; Dann, J. G.; Eliopoulos, E.; Geddes, A. J.; Ogg, D.; North, A. C. T. The Structure of mouse L1210 dihydrofolate reductase-drug complexes and the construction of a model of human enzyme. *FEBS Lett.* **1987**, *218*, 178–184.

(10) (a) Fling, M. E. Unpublished results. (b) Daly, S.; Mastromei, G.; Yacoub, A.; Lorenzetti, R. Sequence of a Dihydrofolate Reductase-Encoding Gene from Candida Albicans. *Gene* **1994**, *147*, 115–118.

(11) Whitlow, M.; Howard, A. J.; Stewart, D.; Hardman, K.; Chan, J. H.; Kuyper, L. F.; Baccanari, D. P. Unpublished results. Full details of this work will be reported elsewhere.

(12) Ashton, W. T.; Hynes, J. B. Synthesis of 5-Substituted Quinazolines as Potential Antimalarial Agents. *J. Med. Chem.* **1973**, *16*, 1233–1237.

(13) Friedman, L.; Shechter, H. Dimethylformamide as a Useful Solvent in Preparing Nitriles from Aryl Halides and Cuprous Cyanide. Improved Isolation Techniques. *J. Org. Chem.* **1961**, *26*, 2522–2524.

(14) Hantzch, A.; Vagt, A. Ueber das sogenannte Diazoguanidin. *Justus Liebigs Ann. Chem.* **1900**, *314*, 339–369.

(15) Rosowsky, A.; Chaykovsky, M.; Chen, K. K. N.; Lin, M.; Modest, E. J. 2,4-Diaminothieno[2,3-*d*]pyrimidines as Antifolates and Antimalarials. 1. Synthesis of 2,4-Diamino-5,6,7,8-tetrahydrothianaphtheno[2,3*d*]pyrimidines and Related Compounds. *J. Med. Chem.* **1973**, *16*, 185–188.

(16) Hynes, J. B.; Pathak, A.; Panos, C. H.; Okeke, C. C. Direct Synthesis of 2,4-Diaminoquinazolines from 2-Fluorobenzonitriles. *J. Heterocycl. Chem.* **1988**, *25*, 1173–1177.

(17) Miyashita, M.; Yoshikoshi, A.; Grieco, P. A. Pyridinium p-Toluenesulfonate. A Mild and Efficient Catalyst for the Tetrahydropyranylation of Alcohols. *J. Org. Chem.* **1977**, *42*, 3772–3774.

(18) Davoll, J.; Johnson, A. M. Quinazoline Analogues of Folic Acid. *J. Chem. Soc. C*, **1970**, 997–1002.

(19) Still, W. C.; Kahn, M.; Mitra, A. Rapid Chromatographic Technique for Preparative Separations with Moderate Resolution. *J. Org. Chem.* **1978**, *43*, 2923–2925.

(20) Hunter, R. N.; Davis, S. E.; Kuyper, L. F. Unpublished results.

(21) Richardson, M. D. Opportunistic and pathogenic fungi. *J. Antimicrob. Chemother.* **1991**, *28* (Suppl. A), 1–11.

(22) Edwards, J. E., Jr. Invasive Candida Infections - Evolution of a Fungal Pathogen. *N. Engl. J. Med.* **1991**, *324*, 1060–1062.

(23) Baccanari, D. P. Unpublished results.

(24) (a) Matthews, D. A.; Alden, R. A.; Bolin, J. T.; Filman, D. J.; Freer, S. T.; Hamlin, R.; Hol, W. G. J.; Kisliuk, R. L.; Pastore, E. J.; Plante, L. T.; Xuong, N.; Kraut, J. Dihydrofolate reductace from *Lactobacillus casei*: X-ray structure of the enzyme-methotrexate-NADPH complex. *J. Biol. Chem.* **1978**, *253*, 6946–6954. (b) Bolin, J. T.; Filman, D. J.; Matthews, D. A.; Hamlin, R. C.; Kraut, J. Crystal structures of *Escherichia coli* and *Lactobacillus casei* dihydrofolate reductase refined at 1.7A resolution. I. General features and binding of methotrexate. *J. Biol. Chem.* **1982**, *257*, 13650–13662. (c) Filman, D. J.; Bolin, J. T.; Matthews, D. A.; Kraut, J. Crystal structures of *Escherichia coli* and *Lactobacillus casei* dihydrofolate reductase refined at 1.7A resolution. II. Environment of bound NADPH and implications for catalysis. *J. Biol. Chem.* **1982**, *257*, 13663–13672. (d) Champness, J. N.; Stammers, D. K.; Beddel, C. R. Crystallographic investigation of the cooperative interaction between trimethoprim, reduced cofactor and dihydrofolate reductase. *FEBS Lett.* **1986**, *199*, 61–67.

(25) (a) This work was done prior to the availability of the crystal structure of human DHFR, which has since been solved; see: Oefner, C.; D'Arcy, A.; Winkler, F. K. Crystal structure of human dihydrofolate reductase complexed with folate. *Eur. J. Biochem.* **1988**, *174*, 377–385. (b) Davies, J. F.; Delcamp, T. J.; Prendergast, N. J.; Ashford, V. A.; Freisheim, J. H.; Kraut, J. Crystal Structures of Recombinant Human Dihydrofolate Reductase Complexed with Folate and 5-Deazafolate. *Biochemistry* **1990**, *29*, 9467–9479.

(26) Hansch, C.; Leo, A. J. *Substituent Constants for Correlation Analysis in Chemistry and Biology*; Wiley & Sons: New York, 1979; pp 48–63.

(27) Hitchings, G. H. Mechanism of Action of Trimethoprim-Sulfamethoxazole. *J. Infect. Dis.* **1973**, *128* (Suppl.), S433–S436.

(28) Newman, H.; Angier, R. B. The Synthesis of the Ring-B Sulfur Analog of Dehydrogriseofulvin. *J. Org. Chem.* **1969**, *34*, 1463–1465.

(29) Baccanari, D. P.; Tansik, R. L.; Joyner, S. S.; Fling, M. E.; Smith, P. L.; Freisheim, J. H. Characterization of *Candida albicans* Dihydrofolate Reductase. *J. Biol. Chem.* **1989**, *264*, 1100–1107.

(30) Prendergast, N. J.; Delcamp, T. J.; Smith, P. L.; Freisheim, J. M. Expression and Site-directed Mutagenesis of Human Dihydrofolate Reductase. *Biochemistry* **1988**, *27*, 3664–3671.

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