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J. Comb. Chem., **2004**, 6 (5), 776-782• DOI: 10.1021/cc049953+ • Publication Date (Web): 29 June 2004

An Expedient and Facile One-Step Synthesis of a Biguanide Library by Microwave Irradiation Coupled with Simple Product Filtration. Inhibitors of Dihydrofolate Reductase

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Received February 17, 2004

It has been demonstrated previously by us that guanide-containing compounds (**1** and **2**) can inhibit significantly dihydrofolate reductase (DHFR). In this report, we have produced an array of alkyl- and aryl-based biguanide compounds using microwave irradiation. Further, we have demonstrated the use of TMSCl for the first time as an excellent and practical catalyst for the formation of alkyl and aryl biguanides. Using these methods, we prepared a 60-compound collection, of which one compound (**21g**) showed approximately one-half of the inhibitory activity of the parent compound **2**.

Introduction

Escherichia coli (*E. coli*) dihydrofolate reductase (DHFR) catalyzes the NADPH-dependent reduction of dihydrofolate to tetrahydrofolate and is the target of the antibiotic trimethoprim. Tetrahydrofolate, and other reduced folates, are required in bacteria for the biosynthesis of purines, thymidylate, panthothenate, and some amino acids.[1] Antibacterial synergism with sulfonamides, which target folate synthesis in bacteria, has led to worldwide use of "trimethoprim-sulfa" to treat infections as disparate as community-acquired respiratory and urinary tract infections to opportunistic infections such as pneumonia in hospital settings. Because of the importance of DHFR as an antibacterial target, the structure, mechanism, and inhibition of the *E. coli* enzyme have been the subject of intensive study. Significant achievements include comprehensive studies of inhibition by diaminobenzylpyrimidines (such as trimethoprim),[2] a complete kinetic and thermodynamic description of the reaction pathway,[3] and an analysis of six isomorphous crystallographic structures to produce a movie depicting loop and subdomain movements along the predominant reaction pathway.[4]

Most recently, we reported on a high-throughput screen of 50 000 structurally diverse small molecules against *E. coli* dihydrofolate reductase to detect new inhibitors.[5] This revealed nine molecules that were competitive with dihydrofolate, which had not been characterized previously as inhibitors of DHFR. These inhibitors fell into three broad structural classifications: 2,4-diaminoquinazolines, 2,4 diaminopyrimidines, and molecules without diaminoheterocyles. Among this latter group of molecules were two particularly potent guanyl-containing molecules: compounds **1**, a guanyl thiourea, and **2**, a guanyl guanidine (K_i values of 26 and 65 nM, respectively). In this present study, we have sought to explore the structure/activity relationship (SAR) of these compounds, as a first step, against bacterial DHFR with the synthesis and testing of a series of structurally related compounds.

In designing a route to prepare analogues of **2**, we were keen to incorporate microwave irradiation that can enhance greatly reaction rates as we have reported in the preparation of several structurally diverse molecular libraries.[6] Despite faster reactions though, microwave chemistry in a library preparation context still suffers from sample handling issues that hamper it from significantly accelerating the overall library preparation process. For example, the special reaction vials are sealed and must be de-capped, and the solutions inside must be worked up one at a time, which greatly diminishes some of the advantage of working with microwave irradiation. Significantly though, when compared to conventionally heated reactions, microwaved reactions often proceed with a higher percent conversion for the desired reaction, which leads to cleaner crude product mixtures.[7] We believe that, with the proper chemistry development, this advantage can be exploited to regain some of that efficiency lost by the "one at a time" nature of performing microwaved reactions.[8] The way that we have chosen to exploit this advantage is by the preparation of crystalline products that would be pure enough upon filtration of the crude reaction mixture to enter directly into the biological evaluation (screens).

Results

Library Preparation of Aryl-Based Biguanide Compounds. The formation of biguanide compounds by the acid-catalyzed addition of an amine to dicyandiamide is well known.[9] We believed that we could implement a microwave irradiation strategy to enhance the rate of these often slow reactions. This would be especially useful with electron poor

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10.1021/cc049953+ CCC: $27.50
Published on Web 06/29/2004

Scheme 1

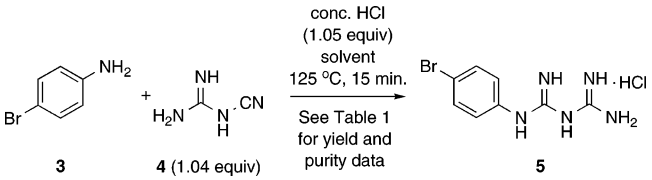
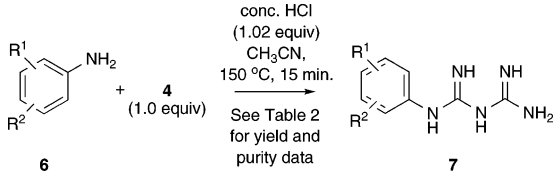


Table 1. Solvent Influence on Reaction Rate and Biguanide Precipitation

entry	solvent	yield (%)[a,b]	LC/MS (%)[c]
1	H_2O	72	99
2	MeOH	51	99
3	EtOH	72	99
4	PrOH	74	99
5	BuOH	77	99
6	*i*PrOH	81	99
7	toluene	77	87
8	dioxane	79	98
9	THF	81	99
10	acetone	22	99
11	CH_3CN	90	98

[a] Conditions: 4-bromoaniline (**3**, 200 mg, 1.13 mmol), dicyandiamide (**4**, 100 mg, 1.18 mmol), concentrated HCl (100 μL, 1.20 mmol), solvent (500 μL), microwave irradiation 125 °C, 15 min. [b] In all cases, a precipitate was observed. The solid was collected and washed twice with 500 μL of solvent, except for runs in water where the solid was washed twice with EtOH instead. [c] Purity was determined by HPLC at 230 nm.

Scheme 2



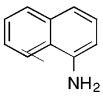
anilines whose structures (e.g., **2**) are particularly germane to DHFR inhibition (vide supra).

We began by optimizing the reaction temperature, which involved irradiating equal amounts of 4-bromoaniline (**3**) and dicyandiamide (**4**) in aqueous acid (concentration of aniline 3 M) for 15 min at different power settings (see Scheme 1 for structures). It was discovered that 150 °C was the optimum temperature for reaction rate and product recovery as reactions heated beyond this point led to decomposition. Thus, library production runs were performed at this temperature and typically for 15 min as most reactions that led to product were generally complete by this time in any case.

Next, we varied the solvent to optimize not only the reaction rate, but to determine which one(s) would lead to a high recovery of clean product following product crystallization (see Scheme 1 and Table 1). With the exception of entry 8, which produced a clean but unidentified side product, biguanide purity was very high in most solvents, and the best recovery was recorded in acetonitrile (entry 11).

With these results in hand, we set out to prepare a collection of biguanide compounds to test for activity against DHFR, and the results are summarized in Scheme 2 and Table 2. A number of compatibility issues were spotted right away that did not surface in our library rehearsal. The reaction does not lead to clean product formation with

Table 2. The Attempted Preparation of a 41-Member Aniline-Based Biguanide Library

entry	R_1	R_2	product number	yield (%)	LC/MS (%)[a]
1	H	H	**7a**	85	96
2	2-Me	3-Me	**7b**	85	99
3	2-Me	4-Me	**7c**	83	98
4	2-Et	6-Et	**7d**	68	88
5	2-*t*Bu	H	**7e**	2	35
6	4-OMe	H	**7f**	79	99
7	2-F	H	**7g**	75	99
8	3-F	H	**7h**	77	99
9	4-F	H	**7i**	80	99
10	2-Br	H	**7j**	67	99
11	4-Br	H	**5**	80	99
12	4-Cl	H	**7k**	78	99
13	2-Cl	3-Cl	**7l**	54	93
14	3-Cl	5-Cl	**7m**	55	86
15	3-Cl	4-Cl	**7n**	76	99
16	3-Cl	4-OMe	**7o**		
17	3-Cl	4-OH	**7p**		
18	2-Cl	5-NO_2	**7q**	55	58
19	2-Cl	4-NO_2	**7r**	41	70
20	3-NO_2	4-Cl	**7s**	69	89
21	4-I	H	**7t**	74	94
22	2-CF_3	H	**7u**	6	13
23	3-CF_3	H	**7v**	45	99
24	4-CF_3	H	**7w**	65	99
25	2-OH	H	**7x**		
26	4-OH	H	**7y**		
27	2-CH_2OH	H	**7z**		
28	2-SH	H	**7aa**		
29	4-phenylaza	H	**7bb**	82	93
30	2-COOH	H	**7cc**	74	56
31	3-COOH	H	**7dd**		
32	4-COOH	H	**7ee**		
33	3-COOH	4-OH	**7ff**		
34	4-COOEt	H	**7gg**	69	99
35	4-COMe	H	**7hh**	77	94
36	2-NO_2	H	**7ii**		
37	4-NO_2	H	**7jj**	36	88
38	2-NO_2	4-OH	**7kk**		
39	4-CN	H	**7ll**	53	58
40	4-SO_2NH_2	H	**7mm**		
41			**7nn**	66	93

[a] Purity was determined by HPLC at 230 nm.

substrates containing alcohol (entries 17, 25, 26, 27, 33, 38), amine (data not shown), or thiol groups (entry 28), but rather black product mixtures were obtained, suggesting that decomposition had taken place. The same was the case for any free carboxylic acid (entries 30−33), but esters provide very good results (entry 34). As anticipated, anilines equipped with electron-withdrawing groups had lower recovery, although many of these still gave rise to compounds of acceptable purity with which to do the biological screen (e.g., entries 23, 24, 34, 35, 37), and a good result was also obtained with 1-naphthylamine (entry 41).

Library Preparation of Alkyl- and Other Biguanide Compounds. Despite the moderate success seen with the aryl-based systems, initial attempts to apply those optimized protic acid conditions to primary and secondary alkylamines led to widely varying results. New conditions were developed on benzylamine (**8**), as the representative amine, and the data suggested that alkylamines required lower substrate concen-

Scheme 3

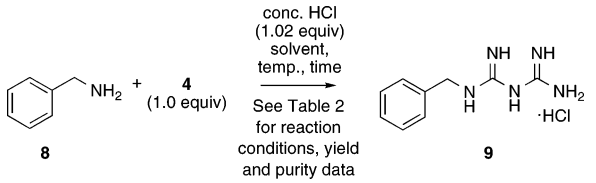


Table 3. Optimization of Biguanide Formation with Benzylamine and Dicyandiamide

entry	reaction time (min)	temp (°C)	solvent	conc (mol L^{-1})	yield (%)	LC/MS (%)[a]
1	15	125	*i*PrOH	1.9		
2	15	150	*i*PrOH	1.9		
3	15	150	CH_3CN	1.9		
4	15	160	CH_3CN	1.9	53	95
5	15	170	CH_3CN	1.9	48	99
6	120	170	CH_3CN	1.9		
7	15	170	BuOH	1.9	47	99
8	30	170	BuOH	1.9		
9	60	170	BuOH	1.9		
10	30	165	THF	1.9	47	98
11	10	170	CH_3CN	1.9	25	95
12	20	170	CH_3CN	1.9	49	99
13	25	170	CH_3CN	1.9	57	97
14	30	170	CH_3CN	1.9	24	99
15	20	170	CH_3CN	3.2	56	99
16	20	170	CH_3CN	1.3	35	99
17	20	170	CH_3CN	1.0	58	99
18	20	170	CH_3CN	0.7	29	99
19	5	200	CH_3CN	0.54	22	99

[a] Purity was determined by HPLC at 230 nm.

tration and higher temperatures to ensure good product recovery of the crystalline product (see Scheme 3 and Table 3).

We set out to produce an array of amine-based biguanides using dicyandiamide (1 equiv) in the presence of concentrated aqueous HCl (1 equiv) in acetonitrile (substrate concentration: 1 mol L^{-1}) at 170 °C for 20 min (see Figure 2 for representative amines, i.e., structures **10**−**17**). The production of alkylbiguanides using acid catalysis is known, but the reaction conditions are significantly more harsh.[9b] Initially, we envisioned that the alkylamines would be more nucleophilic, and thus more reactive than their aniline counterparts, and that should improve reaction rate and yield. In fact, the amines were much more nucleophilic, leading in some cases to unexpected and undesired reactivity. For example, in some instances, reaction of the amine occurred at the guanidine end of dicyandiamide, producing **18**, while in other situations a second equivalent of the amine reacted with the initial desired product, giving rise to symmetrical products **19**.

At this stage, it occurred to us that we needed to consider changing more than just the conditions of these protic acid-catalyzed reactions to allow this transformation to show the generality that we desired across a variety of amine substrates. It has been reported that amines can react with nitriles to form guanidines in the presence of TMS−OTf,[10] but difficulty in handling this highly reactive and sensitive catalyst would make library production with it impractical. Interestingly, TMSCl, which can be handled and dispensed easily, has not been reported as a catalyst for this or other analogous transformations. In a preliminary set of experiments, reaction with TMSCl catalyst was attempted, and the results are shown in Scheme 4.

Yields and purity were very promising, so we proceeded to optimize concentration, temperature, and reaction time. The reaction concentration was varied from 0.25 to 1.0 M, and product recovery did not vary significantly. Reaction rate and product recovery seemed to be optimal at 150 °C. As compared to the protic conditions, reaction rate was greatly enhanced under TMSCl catalysis. The reaction provided significant product recovery (>65%) after only 60 s, and it diminished as the reaction was heated further, mainly due to over-reaction at the guanidine site of the desired product giving rise to compounds resembling **19**. Thus, using the optimized conditions outlined in Scheme 5, a collection of biguanides was prepared easily by microwave heating and simple filtration, and the data are summarized in Table 4. It is noteworthy that with these conditions, additional nitrogen atoms (entry 2) or hydroxy functions (entry 5) are now tolerated.

Evaluation of Biguanide Compounds as Inhibitors of DHFR. DHFR activity was assayed continuously in 96-well microplates by measuring the decrease in absorbance at 340 nm associated with the oxidation of NADPH to NADP.[11] Reactions (200 μL) were performed in triplicate at 25 °C for 10 min and consisted of 50 mM Tris-HCl pH 7.5, 40 μM NADPH, 30 μM dihydrofolate (DHF), 0.01% (w/v) Triton X-100, 10 mM β-mercaptoethanol, and were initiated with 5 nM DHFR. Biguanide compounds were solubilized in 100% DMSO to a final concentration of 10 mM and tested at half log concentrations of 0.0003, 0.001, 0.003, 0.01, 0.03, 0.1, 0.3, 1, 3, 10, 30, and 100 μM where DMSO never exceeded 1% (v/v) in the final reactions. Inhibition of DHFR by the biguanide variants was negligible as maximal loss of activity was ~45% at 100 μM for the most potent compound (**21g**, Table 5) as compared to 97.1% for the parent compound (compound **2**, Figure 1) at the same concentration.

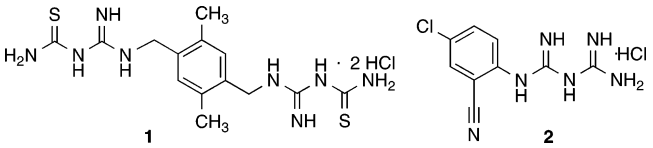


Figure 1. Effective nanomolar binders of DHFR that contain guanidine moieties.

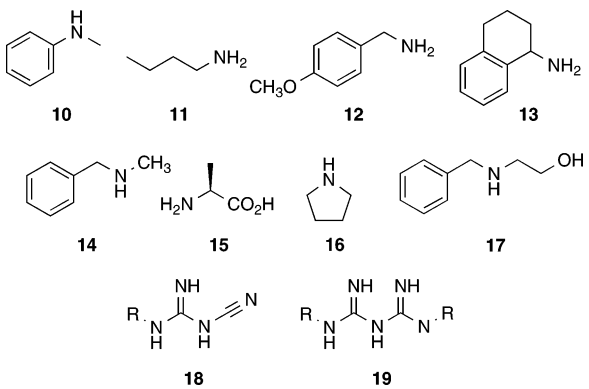
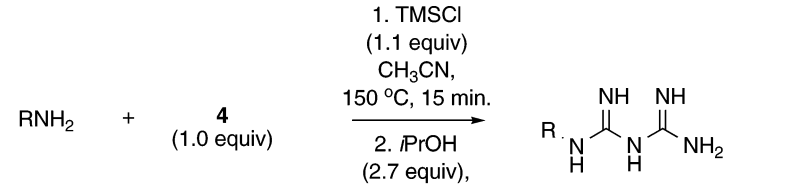


Figure 2. Sample amines reacted with dicyandiamide (**4**) under optimized protic acid conditions for the library of alkylamine biguanide derivatives.

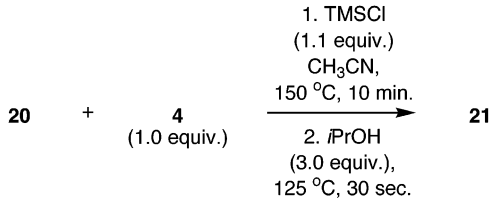
Scheme 4



8, R = benzyl
20a, R = 4-fluorophenyl
20b, R = 2-((3,4-dimethoxy)phenyl)ethyl

9, R = benzyl (69%)
21a, R = 4-fluorophenyl (74%)
21b, R = 2-((3,4-dimethoxy)phenyl)ethyl (75%)
Purities all over 97% (LCMS, UV 230 nm)

Scheme 5



In conclusion, a small array of biguanide compounds was prepared for screening purposes against bacterial DHFR using microwave irradiation. It was found that biguanide products were obtained readily from aniline derivatives under protonic acid catalysis except in cases where the aniline was substituted with a free X−H bond (where X = O, N, or S) or carboxylic acid moiety. This problem was overcome by simply protecting these groups. Alkylamines could not be reacted controllably with dicyandiamide (**4**) under these same conditions, and in most cases over-reaction at the guanidine site on the intermediate desired product took place. However, more controlled reactivity was achieved using TMSCl catalysis. Interestingly, while the reactions actually proceeded kinetically faster under TMSCl conditions, there was significantly less over-reaction, leading to compounds resembling **19**. This represents a new and robust set of reaction conditions to prepare biguanide compounds in a molecular library format.

Compounds **7** and **21**, derivatives of compound **2** lacking the cyano functionality, were screened against DHFR, and it was found that they showed little to no inhibitory activity toward DHFR. This suggests that the cyano group is critical for the inhibitory action of this compound.

Experimental Section

General Methods. All reagents were purchased from Aldrich Chemical Co. and used without further purification. Reactions were carried out under microwave irradiation using a Smith Synthesizer (Personal Chemistry). The machine consisted of a continuous focused microwave power delivery system (0−300 W) that reaches and maintains a preselected temperature. Reactions were performed with stirring in crimped, septum-sealed glass microwave vessels (5 mL). Measured reaction times commence when the solution reaches the selected temperature (i.e., warming and cooling cycles are not included). *Caution: The solvent is heated well above its boiling point, so all necessary precautions should be taken when performing such experiments.* Vessels designed to withhold elevated pressures must be used, and solution temperatures must be allowed to fall below their boiling points before opening the tubes.

NMR spectra were recorded in DMSO-d_6 at 400 and 100.6 MHz for proton and carbon spectra, respectively. Carbon NMR spectral data were acquired using the APT (attached proton test) pulse sequence which displays positive signals (+) for nonproton bound carbons (e.g., carbonyl or quaternary carbons) and carbons that are attached to an even number of protons. Signals for carbons attached to an odd number of protons are negative (−). Proton chemical shifts are listed relative to residual DMSO−H (δ 2.49), and ^{13}C NMR spectra are reported relative to the C−D heptet resonance (δ 39.5 middle peak). ^{1}H NMR data are reported for compounds with purity equal to or greater than 90%, whereas ^{13}C NMR data were reported only for compounds with purity equal to or greater than 95%.

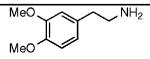
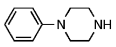
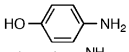
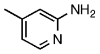
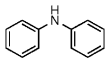
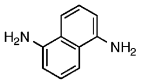
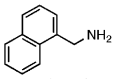
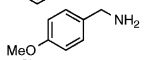
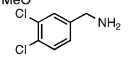
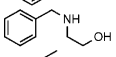
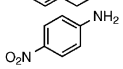
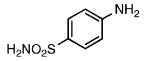
Mass spectra were obtained using a PE SCIEX API 2000 triple quadrupole MS with turboionspray ionization. All mass spectra were full-scan experiments (mass range 50−500 amu). The HPLC system was Shimadzu with an LC-8A pump and an SPD-10A VP UV−vis system (λ = 230 nm) with ZorBAX-CN (150 × 4.6 mm) with a mobile phase consisting of acetonitrile:H_2O (70:30) with 0.1% TFA.

General Procedure for the Synthesis of the Biguanide Library via Protic Acid Catalysis. Into a 5 mL microwave vessel was added CH_3CN (1 mL) followed by the aniline (1.18 mmol) and dicyandiamide (**4**, 101 mg, 1.19 mmol, 1.01 equiv). After the vial was sealed and crimped, concentrated HCl (100 μL, 120 mmol, 1.02 equiv) was added slowly via the robotic dispenser (flow rate 100 μL/min) of the Smith Synthesizer. The mixture was stirred for 30 s and then irradiated at 150 °C for 15 min. After the mixture cooled to approximately 50 °C, the biguanide hydrochloride salt began to precipitate. The solid was collected by filtration and washed with CH_3CN (2 × 1 mL). In some instances, the crude mixture was diluted by 1 mL of CH_3CN to facilitate the filtration process.

4-Bromophenylbiguanide, HCl (5). Yield, 80%; Purity (LC/MS), 99%; ^{1}H NMR (DMSO) δ 9.73 (bs, 1H, D_2O exchange), 7.46 (d, J = 8.5 Hz, 2H), 7.34−7.32 (m, 6H, D_2O exchange for 4H), 7.04 (bs, 2H, D_2O exchange); ^{13}C NMR (DMSO) δ 161.4 (+), 154.7 (+), 138.2 (+), 131.3 (−), 122.3 (+), 114.7 (−).

Phenylbiguanide, HCl (7a). Yield, 85%; Purity (LC/MS), 96%; ^{1}H NMR (DMSO) δ 9.77 (bs, 1H, D_2O exchange),

Table 4. Reaction of a Variety of Amines with Dicyandiamide (**4**) under TMSCl Catalysis

Entry	amine	Product Number	Yield (%)	LCMS (%)
1		**21a**	77	94
2		**21b**	74	89
3		**7e**	36	86
4		**7u**	0[a]	-
5		**21c**	96	98
6		**21d**	0[b]	-
7		**7cc**	57[c]	58
8		**21e**	45	58
9		**21f**	97[d]	63
10		**21g**	67	99
11		**21h**	70	98
12		**21i**	76	99
13		**21j**	25	62
14		**21k**	72	97
15		**21l**	0[b]	-
16		**21m**	81[f]	60
17		**7jj**	74[f]	70
18		**21n**	0[e]	-
19		**21o**	64	99

[a] No precipitate was formed. [b] A precipitate was obtained; however, it did not contain any biguanide product. [c] Cyclized product was observed. [d] The reaction was performed with 2 equiv of both **4** and TMSCl at 160 °C for 10 min. [e] The reaction was performed at 150 °C for 15 min. [f] The reaction was performed at 160 °C for 10 min.

7.36−7.26 (m, 8H, D_2O exchange for 4H), 7.06−7.01 (m, 3H, D_2O exchange for 2H); ^{13}C NMR (DMSO) δ 161.1 (+), 155.2 (+), 138.6 (+), 128.5 (−), 123.2 (−), 120.7 (−).

2,3-Dimethylphenylbiguanide, HCl (7b). Yield, 85%; Purity (LC/MS), 99%; ^1H NMR (DMSO) δ 9.14 (bs, 1H, D_2O exchange), 7.11−6.99 (m, 9H, D_2O exchange for 6H), 2.23 (s, 3H), 2.11 (s, 3H); ^{13}C NMR (DMSO) δ 160.6 (+), 157.1 (+), 136.9 (+), 135.9 (+), 131.4 (+), 127.0 (−), 125.2 (−), 124.1 (−), 20.0 (−), 14.0 (−).

Table 5. Inhibitory Activity of Biguanide Derivatives against *E. coli* DHFR

entry	compound	percent remaining DHFR activity[a]
1	**7a**	85.9
2	**7b**	89.7
3	**7c**	82.3
4	**7d**	82.0
5	**7f**	94.6
6	**7g**	88.3
7	**7h**	90.0
8	**7i**	91.0
9	**7j**	89.7
10	**7k**	89.9
11	**7l**	84.7
12	**7m**	94.5
13	**7o**	84.6
14	**7t**	94.0
15	**7u**	91.7
16	**7w**	89.3
17	**7x**	88.5
18	**7bb**	82.1
19	**7gg**	95.7
20	**7hh**	91.4
21	**9**	86.1
22	**21a**	80.3
23	**21b**	84.3
24	**21g**	54.5
25	**21h**	68.3
26	**21i**	72.2
27	**21k**	85.9
28	**21o**	92.8

[a] Inhibition expressed as percent remaining DHFR activity at 100 μM biguanide compound and 30 μM DHF.

2,4-Dimethylphenylbiguanide, HCl (7c). Yield, 83%; Purity (LC/MS), 98%; ^1H NMR (DMSO) δ 9.01 (bs, 1H, D_2O exchange), 7.16 (d, J = 7.8 Hz, 1H), 7.09 (bs, 2H, D_2O exchange), 7.02 (bs, 5H, D_2O exchange for 4H), 6.95 (d, J = 7.8 Hz, 1H), 2.23 (s, 3H), 2.18 (s, 3H); ^{13}C NMR (DMSO) δ 160.5 (+), 157.0 (+), 134.4 (+), 133.4 (+), 132.1 (+), 130.7 (−), 136.5 (−), 125.8 (−), 20.4 (−), 17.8 (−).

4-Methoxyphenylbiguanide, HCl (7f). Yield, 79%; Purity (LC/MS), 99%; ^1H NMR (DMSO) δ 9.52 (bs, 1H, D_2O exchange), 7.23 (d, J = 8.6 Hz, 2H), 7.16 (bs, 4H, D_2O exchange), 6.99 (bs, 2H, D_2O exchange), 6.87 (d, J = 8.6 Hz, 2H), 3.71 (s, 3H); ^{13}C NMR (DMSO) δ 160.8 (+), 155.9 (+), 155.8 (+), 131.2 (+), 123.2 (−), 113.8 (−), 55.2 (−).

2-Fluorophenylbiguanide, HCl (7g). Yield, 75%; Purity (LC/MS), 99%; ^1H NMR (DMSO) δ 9.19 (bs, 1H, D_2O exchange), 7.69 (m, 1H), 7.36 (bs, 4H, D_2O exchange), 7.23 (bs, 3H, D_2O exchange for 2H), 7.13 (bs, 2H); ^{13}C NMR (DMSO) δ 161.4 (+), 155.5 (+), 154.3 (+, d, J = 245.5 Hz), 126.0 (+, d, J = 12.2 Hz), 125.3 (−), 125.2 (−), 124.2 (−), 115.4 (−, d, J = 20.1 Hz).

3-Fluorophenylbiguanide, HCl (7h). Yield, 77%; Purity (LC/MS), 99%; ^1H NMR (DMSO) δ 10.03 (bs, 1H, D_2O exchange), 7.39−7.27 (m, 6H, D_2O exchange for 4H), 7.09 (bs, 3H, D_2O exchange for 2H), 6.82 (t, J = 7.7 Hz, 1H); ^{13}C NMR (DMSO) δ 162.0 (+, d, J = 241.3 Hz), 161.4 (+), 154.6 (+), 140.8 (+, d, J = 12.1 Hz), 130.1 (−, d, J = 10.3 Hz), 115.9 (−), 109.2 (−, d, J = 21.7 Hz), 107.0 (−, d, J = 26.6 Hz).

4-Fluorophenylbiguanide, HCl (7i). Yield, 80%; Purity (LC/MS), 99%; ^{1}H NMR (DMSO) δ 9.75 (bs, 1H, D_2O exchange), 7.38−7.29 (m, 6H, D_2O exchange for 4H), 7.13 (t, J = 8.6 Hz, 2H), 7.04 (bs, 2H, D_2O exchange); ^{13}C NMR (DMSO) δ 161.2 (+), 158.3 (+, d, J = 239.5 Hz), 155.3 (+), 134.9 (+), 122.9 (−, d, J = 10.5 Hz), 115.1 (−, d, J = 22.8 Hz).

2-Bromophenylbiguanide, HCl (7j). Yield, 67%; Purity (LC/MS), 99%; ^{1}H NMR (DMSO) δ 8.88 (bs, 1H, D_2O exchange), 7.62 (d, J = 8.0 Hz, 1H), 7.58 (d, J = 7.9 Hz, 1H), 7.36−7.27 (m, 7H, D_2O exchange for 6H), 7.09 (t, J = 7.5 Hz, 1H); ^{13}C NMR (DMSO) δ 161.5 (+), 155.7 (+), 136.3 (+), 132.4 (−), 127.7 (−), 127.4 (−), 126.5 (−), 117.7 (+).

4-Chlorophenylbiguanide, HCl (7k). Yield, 78%; Purity (LC/MS), 99%; ^{1}H NMR (DMSO) δ 9.86 (bs, 1H, D_2O exchange), 7.38−7.34 (m, 8H, D_2O exchange for 4H), 7.06 (bs, 2H, D_2O exchange); ^{13}C NMR (DMSO) δ 161.3 (+), 154.8 (+), 137.8 (+), 128.4 (−), 126.7 (+), 122.0 (−).

2,3-Dichlorophenylbiguanide, HCl (7l). Yield, 54%; Purity (LC/MS), 93%; ^{1}H NMR (DMSO) δ 9.12 (bs, 1H, D_2O exchange), 7.63 (bs, 1H), 7.38−7.33 (m, 8H, D_2O exchange for 6H).

3,4-Dichlorophenylbiguanide, HCl (7n). Yield, 76%; Purity (LC/MS), 99%; ^{1}H NMR (DMSO) δ 10.11 (bs, 1H, D_2O exchange), 7.76 (s, 1H), 7.51 (d, J = 8.2 Hz, 1H), 7.47 (m, 4H, D_2O exchange), 7.31 (d, J = 8.2 Hz, 1H), 7.11 (bs, 2H, D_2O exchange); ^{13}C NMR (DMSO) δ 161.6 (+), 154.3 (+), 139.2 (+), 130.7 (−), 130.4 (+), 124.3 (+), 121.2 (−), 120.0 (−).

4-Iodophenylbiguanide, HCl (7t). Yield, 74%; Purity (LC/MS), 93%; ^{1}H NMR (DMSO) δ 9.72 (bs, 1H, D_2O exchange), 7.61 (d, J = 8.5 Hz, 2H), 7.32 (bs, 4H, D_2O exchange), 7.20 (d, J = 8.5 Hz, 2H), 7.03 (bs, 2H, D_2O exchange).

3-Trifluoromethylphenylbiguanide, HCl (7v). Yield, 45%; Purity (LC/MS), 99%; ^{1}H NMR (DMSO) δ 10.14 (bs, 1H, D_2O exchange), 7.80 (bs, 1H, D_2O exchange), 7.61 (d, J = 8.0 Hz, 1H), 7.53−7.45 (m, 5H, D_2O exchange for 3H), 7.35 (d, J = 7.6 Hz, 1H), 7.13 (bs, 2H, D_2O exchange); ^{13}C NMR (DMSO) δ 161.6 (+), 154.6 (+), 139.8 (+), 129.7 (−), 129.3 (+, d, J = 31.5 Hz), 124.0 (+, d, J = 272.7 Hz), 123.8 (−), 119.1 (−, bd, J = 3.9 Hz), 116.3 (−, bd, J = 4.6 Hz).

4-Trifluoromethylphenylbiguanide, HCl (7w). Yield, 65%; Purity (LC/MS), 99.3%; ^{1}H NMR (DMSO) δ 10.21 (bs, 1H, D_2O exchange), 7.64−7.50 (m, 8H, D_2O exchange for 4H), 7.16 (bs, 2H, D_2O exchange); ^{13}C NMR (DMSO) δ 161.6 (+), 154.3 (+), 142.7 (+), 125.8 (−), 123.1 (+), 122.7 (+, d, J = 32.7 Hz), 119.8 (−).

4-(Phenyl-aza)phenylbiguanide, HCl (7bb). Yield, 82%; Purity (LC/MS), 93%; ^{1}H NMR (DMSO) δ 10.23 (bs, 1H, D_2O exchange), 7.86−7.83 (m, 4H), 7.66−7.50 (m, 9H, D_2O exchange for 4H), 6.68 (bs, 2H, D_2O exchange).

Ethyl 4-Biguanidebenzoate, HCl (7gg). Yield, 69%; Purity (LC/MS), 99%; ^{1}H NMR (DMSO) δ 10.06 (bs, 1H, D_2O exchange), 7.87 (d, J = 7.8 Hz, 2H), 7.52 (d, J = 7.8 Hz, 2H), 7.47 (bs, 4H, D_2O exchange), 7.12 (bs, 2H, D_2O exchange), 4.27 (q, J = 7.0 Hz, 2H), 1.29 (t, J = 7.0 Hz, 3H); ^{13}C NMR (DMSO) δ 165.3 (+), 161.6 (+), 154.2 (+), 143.6 (+), 130.0 (−), 123.6 (+), 119.1 (−), 60.3 (+), 14.2 (−).

4-Acetylphenylbiguanide, HCl (7hh). Yield, 77%; Purity (LC/MS), 94%; ^{1}H NMR (DMSO) δ 10.16 (bs, 1H, D_2O exchange), 7.88 (d, J = 8.4 Hz, 2H), 7.52 (d, J = 8.4 Hz, 2H), 7.49 (bs, 4H, D_2O exchange), 7.16 (bs, 2H, D_2O exchange), 2.50 (s, 3H).

Naphthylbiguanide, HCl (7nn). Yield, 66%; Purity (LC/MS), 92%; ^{1}H NMR (DMSO) δ 9.68 (bs, 1H, D_2O exchange), 8.13 (d, J = 6.5 Hz, 1H), 7.94−7.92 (m, 1H), 7.76 (d, J = 7.8 Hz, 1H), 7.57−7.46 (m, 4H), 7−25−7.15 (bm, 6H, D_2O exchange).

General Procedure for the Synthesis of the Biguanide Library in the Presence of TMSCl Catalyst. Into a 5-mL microwave vessel were added the amino derivative (0.6 mmol) and dicyandiamide (**4**, 51 mg, 0.60 mmol, 1.0 equiv). After the vial was sealed and crimped, CH_3CN (800 μL) was added, and this was followed by the slow addition of a 2 N solution of TMSCl (330 μL, 0.66 mmol, 1.1 equiv) in CH_3CN via the robotic dispenser (flow rate 2 mL/min) of the Smith Synthesizer. The mixture was stirred for 10 s and then irradiated for the time and at the temperature listed in Table 4. After the mixture was cooled to approximately 50 °C, *i*PrOH (138 μL, 1.80 mmol, 3.0 equiv) was added via the robotic dispenser (flow rate 1 mL/min). The mixture was stirred for 10 s and then irradiated a second time at 125 °C for 30 s. Upon cooling, the biguanide hydrochloride salt precipitated, and it was collected, washed with CH_3CN (2 × 1 mL), and dried in vacuo. It should be noted that certain peaks do not show up in the carbon NMR spectra for all of the compounds reported below in this series. The proton NMR spectra and LC/MS spectra are all fine and correlate well with the proposed structures. We have observed this phenomenon with other related compounds in the past, especially those with benzylic sulfur- or nitrogen-based groups at this site.

Benzylbiguanide, HCl (9). Yield, 68%; Purity (LC/MS), 99%; ^{1}H NMR (DMSO) δ 7.83 (bs, 1H, D_2O exchange), 7.42−7.17 (m, 6H, D_2O exchange for 1H), 7.01 (s, 5H, D_2O exchange), 4.34 (d, 2H, J = 5.9 Hz, s with D_2O); ^{13}C NMR (DMSO) δ 158.3 (+), 138.6 (+), 128.3 (−), 127.2 (−), 126.9 (−), 44.1 (+), one signal is missing.

2-((3,4-Dimethoxy)phenyl)ethylbiguanide, HCl (21a). Yield, 75%; Purity (LC/MS), 99%; ^{1}H NMR (DMSO) δ 7.29−6.99 (m, 7H, D_2O exchange), 6.87−6.84 (m, 2H), 6.74 (d, 2H, J = 8.2 Hz), 3.74 (s, 3H), 3.71 (s, 3H), 3.30 (m, 2H, t with D_2O, J = 7.3 Hz), 2.69 (t, 2H, J = 7.3 Hz); ^{13}C NMR (DMSO) δ 148.6 (+), 147.3(+), 131.3 (+), 120.5 (−), 112.8 (−), 112.0 (−), 55.5 (−), 43.0 (+), three signals are missing.

1-Naphthalenemethylbiguanide, HCl (21g). Yield, 67%; Purity (LC/MS), 99%; ^{1}H NMR (DMSO) δ 8.05 (d, 1H, J = 8.0 Hz), 7.97 (d, 1H, J = 7.7 Hz), 7.89−7.86 (m, 1H), 7.60−7.46 (m, 4H), 7.15 (bs, 6H, D_2O exchange), 4.82 (d, 2H, J = 5.0 Hz, s with D_2O), a broad pick with D_2O exchange is observed bellow signal between 8.05 and 7.86 (1H); ^{13}C NMR (DMSO) δ 133.2 (+), 130.7 (+), 128.5 (−),

127.8 (−), 126.4 (−), 125.8 (−), 125.4 (−), 125.2 (−), 123.3 (−), four signals are missing.

4-Methoxybenzylbiguanide, HCl (21h). Yield, 70%; Purity (LC/MS), 98%; ^{1}H NMR (DMSO) δ 7.23 (d, 1H, J = 8.3 Hz), 6.89 (d, 1H, J = 8.3 Hz), 4.82 (d, 2H, J = 5.2 Hz, s with D_2O), 3.73 (s, 3H), a broad pick with D_2O exchange is observed between 9 and 6 (7H); ^{13}C NMR (DMSO) δ 158.5 (+), 128.9 (−), 113.8 (−), 55.1 (−), four signals are missing.

3,4-Dichlorobenzylbiguanide, HCl (21i). Yield, 76%; Purity (LC/MS), 99%; ^{1}H NMR (DMSO) δ 7.93 (bs, 1H, D_2O exchange), 7.58 (d, 1H, J = 8.5 Hz), 7.54 (s, 1H), 7.29 (d, 1H, J = 8.5 Hz), 7.12 (bs, 6H, D_2O exchange), 4.82 (d, 2H, J = 5.9 Hz, s with D_2O); ^{13}C NMR (DMSO) δ 130.9 (+), 130.4 (−), 129.5 (+), 129.2 (−), 127.6 (−), four signals are missing.

1,2,3,4-Tetrahydro-1-naphthylbiguanide, HCl (21k). Yield, 72%; Purity (LC/MS), 97%; ^{1}H NMR (DMSO) δ 7.68 (bs, 1H, D_2O exchange), 7.38−7.08 (m, 10H, D_2O exchange for 6H), 4.86 (bs, 1H), 2.78−2.66 (m, 2H), 1.95−1.83 (m, 2H), 1.77−1.70 (m, 2H); ^{13}C NMR (DMSO) δ 157.9 (+), 136.9 (+), 128.7 (−), 128.3 (−), 126.9 (−), 125.8 (−), 29.8 (+), 28.6 (+), 19.7 (+), three signals are missing.

***N*-Methylbenzylbiguanide, HCl (21o).** Yield, 64%; Purity (LC/MS), 99%; ^{1}H NMR (DMSO) δ 7.37−7.26 (m, 7H, D_2O exchange for 2H), 6.88 (s, 4H, D_2O exchange), 4.57 (s, 2H), 2.85 (s, 3H); ^{13}C NMR (DMSO) δ 159.3 (+), 158.7 (+), 136.9 (+), 128.5 (−), 127.4 (−), 127.3 (−), 52.3 (+), 35.1 (−).

Supporting Information Available. Experimental data and MS traces. This material is available free of charge via the Internet at http://pubs.acs.org.

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CC049953+