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Synthesis and antimicrobial activity of N^1-benzyl or N^1-benzyloxy-1,6-dihydro-1,3,5-triazine-2,4-diamines

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ABSTRACT

The emergence and spread of multidrug-resistant strains of *Staphylococcus aureus* and *Mycobacterium tuberculosis* are generating a threat to public health worldwide. In the current study, a series of N^1-benzyl and N^1-benzyloxy-1,6-dihydro-1,3,5-triazine-2,4-diamine derivatives were synthesized and investigated for their antimicrobial activity against *S. aureus,* and *Mycobacterium smegmatis* which is taxonomically related to *M. tuberculosis.* Most of the compounds exhibited good activity against *M. smegmatis* as determined by comparison of diameters of the zone of inhibition of test compounds and standard antibiotics. Compound **7o** showed potent antimycobacterial activity against *M. smegmatis* without mammalian DHFR inhibition liability. The results from this study indicate that 1-benzyl derivatives of 1,6-dihydro-1,3,5-triazine-2,4-diamines may be used as lead compounds for the discovery of antimycobacterial agents.

The 2,4-diamino-1,3-diaza (**1**, Fig. 1) is a common pharmacophoric feature of many antifolates that exhibit antibacterial activity against pathogens such as gram-positive bacteria,[1] *Mycobacterium tuberculosis*,[2,3] and *Mycobacterium avium* which are known to cause opportunistic infections in patients with compromised immune systems.[4–7] For instance, the structural motif is embedded in the structure of diaminopyrimidines like trimethoprim and iclaprim. Trimethoprim (TMP, **2**, Fig. 1), is an antibiotic that has been widely used for over four decades in medical practice,[8] and, most recently, iclaprim (**3**, Fig. 1), has completed a phase III clinical trial against complicated skin and skin structure infection with promising results.[9] Studies have also shown that the biguanide PS-15 (**4**) and its metabolite, WR99210 (**5**), which possesses the same 2,4-diamino-1,3-diaza pharmacophore, have been found to exhibit reasonable in vitro inhibitory activity against *M. tuberculosis* and other mycobacteria.[3,6,7] A ternary complex crystal structure of *M. tuberculosis* dihydrofolate reductase (DHFR) with $NADP^+$ and Br-WR99210 (**6**) was determined in a separate study.[10] In the study, structural comparison of the ternary complex with human DHFR has identified a striking difference in the binding site region near the N^1 and two methyl groups of Br-WR99210 (Fig. 1), where a glycerol molecule binds in a pocket of the complex and this pocket is essentially filled with hydrophobic side-chains in human DHFR.[10] The differences provided opportunities for designing new selective antimycobacterial agents.

In the light of these reports, a series of dihydro-1,3,5-triazines embedded with the 2,4-diamino-1,3-diaza pharmacophore was designed for synthesis. The library of compounds contains derivatives of dihydro-1,3,5-triazine-2,4-diamine that carry various bulky alkyl substitution at the above mentioned C-2 position, and in addition, some of the compounds have either a benzyl or benzyloxy bridge that is connected to the triazine ring at its N-1 position. Thus, compounds **7a**–**7w** were synthesized to structurally mimic the conventional 2,4-diaminopyridine of TMP or iclaprim due to a similar benzyl substitution. Whereas compounds **8a**–**8g** were designed with a N-benzyloxy substitution at N-1 position in an attempt to probe steric and electronic tolerance of the side chain. The designed compounds were synthesized and evaluated for their antimicrobial activity against a panel of bacteria including *Staphylococcus aureus,* and *Mycobacterium smegmatis* which is taxonomically related to *M. tuberculosis*. At the same time, DHFR inhibitory assay was conducted to determine the antifolate activity against mammalian DHFR. This study was conducted to evaluate whether the compounds would interfere with mammalian DHFR which could lead to potential side effect liability if inhibition against bacterial and mammalian DHFR was not selective.[11]

The synthesis of **7a**–**7e** was reported in our previous publication,[12] and the synthetic route to **7a**–**7e** was applied for the preparation of **7f**–**7w** as described in Scheme 1. Reaction of the substituted benzylamine hydrochloride and cynoguanidine at 170–180 °C for 30 min provided biguanide hydrochlorides, which

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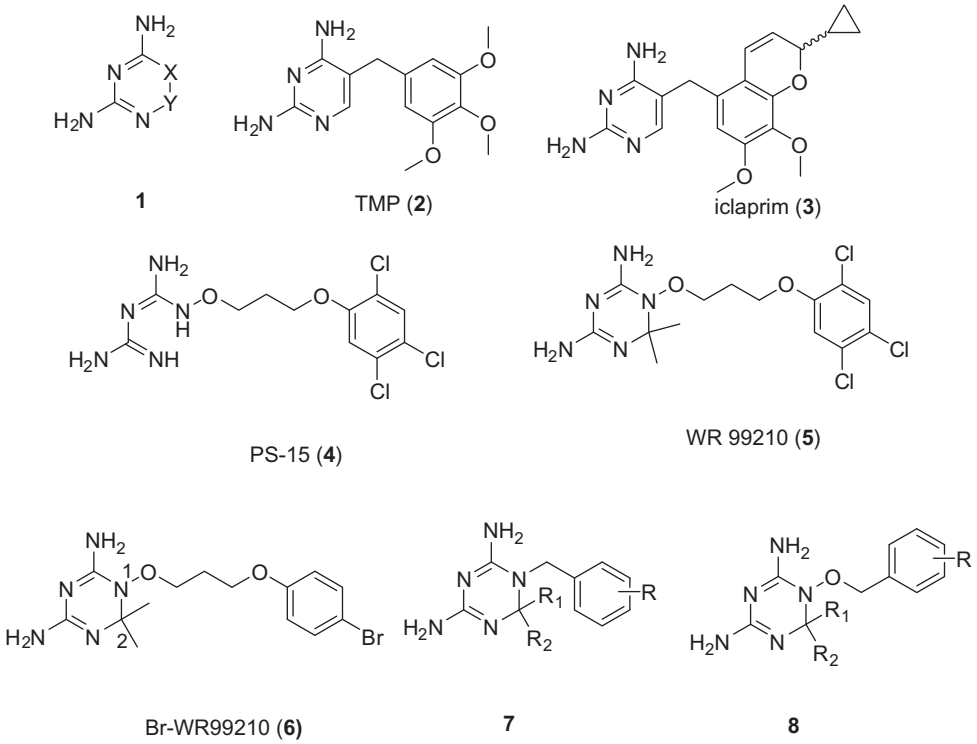
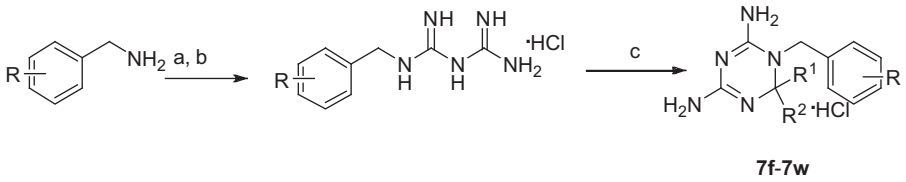
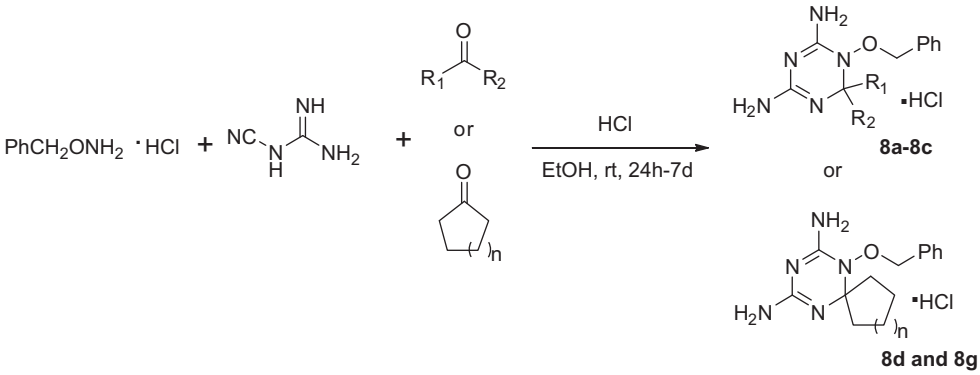


Figure 1. Structures of some antibacterial or antimycobacterial agents with 2,4-diamino-1,3-diaza pharmacophore.



Scheme 1. Synthesis of **7f**–**7w**. Reagents and conditions: (a) HCl, ether, rt; (b) cynoguanidine, 170–180 °C; (c) ketones, 2,2-dimethoxypropane, concd. HCl, ethanol, reflux.

were subjected to HCl-catalyzed cyclocondensation with various ketones to afford the target compounds **7f**–**7w**. The progress of the reaction was followed by the observation of shifts in ultraviolet absorption spectrum of the reaction mixture, or by the measurement of biguanide disappearance which was accomplished by means of the colored copper complex formed characteristically by biguanide with cuprammonium ion.[13] The synthesis of **8a**–**8d** and **8g** was accomplished using a reported procedure[14] for preparation of **8e** and **8f** as described in Scheme 2. Under room temperature, a three component synthesis was carried out to give the targeted compounds with a spectrum of reaction time using different ketone or aldehyde reagents. The relative reaction speed of ketone or aldehyde reagents employed was observed in the following trend: acetone (24 h) = methyl ethyl ketone (24 h) > cycloheptanone (48 h) > *c*-propyl formaldehyde (120 h) > cyclopentanone (7 d) = cyclohexanone (7 d) > cyclobutanone (8 d). The details of synthetic procedures and structural characterizations are provided in Supplementary data.



Scheme 2. Synthesis of 1-benzyloxy-1,3,5-triazines.

Table 1
Inhibition of *S. aureus*, *M. smegmatis* and bovine DHFR by test compounds and TMP

No.	R	R_1	R_2	Zone ratio[a,c] (100 μg)		DHFR[c] (IC_{50})
				S. aureus[b]	*M. smegmatis*[b]	
7a	H	CH_3	CH_3	–[d]	–	19.5[12]
7b	4′-CH_3	CH_3	CH_3	0.30	–	24.6[12]
7c	4′-OCH_3	CH_3	CH_3	–	–	58.8[12]
7d	4′-Cl	CH_3	CH_3	0.31	–	29.9[12]
7e	3′,4′-diCl	CH_3	CH_3	0.35	0.5	12.8[12]
7f	4′-F	CH_3	CH_3	–	–	21.8
7g	3′,4′,5′-triOMe	CH_3	CH_3	–	0.8	>100
7h	4′-CH_3	CH_3	C_2H_5	–	–	>100
7i	4′-OCH_3	CH_3	C_2H_5	–	–	>100
7j	4′-Cl	CH_3	C_2H_5	0.19	–	>100
7k	4′-Cl	CH_3	*c*-Pr	0.3	0.35	>100
7l	4′-CH_3	$-(CH_2)_4-$		0.15	0.17	>100
7m	4′-OCH_3	$-(CH_2)_4-$		0.18	0.53	>100
7n	4′-Cl	$-(CH_2)_4-$		0.25	0.27	>100
7o	3′,4′-diCl	$-(CH_2)_4-$		0.51	0.98	>100
7p	4′-CH_3	$-(CH_2)_5-$		0.18	0.45	>100
7q	4′-OCH_3	$-(CH_2)_5-$		0.15	0.41	>100
7r	4′-F	$-(CH_2)_5-$		0.11	0.24	>100
7s	4′-Cl	$-(CH_2)_5-$		0.3	0.67	>100
7t	3′,4′-diCl	$-(CH_2)_5-$		0.18	0.56	>100
7u	3′,4′,5′-triOMe	$-(CH_2)_5-$		–	–	>100
7v	3′,4′-diCl	$-(CH_2)_6-$		0.43	0.85	>100
7w	4′-CH_3	$-(CH_2)_6-$		0.18	0.66	>100
8a	H	CH_3	CH_3	0.51	2.5	0.069
8b	H	CH_3	C_2H_5	ND[d]	ND	0.155
8c	H	H	c-Pr	ND	ND	0.548
8d	H	$-(CH_2)_3-$		ND	ND	0.134
8e	H	$-(CH_2)_4-$		0.55	2.5	0.173[14]
8f	H	$-(CH_2)_5-$		0.74	2.5	1.79[14]
8g	H	$-(CH_2)_6-$		0.21	2.0	5.33
TMP				1.09	–	>100

[a] Zone ratio $= \frac{\text{inhibition zone of test compound}}{\text{inhibition zone of standard}}$.
[b] Standard drugs for the calculation: *S. aureus*: cephaloridine disk, *M. smegmatis*: Streptomycin disk.
[c] The results are averages of three separate experiments.
[d] ND = not determined, '–' = not active.

Compound **7a**–**7w** and **8a**–**8g** were screened at the loading of 100 μg per disc against a panel of bacteria (namely *S. aureus* ATCC 6538P, *Pseudomonas aeruginosa* ATCC 9027, *M. smegmatis* ATCC 607, *Bacillus subtilis* ATCC 6633, *Klebsiella aerogenes* ATCC 9621, *Escherichia coli* ATCC 25922) and two fungus (*Candida albicans* ATCC 10231 and *Aspergillus niger* ATCC 16404) using the paper disc diffusion method,[15] and compared with TMP tested in the same in vitro model. DHFR inhibition assay was used to determine whether the mammalian antifolate activity of target compounds was present, and bovine DHFR was used as the mammalian standard. The zone ratios which were used to express the comparative efficiency to the standard drugs and DHFR inhibitory activity are summarized in Table 1. In general, only *S. aureus* and *M. smegmatis* were sensitive to most of the tested compounds. All these compounds showed relatively weaker activity against Gram positive bacteria *S. aureus* than positive control, cephaloridine with the zone ratio <1, and none of these compounds showed superiority to TMP which demonstrated about equal potency to the cephaloridine (zone ratio = 1.09). These compounds exhibited more potent activity against *M. smegmatis* than those against *S. aureus*. N-Benzyloxy substituted compounds **8a**–**8f** were found to be the most active compounds against *M. smegmatis* in the both series with the zone ratios of 2.5. However, compounds **8a**–**8f**, bearing a N-benzyloxy side chain, proved to have potent mammalian DHFR inhibitory activity, with IC_{50} values ranged from 0.069 μM to 1.79 μM. DHFR inhibitory activity decreased as the size of the substituent at C-2 of triazine increased. Substitution at C-2 by the very bulky seven membered spiro ring as in compound **8g** led to a still fairly good DHFR inhibitory activity (IC_{50} = 5.33 μM). However, the activity still suggested an unwanted side effect liability as antimicrobial agents. Thus, the potential of **8a**–**8f** as leads for antimycobacterial agents was limited by the toxicity in the DHFR assay. Among the benzyl substituted series **7a**–**7w**, compound **7o** was the best with the zone ratio of 0.98, comparable to the positive control, streptomycin. Moreover, derivatives **7g**–**7w** and **7o** were found to be devoid of DHFR inhibitory activity (IC_{50} >100 μM). On the basis of these observation, compound **7o**, showed a promise as a lead in searching new antimycobacterial agents, for its good activity against *M. smegmatis* and it did not possess side-effect liability through mammalian DHFR inhibition. The preliminary results suggested that the bulky spiro cycloalkyl group at C-2 position was not well tolerated in their inhibitory activity against mammalian DHFR in both series, and it corroborated the study that the exact position for the drug-enzyme binding was filled with hydrophobic side-chains in the human DHFR, which provides the opportunity to design new selective antimycobacterial agent.[10]

In conclusion, a series of dihydro-1,3,5-triazines bearing 2,4-diamino-1,3-diaza pharmacophore was synthesized and screened against microbes and bovine DHFR. Compound **7o** apparently have better inhibitory activity against *M. smegmatis* compared to TMP or standard drug without mammalian DHFR inhibition liability. Herein, these results suggest that N^1-benzyl-1,3,5-triazines, based on the unique 2,4-diamino-1,3-diaza pharmacophore, may represent promising lead candidates that are worthy of further development into potential antimycobacterial agents.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.06.125.

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