Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters 14 (2004) 3161–3164
Bioorganic & Medicinal Chemistry Letters

A new 2-carbamoyl pteridine that inhibits mycobacterial FtsZ

R. C. Reynolds,* S. Srivastava, L. J. Ross, W. J. Suling and E. L. White

Drug Discovery Division, Southern Research Institute, 2000 Ninth Avenue South, Birmingham, AL 35205, USA

Received 11 December 2003; revised 6 April 2004; accepted 6 April 2004

Abstract—The preparation of a new 2-carbamoyl pteridine, its activity data against FtsZ from *M. tuberculosis* (Mtb), and in vitro antibacterial data against Mtb strain H37Ra are presented.

Resistance to many of the current antibacterial agents is now commonplace, necessitating a proactive effort to identify new targets and new pharmacophores for the development of entirely different antibacterial drugs.[1] This situation is especially true for the treatment of tuberculosis where current antitubercular agents have been on the market for 40 or more years and significant resistance has developed on a worldwide scale.[2] Single drug resistant *M. tuberculosis* (Mtb) bacilli are commonplace and multiple drug resistant forms of the disease have been reported throughout the world.[2]

New targets for tuberculosis are continually being identified from existing genomic data and new screening techniques.[3] One target that has received considerable attention in other bacteria is the bacterial tubulin homolog FtsZ.[4] This protein has the hallmark GTPase site typical of tubulin-like proteins and is considered to be the ancestral homolog of eukaryotic tubulins. Functionally, the protein differs from its distant relative. Although FtsZ is also an essential cell division protein, its primary role is in the formation of the contractile Z-ring required for bacterial septation, not separation of chromosomes during mitosis as is the case with its mammalian counterpart. While FtsZ retains only limited sequence similarity to tubulin, the three-dimensional X-ray structure shows a high degree of similarity to the structure of α and β tubulin determined by electron microscopy.[5]

Very little work has been reported relating to inhibitors of bacterial FtsZ in general.[4,6] As part of a large screening effort to identify potential antitubercular agents, we have reported screening and activity data for a class of compounds known to generally inhibit human tubulin polymerization, the 2-alkoxycarbonylaminopyridines.[6] In addition to screening archived compounds that were prepared as potential tubulin inhibitors, several compounds from our antimalarial drug design program were screened based on their structural similarity to the antitubulin compounds.[7] One antimalarial precursor, **1** (Fig. 1), was found to be a potent inhibitor of bacterial growth in vitro, and was also found to inhibit polymerization of FtsZ from Mtb in vitro.[6] The history of this compound and its established activity against the target was the basis for considering this class of compounds as our lead class for the design of selective antitubercular agents that target FtsZ polymerization in Mtb.

From a drug design perspective, this compound has advantages and disadvantages. Compound **1** generally fits within acceptable parameters for an orally active, synthetic drug based on the Lipinski rules.[8] The molecular weight of this compound is, however, slightly over 500. Hence, in terms of modification to improve target activity, this compound has a rather high starting molecular weight. As well, the calculated log *P* exceeds the acceptable range, but these compounds readily form hydrochloride salts that will alter their physical characteristics, including solubility and permeability. Currently, there are no other reported FtsZ inhibitors that closely fit these rules. Furthermore, the deazapteridine nucleus is an accepted pharmacophore for medicinal chemistry elaboration.[9] Finally, the preparation of **1** starts with a relatively expensive starting material, chelidamic acid, and requires six steps to obtain the critical intermediate in a poor overall yield (Fig. 1). The

Keywords: FtsZ; Anti-bacterial; Tuberculosis.
* Corresponding author. Tel.: +1-205-581-2454; fax: +1-205-581-2093; e-mail: reynolds@sri.org

0960-894X/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2004.04.012

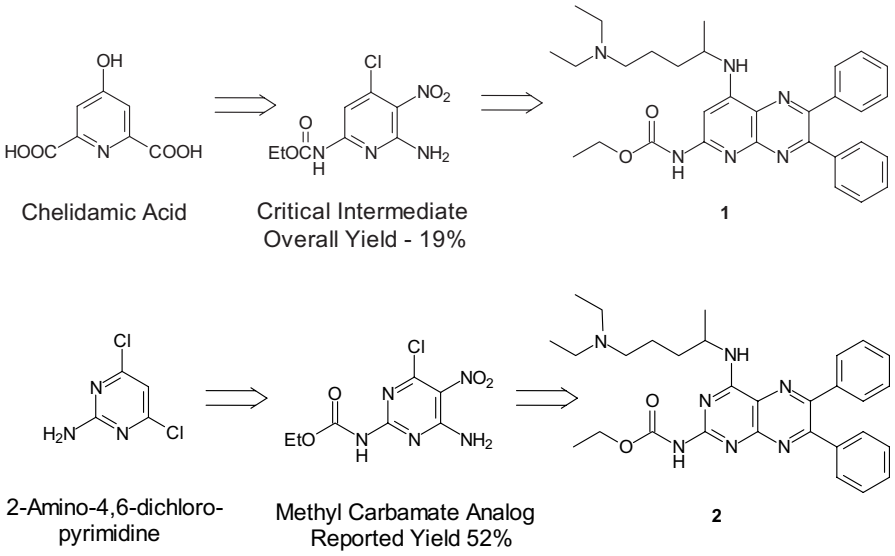


Figure 1. Abbreviated comparison of synthetic routes for **1** and the new analog, compound **2**.

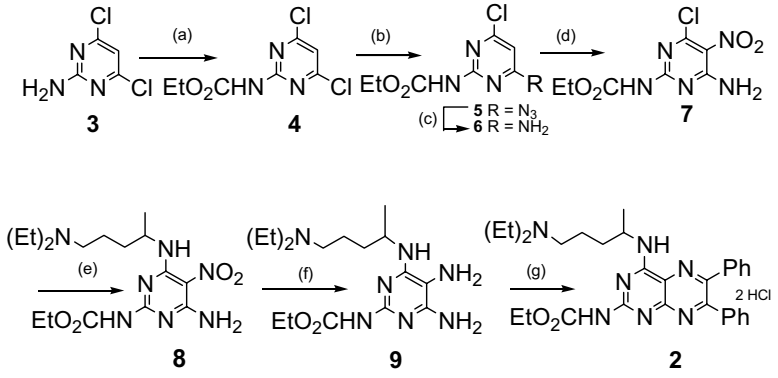
chemistry to produce analogs is both expensive and difficult; two steps have low yields with purification problems.[10]

Hence, we were interested in preparing analogs that were similar and easier to synthesize in higher overall yields using less expensive starting reagents. Our goal was to find a similar pharmacophore to the 3-deazapteridine unit in **1** that had similar activity versus the target yet was easier to synthesize, allowing the ready and inexpensive preparation of numerous analogs for structure/activity analysis.

An obvious solution was to prepare the full pteridine analog of **1** (**2**, Fig. 1). Such an analog is very similar to **1**. Synthetically, one could start with the readily available, inexpensive 2-amino-4,6-dichloropyrimidine. In fact, the methyl analog of the desired intermediate has been reported in four steps with an overall yield of 52%[11] as compared to the critical intermediate required for **1** prepared in six steps with only a 19% overall yield.[7] This intermediate would be easily elaborated into a wide variety of analogs required for a full medicinal chemistry analysis.

The new 2,4,6,7-tetrasubstituted pteridine (**2**) was synthesized as outlined in Scheme 1.[12] Commercially available starting material, 2-amino-4,6-dichloropyrimidine (**3**), was refluxed with oxalyl chloride in benzene followed by addition of ethyl alcohol to provide the 4,6-dichloro-2-pyrimidyl urethane (**4**).[13] Compound **4** was refluxed with NaN_3 in 2-propanol and water to give 4-azido pyrimidine (**5**), and subsequent hydrogenation of **5** with H_2 over Raney Nickel under atmospheric pressure gave the corresponding 4-aminopyrimidine (**6**). Nitration of **6** by the procedure of O'Brien et al.[14] provided the 5-NO_2-substituted pyrimidine (**7**). Displacement of the 4-chloro group of **7** with 2-amino-5-diethylaminopentane gave (**8**), followed by reduction of the nitro group with hydrogen over Raney Nickel under atmospheric pressure gave (**9**), and immediate condensation of the latter with benzil in ethanol by the method of Temple et al.,[15] under nitrogen atmosphere gave the desired 2,4,6,7-tetrasubstituted pteridine (**2**).

The minimum inhibitory concentration (MIC) of **2** for Mtb H37Ra was determined to be 2 μg/mL using a colorimetric (Alamar blue) microdilution broth assay.[16]



Scheme 1. (a) $(COCl)_2$, benzene/EtOH, reflux; (b) NaN_3, 2-propanol, H_2O; (c) Ra-Ni, H_2; (d) HNO_3–H_2SO_4; (e) 2-amino-5-diethylaminopentane, Et_3N; (f) Ra-Ni, H_2, EtOH; (g) Benzil, EtOH, reflux, MeOH, HCl.

Table 1[a]

Inhibition of Mtb FtsZ compound	Mtb FtsZ		Tubulin
	Polymerization ID_{50} (μM)	GTP hydrolysis	Polymerization (100 μM)
1	34.2 ± 2.5	35% at 100 μM	No inhibition
2	38.1 ± 4.1	23% at 200 μM	24%

[a] Each compound was analyzed at least three times per assay. For the FtsZ polymerization assay the means ± the standard deviations are reported.

This was eightfold greater than the MIC of 0.25 μg/mL reported previously[6] for the deaza analog **1**.

Compound **2** was also examined for its ability to inhibit the target, Mtb FtsZ, and its homolog tubulin (Table 1). Methods for the assays have been described in detail previously.[6] The new compound is fully as potent in inhibiting the polymerization of FtsZ as the 3-deazapteridine **1**. Why the compound is less potent in inhibiting GTP hydrolysis is not yet clear. Although GTP hydrolysis and polymerization are linked, the details of their kinetic coupling have not been determined.

In closing, we have reported an efficient synthesis of an analog (**2**) of the only reported synthetic compound (**1**) shown to effectively inhibit FtsZ polymerization. This analog is as potent an inhibitor of FtsZ polymerization as the original lead compound suggesting that the full pteridine nucleus is an acceptable substitution. Interestingly, this analog appears to be less effective than our lead compound (**1**) against whole Mtb in vitro. One possibility is that this compound does not permeate the bacterial cell wall as effectively as the lead agent. For our purposes, however, the increased efficiency of preparing these types of analogs suggests that our primary SAR versus FtsZ polymerization should proceed in the full pteridine series. Once active, improved analogs/substitutions are identified, it would be prudent to make the 3-deaza analogs for a comparison of in vitro antibacterial activity.

Acknowledgements

This work was supported by a grant from the National Institutes of Health 1R01AI50470.

References and notes

1. Projan, S. J. *Curr. Opin. Microbiol.* **2003**, *6*, 427–430.
2. Butler, D. *Nature* **2000**, *406*, 670–672.
3. Kremer, L.; Besra, G. S. *Expert Opin. Invest. Drugs* **2002**, *11*, 1033–1049.
4. Wang, J.; Galgoci, A.; Kodali, S.; Herath, K. B.; Jayasuriya, H.; Dorso, K.; Vicente, F.; Gonzalez, A.; Cully, D.; Bramhill, D.; Singh, S. *J. Biol. Chem.* **2003**, *278*, 44424–44428.
5. Nogales, E.; Wolf, S. G.; Downing, K. H. *Nature* **1998**, *391*, 199–203.
6. White, E. L.; Suling, W. J.; Ross, L. J.; Seitz, L. E.; Reynolds, R. C. *J. Antimicrob. Chemother.* **2002**, *50*, 111–114.
7. Temple, C.; Rose, J. D.; Elliot, R. D.; Montgomery, J. A. *J. Med. Chem.* **1968**, *11*, 1216–1218.
8. Lipinski, C. A. *J. Pharmacol. Toxicol. Meth.* **2000**, *44*, 235–249.
9. Werbel, L. M. In *Folate Antagonists as Therapeutic Agents. 1. Biochemistry, Molecular Actions, and Synthetic Design*; Sirotnak, F. M.; Burchall, J. J.; Ensminger, W. D.; Montgomery, J. A., Eds.; Academic: New York, 1984; pp 261–287.
10. Elliot, R. D.; Temple, C.; Montgomery, J. A. *J. Org. Chem.* **1966**, *31*, 1890–1894.
11. Temple, C.; Renner, G. A. *J. Med. Chem.* **1992**, *35*, 4809–4812.
12. *N-Substituted 4,6-dichloro-2-pyrimidinyl carbamic acid ethyl ester* **4**: A solution of 13.8 mL of oxalyl chloride in 100 mL of dry C_6H_6 was added dropwise with stirring to a solution of 5 g (30 mmol) of 2-amino-4,6-dichloropyrimidine (**3**) in 700 mL of dry C_6H_6, and the mixture was refluxed for 3 h under argon until hydrogen chloride and carbon monoxide evolution ceased, then cooled, and the clear solution evaporated to a semisolid. The semisolid was dissolved in benzene (450 mL), a solution of absolute ethanol (1.24 mL) in 180 mL of dry benzene was added, and the mixture was allowed to stand overnight. The solvent was removed under vacuum and the residue was stirred 5–10 min with boiling petroleum ether and evaporated to dryness. The insoluble oil was purified through column chromatography by using 0.5% MeOH:$CHCl_3$. Fractions containing compound were concentrated under vacuum to give white solid; yield 4.4 g (61.4%); mp 117–119 °C; TLC (5% MeOH:$CHCl_3$) $R_f = 0.73$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.78 (br s, 1H, NH), 7.06 (s, 1H), 4.29 (q, 2H), 1.33 (t, 3H, $J = 7.2$ Hz); MS m/z $(M+H)^+$ 236, $(M+Na)^+$ 258; HRMS calcd for $C_7H_7Cl_2N_3O_2(M+Na)^+$ 257.9807, found 257.9813.
Ethyl[4-azido-6-chloropyrimidin-2-yl]carbamate (**5**): To a suspension of *N*-ethyl carbamate of 2-amino-4,6-dichloropyrimidine (**4**) (0.27 g, 1 mmol) in 2-propanol (15 mL) was added a solution of NaN_3 (0.071 g, 1 mmol) in water (0.8 mL), and the mixture was heated at reflux for 1.5 h and cooled to room temperature. The mixture was collected, dried over anhydrous sodium sulfate, filtered, and concentrated at reduced pressure. The obtained residue was purified by column chromatography using 0.5% MeOH in $CHCl_3$. Fractions containing compound were concentrated under vacuum to give a solid; yield 150 mg (60%); mp 127–130 °C; TLC (5% MeOH:$CHCl_3$) $R_f = 0.75$; ^{1}H NMR (300 MHz,$CDCl_3$) δ 6.49 (s, 1H), 4.28 (q, 2H, OCH_2), 1.33 (t, 3H, $J = 6.9$ Hz, Me); MS m/z $(M+H)^+$ 243, $(M+Na)^+$ 265; HRMS calcd for $C_7H_7ClN_6O_2$ $(M+Na)^+$ 265.0211, found 265.0208.
Ethyl[4-amino-6-chloropyrimidin-2-yl] carbamate (**6**): A partial solution of ethyl[4-azido-6-chloropyrimidin-2-yl] carbamate (**5**) (2.1 g, 8.6 mmol) in EtOH (250 mL) was hydrogenated for 3 h at room temperature and atmospheric pressure in the presence of Ra-Ni (2.1 g, washed three times with water and two times with EtOH) to give a clear supernatant. The catalyst was removed by filtration (celite), and the filtrate was evaporated to dryness at

reduced pressure to afford the compound; yield 1.8 g (96%); mp 156–158 °C; TLC (5% MeOH:$CHCl_3$) $R_f = 0.4$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.92 (s, 1H), 6.16 (s, 1H), 5.50 (s, 2H), 4.24 (q, 2H, OCH_2), 1.30 (t, 3H, $J = 6.9$ Hz, Me); MS m/z $(M+H)^+$ 217, $(M+Na)^+$ 239; HRMS calcd for $C_7H_9ClN_4O_2$ $(M+Na)^+$ 239.0306, found 239.0308.

Ethyl[4-amino-6-chloro-5-nitropyrimidin-2-yl] carbamate (**7**): To a solution of fuming HNO_3 (1.2 mL, $d = 1.49$) in concd H_2SO_4 (5.6 mL) was added the ethyl[4-amino-6-chloropyrimidin-2-yl] carbamate (**6**) (1.66 g, 7.6 mmol) portionwise over 0.5 h, while maintaining the temperature at 30–35 °C. The resulting solution was stirred for 1 h at 30–35 °C and added dropwise to crushed ice. The pale yellow precipitate was collected by filtration, resuspended in H_2O, and neutralized (at 0–5 °C) to pH 8 with NH_4OH. The precipitate of crude was collected byfiltration, washed with water (2×10 mL) and purified on a short flash column (EtOAc) to afford the nitrated product; yield 0.42 g (21%); TLC (5% MeOH:$CHCl_3$) $R_f = 0.6$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.27 (q, 2H, OCH_2), 1.33 (t, 3H, $J = 6.9$ Hz, Me); MS m/z $(M+Na)^+$ 284; HRMS calcd for $C_7H_8ClN_5O_4$ $(M+Na)^+$ 284.0157, found 284.0145.

Ethyl-{4-Amino-6-[[4-(diethylamino)-1-methylbutyl]amino]-5-nitropyrim-idin-2-yl}carbamate (**8**): A hot solution of ethyl [4-amino-6-chloro-5-nitropyrimidin-2-yl] carbamate (**7**) (0.1 g, 0.38 mmol), 2-amino-5-diethylamino-pentane (0.09 mL, 0.45 mmol), and triethylamine (0.05 mL) in THF (2 mL) was refluxed for 3 h, cooled to room temperature, and evaporated to dryness at reduced pressure, purified on a short silica gel column using 5% MeOH:$CHCl_3$:1% NH_4OH as eluent. The fractions containing compound were concentrated in vacuo; yield 0.06 g (41%); TLC (10% MeOH:$CHCl_3$:1% NH_4OH) $R_f = 0.5$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.42 (m, 1H), 4.24 (q, 2H), 2.77–2.67 (m, 6H, 3×CH_2), 1.66–1.60 (m, 4H, 2×CH_2), 1.34–1.27 (m, 6H, 2×CH_3), 1.14 (t, 6H, $J = 7.2$ Hz); MS m/z $(M+Na)^+$ 383; HRMS calcd for $C_{16}H_{29}N_7O_4$ $(M+H)^+$ 384.2353 found 384.2361.

2-Carbamic acid, 4-[[4-(diethylamino)-1-methyl-butyl]-amino-6,7-diphenyl-ethyl ester, pteridine dihydrochloride (**2**): A solution of ethyl-{4-amino-6-[[4-(diethylamino)-1-methylbutyl]amino]-5-nitropyrimidin-2-yl}carbamate (**8**) in EtOH (15 mL) was hydrogenated at room temperature and 1 atm pressure in the presence of Ra-Ni (0.4 g, weighed wet, washed 3×H_2O and 2×EtOH) for 8 h, and TLC was checked. On completion of the reaction, the catalyst solution was filtered through celite, and the filtrate was evaporated to dryness yielding a quantitative amount of diamine (**9**) as a yellow oil. To the stirred solution of the diamine (**9**) (0.276 g, 0.78 mmol) in EtOH (15 mL), benzil (0.164 g, 0.78 mmol) was added. The clear, yellow solution was stirred at room temperature for 24 h then refluxed for 12 h under N_2 atmosphere. The solvent was removed in vacuo, leaving a resinuous mass, which was purified with a short silica gel column using 2% MeOH:$CHCl_3$:1% NH_4OH as eluent. The fractions containing compound were concentrated under vacuum. HCl in diethylether was added dropwise in the stirred solution of compound in dry methanol (5 mL). The solvent was concentrated in vacuo and the solid that was obtained was dried under high vacuum (P_2O_5, 6 h, 78 °C); yield 0.3 g (74.6%); mp 165–170 °C; TLC (10% MeOH:$CHCl_3$:1% NH_4OH) $R_f = 0.6$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.52–7.28 (m, 1H), 4.60–4.48 (m, 1H), 4.31–4.24 (q, 1H), 2.75–2.55 (br s, 6H, NCH_2), 1.69–1.66 (m, 4H, CH_2), 1.38–1.32 (m, 6H, CH_3), 1.10 (m, 6H, CH_3); MS m/z $(M+H)^+$ 528; Anal. Calcd for $C_{30}H_{37}N_7O_2{\cdot}2HCl{\cdot}2H_2O$: C, 56.60; H, 6.80; N, 15.40. Found: C, 56.74; H, 6.77; N, 15.40.

13. Boldyrev, I. V.; Valdimirtsev, I. F.; Romanenko, E. A.; Korzhenevskaya, N. G.; Titov, E. V.; Cherkasov, V. M. *Chem. Heterocycl. Compd. USSR* **1977**, 1006–1009.
14. O'Brien, D. E.; Cheng, C. C.; Pfleiderer, W. *J. Med. Chem.* **1966**, *9*, 573–575.
15. Temple, C.; Laster, G. A.; Rose, D. J.; Montgomery, A. J. *J. Heterocycl. Chem.* **1970**, *7*, 1195–1202.
16. Suling, W. J.; Seitz, L. E.; Pathak, V.; Westbrook, L.; Barrow, E. W.; Zywno-Van-Ginkel, S.; Reynolds, R. C.; Piper, J. R.; Barrow, W. W. *Antimicrob. Agents Chemother.* **2000**, *44*, 2784–2793.