Bioorganic & Medicinal Chemistry Letters 23 (2013) 1279–1284

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Structural analysis of the active sites of dihydrofolate reductase from two species of *Candida* uncovers ligand-induced conformational changes shared among species

Janet L. Paulsen, Kishore Viswanathan, Dennis L. Wright *, Amy C. Anderson *

Department of Pharmaceutical Sciences, University of Connecticut, 69 N. Eagleville Rd., Storrs, CT 06269, USA

ARTICLE INFO

Article history:
Received 4 October 2012
Revised 20 December 2012
Accepted 2 January 2013
Available online 11 January 2013

Keywords:
Antifolate
DHFR
Protein flexibility
X-ray crystallography
Molecular dynamics

ABSTRACT

A novel strategy for targeting the pathogenic organisms *Candida albicans* and *Candida glabrata* focuses on the development of potent and selective antifolates effective against dihydrofolate reductase. Crystal structure analysis suggested that an essential loop at the active site (Thr 58-Phe 66) differs from the analogous residues in the human enzyme, potentially providing a mechanism for achieving selectivity. In order to probe the role of this loop, we employed chemical synthesis, crystal structure determination and molecular dynamics simulations. The results of these analyses show that the loop residues undergo ligand-induced conformational changes that are similar among the fungal and human species.

The severe health risks presented by systemic fungal infections continue to produce significant increases in mortality and morbidity. *Candida* spp. are primary pathogenic organisms in systemic infections with *Candida albicans* accounting for approximately 50–70% of infections.[1,2] However, *Candida glabrata*, associated with a high rate of mortality, now accounts for an increasing proportion of infections.[3,4] Additionally, *C. glabrata* is less sensitive to commonly used antifungal agents such as fluconazole and other azoles.[1,5] The development of new classes of antifungal agents with good potency against these *Candida* species is a high priority.

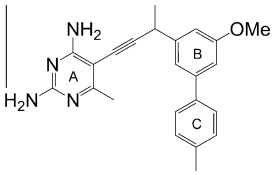
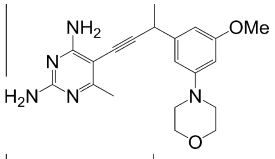
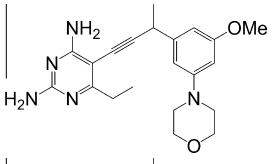
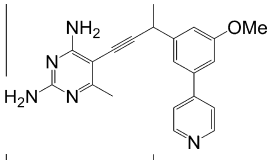
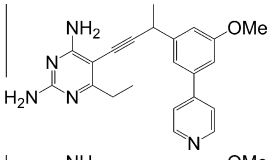
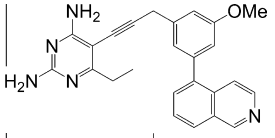
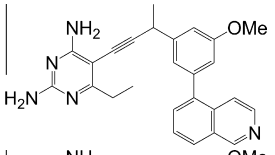
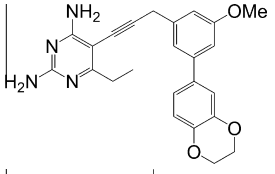
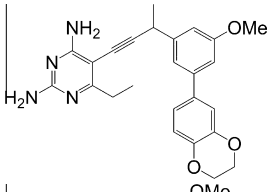
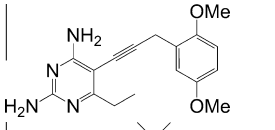
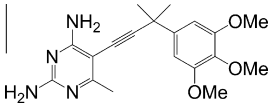
The vast majority of the current antifungal agents target the fungal cell wall or its biosynthesis, leaving many of the essential metabolic functions unexplored as therapeutic targets. One such essential enzyme, dihydrofolate reductase (DHFR), has long been appreciated as an effective target for antimicrobial therapy. As DHFR is also essential to human cells, an effective antifungal antifolate design must take advantage of differences in the pathogenic enzyme relative to the human enzyme. Over the past several years we have focused on the development of a novel class of antifolates characterized by a conserved diaminopyrimidine moiety linked through a propargylic spacer to a variable hydrophobic domain.[6–11] Leads such as compound **1** (shown in Table 1) have been shown to function as inhibitors of *C. glabrata* (CgDHFR) and *C. albicans* DHFR (CaDHFR) as well as exhibit antifungal activity (MIC values of 1.5 μg/mL) and thus are interesting as potential antifungal therapeutics.[7,8,11] We have reported several crystal structures of CgDHFR and CaDHFR bound to NADPH and several members of this lead series.[7,8,11] Analysis of these crystal structures has strongly suggested that residues in a loop at the fungal active site (Thr 58-Phe 66) may be displaced further from the active site relative to the analogous residues in the human structure (Thr 56-Asn 64), a property that may be advantageous for gaining selectivity in inhibitor design.

In order to investigate whether this critical loop truly plays an active role in determining the affinity of the propargyl-linked antifolates for the fungal and human enzymes, here we present an analysis of existing crystal structures of CgDHFR and CaDHFR bound to the propargyl-linked antifolates as well as four new ternary crystal structures of CgDHFR and CaDHFR bound to a recent generation of inhibitors possessing a heterocyclic moiety intended to improve solubility.[12] We then designed and synthesized four additional propargyl-linked antifolates with a bulkier, fused ring system intended to especially probe the flexibility of the loop. Crystal structures of CgDHFR and CaDHFR with one of these compounds emphasize the ligand-induced conformational changes observed with these more sterically demanding inhibitors. Finally, we carried out a detailed molecular dynamics study that focused on the loop regions of both the fungal and human enzymes. These MD results show that this loop in the active site is particularly flexible and while the loop undergoes ligand-induced conformational changes, these changes are similar across the three species.

* Corresponding authors. Tel.: +1 860 486 6145/9451; fax: +1 860 486 6857.
E-mail addresses: dennis.wright@uconn.edu (D.L. Wright), amy.anderson@uconn.edu (A.C. Anderson).

0960-894X/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmcl.2013.01.008

Table 1
Enzyme inhibition of CaDHFR, CgDHFR and human DHFR with propargyl-linked antifolates

ID	Structure	CaDHFR IC_{50} (nM) (selectivity)	CgDHFR IC_{50} (nM) (selectivity)	huDHFR IC_{50} (nM)
1		22 ± 1 (64)	0.60 ± 0.27 (2350)	1410 ± 15
2		21 ± 4 (19)	20 ± 3 (20)	400 ± 40
3		23 ± 4 (11)	22 ± 2 (11)	250 ± 4
4		61 ± 3 (25)	97 ± 9 (15)	1500 ± 80
5		60 ± 2 (22)	89 ± 8 (15)	1300 ± 10
12		71 ± 20 (0.84)	16 ± 1 (3.8)	60 ± 6
13		17 ± 5 (2.6)	11 ± 1 (4.1)	45 ± 3
18		78 ± 2 (1.8)	19 ± 4 (7.4)	140 ± 9
19		23 ± 2 (11)	18 ± 3 (14)	260 ± 20
20		100 ± 7 (13)	8.2 ± 1.3 (156)	1280 ± 15
21		33 ± 14 (9)	11 ± 2 (26)	290 ± 17

Over the past several years, we have reported eight CgDHFR and three CaDHFR crystal structures bound to NADPH and antifolates possessing a pyrimidinyl ring linked through the propargyl bridge to substituted monophenyl or biphenyl systems.[7,8,11] Analysis of these structures has been critical to improving the potency and selectivity of the inhibitors for the fungal enzymes. While there