BIOINFORMATION
Discovery at the interface of physical and biological sciences

open access www.bioinformation.net Views & Challenges

Volume 9(13)

Sitagliptin does not inhibit the M1 alanyl aminopeptidase from *Plasmodium falciparum*

Sheena McGowan

Department of Biochemistry and Molecular Biology, Monash University, Clayton Campus, Australia; Email: sheena.mcgowan@monash.edu

Received May 31, 2013; Accepted June 01, 2013; Published July 17, 2013

The M1 alanyl aminopeptidase from *Plasmodium falciparum* has been shown to be an essential hemoglobinase enzyme, catalyzing the final stages of hemoglobin break-down within intra-erythrocytic parasites [1]. Recently there has been much interest in this protease as a potential drug target for the development of novel antimalarials **[1-13]**. In a recent report, Krishnamoorthy and Achary propose that Sitagliptin may serve as a potent competitive inhibitor of the M1 alanyl aminopeptidase enzyme from *Plasmodium falciparum* **[14]**. The molecule of interest, the M1 alanine aminopeptidase or *Pf*A-M1, has been well studied and characterized by our group and others (For examples of recent papers, see **[1-13]**). To date, multiple published X-ray crystal structures and models are available of inhibitors bound to the active site of *Pf*A-M1. Krishnamoorthy and Achary report a docking analysis of the enzyme with "about 100 low molecular weight protease inhibitors …". The docking results were validated by inclusion of specific substrate (Ala-β-naphthylamide) from which they calculated an *in silico* K_m value that was closely correlated with experimental data. Unfortunately, this correlation with experimental data was not observed with the selected positive inhibitor control, Bestatin. The interaction / inhibition of *Pf*A-M1 by Bestatin has been reported previously **[1, 9, 12, 15]** and the dipeptide analog has an *in vitro* K_i in the nM range for *Pf*A-M1. The *in silico* value calculated was ~ 100 µM, indicating that the parameters defined for docking were likely inadequate for the cation-occupied active site. The article states that Sitagliptin is the most potent *in silico* inhibitor with a $K_{i(avg)}$ of 2 uM (however only 8/100 docking results were provided in Supplementary Table 1). We have completed an *in vitro* analysis of the effect of Sitagliptin on the activity of both *Pf*A-M1 and the second neutral aminopeptidase, *Pf*A-M17. Aminopeptidase activity assays were carried out in 200 µl total volume in 50 mM Tris-HCl pH 8.0, at 37 ºC (with the addition of 2 mM $CoCl_2$ for *Pf*A-M17). Following a 10 min incubation of enzyme and Sitagliptin (0 – 0.5 mM), reactions were initiated by addition of fluorigenic substrate (L-Leucine-7-amido-4-methylcoumarin). Progress curves were monitored using a spectrofluorimeter until a final steady-state velocity was reached. We determined the inhibitory kinetics via K_i values from Dixon plots of $1/v_s$ versus inhibitor concentration when $[S] << K_M$. We report here that this compound had no effect on the aminopeptidase activity of either enzyme. *In silico* drug docking suffers from several problems, including modeling the physics of the system, solvent effects, dynamics, and the difficulty in accurately ranking the docked results, and therefore relies critically on validation by experiment. It is vital that we rigorously test hypotheses generated from webservers to ensure that these algorithms continue to improve in their accuracy and hence usefulness. Blanket statements about efficacy from untested *in silico* studies will confound the literature and waste precious resources by following up on false positives identified in poorly controlled *in silico* studies. Computational biology has an important role to play in research and drug design; however, it is absolutely vital that we apply critical evaluation of results obtained to ensure that it becomes a robust method in the future.

References:

[1] Harbut MB *et al. Proc Natl Acad Sci U S A*. 2011 **108**: 526 [PMID: 21844374]
[2] Azimzadeh O *et al. Malar J*. 2010 **9:** 189 [PMID: 20591164]
[3] Dalal S & Klemba M, *J Biol Chem*. 2007 **282**: 35978 [PMID: 17895246]
[4] Dalal S *et al. Mol Biochem Parasitol*. 2012 **183**: 70 [PMID: 22348949]
[5] Deprez-Poulain R *et al. J Med Chem*. 2012 **55**: 10909 [PMID: 23176597]

[6] Flipo M *et al. J Med Chem*. 2007 **50**: 1322 [PMID: 17326615]
[7] Gardiner DL *et al.* In Proteinases as Drug Targets, Dunn B (Ed.) Royal Society Chemistry: Oxford, 2012 **1**: 169
[8] McGowan S *et al. Proc Natl Acad Sci U S A*. 2010 **107**: 2449 [PMID: 20133789]
[9] McGowan S *et al. Proc Natl Acad Sci U S A*. 2009 **106**: 2537 [PMID: 20133789]
[10] Skinner-Adams TS *et al. Antimicrob Agents Chemother*. 2012 **56**: 3244 [PMID: 22450967]
[11] Skinner-Adams TS *et al. Trends Biochem Sci*. 2010 **35**: 53 [PMID: 19796954]
[12] Velmourougane G *et al. J Med Chem*. 2011 **54:** 1655 [PMID: 21366301]
[13] Kannan Sivaraman K *et al. J Med Chem*. 2013 epub
[14] Krishnamoorthy M & Achary A, *Bioinformation*. 2013 **9**: 293 [PMID: 23559748]
[15] Harbut MB *et al. Bioorg Med Chem Lett*. 2008 **18**: 5932 [PMID**:** 18823778]

Edited by P Kangueane
Citation: McGowan, Bioinformation 9(13): 661-662 (2013)