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Rapid Communication

Inhibitors of *Escherichia coli* serine acetyltransferase block proliferation of *Entamoeba histolytica* trophozoites

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Abstract

The protozoan parasite *Entamoeba histolytica* is the etiologic agent of amebiasis, a major global public health problem, particularly in developing countries. There is an effective anti-amoebic drug available, however its long term use produces undesirable side effects. As *E. histolytica* is a micro-aerophilic organism, it is sensitive to high levels of oxygen and the enzymes that are involved in protecting against oxygen-stress are crucial for its survival. Therefore serine acetyltransferase, an enzyme involved in cysteine biosynthesis, was used as a target for identifying potential inhibitors. Virtual screening with *Escherichia coli* serine acetyltransferase was carried out against the National Cancer Institute chemical database utilizing molecular docking tools such as GOLD and FlexX. The initial analysis yielded 11 molecules of which three compounds were procured and tested for biological activity. The results showed that these compounds partially block activity of the *E. coli* enzyme and the growth of *E. histolytica* trophozoites but not mammalian cells.

Keywords: *Entamoeba histolytica*; Serine acetyltransferase; GOLD; FlexX; NCI; Amoebiasis

Amoebiasis caused by *Entamoeba histolytica*, an early branching protozoan parasite, remains one of the major public health problems in the developing world. There are an estimated 50 million infected people world-wide, leading to 50,000 to 100,000 deaths annually (Stanley, 2003). Nitroimidazoles are the drugs of choice for treatment of infections caused by a number of anaerobic or micro-aerophilic microorganisms including *E. histolytica* (Freeman et al., 1997). However, some of these have been shown to be mutagenic in bacteria, carcinogenic in rodents (Rustia and Shubik, 1972; Shubik, 1972; Legator et al., 1975) and are not well tolerated by many humans. Therefore there is a need for identifying new anti-amebic agents (Ali and Nozaki, 2007).

The biosynthetic pathway of the sulphur-containing amino acid cysteine has been demonstrated to be essential for growth and various cellular activities of *E. histolytica* (Diamond et al., 1978; Gillin and Diamond, 1980b). Apart from being used as a precursor for protein synthesis, cysteine may compensate for the lack of glutathione, a major component of oxidative stress resistance in many organisms (Fahey et al., 1984; Loftus et al., 2005). It is also needed in the attachment to matrix, elongation and mobility of *E. histolytica* cells (Gillin and Diamond, 1980a, 1981). Therefore, a possible strategy for inhibiting *E. histolytica* growth could be to block cysteine biosynthesis (Ali and Nozaki, 2007). Serine acetyltransferase (SATase), an enzyme in the cysteine biosynthetic pathway, has been demonstrated to play a central role in controlling the intracellular cysteine concentration in *E. histolytica* (Nozaki

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et al., 1999) and is therefore a potential target for the design of new inhibitors.

In humans, cysteine is produced solely from methionine and serine which are obtained from food using the reverse *trans*-sulphuration pathway through *S*-adenosylmethionine, homocysteine and cystathionine as intermediates. On the other hand, plants and bacteria are capable of incorporating inorganic sulphur into organic compounds through a sulphur assimilatory cysteine biosynthetic pathway. Thus animals that produce cysteine via a reverse *trans*-sulphuration pathway lack a sulphur assimilatory pathway (Nozaki et al., 1999). *Entamoeba histolytica* is similar to plants and bacteria as cysteine is synthesised in this organism from inorganic sulphur. Since SATase is one of the key enzymes for the biosynthesis of cysteine in *E. histolytica* but not in humans, it may serve as a potential novel target for developing new chemotherapeutic agents. The present report describes results of in silico screening of a chemical compound library against *Escherichia coli* SATase and preliminary data of some of the selected compounds on the proliferation of *E. histolytica* cells.

SATase, an enzyme which catalyses the acetylation of L-serine to *o*-acetyl-L-serine exists as a dimer of trimers (Hindson et al., 2000). *Escherichia coli* SATase (PDB id: 1T3D) and the *E. histolytica* (Accession No. BAA82868) homolog share 49% similarity at the amino acid level. The active site of *E. coli* SATase is located between the interface of the two subunits (termed A and B) in each trimer. The target binding site was derived from the crystal structure of the complex involving the two subunits and cysteine. The amino acids residues 92–98 and 157–160 of chain A and 184–193 of chain B defined the active site (Pye et al., 2004). The National Cancer Institute (NCI) database (Shi et al., 2000; Voigt et al., 2001) containing organic and inorganic structures was downloaded from the website http://cactus.nci.nih.gov/ncidb2/download.html in three-dimensional (3D) SDF-file format and then converted to mol2 format using the program Babel (http://sourceforge.net/projects/openbabel/). The NCI database was searched by using GOLD (Jones et al., 1997) and FlexX (Rarey et al., 1996) programs. The molecules were ranked according to their scores, calculated by using the energy function in the respective programs. The top 500 candidate molecules with the best scores from both programs were considered as possible SATase interacting molecules for further study. Since docking programs are likely to generate a number of false positives (Schneider and Bohm, 2002), a list of 11 molecules, common among the top 500 ranked compounds according to scoring functions of both GOLD and FlexX programs, were selected for biological testing (Table 1). Not all compounds in the NCI library are freely available and therefore the three compounds obtained were subjected to further studies (C1-3, Fig. 1).

Escherichia coli SATase was partially purified following a modified procedure reported earlier, before assaying for the activity (Kredich and Tomkins, 1966). Briefly, *E. coli* DH5α cells were grown in Luria Broth medium and then collected by centrifugation. The pellet was suspended in 0.01 M Tris–Cl, pH 7.5. The cells were sonically disrupted and debris together with unbroken cells were removed by centrifugation. The supernatant containing the crude extract was subjected to streptomycin sulphate (10%) and ammonium sulphate (70%) precipitation. The precipitated protein was dissolved in 0.01 mM Tris–Cl, pH 7.5, and desalted on a G-25 column (12 × 0.8 cm) pre-equilibrated with extraction buffer. The eluted protein was used in the

Table 1
Eleven compounds selected via screening of National Cancer Institute chemical library using molecular docking tools Gold and FlexX

No.	Compound ID	Gold score	FlexX score
1	NCI 128884[a]	49.91	−22.63
2	NCI 29607[a]	51.28	−21.90
3	NCI 653543[a]	58.09	−23.66
4	NCI 201774	57.71	−21.97
5	NCI 307161	51.22	−22.63
6	NCI 320958	52.16	−24.34
7	NCI 516772	56.90	−23.51
8	NCI 623721	58.23	−23.45
9	NCI 649118	51.75	−25.14
10	NCI 652915	50.53	−21.89
11	NCI 675993	50.12	−21.89
12	Cysteine	21.46	−19.36

[a] The three compounds procured for bioassay.

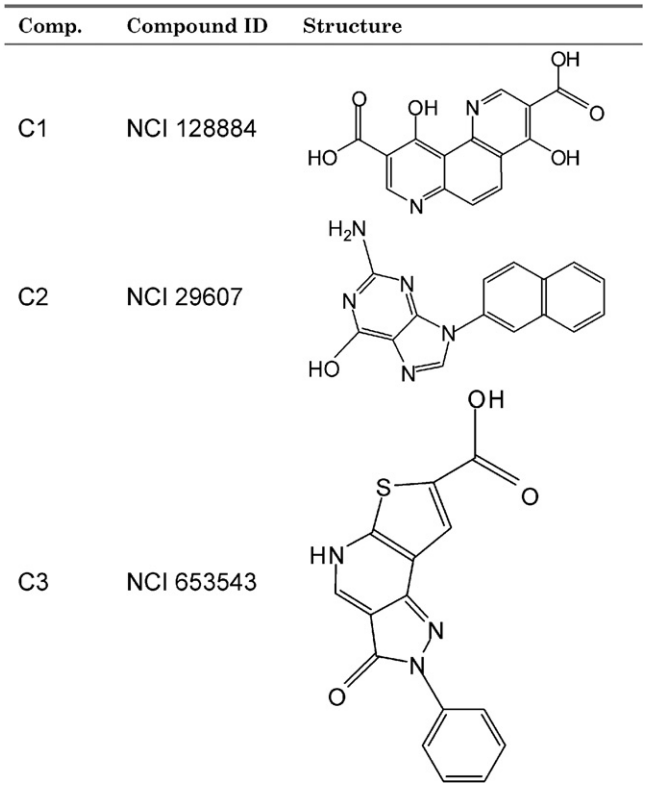

Fig. 1. Chemical structure of the three putative inhibitors of serine acetyltransferase identified by virtual screening and used for biological assays.