Computational studies on NBDHEX as *Giardia duodenalis* thioredoxin reductase (*g*TrxR) inhibitor

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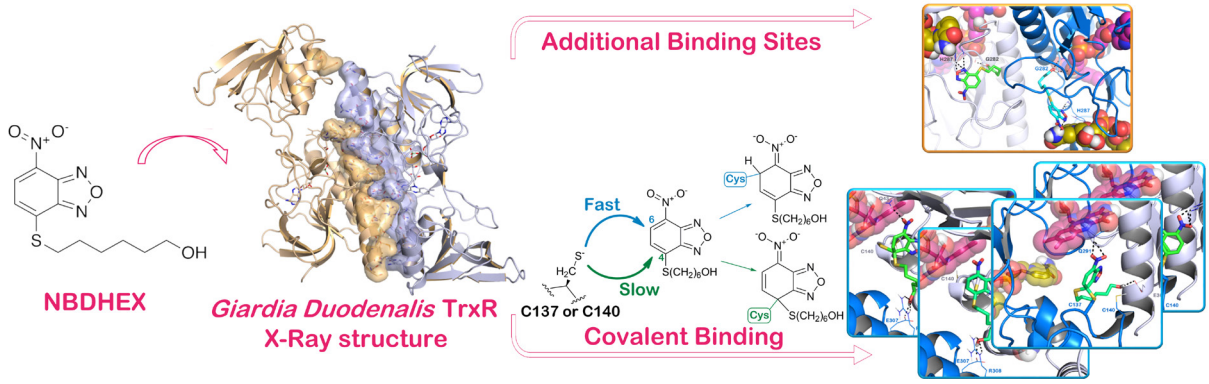
Giardia duodenalis is a microaerophilic parasite that colonizes the upper fractions of the humans' intestine. *Giardia* infection is a major responsible to diarrheal disease worldwide. [1] Nitroheterocycles (e.g. metronidazole) or benzimidazoles (e.g. albendazole) are the most commonly used therapeutic agents. Unfortunately, low compliance or resistance phenomena reduce their efficacy. We discovered that the antitumoral drug 6-(7-nitro-2,1,3-benzoxadiazol-4-ylthio)hexanol (NBDHEX) is active against *G. duodenalis* trophozoites. Administration of NBDHEX to parasite cells and *in vitro* led to the formation of covalent adducts with catalytic cysteines (C137 and C140) of thioredoxin reductase (*g*TrxR), a key component of *Giardia* redox system. Moreover, NBDHEX is modified by *g*TrxR *in vitro,* by nitroreduction. *g*TrxR provides to the parasite efficient defenses against reactive oxygen species (ROS) and it is a target of 5-nitroimidazole drugs contributing to their metabolism. However, the exact mechanism involved in the *g*TrxR inhibition mediated by these compounds is yet to be defined. The definition of the structural determinants of activity against gTrxR could be important for the identification of novel drugs endowed with an innovative mode of action. With this aim, the crystal structure of *g*TrxR was solved and the potential mechanism of inhibition of NBDHEX was analyzed *in silico*.



The crystallographic structures were employed in a comprehensive *in silico* analysis to gain insight into the mechanism governing the inhibitory activity of NBDHEX. Covalent docking was performed in Maestro suite 2015 adopting the Covalent Docking protocol (CovDock). [2] The binding mode of covalently bound NBDHEX was analyzed and the reaction SMARTS pattern was customized to obtain a reliable reaction for NBDHEX. Moreover, by means of Sitemap (SiteMap, version 3.4, Schrödinger, LLC, Release 2015), we identified an accessory binding site, supported by previous biochemical studies. Except for metronidazole, none of the drugs currently used against *Giardia* has *g*TrxR as the primary target. In this scenario, NBDHEX is an extremely interesting compound, being activated by *g*TrxR and, at the same time, inhibiting the enzyme itself. Our study paves the way for the rational design of optimized ligands with improved efficacy against *Giardia* infection and sharing the same mechanism of NBDHEX.

[1] U. Ryan, S.M. Cacciò. Zoonotic potential of Giardia, *Int J Parasitol*, **2013**, *43*, 943-956.
[2] Zhu, K.; *et al*. Docking covalent inhibitors: A parameter free approach to pose prediction and scoring, *J Chem Inf Model*, **2014**, *54*, 1932−1940