# PHENOTYPIC HITS IN ANTI-*TRYPANOSOMA* DRUG DISCOVERY

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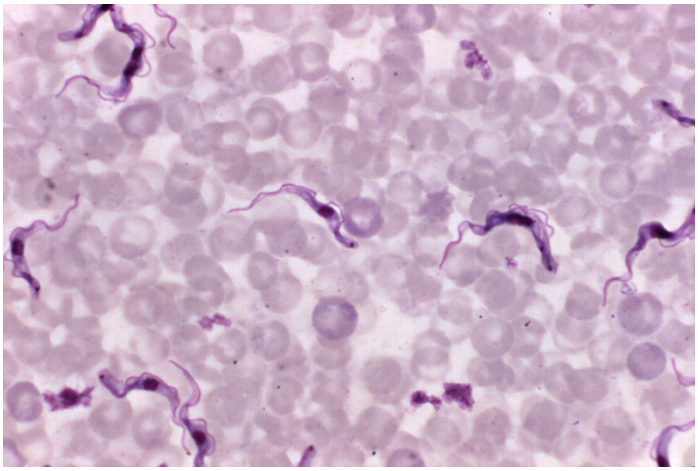

*Phenotypic screening is a powerful approach in neglected tropical disease drug discovery. Hence, we tested a focused natural naphthoquinone-inspired library against trypanosomatid cells, providing a promising hit compound with a multitarget mechanism of action*

***Hit* fenotipici nella ricerca di nuovi farmaci anti-*Trypanosoma***
L'approccio fenotipico ha grandi potenzialità nella ricerca di nuovi farmaci per le malattie tropicali dimenticate. In quest'ottica, una piccola libreria di naftochinoni derivati da prodotti naturali è stata sintetizzata e testata su tripanosomi interi, conducendo all'identificazione di un *hit* con profilo d'azione *multitarget*.

At the beginning of the third millennium neglected tropical diseases (NTDs) still represent a significant health burden worldwide, especially in the poorest and rural regions of the globe. In particular, maladies caused by trypanosomatid parasites are among the NTDs with the highest rates of death and are indicated by the World Health Organization (WHO) in the number of the most challenging ones. Specifically, *Trypanosoma cruzi* and *Trypanosoma brucei* parasites are responsible for highly infective and lethal maladies, such as Chagas' disease in Latin America and sleeping sickness in sub-Saharan Africa, respectively[1,2]. The majority of the currently available medicines to treat these diseases, being decades old and suffering from poor efficacy, high toxicity, and increasing resistance, by no means reflects the clinical need. Accordingly, NTDs are a huge health emergency, which requires remarkable efforts in the search for effective therapies.

In this respect, fast phenotypic screening, having the advantage of identifying compounds which are active against the whole parasite, and circumventing pharmacokinetic issues, has been undergone a resurgence in the last years[3]. Recently, it has resulted in the identification of a number of compounds currently in preclinical and clinical phases[4]. In this respect, the majority of new molecular entities approved by the FDA between 1999 and 2008 were identified by phenotypic screening (37% versus 23% discovered by target-based approaches). However, as target-based approaches overbear phenotypic screening during this time frame, the success of this latter one is even undervalued[4].

Furthermore, compounds resulting from phenotypic assays may affect multiple proteins or pathways in the organism, showing a multitarget mechanism of actions and providing new molecular targets, which would not be identified in a more conventional target-based approach[4].

Accordingly, a possible integrated strategy in drug discovery for NTDs could be the combination of parallel synthesis of small molecules libraries with fast phenotypic assays.

To develop reduced size libraries with high hit rates for screening purposes, natural product-derived and -inspired collection concepts are particularly suitable, because they recognize natural product fragments as evolutionarily selected and biologically pre-validated frameworks for compound collection development[5]. Naphthoquinones and other related quinones have been reported as one of the major natural product classes with remarkable activity against trypanosomatid parasites. For instance, lapachol (2-hydroxy-3-(3-methyl-2-butenyl)-1,4-naphthoquinone) shows a significant anti-trypanosomal potency, without arising any relevant health concerns[6]. In

this field, we developed a focused library of 16 natural-inspired 1,4-naphthoquinone and 1,4-anthraquinone derivatives with a favourable anti-trypanosomatid profile in phenotypic whole cell assays. 2-Phenoxy-1,4-naphthoquinone (B6), showing an $ED_{50}$ of 80 nM against *Trypanosoma brucei rhodesiense* (*T.b.r.*), and a selectivity index (ratio of the compound's $ED_{50}$ values on mammalian cell lines and trypanosomes) of 74, turned out as the most interesting derivative of the series and a promising anti-trypanosomatid hit compound[7]. With B6 in hand, we aimed at detecting its putative target(s). In this respect, we considered chemical proteomics as a powerful method to fish out targets from cell lysates using an immobilized derivative of the selected hit compound on an affinity chromatography column (Fig. 1).

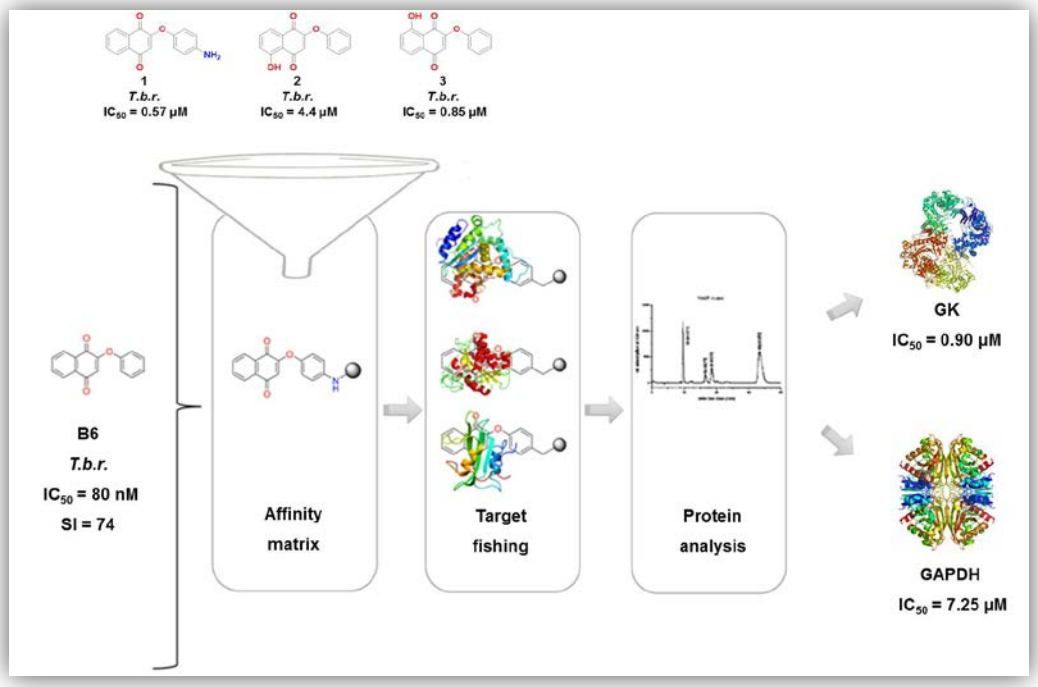


*Fig. 1*
*Chemical proteomics workflow*

Accordingly, we tried to modify B6 structure, introducing different reactive groups (i.e. hydroxyl and amino functionalities) for a covalent linkage to the solid matrix, without compromising its biological activity. Among the synthesized congeners **1**-**3** (Fig. 1), **1** retained the anti-*Trypanosoma* profile of the parent compound and was selected for further affinity-based target identification studies. This led to two putative targets, namely glycosomal glycerol kinase (*Tb*GK) and glycosomal glyceraldehyde-3-phosphate dehydrogenase (*Tb*GAPDH). B6 resulted a micromolar inhibitor of both enzymes in subsequent biochemical assays ($IC_{50}$ = 0.90 µM and 7.25 µM on GK and GAPDH, respectively), but only GAPDH, a vital parasitic enzyme and a well-validated molecular target for NTD drug discovery, was pursued further. Accordingly, GAPDH inhibition by B6 has been deeply investigated through kinetic and docking studies, suggesting a covalent cysteine trapping mechanism[8].

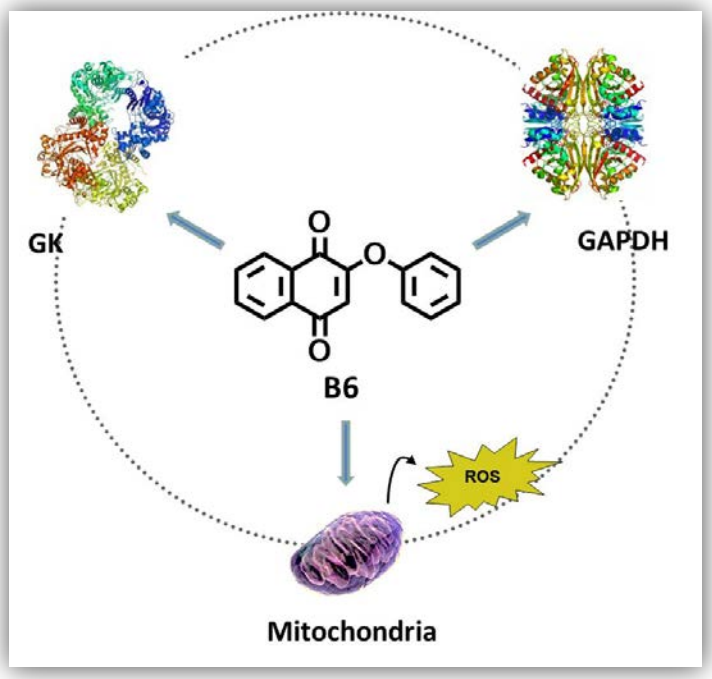


*Fig. 2*
*B6 multitarget mechanism of action*

However, to completely account for the cell-based nanomolar profile of B6, and acknowledged that chemical proteomics is not meant for non-protein target identification, other mechanisms of action are envisaged. In particular, considering that quinones and naphthoquinones are known to interact with the mitochondrial respiratory chain, oxygen consumption and oxygen radicals (ROS) production were also examined. Interestingly, B6 resulted able to generate ROS in permeabilized parasites and trypanosoma mitochondria, a mechanism that may additionally contribute to its marked trypanocidal potency. Overall, B6 showed a multitarget profile (Fig. 2), which provides a molecular explanation of its nanomolar anti-*Trypanosoma* activity[8].

From what we learned from this investigation, phenotypic screening is a truly useful and high-hit rate approach in NTD drug discovery, but the subsequent target fishing step could result in a demanding challenge. Nevertheless, elucidation of specific target(s) of phenotypic hits is necessary to understand underlying mechanisms and to progress active molecules into the following structure-based hit-to-lead and lead optimization steps[9].

By weighing the pros and cons, we still consider the phenotypic strategy as a promising option in the med-chem toolbox.

**REFERENCES**

[1]A. Cavalli *et al., FEMS Immunol. Med. Microbiol.,* 2010, **58**, 51.
[2]A. Cavalli, M.L. Bolognesi, *J. Med. Chem.,* 2009, **52**, 7339.
[3]I.H. Gilbert, *J. Med. Chem.,* 2013, **56**, 7719.
[4]M.L. Sykes, V.M. Avery, *J. Med. Chem.,* 2013, **56**, 7727.
[5]R. Breinbauer *et al., Angew. Chem. Int. Ed. Engl.,* 2002, **41**, 2879.
[6]K. Krohn *et al., Arkivoc Special Issue Reviews and Accounts,* 2007, 145.
[7]M.L. Bolognesi *et al., Bioorg. Med. Chem. Lett.,* 2008, **18**, 2272.
[8]S. Pieretti *et al,. PLoS Negl. Trop. Dis.,* 2013, **7**, e2012.
[9]J. Lee, M. Bogyo, *Curr. Opin. Chem. Biol.,* 2013, **17**, 118.