IN SILICO PREDICTION OF PHARMACOKINETIC PARAMETERS AND EVALUATION OF PEROXIDASE ACTIVITY IN A SERIES OF 3-METHYL-1,6-DIPHENYL-1*H*-PYRAZOLO[3,4-*b*]PYRIDINE COMPOUNDS

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Introduction

The treatment of Chagas disease is based only on two drugs, benznidazole and nifurtimox, which are poorly active in the chronic phase of the disease besides their severe side effects. In the search of new trypanocidal compounds with antichagasic potential, our research group developed a series of 3-methyl-1,6-diphenyl-1*H*-pyrazolo[3,4-*b*]pyridine derivatives (Figure 1) that showed promising activity against the amastigote forms of *T. cruzi in vitro* (Soares et al., 2018). However, taking into account that many compounds that are promising in *in vitro* tests do not demonstrate the same pharmacological profile in *in vivo* assays, which are usually associated with problems in the pharmacokinetic phase, the prediction of ADME processes is extremely important in the search for a drug candidate (Pereira, 2007; Stepan et al., 2013). On the other hand, since phenols can act as substrates or inhibitors of heme-enzymes, they can be linked to the overall enzymatic rate (Le; Zhao; Frazen, 2014). Among heme-enzymes, peroxidases are enzymes with a more exposed heme group at the catalytic site. In view of a promising potential of these compounds, in this work we analyzed some *in silico* estimation of pharmacokinetic parameters and the affinity of these compounds with enzymes that have the heme group.

Method

The affinity of the compounds with heme-enzymes was evaluated on the peroxidase enzyme, in collaboration with Dr. Pereira, L.M. and Dr. Albuquerque, S. from FCFRP-USP. The enzyme was incubated with the compounds at a concentration of 100 µM and the activity of the enzyme was monitored by measuring the optical density (OD) using a spectrophotometer (Pereira, 2013). The *in silico* studies started by building the structures of the compounds LQMed 521-528 using the ChemDraw® JS editor (https://chemdrawdirect.perkinelmer.cloud /js/sample/index.html) and then converted into the SMILES format. The pharmacokinetic predictions were performed on the web servers SWISSADME (Daina et al, 2017) and admetSAR (Yang et al, 2019).

Results / Discussion

The evaluation of the compounds LQMed 521-528 on enzyme peroxidase is shown on the enzyme activity curve (Figure 1), constructed through absorbance measurements as a function of time. The growth curve showed that these compounds did not inhibit the activity of the enzyme at a 100 µM concentration. Since pharmacokinetic parameters provide a general indication of the drug's behavior in the body, some *in silico* studies have been carried out with the compounds LQMed 521-528. The log P values predicted for these compounds are within the desired range of log P for drugs according to 'Lipinski's criteria for oral administration, and all compounds were identified as having a high probability of gastro-intestinal (GI) absorption (Table 1). In addition, the compounds were not identified as P-glycoprotein (P-gp) substrates in the *in silico* studies (Table 1). Since P-gp is a transmembrane permeability glycoprotein that

functions as a carrier-mediated primary active efflux transporter, bioavailability can be lowered or resistance induced when drugs are P-gp substrates (Srivalli, Lakshmi, 2012).

Figure 1. Effect of the LQMed 521-528 compounds at a 100 µM concentration on the peroxidase activity.

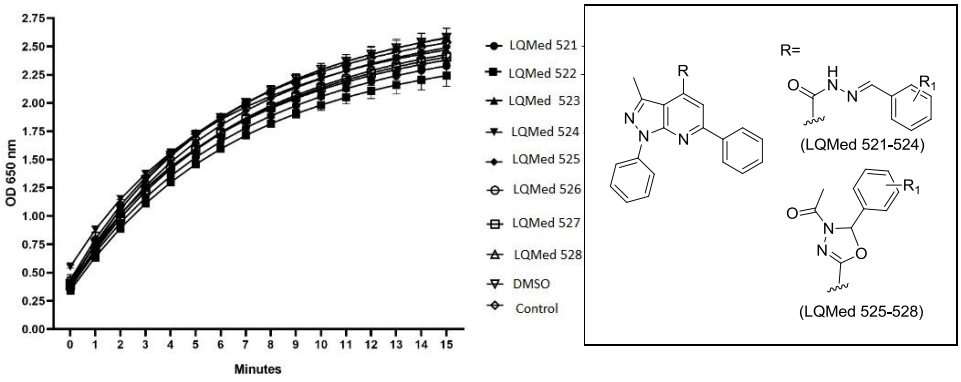

Table 1: Predicted pharmacokinetic properties of LQMed 521-528 compounds. GI absorption and P-gp substrate by consensus of SWISSADME and admetSAR. Log P by SWISSADME server.

	R_1	Lipophilicity (Log $P_{o/w}$[a])	GI absorption	P-gp substrate
LQMed 521	3-OH	4.36	High (1.0000)	No (0.6465)
LQMed 522	3,4-di-OH	3.96	High (0.9944)	No (0.5426)
LQMed 523	3-OH-4-OCH_3	4.36	High (0.9920)	No (0.5461)
LQMed 524	2-OH	4.37	High (0.9975)	No (0.6753)
LQMed 525	4- $OC(O)CH_3$	4.75	High (0.9953)	No (0.6892)
LQMed 526	3- $OC(O)CH_3$	4.77	High (0.9953)	No (0.6892)
LQMed 527	2-$OC(O)CH_3$	4.77	High (0.9925)	No (0.7089)
LQMed 528	3,4-di-$OC(O)CH_3$	4.69	Low (0.9928)	No (0.6616)

[a] The consensus log $P_{o/w}$ is the arithmetic mean of the values predicted by the five proposed methods (XLOGP3, WLOGP, MLOGP, SILICOS-IT, iLOGP).

Conclusion

In this study, we evaluated pharmacokinetic properties, such as lipophilicity, GI absorption and P-gp substrate, for a serie of 3-methyl-1,6-diphenyl-1*H*-pyrazolo[3,4-*b*]pyridine derivatives that showed promising trypanocidal activity. The derivatives exhibited results within the desirable criteria for drugs. The enzymatic assay showed that these compounds do not inhibit the peroxidase enzyme, being unlikely to inhibit human enzymes, which is a positive aspect regarding their toxicity.

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