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New 1,6-diphenyl-1*H*-pyrazolo[3,4-*b*]pyridines Derivatives with Potent Antichagasic Activity

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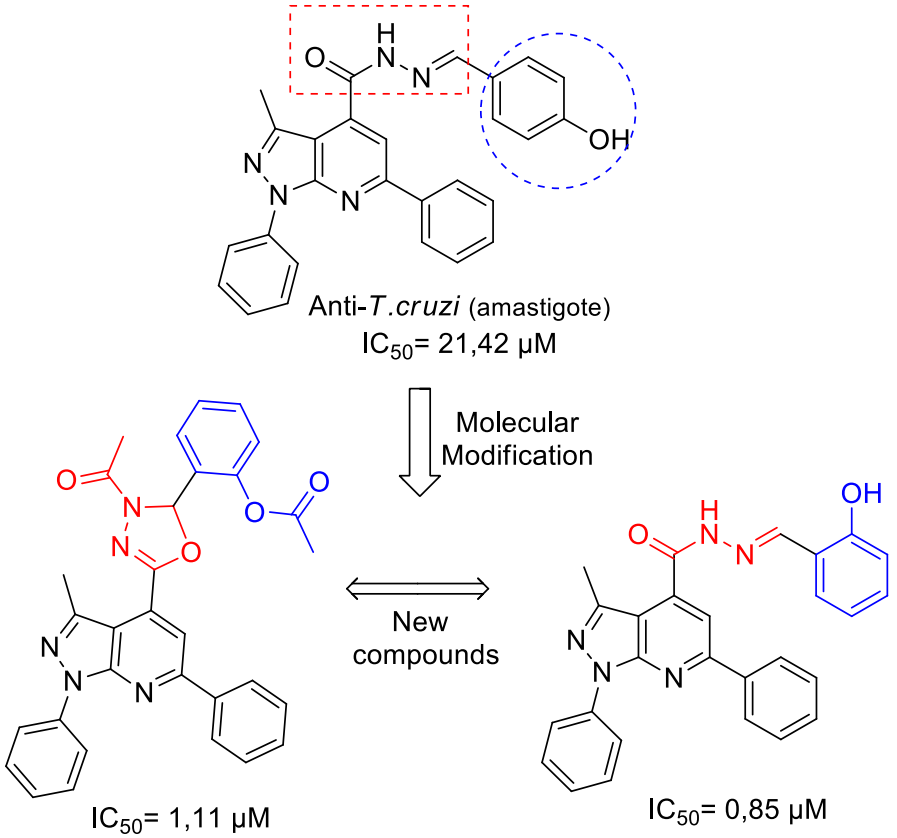
Graphical Abstract

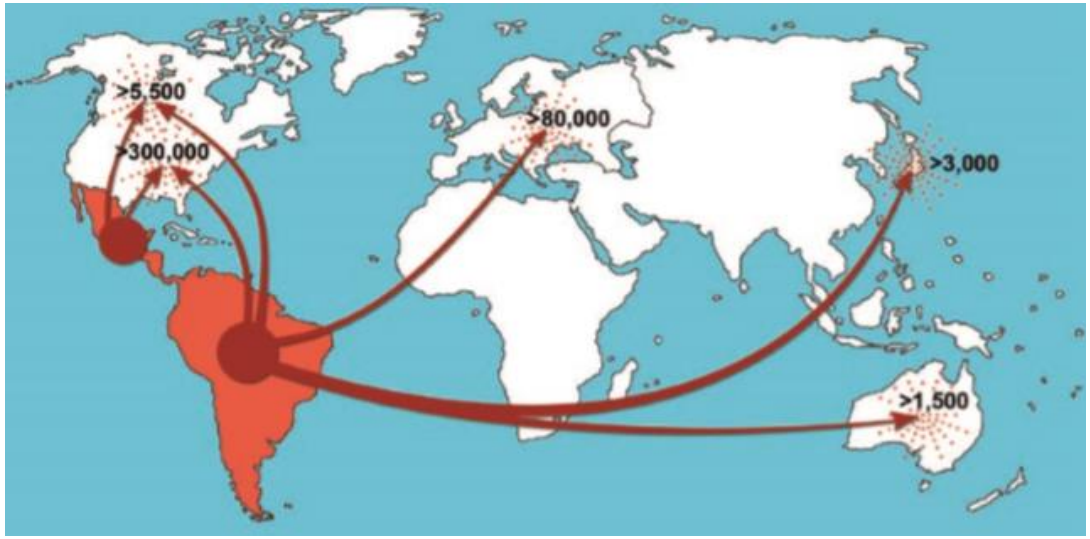


Abstract: Chagas' disease is an infection caused by the protozoan *Trypanosoma cruzi* that represents a major public health threat in Latin America. Previously we developed 4-carbohydrazide derivatives of 1*H*-pyrazolo[3,4-*b*]pyridine as antichagasic agents, which the hit compound was the *N'*-4-hydroxybenzylidene-carbohydrazide derivative. In order to verify the influence of the substituent position and the carbohydrazide moiety replacement for the 1,3,4-oxadiazoline moiety, herein we described the synthesis and *in vitro* evaluation of trypanocidal activity and cytotoxicity of eleven new 1,6-diphenyl-4-(substituted)-1*H*-pyrazolo[3,4-*b*]pyridine derivatives. All the new 1,6-diphenyl-3-methyl-4-(substituted)-1*H*-pyrazolo[3,4-*b*]pyridine derivatives were obtained with yields ranging from 70 to 95% and had their structures elucidated by spectroscopic methods. These compounds were evaluated *in vitro* against intracellular amastigote form of *T. cruzi*, using the benznidazole drug as the positive control and had their cytotoxicity profiles determined on LLCMK$_2$ mammalian cells. Among the new compounds, the *N'*-2-hydroxybenzylidene-carbohydrazide and 2-(*N'*-acetyl-1,3,4-oxadiazolin-2-yl)-phenyl acetate derivatives showed the most significant antichagasic activities and low cytotoxicity profile in comparison to benznidazole drug. The results suggest that the 2-substituted position of the phenyl group connected to the carbohydrazide or oxadiazoline moieties play an important role for the antichagasic activity of 1,6-diphenyl-4-(substituted)-1*H*-pyrazolo[3,4-*b*]pyridines compounds. Furthermore, our results indicate a bioisosteric replacement of carbohydrazide moiety by the 1,3,4-oxadiazoline ring.

Keywords: Chagas disease; *Trypanosoma cruzi*; 1*H*-pyrazolo[3,4-*b*]pyridine.

Introduction

- Chagas disease, also known as american trypanosomiasis, is an infection caused by the protozoan *Trypanosoma cruzi* responsible for a burden of 6 million infections and 7 thousand death globally each year[1].
- Although it is endemic in 21 counstries across Latin America, on last decades Chagas disease has been spread to non-endemic areas due to migration[2].

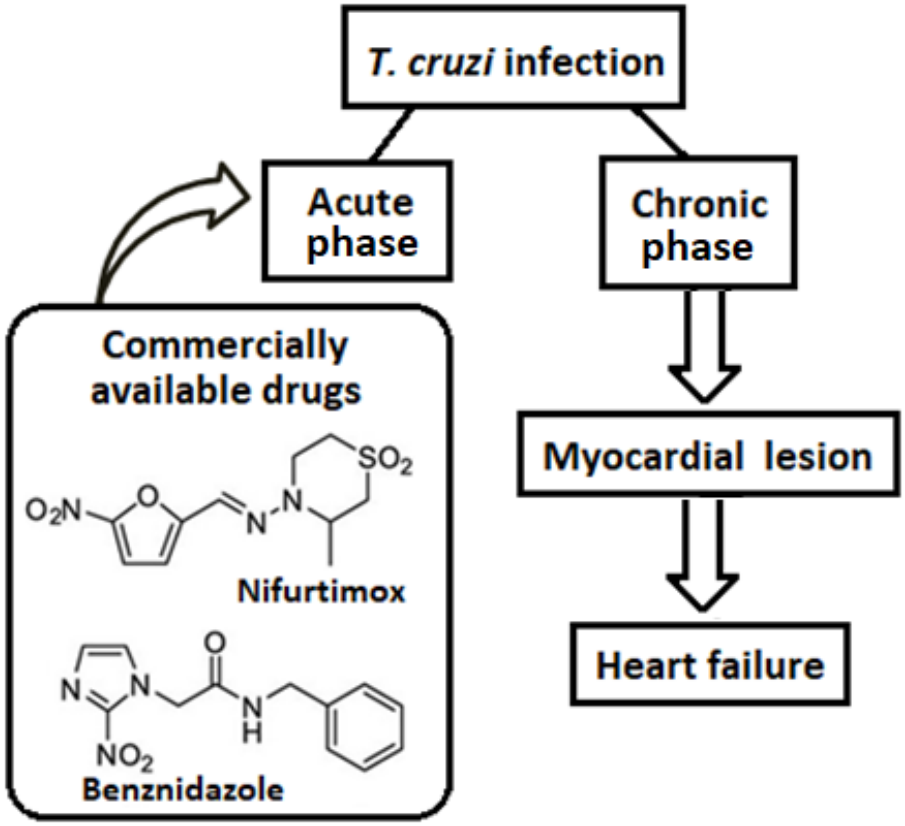


Immigration Routes from Latin America and an estimate of the number of infected people.[3]

[1] WHO. **Chagas disease (American trypanosomiasis)**. Available in: <http://www.who.int/mediacentre/factsheets/fs340/en/>. Acess in: 22 out. 2018. [2] CHATELAIN, E. **Comput. Struct. Biotechnol. J.**, 15, 98–103, 2017. [3] COURA, J. R.; VIÑAS, P. A. **Nature**, 465, S6–S7, 2010.

Introduction

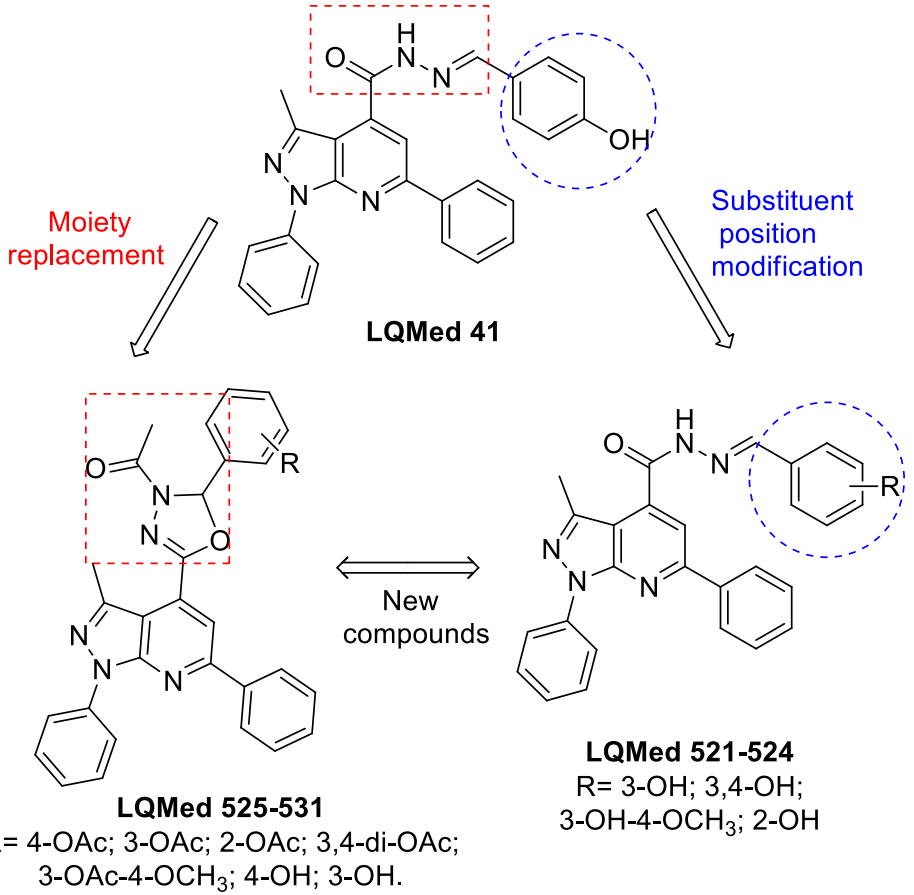
- Chagas disease is considered one of the main causes of heart failure in Brazil, that is highly associated with persistence of amastigote form of *T. cruzi* in cardiac muscle of chronically infected patients[4,5].
- The development of new drugs against *T. cruzi* is still required since the only two drugs (nifurtimox and benznidazole) currently used cause severe side effects and are not effective in chronic infection[5].



[4] CHATELAIN, E. **J Biomol Screen**, 20 (1), 22–35, 2015. [5] BERMUDEZ, J. et al. **Acta Trop.**, 156, 1–16, 2016.

Introduction

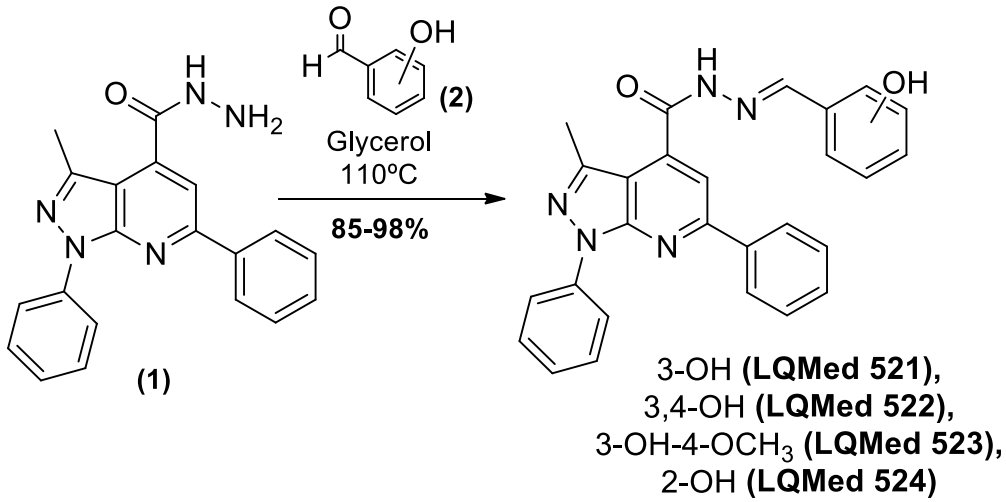
- The hit compound from previous work was the *N'*-4-hydroxybenzylidene-carbohydrazide derivative[6] (LQMed 41).
- In order to verify the influence of the substituent position and the replacement of carbohydrazide moiety for 1,3,4-oxadiazoline moiety, herein we described the synthesis and *in vitro* evaluation of trypanocidal activity and cytotoxicity of 11 new 1,6-diphenyl-4-(substituted)-1*H*-pyrazolo[3,4-*b*] pyridine derivatives.



[6] DIAS, L.R.S. et al. **Bioorg. Med. Chem.**, 15(1), 211, 2007.

Results and discussion

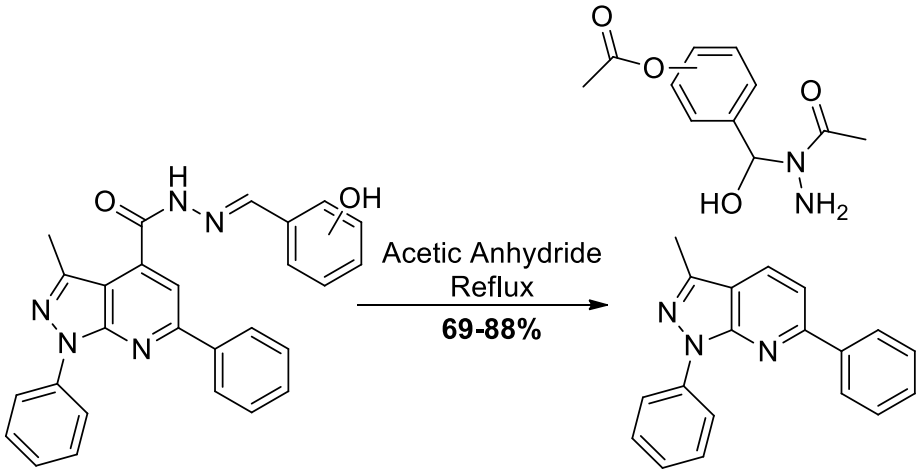
- All new compounds were purified by recrystallization and their structures were elucidated by spectroscopic methods (IR, 1D and 2D NMR (^{1}H and ^{13}C) and HRMS).
- The new compounds were synthesized using the 4-carbohydrazide-1,6-diphenyl-3-methyl-1*H*-pyrazolo[3,4-*b*]pyridine (1)[6] as the key intermediate.
- The reaction between 1 and hydroxybenzaldehydes (2) in a green solvent medium (glycerol)[7] furnished the *N'*-benzylidene-carbohydrazide derivatives (LQMed 521-524).



[6] DIAS, L.R.S. et al. **Bioorg. Med. Chem.**, 15(1), 211, 2007. [7] RIBEIRO, J. L. S. Master's Dissertation. Universidade Federal Fluminense, 2017.

Results and discussion

- The 1,3,4-oxadiazoline derivatives (LQMed525-529) were synthesized from *N'*-benzylidene-carbohydrazides (LQMed41, 521-524) by treatment with refluxing acetic anhydride. [8-10]
- The LQMed525-529 compounds have the hydroxyl group replaced by the acetyl group.



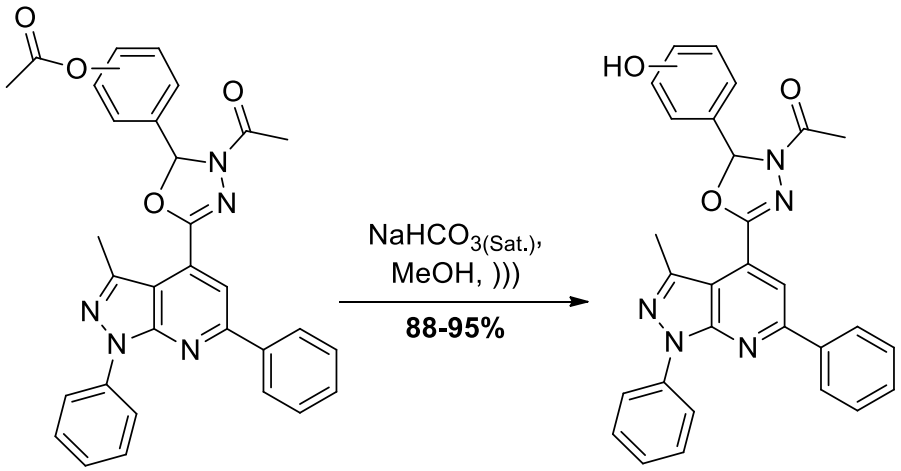
(LQMed 41, 521-524)

4-OC=OCH$_3$ **(LQMed 525),**
3-OC=OCH$_3$ **(LQMed 526),**
2-OC=OCH$_3$ **(LQMed 527),**
3,4-OC=OCH$_3$ **(LQMed 528),**
3-OC(O)CH$_3$-4-OCH$_3$ **(LQMed 529)**

[8] KOÇYIĞIT-KAYMAKÇIOĞLU, B. et al. **Med. Chem. Res.**, 21(11), 3499, 2012. [9]MALLIKARJUNA, B.P. et al. **Eur. J. Med. Chem.**, 44(11), 4739, 2009. [10] MOREIRA OSÓRIO, T. et al. **Bioorg. Med. Chem. Lett.**, 22(1), 225, 2012.

Results and discussion

- Additional hydrolysis step was carried out in order to obtain two new hydroxylated 1,3,4-oxadiazoline derivatives (LQMed530 and 531) from the corresponding acetylated compounds (LQMed525 and 526). In this step, ultrasound was used for improving reaction time and yields[11].



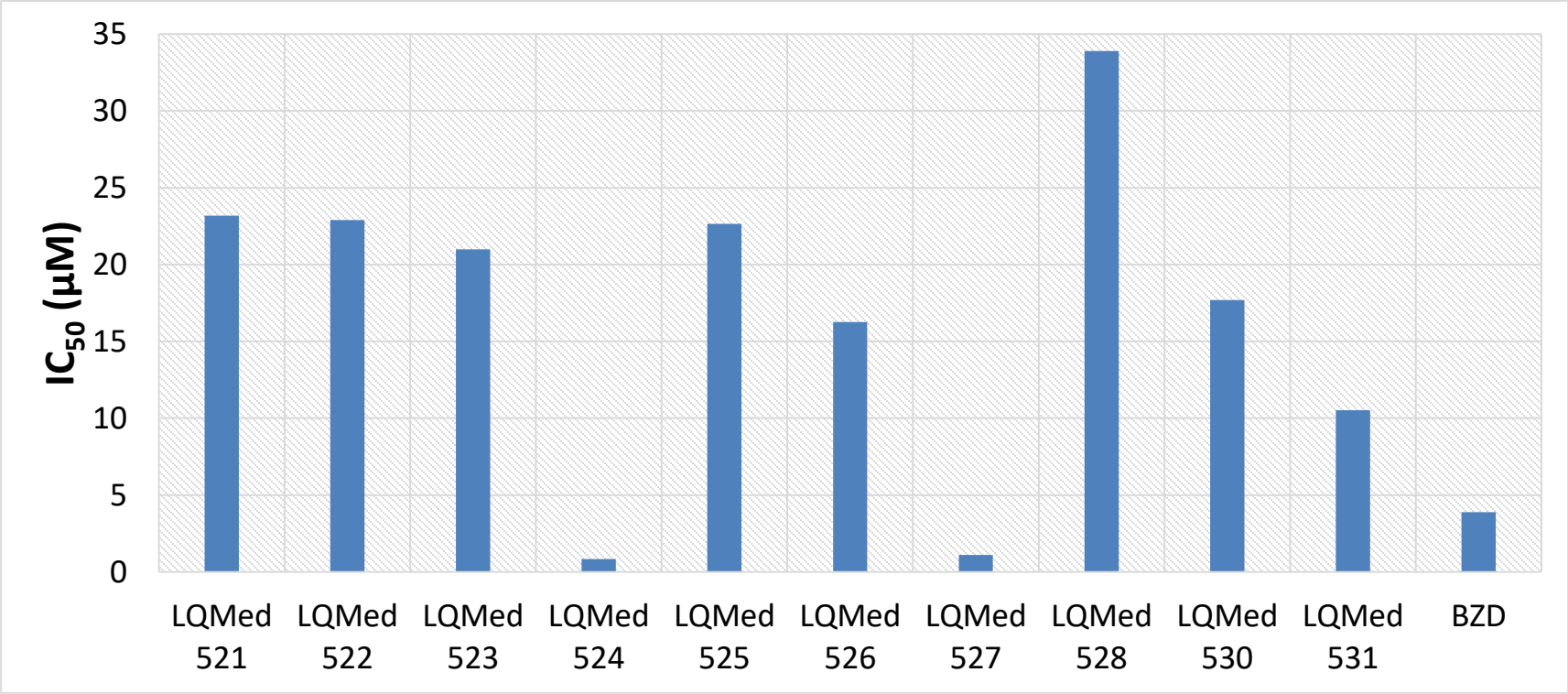
(LQMed 525, 526)

4-OH **(LQMed530)**
3-OH **(LQMed531)**

[11] PACCOLA, C. E. T et al. **J. Phys. Chem. C**, 119 (33), 19162–19170, 2015.

Results and discussion

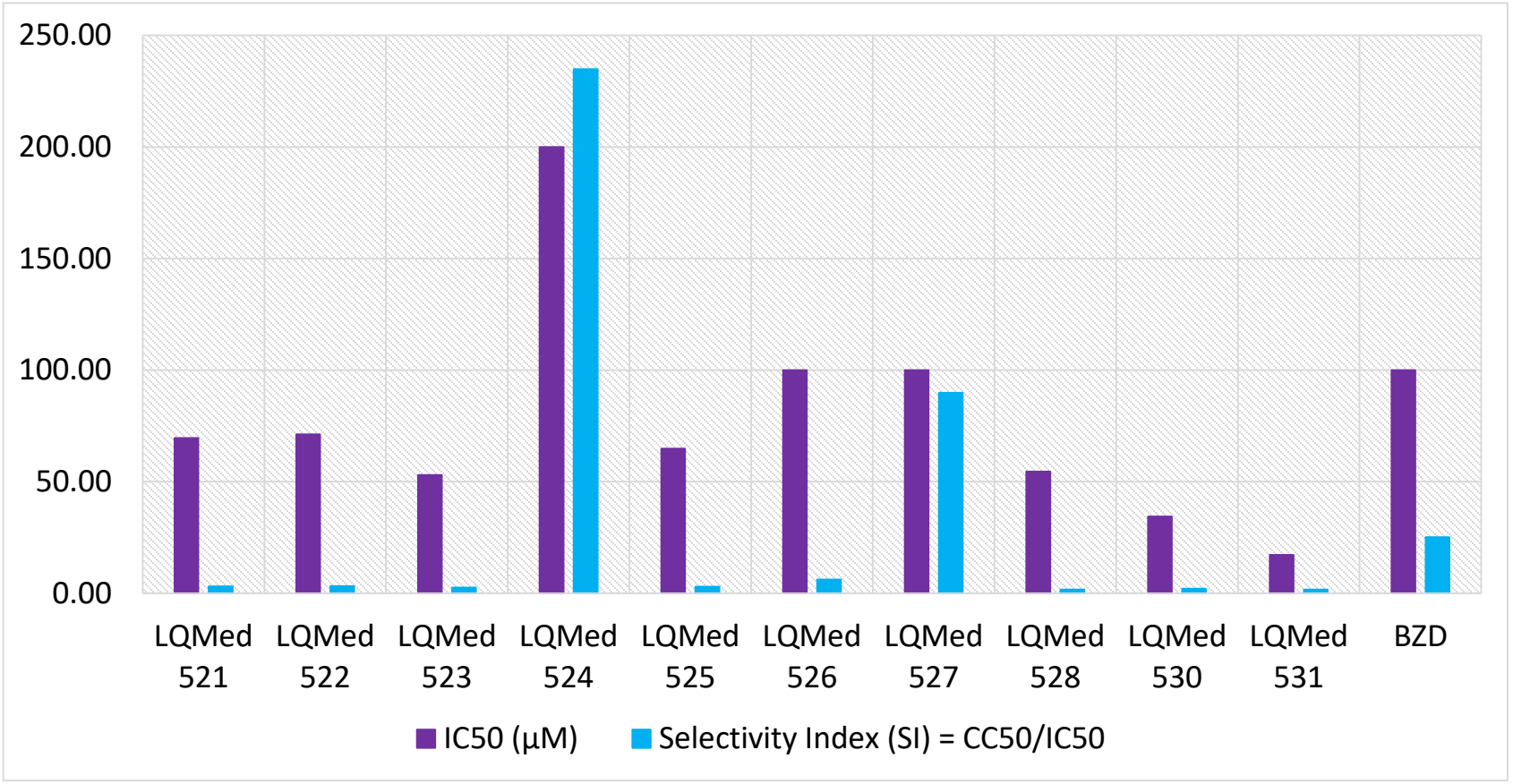
❖ Trypanocidal activity evaluation against the amastigote form of *T. cruzi.*



- BZD = Benznidazole drug
- The low solubility of LQMed529 (R= 3-OAc-4-OCH_3) in water and DMSO has prevented its evaluation.

Results and discussion

❖ Cytotoxicity on LLCMK$_2$ mammalian cells and Selectivity Index.



▪ BZD = Benznidazole drug

Conclusions:

- The new 1,6-diphenyl-3-methyl-4-(substituted)-1*H*-pyrazolo[3,4-*b*]pyridine derivatives (LQMed521-531) were synthesized in good yields and were evaluated *in vitro* for trypanocidal activity and cytotoxicity profile;
- Among the new compounds, the *N'*-2-hydroxybenzylidene-carbohydrazide derivative (LQMed524) and the 2-(*N'*-acetyl-1,3,4-oxadiazolin-2-yl)-phenyl acetate derivative (LQMed527) have exhibited higher activities against *T. cruzi* and low cytotoxicity profile in comparison with benznidazole drug;
- The results showed that the 2-substituted position of phenyl group connected to the carbohydrazide or oxadiazoline moiety plays an important role for the antichagasic activity of this class of compounds;
- Furthermore, these results indicate a nonclassical bioisosteric replacement of the carbohydrazide moiety by the 1,3,4-oxadiazoline ring;
- The findings showed that LQMed524 and 527 are promising drug candidates for the treatment of chronic phase of the Chagas disease.

Acknowledgments

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