The 7th International Electronic Conference on Medicinal Chemistry (ECMC 2021)
01–30 NOVEMBER 2021 | ONLINE

Benzoic acid derivatives as prodrugs for the treatment of tuberculosis

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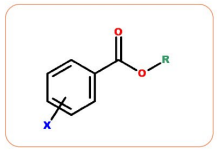
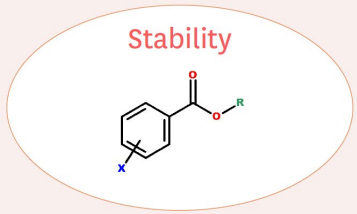
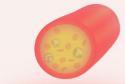
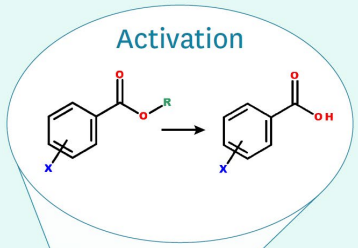
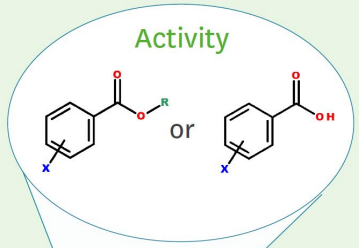


X	R
4-Cl	H
4-NO_2	Pr
3,5-Cl	Ph
3,5-NO_2	He
2,6-Cl	









Abstract:

The emergence of resistance against to the first line anti-TB drugs, such as pyrazinamide, has led to the need for new approaches. One interesting approach is the use of **prodrugs** that often have showed **improved biological activities** over drugs with poor absorption or difficulty to cross membranes.

Previous studies demonstrate that weak acids like benzoic acid, present antimycobacterial activity, which increases at acidic pH. Moreover, esters of those acids revealed to be a viable alternative since they may diffuse more easily through the cell membranes. Previously we showed that **mycobacteria can easily activate esters of benzoic acid** by conversion to the corresponding acid.

Since Zhang postulated that the activity of the acids could be dependent on their pKa, we set up to synthesize a library of benzoates with different electron withdrawing groups (4-chloro, 2,6-dichloro, 4-nitro, 3,5 dinitro), to modulate pka and different alkoxy substituents (propyl, hexyl and phenyl) to modulate their lipophilicity. **We tested the activity of the esters and the free acids against mycobacteria and the activation of the esters by mycobacterial enzymes**. Since the benzoates must survive the transport phase, we also studied the stability of the compounds in buffer and in plasma.

We concluded that **all the benzoates could be activated by mycobacterial enzymes** and the **esters presented higher activity than the corresponding free acids**, with the nitrobenzoates, and specially the **dinitrobenzoates**, showing very interesting **antitubercular activity** that deserve further exploration. These results do not show a correlation between the activity and the pKa of the acids.

Keywords: benzoates; esterases; prodrugs; tuberculosis; weak acids.

Introduction

The emergence of resistance to the first line anti-TB drugs, such as pyrazinamide, has led to the **need for new approaches**.

One interesting approach is the use of **prodrugs** that often have showed **improved biological activities** over drugs with poor absorption or difficulty to cross membranes.

A known example is pyrazinoic acid. Pyrazinoic acid is active against *Mycobacterium tuberculosis,* unfortunately it has difficulties in absorption and penetration into the bacteria.

However, if it is transformed into the **amide derivative** (pyrazinamide), the compound **penetrates easily into the cells** and the **activity increases** significantly.

Inside the bacteria pyrazinamide is activated into pyrazinoic acid that is free to act.

We and others have developed ester prodrugs alternative to pyrazinamide based also in the molecule of pyrazinoic acid.

Many ester derivatives suffer from poor plasma stability, but we managed to obtain **ester prodrugs of pyrazinoic acid** that **are stable to plasma esterases** but can **be activated by mycobacterial enzymes**.

- Simões, M. F.; Valente, E.; Gómez, M. J. R.; Anes, E.; Constantino, L. Lipophilic Pyrazinoic Acid Amide and Ester Prodrugs Stability, Activation and Activity against M. Tuberculosis. *Eur. J. Pharm. Sci.* **2009**, *37* (3–4), 257–263. https://doi.org/10.1016/j.ejps.2009.02.012.

- Pires, D.; Valente, E.; Simões, M. F.; Carmo, N.; Testa, B.; Constantino, L.; Anes, E. Esters of Pyrazinoic Acid Are Active against Pyrazinamide-Resistant Strains of Mycobacterium Tuberculosis and Other Naturally Resistant Mycobacteria in Vitro and Ex Vivo within Macrophages. Antimicrob. Agents Chemother. **2015**, 59 (12), 7693–7699. https://doi.org/10.1128/AAC.00936-15.

Other weak acids besides pyrazinoic acid present antimycobacterial activity.

Previously we showed that:

- Simple benzoate esters present antimycobacterial activity,
- Mycobacteria can activate benzoate esters with different alkoxy substituents,
- The activation of the compounds and their stability in plasma and in liver could be modulated by modification of the alkoxy substituents.

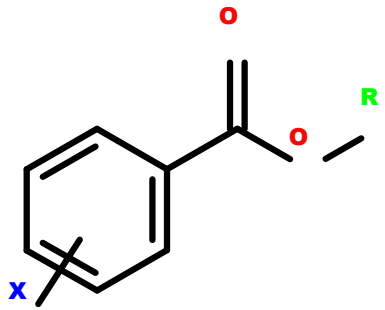
Since Zhang postulated that the antimycobacterial activity of the acids could be dependent on their pKa, we set up to synthesize and obtain a library of benzoic acids and corresponding esters with different electron withdrawing groups to modulate their acidity, and different alkoxy groups.

Valente, E.; Testa, B.; Constantino, L. Activation of Benzoate Model Prodrugs by Mycobacteria. Comparison with Mammalian Plasma and Liver Hydrolysis. *Eur. J. Pharm. Sci.* **2021**, *162*, 105831. https://doi.org/10.1016/j.ejps.2021.105831.

Zhang, Y.; Zhang, H.; Sun, Z. Susceptibility of Mycobacterium Tuberculosis to Weak Acids. *J. Antimicrob. Chemother.* **2003**, *52* (1), 56–60. https://doi.org/10.1093/JAC/DKG287.

Results and discussion

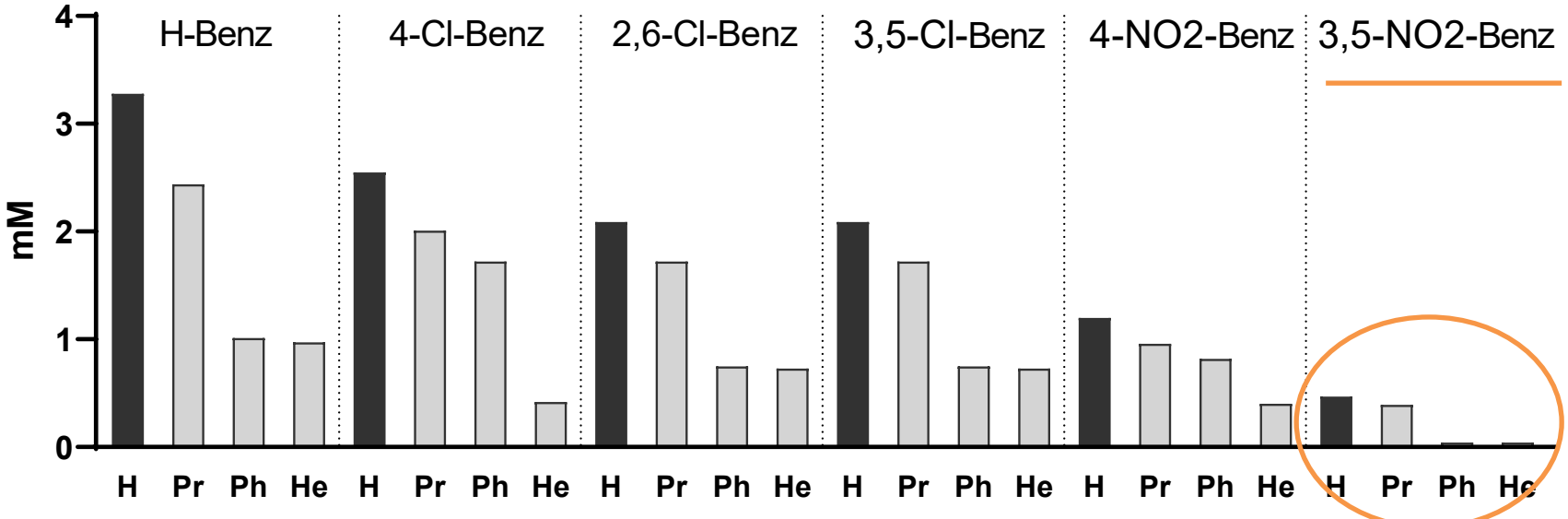
In the present work we tested the **activity** of the compounds against mycobacteria and tested their **activation** by mycobacterial enzymes



X	R
4-Cl	Pr
4-NO_2	He
3,5-Cl	Ph
3,5-NO_2	H
2,6-Cl	

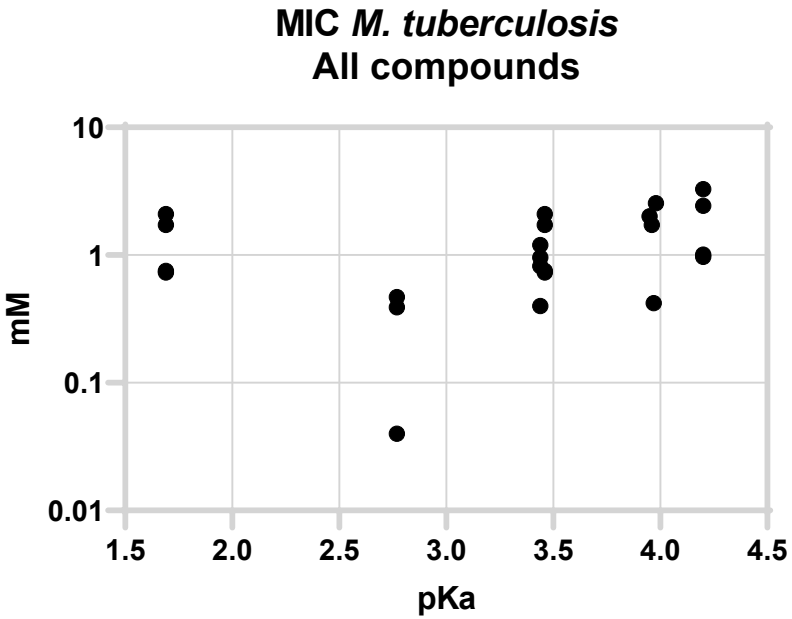
Since the compounds must survive mammalian enzymes, we also studied the **stability of the compounds in human plasma**.

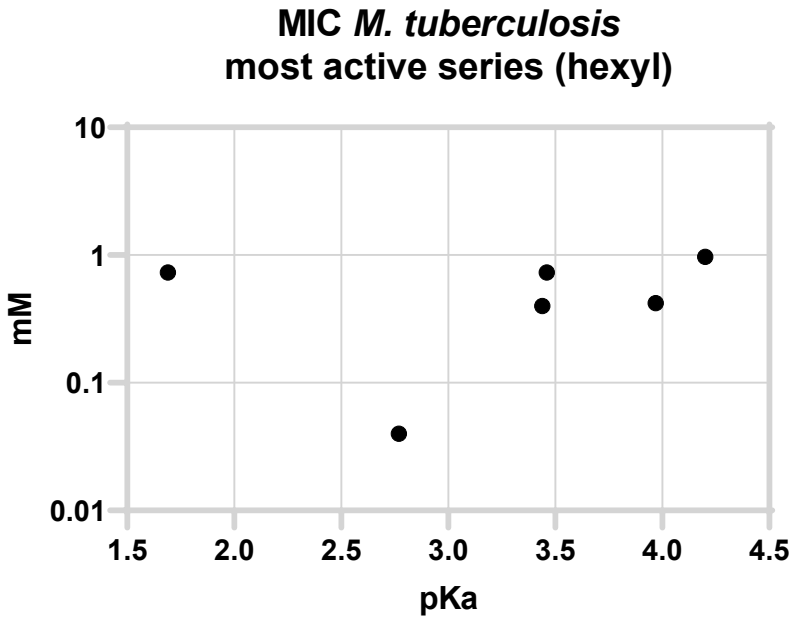
Aromatic substituent



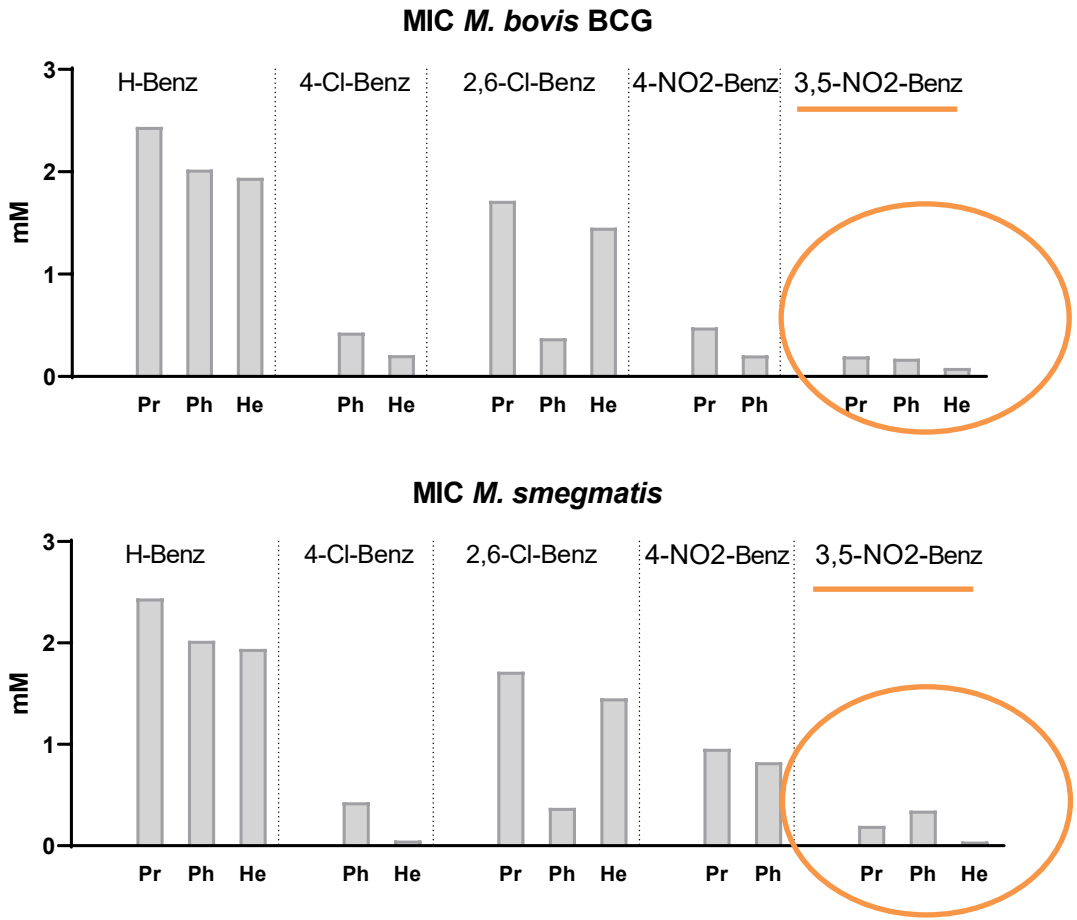
Alkoxy group in ester
(H= free acid)

The activity of the compounds against *M. tuberculosis* was not correlated with pka of the acids

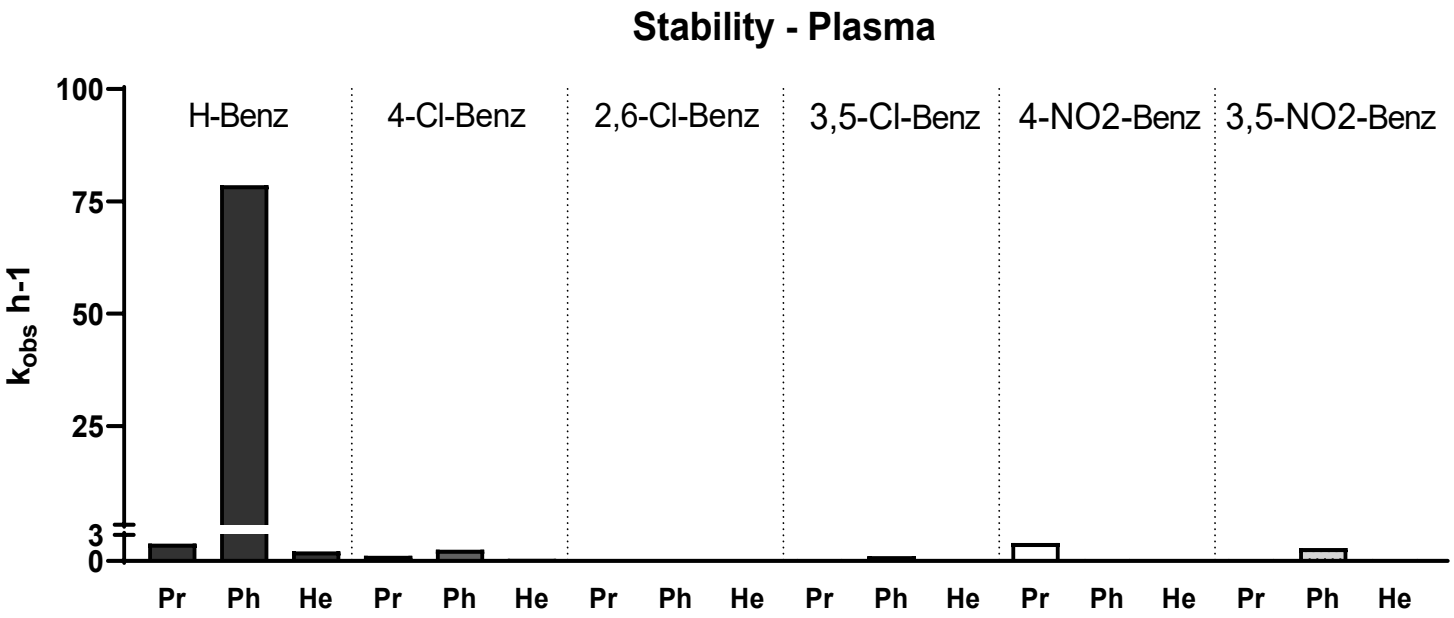




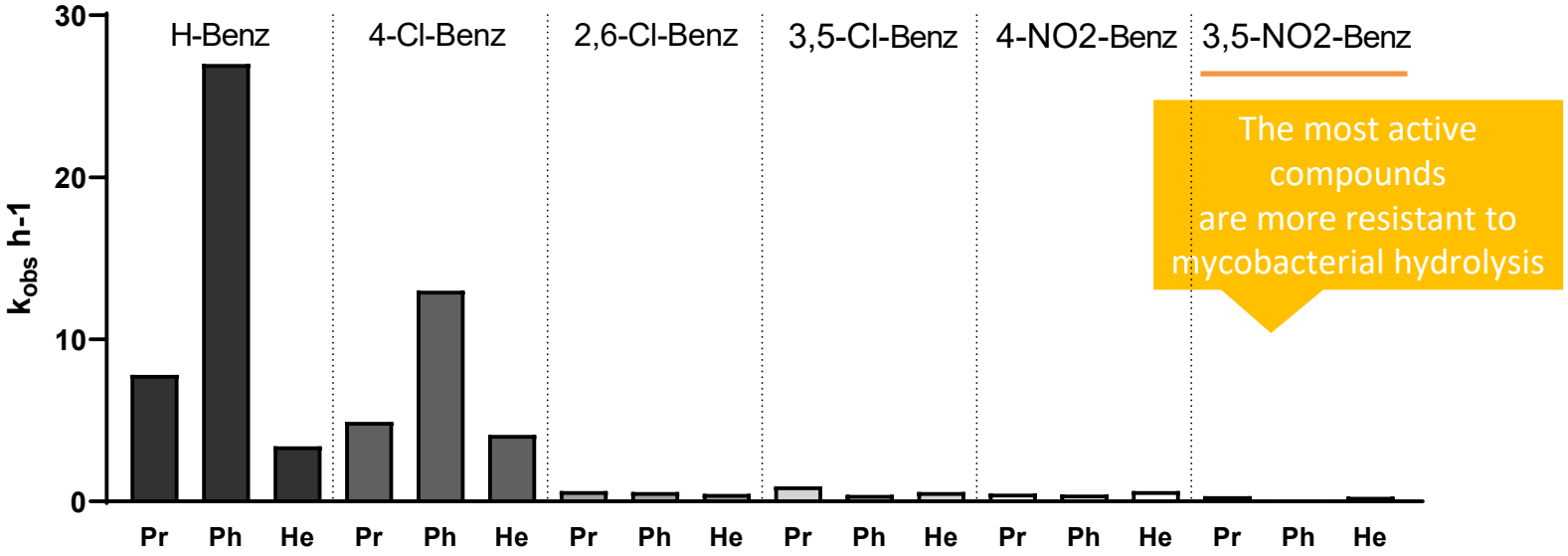
Activity against against *M bovis BCG* and *M smegmatis* followed the same pattern observed with *M. tuberculosis*.



The introduction of electron withdrawing groups reduced the k_{obs} of the hydrolysis and thus improved the stability of the compounds in plasma



Activation in mycobacterial homogenate,



Method: Valente, E.; Simões, M. F.; Testa, B.; Constantino, L. Development of a Method to Investigate the Hydrolysis of Xenobiotic Esters by a Mycobacterium Smegmatis Homogenate. J. Microbiol. Methods 2011, 85 (2), 98–102. https://doi.org/10.1016/j.mimet.2011.02.003.

Conclusions

- Activity was not correlated with the **pKa** of the free acid.
- The **3,5-dinitrobenzoates** presented the highest activities of the series.
- **3,5-dinitrobenzoates** are hydrolysed slowly to the corresponding carboxylic acid in mycobacterial homogenate, suggesting that they are **not acting as prodrugs** of a weak acid but may be **active "*per se*"**.
- The **3,5-dinitrobenzoates are very stable in plasma** suggesting that they can **survive transport** phase in plasma.
- We will study if we could **improve the activity** of the series by **fine tunning the alkoxy group**.

Acknowledgments

This work was supported from grant PTDC/SAU-INF/28080/2017 from Fundação para a Ciência e Tecnologia (FCT) and had also financial support from FCT (via ImedULisboa) from projects UIDB/04138/2020 and UIDP/04138/2020.