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Design, Synthesis and Biological Activity of Furoxan Derivatives Against Multidrug-Resistant Tuberculosis

Guilherme Fernandes [1,2]*, Paula Souza [2], Chung Man Chin [2], Fernando Pavan [2] and Jean Leandro dos Santos [1,2]

[1] Institute of Chemistry, UNESP – Univ Estadual Paulista, Araraquara, Brazil;

[2] School of Pharmaceutical Sciences, UNESP – Univ Estadual Paulista, Araraquara, Brazil.

* Corresponding author: guilhermefelipe@outlook.com

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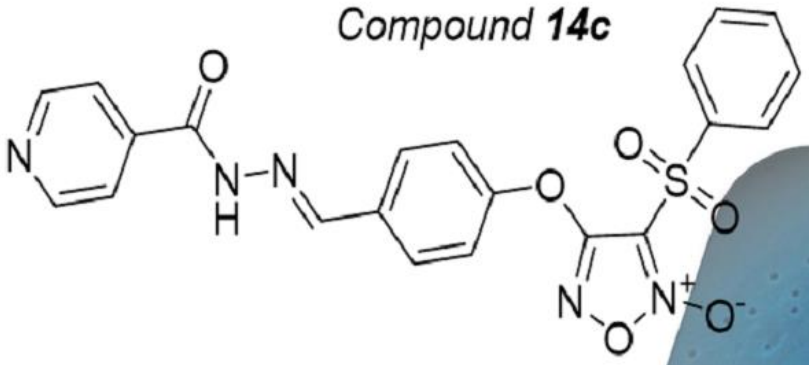
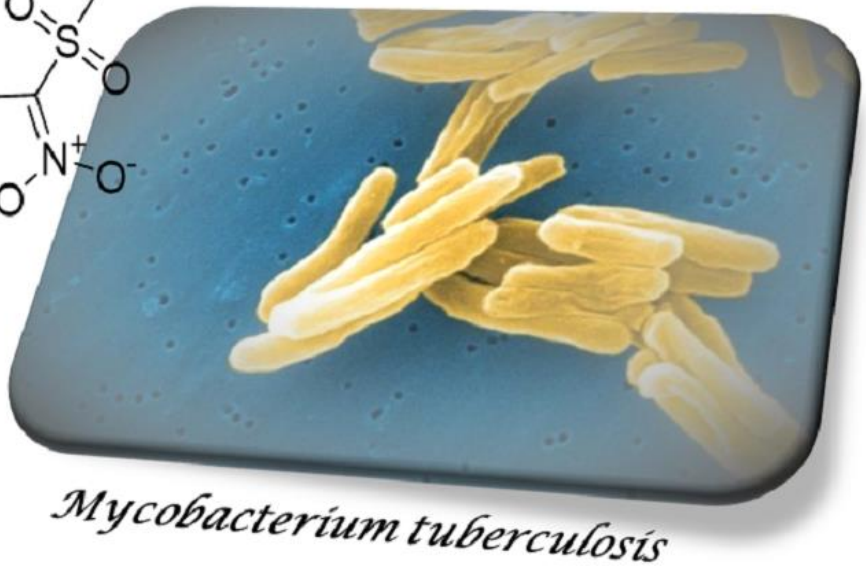
Design, Synthesis and Biological Activity of Furoxan Derivatives Against Multidrug-Resistant Tuberculosis

Compound **14c**



- MIC_{90} = 1.03 μM ($H_{37}Rv$)
- MIC_{90} = 7.0 μM (MDR-TB)
- IC_{50} = 43.01μM (MRC-5)
- $SI_{(H37Rv)}$ = 20.29 (MRC-5)



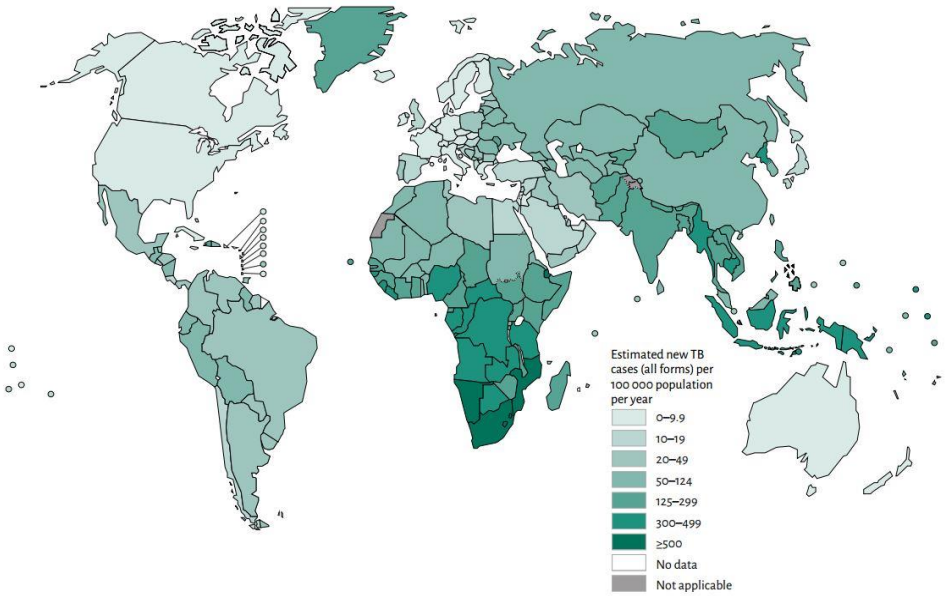
Mycobacterium tuberculosis

Abstract: Tuberculosis remains a serious health problem responsible to cause millions of deaths annually. The scenario becomes alarming when it is evaluated that the number of new drugs does not increase proportionally to the emergence of resistance to the current therapy. Furoxan derivatives, known as nitric oxide donors, have been described to exhibit a wide range of biological activities, including antitubercular. Herein, a novel series of twelve hybrid furoxanyl derivatives were designed, synthesized and evaluated *in vitro* against *Mycobacterium tuberculosis* H37Rv using the REMA method. The furoxan derivatives have exhibited MIC_{90} values ranging from 1.03 to 62 µM. For most active compounds, the selectivity index ranged from 3.78 – 52.74 in MRC-5 cells. Furthermore, these most active compounds were also evaluated against a clinically isolated multi-drug resistant strain (isoniazid, rifampicin, streptomycin and etambutol) and exhibited MIC_{90} values ranging from 1.44 to 5.63 µM. In addition, The amount of nitric oxide (NO) released was indirectly detected by Griess reaction through the measurement of nitrites in the medium. All compounds were able to release NO at levels ranging from 0.16 – 44.23%. In conclusion, furoxan derivatives were identified as new promising compounds useful to treat sensitive and resistant tuberculosis.

Keywords: furoxan; tuberculosis; antituberculosis agents.

Tuberculosis

Mycobacterium tuberculosis

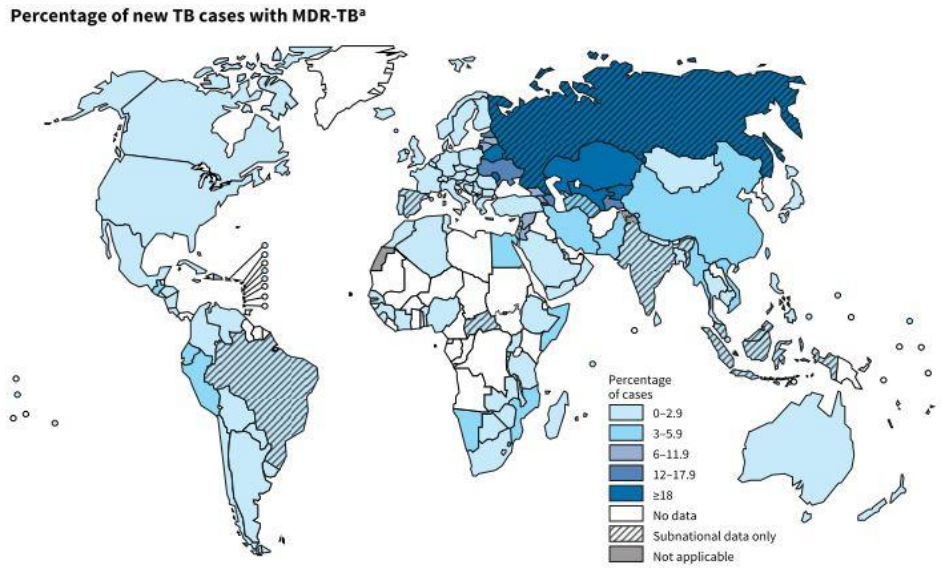


WORLD HEALTH ORGANIZATION. **Global tuberculosis report 2015**

- Infectious disease responsible for the largest number of deaths worldwide
- 1.5 million deaths in 2014
- 9.6 million new cases in 2014
- 12% of new cases in HIV-positive patients
- One third of the world's population infected

Multidrug-Resistant Tuberculosis

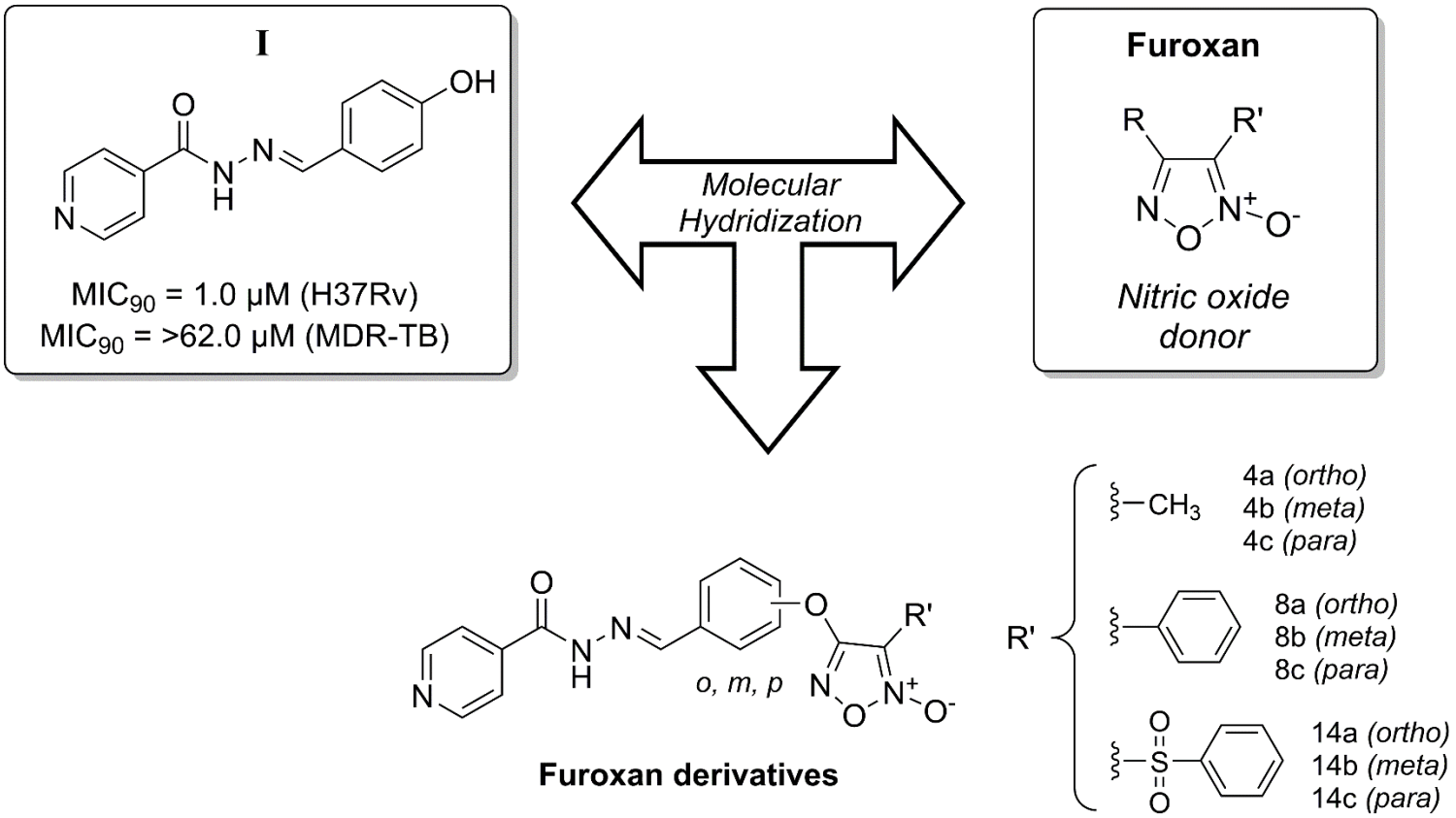
Extensive treatment and several side effects



- 480,000 cases of MDR-TB incidents in 2014
- 190,000 deaths from MDR-TB and 2014
- Only 50% of patients were successfully treated in 2014
- 9.7% of MDR-TB were in fact XDR-TB

WORLD HEALTH ORGANIZATION. **Global tuberculosis report 2015**

Structural Design



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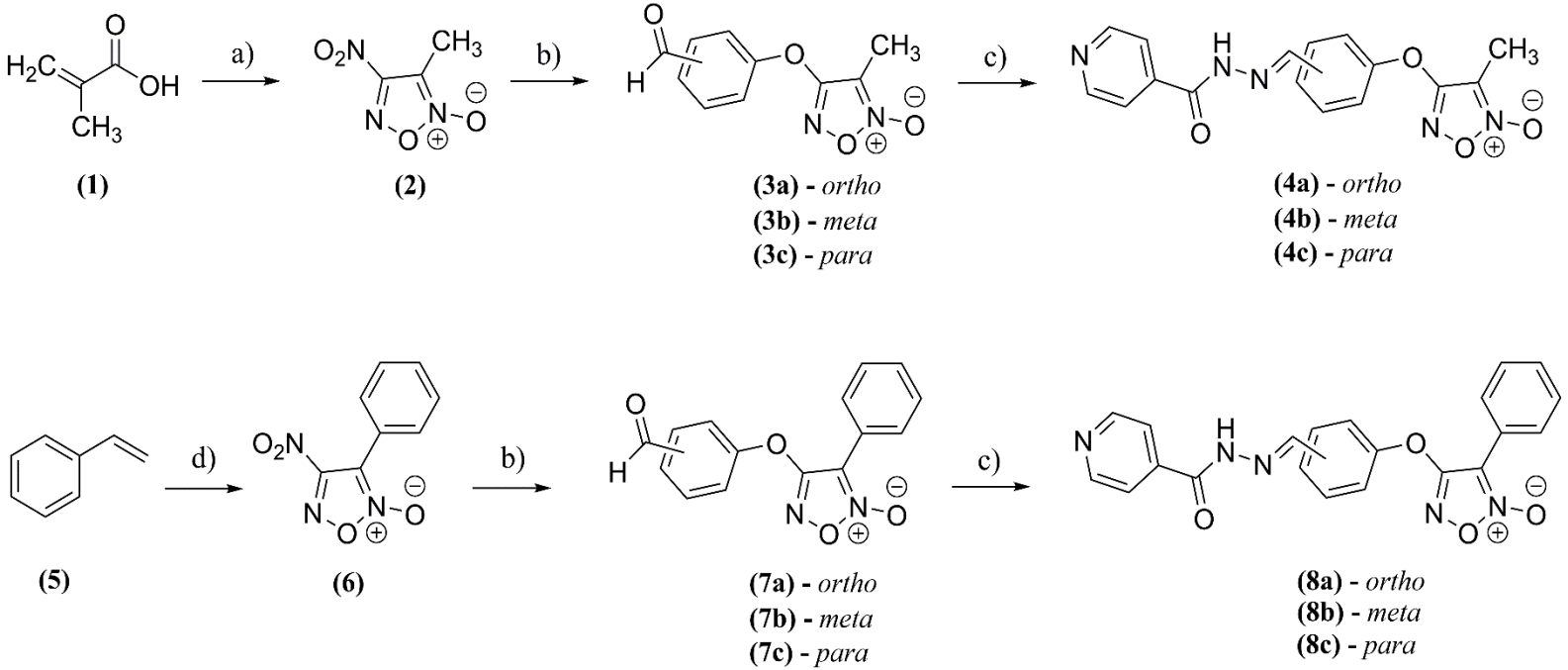
Chemistry



Scheme 1. Reagents and conditions: **(a)** 1,2-dichloroethane, H_2SO_4 60%, $NaNO_2$, 50 °C, 30 min; **(b)** 2, 3 or 4- hydroxybenzaldehyde, 1,8-diazabicycloundec-7-ene (DBU), anhydrous dichloromethane, r.t., 2 h; **(c)** isonicotinic hydrazide, ethanol, acetic acid, r.t., 12 h; (**d**) acetic acid, hydrochloric acid, dichloromethane, $NaNO_2$, r.t, 12 h.

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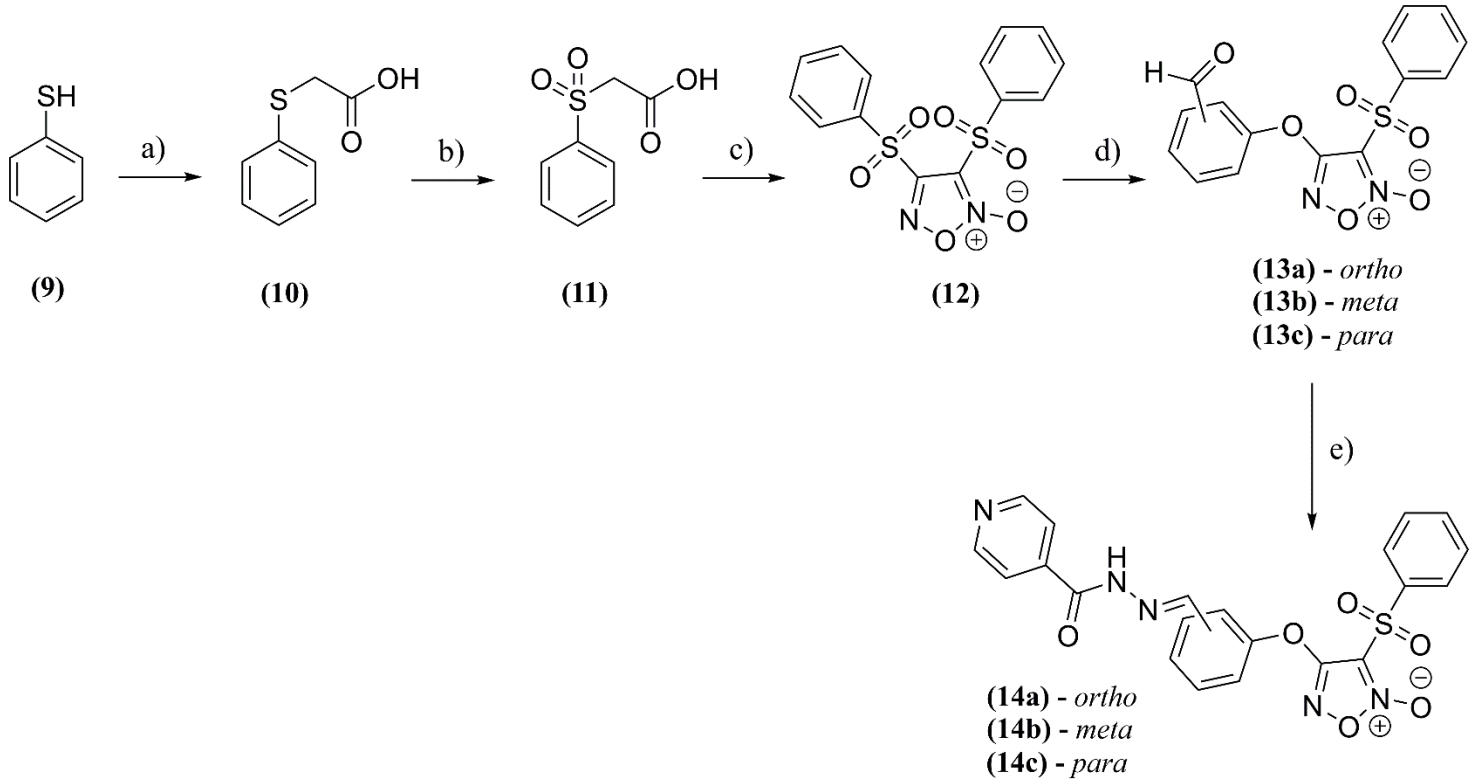
Chemistry



Scheme 2. Reagents and conditions: **(a)** monochloroacetic acid, NaOH, H_2O, 110 °C, 3 h; **(b)** hydrogen peroxide 30%, acetic acid, r.t., 24 h; **(c)** fuming nitric acid, acetic acid, 110 °C, 1 h; **(d)** 2, 3 or 4- hydroxybenzaldehyde, 1,8-diazabicycloundec-7-ene (DBU), anhydrous dichloromethane, r.t., 2 h; **(e)** isonicotinic hydrazide, ethanol, acetic acid, r.t., 12 h**.**

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Results and discussion

Compound	MIC_{90} (µM) – $H_{37}Rv$	IC_{50} (µM) for MRC-5	SI	MIC_{90} (µM) – MDR-TB [a]	LogP
4a	> 62.0	-	-	-	1.4
4b	> 62.0	-	-	-	1.3
4c	> 62.0	-	-	-	1.3
8a	> 62.0	-	-	-	2.7
8b	> 62.0	-	-	-	2.9
8c	11.82	623.44	52.74	24.3	2.9
14a	8.60	34.40	3.78	50.0	2.2
14b	1.61	30.10	14.13	21.3	2.3
14c	1.03	43.01	20.29	7.0	2.1
rifampicin	0.5	-	-	Res	-
Isoniazid	0.11	-	-	Res	-

[a] Resistance to isoniazid, rifampicin, streptomycin and ethambutol. Res, resistant.

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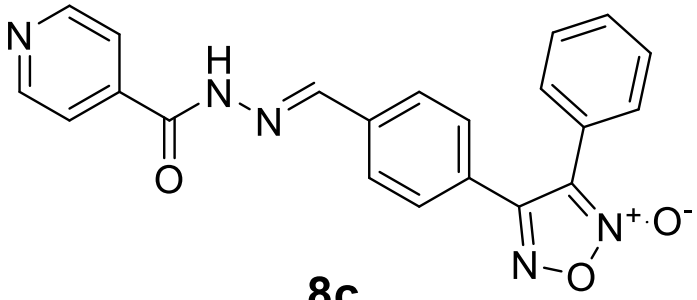
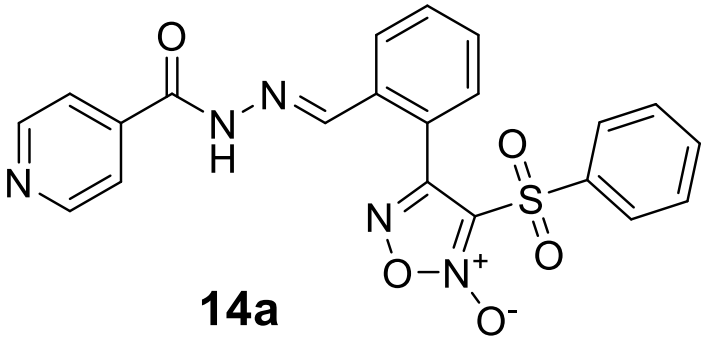
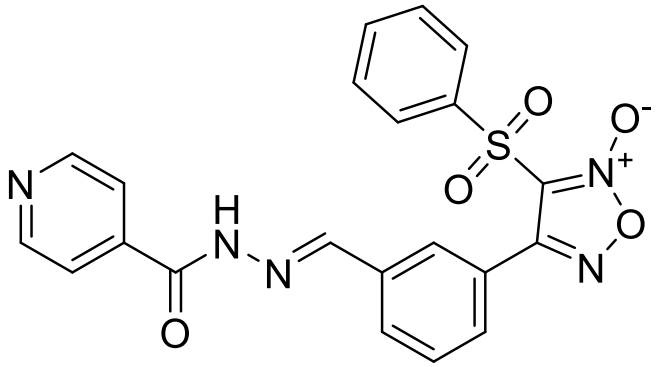
Results and discussion



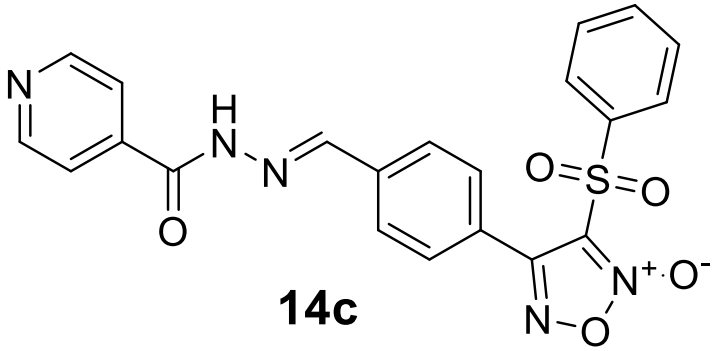
8c



14a



14b



14c

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Results and discussion

- Furoxan derivatives methyl (**4a-c**) and phenyl (**8a-b**) did not exhibit activity against MTB (MIC_{90} superior to 62 µM);
- Phenylsulfonyl furoxan series: *ortho* (**14a**), *meta* (**14b**) and *para* (**14c**) have shown promising activity against MTB with MIC_{90} values below 8.6 mM;
- The MIC_{90} values of these four compounds (**14a-c**; **8a**) were greater than several first and second line antitubercular drugs, such as pyrazinamide (>48 µM), cycloserine (245 µM) and kanamycin (3.4 µM);
- Compounds (**8c**; **14a-c**) showed MIC_{90} values ranging from 7.0 to 50.0 µM against a clinical isolate MDR strain, being the compound **14c** (MIC_{90} 7.0 µ M) the most promising among the series;

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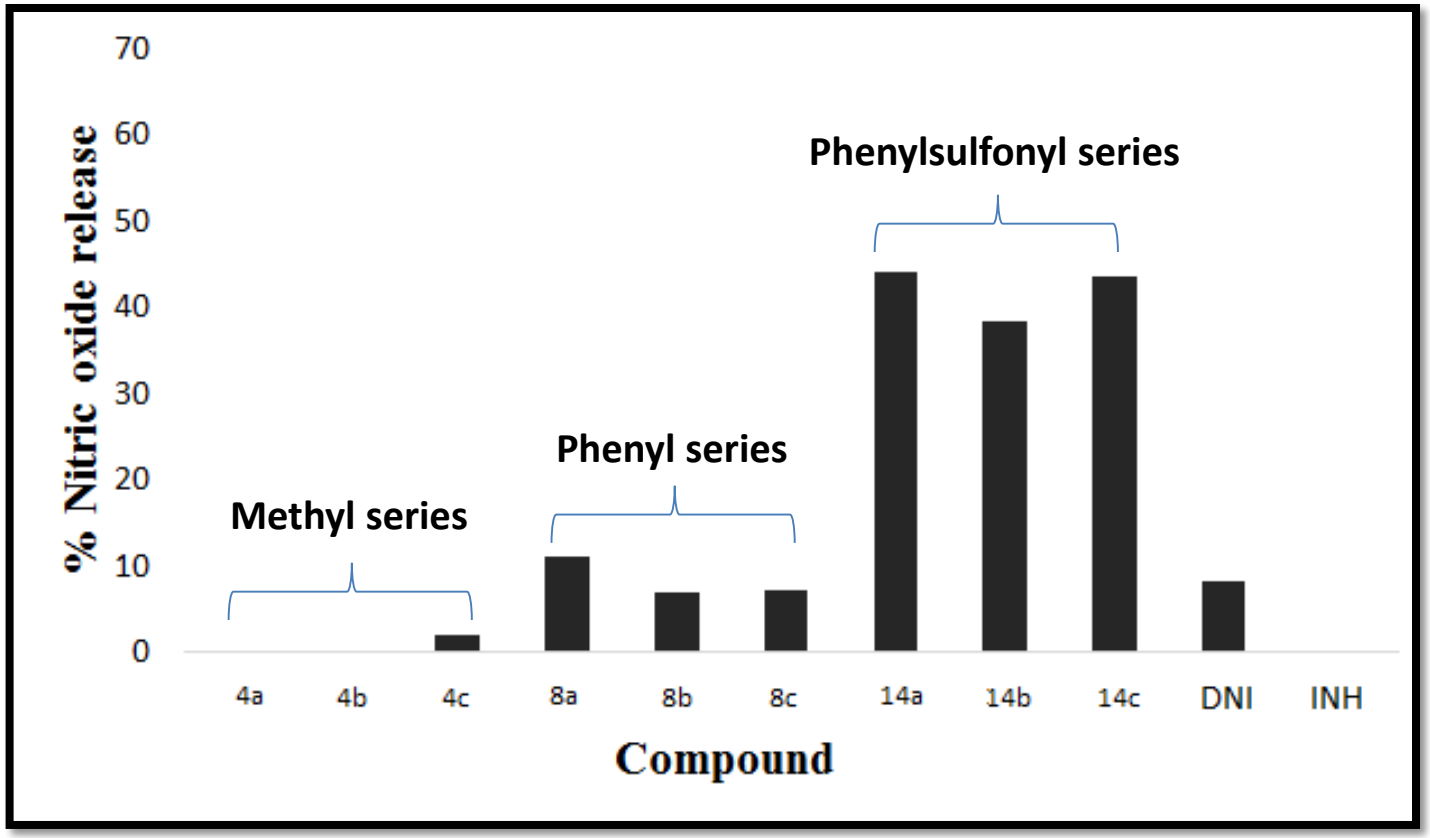
Results and discussion

- Nitric oxide release



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DNI, isosorbide dinitrate (positive control); INH, isoniazid.

Results and discussion

- All furoxan compounds were capable to generate nitrite in the medium at values ranging from of 0.16% - 44.23%;

- Our findings appoint that the antitubercular activity seems to be related in part, to the ability to release nitric oxide by the furoxan subunit;

- It was observed that phenylsulfonyl series (**14a-c**) showed the best antitubercular activity and generated high levels of nitric oxide, while the methyl series (**4a-c**), with low NO-release profile, demonstrated inferior antitubercular activity.

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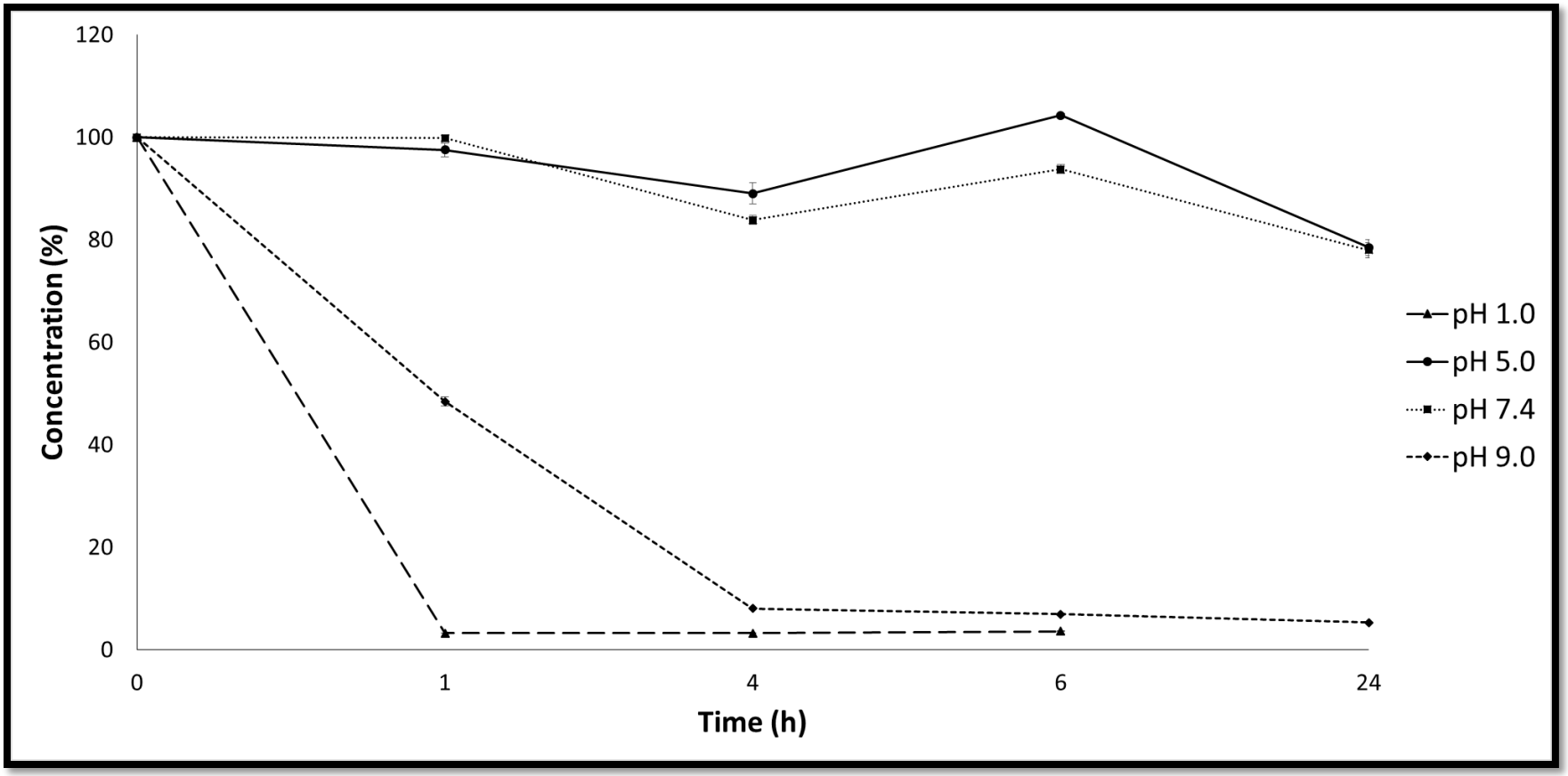
Results and discussion

- *In vitro* stability study



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Results and discussion

- The most promising compound (**14c**) was selected to analyze its chemical stability using an *in vitro* assay. We carried out the stability study under four conditions (pHs 1.0, 5.0, 7.4 and 9.0);
- Compound **14c** were unstable at pH 1.0 and 9.0 being degraded around 90% and 50% after the first hour, respectively;
- It was not detected significant chemical degradation at pH 5.0 (0%) and 7.4 (15%) after 6 h for compound **14c**;
- After 24 h, a reduction of 20% was observed at both pHs 5.0 and 7.4, showing a relative stability of compound **14c** in these pHs values.

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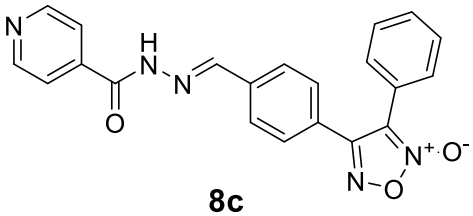
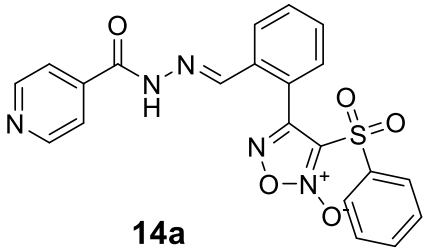
Conclusions

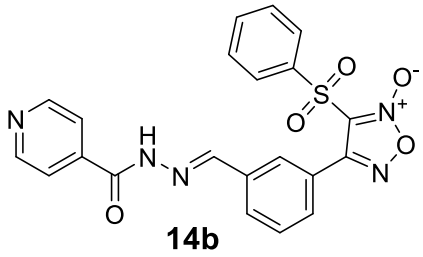
- Compounds (**8c** and **14a-c**) showed MIC_{90} values ranging from 1.03 to 11.82 µM and SI ranging from 3.78 to 52.74 (MRC-5);
- Compounds (**8c** and **14a-c**) presented activity against a clinical isolate MDR-TB strain with MIC_{90} values ranging from 7.0 to 50.0 µM;
- *In vitro* hydrolysis studies have demonstrated that compound **14c** is stable at pH 5.0 and 7.4 until 6 h;
- The results described here pointed out compounds **8c** and **14a-c** as novel lead compounds for the treatment of TB infection, including against resistant strain.



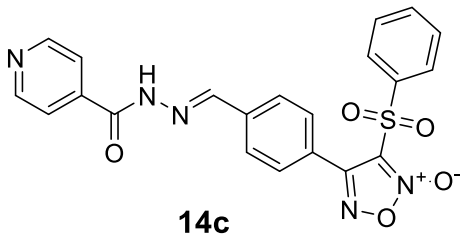
8c



14a



14b



14c

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Acknowledgments

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