3rd International Electronic Conference on Medicinal Chemistry
1-30 November 2017
chaired by Dr. Jean Jacques Vanden Eynde
sponsored by pharmaceuticals

Synthesis and Anti-*Mycobacterium tuberculosis* Activity of *N*-oxide Containing Heterocycles

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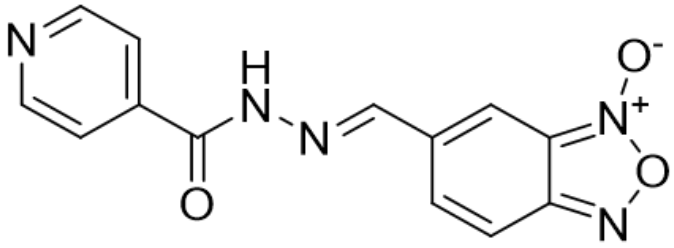
Synthesis and Anti-*Mycobacterium tuberculosis* Activity of *N*-oxide Containing Heterocycles



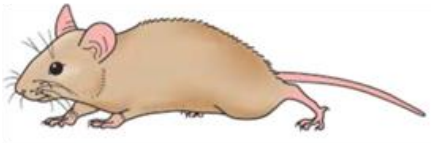
Compound 8

In vitro

MIC_{90} (Active) = 1.10 µM
MIC_{90} (Dormant) = 6.62 µM
MIC_{90} (INH-Resistant) = 8.59 µM
MIC_{90} (RMP-Resistant) = 3.78 µM
MIC_{90} (BDQ-Resistant) = 1.20 µM
IC_{50} (MRC-5) = 519.20 µM

In vivo

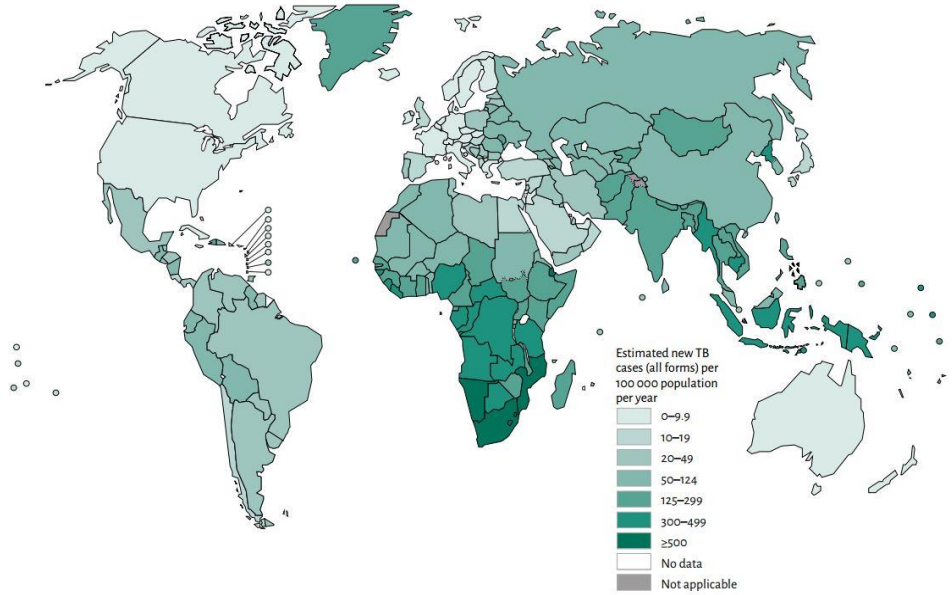
Sterilization



Abstract: Tuberculosis, caused by the *Mycobacterium tuberculosis* (*Mtb*), is the infectious disease responsible for the highest number of deaths worldwide. Herein, 22 new *N*-oxide-containing compounds were synthesized followed by in vitro evaluation of their antitubercular potential against *Mtb*. The compounds demonstrated MIC_{90} values ranging from 0.40 to 62 µM. Among the different heterocyclic compounds containing *N*-oxide, the benzofuroxan derivative 8 was found to be the most promising compound, with MIC_{90} values of 1.10 and 6.62 µM against active and non-replicating *Mtb*, respectively. Compound 8 was also active against monoresistant strains. Moreover, we performed *in vivo* experiments to confirm the safety and efficacy of compound 8; the compound was found to be orally bioavailable and highly effective leading to the reduction of the number of *Mtb* to undetected levels in a mouse model of infection. Microarray-based initial studies on the mechanism of action revealed an upregulation of a number of transcripts encoding proteins belonging to both small and large subunits of the ribosome, suggesting that compound 8 blocked the process of translation. Altogether, these results indicated benzofuroxan derivative 8 to be a promising lead compound for the development of a novel chemical class of antitubercular drugs.

Keywords: furoxan; benzofuroxan; quinoxaline 1,4-di-*N*-oxide; tuberculosis; antituberculosis agents.

Tuberculosis



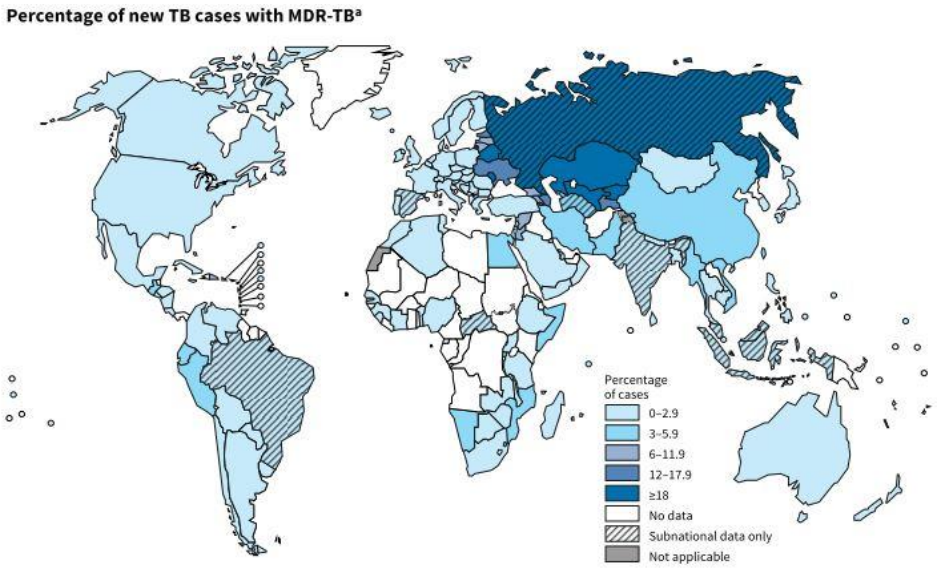
WORLD HEALTH ORGANIZATION. **Global tuberculosis report 2016**

Mycobacterium tuberculosis

- Infectious disease responsible for the largest number of deaths worldwide
- 2 million deaths in 2015
- 9.6 million new cases in 2015
- 12% of new cases in HIV-positive patients
- One third of the world's population infected

Multidrug-Resistant Tuberculosis

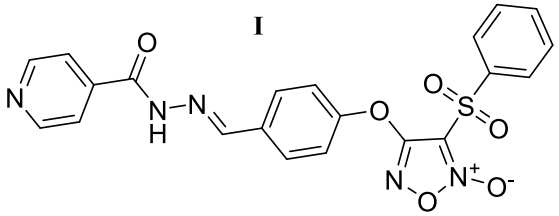
Extensive treatment and several side effects



- 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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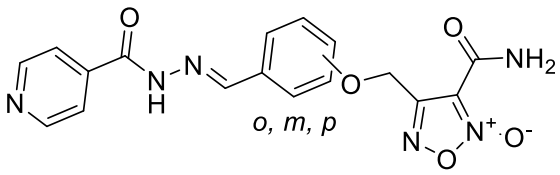
Eur. J. Med. Chem., v. 123, p. 523-531, **2016.**

MIC_{90} = 1.0 μM (H37Rv)
IC_{50} = 43.0 μM (MRC-5)

Series 1

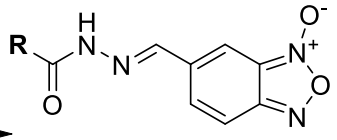


o, m, p

Furoxan derivatives

ortho - **4a**
meta - **4b**
para - **4c**

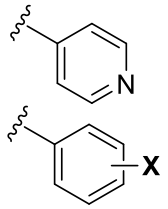
Series 2



(**8-17**)

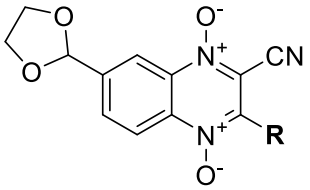
Benzofuroxan derivatives

R =



X = H, *t*-butyl, NO_2, OH, NH_2

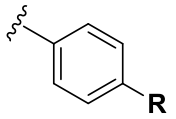
Series 3



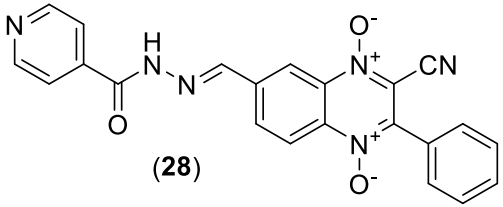
(**19-26**)

Quinoxaline 1,4-di-*N*-oxide derivatives

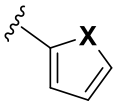
R =



R = H, CH_3, Cl, F, OCH_3, OCF_3



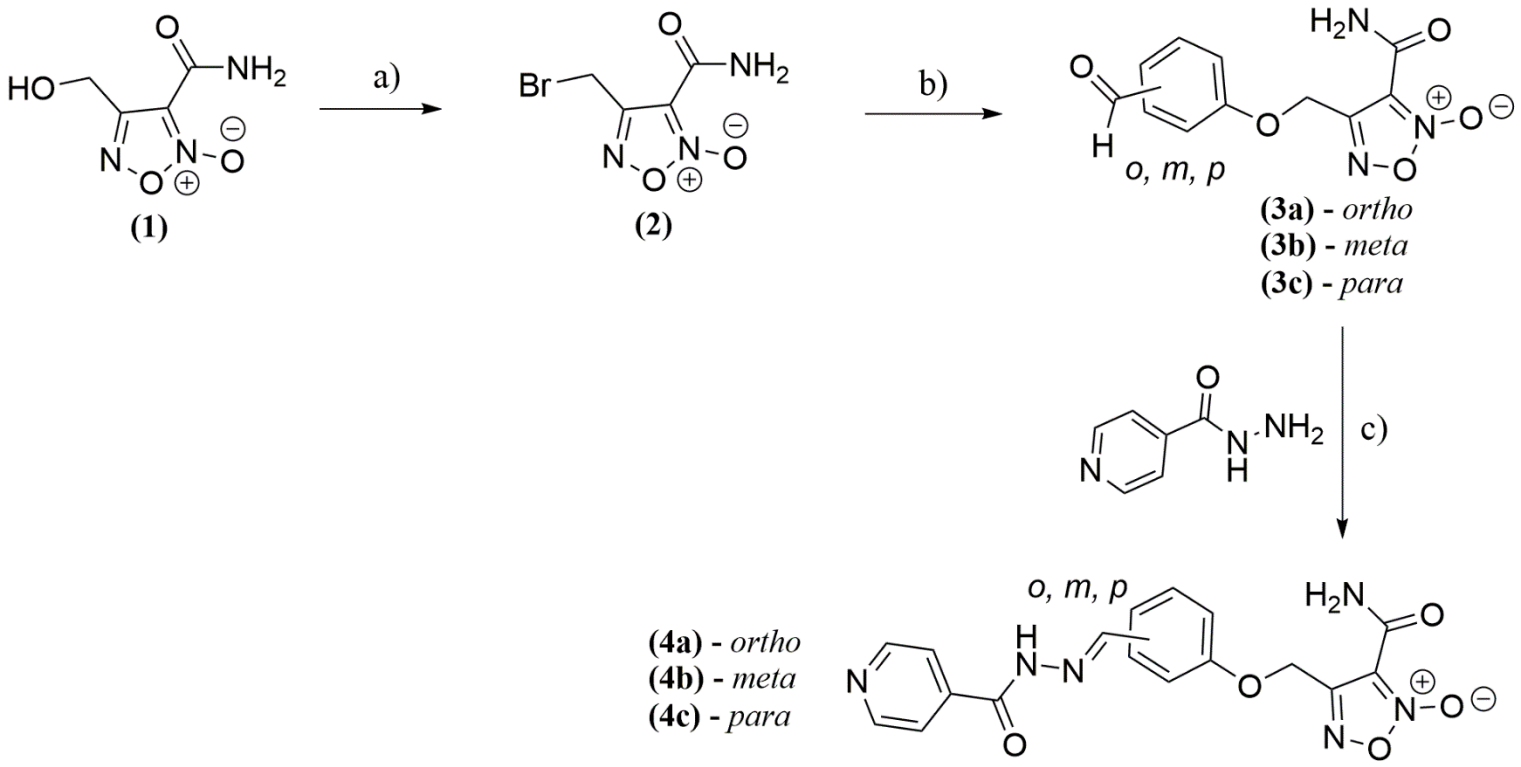
(**28**)



X = O, S

FERNANDES, G.F. *et al*. Design, Synthesis, and Characterization of N-Oxide-Containing Heterocycles with in Vivo Sterilizing Antitubercular Activity. **J. Med. Chem.** 2017.

Chemistry



Scheme 1. Reagents and conditions: **(a)** thionyl bromide, DMF, r.t., 30 min; **(b)** DBU, 2, 3 or 4-hydroxybenzaldehyde, DCM, r.t., 1 h; **(c)** ethanol, acetic acid, r.t., 12 h.

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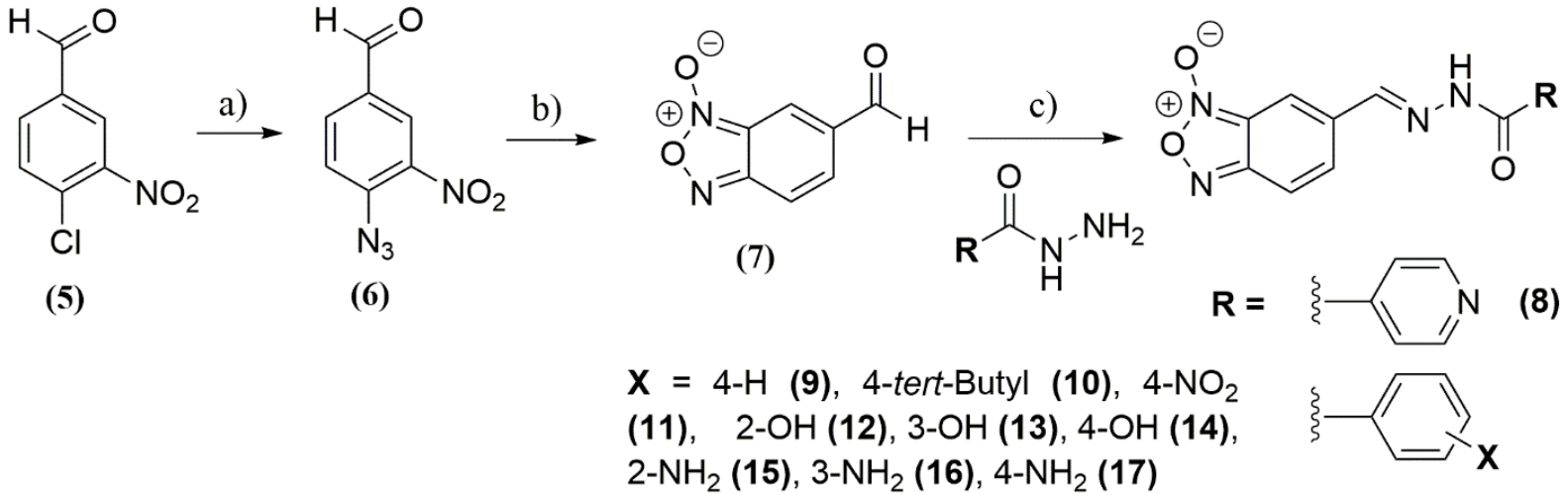
Chemistry



Scheme 1. Reagents and conditions: **(a)** NaN_3, DMSO, 75 ºC, 1 h; **(b)** toluene, reflux, 2h; **(c)** aromatic hydrazide, ethanol, acetic acid, r.t., 12 h.

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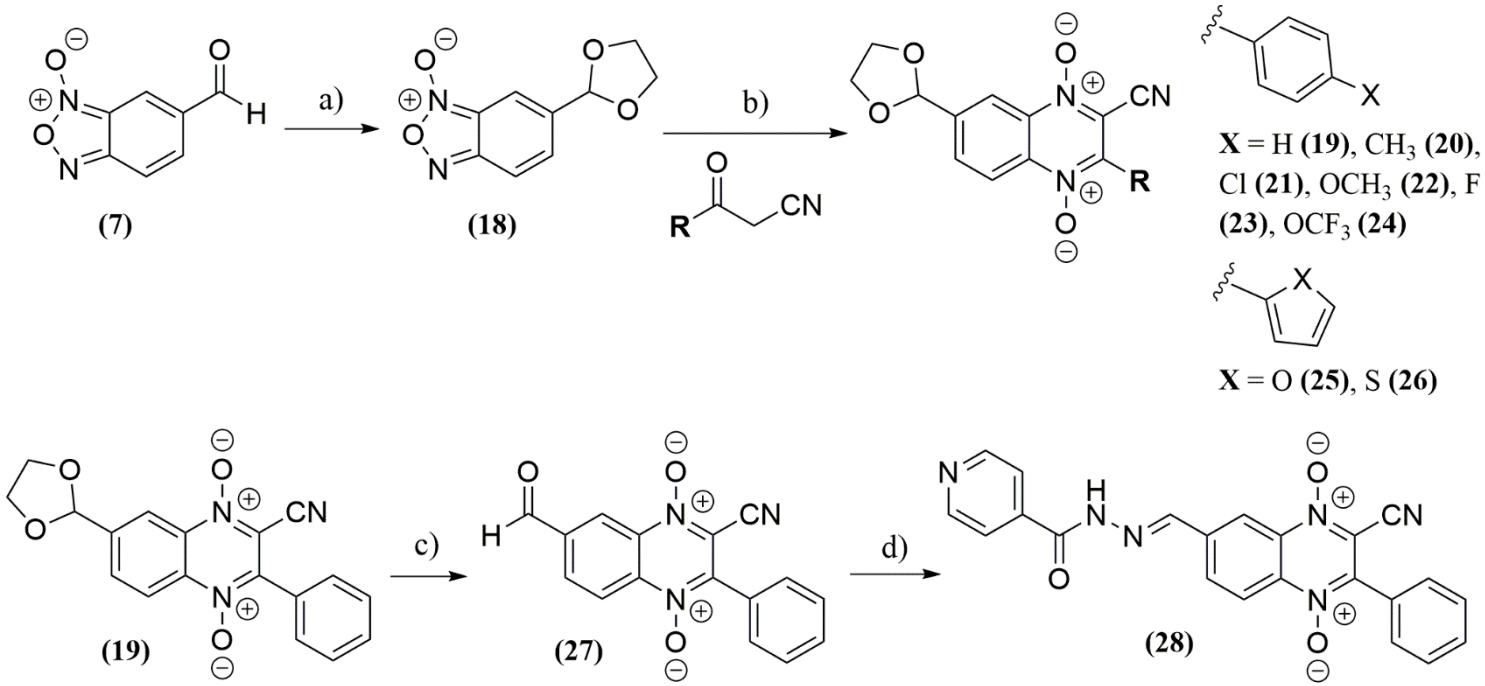
Chemistry



Scheme 1. Reagents and conditions: **(a)** toluene, ethylene glycol, *p*-toluenesulfonic acid, reflux, 12 h; **(b)** DCM, K_2CO_3, 40 ºC, 96 h; **(c)** acetone, HCl, r.t., 48 h; **(d)** isonicotinohydrazide, ethanol, acetic acid, r.t., 12 h.

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Results and discussion

➢ *Initial screening*

Compound	MIC_{90} (µM) – $H_{37}Rv$	IC_{50} (µM) for MRC-5	SI	MIC_{90} (µM) – Dormant TB	LogP
4a	0.42	854.00	2033.30	7.72	1.3
4b	0.40	1281.90	3204.70	4.20	1.3
4c	0.43	1159.50	2696.50	2.04	1.3
8	1.10	519.20	472.0	6.62	1.5
9	8.30	130.40	15.60	-	2.2
10	3.90	25.20	6.30	-	3.8
11	5.29	-	-	-	0.9
12	> 62.0	-	-	-	1.3
13	> 62.0	-	-	-	1.2
14	> 62.0	-	-	-	1.2
15	12.30	122.40	9.90	-	2.0
16	17.80	82.10	4.60	-	1.4
17	10.66	841.0	78.90	>10.0	1.2

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Results and discussion

➢ *Initial screening*

Compound	MIC_{90} (µM) – $H_{37}Rv$	IC_{50} (µM) for MRC-5	SI	MIC_{90} (µM) – Dormant TB	LogP
19	30.80	31.90	0.90	-	0.7
20	16.50	17.20	1.10	-	1.6
21	16.20	12.60	0.80	-	1.8
22	12.00	15.00	1.20	-	1.4
23	24.30	21.80	0.90	-	1.3
24	15.40	66.80	4.30	-	2.2
25	5.20	35.70	6.80	-	2.0
26	12.10	17.30	1.40	-	1.9
28	39.70	21.00	0.50	-	1.0
Isoniazid	0.1	-	-	-	-
Rifampicin	0.1	-	-	-	-

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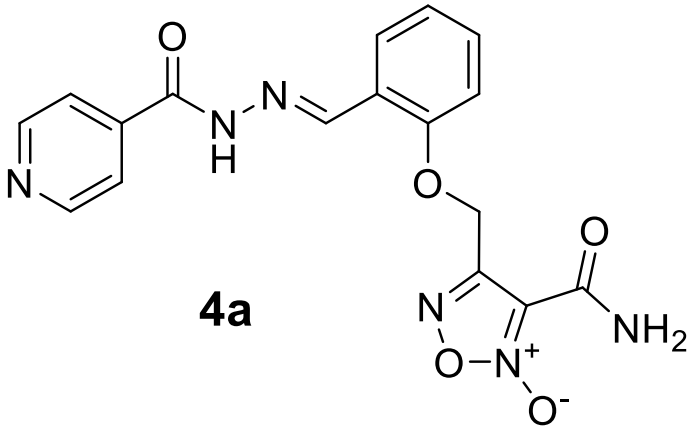
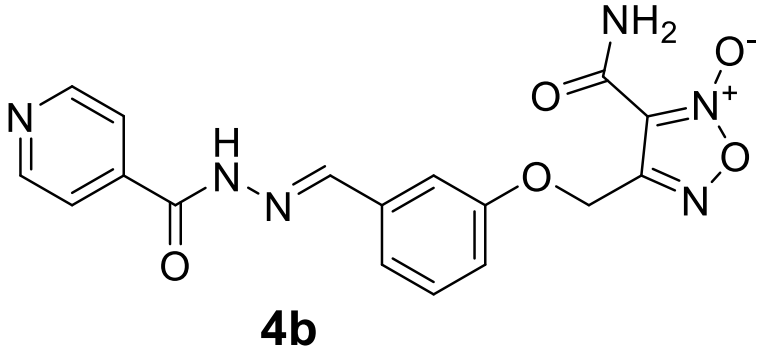
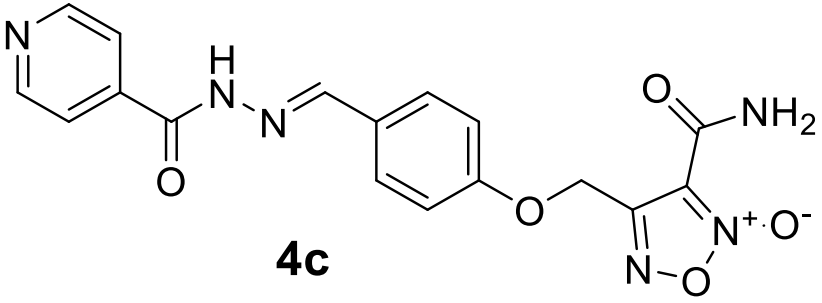
Results and discussion



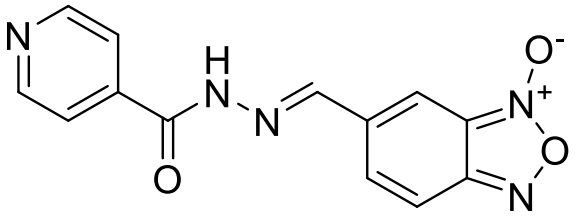
4a



4b



4c



8

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Results and discussion

- Benzofuroxan derivatives **9**-**17** and quinoxaline derivatives **19**-**28** were not active against MTB or were very cytotoxic;
- Amide furoxan series: *ortho* **4a**, *meta* **4b** and *para* **4c** have shown promising activity against MTB with MIC_{90} values below 0.43 µM. The same was observed for benzofuroxan derivative **8**, which presented MIC_{90} value of 1.1 µM;
- The MIC_{90} values of these four compounds (**4a-c**; **8**) were greater than several first and second line antitubercular drugs, such as pyrazinamide (>48 µM), cycloserine (245 µM) and kanamycin (3.4 µM);
- Additionally, these four compounds (**4a-c**; **8**) showed activity against dormant MTB with MIC_{90} values ranging from 2.04 – 7.72 µM.

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Results and discussion

➢ *Monoresistant strains*

Compound	MIC_{90} (µM) – INH resistant	MIC_{90} (µM) – RMP resistant	MIC_{90} (µM) – MOX resistant	MIC_{90} (µM) – BDQ resistant	MIC_{90} (µM) – CAP resistant	MIC_{90} (µM) – SM resistant
4a	>261.71	0.44	0.81	0.81	>261.71	27.4
4b	>261.71	2.31	1.22	2.56	>261.71	>261.71
4c	>261.71	1.99	0.66	6.38	>261.71	>261.71
8	8.59	3.78	5.72	1.20	15.25	16.98
RFP	0.01	>1.00	0.10	0.04	0.21	0.03
INH	>5.0	0.35	0.28	0.23	>5.00	>5.00
MOX	0.23	0.12	>8.00	0.26	0.35	0.36
BDQ	0.01	0.01	0.06	1.70	0.06	0.06
CAP	-	-	-	-	60.46	1.72
SM	-	-	-	-	2.55	>100

[a] RIF = rifampicin; INH = isoniazid; MOX = moxifloxacin; BDQ = bedaquiline; CAP = capreomycin; SM =streptomycin.

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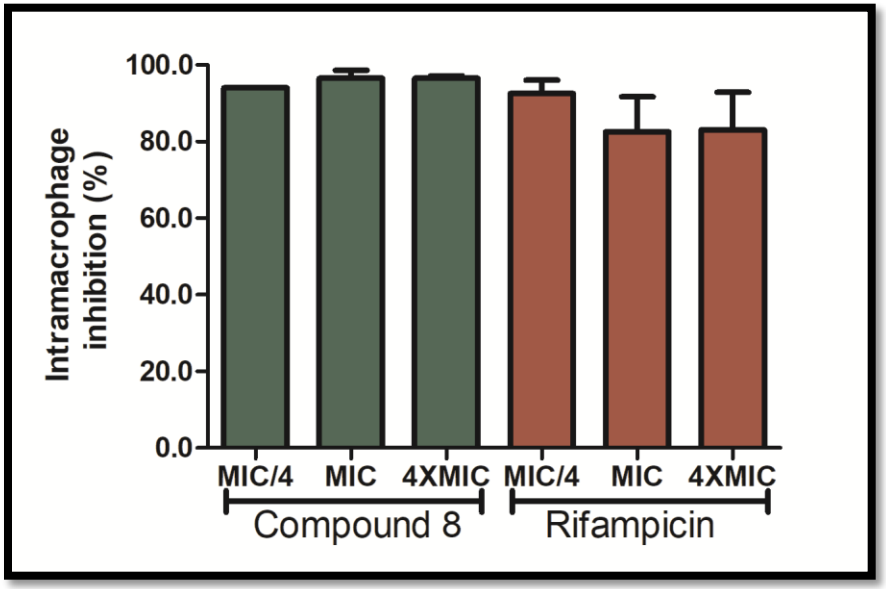
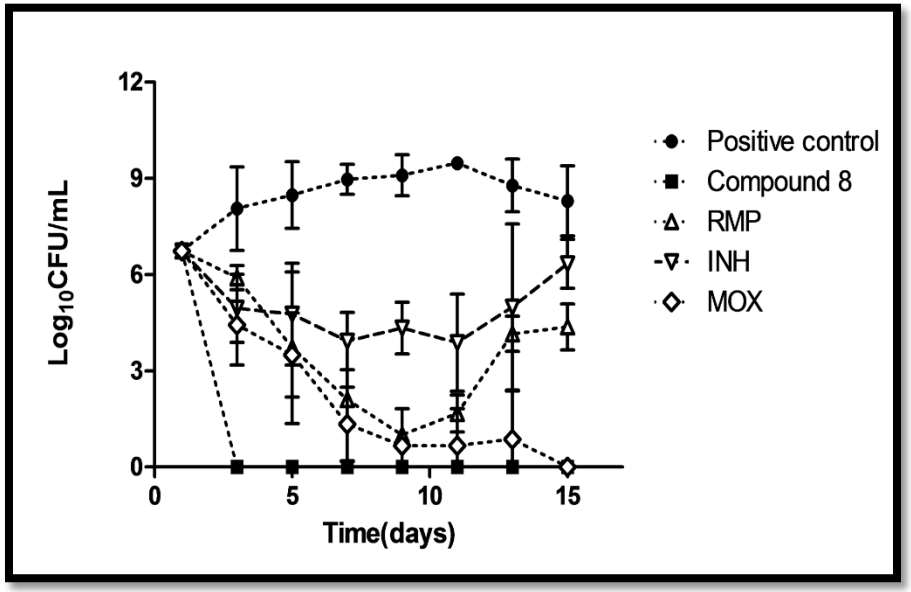
Results and discussion

➢ *Further evaluation*



❖ Intramacrophage activity of compound **8**



❖ Time-kill curves of compound **8**

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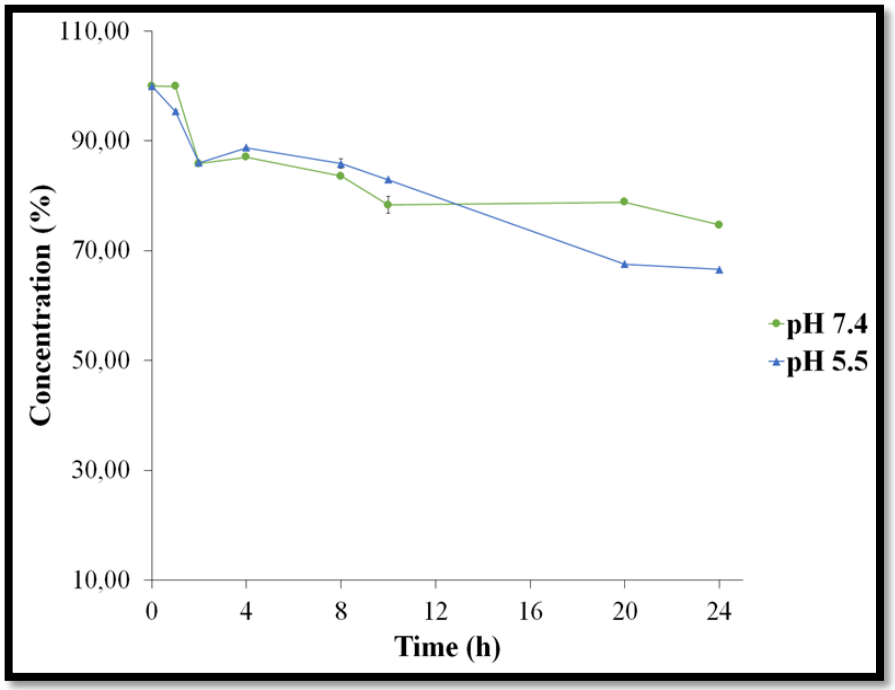
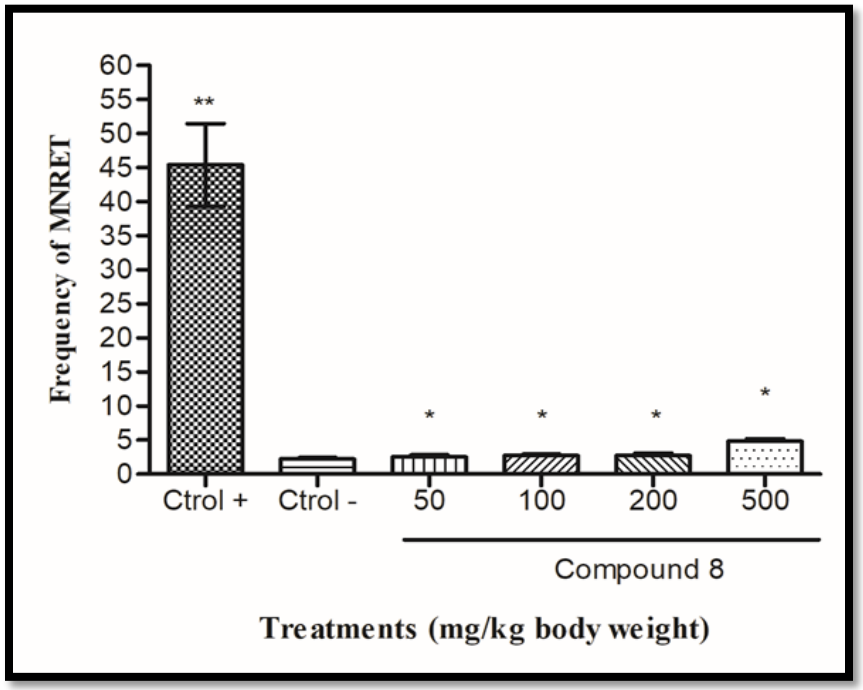
Results and discussion

➢ *Further evaluation*





❖ *In vitro* chemical stability of compound **8**

❖ *In vivo* micronucleus assay for compound **8**

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Results and discussion

- Intramacrophage inhibition assay revealed that benzofuroxan derivative **8** exhibited a high intracellular inhibition at all concentrations tested (around 90%);
- Time-kill kinetic experiments showed that compound **8** is bactericidal with an early bactericidal effect. Additionally, the benzofuroxan **8** was able to sterilize the cultures after 48 h of exposure;
- Compound **8** was stable at pH 7.4 and 5.5 being degraded around 20% and 30% after 24 hours, respectively;
- Micronucleus assay using mouse peripheral blood reticulocytes showed that compound **8** was not genotoxic at all concentrations tested.

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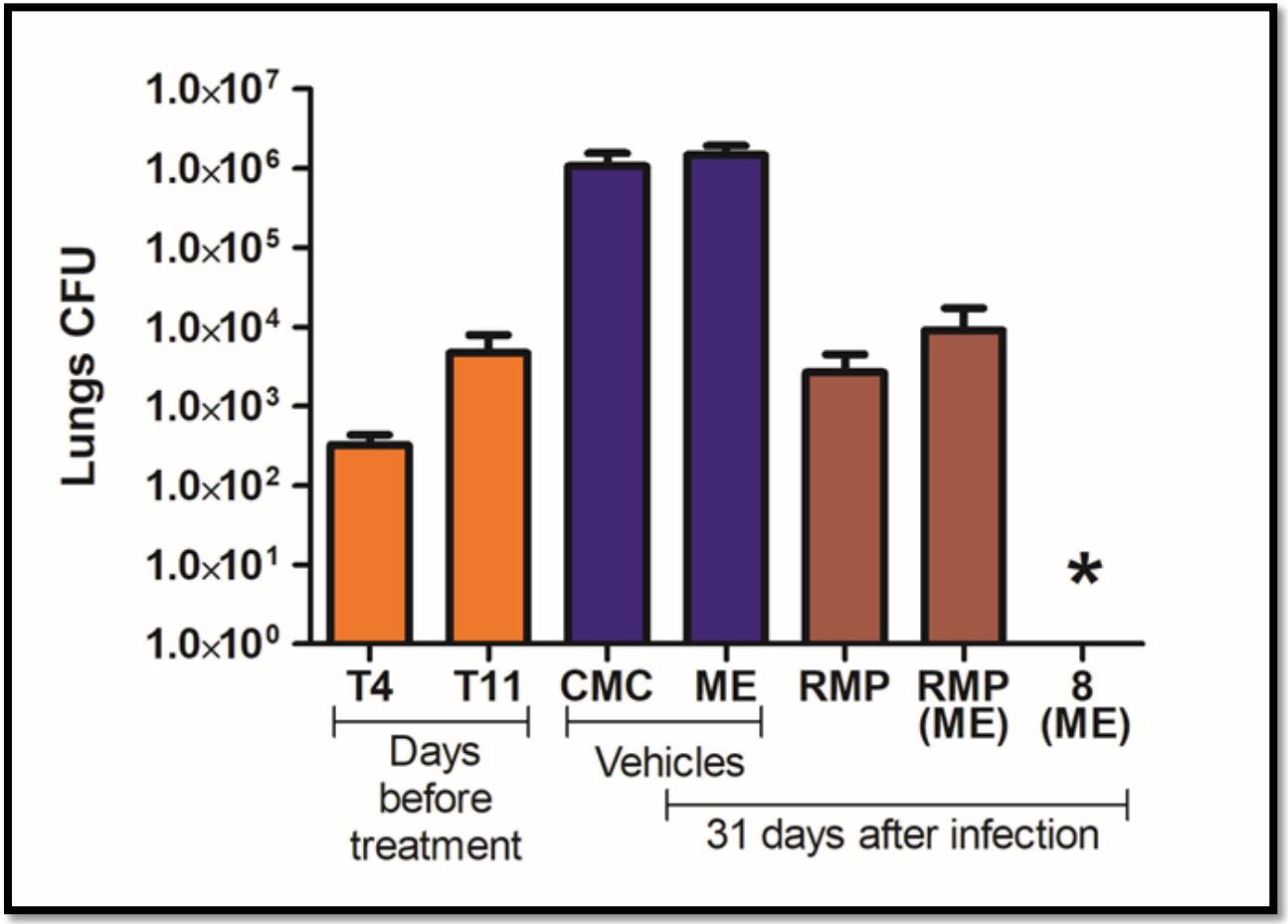
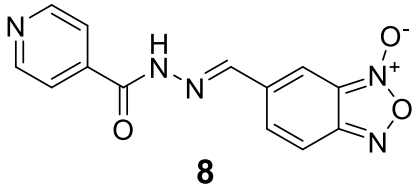
Results and discussion

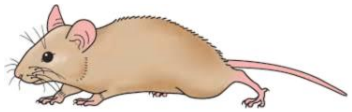
➢ *In vivo efficacy*





8

Microemulsion (ME)



Sterilization

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Conclusions

- Compound **8** showed MIC_{90} value of 1.10 µM against MTB H37Rv and IC_{50} of 519 µM against MRC-5 cells. Additionally, compound **8** was active against dormant *M. tuberculosis* and several monoresistant strains;
- Compound **8** was active against intracelular mycobacteria and showed bactericidal effect in the time-kill experiments. Moreover, compound **8** was stable at pH 7.4 and 5.5 and was not genotoxic in the micronucleus assay;
- *In vivo* infection model revealed that compound 8 was able to sterilize the *M. tuberculosis* from mice lungs;
- The results described herein pointed out compound **8** as a promising lead compound for the treatment of TB infection including against resistant strain.

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Acknowledgments

Lapdesf
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