



## REVIEW PAPER

DOI: <https://doi.org/10.20883/jms.218>

# Some nitroimidazole derivatives as antibacterial and antifungal agents in *in vitro* study

Justyna Żwawiak<sup>a</sup>, Dorota Olender<sup>b</sup>, Lucjusz Zaprutko<sup>c</sup>

Chair and Department of Organic Chemistry, Pharmaceutical Faculty, Poznan University of Medical Sciences, Poland

<sup>a</sup> <https://orcid.org/0000-0002-5420-6499>

<sup>c</sup> <https://orcid.org/0000-0003-1121-6272>

<sup>b</sup> <https://orcid.org/0000-0001-8320-5201>

### ABSTRACT

Nitroimidazoles have wide range of therapeutic uses mainly as anaerobic antibacterials and antiprotozoal agents. Some bicyclic nitroimidazodihydrooxazoles and nitroimidazotetrahydrooxazines are found to be antituberculosis agents. Hence, the biological and chemical properties of mentioned substances are of great interest to scientists. The aim of this review is to show the general knowledge concerning the chemistry and biological activity of some nitroimidazole derivatives, based on experimental studies. The results of biological tests provide many useful information on the effects of particular groups or other structural elements on the level of pharmacological activity. Also, these studies can be helpful in further planning of syntheses of active substances with using nitroimidazole moiety.

**Keywords:** nitroimidazoles, nitroimidazooxazoles, nitroimidazotetrahydropyrimidines, *M. tuberculosis*, antifungals.

## Introduction

Nitroimidazoles make a group of compounds of great commercial and pharmacological importance [1]. These compounds with a nitro group at position 5 are usually more active than the corresponding 4-nitro-derivatives. However, 4-nitroimidazoles exhibit lesser toxicity than 5-nitro-analogues. These effects are remarkable for 2,4- and 2,5-dinitroimidazoles. Moreover, 2-nitroimidazole derivatives are generally more active as radiosensitizers than metronidazole (5-nitro-derivative) [2]. Introduction of an electron accepting group at position 5 in the 4-nitroimidazole ring causes increase in cytotoxic and radiosensitizing activity [3]. Nitroimidazoles are vital particularly in the therapy of disorders caused by bacteria and protozoa. The best known – Metronidazole, is effective against *Bacteroides*, *Fusobacterium*, *Megasphaera*, *Clostridium*, sometimes

*Peptococcus* and *Helicobacter*. Tinidazole was found to be active against *Gardnella*, *Propionibacterium*, *Eubacterium*, *Campylobacter*, *Actinomyces* and *Spirochetes*. Moreover, some bicyclic nitroimidazooxazoles show considerable activity against tuberculosis [4, 5]. A series of bicyclic nitroimidazooxazoles, originally investigated as radiosensitizers for use in cancer chemotherapy, have been found to be active against culture replication *M. tuberculosis* [6, 7]. Compounds containing the imidazo[2,1-*b*][1,3]oxazine ring system have been shown to be active against tuberculosis as well. The most promising compound of this series PA-824, has the MIC of 0.06 µg/ml against *M. bovis* BCG and high activity against *Mtb* H<sub>37</sub>Rv [5, 8].

The aim of this work is to show our contribution to the current knowledge of antibacterial and antifungal activity of synthesized nitroimidazole derivatives.

## Tuberculostatic activity

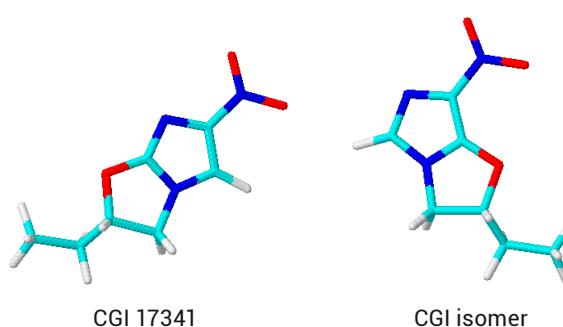
In the work on the syntheses of pharmacologically active nitroimidazole derivatives, a series of eight bicyclic 2,3-dihydro-7-nitroimidazo[5,1-*b*]oxazoles were performed [9].

These compounds were obtained in a one-pot reaction by treating 4,5-dinitroimidazole or 2-methyl-4,5-dinitroimidazole with appropriate oxiranes. Selected compounds were tested for antimycobacterial activity *in vitro*, against *M. tuberculosis* H<sub>37</sub>R<sub>v</sub>, *M. BCG*, *M. avium* and two "wild" strains, isolated from the tuberculous patients. The growth of strains was tested after 21 days. The lowest concentration of the compound investigated, at which no growth of strains was observed, was taken as the MIC. In the concentrations tested, these products showed no activity as well as isoniazid, especially against *Myc. BCG*, *Myc. tbc.* 1676, *Myc. tbc.* 456 and *Myc. avium*. Trying to explain of obtained results, simple analysis of the orientation of molecules of **CGI 17341** and their isomer was performed by PC GAMESS 7.0 program [10] (**Figure 1**). Comparison of these two molecules exhibited similarity in the distances between the oxygen atoms from oxazole rings and the alkyl chains in both of them. However, the distances between the NO<sub>2</sub> groups and the alkyl chains as well as the NO<sub>2</sub> groups and the oxygen atoms from oxazole rings were different and appeared to be an important factor in antitubercular agents. It was possible that the

orientation of the isomeric compound and similar products provided too little space for a receptor.

In the last decade, a considerable interest has been dedicated to potential drugs against *M. tuberculosis* [11, 12], including several bicyclic nitroimidazole derivatives characterized by significant MIC values, e.g. mentioned above pre-tomanid (PA-824) and delamanid (OPC-67683) (**Figure 2**). Nowadays, these compounds are undergoing clinical trials.

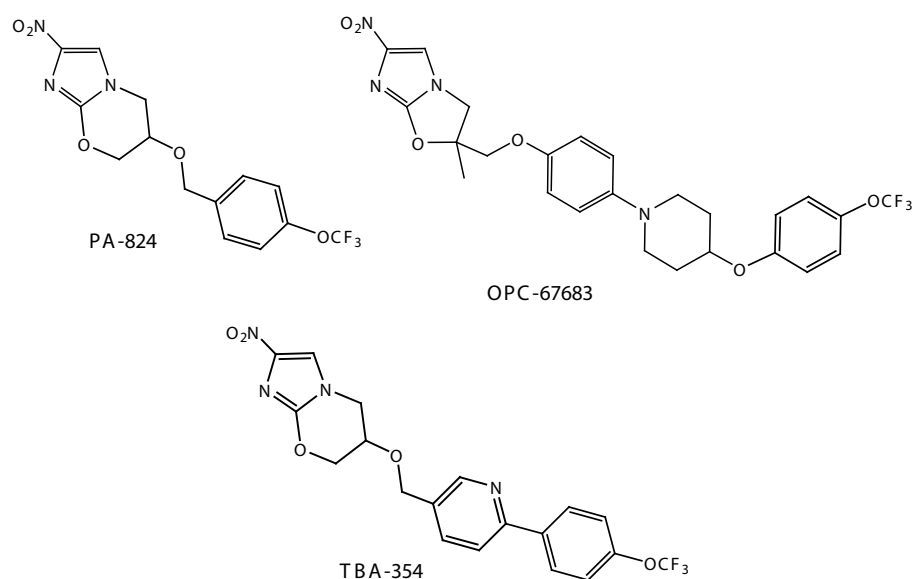
Other compounds e.g. TBA-354 emerged as the preferred candidate from a number of analogues that were extensively evaluated for activ-



The distances between essential groups or atoms:

	CGI 17341	CGI isomer
NO <sub>2</sub> – O (from oxazole ring)	4.67458 Å	3.28392 Å
NO <sub>2</sub> – CH <sub>3</sub>	7.11406 Å	6.05847 Å
O (from oxazole ring) – CH <sub>3</sub>	2.98517 Å	2.97649 Å

**Figure 1.** Analysis of the orientation of molecules of CGI 17341 and their isomer



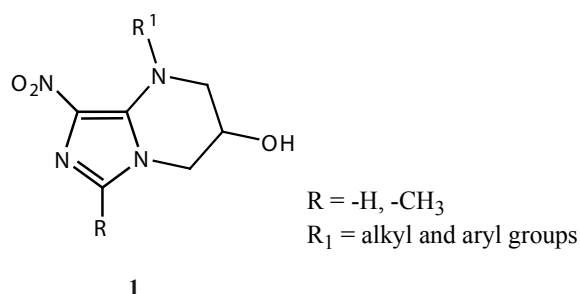
**Figure 2.** Structures of bicyclic nitroimidazoles as antitubercular drug candidates

ity in mouse models of chronic TB, and for pharmacokinetic, genetic and safety profiling. TBA-354 has recently been approved by the US Federal Drug Administration for clinical trial [13].

The initial Structure – Activity Relationship (SAR) studies have revealed that the replacement of the oxygen atom at C(9) position of tetrahydrooxazine ring from PA-824 with a methylene group results in the loss of antitubercular aerobic and anaerobic activities [14]. However, the C(9) position oxygen of the tetrahydrooxazine ring of PA-824 can be replaced by either nitrogen or sulfur with no significant reduction in MIC value in aerobic conditions, in comparison with MIC of the parent nitroimidazooxazine [15]. These results have encouraged us to synthesise a set of bicyclic, heterocyclic compounds, which are structural isomers of the structure of PA-824.

We decided to prepare 3-hydroxy-8-nitroimidazo[5,1-*b*]-1,4,5,6-tetrahydropyrimidine core in which a six-membered ring is connected with N-1 and C-5 atoms of nitroimidazole moiety, but not with N-1 and C-2 atoms [16]. Consequently, the oxazine part of the molecule was replaced with a diazine ring (**Figure 3**).

Moreover, these compounds, in most cases,



**Figure 3.** The structure of 3-hydroxy-8-nitroimidazo[5,1-*b*]-1,4,5,6-tetrahydropyrimidines

exhibited favorable calculated SAR parameters: rather high octanol – water partition coefficient (Log P) and low values (below 140 Å<sup>2</sup>) of Polar Surface Area (PSA) that is linked closely to higher bioavailability. This fact strongly prompted us to study 3-hydroxy-8-nitroimidazo[5,1-*b*]-1,4,5,6-tetrahydropyrimidines as potential new tuberculostatic substances. Some of these products were tested against *M. tuberculosis* 2441, 9656, 14023 – SM (streptomycin), INH (isoniazid), RFP (rifampicin) resistant and *M. tuberculosis* 5318 – SM, INH, RFP, EMB (ethambutol) resistant in

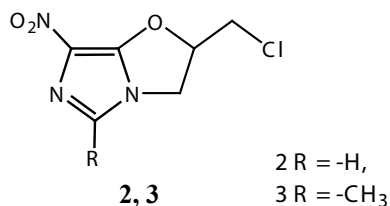
*in vitro* assays. Finally, biological assays *in vitro* were performed to determine the effects of chosen products on *M. tuberculosis* species. These new compounds were tested against four drug resistant strains: *M. tuberculosis* 5318 (res. SM, INH, RMP, EMB), *M. tuberculosis* 2441, 9656 and 14023 (res. SM, INH, RMP) using *M. tuberculosis* H<sub>37</sub>R<sub>v</sub> as a control to validate the assay results. The measured MIC's showed no inhibition activity even at the highest concentrations. For all products tested the MIC values were higher than 25 µg/mL.

While PA-824 and 3-hydroxy-8-nitroimidazo[5,1-*b*]-1,4,5,6-tetrahydropyrimidine core have structural similarities, their bioactivities against *M. tuberculosis* are completely different. As proved, binding a six-membered ring to the N-1 and C-5 atoms of nitroimidazole moiety, in contrast to N-1 and C-2 substitution, totally abrogated antitubercular activity. The lack of inhibition is probably connected with the specific orientation of this bicyclic molecule that may interfere with formation of e.g. hydrogen bonds with active sites of receptors. Moreover, they have different stereochemistry than PA-824 [17].

## Antifungal properties

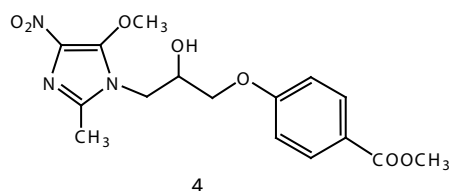
In our earlier works [16,18], substitution of the halogen atom in the –CH<sub>2</sub>X group in 2-chloromethyl-7-nitroimidazo[5,1-*b*]-2,3-dihydrooxazole system with phenols [18] and primary amines [16] has been described. The main feature of these syntheses is dihydrooxazole ring opening reaction. This mechanism has been used for forming new nitroimidazole derivatives with thiophenol and secondary amine moieties as a result of nucleophilic substitution reaction in 2-chloromethyl-7-nitroimidazo[5,1-*b*]-2,3-dihydrooxazole (**2**) and 2-chloromethyl-5-methyl-7-nitroimidazo[5,1-*b*]-2,3-dihydrooxazole (**3**) [19] (**Figure 4**).

Treatment of the nitroimidazodihydrooxazole with equimolar amount of amine or thiophenol furnished the products with one amino or thiopheno- group substituted at C-5 position of nitroimidazole ring. Increasing this ratio to 4 equivalents of nucleophile lead to form respective derivatives with two newly introduced cyclic moieties – the first one at C-5 position and the second one – at N-1 alkyl chain, as a result of



**Figure 4.** The structure of 7-nitroimidazo[5,1-*b*]-2,3-dihydroxazoles

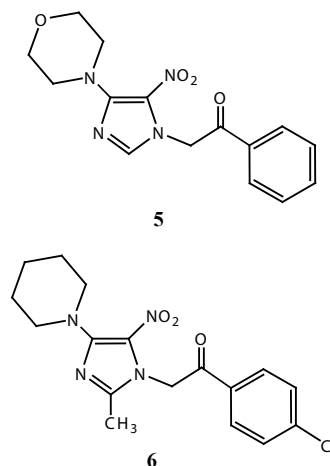
nucleophilic substitution of chlorine atom. It was found that some of these compounds are highly active against *Trichoderma viride*. Antifungal activity of these compounds was observed even after 21 days of the study. Additionally, moderate anti- *Trichoderma viride* activity was observed for few products. One compound (**4**) (**Figure 5**) showed the high efficiency relative to *Aspergillus niger*, *Penicillium funiculosum* and *Paecilomyces variotti* after 4 days of exposure. The results of visual assessment on 7<sup>th</sup> and 14<sup>th</sup> day showed that fungistatic properties decreased significantly. This fact can suggest diffusion of drug into malt agar. Among products tested, compound **4** was the most effective nitroimidazole derivative against mixture of fungi. Higher effectiveness probably was induced by the presence of methyl group at C-2 position of imidazole ring.



**Figure 5.** The structure of nitroimidazole derivative with the highest antifungal activity

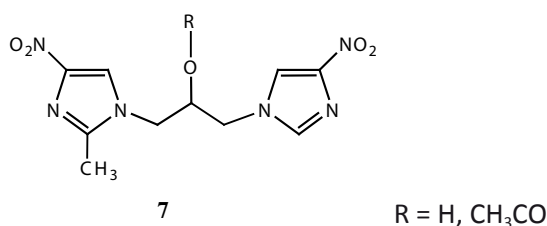
Some N-substituted nitroimidazole derivatives containing phenacyl group were tested for their antifungal activity. In the studies were used the standard nutrient method against *Sclerophoma pityophila*. The results of tests were expressed as the ED<sub>50</sub> (substances concentrations retarding the fungal growth rate by 50 percent in comparison with plates where the agent studied was absent), and the effective dose ED<sub>100</sub> (substances concentrations retarding the fungal growth rate by 100 percent in comparison with plates where the agent studied was absent). As it was shown in the cited work [20], all compounds are weakly

active against the fungi used. On the other hand, N-phenacyl derivatives **5** and **6** (**Figure 6**) showed high fungistatic activity (ED's < 25) against *S. pityophila*. High effectiveness was induced by the displacement of nitro group at 4-position on the imidazole ring to morpholine or piperidine rest. Also, the presence of a chlorine atom at 4-position on the phenyl ring on the N-phenacyl moiety influences the increase in antifungal activity.



**Figure 6.** The structures of N-phenacyl nitroimidazole derivatives with high fungistatic activity

Also, some derivatives of 1,3-bis-(1-imidazolyl)-2-hydroxy- (or acetoxy-) propane, for which synthesis was described in one of earlier works [21], now favorably undergoing antifungal test. The most effective among them is unsymmetrical acetoxy- derivative **7** (**Figure 7**).



**Figure 7.** The structure of derivatives of 1,3-bis-(1-imidazolyl)-2-acetoxypropane

## Conclusions

As shown results of our different chemical and biological studies, nitroimidazole derivatives are important group of bioactive compounds. Sev-

eral substituted nitroimidazoles are of considerable pharmacological significance, particularly as antifungal agents. Our results provide important information on the effects of certain substituents and structural elements on the occurrence of certain activity or lack thereof. Moreover these studies can be helpful in future planning of syntheses of active drugs with using nitroimidazole scaffold.

### Acknowledgements

#### Conflict of interest statement

The authors declare no conflict of interest.

#### Funding sources

There are no sources of funding to declare.

### References

1. Mital A. Synthetic Nitroimidazoles: Biological Activities and Mutagenicity Relationships. *Sci Pharm*. 2009 Aug;77:497–520.
2. Mukherjee T, Boshoff H. Nitroimidazoles for the treatment of TB: past, present and future. *Future Med Chem*. 2011 Nov;3(11):1427–1454.
3. Boyer JH. Nitroazoles. VCH Publishers Inc, Florida. 1986,165–166.
4. Clifton EB, Boshoff HIM, Dowd CF. Prospects for clinical introduction of nitroimidazole antibiotics for the treatment of tuberculosis. *Curr Pharm Design*. 2004 Oct;10:3239–3262.
5. Adib M, Sheibani E, Mostofi M, Ghanbary K, Bijanzadeh HR. Efficient highly diastereoselective synthesis of 1,8a-dihydro-7H-imidazo[2,1-b][1,3]oxazines. *Tetrahedron*. 2006 Apr; 62:3435–3438.
6. Agrawal KC, Bears K, Sehgal RK, Brown JN, Rist PE, Rupp WD. Potential radiosensitizing agents. Nitroimidazoles. *J Med Chem*. 1979;22:583–586.
7. Nagarajan K, Shankar R, Rajappa S, Shenoy ST, Costa-Pereira R. Nitroimidazoles XXI. 2,3-dihydro-6-nitroimidazo[2,1-b]oxazoles with antitubercular activity. *Eur J Med Chem*. 1989;24:631–633.
8. Otera J, Orita A. US Patent No. 7, 115, 736 B2 (2006).
9. Żwawiak J, Olender D, Zwolska Z, Augustynowicz-Kopec E, Zaprutko L. Synthesis of 2,3-dihydro-7-nitroimidazo[5,1-b]oxazoles as potential tuberculostatic agents. *Acta Pol Pharm*. 2008 Apr;65:229–233.
10. Nemukhin AV, Grigorenko BI, Granovsky AA. Molecular modeling by using the PC GAMESS program: From diatomic molecules to enzymes. *Moscow University Chemistry Bulletin*. 2004;45,75–102.
11. Thompson AM, Blaser A, Anderson RF, Shinde SS, Franzblau SG, Ma Z et al. Synthesis, reduction potentials, and antitubercular activity of ring A/B analogues of the bio-reductive drug (6S)-2-nitro-6-[4-(trifluoromethoxy)benzyl]oxy}-6,7-dihydro-5H-imidazo[2,1-b][1,3]oxazine (PA-824). *J Med Chem*. 2009 Feb;52:637–645.
12. Bhaumik K, Akamanchi KG. 2,4-Dinitroimidazole: Microwave assisted synthesis and use in synthesis of 2,3-dihydro-6-nitroimidazo[2,1-b]oxazole analogues with antimycobacterial activity. *J Heterocyclic Chem*. 2004 Jan;41:51–55.
13. Denny WA. TBA-354: A new drug for the treatment of persistent tuberculosis. *Chemistry in New Zealand* 2015;January:18–22.
14. Kim P, Zhang L, Manjunatha UH, Singh R, Patel S, Jiricek J et al. Structure-activity relationships of antitubercular nitroimidazoles. 1. Structural features associated with aerobic and anaerobic activities of 4- and 5-nitroimidazoles. *J Med Chem*. 2009 Mar;52:1317–1328.
15. Kim P, Kang S, Boshoff HI, Jiricek J, Collins M, Singh R et al. Structure-activity relationships of antitubercular nitroimidazoles. 2. Determinants of aerobic activity and quantitative structure-activity relationships. *J Med Chem*. 2009 Mar;52:1329–1344.
16. Zaprutko L, Żwawiak J, Zwolska Z, Augustynowicz-Kopec E, Bartoszak-Adamska E, Nowicki W. Synthesis, structure and biological evaluation of novel bicyclic nitroimidazole derivatives. *Arch Pharm Chem Life Sci*. 2012 Dec;345:463–467.
17. Żwawiak J, Kujawski J, Zaprutko L. Structure – tuberculostatic activity evaluation among isomeric bicyclic nitroimidazoles. *Acta Pol Pharm*. 2017; in press.
18. Żwawiak J, Gzella A, Zaprutko L. Reactions of nitroimidazodihydrooxazole with alcohols and phenols in the presence of potassium carbonate. *Pol J Chem*. 2009 Mar;83:1309–1315.
19. Żwawiak J, Perdoch W, Mazela B, Zaprutko L. Synthesis and antifungal properties of some nitroimidazole derivatives. *Int J Med Pharm Res*. 2014 Aug;2:622–633.
20. Olender D, Żwawiak J, Lukianchuk V, Lesyk R, Kropacz A, Fojutowski A, Zaprutko L. Synthesis of some N-substituted nitroimidazole derivatives as potential antioxidant and antifungal agents. *Eur J Med Chem*. 2009 May;44:645–652.
21. Tułeczki J, Zaprutko L. Synteza związków pochodnych 2-propanolu z układem nitroimidazolu. *Acta Pol Pharm*. 1984;3:281–292.

Acceptance for editing: 2019-03-13  
Acceptance for publication: 2019-03-29

#### Correspondence address:

Justyna Żwawiak  
Department of Organic Chemistry,  
Pharmaceutical Faculty,  
Poznan University of Medical Sciences, Poland  
6 Grunwaldzka Street, 60-780 Poznań, Poland  
phone: +48 618546678, fax: +48 618546680  
e-mail: jzwawiak@ump.edu.pl