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

Duel Anti-Inflammatory and Anti-Bacterial Effects of Phenylhydrazide and Phenylhydrazone Derivatives

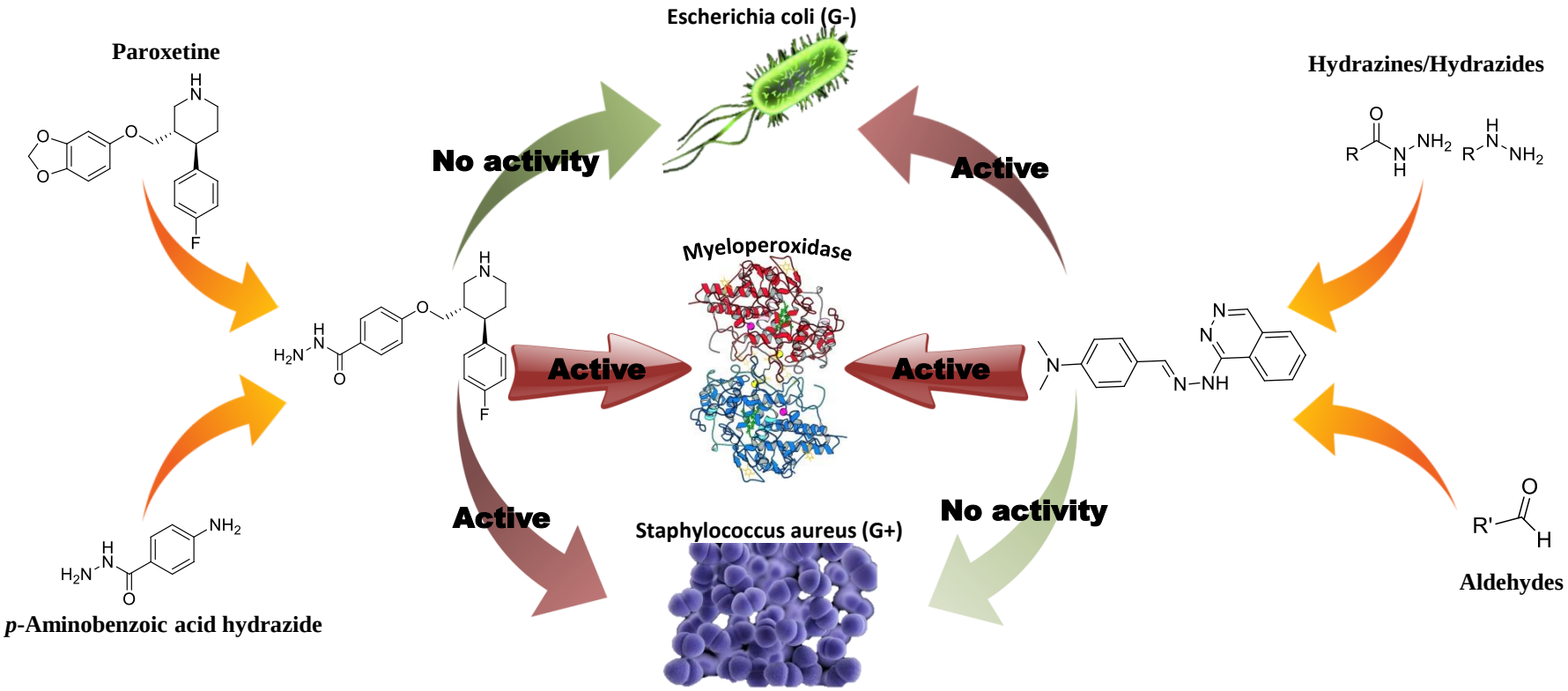
Jalal Soubhye[1,*], Franck Meyer [2], Pierre Van Antwerpen[1], Véronique Fontaine[3], Michel Gelbcke[2] and François Dufrasne[2]

[1] Pharmacognosie, Bioanalyse et Médicaments, Faculty of pharmacy, Université Libre de Bruxelles (ULB), Boulevard du Triomphe, 1050 Bruxelles, Belgium;
[2] Microbiology, Bioorganic and Macromolecular Chemistry, Faculty of Pharmacy, Université Libre de Bruxelles, Campus de la Plaine, Boulevard du Triomphe, 1050 Bruxelles, Belgium.

* Corresponding author: jsoubhye@ulb.ac.be

ULB UNIVERSITÉ LIBRE DE BRUXELLES

Duel Anti-Inflammatory and Anti-Bacterial Effects of Phenylhydrazide and Phenylhydrazone Derivatives



Abstract:
The implementation of dynamic combinatorial libraries allowed to obtain several compounds derived from aromatic hydrazone with high activity on MPO. These inhibitors were found to be reversible and irreversible inhibitors of MPO at the nanomolar level. Docking experiments highlighted the interaction between the most active ligands and MPO, and further kinetic studies defined the mode of inhibition of these compounds. *In vivo* evaluation showed that one dose of irreversible inhibitors is able to suppress the activity of MPO after inducing inflammation.
Starting from benzoic acid hydrazide and paroxetine, a new series of compounds were designed and synthesized. These compounds have shown very high activity on MPO with IC_{50} of 12-900 nM. The mechanism of action experiments has demonstrated that these inhibitors inhibit MPO irreversibly.
Finally, hydrazide and hydrazine derivatives were tested as anti-bacterial agents. Surprisingly, all hydrazone derivatives showed high activity against Gram (-) bacteria and low activity on Gram (+). In contrast, hydrazide derivatives showed very high potency against Gram (+) but no effect was found on Gram (-).
Keywords: Anti-Inflammatory; Anti-Bacterial; Myeloperoxidase; Phenylhydrazide; Phenylhydrazone

Introduction

Inflammation

Inflammation is an ensemble of complex of biological defensive reactions carried out by the organism against harmful stimuli:

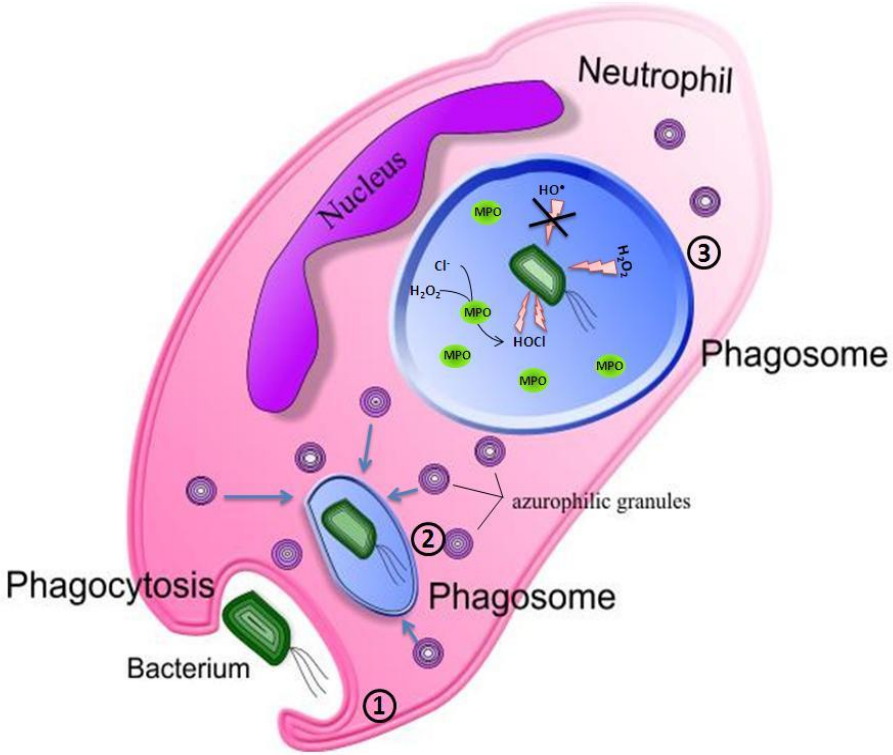
- Pathogens
- Irritants
- Damaged cells

Cardinal signs of inflammation are:

- Pain
- Redness
- Immobility (loss in function),
- Swelling
- Heat

Introduction

Myeloperoxidase

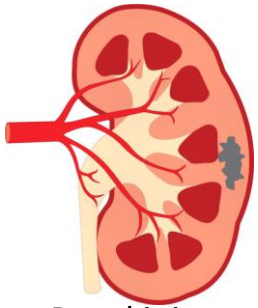


- The heme enzyme myeloperoxidase is a lysosomal protein that plays an important role in innate immunity system. It is expressed in neutrophils and stored in their azurophilic granules.
- After phagocytosis of pathogens by the neutrophils, MPO produces a powerful oxidizing agent HOCl from $H_2O_2$and Cl^- which leads to the oxidation (degradation) of biomolecules of pathogens in the phagosome.

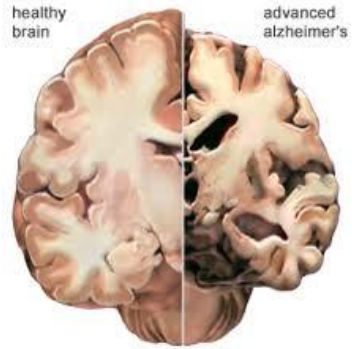
Klebanoff. *J.Leukoc.Biol.* **2005**, 77

Introduction

Myeloperoxidase and inflammatory syndromes

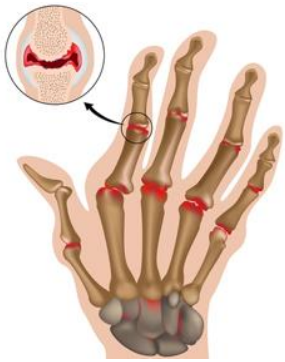


Renal injury

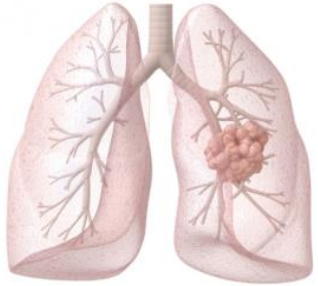


Alzheimer

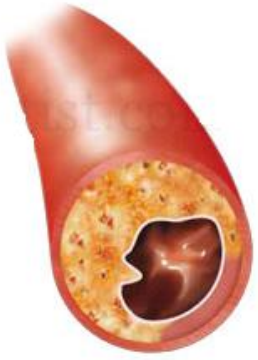
- In some cases, MPO is released from neutrophils producing HOCl in the circulation which results in oxidative damages for the host tissues.
- These damages sometimes contribute to the development of injuries in several organs or systems such as kidney, central nervous system, articulations, lung and cardiovascular system



Rheumatoid arthritis



Lung injury

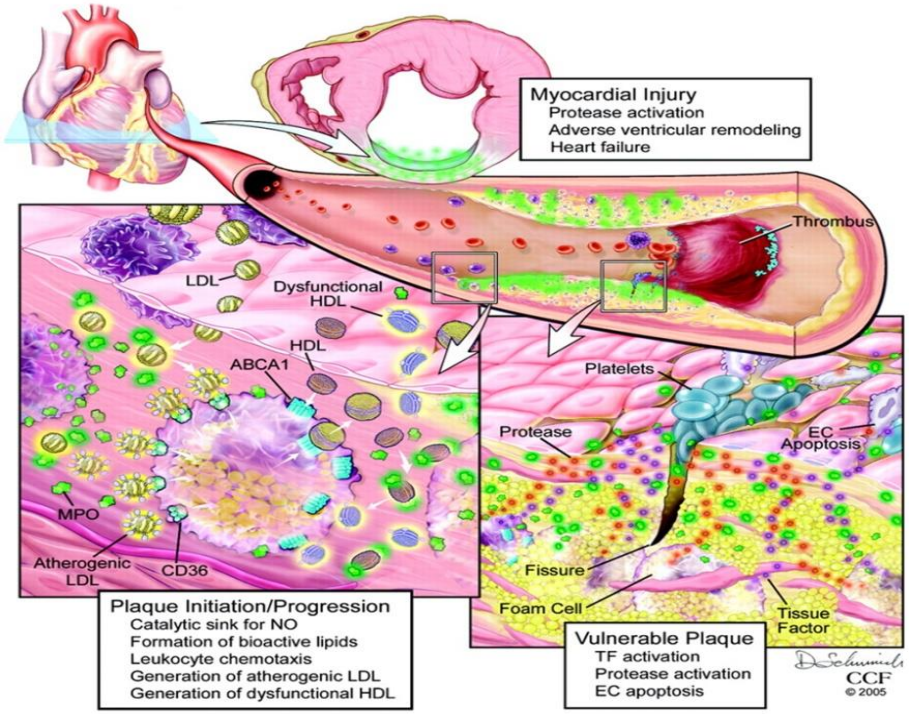


Atherosclerosis

Klebanoff. *J.Leukoc.Biol.* **2005**, 77

Introduction

Myeloperoxidase and atherosclerosis



The close relation between MPO activity and cardiovascular diseases prompted the study of the roles of MPO in atherosclerosis. It is found that MPO contributes to development of atherosclerosis by several effects:

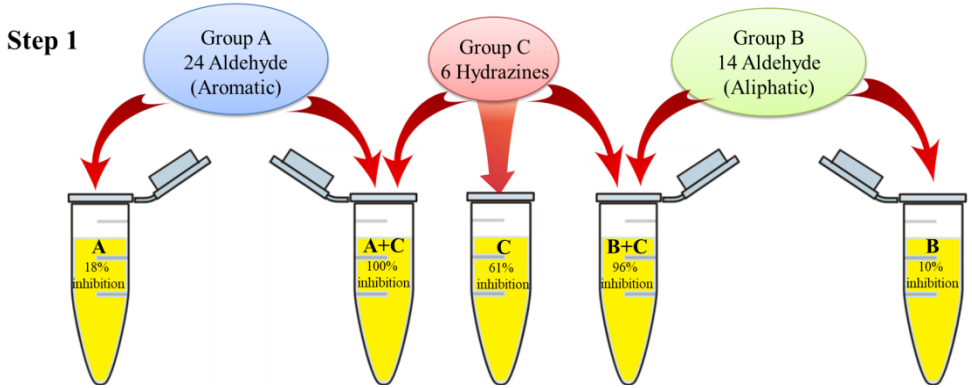
- Oxidation of low-density lipoproteins (LDLs) → inflammatory response in monocytes → foam cells.
- oxidation of high-density lipoproteins (HDLs) → decrease in capacity in removing the cholesterol from atherosclerotic lesions.
- Dysfunction of endothelial → vulnerable plaques.

Nicholls and Hazen. *Arteriosclerosis, thrombosis, and vascular biology*. **2005**, 25

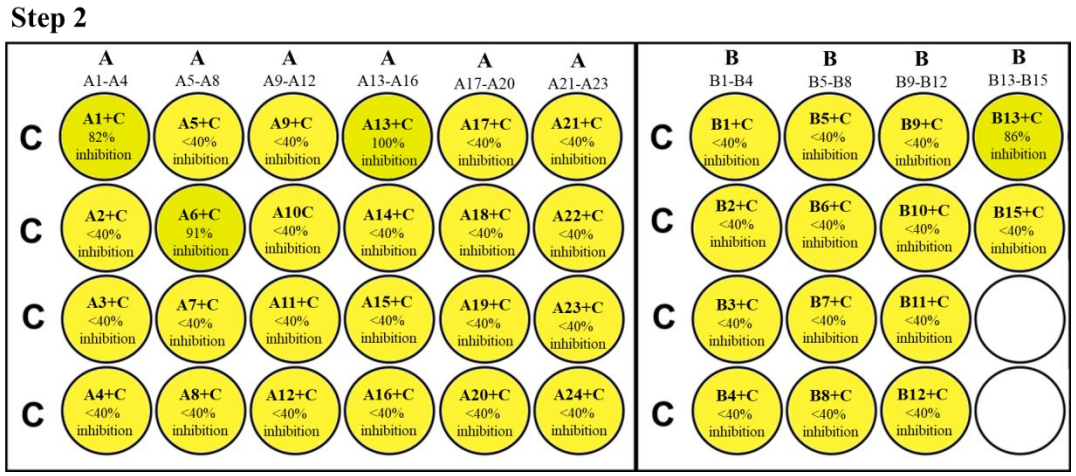
Results and discussion (Part 1)

Design of Reversible and Irreversible Myeloperoxidase Inhibitors: from Dynamic Combinatorial Library

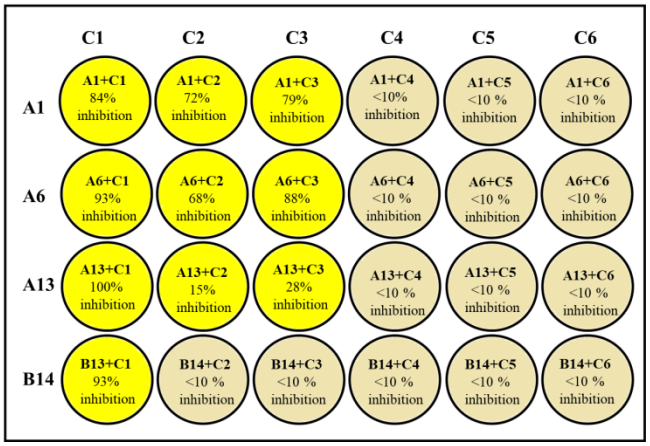
Combinatorial procedure



Step 1 : Tube A contains the 24 aldehydes of group A and MPO, tube B contains the 14 aldehydes of group B and MPO, tube C contains the 6 hydrazines of group C and MPO, tube A-C contains the 23 aldehydes of group A and the 6 hydrazines of group C and MPO, tube B-C contains the 15 aldehydes of group B and the 6 hydrazines of group C and MPO.



Step 2 : in a 96-well plate the group C was added to one of the compounds of group A and B.

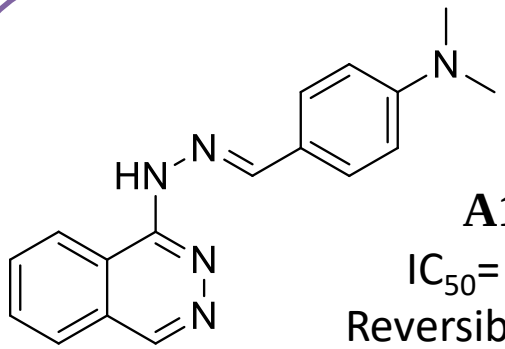


Step 3 : one of the best aldehydes was added to one of the hydrazine compounds.

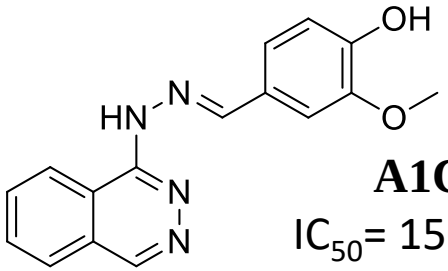
Results and discussion (Part 1)

Design of Reversible and Irreversible Myeloperoxidase Inhibitors: from Dynamic Combinatorial Library

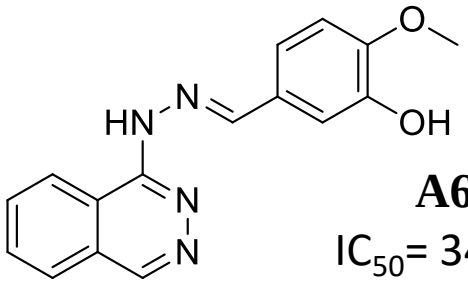
The best inhibitors



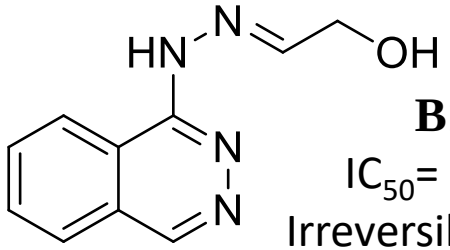
A13C1
IC_{50}= 80 nM,
Reversible inhibitor



A1C1
IC_{50}= 150 nM,
Irreversible inhibitor



A6C1
IC_{50}= 340 nM,
Irreversible inhibitor

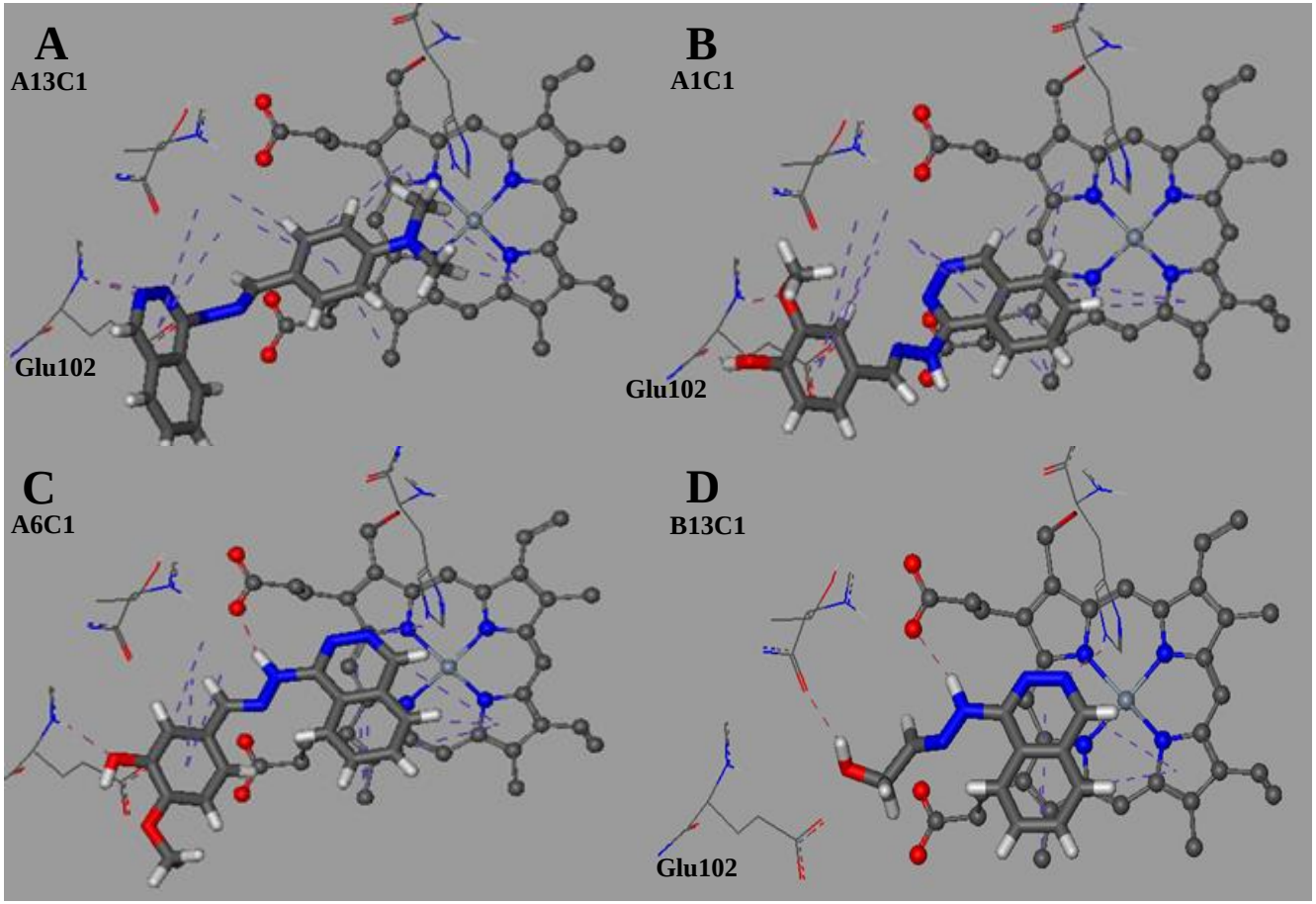


B13C1
IC_{50}= 110 nM,
Irreversible inhibitor

Results and discussion (Part 1)

Design of Reversible and Irreversible Myeloperoxidase Inhibitors: from Dynamic Combinatorial Library

Docking experiments for the best inhibitors



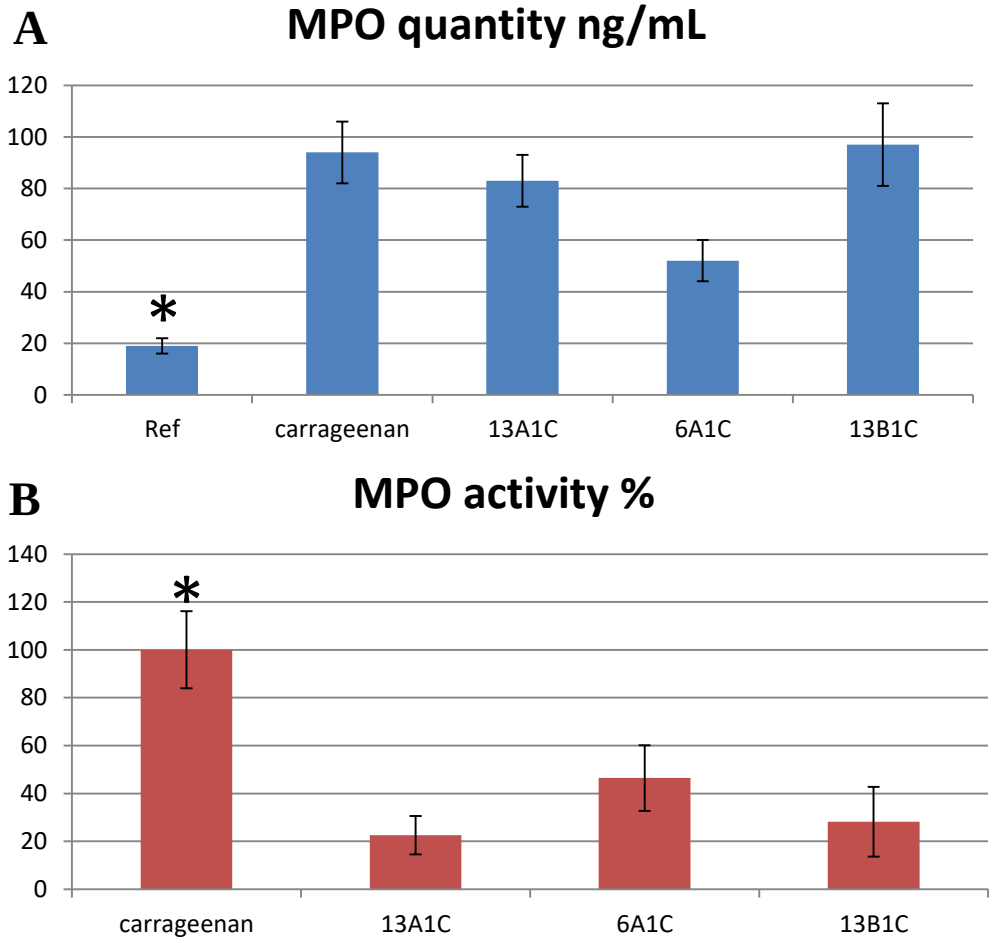
Comparison of best-scored docking poses of the hydrazones derived from hydralazine. (A) Compound **A13C1** : stacking pose on the heme (phenyl of the aldehyde; for other derivatives this is the aromatic ring of hydralazine). (B) compound **A1C1**, (C) compound **A6C1**, (D) compound **B13C1**.

Results and discussion (Part 1)

Design of Reversible and Irreversible Myeloperoxidase Inhibitors: from Dynamic Combinatorial Library

***In vivo* test**

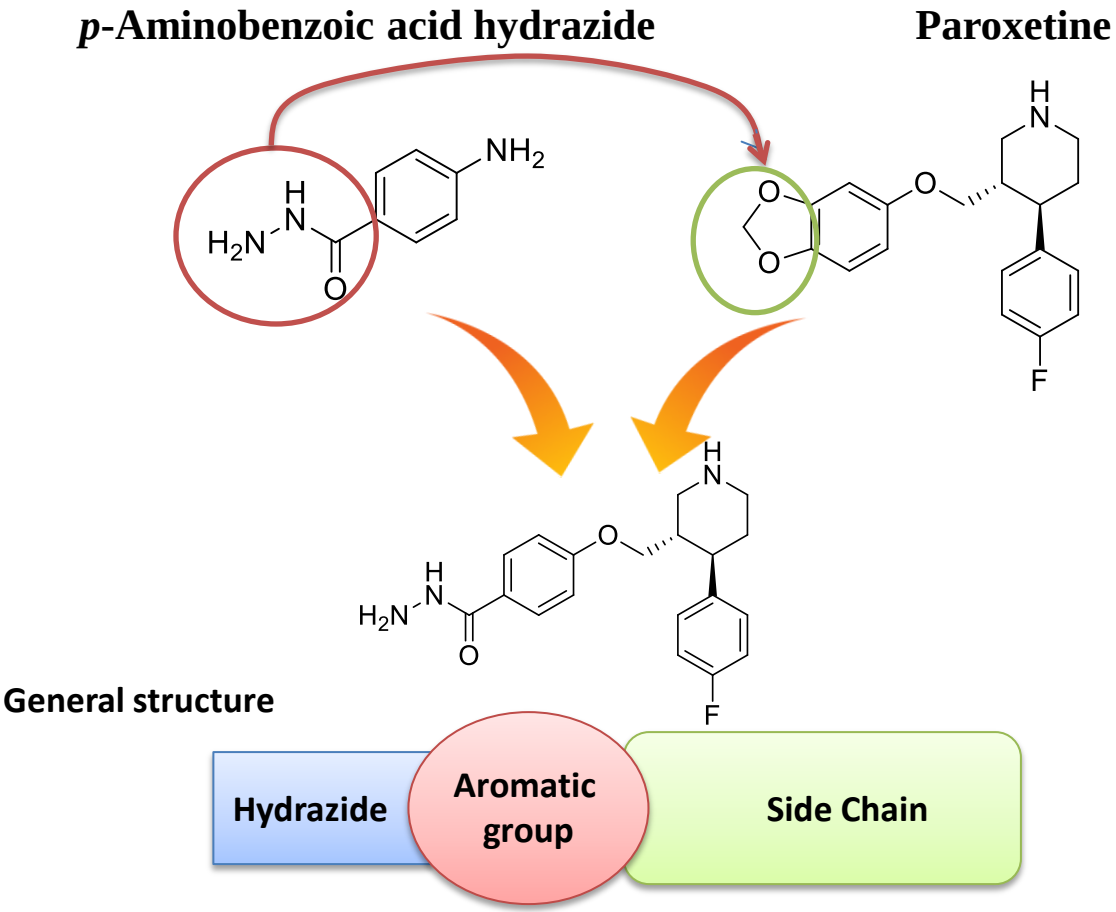
Determination of MPO concentration collected in the peritoneal liquid of rats (top). Measurement of MPO activity collected in peritoneal liquid after 48 h of drug administration (bottom). (*) MPO concentration in ref group is significantly lower than the other groups, and the activity of MPO in the carrageenan group is significantly higher than in the other groups ($P < 0.001$, Shapiro–Wilk test).



Results and discussion (Part 2)

Ligand-Based Design of Irreversible Myeloperoxidase Inhibitors Starting from Paroxetine and p-Aminobenzoic Acid Hydrazide

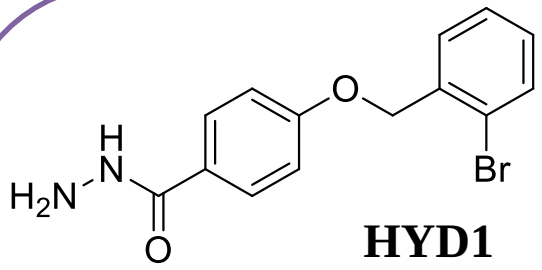
Drug design



Results and discussion (Part 2)

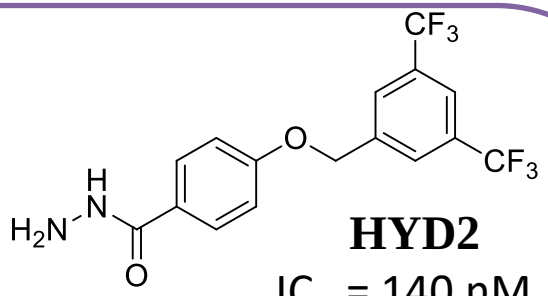
Ligand-Based Design of Irreversible Myeloperoxidase Inhibitors Starting from Paroxetine and p-Aminobenzoic Acid Hydrazide

Results



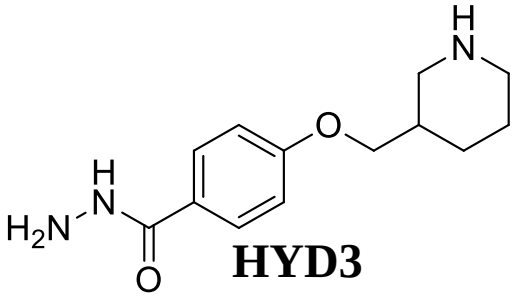
HYD1

IC_{50}= 81 nM,
Irreversible inhibitor



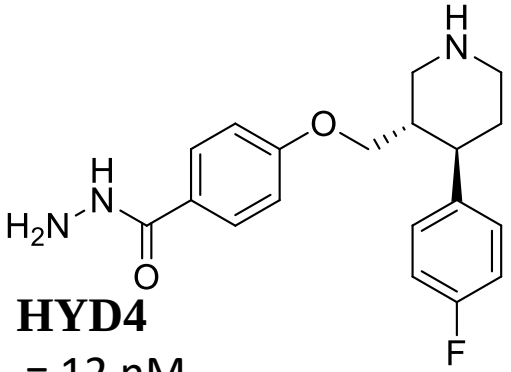
HYD2

IC_{50}= 140 nM,
Irreversible inhibitor



HYD3

IC_{50}= 41 nM,
Irreversible inhibitor



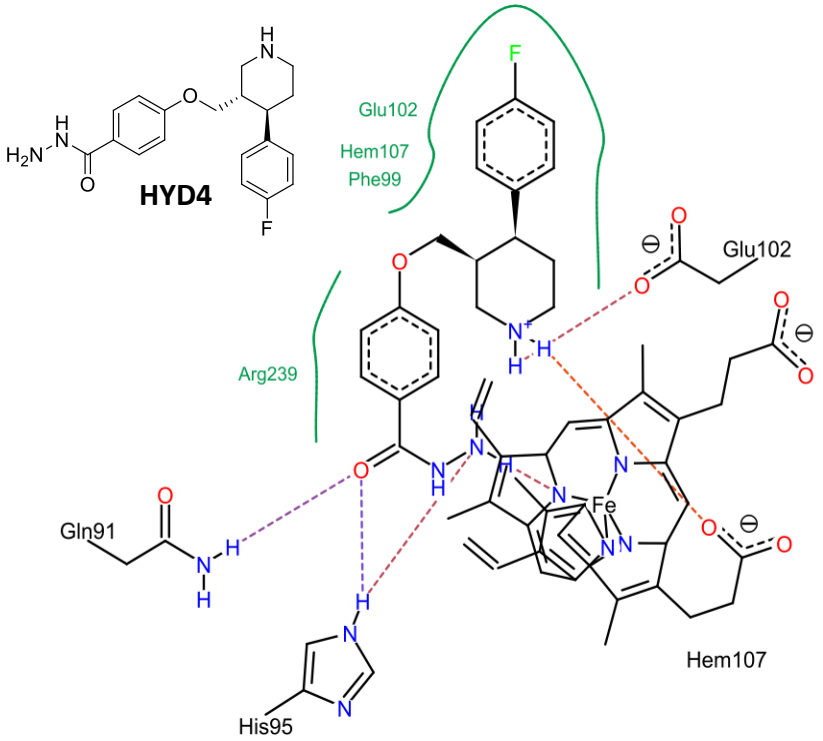
HYD4

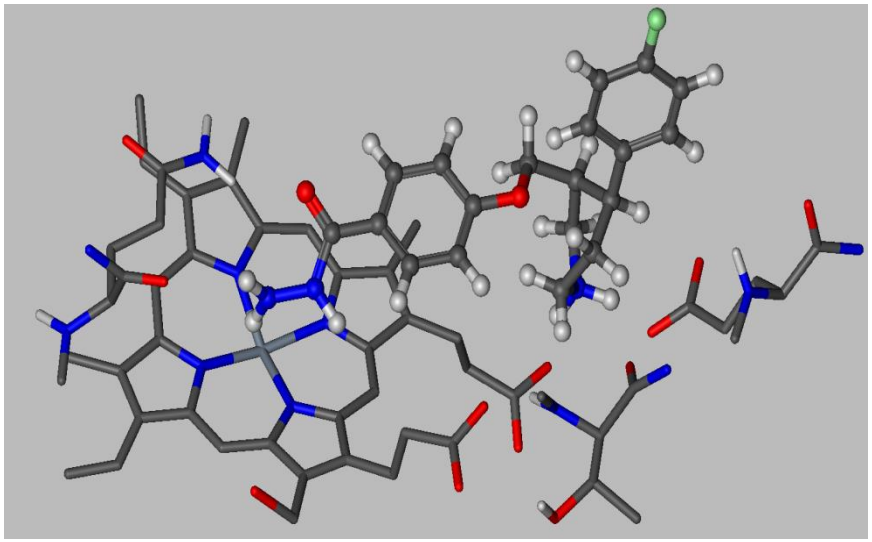
IC_{50}= 12 nM,
Irreversible inhibitor

Results and discussion (Part 2)

Ligand-Based Design of Irreversible Myeloperoxidase Inhibitors Starting from Paroxetine and p-Aminobenzoic Acid Hydrazide

Results





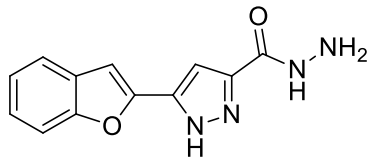
The high activity of **HYD4** can be explained by the high interactions between the inhibitor and the residues of the active site of MPO.

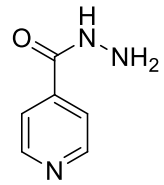
Results and discussion (Part 3)

Anti-Bacterial Effects of The Hydrazone and Hydrazide Derivatives

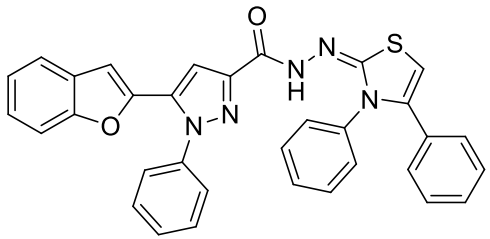
In addition to the anti-mycobacteria, isoniazide, several hydrazide compounds showed anti-bacterial effect.
Our hydrazones and hydrazides were screened on G(+) and G(-) bacteria.

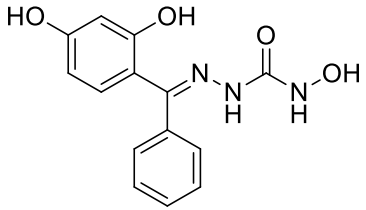
Anti-bacterial hydrazide compounds





Isoniazide



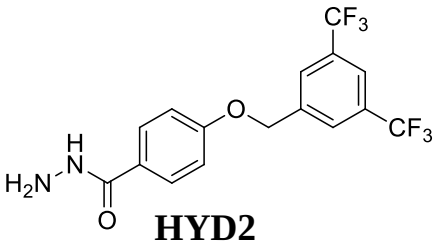


Arch. Pharm. Chem. Life Sci. 2008, 341, 734 – 739; *Iran J Pharm Res.* 2016; 15(Suppl): 29–35.

Results and discussion (Part 3)

Anti-Bacterial Effects of The Hydrazone and Hydrazide Derivatives

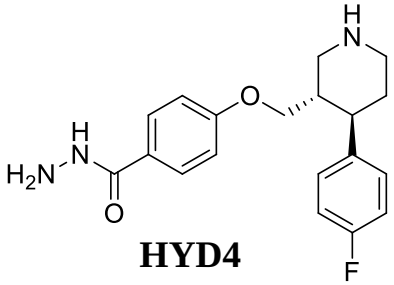
Results



HYD2

E.coli: no activity

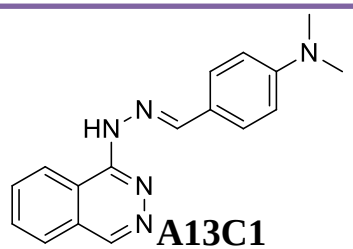
S.aureus: MIC= 60 µg/mL



HYD4

E.coli: MIC= 500 µg/mL

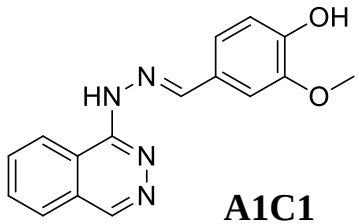
S.aureus: MIC= 15 µg/mL



A13C1

E.coli: MIC= 120 µg/mL

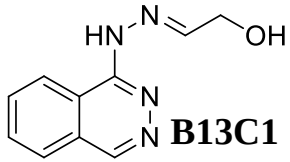
S.aureus: MIC= 500 µg/mL



A1C1

E.coli: MIC= 120 µg/mL

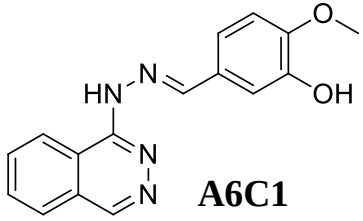
S.aureus: MIC= 500 µg/mL



B13C1

E.coli: no activity

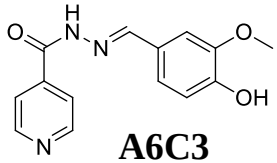
S.aureus: no activity



A6C1

E.coli: MIC= 120 µg/mL

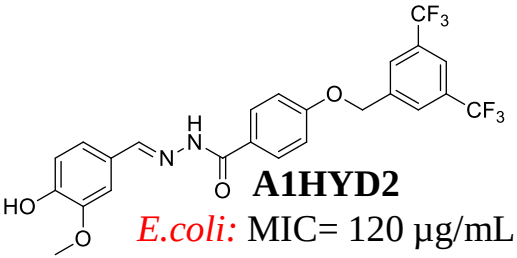
S.aureus: MIC= 500 µg/mL



A6C3

E.coli: MIC= 120 µg/mL

S.aureus: MIC= 500 µg/mL



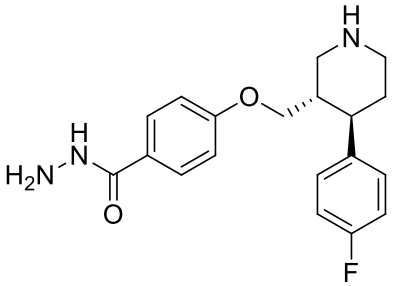
A1HYD2

E.coli: MIC= 120 µg/mL

S.aureus: MIC= 250 µg/mL

Conclusions

- Sevral aryl hydrazones and aryl hydrazides were obtained by dynamic combinatorial chemistry and rational drug desgin as MPO inhibitors.
- Some of these inhibitors showed high potency against MPO at nanomolar range.
- The studies of mechanism of action has demonstrated that these inhibitors are irreversible.
- Screening of these aryl hydrazones and aryl hydrazide on E.coli and S.aureus showed that aryl hydrazones are active against G(-) bacteria while aryl hydrazides are active against G(+) bacteria.
- **HYD4** showed the best activity on both MPO and S.aureus but low activity on G(-).
- The mechanism by which these compounds work as anti-bacterial agents must be determined.



HYD4

E.coli: MIC= 500 µg/mL

S.aureus: MIC= 15 µg/mL

MPO: IC_{50}= 12 nM

Irreversible inhibitor

Acknowledgments

Department of Pharmacognosy, Bioanalysis and Drugs.
Department of Microbiology, Bioorganic and Macromolecular Chemistry.

WBI
Wallonie-Bruxelles International

ULB

fnrs
LA LIBERTÉ DE CHERCHER