Design, Synthesis And Activity Evaluation Of New Irreversible Myeloperoxidase Inhibitors Derived From Benzodioxole

Jalal Soubhye¹,*, Bénédicte Valet¹, Sara Tadrent¹, Iyas Aldib¹, Michel Gelbcke¹, Paul Furtmüller², Jean Nève¹, Christian Obinger², François Dufrasne¹ and Pierre Van Antwerpen¹

¹ Laboratoire de Chimie Pharmaceutique Organique, Faculté de Pharmacie, Université Libre de Bruxelles, Brussels, Belgium.
² Department of Chemistry, BOKU–University of Natural Resources and Life Sciences, Vienna, Austria.

* Corresponding author: E-mail: jsoubhye@ulb.ac.be
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Abstract:
The role of Myeloperoxidase (MPO) in the oxidative damages and the inflammatory syndromes is well documented. Thus, the inhibition of MPO in the circulation can be useful in the treatment of several inflammatory diseases. Some potent reversible MPO inhibitors derived from fluorotryptamine were published. In addition we have reported that the SSRI agent (paroxetine) can irreversibly inhibit MPO at low nanomolar range. With the docking experiments, the important chemical groups in both paroxetine and fluorotryptamine derivatives were determined and general structure of the new series was designed. This general structure consists of dioxole, aromatic ring Ar, hydrogen bond donor HBD and a space between HBD and Ar. Several modifications were applied to study the SAR of this series.
These compounds were synthesized and tested in vitro. It is found that the IC$_{50}$ of the compounds with amine are the lowest values among all the functional groups (IC$_{50}$ = 10-60 nM), that 5 carbons on the side chain give the best activity. Dioxole group is very important for the activity and the irreversibility. The in vitro test of these compounds on SERT improved the selectivity vs SERT.

Keywords: Myeloperoxidase; Irreversible Inhibitor; Benzodioxole; paroxetine
Introduction

MPO, EC 1.11.2.2
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 \( \text{H}_2\text{O}_2 \) and \( \text{Cl}^- \) which leads to the oxidation (degradation) of biomolecules of pathogens in the phagosome.

Klebanoff. *J.Leukoc.Biol.* **2005**, 77
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.
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
The goal of the study

Friend

Oxidative damages in pathogens

Killing pathogens

Inside phagosome of neutrophile
Outside neutrophile

Foe

Oxidative damages in host tissues and biomolecules

Inflammatory Syndromes

HOCl

MPO

MPO Inhibitor

The goal of the study
The goal of the study

5F4C\(^{(1)}\)

IC\(_{50}\) = 12nM
Reversible Inhibitor
Not Selective

5F3CA\(^{(2)}\)

IC\(_{50}\) = 18nM
Reversible Inhibitor
Selective

HX1\(^{(3)}\)

IC\(_{50}\) = 5nM
Reversible Inhibitor
Selective ??

TX1\(^{(4)}\)

IC\(_{50}\) = 500nM
Irreversible Inhibitor
Selective

Paroxetine\(^{(5)}\)

IC\(_{50}\) = 20nM
Irreversible Inhibitor
Not Selective

(1) Soubhye et al. *J.Med.Chem.* 2010, 53; (2) Soubhye et al. *J.Med.Chem.* 2013, 56; (3) Forbes et al. *J.Bio.Chem.* 2013, 288;
(4) Ward et al. *Biochemistry.* 2013, 52; (5) Soubhye et al. *J.Pharm.Pharmacol*, 2014, 66.
Drug design
HBD: hydrogen bond donor

General structure of the benzodioxole series
Drug design

Chain length

Functional group
### Drug design

#### Cyclic functional group

| ![Chemical Structure](image1.png) | ![Chemical Structure](image2.png) | ![Chemical Structure](image3.png) | ![Chemical Structure](image4.png) |
|----------------------------------|----------------------------------|----------------------------------|----------------------------------|

#### Bridge

| ![Chemical Structure](image5.png) | ![Chemical Structure](image6.png) | ![Chemical Structure](image7.png) |
|----------------------------------|----------------------------------|----------------------------------|

#### Dioxole

| ![Chemical Structure](image8.png) | ![Chemical Structure](image9.png) | ![Chemical Structure](image10.png) |
|----------------------------------|----------------------------------|----------------------------------|
Chemistry
Synthesis of the compounds with amine, amide and nitrile with different chain length

NaH (60% in mineral oil) was suspended in a solution of sesamol with DMF, after 10 minutes the bromonitrile derivative was added.

For the compounds with short chain length: 1 equivalent sesamol ≠ 3 equivalent bromonitrile derivative
For the compounds with long chain length (n= 4 and 5): 1 equivalent sesamol ≠ 1 equivalent bromonitrile derivative.

After 2h of the reaction in tert-butanol, H₂O was added.

The mixture was kept stirring 15 minutes.
The time of stirring with water is very important.

The purification was achieved by acid/base extraction.
Chemistry

Synthesis of the compounds with hydroxyl, substituted amine and Cyclic functional group

The bromo-alcohol derivative was added to the solution of sesamol and potassium tert-butoxide very slowly (over 1h)

n= 2  20% yield
n= 3  36% yield
n= 4  25% yield

TEA: Triethanolamine
RT: Room temperature
90-95% yield

40-90% yield
Chemistry

Changing the bridge and the dioxole

The obtained compounds were dissolved in DMSO with NaN₃ (5h, 100°C). The obtained azido compounds were hydrogenated by Pd/C in ethanol under H₂ 60 psi.

This compound was obtained by the the same procedure as for the amino compounds.

After the reaction was finished the reagent was evaporated.
Results and discussion
Results and discussion

In vitro test and SAR study

**Chain length:** 5 carbons on the side chain gives the best activity for the compounds with amine group while for the amide and nitrile the best compounds are those with 4 carbons.

![Chemical structures](image)

- Chain length: 5 carbons on the side chain gives the best activity for the compounds with amine group while for the amide and nitrile the best compounds are those with 4 carbons.

**Functional group:** the effect of the functional group is as following: \(-\text{NH}_2\) > \(=\text{NH}\) > \(=\text{N}\) > -CONH_2 > -CN > -OH > -Cl > -CH_3. And \(=\text{N}^+\) has no activity.

**Cyclic functional group:** among piperazine, morpholine and pyrrolidine, the piperazine gives the best activity with the same activity of the compound with \(=\text{NH}\).
**Results and discussion**

In vitro test and SAR study

**Bridge:** the best activity was shown when the bridge is ether. When the bridge is ester or amide the activity is lost.

![No activity](image1.png)  \[ \text{IC}_{50} = 19 \text{ nM} \]

**Dioxole:** the compounds unsubstituted on the carbon of dioxole have the best activity. The compound without dioxole (dihydroxyl) has no activity.

![IC\(_{50}\) = 20 nM](image2.png)  \[ \text{IC}_{50} > 1000 \text{ nM} \]  \[ \text{Not active} \]
Results and discussion

In vitro test and SAR study

Docking could explain all the results except losing the activity in the compound without dioxole (dihydroxyl), the compound with ester bridge and the compound with amide bridge. The compounds that feature hydrogen bond or salt bridge with Glu102 have high potency.

SERT inhibition: in vitro test of all the synthetic compounds showed that these compounds have no activity on SERT, so our new inhibitors are selective for MPO.

SERT: serotonin transporter
Results and discussion

Mechanism of action

Chlorination pathway
- HOCl
- k2
- Cl⁻
- MPO^{3+} + H_2O_2
- k1
- Fe(III)₅ Por
- Compound I
  - Fe(IV)=O Por
- Compound II
  - Fe(IV)=O Por

Peroxidation pathway

Absorbance
- Wavelength (nm)
- Time in s
- 250, 350, 450, 550, 650, 750

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Results and discussion

Mechanism of action

![Graph showing the effect of inhibitors on MPO activity]

In order to improve the irreversible inhibitory effect of our new compounds on MPO, several concentrations of (the best new inhibitor, paroxetine and potent reversible inhibitor) were incubated with fixed amounts of MPO for 1h. After 1h, the activity of MPO was measured. It is found that when: the concentration of our new inhibitor is 50 times higher than this of MPO, the inhibition is 100%, the concentration of our paroxetine is 100 times higher than this of MPO, the inhibition is 100%. But with the reversible inhibitor, the inhibitory effect cannot reach at 100%.
Conclusions

- We developed the first potent irreversible inhibitors of MPO that inhibit the enzyme at nanomolar range. These inhibitors are derived from benzodioxole.

- The compounds that have amine on the side chain have the best activity.

- Five carbons between the bridge and the amine give the best activity.

- Ether group as a bridge between aromatic group and alkyl chain gives the best activity.

- The compound which has not dioxole reacts with both Compound I and Compound II of MPO in very fast way, so this molecule cannot cause accumulation of the inactive form of MPO (Compound II). This makes the compound with no activity.

- The most potent inhibitor among the synthesized compound has IC$_{50}$ of 13 nM.
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Laboratoire de Chimie Pharmaceutique Organique [Therapeutic Chemistry]

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