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

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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 H₂O₂ and Cl⁻ which leads to the oxidation (degradation) of biomolecules of pathogens in the phagosome.
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.

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

The goal of the study

**Friend**
- Oxidative damages in pathogens
- Killing pathogens

**Foe**
- Oxidative damages in host tissues and biomolecules
- Inflammatory Syndromes

Inside phagosome of neutrophile
Outside neutrophile

- HOCl
- MPO
- MPO Inhibitor
The goal of the study

- **5F4C**<sup>(1)</sup>
  - IC<sub>50</sub> = 12nM
  - Reversible Inhibitor
  - Not Selective

- **5F3CA**<sup>(2)</sup>
  - IC<sub>50</sub> = 18nM
  - Reversible Inhibitor
  - Selective

- **HX1**<sup>(3)</sup>
  - IC<sub>50</sub> = 5nM
  - Reversible Inhibitor
  - Selective ??

- **TX1**<sup>(4)</sup>
  - IC<sub>50</sub> = 500nM
  - Irreversible Inhibitor
  - Selective

- **Paroxetine**<sup>(5)</sup>
  - IC<sub>50</sub> = 20nM
  - Irreversible Inhibitor
  - Not Selective

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Drug design
Drug design

HBD: hydrogen bond donor

General structure of the benzodioxole series
Drug design

Chain length

<table>
<thead>
<tr>
<th>[\text{Dioxole} \quad \text{Aromatic ring} \quad \text{Bridge} \quad \text{Alkyl side chain} \quad \text{Functional group}]</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1.png" alt="Chemical structures" /></td>
</tr>
</tbody>
</table>

Functional group

<table>
<thead>
<tr>
<th><img src="image2.png" alt="Chemical structures" /></th>
</tr>
</thead>
</table>
## Drug design

### Cyclic functional group

<table>
<thead>
<tr>
<th>Structure</th>
<th>Structure</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1" alt="Structure 1" /></td>
<td><img src="image2" alt="Structure 2" /></td>
<td><img src="image3" alt="Structure 3" /></td>
</tr>
</tbody>
</table>

### Bridge

<table>
<thead>
<tr>
<th>Structure</th>
<th>Structure</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image4" alt="Structure 1" /></td>
<td><img src="image5" alt="Structure 2" /></td>
<td><img src="image6" alt="Structure 3" /></td>
</tr>
</tbody>
</table>

### Dioxole

<table>
<thead>
<tr>
<th>Structure</th>
<th>Structure</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image7" alt="Structure 1" /></td>
<td><img src="image8" alt="Structure 2" /></td>
<td><img src="image9" alt="Structure 3" /></td>
</tr>
</tbody>
</table>
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
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).

- 
  \[ \text{n} = 2 \] 
  20% yield
- 
  \[ \text{n} = 3 \] 
  36% yield
- 
  \[ \text{n} = 4 \] 
  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 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)

<table>
<thead>
<tr>
<th>Chain length</th>
<th>Activity IC₅₀</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chain length 5 with amine group</td>
<td>13 nM</td>
</tr>
<tr>
<td>Chain length 5 with amide group</td>
<td>31 nM</td>
</tr>
<tr>
<td>Chain length 5 with nitrile group</td>
<td>52 nM</td>
</tr>
</tbody>
</table>

**Functional group:** the effect of the functional group is as following: \(-\text{NH}_2\) > \(=\text{NH}\) > \(=\text{N}\) > \(-\text{CONH}_2\) > \(-\text{CN}\) > \(-\text{OH}\) > \(-\text{Cl}\) > \(-\text{CH}_3\). 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 \(=\text{NH}\).

![Chemical structures](image)

<table>
<thead>
<tr>
<th>Functional group</th>
<th>Activity IC₅₀</th>
</tr>
</thead>
<tbody>
<tr>
<td>Piperazine</td>
<td>22 nM</td>
</tr>
</tbody>
</table>
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.

![Chemical structure](image1.png)

- No activity
- IC$_{50}$ = 19 nM

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

![Chemical structure](image2.png)

- IC$_{50}$ = 20 nM
- IC$_{50}$ > 1000 nM
- 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.

\[ \Delta G = -22.9 \text{ kcal} \]
\[ \Delta G = -13.1 \text{ kcal} \]
\[ \Delta G = -20.6 \text{ kcal} \]

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

\[ \text{HOCl} \xrightarrow{k_2} \text{Cl}^- \]

\[ \text{MPO}^{3+} + \text{H}_2\text{O}_2 \xrightarrow{k_1} \text{Compound I (Fe(IV)=O, Por^{4+})} \]

\[ \text{Compound I (Fe(IV)=O, Por^{4+})} \xrightarrow{k_4} \text{Compound II (Fe(IV)=O, Por)} \]

\[ \text{Compound II (Fe(IV)=O, Por)} \xrightarrow{k_3} \text{Peroxidation pathway} \]

Absorbance

1st International Electronic Conference on Medicinal Chemistry
2-27 November 2015
Results and discussion

Mechanism of action

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%. 
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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