EXPERT OPINION ON THERAPEUTIC PATENTS
https://doi.org/10.1080/13543776.2020.1780210

Taylor & Francis
Taylor & Francis Group

REVIEW

A patent review of myeloperoxidase inhibitors for treating chronic inflammatory syndromes (focus on cardiovascular diseases, 2013-2019)

Jalal Soubhye[a], Pierre Van Antwerpen[a] and François Dufrasne[b]

[a]Department of Pharmacognosy, Bioanalysis and Drug Discovery, Faculty of Pharmacy, Universite Libre De Bruxelles (ULB), Bruxelles, Belgium; [b]Microbiology, Bioorganic and Macromolecular Chemistry, Faculty of Pharmacy, Universite Libre De Bruxelles, Bruxelles, Belgium

ABSTRACT

Introduction: Myeloperoxidase (MPO) is an immune enzyme found in neutrophils and macrophages. It produces the highly oxidative compound HOCl from H_2O_2 and Cl^- ions inside the phagosome of the neutrophil. Leakage of the enzyme outside the cell causes oxidative damages for the biomolecules promoting many inflammatory diseases such as atherosclerosis. Thus, there is a real interest to develop potent inhibitors of MPO as non-steroidal anti-inflammatory agents. This review highlights the several published MPO inhibitors, their activity, and the challenges met during the development of these compounds.

Areas covered: This article covers the patent literature published on MPO inhibitors from 2013 to 2019, as well as the potential use of these compounds as therapeutics for inflammatory syndromes, especially that plays an important role in the initiation and progression of atherosclerosis.

Expert opinion: To date, many MPO inhibitors with different structures have been studied, many of which have prominent inhibitory activities. Furthermore, the specificity of these drugs offers hope for the targeted therapy of inflammatory syndromes. Although many data have proved that MPO can contribute to several chronic inflammatory syndromes, the usefulness of MPO inhibitors in preventing and treating inflammatory disorders is still under investigation.

ARTICLE HISTORY
Received 8 March 2020
Accepted 5 June 2020

KEYWORDS
Myeloperoxidase; chronic inflammatory syndromes; atherosclerosis; oxidative damages

1. Introduction

Neutrophils are the major immune cells in innate immunity that neutralize foreign antigen [1]. After the phagocytosis of the antigen by neutrophils, lysosomal enzymes are released into the phagosome. This process is usually accompanied by the production of highly reactive oxygen species (ROS) which have strong microbicidal properties [2]. Among these lysosomal enzymes, myeloperoxidase (MPO, EC,1.11.2.2) is one of the most abundant proteins in neutrophils. It is stored in azurophilic granules of the neutrophils accounting for 5% of the dry weight of these white cells [3]. In addition, MPO is found in lysosomes of monocytes [4]. Due to its high oxidative product, hypochlorous acid (HOCl), this enzyme plays a major role in killing the pathogens through neutrophils and monocytes [5].

Myeloperoxidase is a heme enzyme with a green color [6]. It consists of two subunits that are attached together by a disulfide bond [7]. Each subunit binds covalently with the heme group leading to two active sites. It has been determined that each subunit of MPO presents a calcium-binding site and five putative sites of asparagine-linked glycosylation (Asn157, Asn189, Asn225, Asn317, and Asn563) which play important roles in the dimerization of the enzyme and stabilization of the dimer. Analysis of the protein structure has shown also the proximal His336 and distal His95 which play a very important role in the enzymatic activity [8,9].

1.1. Activity of MPO

Myeloperoxidase uses H_2O_2 to oxidize chloride (Cl^-), bromide (Br^-), iodide (I^-), and the pseudohalide thiocyanate (SCN^-) (but not fluoride) to their respective hypo(pseudo)halous acids [10]. The halide oxidation starts by the reaction of ferric-MPO (Por-Fe^{3+}) with H_2O_2 to form Compound I ($^{\bullet+}$Por-Fe (IV) = O), which contains two oxidizing equivalents more than the resting enzyme. Compound I directly oxidizes the halide to recover the native enzyme by a two-electron process. These reactions are called the halogenation cycle (Figure 1) [11].

In the presence of exogenous or endogenous electron donors (AH_2) with a high affinity toward MPO, MPO-Compound I is converted to a ferryl(IV) state (MPO-Compound II) which is inactive in the oxidation of the halogens. MPO-Compound II can be reduced via a one-electron process in the presence of another electron donor recovering the native MPO. These reactions belong to the so-called peroxidation cycle (Figure 1) [12]. The difference in standard reduction potential $E^{o\prime}$ of relevant redox MPO couples can explain the difference in the oxidative activity of each state of MPO where it has been found that $E^{o\prime}$ (Compound I/Compound II) = 1.35 V and $E^{o\prime}$ (Compound I/native MPO-Fe^{3+}) is 1.16 V, whereas the value of $E^{o\prime}$ (Compound II/native MPO-Fe^{3+}) = 0.97 V [13,14].

Despite that Cl^- has a lower affinity toward MPO than Br^-, I^-, and SCN^-, chloride is considered as the physiological

CONTACT Jalal Soubhye jsoubhye@ulb.ac.be Department of Pharmacognosy, Bioanalysis and Drug Discovery, Faculty of Pharmacy, Universite Libre De Bruxelles (ULB), 1050, Bruxelles, Belgium

This article was originally published with errors, which have now been corrected in the online version. Please see Correction (http://dx.doi.org/1804654)

Article highlights

- MPO plays a significant role in atherosclerosis and other chronic inflammatory syndromes.
- The evidence which implicated MPO in atherosclerosis made the enzyme a promising target for treating and preventing CVD.
- Many new MPO inhibitors have been developed and approved, some of which have entered clinical trials.
- MPO inhibitors can have significant application prospects if their associated limitations are overcome.

This box summarizes key points contained in the article.

substrate for MPO because of its high concentration in plasma and in phagosomes compared to the other (pseudo)halogens. However, in heavy smokers, the concentration of thiocyanate is significantly increased and the kinetic rate of its oxidation is the same as for chloride [10].

1.2. Role of MPO in pathology

1.2.1. MPO and oxidative damages

During inflammation, MPO can be released outside the neutrophils causing oxidative damages for the host tissues [5]. These damages can be caused by two processes: 1) producing HOCl which oxidizes the biomacromolecules such as DNA, RNA, proteins, lipoproteins [15]; 2) direct oxidation by MPO of which some amino acids and hormones can be substrates (ex: tyrosine and serotonin) [16]. HOCl produced by MPO is able to oxidize a large variety of biomolecules by chlorination and/or oxidation [17]. It oxidizes sulfhydryl groups in proteins causing their inactivation. This oxidation can form disulfide bonds that can result in the crosslinking of proteins. However, oxidation of Cys by HOCl gives cysteic acid and cysteine [18]. Hypochlorous acid can readily react with the amino acids which have an amine side chain (ex. Lys) resulting in a chloramine compound. Tyr can be oxidized by MPO giving either o,o'-dityrosine (di-tyr) via direct oxidation or chlorotyrosine (Cl-tyr) via HOCl where Cl^{-}tyr is considered as the marker of MPO oxidation [19]. HOCl can attack the free amino acids as well as the residues of amino acids in proteins [17]. In addition, the nitrogen atoms of nucleosides are readily oxidized by HOCl causing damages in DNA and RNA [20]. Reactions of unsaturated fatty acids and cholesterol with HOCl are also possible that generate lipid hydroperoxide and cholesterol chlorhydrin [21,22].

Many studies have proved that MPO contributes in systemic inflammatory response syndrome implicated in several disorders such as atherosclerosis [23], Alzheimer disease [24], Parkinson disease [25,26], Huntington disease [27], rheumatoid arthritis [28], and several types of cancer [29–34].

1.2.2. Role of MPO in atherosclerosis

It has been proposed that the oxidative stress contributes to pathogenesis and progression of atherosclerosis via complex processes and mechanisms. Oxidative damages play an important role in cardiovascular diseases and also reduce the physiological functions that may adjust myocardiocytes. It has been demonstrated that the atherosclerotic lesion formation is accelerated by the oxidative stress induced by inflammation,

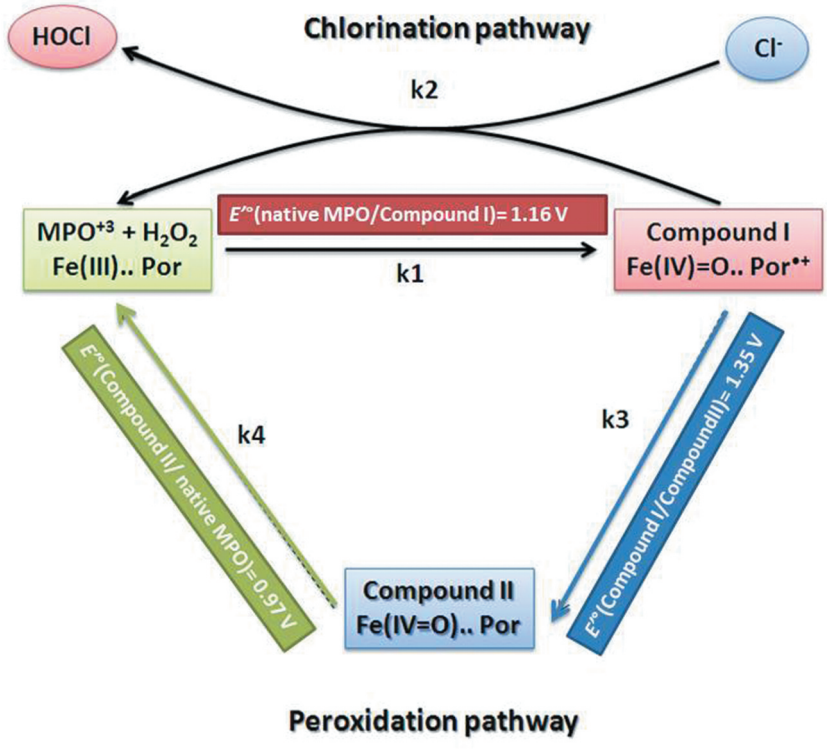

Figure 1. The formation of MPO-Compound I and II. Compound I is generated by oxidation of MPO by H2O2 by a two-electron process (k1). The standard reduction potential of this reaction is E°'= 1.16 V. The reaction of Compound I with Cl- gives HOCl (k2). In the presence of electron donor HA, Compound I is reduced to Compound II by one-electron process (k3, E°'= 1.35 V). Compound II may be reduced by other electron donor AH to give native MPO (k4, E°'= 0.97 V).

mitochondria, autophagy, apoptosis, and epigenetics [35]. Recent studies had indicated that reactive oxygen species (ROS) and reactive nitrogen species (RNS) produced by oxidase and peroxidase enzymes play different roles in atherogenesis [36,37]. They are implicated in endothelial dysfunction, neovascularization, vascular proliferation, apoptosis, matrix degradation, inflammation, and thrombosis [35]. MPO is one of these enzymes which contribute to ROS production and therefore oxidative stress [5].

The role of MPO in atherosclerosis is well documented. It has been reported that MPO contributes to LDL oxidation that leads to MPO-modified LDL (MoxLDL), and HDL. The modification of HDL reduces its protective role against cholesterol accumulation Endothelial cells can be also modified by MPO, causing dysfunction of these cells and development of vulnerable plaques (Figure 2) [38].

1.2.2.1. Oxidation of LDL. Many modifications in LDL produced by MPO have been reported. Tyrosine residue in LDL can be oxidized by HOCl to give chlorotyrosine, which is found abundantly within lipid species in human atherosclerotic lesions. Myeloperoxidase can use Tyr residues as substrates so generating tyrosyl radical which can easily lead to o,o′-dityrosine found in oxLDL [39].

In addition to HOCl, MPO can produce reactive nitrogen species including $NO^{2\bullet}$ and $NO2^{\bullet-}$ that readily react with Tyr residues in Apo B-100 to give NO_2Tyr causing LDL to be atherogenic. The nitrated form of LDL, termed 'NO_2LDL,' is selectively recognized by the scavenger receptor CD36, a major participant in the formation of foam cells and atherosclerotic lesion development [23].

1.2.2.2. Oxidation of HDL. The data obtained from HDL isolated from human atheroma proved that MPO selectively binds to a certain region on helix 8 of apolipoprotein A-I the major lipoprotein in HDL, and ClTyr and NO_2Tyr are found in relatively large amount in HDL isolated from humans suffering from atherosclerosis. The extent of this modification was found to correlate with the frequency of coronary artery disease [23]. It is has been observed that the enrichment of apolipoprotein A-I with NO_2Tyr and ClTyr is associated with a reduction in its ability to promote ATP-binding cassette transporter (ABCA1)-dependent cholesterol efflux. Thus, MPO causes the accumulation of cholesterol in cells of the artery wall. These findings evidence the participation of MPO in the initiation and/or propagation of CVD [23].

1.2.2.3. Dysfunction of endothelial cells. It is well known that the dysfunction of the endothelial cells is one of the earliest changes of atherogenesis. This stage is characterized by the abnormal vascular reactivity and expression of various proinflammatory and prothrombotic factors. The key role of MPO in the endothelial cell dysfunction involves a limitation in NO bioavailability [23]. It has been demonstrated that MPO reduces the bioavailability of NO by two processes: 1) MPO can promote endothelial dysfunction in a direct way via NO

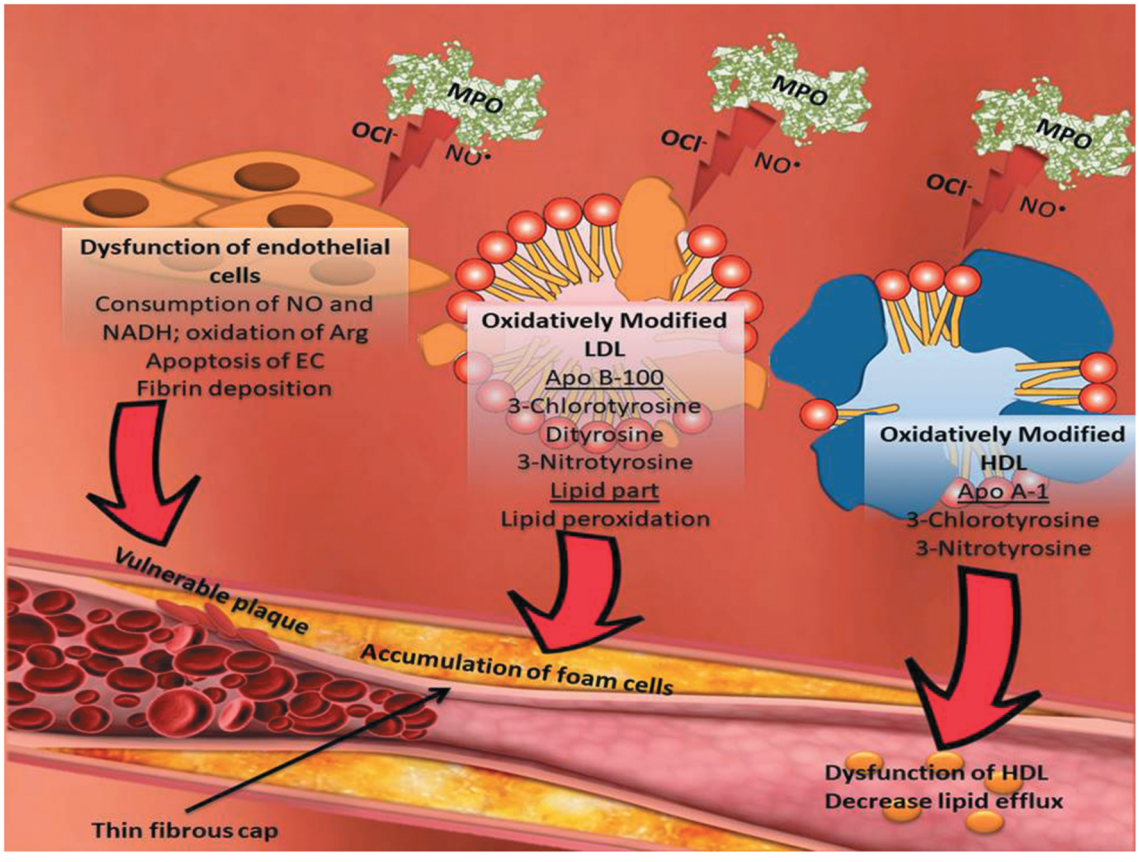

Figure 2. Myeloperoxidase and development of atherosclerosis. Myeloperoxidase contributes to endothelial dysfunction, modification of LDL and HDL.

consumption by using it as a substrate [40]; 2) MPO-generated oxidants are capable to inhibit the activity of NO-synthase [41].

The role of endothelial cells of arteries in fibrinolysis and inhibition of coagulation has been well established. However, when fibrin deposes on the endothelial cell surface, their permeability increases causing subendothelial lipid accumulation and foam cell formation [42].

Because MPO is implicated in several chronic diseases, inhibiting its activity in the circulation can be exploited to control the development of many inflammatory disorders, especially, atherosclerosis, Alzheimer disease, and Parkinson disease, and thus this enzyme could be an important target for the therapy of these diseases [38,43,44].

1.3. Strategies for finding MPO inhibitors

Because MPO is an immune enzyme that acts inside neutrophils, the only adverse effect expected for inhibitors is an impairing of the activity of neutrophils against pathogens [23]. However, MPO is packed in azurophilic granules resulting in the protection of this enzyme from the extracellular environment changes [45]. Thus, the effect of the inhibitors on MPO activity is thought to be easily reduced by targeting only the extracellular enzymes that are not important in pathogen killing and rather implicated in the host damages. Relatively polar MPO inhibitors cannot enter the neutrophils and are considered to potentially inhibit only extracellular enzymes [38]. In order to find and design such these inhibitors, three strategies have been applied:

The first strategy is designing small compounds that have a reduction potential of 0.97 V $\ll E^{\circ\prime}$ ($A^{\bullet}$/AH) <1.35 V. These molecules can be easily oxidized by MPO-Compound I and give the inactive state MPO-Compound II. Simultaneously, they cannot reduce MPO-Compound II and lead to the accumulation of this inactive form of MPO. The main problem of these inhibitors is that when they are used in vivo, several biomolecules can serve as substrates for MPO and reduce MPO-Compound II to recover the native enzyme. Thus, these inhibitors loose their activity *in vivo* [46].

The second strategy focuses on small compounds that have a high affinity for the active site of MPO. These compounds are designed to do strong interactions with the residues of the active site of the enzyme. Glu102 is considered the essential amino acid that does a salt bridge or/and hydrogen bond with the inhibitor. Additionally, the inhibitor must have a reduction potential $E^{\circ\prime}$ ($A^{\bullet}$/AH) <1.35 V to be able to reduce MPO-Compound I to MPO-Compound II. When this kind of molecule generates the inactive state of the enzyme, it keeps its interactions with the active site of MPO so preventing other substrates to enter the active site, and causing accumulation of MPO-Compound II with competitive inhibition. Indeed, several potent MPO inhibitors have been obtained by this method including aminoalkyl-indole compounds and aryl hydroxamic acid derivatives [47–50].

The third strategy is based on the design of small compounds that have a relatively high affinity toward MPO and do covalent bonds after their oxidation by the enzyme. These molecules are irreversible MPO inhibitors acting through the degradation of the heme group. Most of the patented inhibitors belong to this family [38].

2. Discussion of selected patents

For two decades, MPO has attracted the attention of the medicinal chemists who have been trying to find inhibitors for treating and preventing several inflammatory diseases. Although no MPO inhibitor is used clinically up to now, some of these inhibitors are in pre-clinical and clinical trial stages. A comprehensive review of MPO inhibitor-related invention patents during the specified period has revealed significant trends (Table 1); accordingly, we classify these inhibitors into the following groups depending on their scaffold.

Table 1. The patents published on MPO inhibitors, patent numbers, activity, and toxicity.

Compound	Class of compound	Patent N°	Year	IC_{50} μM	Toxicity
AZD3241 (Verdiperstat)	Thioxanthine	WO/2003/089430 WO/2006/062465	2003	0.63	No adverse effects have been observed in human at 600 mg/d (oral administration)
AZD5904		WO/2007/142,577 WO/2009/025618		0.2	No adverse effects have been observed in human at 325 mg/d (oral administration)
Compound 3	Alkyl-5-Fluoroindole	WO/2010/136,546	2010	0.005	No adverse effects have been observed in rats at 10 mg/kg
Compound 4	Alkyl-5-Fluoroindole	EP2682119	2013	0.018	No adverse effects have been observed in rats at 50 mg/kg
PF06282999	Thiopyrimidinone	WO/2016/178,113	2016	1.9	Serious adverse effects at 350 mg/d
Compound 7	Thioxodihydroquinazolinone	US20160243121	2016	0.1	-
AZD4831	Thioxanthine	WO/2016/087338	2016	0.007	No adverse effects have been observed in human at 405 mg/d (oral administration)
KYC	Tri-peptide	EP2485750B1	2017	-	No adverse effects have been observed in murine at 0.3 mg/kg/d
Compound 1 and 2	Triazolopyridine Macrocyclic	WO/2018/005336	2018	0.1–0.001	-
PIC1	Peptide	WO/2019/139,886	2019	-	No adverse effects have been observed in mice at 10 mg/kg
Compounds 8–11	Triazolopyridine	US20190031623	2019	0.005–0.03	No adverse effects have been observed in rats at 250 mg/d

2.1. Triazolopyridine macrocyclic inhibitors

Recently, macrocyclic compounds derived from triazolopyridine have been designed as MPO inhibitors. Figure 3 shows the general structure of these new inhibitors. The activity of these compounds *in vitro* reached the nanomolar level (IC_{50} values ranging between 1 and 100 nM). It has been found that these inhibitors can inactive MPO in reversible model. However, no compounds among these inhibitors have been tested *in vivo*, and, therefore, the pharmacokinetics and absorption/distribution/metabolism/elimination/toxicity (ADME-T) data profile are not available [51].

2.2. Peptide-based inhibitors

Starting from the amino acid sequence of the capsid of human astrovirus (787 amino acid residues), a small polypeptide (15 amino acids; IALILEPICCQERAA, Figure 4A) has been designed and synthesized for inhibiting the classical pathway of the complement. In order to enhance the solubility of this peptide, it was grafted with a 24mer monodisperse PEG moiety on the C terminus to give a Peptide Inhibitor of Complement C1 (**PIC1**) [52]. This polypeptide has shown good inhibitory activity on MPO *in vitro*. The dual activity of **PIC1** against the complement pathway and MPO makes this peptide a good hit for treating the inflammatory syndromes that cause injury to the lungs especially in patients with a high-risk disease such as cystic fibrosis patients [53].

However, the activity of **PIC1** was at high micromolar ranges (500 µM); therefore, new peptides have been developed starting from **PIC1**. These peptides were substituted with sarcosine or PEG. Peptide **PA-I8Sar** with the sequence IALILEP(Sar)CCQERAA has shown the best activity against MPO. Although **PA-18Sar** showed a very high activity on both complement pathway and MPO compared with **PIC1**, only peptide **PIC1** has been subjected to *in vivo* test in Wistar rats with induced inflammatory reactions [52]. The results revealed that **PIC1** significantly reduced the injury to the lungs as well as the activity of MPO compared to the control rats. **PIC1** also showed prophylactic properties against lung injury. However, the peptidic structures of **PIC1** and **PA-18SAR** prevent the compounds to be absorbed via gastrointestinal tract, and therefore, they cannot be administrated orally, which is considered as a negative feature in drug development [54].

PIC1 was not the first peptide that was used as an MPO inhibitor. The tripeptides N-acetyl lysyltyrosylcysteine amide (**KYC**) (Figure 4B) has showed a good inhibitory activity on MPO. This peptide is a reversible inhibitor which causes accumulation of MPO-Compound II by reducing Compound I and staying into the active site so that it prevents other electron donors to help recovering the native enzyme [55]. **KYC** was tested *in vivo* and the results demonstrated that this tripeptide effectively inhibits the formation of MoxLDL, increases vasodilation in mice with sickle cell disease, inhibits eosinophil infiltration and collagen deposition in asthma mice, and decreases ischemic injury of the heart. In addition, **KYC** may also be capable of scavenging toxic oxidants and free radicals [55,56]. Moreover, it has been reported that due to its MPO inhibitory activity, **KYC** can reduce brain damage after stroke [57] and prevents the progression of multiple sclerosis in a murine model [58]. These peptides were found to be reversible inhibitors for MPO by reversible binding to the active site residues of the enzyme [59].

2.3. Ferulic acid derivatives

Starting from the reversible inhibitor ferulic acid, Liu et al. have developed a new potent reversible inhibitor **INV-315** (the structure did not publish). The effects of **INV-315** on the progression of atherosclerotic lesions and endothelial function have been investigated in $ApoE^{-/-}$ mice models. It has been found that **INV-315** can reduce plaque burden and improved endothelial function in response to acetylcholine. In addition, the administration of this inhibitor resulted in a decrease in superoxide production and nitrotyrosine content in the aorta. However, the effect of **INV-315** on lipid peroxidation which is one of the main atherogenic pathways of MPO has not been shown in this study [60].

Compounds **a** and **b** were other compounds derived from ferulic acid (with IC_{50} of 0.9 and 8 µM, respectively) were developed by Jayaraj et al. in 2019 as reversible MPO inhibitors (Figure 4 C). These compounds have showed good inhibition effects of the oxidation of HDL *in vitro*. It has been demonstrated that in the presence of these compounds, the

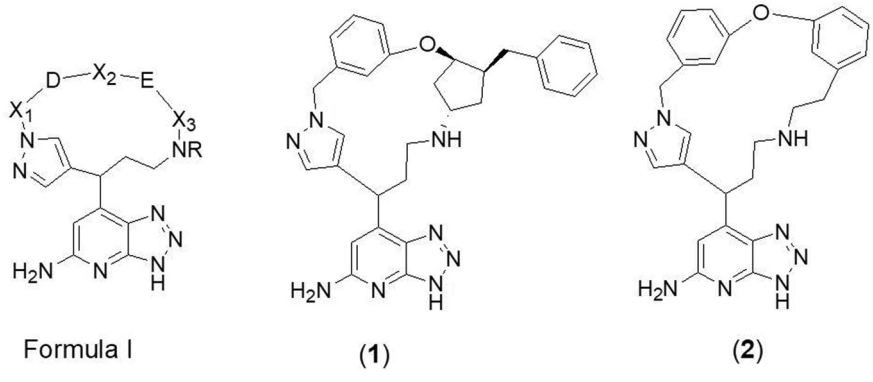

Figure 3. General structure of the macrocyclic inhibitors of MPO (formula I) and the most active compounds in this group (compounds 1 and 2).

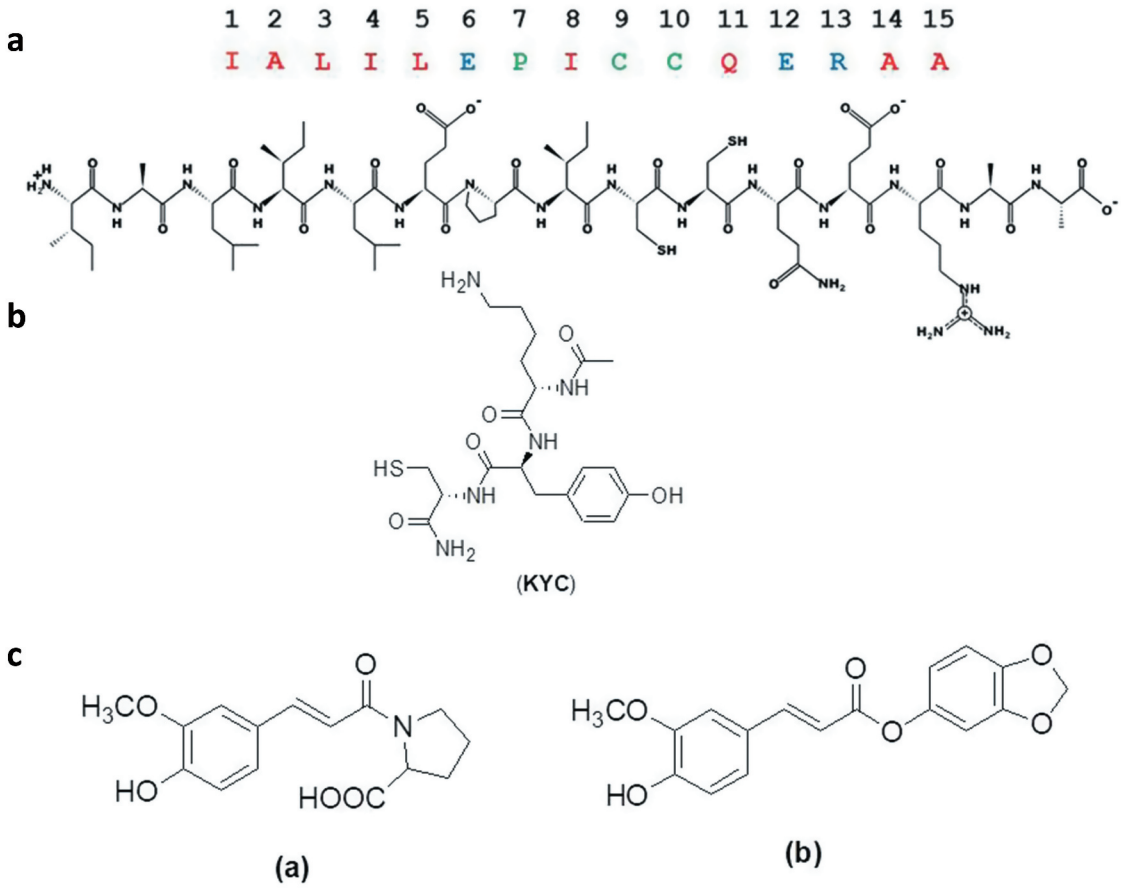

Figure 4. Structures of peptide-based MPO inhibitors, PIC1 (A) and KYC (B), structures of ferulic Acid derivatives (C)

cholesterol efflux of HDL was enhanced, indicating that these two compounds can be useful as cardioprotective agents [61].

2.4. Indole alkylamine derivatives

Tryptamine has been considered as one of the first good reversible inhibitors for MPO. The mechanism of inhibition has been well documented. Tryptamine enters the active site of MPO by doing interactions with its amino acid residues [46,62]. Docking experiments showed that Glu102 plays an important role in the interaction with tryptamine by doing a salt bridge with the amino group of the side chain of tryptamine [63]. The introduction of halogens increases the activity not only by increasing the interactions with the active site but also because the inhibitor becomes a good substrate for MPO-Compound I [46]. Figure 5A illustrates the predicted interactions between 5-fluorotryptamine and MPO. These results led medicinal chemists to develop new MPO inhibitors by structure-based design starting from tryptamine. These researches resulted in very potent MPO inhibitors with an activity at low nanomolar range. Compound **3** (3-(5-aminopentyl)-5-fluoro-1 *H*-indole) inhibits MPO with an IC_{50} of 5 nM and it can also inhibit the LDL oxidation via MPO (Figure 5B). It has been demonstrated that the mechanism of MPO inhibition by compound **3** is the same as by tryptamine. It can enter the active site but with higher affinity and do more interactions than other weaker inhibitors (Figure 5B) [64,65].

In vivo toxicity tests on compound **3** revealed that this molecule has no toxic effect on the organisms when it was administrated at therapeutic concentrations (when 10–50 mg/Kg was injected in rats). However, further pharmacological studies showed that compound **3** can inhibit serotonin-reuptake transporters (SERT) at the same concentration range as MPO IC_{50}. Despite the possibility of using compound **3** in treating depression in patients with high risk of atherosclerosis, selective inhibitors must be developed in order to avoid the adverse effects related to serotonin accumulation [66].

Based on these observations, further pharmacomodulation studies resulted in new selective MPO inhibitors (selectivity was studied only versus SERT). Compound **4** (3-(4-(butylamido))-5-fluoro-1 *H*-indole, Figure 5B) with an amid group instead of an amine in the side chain was shown to have the best activity/selectivity profile with IC_{50} of 18 nM and a selectivity index of 630. Structure–activity relationship (SAR) studies concluded that the amido group reduces dramatically the affinity of the compound toward MPO while keeping its activity on MPO. Compound **4** has been subjected to an *in vivo* toxicity tests and the results showed that this molecule has no remarkable adverse effects and it did not show any tissue damages in the organs at the concentration of treatment [67,68].

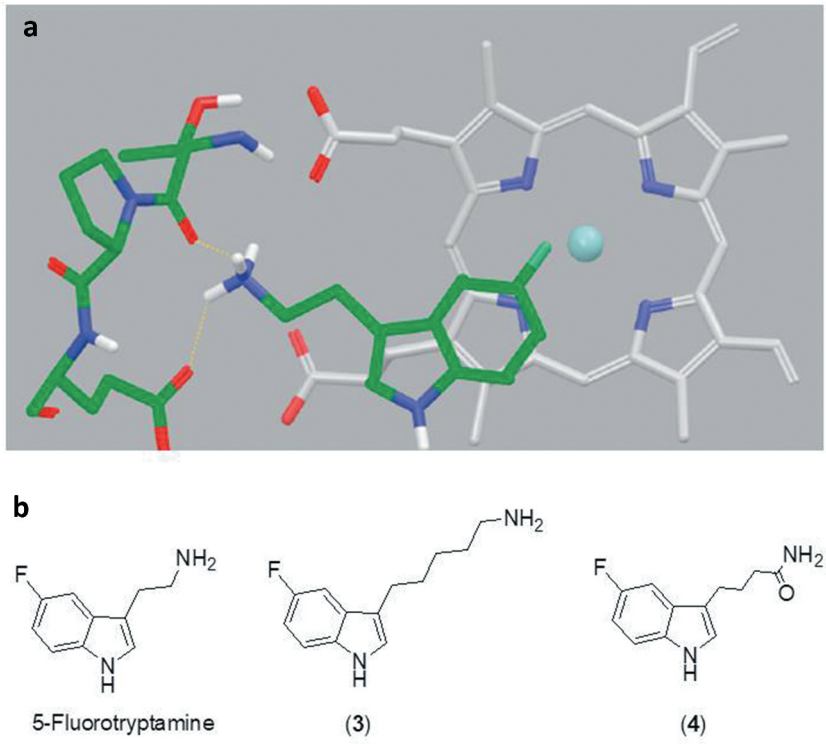

Figure 5. Indole derivatives MPO inhibitors, (A) predicted interaction between 5-fluorotryptamine and the active site of MPO, stacking with pyrrole D of the heme and the ionic interaction with Glu102 [58]. (B) best MPO inhibitors derived from tryptamine.

The pre-clinical studies of compounds **3** and **4** have been stopped in this stage and they are no longer subjected to pre-clinical or clinical trials.

2.5. Thioxanthine derivatives

Thioxanthine has been one of the most studied structures for MPO inhibition [38]. Several inhibitors have been developed by AstraZeneca. These thioxanthine compounds are suicide inhibitors through the covalent bond formation of a thioether bridge between the thioxanthine and the heme group of MPO (Figure 6A) [69,70]. Most of these compounds were shown to be orally available molecules [71,72].

The irreversible inhibitor **AZD5904** (Figure 6), which has an IC_{50} value of 0.2 µM, has been subjected to pre-clinical and clinical trial phase I. It has been demonstrated that **AZD5904** can inhibit MPO in mice and rats after oral administration. It was relatively selective versus the other MPO-related peroxidases (lactoperoxidase LPO and thyroid peroxidase TPO). *In vivo* studies have shown that **AZD5904** reduces the formation of glutathione sulfonamide which is produced by the oxidation and cyclization of glutathione. The oral administration of **AZD5904** to 181 healthy volunteers in single doses (1400 mg) and multiple doses (325 mg) did not lead to an overtly drug-related adverse event. The administration of 300 mg of this molecule yielded blood concentrations peak of 30 µM and an average blood concentration of 12 to 16 µM (greater than its IC_{50} value).

The elimination of **AZD5904** is mainly via renal route; thus, caution is required in patients with impaired renal function. Plasma protein binding is 44% with low blood-brain barrier (BBB) penetration, so it can be used for several inflammatory syndromes such as cardiovascular and pulmonary inflammatory diseases (but not in neurological diseases). Pre-clinical studies on rats have shown that **AZD5904** can reverse the resistance to muscle microvascular insulin in high-fat diet-fed animals. During in vitro studies, **AZD5904** led to inhibition of CYP2C19 (but not the other cytochromes including CYP1A2, CYP2D6, and CYP3A4) and was a P-glycoprotein 1 substrate [73]. The clinical trials on this compound have been stopped at this stage.

Other thioxanthine derivatives were subjected to clinical trials. **AZD3241** is another potent irreversible MPO inhibitor with IC_{50} of 0.63 µM. A pre-clinical trial has pointed out that **AZD3241** is selective for MPO and TPO but equipotent in inhibiting LPO. However, significant selectivity was found against 150 other potential targets including enzymes, receptors, ion channels, and transporters. In vivo studies have shown that the inhibitor decreases the activity of MPO in plasma during the acute peritonitis with a significant protection of the nervous system [74]. Phase I study on **AZD3241** undertaken to determine its pharmacokinetic profile has shown that the compound is an orally available inhibitor, which can be absorbed via gastro-intestinal tract. After the oral administration of 600 mg of **AZD3241**, the molecule is readily absorbed with an area under curve (AUC) ranging from 49.4 to 39.5 µmol.h/L in healthy subjects and patients (multiple system atrophy, MSA) respectively; 4.5 h after its administration, the inhibitor reaches the maximum concentration in plasma (Cmax) with 5.2 and 4.2 µM in healthy persons and MSA patients, respectively. It has been found also that the

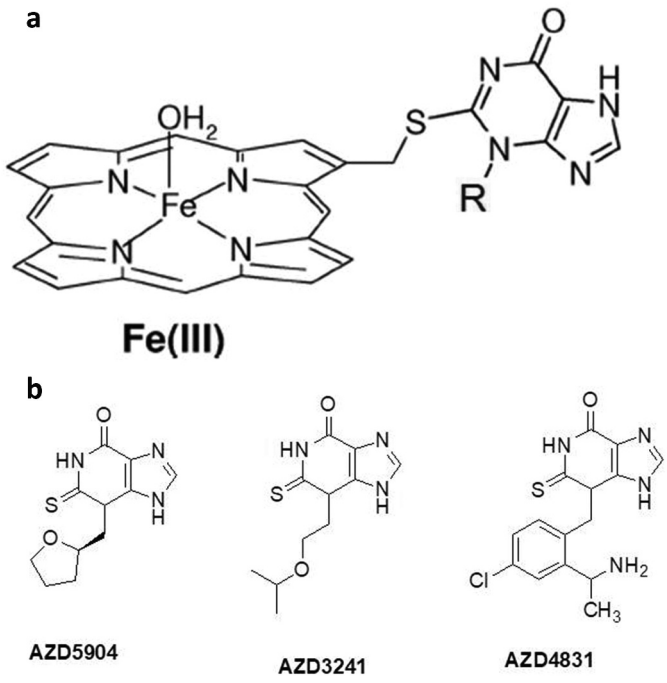

Figure 6. Thioxanthine MPO inhibitors, (A) the covalent bond between thioxanthine and the heme group of MPO. (B) structures of thioxanthines subjected to clinical trials.

administration of this inhibitor with a high-fat food increases the absorption by 69%. The clearance of **AZD3241** is mainly by renal route, but unlike **AZD5904**, this compound can easily penetrate BBB. Thus, the pharmacokinetic profile indicated that the concentration of **AZD3241** in plasma after oral administration is much higher than its IC_{50} value. The main adverse effect upon giving 600 mg of this inhibitor was headache which sometimes led to stop the treatment [74,75].

As **AZD3241** is BBB penetrant, phase II clinical trial has been achieved to test this compound on MSA and Parkinson's disease rather than CVD. Indeed, the results have shown that the treatment of Parkinson's disease patients with 600 mg daily for 8 weeks significantly decreased the microglial activation (one of the main mechanisms of neurodegeneration) in degenerating brain areas. However, despite its activity in suppressing the microglial activation, **AZD3241** failed to reduce the neuronal loss and the motor impairments [76].

AstraZeneca has developed special thioxanthine derivatives (1-[2-(aminomethyl)benzyl]-2-thioxo-1,2,3,5-tetrahydro-4 *H*-pyrrolo[3,2-d]pyrimidin-4-one) as Irreversible MPO inhibitors targeting cardiovascular diseases. **AZD4831** (Figure 6) was chosen to enter clinical trials. It has an IC_{50} value of 7 nM. The pharmacokinetic studies on 40 healthy subjects have indicated that **AZD4831** is easily absorbed after oral administration and distributed rapidly into plasma, to reach its C_{max} between 0.51 and 1.00 h with C_{max} value of 1 µM after giving 135 mg of the compound. A high-fat and high-calorie meal led to a reduced absorption rate by 44% compared to the administration without food intake. It has been found also that **AZD4831** has a long plasma half-life ($t_{1/2}$ = 38–50 h) and the main clearance route is renal [77]. In vitro metabolism studies have indicated that the cytochrome P450 (CYP) enzymes CYP3A4 and CYP3A5 are involved in the metabolism of **AZD4831** [77].

AZD4831 was shown to be well tolerated: no adverse effects have been observed except maculopapular rash but only at high doses (135 mg) and in 33% of the participants [77].

The good ADME-Tox profile of **AZD4831** allows it to go on in phase II clinical trial as a treatment of heart failure. The study has begun in July 2018 with 30 participants (age ≥30 years) and the patients received 30 mg of **AZD4831** via oral administration [78].

2.6. Thioxodihydroquinazolinone compounds

Many thioxodihydroquinazolinone compounds have been designed and synthesized as MPO inhibitors. The pharmacomodulation led to promising MPO inhibitors for use in the treatment of inflammatory diseases including CVS and CNS diseases (Figure 7). Compounds **5, 6**, and **7** showed high inhibitory activity on MPO with IC_{50} of 0.1 µM. The kinetic and reversibility studies indicated that thioxodihydroquinazolinones inhibit MPO reversibly and they are substrates for MPO-Compound I so that these compounds cause accumulation of the inactive form of MPO. Unfortunately, none of these compounds has been subjected to pre-clinical trials or in vivo tests [79,80].

2.7. Triazolopyridine compounds

Starting from triazolopyridine macrocyclic inhibitors and keeping the triazolopyridine moiety, other inhibitors have been designed and synthesized. Structure–activity relationship showed that the substitution on position 7 of triazolpyridine by benzyl or heterocyclic ethers is essential for the activity. In

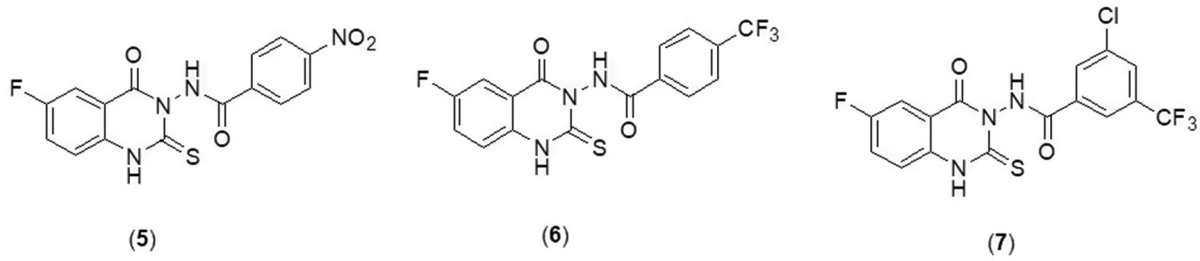

Figure 7. Thioxo-dihydroquinazolin-one compounds

addition, using thioether instead of ether increases the activity and the affinity toward MPO (Figure 8). However, most of the tested compounds with benzyl ether and thioether moieties showed a high potency against MPO at low nanomolar range. Indeed, it has been found that the aromatic group on position 7 of triazolopyridine increases the interaction between the inhibitor and the hydrophobic pocket of the active site of the enzyme. Compounds 7-(2-fluoro-benzyloxy)-3 *H*- [1–3]triazolo[4,5-b]pyridin-5-ylamine (**8**), 7-(2-fluoro-benzylsulfanyl) -3 *H*- [1–3]triazolo[4,5-b]pyridin-5-ylamine (**9**), 7-(2,6-dichloro-benzyloxy)-3 *H*- [1–3]triazolo[4,5-b]pyridin-5-ylamine (**10**) and 7-(2,6-dichloro-benzylsulfanyl)-3 *H*- [1–3]triazolo[4,5-b]pyridin-5-ylamine (**11**) were identified as potent reversible inhibitors with IC_{50} of 30, 9, 7, and 5 nM, respectively. These inhibitors showed good selectivity for MPO versus the other heme-containing oxidizing enzymes including LPO, TPO, EPO, and CYP 3A4. In vivo tests in rats with compound **9** indicated that this inhibitor is orally bioavailable and rapidly distributes into the blood with a serum concentration much higher than IC_{50} after oral administration of a safe dose (the dose which gives good activity without dangerous adverse effects). The in vivo tests also demonstrated that compound **9** reduces the inflammatory status and the oxidative stress by decreasing the reactive oxygen species (ROS). These results make compound **9** a good candidate for pre-clinical trials for inflammatory syndromes treatment [81,82].

2.8. 2-Thiopyrimidinone compounds

From a compound structurally related to thioxanthine, many MPO inhibitors have been developed by Pfizer. Propylthiouracil (**PTU**) which is clinically used for the treatment of hyperthyroidism has been identified as an irreversible MPO inhibitor. SAR on **PTU** has established that electron-rich aromatic moiety instead of a propyl chain and substitution by polar alkyl chains on position N1 are very important for increasing the activity on MPO and decreasing the activity on TPO and LPO [83].

These pharmacomodulation studies have led to compound 2-(6-(5-chloro-2-methoxyphenyl)-4-oxo-2-thioxo-3,4-dihydro-pyrimidin-1(2 *H*)-yl)acetamide (**PF06282999**) (Figure 9). In vitro studies have reported that **PF06282999** is an MPO inhibitor selective versus TPO, LPO, and cytochrome P450 isoforms with an IC_{50} of 1.9 µM [84].

The pre-clinical pharmacokinetic profile of **PF06282999** has been established in rat, mouse, dog, and monkey. After

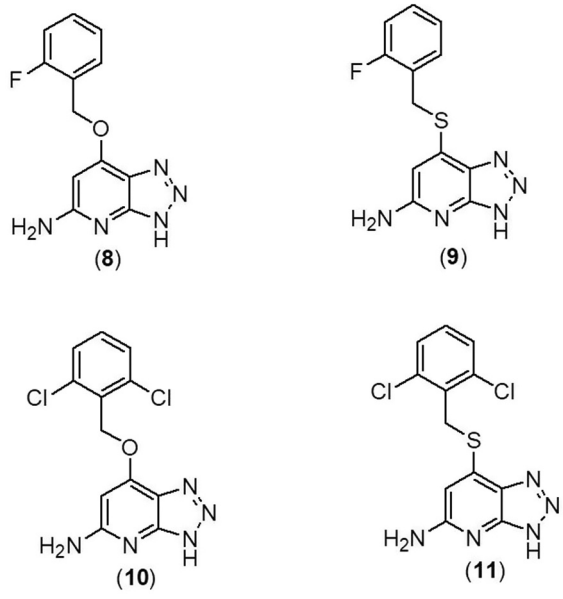

Figure 8. Triazolopyridine compounds

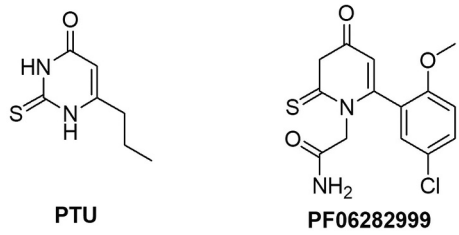

Figure 9. 2-Thiopyrimidinone compounds

oral administration of the inhibitor, the compound is absorbed with a bioavailability of 75% to 100% according to the animal used. In vivo toxicity tests with **PF06282999** revealed that no toxic effects have been observed upon giving an expected therapeutic dose of the inhibitor. In addition, oral dose of **PF06282999** in inflammatory-induced monkey reduced the activity of serum MPO [84].

PF06282999 has been advanced to clinical trials to determine its pharmacokinetics, safety/tolerability. MPO inhibition studies have been pursued due to its safe ADME-TOXprofile as well as its in vivo inhibitory activity of MPO. The investigation of clinical pharmacokinetics of **PF06282999** in phase I has shown that this compound can be absorbed after oral administration of 200 mg to lead to the C_{max} after 1 h. It was eliminated via renal clearance with a half-life of approximately 4.3–4.4 hours and after 24 hours, 75% of the dose was excreted by renal route [85–87].

Despite the high potency and selectivity of **PF06282999** as well as its good ADME profile, the phase I clinical trial was terminated in February 2015 and the study has stopped and will be resumed due to some serious adverse events [88].

3. Conclusion

Despite the importance of MPO in innate immune defense, it has been implicated in a large number of inflammatory diseases including CVD and CNS diseases. Thus, inhibition of circulating MPO can be useful for treating and preventing these inflammatory syndromes especially atherosclerosis, heart failure, and neurodegenerative diseases [89].

Many MPO inhibitors have been developed. Most of these inhibitors exhibited good activity in vitro and in vivo. However, only 2-thioxanthine and 2-thiopyrimidinone were advanced to clinical trials. These compounds are orally available and show high selectivity versus the other peroxidases, CYP254, several receptors, and ion channels. In fact, the clinical trials with these inhibitors have not been completed yet and the usefulness of this type of treatment has not been established until today. However, the in vivo results are promising and hopeful for the development of a new treatment for inflammatory syndromes [90].

4. Expert opinion

This review highlights the interest in MPO inhibitors as potential drugs for treating CVD and other inflammatory syndromes.

The evidence involving the heme enzyme MPO in these diseases has been accumulated for about 20 years. It can oxidize the biomolecules by producing HOCl and by directly reacting with them. HOCl is a highly oxidative compound that is not selective for the pathogens; thus, it triggers a huge spectrum of oxidative damages and therefore can contribute to systemic inflammatory response syndrome promoting and/or contributing to a large number of chronic diseases. Among the chronic syndromes, atherosclerosis is the most studied disorder and the correlation between MPO and this disease has been largely established [5]. The key role of MPO in atherosclerosis is the oxidative modification of LDL. These data attracted more attention to the inhibitors of this enzyme, and such strategy might be helpful for treating and/or preventing atherosclerosis and other inflammatory diseases [91].

For about two decades, many MPO inhibitors have been developed as a new strategy for the treatment of chronic inflammatory diseases. These inhibitors are divided into two categories: reversible inhibitors and irreversible inhibitors. It has been suggested that the circulating MPO in the blood is a leftover of neutrophils and its concentration increases in the inflammatory cases where the high level of MPO in plasma has been found to be a risk factor for CVD [92–94]. Thus, the suicidal inhibitors are preferred rather than the reversible ones as the frequency of drug administration is lower when the drug acts irreversibly [95]. However, besides the activity, the safety profile is the most important property for the inhibitor in order to enter clinical use and with this good tolerance, the compound can be administrated even in a relatively high-dose or high-frequency schedule [96]. Indeed, although several reversible inhibitors were active at nanomolar range with good tolerance (ex. indole alkyl amine and triazolopyridine compounds), only the irreversible compounds were advanced to clinical trials (thioxanthine and thiopyrimidinone compounds).

AZD5904, AZD3241, AZD4831, and **PF06282999** are the only inhibitors that were subjected to clinical trials. The first three compounds belong to the thioxanthine family and the last one to thiopyrimidinone. All of these compounds have the same mechanism of action: a thioether bridge is formed between the heme group of the enzyme and the oxidized thioxantine or thiopyrimidinone (Figure 6A). However, the clinical trials of **PF06282999** have been terminated due to its serious adverse effects [85].

Despite the accumulation of evidence involving MPO in many chronic syndromes, especially atherosclerosis [97], and the activity of these compounds in inhibiting MPO in vivo after

oral administration [98], their usefulness for treating and/or preventing atherosclerosis has not been proved yet and further studies are needed [99]. In fact, the main challenge in studying the effect of the MPO inhibition on atherosclerosis is that this disease needs a long time to develop [100,101]. In addition, atherosclerosis is a multi-factorial syndrome [102–106]; thus, it is difficult to obtain a suitable animal model to realize the *in vivo* studies. It is noteworthy that all pre-clinical tests have been achieved in animals with high expressions of MPO to evaluate the activity of the enzyme in the presence of the inhibitor and on normal animals to determine the tolerance, safety, ADME profiles [107–109].

An attempt to evaluate the interest of MPO inhibition in atherosclerosis has been done on **PF06282999** using low-density lipoprotein receptor (Ldlr) knockout ($Ldlr^{-/-}$) mouse model of atherosclerosis. The results established from this experiment have indicated that MPO inhibition could not improve the atherosclerotic plaque area or leukocyte migration, but rather reduces the inflammatory level of atherosclerotic lesions; thus, MPO inhibition could stabilize the atherosclerotic lesion preventing atherosclerotic plaque rupture [97,110]. In addition, the experiments on **PIC1** have indicated that lung injury could be reduced by MPO inhibitors [52,54]. Moreover, the data established from in vivo experiments using the tandem stenosis model of atherosclerotic plaque instability in apolipoprotein E gene knockout ($Apoe^{-/-}$) mice and by employing 2-thioxanthine compound **AZM198** (structure not disclosed) suggested that the inhibition of MPO can reduce atherosclerotic plaque instability [111]. Giving **INV-315** for mice also has resulted in reduction in atherosclerotic lesions [60]. On the contrary, it has been reported that **AZD3241** could not reduce neurodegeneration and it failed to alter the neuronal loss and the motor impairments [112]. These conflicting results may originate in the fact that these diseases are multifactorial and the response to the treatment by MPO inhibitors depends on the animal model used in experiments.

In summary, the data that implicate MPO in several CVD and CNS diseases have made this enzyme a new target for the treatment and prevention of these syndromes. The most important inhibitors are **AZD5904, AZD3241, AZD4831**, and **PF06282999** since these inhibitors were advanced to clinical trials. However, until now, no inhibitor has passed phase II of clinical trials as their pharmacological properties have not shown satisfactory results that made these molecules move to the higher phases of clinical trials. And some of these compounds have been in repurposing studies while **AZD5904** has been abandoned as a clinical candidate for the treatment of inflammatory conditions involving oxidative stress [113]. Therefore, the true interest of these inhibitors is still under investigation.

According to these data, we believe that the development of potent MPO inhibitors will be continued for using them in the treatment of CVD and other chronic syndromes. By the exception to **AZD4831**, all the inhibitors which passed to clinical trials have an activity at μM range that activity that appears to be insufficient to be able to reduce the deleterious effects of MPO. In fact, the in vitro test of the inhibition of the MPO-based oxidation of LDL has shown that only the compounds which have an activity at low nM range can inhibit the formation of MoxLDL [64,67,91]. Therefore, potent reversible inhibitors may be a good choice to discover novel therapeutic interventions for atherosclerotic cardiovascular and other chronic syndromes.

Funding

This paper was funded by the Universite Libre de Bruxelles.

Declaration of interest

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

Reviewer disclosures

Peer reviewers on this manuscript have no relevant financial or other relationships to disclose.

References

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. Ermert D, Niemiec MJ, Röhm M, et al. Candida albicans escapes from mouse neutrophils. J Leukoc Biol. 2013;94:223–236.
2. Jacobs L, Nawrot TS, de Geus B, et al. Subclinical responses in healthy cyclists briefly exposed to traffic-related air pollution: an intervention study. Environ Health. 2010;9:64.
3. Metzler KD, Fuchs TA, Nauseef WM, et al. Myeloperoxidase is required for neutrophil extracellular trap formation: implications for innate immunity. Blood. 2011;117:953–959.
4. Nauseef WM. Posttranslational processing of a human myeloid lysosomal protein, myeloperoxidase. Blood. 1987;70:1143–1150.
5. Klebanoff SJ. Myeloperoxidase: friend and foe. J Leukoc Biol. 2005;77:598–625.
•• **Comprehensive review on the role of MPO in pathology.**
6. Klebanoff SJ, Kettle AJ, Rosen H, et al. Myeloperoxidase: a front-line defender against phagocytosed microorganisms. J Leukoc Biol. 2013;93(2):185–198.
7. Nauseef WM. Biosynthesis of human myeloperoxidase. Arch Biochem Biophys. 2018;642:1–9.
8. Fiedler TJ, Davey CA, Fenna RE. X-ray crystal structure and characterization of halide-binding sites of human myeloperoxidase at 1.8 A resolution. J Biol Chem. 2000;275:11964–11971.
9. Van Antwerpen P, Slomianny M-C, Boudjeltia KZ, et al. Glycosylation pattern of mature Dimeric Leukocyte and recombinant Monomeric Myeloperoxidase: glycosylation is required for optimal enzymatic activity. J Biol Chem. 2010;285:16351–16359.
10. Furtmüller PG, Burner U, Obinger C. Reaction of Myeloperoxidase compound I with chloride, bromide, iodide, and thiocyanate. Biochemistry. 1998;37:17923–17930.
11. Kettle AJ, Winterbourn CC. The mechanism of myeloperoxidase-dependent chlorination of monochlorodimedon. Biochim Biophys Acta. 1988;957:185–191.
12. Hoogland H, van Kuilenburg A, van Riel C, et al. Spectral properties of myeloperoxidase compounds II and III. Biochim Biophys Acta. 1987;916:76–82.
13. Paumann-Page M, Furtmüller PG, Hofbauer S, et al. Inactivation of human myeloperoxidase by hydrogen peroxide. Arch Biochem Biophys. 2013;539:51–62.
14. Arnhold J, Furtmüller PG, Obinger C. Redox properties of myeloperoxidase. Redox Rep. 2003;8:179–186.

• **The mechanism of MPO oxidation**

15. Pereira WE, Hoyano Y, Summons RE, et al. Chlorination studies. II. The reaction of aqueous hypochlorous acid with alpha-amino acids and dipeptides. Biochim Biophys Acta. 1973;313:170–180.
16. Ximenes VF, Maghzal GJ, Turner R, et al. Serotonin as a physiological substrate for myeloperoxidase and its superoxide-dependent oxidation to cytotoxic tryptamine-4,5-dione. Biochem J. 2009;425:285–293.
17. Fu X, Kassim SY, Parks WC, et al. Hypochlorous acid generated by myeloperoxidase modifies adjacent tryptophan and glycine residues in the catalytic domain of matrix metalloproteinase-7 (matrilysin): an oxidative mechanism for restraining proteolytic activity during inflammation. J Biol Chem. 2003;278:28403–28409.
18. Prütz WA. Hypochlorous Acid Interactions with Thiols, Nucleotides, DNA, and other biological substrates. Arch Biochem Biophys. 1996;332:110–120.
19. Mongirdienė A, Laukaitienė J, Skipskis V, et al. The effect of oxidant hypochlorous acid on platelet aggregation and dityrosine concentration in chronic heart failure patients and healthy controls. Medicina (Kaunas). 2019;55(5):198.
20. Dennisjr W, Olivieri V, Kruse C. The reaction of nucleotides with aqueous hypochlorous acid. Water Res. 1979;13:357–362.
21. Grotto D, Maria LS, Valentini J, et al. Importance of the lipid peroxidation biomarkers and methodological aspects for malondialdehyde quantification. Quím Nova. 2009;32:169–174.
22. Spickett CM, Jerlich A, Panasenko OM, et al. The reactions of hypochlorous acid, the reactive oxygen species produced by myeloperoxidase, with lipids. Acta Biochim Pol. 2000;47:889–899.
23. Nicholls SJ, Hazen SL. Myeloperoxidase and cardiovascular disease. Arterioscler Thromb Vasc Biol. 2005;25:1102–1111..

•• **Important review on the role of MPO in atherosclerosis**

24. Green PS, Mendez AJ, Jacob JS, et al. Neuronal expression of myeloperoxidase is increased in Alzheimer's disease. J Neurochem. 2004;90:724–733.
25. Gellhaar S, Sunnemark D, Eriksson H, et al. Myeloperoxidase-immunoreactive cells are significantly increased in brain areas affected by neurodegeneration in Parkinson's and Alzheimer's disease. Cell Tissue Res. 2017;369:445–454.
26. Choi D-K, Pennathur S, Perier C, et al. Ablation of the inflammatory enzyme myeloperoxidase mitigates features of Parkinson's disease in mice. J Neurosci. 2005;25:6594–6600.
27. Sánchez-López F, Tasset I, Agüera E, et al. Oxidative stress and inflammation biomarkers in the blood of patients with Huntington's disease. Neurol Res. 2012;34:721–724.
28. Fernandes RMSN, da Silva NP, Sato EI. Increased myeloperoxidase plasma levels in rheumatoid arthritis. Rheumatol Int. 2012;32:1605–1609.
29. Saed GM, Ali-Fehmi R, Jiang ZL, et al. Myeloperoxidase serves as a redox switch that regulates apoptosis in epithelial ovarian cancer. Gynecol Oncol. 2010;116:276–281.
30. Nagra RM, Becher B, Tourtellotte WW, et al. Immunohistochemical and genetic evidence of myeloperoxidase involvement in multiple sclerosis. J Neuroimmunol. 1997;78:97–107.
31. Cascorbi I, Henning S, Brockmöller J, et al. Substantially reduced risk of cancer of the aerodigestive tract in subjects with variant–463A of the myeloperoxidase gene. Cancer Res. 2000;60:644–649.
32. Hung RJ, Boffetta P, Brennan P, et al. Genetic polymorphisms of MPO, COMT, MnSOD, NQO1, interactions with environmental exposures and bladder cancer risk. Carcinogenesis. 2004;25:973–978.
33. Pakakasama S, Chen TT-L, Frawley W, et al. Myeloperoxidase promotor polymorphism and risk of hepatoblastoma. Int J Cancer. 2003;106:205–207.
34. Lee JS, Oh TY, Ahn BO, et al. Involvement of oxidative stress in experimentally induced reflux esophagitis and Barrett's esophagus: clue for the chemoprevention of esophageal carcinoma by antioxidants. Mutat Res. 2001;480–481:189–200.
35. Yang X, Li Y, Li Y, et al. Oxidative Stress-Mediated Atherosclerosis: mechanisms and Therapies. Front Physiol. 2017;8:600.
36. Fulton DJR, Barman SA. Clarity on the Isoform-specific roles of NADPH oxidases and NADPH oxidase-4 in Atherosclerosis. Arterioscler Thromb Vasc Biol. 2016;36:579–581.
37. Park J-G, Oh GT. The role of peroxidases in the pathogenesis of atherosclerosis. BMB Rep. 2011;44:497–505.
38. Soubhye J, Aldib I, Delporte C, et al. Myeloperoxidase as a target for the treatment of inflammatory syndromes: mechanisms and structure activity relationships of inhibitors. Curr Med Chem. 2016;23:3975–4008.

•• **Comprehensive review on MPO inhibitors**

39. Malle E, Marsche G, Arnhold J, et al. Modification of low-density lipoprotein by myeloperoxidase-derived oxidants and reagent hypochlorous acid. Biochim Biophys Acta. 2006;1761:392–415.
40. Abu-Soud HM, Hazen SL. Nitric oxide modulates the catalytic activity of myeloperoxidase. J Biol Chem. 2000;275:5425–5430.
41. Harrison DG. Cellular and molecular mechanisms of endothelial cell dysfunction. J Clin Invest. 1997;100:2153–2157.
42. Zouaoui Boudjeltia K, Daher J, Van Antwerpen P, et al. Exposure of endothelial cells to physiological levels of myeloperoxidase-modified LDL delays pericellular fibrinolysis. PLoS ONE. 2012;7:e38810.

• **The role of MPO on fibrinolysis**

43. Ndrepepa G. Myeloperoxidase – A bridge linking inflammation and oxidative stress with cardiovascular disease. Clin Chim Acta. 2019;493:36–51.
44. Malle E, Furtmüller PG, Sattler W, et al. Myeloperoxidase: a target for new drug development? Br J Pharmacol. 2007;152:838–854.
45. Reiding KR, Franc V, Huitema MG, et al. Neutrophil myeloperoxidase harbors distinct site-specific peculiarities in its glycosylation. J Biol Chem. 2019;294:20233–20245.
46. Jantschko W, Furtmüller PG, Zederbauer M, et al. Exploitation of the unusual thermodynamic properties of human myeloperoxidase in inhibitor design. Biochem Pharmacol. 2005;69:1149–1157.
47. Soubhye J, Meyer F, Furtmüller P, et al. Characterization of chemical features of potent myeloperoxidase inhibitors. Future Med Chem. 2016;8:1163–1177.
48. Aldib I, Soubhye J, Zouaoui Boudjeltia K, et al. Evaluation of new scaffolds of myeloperoxidase inhibitors by rational design combined with high-throughput virtual screening. J Med Chem. 2012;55:7208–7218.
49. Bensalem S, Soubhye J, Aldib I, et al. Inhibition of myeloperoxidase activity by the alkaloids of Peganum harmala L (Zygophyllaceae). J Ethnopharmacol. 2014;154:361–369.
50. Aldib I, Gelbcke M, Soubhye J, et al. Novel bis-arylalkylamines as myeloperoxidase inhibitors: design, synthesis, and structure-activity relationship study. Eur J Med Chem. 2016;123:746–762.
51. Smallheer J, Kick E, Valente M, et al. Macrocyclic Inhibitors of Myeloperoxidase. WO/2018/005336, (2018).
52. Hair PS, Enos AI, Krishna NK, et al. Inhibition of complement activation, myeloperoxidase, NET formation and oxidant activity by PIC1 peptide variants. Li Y. editor PLoS ONE. 2019;14:e0226875..
53. Hair PS, Sass LA, Krishna NK, et al. Inhibition of myeloperoxidase activity in cystic fibrosis sputum by peptide inhibitor of complement C1 (PIC1). Palaniyar N. editor PLoS ONE. 2017;12:e0170203..
54. Krishna N, Cunnion K PIC1 inhibition of myeloperoxidase oxidative activity in an animal model. WO/2019/139886 (2019).
55. Zhang H, Jing X, Shi Y, et al. N-acetyl lysyltyrosylcysteine amide inhibits myeloperoxidase, a novel tripeptide inhibitor. J Lipid Res. 2013;54:3016–3029.
56. Hao Z, Yang S, Hao X, et al. Peptide-based myeloperoxidase inhibitors for use in therapy. EP2485750B1 (2010).
57. Yu G, Liang Y, Huang Z, et al. Inhibition of myeloperoxidase oxidant production by N-acetyl lysyltyrosylcysteine amide reduces brain damage in a murine model of stroke. J Neuroinflammation. 2016;13:119.
58. Yu G, Zheng S, Zhang H. Inhibition of myeloperoxidase by N-acetyl lysyltyrosylcysteine amide reduces experimental autoimmune encephalomyelitis-induced injury and promotes oligodendrocyte regeneration and neurogenesis in a murine model of progressive multiple sclerosis:. NeuroReport. 2018;29:208–213.
59. van der Does AM, Hensbergen PJ, Bogaards SJ, et al. The human lactoferrin-derived peptide hLF1-11 exerts immunomodulatory effects by specific inhibition of myeloperoxidase activity. J Immunol. 2012;188:5012–5019.

60. Liu C, Desikan R, Ying Z, et al. Effects of a novel pharmacologic inhibitor of myeloperoxidase in a mouse atherosclerosis model. Schmidt HHHW. editor PLoS ONE. 2012;7:e50767.
61. Jayaraj P, Narasimhulu CA, Maiseyeu A, et al. Methoxyphenol derivatives as reversible inhibitors of myeloperoxidase as potential antiatherosclerotic agents. Future Med Chem. 2020;12:95–110.
62. Ximenes VF, Paino IMM, Faria-oliveira de OMM, et al. Indole ring oxidation by activated leukocytes prevents the production of hypochlorous acid. Braz J Med Biol Res. 2005;38:1575–1583.
63. Van Antwerpen P, Zouaoui Boudjeltia K. Rational drug design applied to myeloperoxidase inhibition. Free Radic Res. 2015;49:711–720.
64. Soubhye J, Prévost M, Van Antwerpen P, et al. Structure-based design, synthesis, and pharmacological evaluation of 3-(Aminoalkyl)-5-fluoroindoles as myeloperoxidase inhibitors. J Med Chem. 2010;53:8747–8759.

- **Development of the most active MPO inhibitors (until today)**

65. Soubhye J, Dufrasne F, Van Antwerpen P 3-Alkyl-5-fluoroindole derivatives as myeloperoxidase inhibitors. WO2010136546 (2010).
66. Soubhye J, Aldib I, Prévost M, et al. Hybrid molecules inhibiting myeloperoxidase activity and serotonin reuptake: a possible new approach of major depressive disorders with inflammatory syndrome: hybrid MPO and 5-HT reuptake inhibitors. J Pharm Pharmacol. 2014. n/a-n/a. doi:10.1111/jphp.12236
67. Soubhye J, Aldib I, Elfving B, et al. Design, synthesis, and structure–activity relationship studies of novel 3-alkylindole derivatives as selective and highly potent myeloperoxidase inhibitors. J Med Chem. 2013;56:3943–3958.
68. Soubhye J, Dufrasne F, Zouaoui Boudjeltia K, et al. Aromatic N-heterocycle derivatives for use as medicine. EP2682119 (2013).
69. A-K T, Sjögren T, Svensson M, et al. 2-thioxanthines are mechanism-based inactivators of myeloperoxidase that block oxidative stress during inflammation. J Biol Chem. 2011;286:37578–37589.

- **Development of the irreversible MPO inhibitors which passed to clinical trials**

70. Ward J, Spath SN, Pabst B, et al. Mechanistic characterization of a 2-thioxanthine myeloperoxidase inhibitor and selectivity assessment utilizing click chemistry–activity-based protein profiling. Biochemistry. 2013;52:9187–9201.
71. Piek A, Koonen DPY, Schouten E-M, et al. Pharmacological myeloperoxidase (MPO) inhibition in an obese/hypertensive mouse model attenuates obesity and liver damage, but not cardiac remodeling. Sci Rep. 2019;9(1):18765..
72. Nordvall G, Tiden A, Hanson S Thioxanthine derivatives as myeloperoxidase inhibitors. WO/2003/089430 (2003).
73. Barrett E, Aylor KW, Chai W, et al. Inhibition of myeloperoxidase and MPO reverses microvascular insulin resistance in high-fat diet (HFD)-fed Rats. Diabetes. 2018;67:22–LB.
74. Tong X, Zhou D, Savage A, et al. Population pharmacokinetic modeling with enterohepatic circulation for AZD3241 in healthy subjects and patients with multiple system Atrophy. J Clin Pharmacol. 2018;58:1452–1460.
75. Jucaite A, Svenningsson P, Rinne JO, et al. Effect of the myeloperoxidase inhibitor AZD3241 on microglia: a PET study in Parkinson's disease. Brain. 2015;138:2687–2700.
76. AZD3241 PET MSA trial, phase 2, randomized,12 week safety and tolerability trial with pet in MSA patients [Internet]. [cited 2017 Sep 25]. Available from: https://clinicaltrials.gov/ct2/show/NCT02388295?term=Myeloperoxidase+inhibitor&draw=2&rank=3.
77. Gan L-M, Lagerström-Fermér M, Ericsson H, et al. Safety, tolerability, pharmacokinetics and effect on serum uric acid of the myeloperoxidase inhibitor AZD4831 in a randomized, placebo-controlled, phase I study in healthy volunteers. Br J Clin Pharmacol. 2019;85:762–770.
78. MPO inhibitor A_Zeneca for HFpEF [Internet]. [cited 2020 Feb 5]. Available from: https://clinicaltrials.gov/ct2/show/NCT03611153.
79. Li Y, Ganesh T, Diebold BA, et al. Thioxo-dihydroquinazolin-one compounds as novel inhibitors of myeloperoxidase. ACS Med Chem Lett. 2015;6:1047–1052.
80. Lambeth JD, Ganesh T, Smith SM, et al. Quinazoline derivatives, compositions, and uses related thereto. US20160243121 (2016).
81. Wurtz NR, Viet A, Shaw SA, et al. Potent triazolopyridine Myeloperoxidase inhibitors. ACS Med Chem Lett. 2018;9:1175–1180.
82. Quoc Viet A, Wurtz NR Thioether triazolopyridine and triazolopyrimidine inhibitors of Myeloperoxidase. US20190031623 (2019).
83. Ruggeri R 2-Thiopyrimidinones. WO/2016/178113 (2016).
84. Ruggeri RB, Buckbinder L, Bagley SW, et al. Discovery of 2-(6-(5-Chloro-2-methoxyphenyl)-4-oxo-2-thioxo-3,4-dihydropyrimidin-1(2 *H*)-yl)acetamide (PF-06282999): A highly selective mechanism-based myeloperoxidase inhibitor for the treatment of cardiovascular diseases. J Med Chem. 2015;58:8513–8528.
85. Dong JQ, Varma MV, Wolford A, et al. Pharmacokinetics and disposition of the thiouracil derivative PF-06282999, an orally bioavailable, irreversible inactivator of Myeloperoxidase enzyme, across animals and humans. Drug Metab Dispos. 2016;44:209–219.
86. Dong JQ, Gosset JR, Fahmi OA, et al. Examination of the human Cytochrome P4503A4 induction potential of PF-06282999, an irreversible Myeloperoxidase inactivator: integration of Preclinical, in Silico, and biomarker methodologies in the prediction of the clinical outcome. Drug Metab Dispos. 2017;45:501–511.
87. Moscovitz JE, Lin Z, Johnson N, et al. Induction of human cytochrome P450 3A4 by the irreversible myeloperoxidase inactivator PF-06282999 is mediated by the pregnane X receptor. Xenobiotica. 2018;48:647–655.
88. Study A To evaluate the safety and effects on the body of an investigational drug using an endotoxin-induced inflammatory response model (POM) [Internet]. Available from: https://clinicaltrials.gov/ct2/show/NCT01965600?term=PF-06282999&draw=2&rank=3.
89. Myeloperoxidase: AY. Its role for host defense, inflammation, and neutrophil function. Arch Biochem Biophys. 2018;640:47–52.
90. Lazarević-Pasti T, Leskovac A, Vasić V. Myeloperoxidase Inhibitors as Potential Drugs. Curr Drug Metab. 2015;16:168–190.
91. Soubhye J, Chikh Alard I, Aldib I, et al. Discovery of novel potent reversible and irreversible Myeloperoxidase inhibitors using virtual screening procedure. J Med Chem. 2017;60:6563–6586.
92. Hasanpour Z, Javanmard S, Gharaaty M, et al. Association between serum myeloperoxidase levels and coronary artery disease in patients without diabetes, hypertension, obesity, and hyperlipidemia. Adv Biomed Res. 2016;5:103.
93. Teng N, Maghzal GJ, Talib J, et al. The roles of myeloperoxidase in coronary artery disease and its potential implication in plaque rupture. Redox Rep. 2017;22:51–73.
94. Zeng L, Mathew AV, Byun J, et al. Myeloperoxidase-derived oxidants damage artery wall proteins in an animal model of chronic kidney disease–accelerated atherosclerosis. J Biol Chem. 2018;293:7238–7249.
95. Soubhye J, Gelbcke M, Van Antwerpen P, et al. From dynamic combinatorial chemistry to in vivo evaluation of reversible and irreversible Myeloperoxidase inhibitors. ACS Med Chem Lett. 2017;8:206–210.
96. Lemke T, Williams D. Principles of drug discovery. Foye's principles of medicinal chemistry. 5th ed. ed. Lippincott, Philadelphia: Williams & Wilkins; 2002.
97. Long L, Song Y. Myeloperoxidase: A promising therapeutic target in prevention of atherosclerosis. Int J Cardiol. 2018;256:15.
98. Wang W, Ren D, Wang C, et al. Inhibition of myeloperoxidase might be a novel therapeutic target in the treatment of atherosclerosis. Int J Cardiol. 2018;256:33.
99. Senders ML, Mulder WJM. Targeting myeloperoxidase in inflammatory atherosclerosis. Eur Heart J. 2018;39:3311–3313.
100. Lusis AJ. Atherosclerosis. Nature. 2000;407:233–241.
101. Insull W. The pathology of Atherosclerosis: plaque development and plaque responses to medical treatment. Am J Med. 2009;122:S3–S14.
102. Aviram M, Fuhrman B. LDL oxidation by arterial wall macrophages depends on the oxidative status in the lipoprotein and in the cells: role of prooxidants vs antioxidants. Mol Cell Biochem. 1998;188:149–159.

103. Rifai N. Tietz Textbook of Clinical Chemistry and Molecular Diagnostics. 6th ed. Saunders, USA; 2017.
104. Breese BB. The use of the fluorescent antibody technic for identification of group A streptococci in pediatric practice. Am J Public Health Nations Health. 1968;58:2295–2305.
105. Li X, Fang P, Li Y, et al. Mitochondrial reactive oxygen species mediate Lysophosphatidylcholine-induced Endothelial cell activation. Arterioscler Thromb Vasc Biol. 2016;36:1090–1100
106. Williams KJ, Tabas I. The response-to-retention hypothesis of early Atherogenesis. Arterioscler Thromb Vasc Biol. 1995;15:551–561.
107. Pulli B, Ali M, Forghani R, et al. Measuring Myeloperoxidase activity in biological samples. Johnson R. editor PLoS ONE. 2013;8:e67976..
108. Hosseini M, Rezvani HR, Aroua N, et al. Targeting Myeloperoxidase disrupts mitochondrial redox balance and overcomes Cytarabine resistance in human Acute Myeloid Leukemia. Cancer Res. 2019;79:5191–5203.
109. Wadghiri YZ, Hoang DM, Leporati A, et al. High-resolution imaging of Myeloperoxidase activity sensors in human cerebrovascular disease. Sci Rep. 2018;8:7687.
110. Roth Flach RJ, Su C, Bollinger E, et al. Myeloperoxidase inhibition in mice alters atherosclerotic lesion composition. Bader M. editor PLoS ONE. 2019;14:e0214150.
111. Rashid I, Maghzal GJ, Chen Y-C, et al. Myeloperoxidase is a potential molecular imaging and therapeutic target for the identification and stabilization of high-risk atherosclerotic plaque. Eur Heart J. 2018;39:3301–3310.
112. Kaindlstorfer C, Sommer P, Georgievska B, et al. Failure of neuroprotection despite microglial suppression by delayed-start Myeloperoxidase inhibition in a model of advanced multiple system Atrophy: clinical Implications. Neurotox Res. 2015;28:185–194.
113. AstraZeneca's open innovation pharmacology toolbox. [Internet]. [cited 2014 Nov]. Available from: https://openinnovation.astrazeneca.com/clinical-compound-bank.html.