Structure-Based Design, Synthesis, and Pharmacological Evaluation of 3-(Aminoalkyl)-5-fluoroindoles as Myeloperoxidase Inhibitors

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Oxidized low-density lipoproteins (LDLs) accumulate in the vascular wall and promote local inflammation, which contributes to the progression of the atheromatous plaque. The key role of myeloperoxidase (MPO) in this process is related to its ability to modify APO B-100 in the intima and at the surface of endothelial cells. A series of 3-(aminoalkyl)-5-fluoroindole analogues was designed and synthesized by exploiting the structure-based docking of 5-fluorotryptamine, a known MPO inhibitor. In vitro assays were used to study the effects of these compounds on the inhibition of MPO-mediated taurine chlorination and oxidation of LDLs. The kinetics of the interaction between the MPO redox intermediates, Compounds I and II, and these inhibitors was also investigated. The most potent molecules possessed a 4- or 5-carbon aminoalkyl side chain and no substituent on the amino group. The mode of binding of these analogues and the mechanism of inhibition is discussed with respect to the structure of MPO and its halogenation and peroxidase cycles.

Introduction

The heme enzyme, myeloperoxidase (EC 1.11.1.7, MPO), is one of the key players in the first line of the nonspecific immune defense system. In the presence of hydrogen peroxide and halide ions, MPO catalyzes the synthesis of hypohalous acids (e.g., hypochlorous acid, HOCl). These oxidizing and halogenating compounds kill and destroy foreign microorganisms. MPO is packed in the azurophilic granules of phagocytes and contribute to tissue damage at sites of inflammation. In addition to its beneficial role in microbial killing, MPO is reported to be involved in a growing number of diseases because of its ability to generate reaction products that can oxidize many types of biomolecule (lipids, proteins, DNA, etc.).

MPO can influence disease pathogenesis in two ways: In the first, MPO is directly involved at the origin of the disease; in the second, MPO acts to worsen symptoms associated with inflammatory conditions. Several of these pathologies present major problems for public health at a global level. To investigate the involvement of MPO in cardiovascular disease, we focused our attention on atherosclerosis. It is well-known that MPO oxidizes the apolipoprotein (APO) B-100 of low-density lipoproteins (LDLs), thereby contributing to the development of atherosclerosis. Indeed, LDLs oxidized by MPO (Mox-LDL) can induce foam cell formation and trigger inflammatory responses in monocytes and endothelial cells. The interaction between MPO and LDL is facilitated by electrostatic interactions as MPO is strongly basic at physiological pH, whereas LDLs are negatively charged. In addition, it has been shown that MPO can oxidize the APO A1 of high-density lipoproteins (HDLs), thus impairing reverse cholesterol transport. Because of these deleterious effects, development of therapeutic strategies aimed at inhibiting MPO is needed. However, despite several attempts, there are still no molecules with sufficient in vitro and/or in vivo inhibiting properties.

We recently assessed the MPO inhibiting effects of commonly used anti-inflammatory drugs. Flufenamic acid (1, Figure 1) was identified as a good candidate, which inhibited both HOCl production and LDL oxidation. However, these results were only observed at high concentrations, rendering flufenamic acid unusable because of its toxic adverse effects at these doses. Analogous compounds were also investigated but none had better inhibitory activity, likely because their negative charge dampened the interaction between MPO and anionic LDLs. We, therefore, hypothesized that positively...
charged compounds could overcome this problem and in addition would allow more favorable interactions with anionic amino acid residues at the active site of MPO.

Jantschko et al. (2005) showed that tryptamine derivatives act as substrates and inhibitors of MPO. Derivatives that carry a metabolically stable substituent on position 5 of the indole ring, such as chlorine (5-chlorotryptamine) or fluorine (5-fluorotryptamine), were shown to be efficient reversible inhibitors. It must be noted that the stability of the aromatic moiety of potential inhibitors is crucial because the indole ring is subject to oxidation by MPO, particularly on position 5. Substitution by electron-withdrawing groups makes the indole moiety very fragile and prone to cleavage between C-2 and C-3. This has been demonstrated with melatonin, which possesses an OMe group on position 5. A promising MPO drug candidate must be a stable molecule and good substrate so that administered doses and the risk of adverse effects can be kept as small as possible.

By exploiting the positions of 5-fluorotryptamine obtained from structure-based docking, we designed analogues that could have additional interactions with the active site of the heme enzyme. Two strategies were followed, namely varying the length of the aminoalkyl side chain and adding substituents of different sizes to the amino group of the side chain. These analogues were then synthesized and their capacity to inhibit recombinant MPO, produced in CHO-cell lines, was measured. We report the effect of these new compounds on MPO-mediated taurine chlorination and LDL oxidation and on the interaction with the “Compound I” and “Compound II” redox intermediates of MPO. We also propose a mechanism for the inhibition.

Results

Docking Experiments. Indole and tryptamine derivatives have been reported to be reversible inhibitors of human MPO with 5-fluorotryptamine being the most effective. These findings prompted us to perform a rational structure-based design of new tryptamine derivatives based on the docking of 5-fluorotryptamine. The reliability of the docking procedure was previously assessed by comparing the docking of salicylhydroxamic acid with its position in the crystal structure of the MPO complex.

The best pose of 5-fluorotryptamine (Figure 2A) featured stacking of the indole 6-membered ring onto the pyrrole ring D of the heme. Stacking was also observed in the crystal structure of the MPO structure in complex with salicylhydroxamic acid. In addition to the stacking, one salt bridge was formed with Glu102 and one hydrogen bond with Thr100. No hydrogen bond was observed between the indole NH group and the protein. Another pose featured a hydrogen bond but at the expense of the stacking. Interestingly, empty pockets remained around the docked ligand and could be exploited to create structural modifications of 5-fluorotryptamine (Figure 2B). In particular, the voids facing Phe99, Phe147, Leu420, Phe407, and Leu406 could be filled with chemical groups so as to form additional hydrophobic interactions between the ligand and the binding site. On the basis of these observations, we developed two strategies for creating the analogues: (i) varying the length of the alkyl side chain and (ii) adding different alkyl or cyclic substituents on the side chain nitrogen. The amino group of 5-fluorotryptamine that bears a positive charge at physiological pH was conserved so as to keep the potential formation of an ionic...
interaction with Glu102 and/or the propionic heme group or to improve the potency of 5-fluorotryptamine, the effect of adding different substituents to the side chain amino group was analyzed. Compounds with linear or branched alkyl group substitutions on the side chain nitrogen had predicted scoring values close to those of unsubstituted compounds (see Table 1). Compound 26 had a score 1 kcal/mol more favorable than 5-fluorotryptamine (4). Its best pose showed hydrogen bonding to the propionic acid group and formation of a salt bridge with Glu102. Hydrophobic contacts mainly involved Phe99, Pro145, Phe146, Phe147, Phe407, Leu415, and Leu420.

We also probed cyclic groups as substituents on the amino group of the side chain in order to introduce new interactions and to reduce or avoid the energy penalty for immobilization of rotatable bonds upon binding of alkyl substituents. Two fragments were used: Pyrrolidinyl and N-methylpiperazinyl moieties. Compound 13 had the most favorable energy (−8.5 kcal/mol). The docked positions of this compound featured stacking with the heme pyrrole ring D, one hydrogen bond with the propionic group, an electrostatic interaction with Glu102 (not a salt bridge), and hydrophobic contacts with Phe407.

The analysis of the binding modes predicted by docking of these analogues and their binding energy values encouraged us to synthesize them for further evaluation.

### Table 1. IC₅₀ Values for the Inhibition of MPO, Predicted Free Energies of Binding Obtained from Docking Experiments

<table>
<thead>
<tr>
<th>no.</th>
<th>n</th>
<th>R₁</th>
<th>R₂</th>
<th>IC₅₀ (µM)</th>
<th>ΔG (kcal/mol)</th>
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<tbody>
<tr>
<td>6</td>
<td>1</td>
<td>H</td>
<td>H</td>
<td>0.9 ± 0.3</td>
<td>−5.3</td>
</tr>
<tr>
<td>7</td>
<td>1</td>
<td>C₂H₅</td>
<td>C₂H₅</td>
<td>0.2 ± 0.2</td>
<td>−6.3</td>
</tr>
<tr>
<td>8</td>
<td>1</td>
<td>N-methylpiperazinyl</td>
<td>1.0 ± 0.1</td>
<td>−7.3</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>H</td>
<td>H</td>
<td>0.20 ± 0.03</td>
<td>−6.5</td>
</tr>
<tr>
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<td>CH₃</td>
<td>H</td>
<td>0.20 ± 0.02</td>
<td>−6.6</td>
</tr>
<tr>
<td>17</td>
<td>2</td>
<td>C₂H₅</td>
<td>H</td>
<td>0.3 ± 0.1</td>
<td>−6.8</td>
</tr>
<tr>
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<td>0.80 ± 0.02</td>
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</tr>
<tr>
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</tr>
<tr>
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<td>CH₃</td>
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<tr>
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<tr>
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<td>−6.7</td>
</tr>
<tr>
<td>25</td>
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<td>C₂H₅</td>
<td>H</td>
<td>0.3 ± 0.1</td>
<td>−6.6</td>
</tr>
<tr>
<td>26</td>
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<td>C₂H₅</td>
<td>H</td>
<td>0.17 ± 0.08</td>
<td>−7.5</td>
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<tr>
<td>27</td>
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<td>C₆H₆</td>
<td>H</td>
<td>1.5 ± 0.5</td>
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<tr>
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<td>CH₃</td>
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<td>C₂H₅</td>
<td>0.35 ± 0.09</td>
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<td>0.32 ± 0.01</td>
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<td></td>
</tr>
<tr>
<td>31</td>
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<td>N-methylpiperazinyl</td>
<td>0.35 ± 0.06</td>
<td>−8.5</td>
<td></td>
</tr>
<tr>
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<td>4</td>
<td>H</td>
<td>H</td>
<td>0.015 ± 0.004</td>
<td>−6.6</td>
</tr>
<tr>
<td>41</td>
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<td>H</td>
<td>H</td>
<td>0.008 ± 0.002</td>
<td>−6.4</td>
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<tr>
<td>48</td>
<td>6</td>
<td>H</td>
<td>H</td>
<td>0.26 ± 0.01</td>
<td>−5.9</td>
</tr>
</tbody>
</table>

*Compounds are classified first following the length of the side chain and the substituents, then, respectively, according the mono- or disubstitution of the amino group. The IC₅₀ value is the mean ± SD of 3 independent experiments.*
Chemistry. Compound 6 was prepared as indicated in Scheme 1. The carbonyl group of the commercially available 5-fluoroindole-3-carboxaldehyde was transformed into the corresponding oxime to give compound 5. Hydrogenation of the latter, catalyzed by Pd/C 10% in the presence of HCl, led to compound 6.

For the series of indoles substituted by aminomethyl moieties, aminoalkylations of commercially available 5-fluoroindole were performed using the Mannich reaction, i.e., in the presence of secondary amines and formaldehyde, resulting in the synthesis of compounds 7 and 8 (Scheme 2).

For the syntheses starting from 5-fluoro-3-(hydroxyalkyl)indoles, a general procedure based on the Fisher indole synthesis was applied using 4-fluorophenylhydrazine hydrochloride and dihydrofuran or dihydropyran for the ring formation step. To improve the results for this step, we performed the reaction described by Campos and colleagues under microwave. 

These conditions led to shorter reaction times and higher yields (for example, 95% versus 72% for the synthesis of 9). The starting alcohols 9 and 10 were transformed into their mesylate derivatives in good yields by adding methanesulfonyl chloride at room temperature in the presence of triethylamine. Mesylate groups were then converted into azidoalkanes to give compounds 13 and 14, which were hydrogenated using Pd/C as catalyst to create compounds 4 and 15 (Scheme 3).

To synthesize compounds 16 to 31, which were substituted on the aliphatic nitrogen atom, mesylate derivatives 11 and 12 were added to the corresponding amines in dioxane at 100 °C (Scheme 4).

To obtain 3-(4-aminobutyl)-5-fluoro-1H-indole oxalate 34, the mesylate group in 12 was converted to nitrile 32, which was further hydrolyzed by KOH in t-BuOH to give carboxamide 33. Reduction of the amide using LiAlH₄ in dioxane led to the desired amine 34 (Scheme 5).

The synthesis of compound 41 followed the same synthetic path as for the indole derivative 34 but started from...
6-hydroxyhexanal 36, which was obtained by the reduction of \( \varepsilon \)-caprolactone 35 using DIBAL-H (Scheme 6). The aldehyde was then subjected to a Fisher reaction with 4-fluorophenylhydrazine hydrochloride, giving the alcohol 37, which was further converted into the mesylate 38. A nucleophilic substitution was performed on compound 38 using NaCN, and the resulting nitrile 39 was then hydrolyzed into the amide 40. After reduction of the latter by LiAlH4, the final compound (41) was isolated as its oxalate salt.

Finally, the fluorotryptamine derivative with 6 methylene groups in the side chain was obtained, starting from cyclooctanone 42 after a Baeyer–Villiger rearrangement and reduction of the resulting lactone 43 into aldehyde 44 (Scheme 7).

**Biological Activity.** A microplate reader was used to monitor inhibition of MPO-mediated taurine chlorination in a high throughput screening mode. Table 1 lists the IC_{50} values of the 3-(aminoalkyl)-5-fluoroindole analogues. The presented IC_{50} values clearly demonstrate that the capacity of the investigated compounds to inhibit MPO-mediated chlorination was modulated by the length of the aminoalkyl side chain on position 3 of 5-fluoroindole, as well as by the alkyl groups substituting its amino group. Compound 41 with a 5-carbon nonsubstituted side chain was the most efficient inhibitor. Lengthening the side chain from \( n = 1 \) to \( n = 5 \) increased the inhibitory potency. With the exception of substitution by dimethyl or pyrrolidinyl groups, substitution of the side chain nitrogen did not improve the inhibition. Compound 27 with a 3-carbon chain and a butyl substituent displayed the weakest activity. A closer analysis of compounds that had the same side chain length but different substituents revealed striking features. Among the molecules with a 2-carbon side chain, compound 22 with a pyrrolidine and compound 20 with a dimethylamino group exhibited the greatest activity. In this series, the size of the alkyl substituents (except for very bulky groups) apparently did not affect the inhibitory potency (compound 19). In the 3-carbon side chain series, the unsubstituted indole 15 had the most inhibitory activity. In this series, the volume of the substituents did not significantly change the interaction with MPO (as for 2-carbon side chain molecules). Finally, among all the fluorotryptamine derivatives, the 1-carbon chain compounds displayed marginal inhibitory activities.
Information).

Oxidation at three concentrations (1000, 100, and 50 nM; see Supporting Information). Other compounds with a 3-carbon side chain also exhibited rather good inhibitory activity (except compound 15). For comparison, indoles with a 2-carbon side chain had less inhibitory potency and compounds with a 1-carbon side chain were weak inhibitors of LDL oxidation.

Two compounds, however, attracted attention. Compound 27 had a poor inhibitory effect on MPO-mediated taurine chlorination but was an excellent inhibitor of LDL oxidation. In contrast, compound 22 had a small effect on LDL oxidation (except at the higher concentration) despite a marked effect on the chlorination activity of MPO.

Two redox intermediates are relevant in the enzymology of MPO, namely Compound I and Compound II. Because tryptamine derivatives have been shown to act as reversible inhibitors and interact with both redox intermediates,\(^a\) it was important to also study the direct interaction of the most efficient inhibitors (compounds 34 and 41) with Compound I and Compound II by sequential-mixing stopped-flow spectroscopy (Figures 5 and 6). Compound I is formed by the reaction of hydrogen peroxide with the ferric state of MPO (Figure 4). Thereby, the O–O bond is cleaved heterolytically, forming a redox intermediate called Compound I. In this fast reaction, Fe(III) is oxidized to an oxoferryl species [Fe(IV)]=O) and the porphyrin to the corresponding \(\pi\)-cation radical (reaction 1 in Figure 4). Compound I is deficient in two electrons and restoration of the ferric state can be accomplished by either direct two-electron reduction by halides (Cl\(^-\) or Br\(^-\)), thereby forming the corresponding hypohalous acids (reaction 2 in Figure 4), or by one electron reduction steps mediated by aromatic electron donors (including indole derivatives) via Compound II [Fe(IV)-OH] (reactions 3 and 4 in Figure 4).\(^{49}\) The halogenation cycle includes reactions 1 and 2, whereas the peroxidase cycle includes reactions 3, 1, and 3, 4. It is important to note that Compound II cannot oxidize chloride or bromide.

Figures 5 and 6 demonstrate the kinetics of the reactions between compound 34 and MPO Compound I and Compound II. Very similar time traces were observed with compound 41 (not shown). The inhibitors acted as one-electron donors to both redox intermediates of MPO. Figure 5 depicts the direct transition of Compound I to Compound II mediated by oxidation of compound 34. The reaction was monophasic (Figure 5B), and from the linear parent bimolecular rates (\(k_0\)) of (1.1 ± 0.1) \(\times 10^7\) M\(^{-1}\) s\(^{-1}\) (compound 34) and (1.0 ± 0.1) \(\times 10^7\) M\(^{-1}\) s\(^{-1}\) (compound 41) were calculated (Figure 5C). The high intercept clearly demonstrated that Compound II was not stable but further transformed. This is highlighted by Figures 6A and 6B that show the direct monophasic transition of Compound II to native MPO mediated by compound 34 with a clear isosbestic point. The apparent bimolecular rate constants (\(k_d\)) were

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**Table 2. IC\(_{50}\) Values for the Inhibition of the Oxidation of LDL Carried Out by MPO/Cl\(^{-}/\)H\(_2\)O\(_2\)**

<table>
<thead>
<tr>
<th>no.</th>
<th>(n)</th>
<th>(R_1)</th>
<th>(R_2)</th>
<th>IC(_{50}) ((\mu)M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>1</td>
<td>H</td>
<td>H</td>
<td>&gt; 1(^\text{a})</td>
</tr>
<tr>
<td>7</td>
<td>1</td>
<td>C(_3)H(_5)</td>
<td>C(_3)H(_5)</td>
<td>&gt; 1(^\text{a})</td>
</tr>
<tr>
<td>8</td>
<td>1</td>
<td>N-methylpiperazinyl</td>
<td></td>
<td>&gt; 1(^\text{a})</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>H</td>
<td>H</td>
<td>0.94(^\text{a})</td>
</tr>
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<td>0.65(^\text{a})</td>
</tr>
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<td>CH(_3)H(_3)</td>
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<td>23</td>
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<td>N-methylpiperazinyl</td>
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<td>0.65(^\text{a})</td>
</tr>
<tr>
<td>15</td>
<td>3</td>
<td>CH(_3)</td>
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<td>0.65(^\text{a})</td>
</tr>
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<td>6</td>
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<td>H</td>
<td>0.58(^\text{a})</td>
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</table>

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\(^{a}\)IC\(_{50}\) values calculated by measuring the inhibition of the LDL oxidation at three concentrations (1000, 100, and 50 nM; see Supporting Information). \(^{b}\)IC\(_{50}\) values were calculated from sigmoid curves obtained by measuring the inhibition with seven concentrations (5.0, 7.5, 10.0, 25.0, 50.0, 500.0, 1000.0 nM).

To measure MPO-dependent LDL oxidation, which is a contributing factor in atherogenesis, an ELISA was recently developed, based on a mouse monoclonal antibody (Mab AG9) that specifically recognizes MPO-oxidized APO B-100 on LDL.\(^{30}\) Table 2 lists the IC\(_{50}\) values for the inhibition of LDL oxidation by the different compounds. Most of the compounds described in this study effectively inhibited LDL oxidation at submicromolar concentrations. Compounds 34 and 41 and, to a lesser extent, 24 and 27, showed the best inhibition profile. Other compounds with a 3-carbon side chain also exhibited rather good inhibitory activity (except compound 15). For comparison, indoles with a 2-carbon side chain had less inhibitory potency and compounds with a 1-carbon side chain were weak inhibitors of LDL oxidation.
360 M$^{-1}$ s$^{-1}$ (compound 34) and 506 M$^{-1}$ s$^{-1}$ (compound 41) (Figure 6C). The $k_3/k_4$ ratios of 30555 and 19762 suggest that Compound II reduction was the rate limiting step in the peroxidase cycle. Both inhibitors were excellent electron donors for Compound I, but poor substrates for Compound II.

**Discussion and Conclusions**

We used structure-based docking of 5-fluorotryptamine (4) in the active site of MPO to design derivatives with potent inhibitory action against MPO (see Table 1 and 2). All the designed derivatives could be docked into the active site pocket. The best poses featured stacking between the indole and the pyrrole moieties although this interaction was not ideal in all cases. The docking energy binding values of these compounds ranged between $-5.3$ and $-8.5$ kcal/mol with a value of $-6.5$ kcal/mol for 5-fluorotryptamine (4). Given the difficulty in evaluating affinity using the scoring functions developed for the docking programs, we could not reliably discriminate between the different ligands, and as a result, all the designed compounds were synthesized and their IC$_{50}$ values were measured.

In unsubstituted compounds, the distance between the indole moiety and the side chain nitrogen atom appeared to be an important parameter for binding to, and inhibition of, MPO. Lengthening the side chain from 1 to 5 carbons increased the inhibitory potency by 2 orders of magnitude. The docked poses provide a tentative explanation for the high IC$_{50}$ value for the 1-carbon side chain; because of the short alkyl chain, the nitrogen group cannot reach the negatively charged Glu102 to form a salt bridge, as observed for the 2-, 3-, 4-, and 5-carbon side chains. However, a further increase to a 6-carbon side chain again increased the IC$_{50}$.

In contrast, in 2- and 3-carbon side chain compounds, the inhibitory potency decreased when the side chain amino group was substituted with linear alkyl substituents of increasing length. In most cases, compounds with an N-substituted side chain showed higher IC$_{50}$ values compared to unsubstituted compounds with the same side chain length. However, compounds with two substituents had more favorable inhibitory properties compared to those with just one linear substituent (e.g., compare compound 19 with compound 21 or compound 27 with compound 29). The docked poses suggest that disubstituted amino groups fit better into the hydrophobic pocket of MPO.

Compounds with cyclic substituents were not potent inhibitors, with the exception of compound 22. The experimental data and the docked binding modes suggest that the steric hindrance on the side chain amino group and the flexibility of the substituents may be important factors for the structure–activity relationship.

There was no clear correlation between the calculated docking binding energies and the IC$_{50}$ values derived from the two in vitro inhibition assays. This illustrates that molecular docking
may be helpful in predicting binding modes of a molecule to an active site but cannot easily be used for ranking the inhibitory capacity. In the present study, the inhibiting molecules were also substrates and their efficiency to interact with MPO Compounds I and II depended also on the redox potential of the bound molecule itself, which is not necessarily correlated with the binding affinity. As a result of the poor scoring correlation, "induced-fit" studies were undertaken for two compounds selected as the least and most potent inhibitors (27 and 34). Docked poses differed only by the positions of their side chains and the scored affinity shifted from $-6.8$ to $-5.8$ and from $-6.6$ to $-7.6$ kcal/mol for compounds 27 and 34, respectively. This observation suggests that "induced-fit" effects could be important for binding of fluorotryptamine analogues.

Inhibition parameters for LDL oxidation are relevant because they reflect the potential in vivo effects of the compounds (see Table 2). One of the most striking observations is that substitution by $N$-methylpipеразинyl in the compounds with 2-carbon side chains increased the inhibitory potency in comparison with structurally closely related compounds in terms of chain length and steric hindrance (for example, compare compounds 22 and 23). Compound 23 was the weakest in its series in the MPO chlorination inhibition assay (see Table 1) but had a very good activity in the inhibition of LDL oxidation (see Table 2). The reason for this sudden increase in LDL oxidation inhibitory activity for compound 23 is unknown and might be ascribed to the presence of a second nitrogen. The second important observation is the very marked activity of compounds 34 and 41, which was corroborated by the inhibition of taurine chlorination. A relationship between inhibition of taurine chlorination and of LDL-oxidation is obvious for the most potent inhibitors. This demonstrates that these different molecules were able to inhibit LDL oxidation in spite of the binding between MPO and LDL.48 Binding of MPO to LDL during LDL oxidation is responsible for the oxidative modification of APO B-100 in plasma. However, this event could also block the catalytic site of the enzyme and, therefore, interfere with enzymatic inhibition by providing some protective effect.16 The heme group of MPO that is responsible for the enzymatic activity is indeed located in a distal hydrophobic cavity with a narrow oval-shaped opening.30 This characteristic might explain the observed differences in the inhibition of MPO-mediated taurine chlorination and LDL oxidation. As far as binding to LDL is concerned, the lipophilicity of potent inhibitors seems to be an important factor. Indeed, the formation of a complex macromolecular structure between MPO and LDL, mainly through electrostatic interactions, may modify the chemical and steric requirements for good penetration of substrates and/or inhibitors into the heme cavity. Parameters such as charge and lipophilicity of compounds have already been evoked to explain similar results.16,30

The transient kinetics study demonstrated that the probed compounds act as electron donors of both relevant redox intermediates of MPO, namely Compound I and Compound II. Detailed kinetic parameters could be elucidated for the most potent inhibitors, compounds 34 and 41. Substrates for MPO (halides or aromatic compounds) have been demonstrated to
bind and deliver electrons at the entrance to the distal heme pocket near the \( \delta \)-meso bridge (between pyrrol ring A and D; see Figure 4),\textsuperscript{49,50} which is supported by the presented docking data. It is reasonable to assume that substrate binding sites at Compounds I and II are identical. This assumption is based on the fact that structural differences in the heme pocket even between (high-spin) ferric MPO and its (low-spin) cyanide complex are negligible.\textsuperscript{50} The cyanide complex of a peroxidase is a suitable model for the (low-spin) oxoferryl-state of Compound I and Compound II.\textsuperscript{50} Consequently, the observed differences between the apparent reaction rates, \( k_3 \) and \( k_4 \), must be the result of differences in the redox properties of Compound I and Compound II. Because of the very high reduction potential of MPO Compound I, both inhibitors were rapidly oxidized, thereby directly reducing Compound I to Compound II.\textsuperscript{49} Compared to Compound I, Compound II is a weaker oxidant, as reflected by high \( k_3/k_4 \) ratios. Similar findings have been reported for 5-fluorotryptamine, suggesting that these compounds act as reversible inhibitors.\textsuperscript{31} Because of their marked efficiency in one-electron reduction of Compound I, they shift MPO from the chlorination cycle to the peroxidase cycle (Figure 4) because Compound II is not able to oxidize chloride or bromide. The MPO-typical high difference in reduction potentials between Compounds I and II (which is not observed in homologous lactoperoxidase or eosinophil peroxidase) guarantees that a high percentage of MPO stays as Compound II and that other human heme peroxidases cannot oxidize these inhibitors. As a result, the new tryptamine analogues decrease the pool of native MPO and Compound I responsible for the oxidative damage and do not interfere with homologous peroxidases.

In conclusion, a series of 3-(aminoalkyl)-5-fluorotyriodole analogues was designed using structure-based docking of 5-fluorotryptamine. These compounds were synthesized and assessed in vitro. Some of these compounds showed considerable potency for inhibition of taurine chlorination and of LDL oxidation mediated by MPO. The more potent molecules possess a 4- or 5-carbon length side chain and no substituent on the side chain nitrogen. These are the first molecules that have been shown to inhibit LDL oxidation in vitro at nanomolar concentrations, thus providing an excellent basis for further development of drugs targeting MPO. The discrepancies observed in the different MPO inhibition assays (Table 1 and Table 2) provide insight into the interaction between MPO and LDLs during LDL oxidation, which can be further studied in vivo experiments.

**Experimental Section**

**Docking Experiments. Protein and Ligand Preparation.** The X-ray structure of human myeloperoxidase complexed to cyanide and thiocyanate (PDB code: 1DNW) was used as the target structure to endeavor the docking studies.\textsuperscript{50} The X-ray water and \( \text{CN}^- \) and \( \text{SCN}^- \) molecules were removed from the active site. The ligand input files were prepared according to the following procedure. The initial 3D structures of the ligands
were generated using Corina, and the ligand partial charges were ascribed using the OPLS force-field as performed by Glide. As part of an effective 3D ligand structure preparation process the Epik program was used to predict \( pK_a \) values and generate protonation states of all ligands. All ligands feature \( pK_a \) values higher than 8.5 for their side chain nitrogen. They were thus docker in their protonated form.

**Docking Procedure.** The Glide (Grid-Based Ligand Docking With Energetics) algorithm approximates a systematic search of positions, orientations, and conformations of the ligand in the receptor binding site using a series of hierarchical filters (www.schrodinger.com). In the present work, the binding region was defined by a 18 \( \times \) 18 \( \overline{A} \) \( \times \) 18 \( \overline{A} \) box centered on the central position of the cyanide in the crystal complex. At most 10 poses were generated for each molecule. The protein structure was prepared using the Protein Preparation Wizard in the Schrödinger software graphical user interface Maestro. Glide XP docking protocol and scoring function were used to score the poses of the different ligands. The default settings of Glide version 4.5 were used for the remaining parameters. Induced-fit protocols were performed for compound 34 and 27. Initial Glide docked poses were obtained in binding sites with Phε99, Glu102, Arg239, and Phe407 mutated into alanine. Side-chain and backbone minimizations as well as docked poses refinement were carried out in structures with the alanine side chains replaced by their wild type corresponding residues. Glide redockings were carried out in these minimized structures.

**Synthesis.** \( ^1H \) and \( ^13C \) NMR spectra were taken on a Bruker Avance 300 spectrometer at 293 K (Bruker, Wissenbourg, France). \( \delta \) are given in ppm relative to TMS, and the coupling constants are expressed in Hz (frequencies for \( ^1H \) and \( ^13C \) respectively 300 and 75 MHz). IR analyses were performed on a Shimadzu IR-470 spectrophotometer and the peaks data are given in \( cm^{-1} \). The mass spectra were obtained on QTOF 6520 (Agilent, Palo Alto, CA, USA), positive mode, TOF, with mass range 100-1600 Daltons. The mass accuracy is less than 1 ppm. \( ^1H \) and \( ^13C \) NMR spectra were taken on a Bruker Avance 300 spectrometer at 293 K (Bruker, Wissembourg, France). The chemical shifts are given in ppm, and are relative to residual protodeuterated \( CDCl_3 \) solutions. The coupling constants are given in Hz.

**General Procedures.** For the synthesis of compounds 2, 4, 15, and 48, Palladium on charcoal 10% (50 mg) was added to a solution of azide derivative (4.6 mmol) in EtOH (20 mL). The suspension was stirred under H\(_2\) (4.1 bar) overnight. After filtration on Celite, the solvent was evaporated under reduced pressure. The residue was dissolved in ether, extracted with 0.1 M HCl, and the resulting solution was washed with ether. A solution of 1 M NaOH was added (pH \( \approx 10 \)), and the mixture was extracted with diethyl ether. The organic layer was washed with water, dried over Na\(_2\)SO\(_4\), and evaporated under reduced pressure. For compound 4, a saturated solution of anhydrous oxalic acid in ether was added to the ethereal solution and the resulting solid was filtered, washed with ether, and dried under vacuum.

**General Procedures for the Synthesis of Compounds 7 and 8.** To a solution of 5-fluoroindole (200 mg, 1.6 mmol) in dioxane (5 mL) was added a mixture of the amine (1.6 mmol) and formic acid (145 \( \mu L \), 735 mmol/l) in dioxane/formic acid 1:1 (6 mL). The resulting mixture was stirred at room temperature overnight and the solvent was evaporated under reduced pressure. To the residue, a solution of 0.1 M KOH (30 mL) and EtOAc were added. Then the organic layer was washed with water, dried with Na\(_2\)SO\(_4\), and evaporated under vacuum. The residue was dissolved in diethyl ether and extracted with 0.1 M HCl. The aqueous layer was rendered alkaline with 1 M KOH (pH \( \approx 10 \)) and extracted with diethyl ether. The organic layer was dried on Na\(_2\)SO\(_4\) and evaporated.

**General Procedures for the Synthesis of Compounds 9, 10, 37, and 45.** A solution of 4-fluorophenylhydrazidine hydrochloride (1 g, 6.9 mmol) in 4 wt % aqueous H\(_2\)SO\(_4\) (10 mL) and DMA (10 mL) was heated at 100°C under microwave. To this solution was added dihydrofuran, dihydropropyran, or the corresponding hydroxyaldehyde (6.9 mmol) dropwise over 2 min. Then the reaction was heated at 175°C (temperature ramp from room temperature to 100°C over 8 min, then addition of the reagent, heating to 175°C over 8 min and maintaining this temperature for 30 min, 1000 W). The reaction mixture was cooled to room temperature and extracted two times with EtOAc (25 mL), and the organic layer was washed with water and evaporated. The crude material was purified by column chromatography (elucent CH\(_2\)Cl\(_2\)/EtOAc 4:1).

**General Procedures for the Synthesis of Compounds 11, 12, 38, and 46.** A solution of alcohol (5.5 mmol) in CH\(_2\)Cl\(_2\) (25 mL) was cooled with an ice bath. To this solution TEA (0.44 mL, 5.8 mmol) and methanesulfonyl chloride (0.77 mL, 5.5 mmol) were added. The solution was stirred at room temperature for 2 h. Then it was washed with a saturated solution of NaHCO\(_3\) and H\(_2\)O and then dried over Na\(_2\)SO\(_4\) and filtered. The solvent was evaporated under reduced pressure. The crude material was purified by flash chromatography (CH\(_2\)Cl\(_2\)/EtOAc 4:1).

**General Procedures for the Synthesis of Compounds 13, 14, and 47.** To a solution of sulfonate (3.6 mmol) in DMSO (20 mL), Na\(_2\)S\(_2\)O\(_5\) (3.6 mmol) was added, and the solution was stirred at room temperature for 4 h (compounds 13 and 14) or for 1 h at 100°C (compound 47). Then H\(_2\)O (30 mL) and toluene (30 mL) were added. After decantation, the organic layer was washed with water, dried with Na\(_2\)SO\(_4\), filtered, and evaporated under reduced pressure. The residue was purified by column chromatography (CH\(_2\)Cl\(_2\)).

**General Procedures for the Synthesis of Compounds 16—31.** A solution of mesylate 11 or 12 (3.6 mmol) in dioxane (5 mL) was added slowly through an addition funnel to a refluxing solution of the amine (0.26 mol) in dioxane (15 mL) at 100°C. After the addition was completed, the reaction medium was stirred at this temperature for 4 h. After cooling, the mixture was treated with water (20 mL) and extracted with EtOAc (30 mL). The organic layer was dried over Na\(_2\)SO\(_4\) and evaporated to dryness to afford a crude product. The residue was dissolved in 0.1 M HCl. This solution was washed with diethyl ether and rendered alkaline (pH \( \approx 10 \)) with a solution of 1 M NaOH. The mixture was evaporated with ether. The organic layer was washed with water, dried over Na\(_2\)SO\(_4\), and evaporated to afford the crude base as a solid. When bases were obtained as liquids, corresponding tartrate acid salts were prepared according to the following method. After evaporation of ether, the residue was taken up with a hot solution of \( \delta \)-tartaric acid (1 equivalent) in 2-propanol (10 mL). The mixture was cooled and the formed solid was filtered and dried under vacuum.

**General Procedures for the Synthesis of Compounds 32 and 39.** To a solution of NaCN (2 g, 41 mmol) in H\(_2\)O (10 mL), DMA (10 mL) was added and heated at 100°C. Then, a solution of the mesylate (7.4 mmol) in DMA (10 mL) was added dropwise. The reaction was stirred at this temperature for 2 h and then cooled to room temperature and extracted with EtOAc. The organic layer was...
was washed with water, dried on Na₂SO₄, and evaporated. The residue was purified by flash chromatography (CH₂Cl₂/EtOAc 4:1).

**General Procedure for the Synthesis of Compounds 33 and 40.**

To a solution of compound 32 (1.7 mmol) in 11 mL of CH₂Cl₂ was added 5% KOH solution (10 mL). The mixture was filtered through Celite and extracted with EtOAc (30 mL). The organic layer was washed with water, dried on Na₂SO₄ and evaporated. The residue was dissolved in diethylether and a saturated solution of anhydrous oxalic acid in diethylether was added dropwise. The precipitate was filtered, washed with ether, and dried at 40 °C under reduced pressure.

**General Procedure for the Synthesis of Compounds 36 and 44.**

A 1 M Dibal-H solution in CH₂CL₂ (7.8 mL, 7.38 mmol) was added slowly to a solution of the lactone (6.6 mmol) in dry CH₂Cl₂ at 78 °C. After stirring at the same temperature for 1 h, methanol (8.9 mL) was added dropwise and the mixture poured into 0.5 M HCl (200 mL). After stirring for additional 1 h at room temperature, the organic layer was separated. The aqueous phase was washed with diethylether. A 1 M KOH solution was added to the acidic layer (pH ≈ 10) and this was extracted with diethylether. The solvent was dried on Na₂SO₄ and evaporated. The residue was dissolved in diethylether, and a saturated solution of anhydrous oxalic acid in diethylether was added dropwise. The precipitate was filtered, washed with ether, and dried at 40 °C under reduced pressure.

**Inhibition of LDL Oxidation.** LDL oxidation was carried out at 37 °C in a final volume of 500 μL. The reaction mixture contained the following reagents at the final concentrations indicated between brackets pH 7.2 (PBS buffer 1 μg/mL), LDL (1000 μg/mL), 2 μL 1 N HCl (4 mM), one of the drugs at different concentrations, and H₂O₂ (100 μM). The reaction was started after 5 min by cooling the tubes in ice. The assay was performed as described by Mogulinskya et al. in a NUNC maxisorp plate (VWR, Zaventem, Belgium): 200 ng/well of LDL was coated overnight at 4 °C in a sodium bicarbonate pH 9.8 buffer (100 μL). Afterward, the plate was washed with TBS 80 buffer and then saturated during 1 h at 37 °C with the PBS buffer containing 1% BSA (150 μL/well). After washing the wells twice with the TBS 80 buffer, the monoclonal antibody Mab AG9 (200 ng/well) obtained according to a standard protocol and as previously described was added as a diluted solution in PBS buffer with 0.5% BSA and 0.1% of Polysorbate 20. After incubation for 1 h at 37 °C, the plate was washed four times with the TBS 80 buffer and a 3000 times diluted solution of IgG antimouse alkaline phosphatase (Promega, Leiden, The Netherlands) in the same buffer was added (100 μL/well). The wells were washed again four times and a revelation solution (150 μL/well) containing 5 mg of para-nitrophenyl phosphate in 5 mL of diethanolamine buffer was added for 30 min at room temperature. The reaction was stopped with 60 μL/well of 3 N NaOH solution. The measurement of the absorbance was performed at 405 nm with a background correction at 655 nm with a Bio-Rad photometer for a 96-well plate (Bio-Rad laboratories, CA, USA). Results were expressed as IC₅₀.

**Transient State Kinetics.** Material. Highly purified myeloperoxidase of a purity index (A₆₃₀/A₄₅₀) of at least 0.86 was purchased from Planta Natural Products (http://www.planta.at). Its concentration was calculated using ε₄₃₀ = 91 mM⁻¹ cm⁻¹. Hydrogen peroxide, obtained from a 30% solution was diluted and the concentration determined by absorbance measurement at 240 nm where the extinction coefficient is 39.4 M⁻¹ cm⁻¹. Tryptamine derivative stock solutions were prepared in dimethylsulfoxide (DMSO) and stored in dark flasks. Dilution was performed with 200 mM phosphate buffer, pH 7.4, to a final DMF concentration of 2% (v/v) in all assays.

**Pharmacological Tests. Myeloperoxidase Inhibition Assay.** The assay was based on the production of taurine chloramine produced by the MPO/H₂O₂/Cl⁻ system in the presence of a selected inhibitor at defined concentration. The reaction mixture contained the following reagents in a final volume of 200 μL: pH 7.4 phosphate buffer (10 mM PO₄⁻/C₁₀H₂₀O₄), taurine (15 mM), the compound to be tested (up to 20 μM), catalase (8 U/L), and its specific activity (U/mg). The chlorination process is characterized by its protein concentration (mg/mL), its activity (U/mL), and its specific activity (U/mg). The chlorination activity was determined according to Hewson and Hager. Human plasma served for the isolation of LDL by ultracentrifugation according to Havel et al. Before oxidation, the LDL fraction (1.019 < d < 1.067 g/mL) was desalted by two consecutive passages through PD10 gel-filtration columns (Amersham Biosciences, The Netherlands) using PBS buffer. The different steps were carried out in the dark, and the protein concentration was measured by the Lowry assay for both MPO and LDL.

**Determination of LDL Oxidation Inhibition. Preparation of the Recombinant Enzyme and of LDL.** Recombinant MPO was prepared as previously described. Each batch solution is characterized by its protein concentration (mg/mL), its activity (U/mL), and its specific activity (U/mg). The chlorination activity was determined according to Hewson and Hager. Human plasma served for the isolation of LDL by ultracentrifugation according to Havel et al. Before oxidation, the LDL fraction (1.019 < d < 1.067 g/mL) was desalted by two consecutive passages through PD10 gel-filtration columns (Amersham Biosciences, The Netherlands) using PBS buffer. The different steps were carried out in the dark, and the protein concentration was measured by the Lowry assay for both MPO and LDL.
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