Hybrid molecules inhibiting myeloperoxidase activity and serotonin reuptake: a possible new approach of major depressive disorders with inflammatory syndrome

Jalal Soubhyea, Iyas Aldiba, Martine Prévostb, Betina Elfvingc, Michel Gelbckea, Manuel Podreccad, Raphaël Conotteg, Jean-Marie Coletd, Paul G. Furtmüllere, Cédric Delportea,g, Alexandre Rousseauj, Michel Vanhaeverbeekl, Jean Nèvee, Christian Obingeré, Karim Zouaoui-Boudjeltiaf, Pierre Van Antwerpena,g,* and François Dufrasneea,*

aLaboratoire de Chimie Pharmaceutique Organique, Faculté de Pharmacie, bLaboratoire de Structure et Fonction des Membranes Biologiques, Faculté des Sciences, gAnalytical Platform of the Faculty of Pharmacy, Université Libre de Bruxelles, Brussels, dDepartment of Human Biology and Toxicology, Faculty of Medicine and Pharmacy, University of Mons, Mons, hLaboratory of Experimental Medicine, CHU Charleroi, A. Vésale Hospital, Université Libre de Bruxelles, Montigny-le-Tilleul, Belgium, iTranslational Neuropsychiatry Unit, Department of Clinical Medicine, Aarhus University, Aarhus, Denmark and eDepartment of Chemistry, Division of Biochemistry, Vienna Institute of Biotechnology, BOKU – University of Natural Resources and Life Sciences, Vienna, Austria

Keywords
atherosclerosis; depression; irreversible inhibitor; myeloperoxidase; serotonin

Correspondence
François Dufrasne, Laboratoire de Chimie Pharmaceutique Organique, Faculté de Pharmacie, Université Libre de Bruxelles, Université Libre de Bruxelles, Campus plaine, CP 205/5, 1050 Brussels, Belgium.
E-mail: dufrasne@ulb.ac.be

Received November 21, 2013
Accepted February 2, 2014

doi: 10.1111/jphp.12236

*These authors equally contributed to the manuscript.

Abstract

Objectives Major depressive disorder (MDD) is accompanied with an imbalance in the immune system and cardiovascular impairments, such as atherosclerosis. Several mechanisms have been pointed out to underlie this rather unexpected association, and among them the activity of myeloperoxidase (MPO). The aim of our study was to find compounds that inhibit both MPO and serotonin transporter (SERT) for treating MDD associated with cardiovascular diseases.

Methods SERT inhibition was assessed with measuring of [3H]-serotonin uptake using HEK-293 MSR cells. MPO inhibition was determined by taurine chloramine test on 3-(aminoalkyl)-5-fluoroindole derivatives and on clinically relevant antidepressants. All kinetic measurements were performed using a temperature-controlled stopped-flow apparatus (model SX-18 MV). Promising lead compounds were docked onto SERT 3D structure modelled using the LeuT structure complexed to tryptophan (PDB code 3F3A). Their toxicological profile was also assessed.

Key findings 3-(aminoalkyl)-5-fluoroindole derivative with 5 carbons on the side chain and paroxetine showed the best activity on both MPO and serotonin transporter at the nanomolar range. Paroxetine was found to be the first irreversible MPO inhibitor at nanomolar concentrations.

Conclusions Our results put forward the first hybrid molecule (compound 25) and drug (paroxetine) that can be especially used in MDD associated with inflammatory syndrome.

Introduction

Atherosclerosis and depression are quite different pathological entities. The former one is a cardiovascular disease caused by several metabolic disorders, including inflammation, accumulation of fatty compounds in arterial walls and oxidative events. This may lead to fatal complications, such as myocardial infarction and stroke. Depression is clinically characterized by continuous negative mood and anhedonia. The aetiology of depression is very complex and involves several components. In the central nervous system (CNS), the decrease or imbalance of
neurotransmitters in the synaptic cleft, in particular the monoamines norepinephrine, serotonin (5-HT, compound 1, see Figure 1), and dopamine have been considered for the last 50 years as a main cause of depression. Although it cannot explain all cases and stages of the disease, this biogenic amine hypothesis has been the most widely studied mechanism so far.[3] Most interestingly, research papers have shown a rather unexpected relationship between both major depression and atherosclerosis. Among the numerous hypotheses about the correlation between depression and human brain dysfunction, inflammation has become an important issue in understanding some depressive diseases, especially major depressive disorders (MDD).

First, clinical investigations have put forward that dysfunction of the immune system could foster the emergence of MDD along with (i) activation and increased production of immune system-related cells, including leucocytes and neutrophils,[4] as well as (ii) increased levels of interleukin-6 (IL-6) and IL-1Rα.[5] This phenomenon could be partially reversed by antidepressant therapy.[6] Second, it should be noted that, among the numerous elicitors of oxidative stress, the heme enzyme myeloperoxidase (MPO, EC 1.11.2.2) has attracted significant attention due to its possible link with MDD symptoms. MPO has been detected in the serum of depressive patients, as observed by Hazen and colleagues.[7] Furthermore, MPO is also directly involved in the modification of biomolecules in the blood of depressive patients, mainly through its ability to generate hypochlorous acid (HOCl) and derived oxidation products, including organic peroxides, nitrotyrosine and oxidized low-density lipoproteins (LDLs).[8] MPO-derived oxidants have also been reported to be involved in the development of neurodegenerative as well as cardiovascular diseases (especially coronary artery disease), which have a high incidence in patients with MDD.[9]

As far as atherosclerosis is concerned, MPO has been identified as a factor contributing to its development.[10] Furthermore, a recent review shed light on the relationship between this cardiovascular disease, depression and the drugs used to treat the latter.[11] Among the biological processes at the origin of atherosclerosis, oxidative stress[12] together with platelet activation has been pointed out.[13] Regarding blood homeostasis, 5-HT is one of the molecules involved in platelet activation,[14] and 5-HT-specific reuptake inhibitors (SSRIs) have been suggested to decrease this process,[15–18] yielding favourable cardiovascular effects in patients with MDD.[19] The effect of antidepressants and SSRIs on the oxidative stress remains unclear but is rather well described at least for fluoxetine,[20] escitalopram,[21,22] and paroxetine.[23] Furthermore, it is known that 5-HT is one of the best substrates of MPO and is transformed into the following oxidation products: 5-HT dimer and tryptamine-4,5-dione. The latter is known to be neurotoxic[24] as well as implicated in cardiovascular disease.[25] In addition to the production of toxic compounds, the interaction of 5-HT with MPO significantly decreases the concentration of the former, indicating how inflammation might interfere with depression.[26]

A molecule able to act on both pathologies could be valuable to improve the depressive state along with protecting the cardiovascular system in patients with MDD. In modern medicinal chemistry, such molecules are called hybrid drugs, and their discovery has become a great challenge since the 1990s.[27] Designing molecules that include at least two pharmacophores and act at rather low concentrations on at least two different biological systems still remains a very difficult task since both binding sites must accommodate and interact effectively with a single structure. However, the substantial advantage of treating one or even two diseases by acting on two targets is very attractive since drug abuse is a major public health problem due to the cumulative side effects or (dangerous) drug interactions that could follow.[28] Principally, two general methods can be used in the design of hybrid drugs: (i) either linking two different molecules endowed with different activities or (ii) designing one molecule that shares different structural features of several pharmacophores.

In recent papers, we described for the first time a series of 3-(aminoalkyl)-5-fluoroindoles as MPO inhibitors, some of them acting at nanomolar concentrations (see Table 1).[29,30] Analysis of data from the literature showed that one of these compounds and some closely related structural analogues (Figure 1, compounds 2–5) are potent 5-HT reuptake inhibitors (SRIs), leading to the serendipitous discovery of potential dual-targets acting molecules.[31–33] We have then further investigated the pharmacology of 3-(aminoalkyl)-5-fluoroindole derivatives, by (i) assessment of the 5-HT reuptake inhibition, (ii) analysis of the potential binding modes predicted by docking experiments on a 3D modelled structure of the 5-HT transporter and (iii) assessment of...
In addition, MPO inhibition of SSRIs widely used in clinical practice (escitalopram, paroxetine, fluoxetine and fluvoxamine, see Figure 2) was studied to investigate their potential beneficial effects in cardiovascular disorders accompanying severe depression. Finally, transient kinetics experiments were undertaken to analyse the mechanism of action of the possible MPO-inhibiting SSRIs.

Table 1 Synthesized 3-(aminoalkyl)-5-fluoroindoles and SSRIs

<table>
<thead>
<tr>
<th>Compound</th>
<th>n</th>
<th>R1</th>
<th>R2</th>
<th>Ki (mean ± SD in nM)</th>
<th>IC50 (mean ± SD in nM)</th>
<th>Ratio Ki/IC50</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>1</td>
<td>H</td>
<td>H</td>
<td>995 ± 136</td>
<td>900 ± 300</td>
<td>1.1</td>
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<tr>
<td>7</td>
<td>1</td>
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<td>41 ± 11</td>
<td>1000 ± 100</td>
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<tr>
<td>8</td>
<td>2</td>
<td>H</td>
<td>H</td>
<td>47 ± 11</td>
<td>200 ± 30</td>
<td>0.2</td>
</tr>
<tr>
<td>9</td>
<td>2</td>
<td>CH3</td>
<td>CH3</td>
<td>53 ± 11</td>
<td>90 ± 60</td>
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</tr>
<tr>
<td>10</td>
<td>2</td>
<td>H</td>
<td>CH3CH3</td>
<td>39 ± 17</td>
<td>300 ± 100</td>
<td>0.1</td>
</tr>
<tr>
<td>11</td>
<td>2</td>
<td>CH3</td>
<td>CH3CH3</td>
<td>22 ± 5</td>
<td>160 ± 80</td>
<td>0.1</td>
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<tr>
<td>12</td>
<td>2</td>
<td></td>
<td></td>
<td>21 ± 8</td>
<td>40 ± 30</td>
<td>0.5</td>
</tr>
<tr>
<td>13</td>
<td>2</td>
<td>H</td>
<td>CH3CH2CH3</td>
<td>19 ± 8</td>
<td>800 ± 20</td>
<td>0.02</td>
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<tr>
<td>14</td>
<td>2</td>
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<td>CH3CH2CH3</td>
<td>CH3</td>
<td>148 ± 70</td>
<td>0.1</td>
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<tr>
<td>16</td>
<td>3</td>
<td>H</td>
<td>H</td>
<td>3.9 ± 0.8</td>
<td>50 ± 8</td>
<td>0.05</td>
</tr>
<tr>
<td>17</td>
<td>3</td>
<td>H</td>
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<td>11.2 ± 6.6</td>
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</tr>
<tr>
<td>18</td>
<td>3</td>
<td>H</td>
<td>CH3CH3</td>
<td>1.2 ± 0.6</td>
<td>130 ± 90</td>
<td>0.009</td>
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<tr>
<td>19</td>
<td>3</td>
<td>CH3</td>
<td>CH3CH3</td>
<td>21 ± 13</td>
<td>300 ± 100</td>
<td>0.07</td>
</tr>
<tr>
<td>20</td>
<td>3</td>
<td></td>
<td></td>
<td>5.0 ± 0.6</td>
<td>350 ± 90</td>
<td>0.01</td>
</tr>
<tr>
<td>21</td>
<td>3</td>
<td>H</td>
<td>CH3CH2CH3</td>
<td>3.7 ± 1.8</td>
<td>320 ± 10</td>
<td>0.01</td>
</tr>
<tr>
<td>22</td>
<td>3</td>
<td>H</td>
<td>CH3CH2CH3</td>
<td>CH3</td>
<td>214 ± 83</td>
<td>1.3</td>
</tr>
<tr>
<td>23</td>
<td>3</td>
<td></td>
<td></td>
<td>21 ± 1.1</td>
<td>350 ± 60</td>
<td>0.08</td>
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<td>24</td>
<td>4</td>
<td>H</td>
<td>H</td>
<td>5.6 ± 1.3</td>
<td>15 ± 4</td>
<td>0.4</td>
</tr>
<tr>
<td>25</td>
<td>5</td>
<td>H</td>
<td>H</td>
<td>2.6 ± 0.7</td>
<td>8 ± 2</td>
<td>0.3</td>
</tr>
</tbody>
</table>

Escitalopram oxalate 0.39 ± 0.35 Not active –
Fluoxetine hydrochloride 3.4 ± 2.6 Not active –
Paroxetine hydrochloride hemihydrate 0.3 ± 0.2 22 ± 2 0.02
Fluvoxamine maleate 3.7 ± 2.9 Not active –

Results for 5-HT reuptake inhibition expressed as Ki, previous results obtained with myeloperoxidase inhibition expressed as IC50, and the calculated ratios between Ki and IC50.[29] The compounds are first classified according to alkyl side chain length and then increasing size of the substituents on the amino group. SSRI, selective serotonin reuptake inhibitor.
Docking experiments

Docking of 5-HT and of other compounds was performed on hSERT 3D structure. No high resolution three-dimensional (3D) structure of the transporter has been determined so far. A BLAST search performed using the PDB sequences identified the bacterial Aquifex aeolicus leucine transporter LeuT as homologous to hSERT. Therefore, a 3D model was built with MODELLER, using the LeuT structure complexed to tryptophan (PDB code 3F3A) as a template and a sequence alignment produced by Promals3D, which features about 24% sequence identity. The 3D model of hSERT superimposes on the LeuT structure with a root mean square deviation of 1.5 Å for the Cα positions, which is a fairly low value. A Ramachandran plot showed that the model features a stereochemistry for the large proportion of residues consistent with allowed areas in the conformational space, except for six residues that are located in loops and distal to the binding site. The transmembrane (TM) segments, TM1, TM3, TM6 and TM8, which shape the binding pocket, feature a high sequence identity and similarity to LeuT, which reinforces the reliability of the model in this region. Compounds were docked in the binding site of the hSERT 3D model that corresponds to the primary binding pocket of LeuT. The underlying assumption that these molecules bind at this site is sustained by a study reporting that tryptamine analogues are competitive inhibitors of hSERT transport. Protein flexibility was included in the docking process (Induced-fit protocol from Schrödinger Inc., New York, NJ, USA) via flexible side chain residues selected with a 4 Å sphere surrounding the tryptophan ligand, which was modelled together with the hSERT protein. To be exhaustive, it is worth to mention that two new models of 5-HT and dopamine transporters have been published after this manuscript had been submitted.

Transient-state kinetics

All kinetic measurements were performed using a temperature-controlled stopped-flow apparatus (model SX-18 MV) from Applied Photophysics (Leatherhead, UK) with a diode-array detector (Applied Photophysics PD.1) or a monochromator attached to the stopped-flow machine. In a typical sequential-mixing stopped-flow experiment the enzyme solution (2 μM heme) was premixed with a 10-fold excess of H2O2. After a delay time of 20 ms, Compound I (50% hypochromicity at 430 nm) was formed and allowed to react with varying concentrations of substrate in 100 mM phosphate buffer (pH 7.0). The reactions were followed at the Soret maximum of Compound II (456 nm). To determine the kinetics of reduction of Compound II, following of formation and reduction of Compound II was performed in one measurement. The
resulting biphasic curves at 456 nm showed the initial formation of Compound II (exponential increase at 456 nm) and then its subsequent transition to ferric MPO (exponential decrease at 456 nm).

Reactions were analysed by fitting the monophasic time traces using the single-exponential equation, provided by the Applied Photophysics software programme. From the slope of the linear plot of the \( k_{\text{obs}} \) values vs substrate concentrations, the apparent second-order rate constant was estimated. At least three determinations (2000 data points) of pseudo-first-order rate constants (\( k_{\text{obs}} \)) were performed for each substrate concentration (pH 7.0, 25°C), and the mean value was used to calculate the second-order rate constant. All reactions were analysed using the Pro-K simulation programme from Applied Photophysics, which allows the synthesis of artificial sets of time-dependent spectra as well as spectral analysis of enzyme intermediates.

**Inactivation of myeloperoxidase**

These experiments are described in the Supporting Information.

**Toxicology**

All animal experiments were approved by the local ethical committee for animal care of the institution (University of Mons, Mons, Belgium). The most active compound 25 was administered by intraperitoneal injection to Wistar Han male rats (mean weight: 553 ± 41 g). The following concentrations were administered in a solution of ethanol (33%): 1, 10, 100 and 300 mg/kg body weight. Each dose was administered to groups of two rats (1 mg/kg R1 and R2, 10 mg/kg R3 and R4, 100 mg/kg R5 and R6, and 300 mg/kg R7 and R8). Only animals treated with 100, 10 and 1 mg/kg, respectively, were placed in metabolism cages for 144 h. The first two days were used for acclimatization of the animals. The animals received a single dose of the tested compound at time ‘0 h’. Urine was collected daily and blood tests were performed 24 and 96 h after treatment.

**Results**

**5-HT reuptake inhibition**

The 3-((aminoalkyl))-5-fluoroindoles previously reported as MPO inhibitors were tested for their 5-HT reuptake inhibition potency (see Table 1). Variation of the alkyl chain length on unsubstituted compounds resulted in the following observations: (i) homotryptamines from \( n = 3–5 \) (compounds 16, 24, and 25) are more potent inhibitors than fluorotryptamine (8), (ii) compound 6, with one carbon atom chain length between the indole ring and the amino group, features a dramatically reduced inhibition potency.

The structure activity relationships (SAR) can be inferred from inspection of the data presented in Table 1. For identical substituents, compounds with \( n = 3 \) are in most cases more active than those with \( n = 2 \), except \( R_2 = \text{CH}_3 \) (compare compounds 21 and 13). Regarding compounds with \( n = 2 \), substitution with either one \( \text{CH}_2\text{CH}_2\text{CH}_3 \) (13) or two \( \text{CH}_3 \) (11) groups, and the two alicyclic molecules (12 and 15), generated the most potent inhibitors in the series. Compound 14 had the lowest inhibition, perhaps as a result of its longer aliphatic chain substituent. In the series with \( n = 3 \), compounds with no substituents, two identical substituents or alicyclic substituents tend to have higher inhibition potency than their homologs with one alkyl chain substitution. In contrast to the series with \( n = 2 \), an improved inhibition was observed for the compound with a butyl substituent relative to one with a propyl moiety (compare compounds 22 and 14). SAR showed that in the piperazine series (7, 15 and 23), all of the compounds are among the most active ones compared with molecules containing similar alkyl side chains.

**Comparison of 5-HT reuptake and myeloperoxidase inhibition potencies of synthesized compounds**

The SAR of the MPO inhibition potency of the compounds has been already discussed in a previous report (see Table 1). Comparison of inhibition of SERT and MPO activity was made for each compound by calculating the ratio \( K_i \) (SERT inhibition)/IC50 (MPO inhibition). Compounds having a ratio >0.1 and nanomolar activity on both targets retained our attention, and consequently molecules 24 and 25 were selected as hybrid lead candidates.

To find other candidates as SERT/MPO inhibitors, we extended our investigation to the most efficient SSRIs used in clinical practice (Table 1 and Figure 2). MPO inhibition screening was done for escitalopram, fluoxetine, paroxetine and fluvoxamine (Figure 2) using the tauroine chloramine test (see Supporting Information). Only paroxetine inhibited MPO with an IC50 value at 22 ± 2 nM. The other drugs had no effect on the chlorination activity of MPO. Additionally, these SSRIs were probed for their interference with MPO-mediated LDL oxidation. Again, only paroxetine showed a potent inhibition an IC50 value of 35 ± 3 nM.

**Molecular docking**

The binding mode of 5-HT has been investigated by molecular docking in the modelled 3D structure of hSERT. The best-scored predicted positions for 5-HT in the binding pocket feature a salt bridge formed between the amine group and Asp98 as an important interaction for binding.
the transporter. A weak cation–π interaction is also observed with Tyr95. The 5-HT amine hydrogen bonds with the backbone of Ala96 and Phe335. These latter interactions are remarkably similar to those of the tryptophan-bound LeuT crystal structure. A T-shape aromatic interaction is observed between 5-HT and Tyr176.

Molecular docking of the most active compounds both on hSERT and MPO (compounds 24 and 25) was performed in a 3D model of the transporter. It is indeed essential to get insight into the binding mode of these molecules to implement rationale pharmacomodulation. The predicted binding modes on MPO were described in a previous study.[29] The binding modes of compounds 24 and 25 predicted on hSERT are similar (Figure 3a and 3c): the amine side chain forms a salt bridge with Asp98, a weak cation–π interaction with Tyr95, and two hydrogen bonds with Ala96 and Phe335 backbone. Aromatic interactions are also observed with Tyr176 and Phe335. The indole NH also hydrogen bonds with the Tyr175 main chain. Hydrophobic interactions are formed with Leu99, Ile172, Ile179, Phe335 and Phe341.

The importance of Asp98 and Tyr95 for the transport function in monoamine transporters was underscored in mutagenesis experiments.[39,43] Given the difficulty in predicting binding affinity using the scoring functions available in the docking programmes, it is often challenging to quantitatively discriminate the potency of different ligands.

Nevertheless, it is interesting to note that compound 24 and 25 are predicted to bind with a similar affinity score (about −11 kcal/mol) in agreement with the measured $K_i$ values.

Noticably, the binding mode of paroxetine in hSERT features similarities with compound 24 and 25 (Figure 3b). In particular, its amino group forms a salt bridge with Asp98, a cation–π interaction and one hydrogen bond with Phe335. The fluorophenyl moiety makes interactions with other aromatic residues: Tyr95, Tyr176 and Phe341, and also with Val343. The benzodioxole moiety forms a π–π interaction with Tyr176, and also interacts with Leu99, Ile179 and Phe335. The affinity score of paroxetine ($\Delta G = −13.7$ kcal/mol) is about 2 kcal/mol higher than that of compounds 24 and 25 ($\Delta G = −11.8$ and −11.4 respectively).

The binding mode of paroxetine in MPO active site presents strong similarities with that of compound 24 and 25, and with fluorotryptamines (Figure 3e). The best pose of paroxetine features a stacking of the six-membered ring benzodioxole onto the pyrrole ring D. Its five-membered ring positions itself between the iron pocket and His95, allowing one of its oxygen to hydrogen bond to Gln91 and Arg239 side chains. In addition to the stacking, one salt bridge is formed by the amino group with Glu102. The fluorophenyl moiety makes interactions with surrounding residues in a pocket at the entrance of the binding site: Phe99, Phe366 and Phe407. The affinity score of
paroxetine $\Delta G = -9.1$ kcal/mol is significantly higher than that of compounds 24 and 25, which amounts to $-6.6$ and $-6.4$ kcal/mol, respectively.\[29\]

**Transient kinetics**

We recently reported that 3-(aminoalkyl)-5-fluoroindole derivatives are reversible inhibitors of MPO. They react with both redox intermediates of MPO, i.e. Compound I and Compound II, but at significantly different rates.\[29\] The reaction is fast with Compound I but very slow with Compound II. This causes accumulation of Compound II, which is outside the chlorination cycle (Figure 4). The latter needs Compound I for the two-electron oxidation of chloride to HOCl. Alternatively, Compound I can be reduced by one-electron donors to Compound II as do these MPO inhibitors very efficiently. Upon mixing Compound I with paroxetine, a fast one-electron reduction occurred, indicated by a red shift of the Soret band to 456 nm, clearly suggesting formation of Compound II (Figure 5a). The reaction was concentration-dependent but showed a saturation behaviour (Figure 5b). The bimolecular rate constant ($k_2$) of this reaction (Figure 4) was calculated from the linear correlation between the $k_{obs}$ values and low paroxetine concentrations ($<20 \mu M$) to be $(6.1 \pm 0.2) \times 10^6$ M/s (Figure 5c). Compound II was not stable but was slowly converted to the resting state. However, while direct reaction of Compound II with 3-(aminoalkyl)-5-fluoroindole derivatives gives full recovery of native MPO (underlining the reversible nature of these inhibitors), reaction of Compound II with paroxetine (Figure 6a) did not allow full recovery of the native state but showed loss of heme absorbance. The extent of recovery of ferric MPO and heme degradation strongly depended on the amount of paroxetine used as electron donor, suggesting that it might act as a suicide inhibitor (Figure 6b). Finally, 500 \mu M paroxetine...
were added to Compound II, which was slowly and partially converted to the resting state (Figure 7a). As $k_1$ (Figure 4) is very low, its exact value could only be estimated using the Pro-K simulation programme (around 20 M/s). The ratio of $k_3/k_4$ (≈305 000) suggests that paroxetine acts as reversible inhibitor promoting the accumulation of Compound II. However, after reaction of MPO with paroxetine, the MPO activity was irreversibly inhibited, suggesting that paroxetine irreversibly interacted with the prosthetic group of MPO during turnover (see Supporting Information).

**Toxicology**

The two animals (R7 and R8) treated with 300 mg of 25 died within 20 min after administration. At necropsy, gross observations showed an early bleeding in the lungs in two individuals. Rats treated with the high dose of compound 25 (R5, R6, 100 mg/kg) stopped eating and drinking until death. Rats treated with low dose (R1 and R2, 1 mg/kg) showed a normal behaviour. Rats treated with 10 mg/kg (R3 and R4) showed different eating habits. During the first 20 min after treatment, all animals seemed prostrate, and had a gasping breath and a swollen nose. Twenty-four hours after treatment, rats treated with low dose behaved normally. Individuals treated with 100 mg/kg appeared severely affected. They were prostrate, breathing quickly and suffered from hypothermia. Four hours after treatment, only rats treated with 100 mg/kg exhibited abnormal behaviour similar to that described above. Seventy-two hours after treatment, the two individuals treated with 100 mg/kg died. The behaviour of other rats showed no sign of abnormality. The surviving rats were killed. Blood and organs (liver, kidney, heart and muscle) were collected and no abnormality was observed. In summary, no observable adverse effects were seen in animals receiving compound 25 at 1 mg/kg, animals treated at 10 mg/kg showed effects but recovered within 48 h, whereas at the high dose (100 mg/kg) the animals died several days after receiving a single dose. Finally, compound 25 has been assessed with the predictive programme Percepta (QSAR approach of toxic effects), and only cardiovascular problems have been reported as possible side effects.

**Discussion**

The major contribution of this study is the discovery of a new class of hybrid molecules acting on two targets: SERT and MPO. Hybrid drugs are not well represented in the armamentarium, with the noticeable exception of drugs treating CNS diseases, such as antipsychotics and antidepressants, that act on several neurotransmitter receptors and transporters, and also some anticancer agents (for instance tyrosine kinases inhibitors). However, the ability of these compounds to act on different targets is generally considered at a first sight more as a lack of selectivity than a real endeavour to design efficient hybrid drugs. As a matter of fact, the targets of hybrid drugs now used in clinical practice have very similar 3D structures or belong to the same protein or enzyme/receptor subfamily (in the above-given examples monoamines receptors and kinases using ATP as cofactor). The case of compounds 24 and 25, and paroxetine, is different: these molecules inhibit two very different proteins, i.e. a heme-containing enzyme (MPO) and a TM transporter (SERT). So it is crucial to understand the molecular characteristics that could explain this double activity.

A comparison of the binding modes predicted by docking in hSERT and MPO active sites shows common structural features in molecules 24, 25 and paroxetine critical for interactions with both target proteins. The pharmacophores derived from these three compounds are summarized in Table 2. Two chemical groups, i.e. the amino group and an aromatic moiety, and their interactions provide a rationale for the nanomolar activity of these compounds in the two binding sites. A protonated amino group on the side chain and an aromatic or heteroaromatic cycle play critical roles in binding to both SERT and MPO. It also appears that the voids around the nitrogen atom are smaller in the MPO than in the SERT binding pocket as shown by the overall low activity obtained in MPO inhibition for compounds featuring substitutions on the amino group, whereas the effect of the same substitution pattern is in most cases less dramatic on SERT inhibition (see compounds with low $K_i/IC_{50}$ ratios, such as 2, 7, 13, 15 to 20, 22 and 23). Some homotryptamines, however, appear as exceptions to this rule (compare compounds 13 and 14, as well as 21 and 22).
The interaction of paroxetine with MPO is twofold. On the one hand, it acts as electron donor for Compound I. On the other hand, it acts as electron donor for Compound II. Because of the huge differences in the apparent bimolecular rate constants ($k_{II}$ and $k_{III}$), it promotes Compound II accumulation (Figure 4). Since the redox intermediate does not participate in the chlorination cycle, paroxetine behaves as a reversible inhibitor of MPO.

**Conclusion**

The serendipitous discovery that newly synthesized 3-(aminoalkyl)-5-fluoroindole derivatives and paroxetine are potent mechanism-based inhibitors of MPO has opened a new field of research in the treatment of MPO-related diseases. Analyses of crystal structures revealed that these inhibitors become X-ray crystallographically visible molecules. The mechanism of irreversible inhibition of MPO is described as a carbene, which forms covalent bonds with the heme cavity. The interaction of paroxetine with MPO is twofold. On the one hand, it acts as electron donor for Compound I. On the other hand, it acts as electron donor for Compound II. Because of the huge differences in the apparent bimolecular rate constants ($k_{II}$ and $k_{III}$), it promotes Compound II accumulation (Figure 4). Since the redox intermediate does not participate in the chlorination cycle, paroxetine behaves as a reversible inhibitor of MPO.

**Table 2**

Common structural features for the pharmacophores of compounds 24 and 25 and paroxetine related to their binding to hSERT and myeloperoxidase active site.

<table>
<thead>
<tr>
<th>Chemical groups</th>
<th>Interactions with binding sites</th>
</tr>
</thead>
<tbody>
<tr>
<td>MPO (in this study)</td>
<td>hSERT</td>
</tr>
<tr>
<td>Salt bridge with Glu102</td>
<td>Hydrogen bond with Tyr176</td>
</tr>
<tr>
<td>Salt bridge with Arg239</td>
<td>Hydrogen bond with Tyr176</td>
</tr>
<tr>
<td>Salt interaction with the heme cavity</td>
<td>-</td>
</tr>
<tr>
<td>Hydrogen bond with Glu102</td>
<td>-</td>
</tr>
<tr>
<td>Hydrogen bond with Arg239</td>
<td>-</td>
</tr>
<tr>
<td>Hydrogen bond with Tyr176</td>
<td>-</td>
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<tr>
<td>Oxygen of the benzodioxole moiety</td>
<td>-</td>
</tr>
<tr>
<td>Hydrogen bond with Gln91 and with Arg239</td>
<td>-</td>
</tr>
<tr>
<td>Amino group of the indole ring</td>
<td>-</td>
</tr>
<tr>
<td>π−π interaction with the heme cavity</td>
<td>-</td>
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<tr>
<td>Cation−π interaction with Phe335</td>
<td>-</td>
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<tr>
<td>Hydrogen bond with Tyr176</td>
<td>-</td>
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<tr>
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</tbody>
</table>

at nanomolar concentrations (MPO: IC50 = 15 ± 4 and 8 ± 2 nM and SRI: Ki = 5.6 ± 1.3 and 2.6 ± 0.7 nM, respectively). Additionally, a new clinical application was demonstrated for the SSRI paroxetine that inhibits the chlorination activity (IC50 = 22 ± 2 nM) and MPO-mediated LDL oxidation (IC50 = 35 ± 3 nM). Thus, these three compounds (compounds 24, 25 and paroxetine) can be considered as starting compounds to develop other SSRIs as hybrid drugs that could also inhibit MPO, and consequently can be used in the treatment of MDD associated with atherosclerotic events. In our newly synthesized indole series, compound 25 is the most promising one with no significant adverse effect at the clinically relevant concentrations.

**Acknowledgements**

M.P. is ‘Maître de Recherche at the Fonds National de la Recherche Scientifique (FRS-FNRS, Belgium). The technical assistance supplied by Pia Høgh Plougmann for [3H]5-HT uptake is gratefully acknowledged. This study was supported by grants from the Belgian Fund for Scientific Research (FRS-FNRS, n°34553.08) as well as from the FER 2007 (ULB). C.D. is a postdoctoral researcher funded by the Fonds National de la Recherche Scientifique (FRS-FNRS). C.O. and P.G.F. are grateful to the Austrian Science Fund (FWF) for supporting this work (grant numbers P20664 and P15660).

**Declarations**

**Conflict of interest**

The Author(s) declare(s) that they have no conflicts of interest to disclose.

**References**

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Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher’s web-site:
Appendix S1. Data for inactivation of myeloperoxidase by paroxetine, taurine chloramine test procedure and data for the inhibitory effect of paroxetine on the chlorination activity of MPO are given.