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A new insight on the hypochlorous acid scavenging mechanism of tryptamine and tryptophan derivatives

Luísa C. Carvalho [a], Mónica S. Estevão [a], Luísa M. Ferreira [a], Eduarda Fernandes [b,*], M. Manuel B. Marques [a,*]

[a] REQUIMTE-CQFB, Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, 2829-516 Caparica, Portugal
[b] REQUIMTE, Departamento de Química, Faculdade de Farmácia, Universidade do Porto, Rua Aníbal Cunha, 164, 4099-030 Porto, Portugal

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ABSTRACT

The reaction mechanisms of hypochlorous acid (HOCl) with several tryptophan and tryptamine derivatives, previously reported to scavenge this powerful oxidant, was investigated to determine whether ionic or radical pathways were involved. For this purpose, the reaction of tryptamine and tryptophan derivatives with HOCl was optimized and some compounds were isolated by HPLC and their structures assigned. In order to prevent possible radical reaction pathway, experiments have been carried in the presence of the radical trap TEMPO (2,2,6,6-tetramethylpiperidine-1-oxyl). The obtained results show that the reaction mechanisms are influenced by the type of structure and that a complex pathway is involved, in which both ionic and radical mechanisms can occur.

In the event of inflammatory processes, reactive oxygen species (ROS) are produced by endothelial cells, Kupffer cells, neutrophils, and also macrophages, as a mechanism of defense against foreign or infectious pathogens.[1–3] When the production of ROS becomes uncontrolled, or sustained for a long time, a wide number of pathologies may evolve, such as rheumatoid arthritis, atherosclerosis, sepsis, and accelerated senescence, and, in an ultimate instance, cancer.[4,5]

Hypochlorous acid (HOCl) is one of the ROS that may be produced in excess during inflammatory processes. Consequently, HOCl plays an important role in the above mentioned diseases and, for that reason, is a potential target for the chemotherapy of inflammation. Activated neutrophils contain the enzyme myeloperoxidase that oxidizes Cl-ions into HOCl, by using H_2O_2 as a co-substrate, according to the following Eq. 1:[6]

$$H_2O_2 + Cl^{\ominus} + H^{\oplus} \rightarrow HOCl + H_2O \quad (1)$$

Further than HOCl, activated neutrophils also release other ROS such as superoxide radical ($O_2^{\cdot-}$), hydrogen peroxide (H^2O_2), singlet oxygen ($^1O_2$) and peroxyl radical ($ROO^{\cdot}$). H_2O_2, $O_2^{\cdot-}$, all contributing to tissue damage at active inflammation sites.[7–9] In addition, during inflammatory processes, ROS are also produced as a consequence of cyclooxygenase activity.[10] The non-steroidal anti-inflammatory drugs (NSAIDs) indomethacin, acemetacin, and etodolac are indole derivatives (Fig. 1), and block prostaglandin synthesis by non-selective inhibition of COX-1 and COX-2 (indomethacin, acemetacin),[11] or by selective inhibition of COX-2 (etodolac).[12]

Despite of the fact that inhibition of prostaglandin synthesis is the primary therapeutic mechanism of NSAIDs, it has been suggested that the anti-inflammatory action of these drugs may be also due, to some extent, to their ability to scavenge ROS, and to inhibit the respiratory burst of neutrophils triggered by various activator agents.[2,3] Recently, the scavenging activity of these and other NSAIDs was evaluated against several ROS using non-cellular in vitro systems.[13] Thus, these effects may represent an additional mechanism for the anti-inflammatory activity of these drugs that should be taken into consideration for their pharmacological activity.

Curiously, regardless of their scavenging activity against $HO^{\cdot}$, $O_2^{\cdot-}$ and $ROO^{\cdot}$, no HOCl scavenging activity has been found for the

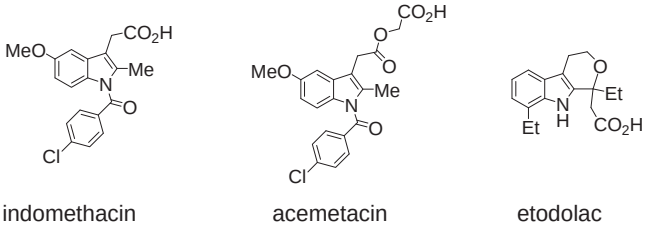

Figure 1. Structure of the NSAIDs indomethacin, acemetacin and etodolac, containing an indole ring as a common scaffold.

* Corresponding authors.
E-mail addresses: egracas@ff.up.pt (E. Fernandes), mmbmarques@dq.fct.unl.pt (M. Manuel B. Marques).

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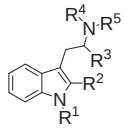


1 R^1 = H, R^2 = $CH_2CHC(Me)_2$, R^3 = CO_2Me, R^4, R^5 = Phth
2 R^1 = $CH_2CHC(Me)_2$, R^2 = H, R^3 = H, R^4 = H, R^5 = H
3 R^1 = $CH_2CHC(Me)_2$, R^2 = H, R^3 = CO_2Me, R^4 = H, R^5 = Ac
4 R^1 = $CH_2CHC(Me)_2$, R^2 = H, R^3 = H, R^4, R^5 = Phth
5 R^1 = H, R^2 = H, R^3 = H, R^4, R^5 = Phth
6 R^1 = $CH_2CHC(Me)_2$, R^2 = H, R^3 = CO_2Me, R^4, R^5 = Phth
7 R^1 = $CH_2CHCHPh$, R^2 = H, R^3 = CO_2Me, R^4 = H, R^5 = Ac
8 R^1 = H, R^2 = H, R^3 = CO_2H, R^4 = H, R^5 = H
9 R^1 = H, R^2 = H, R^3 = H, R^4 = H, R^5 = H
10 R^1 = H, R^2 = H, R^3 = CO_2Me, R^4 = H, R^5 = H
11 R^1 = H, R^2 = H, R^3 = CO_2H, R^4 = H, R^5 = Ac
12 R^1 = H, R^2 = H, R^3 = CO_2Me, R^4, R^5 = Phth
13 R^1 = $CH_2CHC(Me)_2$, R^2 = H, R^3 = CO_2H, R^4 = H, R^5 = H
14 R^1 = H, R^2 = $CH_2CHC(Me)_2$, R^3 = CO_2H, R^4 = H, R^5 = H
15 R^1 = $CH_2CH_2CH(Me)_2$, R^2 = H, R^3 = H, R^4, R^5 = Phth
17 R^1 = $CH_2CH_2CH_2Ph$, R^2 = H, R^3 = CO_2Me, R^4 = H, R^5 = Ac
18 R^1 = H, R^2 = $CH_2CH_2CH(Me)_2$, R^3 = CO_2Me, R^4, R^5 = Phth

Figure 2. Substitution pattern of a previously reported indole library showing HOCl scavenging activity.[16]

indole-derived NSAIDs (indomethacin, acemetacin, and etodolac). However, it is known that HOCl reacts with melatonin,[14,15] which is an indole hormone and one of the most recognized natural antioxidants. In addition, we have recently reported a strong HOCl scavenging activity for several compounds from an indole based library (Fig. 2).[16] We have also explored the electrochemical profile of these compounds,[17] on the expectation that a correlation between the scavenging activity and the oxidation potential would contribute to clarify the HOCl scavenging mechanism(s) of these compounds. However, the correlation between the scavenging activity and Epox was poor, suggesting that complex chemical pathways should take place instead of a simple radical reaction involving an electron donating process.

If linked to effective ability to inhibit COX enzymes (under study), the observed HOCl scavenging activity of tested indole derivatives[16] may strongly contribute for their putative anti-inflammatory potential. This idea stimulated us to perform experiments to elucidate the reaction mechanism(s) of some of the active compounds with HOCl, and to understand the structure–activity relationship, for future structural modification and therefore the development of compounds with improved potency.

Several experiments were performed to establish the conditions that allowed monitoring the reaction of indole derivatives with HOCl, as well as detection of the products formed. The first attempts were carried using indole as a model system, in order to optimize the conditions for monitoring the reaction, such as solvent, reaction temperature, and time. The choice of an organic solvent (in a buffer medium) and a lower temperature were mandatory for the reaction control due to the powerful reactivity of HOCl as oxidizing agent. Thus, reactions were carried in acetonitrile, in the presence of phosphate buffer solution (pH 7), and the HOCl solution,[13,18] pH 6.2 (1.5 equiv), at 0 °C. Of note, the reaction mixtures obtained with indole were extremely complex and difficult to analyze.

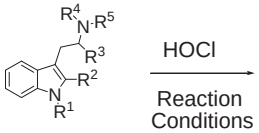
Subsequently, indolic compounds were selected from the library, the choice being done on the basis of their HOCl scavenging potency. Accordingly, from the tryptamine derivatives, compounds **2**—the less reactive and **9**—the most reactive, were selected. From the tryptophan derivatives, compounds **8** (the most reactive), **10**, **11** (the less reactive—$IC_{50} \approx 50$) and **13** were selected. These compounds were exposed to HOCl, the reaction was monitored by TLC, and the products isolated were purified by HPLC (Table 1).[19]

Only compounds **9** and **11** generated a mixture from which it was possible to isolate and characterize some of the reaction products. Compounds **19** and **20** were the major products derived from the mixtures of HOCl with compounds **9** and **11**, respectively (Scheme 1).[20] All the other compounds afforded complex co-eluting mixtures, making impossible to detect any isolable product, and/or the products decomposed during HPLC purification (Table 1).

Since the reaction with tryptamine (**9**) and *N*-acetyl tryptophan methyl ester (**11**) gave rise to chlorinated products either on the side chain (**19**), or at the aromatic ring (**20**), respectively, the reaction with HOCl was next investigated in the presence of the radical trap TEMPO (2,2,6,6-tetramethylpiperidine-1-oxyl). Compound **11** was the only one that afforded a product stable enough to allow isolation and structure assignment (compound **21**, Scheme 1).[21] The structures for the isolated compounds were proposed on the basis of ^1H, ^{13}C NMR (also 2D experiments), and mass spectrometry assignment. Unfortunately, compounds **20** and **21** decomposed after 24 h, and further investigation of these structures was not possible.

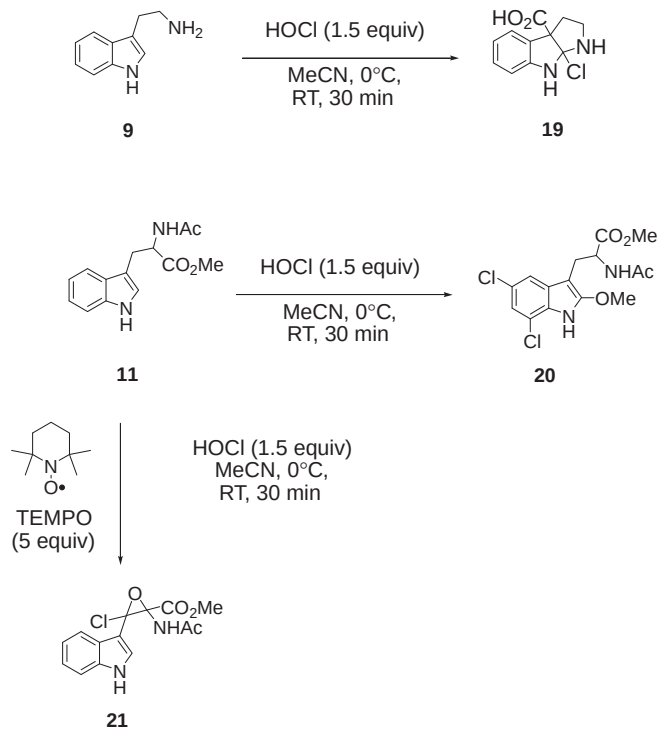
The indole framework is widely identified as a privileged structure or pharmacophore,[22] and one of the most promising endow-

Table 1
Reaction of indole derivatives **2**, **8**–**11** and **13** with HOCl

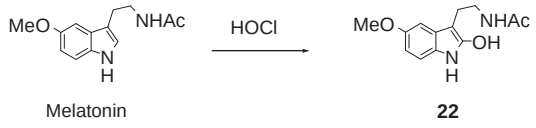


Entry	Compound	R^1	R^2	R^3	R^4, R^5	Reaction conditions[a] (TEMPO)	Product (Yield%)
1	**2**	$CH_2CH(Me)_2$	H	H	H,H	—	b
2	**8**	H	H	CO_2H	H,H	—	c
3	**9**	H	H	H	H, H	—	**19 (2)**
4	**9**	H	H	H	H, H	5 equiv	d
5	**10**	H	H	CO_2Me	H, H	—	d
6	**11**	H	H	CO_2Me	H, Ac	—	**20 (8.6)**
7	**11**	H	H	CO_2Me	H, Ac	5 equiv	**21 (2.6)**
8	**13**	$CH_2CH(Me)_2$	H	CO_2H	H, H	—	e

— Absence of TEMPO.
[a] All the experiments were carried in MeCN, with 1.5 equiv of HOCl.
[b] After 30 min the starting material **2** was the major component.
[c] Compound **8** was not soluble under the reaction conditions.
[d] Decomposition of the products during HPLC purification.
[e] The product was an insoluble oil.



Scheme 1. Reaction of HOCl with tryptamine (**9**) and tryptophan derivative **11**. Proposed structures for the isolated products **19**–**21**.



Scheme 2. Literature proposed routes for the scavenging of HOCl by melatonin.[14]

ments of indole derivatives is their antioxidant potential.[23] In addition, its substitution pattern has been reported to influence the antioxidant activity and its efficiency in biological systems.[23] In particular, the indolic nitrogen, is described as the active redox center of indoles.[24,25] Similarly, the high reactivity of melatonin with reactive species is probably due to the presence of an electron-rich aromatic ring system, reacting as an electron donor, to form the melatoninyl cation radical, or by providing an electrophilic radical addition at the C-3 position of the indole ring.[26]

However, when a cyclic voltammetric study was undertaken on the studied library, no visible correlation could be observed with the HOCl scavenging activity displayed by these compounds (data not shown). Since the NSAIDs containing the indole skeleton are not able to scavenge HOCl, the interesting experimental behavior observed for the tested compounds prompted us to explore more deeply the mechanism involved.

Although the mechanism of HOCl reactivity has been explored in the literature, it is not possible to obtain a comprehensible answer concerning the mechanism involved in the reaction with HOCl. While Mason and co-workers concluded that the reactivity of HOCl occurs via radical processes,[24] other authors considered ionic mechanisms,[27–32] and in fact, possible transition states for several ionic reaction pathways have been calculated.[33] Radical dissociation of HOCl has only been discussed under photolytic catalysis.[34]

In the hope of getting a clear answer, several experiments were undertaken and, from the obtained results, it was possible to conclude that TEMPO prevents chlorination in the aromatic ring of compound **11**, which then might occur via a radical mechanism (compound **20**). In the absence of TEMPO, both the tryptamine (**9**) and tryptophan derivative **11** gave halogenated compounds. Tryptamine gives compound **19**, which might result either form a radical or ionic mechanism. On the other hand, for tryptophan derivative **20**, the position of chlorination suggests a radical mechanism. Nevertheless, in compound **21**, no chlorination of the aromatic ring was observed, with the product formed being chlorinated only at the side chain.

The results obtained indicate that both mechanisms (radical and ionic) can be involved in the reaction between tryptamine/tryptophan with HOCl. This would explain the observed lack of a good correlation between the oxidation potential and scavenging activity of HOCl by the tested indoles.

In the case of melatonin, its HOCl scavenging mechanism has been described.[14,15] However, the reaction of HOCl with melatonin was proposed to proceed via nucleophilic addition of water (solvent) at the C-2 position that, after loss of the proton, followed by dehydrohalogenation, gave the 2-hydroxyindole compound (**22**) (Scheme 2).

In conclusion, this study demonstrates that the reaction of indole compounds with HOCl is complex and involves both radical and ionic mechanisms.

Acknowledgments

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19. Melting points were determined on a Reichert Thermovar apparatus and are uncorrected. Ordinary mass spectra were recorded on a Fisons TRIO 2000 or AEI MS-9 spectrometers. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker ARX 400 spectrometer. Chemical shifts are reported relative to tetramethylsilane as the internal reference (δ_H 0.00 ppm) for ^{1}H NMR spectra and to $CDCl_3$ (δ_C 77.00 ppm) for ^{13}C NMR spectra. High resolution mass spectra were recorded on an AutoSpecQ spectrometer. IR spectra were run on a FT Perkin–Elmer 683 instrument, with absorption frequencies expressed in reciprocal centimeters. Thin-layer chromatography was performed on Merck silica gel 60 F254 plates and PTLC on 0.5 mm thick plates. Column chromatography was carried out on Merck silica gel 60 (70–230 mesh).
General procedure: To a solution of tryptamine (**9**) or tryptophan methyl ether (**10**) (100 mg) in acetonitrile (1 mL) in the presence of phosphate buffer solution (pH 7), was added dropwise a freshly prepared solution of HOCl pH 6.2 (1.5 equiv), at 0 °C. The reaction was slowly warmed to room temperature, and stirred for 30 min. After this time, the reaction was stopped by addition of

$NaHCO_3$ solution, and extracted twice with AcOEt. The combined organic layers were dried over $NaSO_4$ and evaporated.

20. *Physical and analytical data of the isolated compounds*: 8a-chloro-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-*b*]-3a-carboxylic acid (**19**). Compound **19** was isolated by preparative chromatography (AcOEt/MeOH 5%), as a light brown solid (2%): mp: 238 °C (dec.). IR (KBr) ν_{max}: 3270, 1723, 1621, 1473, 1089, 755. ^{1}H NMR ($(CD_3)_2CO$) δ = 9.59 (1H, s), 7.45 (1H, d, *J* = 7.5 Hz), 7.32 (1H, t, *J* = 7.7 Hz), 7.08 (1H, td, *J* = 7.6 Hz), 6.96 (1H, d, *J* = 7.7 Hz), 6.66 (1H, s), 3.91–3.84 (1H, m), 3.50–3.44 (1H, m), 2.35–2.27 (1H, m), 2.20–2.14 (1H, m). ^{13}C NMR ($(CD_3)_2CO$) δ = 175.7, 152.4, 142.3, 131.3, 129.5, 125.3, 123.4, 111.1, 80.0, 36.3, 27.9. ESI-TOF: 241.0589 (M+1), 158.0621 [(M+1)−(·Cl+COOH)], 130.0676 [(M+1)−C_2H_4]. Methyl 2-acetamido-3-(5,7-dichloro-2-methoxy-1*H*-indol-3-yl)propanoate (**20**). The residue was purified by HPLC. Separation gradient system: A (H_2O; pH 2.5)/B (acetonitrile); A/B, 75:25 (0 min)—50:50 (25 min)—0:100 (26 min)—0:100 (26 min)—75:25 (35 min); flow-rate, 3 mL/min. Compound **20** (retention time 30 min) was isolated as a yellow oil (8.6%). IR (KBr) ν_{max}: 3337, 2954, 2918, 2850, 1736, 1655, 1465, 1171, 731. ^{1}H NMR ($CDCl_3$) δ = 8.20 (1H, s), 7.21 (1H, s), 7.07 (1H, s), 6.11 (1H, d, *J* = 7.6 Hz), 4.83 (1H, m), 4.11 (3H, s), 3.70 (3H, s), 3.18 (2H, dd, *J* = 5.4, 14.8 Hz), 1.94 (3H, s). ^{13}C NMR ($CDCl_3$) δ = 172.1, 169.9, 130.2, 126.1, 120.1, 115.9, 91.1, 77.2, 52.7 (3× C), 29.7, 23.1 (2× C). ESI-TOF: 361.0339 (M+1), 327.0223 ((M+1)−$CH_3O^{\cdot}$), 213.9703.
21. Methyl 2-acetamido-3-chloro-3-(1*H*-indol-3-yl)oxirane-2-carboxylate (**21**). To a solution of tryptophan methyl ester (100 mg) in acetonitrile (1 mL) in the presence of phosphate buffer solution (pH 7), was added TEMPO (5 equiv). Then, a freshly prepared solution of HOCl pH 6.2 (1.5 equiv), was added dropwise at 0 °C. The reaction was slowly warmed to room temperature, and stirred for 30 min. After this time, the reaction was stopped by addition of $NaHCO_3$ solution, and extracted twice with AcOEt. The combined organic layers were dried over $NaSO_4$ and evaporated. The residue was purified by HPLC. Separation gradient system: A (H_2O; pH 2.5)/B (acetonitrile); A/B, 75:25 (0 min)—50:50 (25 min)—0:100 (26 min)—0:100 (28 min)—75:25 (35 min); flow-rate, 3 mL/min. Compound **21** (retention time 26 min) was isolated as a yellow oil (2.6%): IR (NaCl) ν_{max}: 3394, 1711, 1695, 1445, 1296, 1164, 745. ^{1}H NMR ($(CD_3)_2CO$) δ = 9.25 (1H, s), 7.72 (1H, d, *J* = 6.4 Hz), 7.54 (1H, s), 7.43 (1H, d, *J* = 7.9 Hz), 7.29–7.20 (4H, m), 3.84 (3H, s), 2.79 (3H, s). ^{13}C NMR ($(CD_3)_2CO$) δ = 171.6, 161.6, 143.5, 139.3, 123.1, 121.2, 120.9, 120.1, 119.7, 119.5, 112.3, 107.6, 51.8, 25.7. ESI-TOF: 309.0850 (M+1), 279.0741 [(M+1)−(2× CH_3)], 183.0551.
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