ω-(5-Phenyl-2H-tetrazol-2-yl)alkyl-substituted hydrazides and related compounds as inhibitors of amine oxidase copper containing 3 (AOC3)

Florian Galster | Timo Pöstges | Walburga Hanekamp | Matthias Lehr

Abstract
Amine oxidase copper containing 3 (AOC3), also known as plasma amine oxidase, semicarbazide-sensitive amine oxidase, or vascular adhesion protein-1, catalyzes the oxidative deamination of primary amines to aldehydes using copper and a quinone as cofactors. Because it is involved in the transmigration of inflammatory cells through blood vessels into tissues, AOC3 is thought to play an important role in inflammatory diseases. Therefore, inhibitors of this enzyme could lead to new therapeutics for the treatment of inflammation-related diseases. Recently, 6-(5-phenyl-2H-tetrazol-2-yl)hexan-1-amine was found to be a tight-binding substrate of AOC3. To obtain novel inhibitors of the enzyme, the amino group of this substrate was replaced with functional groups that occur in known AOC3 inhibitors, such as hydrazide or glycine amide moieties. In addition, derivatives of the compounds obtained in this way were prepared. The obtained hydrazide 5, which proved to be the most effective, was subjected to further structural modifications. Selected hydrazides were evaluated for selectivity toward some other amine oxidases.

KEYWORDS
5-phenyltetrazole, amine oxidase copper containing 3, glycine amide, hydrazide, inhibitor

1 | INTRODUCTION

The copper-dependent amine oxidases are copper- and quinone-dependent enzymes that catalyze the oxidative deamination of primary amine groups to aldehydes with the simultaneous release of hydrogen peroxide and ammonia.[1] They are divided into two subgroups depending on their organic cofactors. On the one hand, these are the topaquinone-containing enzymes with the representatives AOC1, AOC2, and AOC3, and on the other hand, the lysine tyrosylquinone-containing lysyl oxidases (LOX).

The best-studied topaquinone-containing amine oxidase is AOC3 (amine oxidase copper containing 3). In contrast to the flavine-dependent monoamine oxidases A and B (MAO-A and MAO-B), which have been in the focus of pharmacotherapy for more than 40 years, AOC3 has remained in the shadows for a long period of time.[2] The vast number of alternative and ambiguous names for AOC3 reflects the limited understanding of its (patho)physiological significance in the past. Thus, it has been referred to as benzyamine oxidase, chlorgiline-resistant amine oxidase, plasma amine oxidase, primary amine oxidase, semicarbazide-sensitive amine oxidase, serum monoamine oxidase, and vascular adhesion protein-1. Only the
discovery that certain diseases are associated with altered AOC3 levels has led to increased interest in AOC3 as a therapeutically relevant drug target over the past 25 years.

In addition to a membrane-bound form, there is also a circulating one in plasma. This is probably the result of proteolytic cleavage of the membrane-bound AOC3 protein, especially from vascular endothelial cells.[1] In various diseases, such as inflammatory liver diseases and diabetic retinopathy, markedly elevated concentrations of the enzyme, whose physiological substrate is not yet clearly known, are found in blood plasma.[4-6] AOC3 is also of particular importance in inflammatory processes. It is involved in the transmigration of leukocytes from the blood to the inflamed tissue, where these cells amplify the inflammatory response.[7-12] Inhibitors of AOC3 could, therefore, serve as novel anti-inflammatory drugs, among other things.

The enzymatic catalytic mechanism of AOC3 action is described as a so-called "ping-pong mechanism", which is divided into two half-reactions. In the first reductive half-reaction, the oxidation of the amine to the aldehyde takes place after the formation of a Schiff base. In the subsequent oxidative half-reaction involving copper ions, the oxidative regeneration of the amine oxidase cofactor takes place with the reduction of oxygen to hydrogen peroxide.[1,13] A number of inhibitors of AOC3 have been described in the literature.[14-18] such as hydrazides like 2,21] glycine amides like 3,22,23] and 3-fluoroallylamines like BI 1467335 (Figure 1).[24-26] The latter compound was even investigated in clinical trials by Boehringer Ingelheim for the treatment of nonalcoholic steatohepatitis[27] and nonproliferative diabetic retinopathy.[28] However, these were terminated in 2020, in particular, because of the risks of drug interactions. Nevertheless, AOC3 is still considered a very interesting target for the development of innovative pharmaceuticals.

We have recently described an assay for the investigation and characterization of AOC3 inhibitors that uses 6-(5-phenyl-2H-tetrazol-2-yl)hexan-1-amine (1) as a novel substrate.[29] This compound binds much more strongly to the enzyme than the also non-physiological benzylamine substrate, which is usually used in AOC3 assays. Starting from the tightly binding hexanamine substrate 1, now novel inhibitors of AOC3 should be obtained by replacing its amine function with functional groups present in known inhibitors of AOC3. For this purpose, corresponding hydrazide and glycine amide derivatives were to be prepared first. These should then be varied structurally if they are effective. The most potent of the developed AOC3 inhibitors should also be investigated for inhibition of the related amine oxidases AOC1 (also referred to as diamine oxidase), MAO-A, and MAO-B.

2 | RESULTS AND DISCUSSION

2.1 | Chemistry

The synthesis of the heptane hydrazide 5 was carried out as shown in Scheme 1. First, phenyltetrazole was reacted with ethyl 7-bromoheptanoate in acetonitrile in presence of the base potassium carbonate. The obtained tetrazoyl-substituted heptanoic acid ethyl ester 4 was converted to the desired hydrazide 5 upon hydrazinolysis in ethanol. For the synthesis of the semicarbazide 9, hexan-1-amine 1 was reacted with diphenyl carbonate and the resulting phenyl carbamate 8 was treated with hydrazine hydrate (Scheme 2). The corresponding thiosemicarbazide 11 was prepared by first reacting the amine 1 with carbon disulfide. The obtained isocyanate 10 was then subjected to hydrazinolysis to give the target compound (Scheme 2).

The preparation of the glycine amide derivative 7 started from amine 1. Reaction with Boc-protected glycine in presence of N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide (EDC) and 1-hydroxybenzotriazole yielded the Boc-protected glycine amide 6, which was converted to 7 by treatment with trifluoroacetic acid in dichloromethane (Scheme 2). For the synthesis of the homologous 3-aminopropanamide and 4-aminobutanamide derivatives 13 and 15, amine 1 was reacted with EDC-activated and benzoxycarbonyl-protected 3-aminopropanoic acid and 4-aminobutyric acid, respectively, followed by cleavage of the protecting group by catalytic hydrogenation (Scheme 2).

N-(2-Aminoethyl)hexanamide 17 was prepared by reacting the ethyl ester group of the hexanoic acid ester intermediate 16 with ethylenediamine in ethanol (Scheme 1). For the synthesis of the homologous N-(3-aminopropyl)hexanamide 19, ester 16 was first treated with tert-butyl N-(3-aminopropyl)carbamate. Subsequently, the Boc protecting group of the obtained N-aminopropyl-substituted amide 18 was cleaved with HCl in cyclopentyl methyl ether and ethyl acetate (Scheme 1).

The syntheses of the compounds in which the amino group of hexanamine 1 was incorporated into a ring system all started from the bromohexane-substituted phenyltetrazole 20 (Scheme 3). The reaction of 20 with Boc-protected 2-oxopyrrolidin-3-amine and 5-oxopyrrolidin-3-amine after deprotonation with sodium hydride followed by cleavage of the Boc group with HCl in cyclopentyl methyl ether and ethyl acetate led to the aminopyrrolidone derivatives 22 and

![Figure 1](image-url)
**Scheme 1** Synthesis of compounds 5, 17, 18, and 19. Reagents and conditions: (a) 4: Ethyl 7-bromoheptanoate, potassium carbonate, acetonitrile, reflux, 5 h; 16: ethyl 6-bromohexanoate, potassium carbonate, acetonitrile, reflux, 6.5 h; (b) hydrazine monohydrate, ethanol, reflux; (c) ethylenediamine, ethanol, reflux, 32 h; (d) tert-butyl N-(aminopropyl)carbamate, 90°C, 7 days; (e) ethyl acetate, HCl (4 M) in cyclopentyl methyl ether, room temperature, 4 h.

**Scheme 2** Synthesis of compounds 7, 9, 11, 13, and 15. Reagents and conditions: (a) N-(tert-Butoxycarbonyl)glycine, 1-hydroxybenzotriazole, N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC HCl), DMF, room temperature, 43 h; (b) trifluoroacetic acid, dichloromethane, room temperature, 3 h; (c) diphenyl carbonate, THF, water, room temperature, 2 h; (d) hydrazine monohydrate, 1,2-dimethoxyethane, 80°C, 5 h; (e) carbon disulfide, triethylamine, ethanol, room temperature, 2 h followed by di-tert-butyl dicarbonate, 4-dimethylaminopyridine, ethanol, room temperature, 45 min; (f) hydrazine monohydrate, dichloromethane, room temperature, 2 h; (g) 3-(benzoyloxycarbonylamino)propanoic acid (in case of 12) or 4-(benzoyloxycarbonylamino)butyric acid (in case of 14), 1-hydroxybenzotriazole, EDC HCl, DMF, room temperature, 20 or 22 h; (h) H2, palladium on charcoal, THF, methanol, room temperature, 4 h.
24. The aminomethyl tetrazoles 27 and 28 and aminomethyl imidazole 30, respectively, were similarly prepared from 20 using the corresponding Boc-protected heterocycles (Scheme 3). The other hydrazides were synthesized from phenyltetrazole in the same manner as hydrazide 5 using the corresponding bromine-substituted ester derivatives.

2.2 | Biology

To determine AOC3 inhibition, the substrate 6-(5-phenyl-2H-tetrazol-2-yl)hexan-1-amine (1) was incubated with AOC3 from bovine plasma in the presence or absence of test substances and the resulting aldehyde was measured after reaction with Tris as an oxazolidine derivative by high-performance liquid chromatography (HPLC) and UV detection at 238 nm. The determinations were performed both without and with preincubation of the enzyme with the potential inhibitor. The assays for inhibition of AOC1, MAO-A, and MAO-B were performed in an analogous manner, using 4-(5-phenyl-2H-tetrazol-2-yl)butan-1-amine as substrate in the case of the MAO enzymes. With these enzymes, only the experiments were performed in which the enzyme and the test compound were preincubated for a certain time before the addition of the substrate.

Evaluation of AOC3 inhibition without preincubation of the enzyme with the inhibitor showed that heptanethyldrazide 5 inhibited AOC3 about three times more strongly than the known hydrazide-based inhibitor 2 (IC50: 3.5 vs. 11 µM) (Table 1). As hydrazides were described to covalently inactivate the enzyme via the formation of Schiff bases with the topaquinone cofactor, it was also investigated how the IC50 values changed when the enzyme was preincubated with the inhibitor for 15 min before the addition of the substrate. Under these conditions a considerable increase in activity by a factor of about 40–50 for both hydrazides could be observed, demonstrating the covalent inhibition mechanism. The reason for this effect on inhibitory potency can be seen in the fact that the formation of covalent bonds takes a certain amount of time and the inhibitors, therefore, have more time to switch off the enzyme when preincubated with it, resulting in reduced enzymatic activity. The large difference in IC50 inhibition values obtained when performing the measurement without and with preincubation indicates a relatively low initial competitive binding affinity accompanied by high chemical reactivity of the hydrazides.

Unlike the hydrazides studied, the prepared glycine amide derivative 7 was significantly less effective than the glycine amide reference inhibitor 3. Both with and without preincubation with the AOC3, an IC50 of about 6 µM was measured for 7. This result indicates that 7 does not interact in a covalent manner with the enzyme. In contrast, an
**TABLE 1**  Inhibition of amine oxidase copper containing 3 (AOC3) by hydrazides and glycine amides depending on the test conditions.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Inhibition of AOC3, IC$_{50}$ (µM)$^a$</th>
<th>Without preincubation$^b$</th>
<th>With preincubation$^c$</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>3.5</td>
<td>0.069</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>6.0</td>
<td>6.3</td>
<td></td>
</tr>
<tr>
<td>2 (reference hydrazide)</td>
<td>11 ± 1.2</td>
<td>0.29 ± 0.01</td>
<td></td>
</tr>
<tr>
<td>3 (reference glycine amide)</td>
<td>0.72 ± 0.14</td>
<td>0.37 ± 0.14</td>
<td></td>
</tr>
</tbody>
</table>

$^a$In the case of 5 and 7: IC$_{50}$ values are the means of two independent determinations, errors are within ±20%; in the case of 2: IC$_{50}$ values are the means ± standard deviations, n = 3; in the case of 3: IC$_{50}$ values are the means ± standard deviations, n = 5.

$^b$Enzyme reaction was started by addition of the enzyme solution to a mixture of substrate and inhibitor.

$^c$Enzyme solution was preincubated with the inhibitor for 15 min at 37°C before the substrate was added.

**TABLE 2**  Inhibition of amine oxidase copper containing 3 (AOC3) by different hydrazide- and glycine amide-derived compounds depending on the test conditions.

<table>
<thead>
<tr>
<th>Compound</th>
<th>n</th>
<th>R</th>
<th>Inhibition of AOC3 (% Inhibition at 10 µM)$^a$</th>
<th>Without preincubation$^b$</th>
<th>With preincubation$^c$</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>6</td>
<td>-CONHNH$_2$</td>
<td>76 ± 4</td>
<td>92 ± 2</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>6</td>
<td>-NHCONHNH$_2$</td>
<td>45 ± 3</td>
<td>98 ± 2</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>6</td>
<td>-NHCSNHNH$_2$</td>
<td>12 ± 3</td>
<td>87 ± 1</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>6</td>
<td>-NHCOCH$_2$NH$_2$</td>
<td>60 ± 3</td>
<td>61 ± 5</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>6</td>
<td>-NHCO(CH$_2$)$_2$NH$_2$</td>
<td>N.a.</td>
<td>N.a.</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>6</td>
<td>-NHCO(CH$_2$)$_2$NH$_2$</td>
<td>N.a.</td>
<td>N.a.</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>5</td>
<td>-CONH(CH$_2$)$_2$NH$_2$</td>
<td>N.a.</td>
<td>N.a.</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>5</td>
<td>-CONH(CH$_2$)$_2$NH$_2$</td>
<td>N.a.</td>
<td>N.a.</td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>6</td>
<td>3-amino-2-oxopyrrolidin-1-yl</td>
<td>N.a.</td>
<td>N.a.</td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>6</td>
<td>4-amino-2-oxopyrrolidin-1-yl</td>
<td>N.a.</td>
<td>N.a.</td>
<td></td>
</tr>
<tr>
<td>27</td>
<td>6</td>
<td>5-(aminomethyl)-1H-tetrazol-1-yl</td>
<td>33 ± 2</td>
<td>26 ± 4</td>
<td></td>
</tr>
<tr>
<td>28</td>
<td>6</td>
<td>5-(aminomethyl)-2H-tetrazol-2-yl</td>
<td>20 ± 8</td>
<td>N.a.</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>6</td>
<td>2-(aminomethyl)-1H-imidazol-1-yl</td>
<td>18 ± 3</td>
<td>N.a.</td>
<td></td>
</tr>
</tbody>
</table>

(Continues)
approximately twofold higher inhibitory activity was observed for the reference inhibitor 3 when preincubated with the enzyme. Accordingly, there could be a covalent mechanism of inhibition, which is also postulated in the literature for this compound.\[22\]

Next, the hydrazide group of compound 5 was varied. In addition to the corresponding semicarbazide 9, the thiosemicarbazide 11 was also prepared. Both derivatives proved to be less effective than the starting hydrazide 5. While an inhibition value of 76% was determined for 5 at a concentration of 10 µM in the test without preincubation, this was only 45% for the semicarbazide 9 and even only 12% for the thiosemicarbazide 11 (Table 2). In the case of preincubation of enzyme and inhibitor, as expected, an increase in activity was observed in each case, whereby ultimately the same order was observed with regard to efficacy. Thus, the IC_{50} values of compounds 5, 9, and 11 were 0.069, 0.18, and 2.3 µM, respectively.

Table 2 (Continued)

<table>
<thead>
<tr>
<th>Compound</th>
<th>n</th>
<th>R</th>
<th>Inhibition of AOC3 (% Inhibition at 10 µM)(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Without preincubation(^b)</td>
</tr>
<tr>
<td>2 (reference)</td>
<td>1</td>
<td>11 ± 1.2</td>
<td>0.29 ± 0.01</td>
</tr>
<tr>
<td>3 (reference)</td>
<td>1</td>
<td>0.72 ± 0.14</td>
<td>0.37 ± 0.14</td>
</tr>
</tbody>
</table>

\(^a\)Values are means ± standard deviations (n = 3, in the case of 7, 27, 28, and 30: n = 4); N.a. means not active at 10 µM (n = 2).

\(^b\)Enzyme reaction was started by the addition of the enzyme solution to a mixture of substrate and inhibitor.

\(^c\)Enzyme solution was preincubated with the inhibitor for 15 min at 37°C before the substrate was added. IC_{50} for 5, 9, and 11: 0.069, 0.18, and 2.3 µM (means two independent determinations, errors are within ±20%).

Table 3

Inhibition of amine oxidase copper containing 3 (AOC3) by various hydrazide compounds.

<table>
<thead>
<tr>
<th>Compound</th>
<th>n</th>
<th>Inhibition of AOC3, IC_{50} (µM)(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Without preincubation(^b)</td>
</tr>
<tr>
<td>32</td>
<td>1</td>
<td>0.83</td>
</tr>
<tr>
<td>34</td>
<td>2</td>
<td>5.5</td>
</tr>
<tr>
<td>36</td>
<td>3</td>
<td>&gt;10(^d)</td>
</tr>
<tr>
<td>38</td>
<td>4</td>
<td>&gt;10(^e)</td>
</tr>
<tr>
<td>40</td>
<td>5</td>
<td>-(^f)</td>
</tr>
<tr>
<td>5</td>
<td>6</td>
<td>3.5</td>
</tr>
<tr>
<td>42</td>
<td>7</td>
<td>5.7</td>
</tr>
<tr>
<td>44</td>
<td>9</td>
<td>9.0</td>
</tr>
</tbody>
</table>

\(^a\)IC_{50} values are the means of two independent determinations, errors are within ±20%; in the case of 2: IC_{50} values are the means ± standard deviations, n = 3.

\(^b\)Enzyme reaction was started by the addition of the enzyme solution to a mixture of substrate and inhibitor.

\(^c\)Enzyme solution was preincubated with the inhibitor for 15 min at 37°C before the substrate was added.

\(^d\)Twenty-six percent inhibition at 10 µM.

\(^e\)Thirty-six percent inhibition at 10 µM.

\(^f\)No evaluation is possible because, at the test substance concentrations of 10 and 3.3 µM in the high-performance liquid chromatography chromatogram, the peak of the enzyme product is affected by the peak of the test substance due to similar retention times.
The glycine amide head group of 7 was also subjected to various structural variations. First, the methylene group between the terminal amino group and the amide function was replaced by an ethylene or propylene spacer, resulting in compounds 13 and 15. Analogous inverse amides of the latter two compounds were also prepared (17 and 19). Furthermore, the glycine amide group was rigidified by incorporation into a pyrrolidone ring (22 and 24). Finally, derivatives were synthesized in which the carbonyl group is formally replaced by an imino group and the two nitrogen atoms are simultaneously incorporated into a tetrazole or imidazole ring (27 and 30). Compound 28, a configurational isomer of 27, was also evaluated. Measurement of AOC3 inhibition revealed that all of the open-chain glycine amide derivatives as well as the two pyrrolidone derivatives did not inhibit the enzyme at the highest test concentration of 10 µM. In the case of the aminomethyl substituted azoles 27, 28, and 30, a low inhibitory activity was found at this concentration, provided that substrate and inhibitor came into contact with the enzyme at the same time. However, the inhibitory effect decreased or was even lost when the enzyme and the inhibitor were preincubated. This indicates that the substances are substrates of AOC3 that competitively displace the assay substrate 1 and thus reduce its turnover. In fact, degradation of the compounds 27, 28, and 30 could be observed when they were incubated with the enzyme alone. For compounds 27 and 30, where the aminomethyl group is located in the immediate vicinity of the hexane chain, only 70%–80% of the original amount of substance was present after 15 min, as measured by HPLC/mass spectrometry (MS). In the case of tetrazole 28, in which the aminomethyl group is less sterically hindered, even less than 5% was detectable after this time. This substance is, therefore, a very good substrate for AOC3. In all cases, the corresponding aldehyde or its hydrate could be detected in the reverse phase (RP)-HPLC chromatograms with somewhat longer retention times. Of the derivatives of AOC3 substrate 1 investigated so far in this study, hydrazide 5 proved to be the most effective inhibitor of the enzyme. Therefore, this compound was finally subjected to further structural variations. Thus, the hexyl spacer between the hydrazide group and tetrazole ring was successively shortened from six to one and lengthened to seven carbon atoms. In addition, a derivative of 5 was prepared in which a part of the alkyl chain was replaced by a more rigid phenoxy residue. With the exception of acetohydrazide derivative 32, whose IC50 was 0.83 µM, all hydrazides had IC50 values greater than 3 µM when enzyme and inhibitor were not preincubated (Table 3). With appropriate preincubation, the inhibitory effect increased markedly, as indicated by the significantly lower IC50 values. The compounds with C1, C5, C6, and C7 spacers (32, 40, 5, and 42) all had IC50 values below 0.10 µM. However, there was a marked difference between the dose–response curve of 32 and that of the other three compounds 40, 5, and 42, which have a significantly longer alkyl spacer between the tetrazole ring and the hydrazide functionality. While the latter hydrazides showed a typical sigmoidal curve, that of 32 was more linear (Figure 2). Furthermore, the curve for acetohydrazide 32 in the IC50 range was much flatter than for the three other hydrazides. The reasons for these differences remain to be elucidated. Replacing the C7 spacer of 42 with a more rigid ethoxyphenyl residue did not change the activity significantly, as shown by the inhibition data of compound 44.

Finally, hydrazides 5 and 32 as well as the hydrazide reference inhibitor 2 were also investigated for their selectivity toward other amine oxidases. In all cases, the enzyme and the inhibitor were preincubated for 15 min before substrate addition. It was shown that all three substances have no influence on MAO-A and MAO-B.

![Dose–response curves of the heptanehydrazide derivative 5 (a) and acetohydrazide derivative 32 (b); data points are means of duplicates or triplicates.](Image 311x251 to 545x614)

**FIGURE 2** Dose–response curves of the heptanehydrazide derivative 5 (a) and acetohydrazide derivative 32 (b); data points are means of duplicates or triplicates.

**TABLE 4** Inhibition of amine oxidase copper containing 3 (AOC3) and AOC1 by selected hydrazides.

<table>
<thead>
<tr>
<th>Compound</th>
<th>AOC3 Inhibition at 1 µM (%)</th>
<th>AOC1 Inhibition at 1 µM (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>90 ± 3</td>
<td>72 ± 7</td>
</tr>
<tr>
<td>32</td>
<td>79 ± 5</td>
<td>59 ± 5</td>
</tr>
<tr>
<td>2 (reference)</td>
<td>80 ± 3</td>
<td>55 ± 3</td>
</tr>
</tbody>
</table>

*Inhibitors and enzymes were preincubated for 15 min before starting the enzyme reaction by addition of substrate. The inhibition values are the means ± standard deviations of three independent determinations.*
at a test concentration of 10 μM. In contrast, AOC1, which like AOC3 has a topaquinone cofactor, is inhibited by these compounds. However, the inhibition values, measured at 1 μM, are somewhat lower toward AOC1 than AOC3 (Table 4). It should be noted that the literature describes that the reference inhibitor 2 does not inhibit AOC1 at appropriate concentrations.\textsuperscript{[17]}

3 | CONCLUSION

In summary, it can be stated that potent inhibitors of AOC3 with hydrazide function could be produced within the scope of this study. Future studies will include additional molecular variations of glycine amide inhibitor 7 and semicarbazide inhibitor 9, which have also been shown to affect the activity of this pathophysiologically relevant enzyme.

4 | EXPERIMENTAL

4.1 | Chemistry

4.1.1 | General

Column chromatography was performed on silica gel 60, particle size 0.040–0.063 mm, from Macherey and Nagel. Melting points (mp) were determined on a Büchi B-540 apparatus and are uncorrected. \(^1\)H NMR spectra (see the Supporting Information) were recorded on a DD2 spectrometer (400 MHz) or a DD2 spectrometer from Agilent. The high-resolution mass spectra (HRMS) were recorded on a Bruker microTOF-Q II spectrometer applying electrospray (ESI) or atmospheric pressure chemical ionization (APCI). The purity of the target compounds were determined by RP-HPLC with UV-detection. The samples were prepared by mixing 20 μl of a 5 mM solution of the respective compound in dimethyl sulfoxide (DMSO) with 180 μl of acetonitrile. Five microliters of the solutions were injected into the HPLC system. Separation was performed using a Nucleosil 100 RP18 3 column (3 mm inside diameter x 125 mm, particle size 3 μM) at a flow rate of 0.4 ml/min and a run time of 30 min. In the case of hydrazides, semicarbazide 9 and thiosemicarbazide 11 the mobile phase consisted of acetonitrile/aqueous Tris buffer (10 mM of hydrazides, semicarbazide or 50 mM, adjusted to pH 8.5 with dilute HCl at 20°C) with a gradient running from 5% (v/v) (hydrazides) or 18% (v/v) of acetonitrile in acetonitrile (80 ml) was heated for 5 h under reflux. The reaction mixture was cooled to room temperature, diluted with ethyl acetate, and filtered. After addition of some silica gel, the solvent was distilled off. The residue was placed on a silica gel column and eluted with cyclohexane/ethyl acetate (gradient from 8:2 to 7:3) to give 4 (1.52 g, 84%) as an oil. Besides, isomeric ethyl 7-(5-phenyl-1H-tetrazol-1-yl)heptanoate (4a) was obtained as a by-product (229 mg, 13%). 4: \(C_{16}H_{22}N_{4}O_{3}\) (302.4); \(^1\)H NMR (400 MHz, CDCl3) \(\delta\) (ppm) 1.24 (t, \(J = 7.2\) Hz, 3H), 1.33–1.46 (m, 4H), 1.58–1.69 (m, 2H), 2.01–2.12 (m, 2H), 2.29 (t, \(J = 7.4\) Hz, 2H), 4.12 (q, \(J = 7.2\) Hz, 2H), 4.65 (t, \(J = 7.1\) Hz, 2H), 7.43–7.53 (m, 3H), and 8.11–8.18 (m, 2H); HRMS (APCI, direct probe) m/z [M+H]\(^+\)\(\) calc.: 303.1816, found: 303.1831. 4a: \(C_{16}H_{22}N_{4}O_{3}\) (302.4); \(^1\)H NMR (400 MHz, CDCl3) \(\delta\) (ppm) 1.24 (t, \(J = 7.1\) Hz, 3H), 1.27–1.37 (m, 4H), 1.53–1.61 (m, 2H), 1.89–1.98 (m, 2H), 2.25 (t, \(J = 7.4\) Hz, 2H), 4.11 (q, \(J = 7.1\) Hz, 2H), 4.41 (t, \(J = 7.3\) Hz, 2H), 7.54–7.61 (m, 3H), and 7.64–7.68 (m, 2H); HRMS (APCI, direct probe) m/z [M+H]\(^+\)\(\) calc.: 303.1816, found: 303.1806.

7-(5-Phenyl-2H-tetrazol-2-yl)heptanethydrazone (5)

A solution of compound 4 (734 mg, 2.43 mmol) in ethanol (7 ml) was treated with hydrazine monohydrate (884 μl, 18.2 mmol) and heated to reflux for 3 h. After the addition of additional hydrazine monohydrate (884 μl, 18.2 mmol), heating to reflux was continued for another 2 h. The mixture was cooled and stirred at room temperature for a further 15 h. Then the solvent and the excess hydrazine monohydrate were distilled off to give 5 (710 mg, quantitative) as a solid. \(C_{16}H_{20}N_{4}O\) (284.4); mp: 96–97°C; \(^1\)H NMR (400 MHz, [\(\text{D}_2\)]DMSO) \(\delta\) (ppm) 1.19–1.35 (m, 4H), 1.47 (q, \(J = 7.2\) Hz, 2H), 1.89–2.05 (m, 4H), 4.13 (s, 2H), 4.72 (t, \(J = 6.9\) Hz, 2H), 7.50–7.62 (m, 3H), 8.01–8.11 (m, 2H), and 8.89 (s, 1H); \(^13\)C NMR (101 MHz, [\(\text{D}_2\)]DMSO) \(\delta\) (ppm) 24.93, 25.45, 27.89, 28.55, 32.35, 52.70, 126.31, 126.99, 129.24, 130.49, 164.01, and 171.49; HRMS (ESI) m/z [M+H]\(^+\)\(\) calc.: 289.1771, found: 289.1779.


To a solution of 6-(5-phenyl-2H-tetrazol-2-yl)hexan-1-amine\textsuperscript{[32]} (245 mg, 1.00 mmol), N-(tert-butoxycarbonyl)glycine (193 mg, 1.10 mmol) and 1-hydroxybenzotriazole (88% [m/m]) (169 mg, 1.10 mmol) in dry DMF (7 ml) was added EDC HCl (211 mg, 1.10 mmol) and the mixture was stirred at room temperature for 43 h. Then ethyl acetate and saturated aqueous sodium hydrogen carbonate solution were added. The organic phase was separated and the aqueous phase was extracted two
times with ethyl acetate. The combined organic phases were washed successively with saturated sodium hydrogen carbonate solution (twice) and brine, dried over sodium sulfate, and filtered. After the addition of some silica gel, the filtrate was concentrated. The residue was placed on a silica gel column and eluted with cyclohexane/ethyl acetate and ethyl acetate (gradient from 8:5 to pure ethyl acetate) to give 6 (332 mg, 82%) as an oil. C_{15}H_{22}N_{6}O (302.4); \( \delta \) ppm: 1.45 (m, 4H), 1.48 (m, 4H), 2.19 (q, J = 6.7 Hz, 2H), 3.51 (t, J = 7.1 Hz, 2H), 5.02 (s, 1H), 7.09–7.14 (m, 2H), 7.18 (t, J = 7.3, 1.0 Hz, 1H), 7.31–7.38 (m, 2H), 7.44–7.53 (m, 3H), and 8.12–8.18 (m, 2H); HRMS (APCI, direct probe) \( m/z \) [M+H\(^+\)]\(^\ast\) calc.: 366.1925, found: 366.1935.

N-[6-(5-Phenyl-2H-tetrazol-2-yl)hexyl]hydrazinecarbamate (9) A solution of 8 (323 mg, 0.88 mmol) in 1,2-dimethoxyethane (2.5 ml) was treated with hydrazine monohydrate (440 mg, 8.79 mmol) and stirred at 80°C for 5 h. The solvent and the excess of hydrazine monohydrate were distilled off and the residue chromatographed on silica gel (dichloromethane/methanol 9:1). The fractions containing the product were almost completely concentrated. After addition of ethyl acetate, 9 precipitated (120 mg, 45%). C_{14}H_{21}N_{7}O (319.4); mp: 122–125°C; \(^1\)H NMR (600 MHz, [D\(_6\)]DMSO) \( \delta \) ppm: 1.26–1.33 (m, 4H), 1.37 (q, J = 6.8 Hz, 2H), 1.96 (q, J = 7.0 Hz, 2H), 2.99 (q, J = 6.7 Hz, 2H), 4.04 (s, 2H), 4.72 (t, J = 7.0 Hz, 2H), 6.28 (s, 1H), 6.83 (s, 1H), 7.52–7.60 (m, 3H), and 8.05–8.09 (m, 2H); \(^{13}\)C NMR (151 MHz, [D\(_6\)]DMSO) \( \delta \) ppm: 25.53, 25.66, 28.70, 29.88, 38.71, 52.72, 126.32, 129.26, 130.50, 160.20, and 164.02; HRMS (APCI, direct probe) \( m/z \) [M+H\(^+\)]\(^\ast\) calc.: 304.1880, found: 304.1866.

2-{6-Isothiocyanato(4-hexyl)‐2-phenyl(2H)tetrazole (10) A solution of 6-5-phenyl(2H-tetrazol-2-yl)hexan-1-amine (323 mg, 1.35 mmol), carbon disulfide (163 µl, 2.7 mmol) and triethylamine (374 µl, 2.7 mmol) in dry ethanol (6 ml) was stirred at room temperature for 2 h. After adding a solution of di-tert-butyl dicarbonate (797 mg, 3.65 mmol) and 4-dimethylaminopropylene (22 mg, 0.18 mmol) in dry ethanol (2 ml), the mixture was stirred at room temperature for another 45 min. Then the solvent was removed in vacuo and the residue chromatographed on silica gel (cyclohexane/ethyl acetate 8:2) to give 10 (290 mg, 75%) as an oil. C_{14}H_{21}N_{3}S (287.4); \(^1\)H NMR (400 MHz, CDCl\(_3\)) \( \delta \) ppm: 1.37–1.56 (m, 4H), 1.67–1.76 (m, 2H), 2.10 (q, J = 7.1 Hz, 2H), 3.51 (t, J = 6.5 Hz, 2H), 4.67 (t, J = 7.0 Hz, 2H), 7.45–7.53 (m, 3H), and 8.12–8.17 (m, 2H); HRMS (APCI, direct probe) \( m/z \) [M+H\(^+\)]\(^\ast\) calc.: 288.1277, found: 288.1296.

Phenyl N-[5-(5-phenyl-2H-tetrazol-2-yl)hexyl]acetamide (8)\(^{29}\) A suspension of powdered diphenyl carbonate (298 mg, 1.39 mmol) in a mixture of THF/water 1:9 (3 ml) was added at room temperature dropwise with stirring a suspension of 6-5-phenyl(2H-tetrazol-2-yl)hexan-1-amine\(^{31}\) (342 mg, 1.39 mmol) in a mixture of THF/water 1:9 (3 ml). After further stirring at room temperature for 2 h, the reaction mixture was diluted with water and exhaustively extracted with ethyl acetate. The combined organic phases were dried over sodium sulfate and the solvent was removed in vacuo. The residue was purified by silica gel chromatography (gradient cyclohexane to cyclohexane/ethyl acetate 7:3) to give 8 (331 mg, 65%) as solid. C_{20}H_{29}N_{3}O_{2} (365.4); mp: 54–56°C; \(^1\)H NMR (400 MHz, CDCl\(_3\)) \( \delta \) ppm: 1.15–1.61 (m, 2H), 2.05–2.13 (m, 2H), 2.32 (q, J = 6.7 Hz, 2H), 4.66 (t, J = 7.0 Hz, 2H), 5.02 (s, 1H), 7.09–7.14 (m, 2H), 7.18 (t, J = 7.4, 1.0 Hz, 1H), 7.31–7.38 (m, 2H), 7.44–7.53 (m, 3H), and 8.12–8.18 (m, 2H); HRMS (APCI, direct probe) \( m/z \) [M+H\(^+\)]\(^\ast\) calc.: 320.1652, found: 320.1665.

Benzyl N-[3-oxo-3-[[6-(5-phenyl-2H-tetrazol-2-yl)hexyl]aminopropyl]carbamate (12) To a solution of 6-5-phenyl(2H-tetrazol-2-yl)hexan-1-amine\(^{32}\) (359 mg, 1.46 mmol), 3-(benzylxoxycarbonyl)propionic acid (359 mg, 1.61 mmol) and 1-hydroxybenzotriazole (88% [m/m], 247 mg, 1.61 mmol) in dry DMF (10 ml) was added EDC HCl (309 mg, 1.61 mmol). After stirring at room temperature for 20 h, the mixture was diluted with half-saturated aqueous sodium hydrogen carbonate solution and extracted exhaustively with ethyl acetate. The combined organic phases were washed twice with saturated aqueous sodium hydrogen carbonate solution, dried over sodium sulfate, and concentrated in vacuo. The residue was chromatographed on silica gel (cyclohexane/ethyl acetate 2:8) to...
yield 12 (521 mg, 79%) as a solid. C_{16}H_{32}N_{6}O_{3} (428.5); mp: 105–107°C; \textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3}) \delta (ppm) 1.33–1.40 (m, 4H), 1.47 (q, J = 6.8 Hz, 2H), 2.05 (q, J = 7.7 Hz, 2H), 2.40 (t, J = 6.0 Hz, 2H), 2.31 (q, J = 6.6 Hz, 2H), 2.47 (t, J = 6.0 Hz, 2H), 6.33 (t, J = 7.1 Hz, 2H), 5.08 (s, 2H), 5.45 (s, 1H), 5.69 (s, 1H), 7.27–7.37 (m, 5H), 7.44–7.52 (m, 3H), and 8.11–8.16 (m, 2H); \textsuperscript{13}C NMR (151 MHz, CDCl\textsubscript{3}) \delta (ppm) 24.99, 26.09, 29.17, 36.36, 41.42, 41.95, 53.06, 126.89, 127.55, 129.00, 130.38, 165.16, and 172.89; HRMS (APCI, direct probe) m/z [M+H\textsuperscript{+}] calcd.: 303.1928, found: 303.1916.

tert-Butyl N-[3-[6-(5-phenyl-2H-tetrazol-2-yl)hexyl]propanamide (18)

6-(5-Phenyl-2H-tetrazol-2-yl)hexan-1-amine\textsuperscript{[23]} (479 mg, 1.95 mmol) was heated with tert-butyl N-(3-aminopropyl)carbamate (11.6 g, 66.6 mmol) at 90°C without solvent for 7 days. The reaction mixture was purified by chromatography on silica gel (cyclohexane/ethyl acetate 2:8) to give 18 (340 mg, 42%) as a solid. C_{16}H_{25}ClN_{6}O (416.5); mp: 81–82°C; \textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3}) 1.39–1.47 (m, 11H), 1.53–1.64 (m, 2H), 1.72 (t, J = 8.2, 6.5 Hz, 2H), 2.03–2.15 (m, 2H), 2.19 (t, J = 7.4 Hz, 2H), 3.14 (q, J = 6.3 Hz, 2H), 3.27 (q, J = 6.2 Hz, 2H), 4.65 (t, J = 7.1 Hz, 2H), 4.89 (s, 1H), 6.22 (s, 1H), 7.41–7.54 (m, 3H), and 8.08–8.18 (m, 2H); HRMS (APCI, direct probe) m/z [M+H\textsuperscript{+}] calcd.: 417.2609, found: 417.2613.

N-(3-Aminopropyl)-6-(5-phenyl-2H-tetrazol-2-yl)hexanamide hydrochloride (19)

A solution 18 (310 mg, 0.74 mmol) in ethyl acetate (6 ml) was treated with a solution of HCl (4 M) in cyclopentyl methyl ether (4 ml) and stirred at room temperature for 4 h. After adding again a solution of HCl (4 M) in cyclopentyl methyl ether (2 ml), the mixture was stirred for another 1 h. The solvent was then distilled off and the residue dispersed in ethyl acetate. The solid was filtered off and dried in vacuo to give 19 (219 mg, 83%). C_{16}H_{25}ClN_{6}O (416.5); \textsuperscript{1}H NMR (400 MHz, D_{2}O (DMSO)) \delta (ppm) 1.23–1.32 (m, 2H), 1.54 (q, J = 7.2 Hz, 2H), 1.68 (q, J = 7.4 Hz, 2H), 1.97 (q, J = 7.7 Hz, 2H), 2.08 (t, J = 7.4 Hz, 2H), 2.69–2.78 (m, 2H), 3.08 (q, J = 6.5 Hz, 2H), 4.72 (t, J = 7.0 Hz, 2H), 7.50–7.60 (m, 3H), and 7.91–8.24 (m, 4H); \textsuperscript{13}C NMR (101 MHz, D_{2}O (DMSO)) \delta (ppm) 24.54, 25.41, 27.32, 28.45, 35.02, 35.44, 36.62, 52.65, 126.30, 126.97, 129.25, 130.50, 164.01, and 172.29; HRMS (APCI, direct probe) m/z [M+H\textsuperscript{+}] calcd.: 317.2077, found: 317.2077.

tert-Butyl N-[2-oxo-1-[6-(5-phenyl-2H-tetrazol-2-yl)hexyl]pyrrolidin-3-yl]carbamate (21)

To a solution of tert-butyl N-(2-oxopyrrolidin-3-yl)carbamate (338 mg, 1.69 mmol) in dry DMF (5 ml) was added sodium hydride (60% dispersion in mineral oil, 68 mg, 1.70 mmol) followed by a solution of 2-(6-bromohexyl)-5-phenyl-2H-tetrazole (20)\textsuperscript{[29]} (523 mg, 1.69 mmol) in dry DMF (5 ml). After stirring at 100°C for 2 h, the cooled mixture was diluted with water and exhaustively extracted with ethyl acetate. The combined organic layers were twice washed with water, dried over sodium sulfate, and concentrated under reduced pressure. The residue was chromatographed on silica gel (cyclohexane/ethyl acetate 4:6) to afford 21 (418 mg, 58%) as a solid. C_{22}H_{32}N_{6}O_{2} (428.5); mp: 77–78°C; \textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3}) \delta (ppm) 1.30–1.48 (m, 13H), 1.53 (q, J = 7.0 Hz, 2H), 1.80–1.89 (m, 1H), 2.07 (q, J = 7.0 Hz, 2H), 2.63 (s, 1H), 3.21–3.38 (m, 4H, 4.14 (s, 1H), 4.65 (t, J = 7.0 Hz, 2H), 5.14 (s, 1H), 7.44–7.52 (m, 3H), and 8.12–8.16 (m, 2H); HRMS (APCI, direct probe) m/z [M+H\textsuperscript{+}] calcd.: 429.2609, found: 429.2625.
A solution of HCl (4 M) in cyclopentyl methyl ether (2 ml) and stirred at room temperature for 2 h. The solvent was then distilled off and the residue dispersed in ethyl acetate. The solid was filtered off and dried in vacuo to give 22 (44 mg, 52%) as a solid. C_{19}H_{20}ClN_{6}O (362.9); \[\text{HRMS (APCI, direct probe)} \delta (m/z) 364.2116, \text{calc.: } 364.2108.\]

\[
\delta (\text{ppm}) 24.01 \text{, } 26.11, 26.14, 26.81, 29.25, 43.23, 44.61, 51.60, 53.17, 126.89, 127.58, 129.04, 130.41, 165.10, \text{ and } 169.77; \text{HRMS (APCI, direct probe) } m/z [M+H]^+ \text{ calc.: } 328.1993, \text{ found: } 328.1955.
\]

tert-Butyl N-[(6-[5-phenyl-2H-tetrazol-2-yl]hexyl)]-1H-tetrazol-5-yl)methyl]carbamate (29)

A solution of 2-[6-bromohexyl]-5-phenyl-2H-tetrazole (20) (136 mg, 0.44 mmol) in acetonitrile (5 ml) was treated with tert-butyl N-[1H-tetrazol-2-ylmethyl]carbamate (79 mg, 0.40 mmol) and potassium carbonate (111 mg, 0.80 mmol) and heated under reflux for 7 h. After addition of ground KOH (22 mg, 0.39 mmol), heating was continued for 15 h. The reaction mixture was cooled to room temperature, filtered, and concentrated under reduced pressure. The residue was chromatographed on silica gel (gradient cyclohexane/ethyl acetate/triethylamine 7:3:0.1 to cyclohexane/ethyl acetate/triethylamine 1:9:0.1) to give 29 (126 mg, 76%) as an oil. C_{22}H_{32}N_{6}O (425.5); \[\text{HRMS (APCI, direct probe)} \delta (m/z) 426.2656, \text{calc.: } 426.2612.\]

\[
\delta (\text{ppm}) 24.98, 25.28, 28.49, 29.23, 31.35, 47.31, 52.66, 120.18, 122.90, 126.30, 126.96, 129.29, 130.54, 139.37, \text{ and } 164.04; \text{HRMS (APCI, direct probe) } m/z [M+H]^+ \text{ calc.: } 326.2088, \text{ found: } 326.2095.
\]

4.2 Biological assays

4.2.1 Inhibition of AOC3

Determination of AOC3 inhibition with or without preincubation of enzyme and test compound was performed as recently described.\[32,34\] 6-(5-Phenyl-2H-tetrazol-2-yl)hexan-1-amine (1) was used as substrate and commercial AOC3 from bovine plasma as enzyme. The aldehyde formed as the product was derivatized with Tris to an oxazolidine derivative, which was determined by HPLC and UV detection at 238 nm. Inhibition of AOC3 was calculated from the
amount of oxazolidine produced in the presence and the absence of a test compound (corrected for blank). IC_{SO} values were determined by probit trans formation.\textsuperscript{[36]}

4.2.2 | Inhibition of AOC1

Inhibition of AOC1 was studied as recently published.\textsuperscript{[34]} Briefly, the commercial enzyme isolated from the porcine kidney was preincubated with the inhibitor for 15 min in potassium phosphate buffer. Subsequently, the enzymatic reaction was started by the addition of the substrate 6-(5-phenyltetrazol-2-yl)hexan-1-amine (I). Determination of the enzymatically formed aldehyde and calculation of the inhibition data was performed in the same manner as for the AOC3 assay described above.

4.2.3 | Inhibition of MAO-A

Inhibition of MAO-A was evaluated as recently described.\textsuperscript{[34]} Briefly, the commercial human recombinant enzyme was preincubated with the inhibitor for 15 min in phosphate-buffered saline containing 0.2% of the detergent Brij35. Subsequently, the enzymatic reaction was started by the addition of the substrate 4-(5-phenyl-2H-tetrazol-2-yl)butan-1-amine. Determination of the enzymatically formed aldehyde and calculation of the inhibition data was performed in the same manner as for the AOC3 assay described above.

4.2.4 | Inhibition of MAO-B

Inhibition of MAO-B was determined as recently described.\textsuperscript{[34]} Briefly, the commercial human recombinant enzyme was preincubated with the inhibitor for 15 min in phosphate-buffered saline (PBS) containing 0.2% of the detergent Triton X-100. Subsequently, the enzymatic reaction was started by the addition of the substrate 4-(5-phenyl-2H-tetrazol-2-yl)butan-1-amine. Determination of the enzymatically formed aldehyde and calculation of the inhibition data was performed in the same manner as for the AOC3 assay described above.

4.2.5 | Degradation of test substances by AOC3

Preparation of the enzyme solution: 150 I.U. (5.7 mg) of AOC3 from bovine plasma (Worthington, delivered by CellSystems) was dissolved in PBS (1000 µl) (prepared from PBS tablets; Sigma-Aldrich; one tablet dissolved in 200 ml of deionized water yields 0.01 M phosphate buffer, 0.0027 M potassium chloride, and 0.137 M sodium chloride, pH 7.4, at 25°C). The obtained solution was diluted with PBS by 1:25 (v/v). Incubation procedure: To a solution of the test compound (0.40 mM) in DMSO (2.5 µl) and DMSO (2.5 µl) was added the diluted solution of bovine AOC3 in phosphate-buffered saline (95 µl). After incubation at 37°C for 15 min, the enzyme activity was terminated by the addition of acetonitrile (100 µl). The samples were cooled in an ice bath for 10 min and centrifuged at 12,000g and 10°C for 5 min. In parallel, controls were prepared by treating mixtures of the test compound solution (0.40 mM) in DMSO (2.5 µl), DMSO (2.5 µl), and PBS (95 µl) in the same way. The extent of degradation was evaluated by RP-HPLC with MS detection. The HPLC/MS system from Shimadzu consisted of two LC-20ADXR HPLC pumps, a SIL-30AC autosampler, and an LCMS-2020 single quad detector. Aliquots of 2 µl were injected onto a HICHROM ACE 3 C\textsubscript{18} column (2.1 mm inside diameter × 100 mm, particle size 3 µm) (Hichrom) protected with a Phenomenex C18 guard column (3 mm inside diameter × 4 mm). Autosampler temperature was 10°C and column oven temperature was set to 20°C. The mobile phase consisted of acetonitrile/water/formic acid 10:90:0.1 (v/v/v) (A) and acetonitrile/water/formic acid 90:10:0.1 (v/v/v) (B). The gradient run from 10% to 95% of solvent B. The flow rate was 0.3 ml/min. Detection was performed in ES+ scan mode. The stability of a test compound was calculated by comparing its peak area in the chromatogram obtained in presence of AOC3 with its peak area in the chromatogram of the control.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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REFERENCES
