5-Selenization of salicylic acid derivatives yielded isoform-specific 5-lipoxygenase inhibitors†

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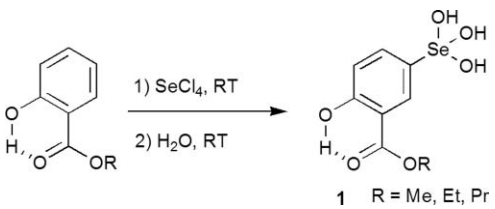
Low molecular weight seleno-organic compounds exhibit glutathione peroxidase (GPx)-like activity; the well-known compound ebselen is being used in clinical trials as a stroke medication. Here, we describe the facile one-step synthesis of novel 5-selenized salicylic acid derivatives using selenium tetrachloride. The products were analyzed by spectroscopic studies including ^{77}Se-NMR and some were subjected to X-ray structure determination. Several products were identified as selective inhibitors of the pro-inflammatory 5-lipoxygenase (LOX) but had little effect on the catalytic activity of 12/15-LOX, which has been implicated in the synthesis of anti-inflammatory mediators. Such isoform-specificity (specificity coefficient >120) has not been reported before for any seleno-organic compound. In addition, synthesis products exhibited GPx-like activity, which was higher than that of ebselen for some derivatives.

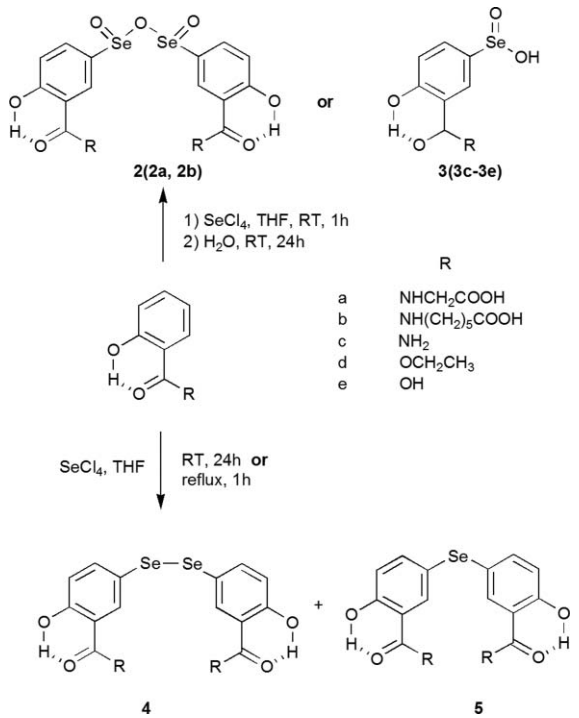
Introduction

GPx is an important constituent of the antioxidative defence system[1] and most mammalian GPx-isoforms bear a selenocysteine at the active site.[2] Many selenium-containing low molecular weight compounds also exhibit GPx activity in the presence of electron donors, and the chemistry of the catalytic reaction has been explored for ebselen[3] and other seleno-organic GPx mimetics.[4,5]

There are several ways to incorporate selenium into bio-organic molecules; selenization of aromatic rings[6,7] is a simple but effective principle. In 1938, 5-selenization of some salicylic acid esters using selenium tetrachloride ($SeCl_4$) was attempted,[8] whereby selenium trihydroxides were initially suggested to be major products (Scheme 1, **1**) that are nowadays regarded as seleninic acids (see Scheme 2).



Scheme 1 First 5-selenization of salicylic acid derivatives by $SeCl_4$.



Scheme 2 Synthetic route for 5-selenization of salicylic acid derivatives.

We have recently extended the synthetic scheme to a salicyloylglycine derivative (Scheme 2, **2a**).[5] This 5-selenization product exhibited a 4-fold higher GPx activity than ebselen,[9] the "gold standard" of seleno-organic compounds. Moreover, it inhibited plant and mammalian 12/15-LOXs at low concentration.[5]

LOXs form a heterogeneous family of lipid-peroxidizing enzymes, which have also been implicated in inflammatory[10] and hyperproliferative[11] diseases. In humans, there are 6 different LOX isoforms originating from separate genes.[12] The isoform 5-LOX is involved in the biosynthesis of leukotrienes, which

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constitute powerful pro-inflammatory mediators,[13] and the 5-LOX inhibitor zileuton has been approved for clinical use.[14] In contrast, anti-inflammatory activities[15] have been reported for 12/15-LOX and epidermal-type LOX has been implicated in skin development.[16] Because of this functional multiplicity, the search for isoform-specific LOX inhibitors is a challenging task in current pharmacological research. Owing to their redox characteristics, several seleno-organic compounds have been identified as LOX inhibitors but isoform-specificity for human isozymes has been not reported.

In this context, we synthesized novel 5-selenized salicylic acid derivatives and tested their GPx-activity as well as their isoform-dependent inhibition of two major LOX isoforms (5-LOX and 12/15-LOX).

Results and discussion

Synthesis

5-Selenization was initiated by reacting salicylic acid derivatives with $SeCl_4$ in tetrahydrofuran (THF), which is known to be a good solvent for $SeCl_4$ (Scheme 2).

Under mild reaction conditions (RT, 1 h) yellow 5-selenium trichlorides were formed,[8] which were further converted in water to white 5-seleninic acids (**3**) or to the corresponding anhydrides (**2**). Here it should be pointed out that we could not observe any formation of 5-selenium trihydroxides (**1**), which had previously been analyzed without using the benefits of modern techniques such as NMR, HR-MS or X-ray crystallography.[8] The crystal data on ethyl ester (**3d**) reveals that a seleninic acid is produced in the reaction. It is interesting, moreover, that salicylic acid amides bearing a free terminal carboxylic group were converted to 5-seleninic acid anhydrides (Scheme 2, **2a** and **2b**), whereas those lacking this structural element yielded 5-seleninic acids (Scheme 2, **3c–e**). This phenomenon will be discussed in the next subsection.

When the reaction mixture (Scheme 2) was refluxed for 1 h or stirred for one day at RT, the 5-selenium trichloride decomposed to form a mixture of yellow diselenide (**4**) and white monoselenide (**5**), which were separated by flash chromatography on silica gel.

X-Ray crystallography[17]

All molecules display an intramolecular hydrogen bond from the OH group to the neighbouring carbonyl function (Fig. 1, 3 and 5; ellipsoids represent 50% probability levels). The packing involves a mixture of classical hydrogen bonds and Se···LO contacts (Fig. 2, 4 and 6); for the latter we have set an arbitrary limit of 3.2 Å, above which the contacts are not considered (the next longest are *ca.* 3.4 Å).

Compound **3c** has a complex three-dimensional packing involving four independent contacts: three hydrogen bonds O2–H02···O1^{i} 2.636(2) Å, N1–H1A···O3^{ii} 2.892(2) Å, N1–H1B···O1^{iii} 2.932(2) Å, and one Se···O3(iv) contact of 3.166(2) Å (symmetry operations are i = ($\frac{1}{2}$ + x, $\frac{3}{2}$ − y, 2 − z), ii = (−x, $\frac{1}{2}$ + y, $\frac{3}{2}$ − z), iii = (−x, $-\frac{1}{2}$ + y, $\frac{3}{2}$ − z), iv = (1 − x, $\frac{1}{2}$ + y, $\frac{3}{2}$ − z)). Fig. 2 shows a two-dimensional ribbon substructure involving the NH_2 groups. The third H bond connects this ribbon to a perpendicularly oriented neighbour, whereas the Se···O3 contact links to a parallel layer.

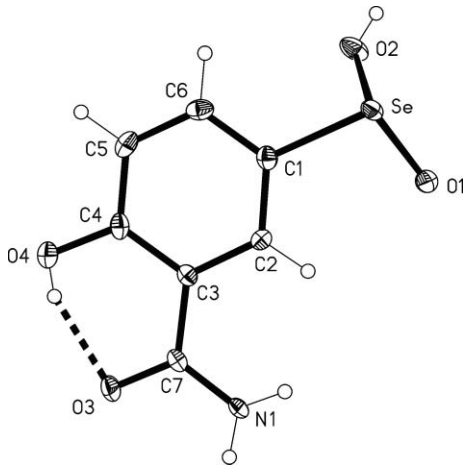


Fig. 1 Structure of compound **3c** in the crystal. Bond lengths (Å) and angles (°) at selenium: Se–O1 1.659(1), Se–O2 1.756(1), Se–C1 1.921(2); O1–Se–O2 104.6(1), O1–Se–C1 101.1(1), O2–Se–C1 93.3(1).

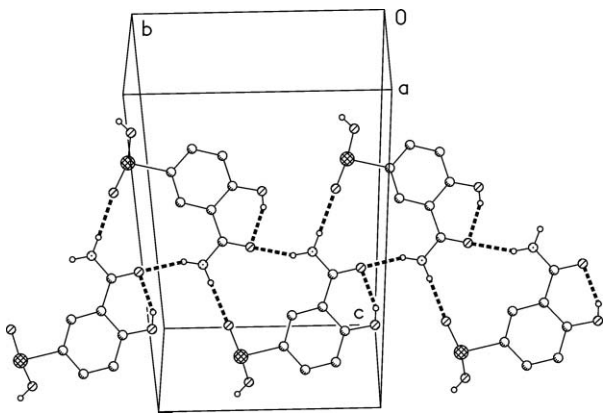


Fig. 2 Packing diagram of compound **3c**.

For compound **3d**, the hydrogen bonds O2–H02···O1^{i} 2.547(2) Å and O5–H05···O4 2.655(2) Å combine with Se···O1^{ii} 3.048(1) Å contact to produce a layer structure parallel to the *xy* plane (Fig. 4), (symmetric operations *i* = (−x, $-\frac{1}{2}$ + y, $\frac{1}{2}$ − z), ii = (−x, $-\frac{1}{2}$ + y, $\frac{1}{2}$ − z)); the aromatic rings lie approximately perpendicular to this plane.

Crystallization of compound **3e** from anhydrous methanol led to single crystals of its seleninic methyl ester derivative **6**. For compound **6**, one hydrogen bond O3–H03···O1^{i} 2.575(2) Å combines with Se···O1^{ii} 2.946(2) Å and Se···O4^{iii} 3.182(1) Å contacts to form a layer structure parallel to the *yz* plane (Fig. 6) (symmetry operations i = (1 − x, $\frac{1}{2}$ + y, $\frac{3}{2}$ − z), ii = (1 − x, 1 − y, 2 − z), iii = (1 − x, $-\frac{1}{2}$ + y, $\frac{3}{2}$ − z)).

The crystallographic data are shown in Table 1.

The methyl esterification mentioned above was also observed in the HR-MS spectrum, in which MeOH was used as a solvent. As seen in Fig. 7, the methyl ester was the main peak (264.96031). NMR and elemental analysis of **3e** proves the possibility of its rapid esterification in MeOH.

This phenomenon indirectly indicates that seleninic acid derivatives with a carboxyl group might have a potential tendency toward esterification and, therefore, salicylic acid amides carrying a free

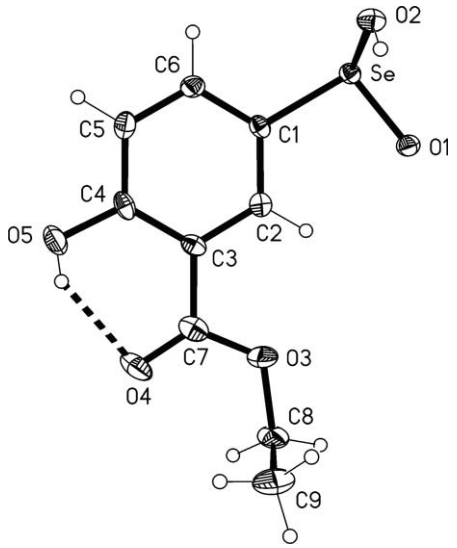


Fig. 3 Structure of compound **3d** in the crystal. Bond lengths (Å) and angles (°) at selenium: Se–O1 1.666(1), Se–O2 1.744(1), Se–C1 1.934(2); O1–Se–O2 105.8(1), O1–Se–C1 98.5(1), O2–Se–C1 99.0(1).

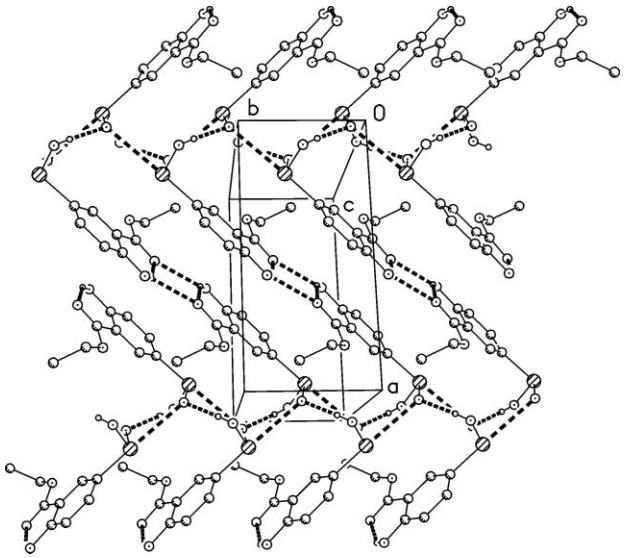


Fig. 4 Packing diagram of compound **3d**.

terminal carboxylic group such as **2a** and **2b** could be produced through self-dehydration (so-called self-esterification).

GPx-like activity

The GPx-like activity of the synthesized compounds was assayed in coupled reductase model[5] with the use of glutathione and *t*-BuOOH as substrates. As expected from the reaction mechanism catalyzed by compound **2a**,[5] 5-seleninic acid and diselenide derivatives (**3c–e**, **4e**) also exhibited a high GPx-like activity (Table 2).

From the comparison of the 5-seleninic acid (**3e**) with the diselenide type (**4e**) it can be seen that if normalized to selenium content, the former is more effective than the latter in GPx-like activity (ratio 2.8 : 2.1), but that these two moieties are certain to be major active components[5] in the catalytic cycle of 5-selenized salicylic acid derivatives. The inactivity of monoselenide

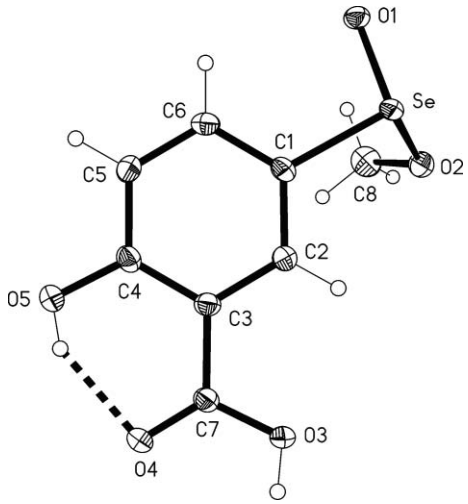


Fig. 5 Structure of compound **6** in the crystal. Bond lengths (Å) and angles (°) at selenium: Se–O1 1.666(1), Se–O2 1.774(2), Se–C1 1.927(2); O1–Se–O2 104.8(1), O1–Se–C1 100.6(1), O2–Se–C1 97.0(1).

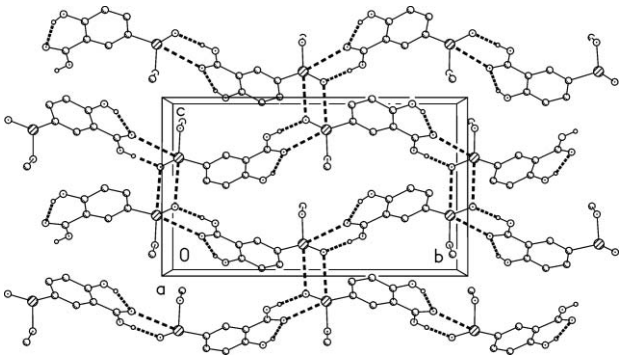


Fig. 6 Packing diagram of compound **6**.

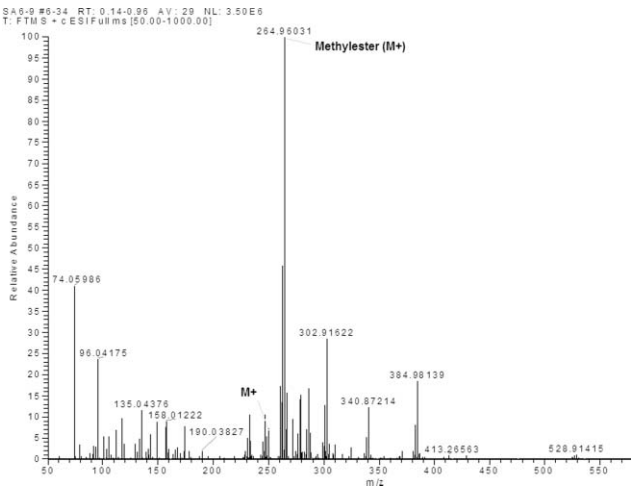


Fig. 7 HR-MS spectrum of **3e** in MeOH.

5e was predicted from previous work.[5] The lack of the activity for compound **2b** suggests that a long aliphatic chain may inhibit the catalytic function, which could bring about a new concept in understanding the mechanism of selenium-containing GPx mimics.

Table 1 Crystallographic data for compounds **3c**, **3d** and **6**

Compound	**3c**	**3d**	**6**
Formula	$C_7H_7NO_4Se$	$C_9H_{10}O_5Se$	$C_8H_8O_5Se$
M_r	248.10	277.13	263.11
Habit	Colourless needle	Colourless needle	Colourless prism
Cryst. size/mm	0.16 × 0.12 × 0.10	0.2 × 0.1 × 0.1	0.12 × 0.11 × 0.09
Crystal system	Orthorhombic	Monoclinic	Monoclinic
Space group	$P2_12_12_1$	$P2_1/c$	$P2_1/c$
Cell constants:			
a/Å	4.9234(2)	11.9653(4)	5.1610(2)
b/Å	9.5712(2)	5.1029(2)	17.4964(8)
c/Å	17.3190(6)	17.4167(8)	10.2112(6)
α (°)	90	90	90
β (°)	90	110.947(4)	90.578(4)
γ (°)	90	90	90
V/Å^3	816.12	993.14	922.01
Z	4	4	4
D_x (Mg m^{-3})	2.019	1.853	1.895
μ/mm^{-1}	4.6	3.8	5.5
F(000)	488	552	520
T/°C	−173	−173	−173
Radiation, λ/Å	Mo-Kα, 0.71073	Mo-Kα, 0.71073	Cu-Kα, 1.54184
$2\theta_{max}$	60	58	151
Refl. measured	18 703	22 715	16 694
Refl. indep.	2338	2658	1898
R_{int}	0.038	0.037	0.035
Parameters	134	154	136
Restraints	2	16	0
w$R(F^2$, all refl.)	0.035	0.046	0.062
$R(F, >4\sigma(F))$	0.020	0.021	0.025
S	0.93	0.93	1.12
max. $\Delta\rho$/e Å^{-3}	0.56	0.54	0.38

Table 2 The rate of NADPH oxidation (υ_0) in presence of synthesized compounds (10 μM) and their GPx-like activity relative to ebselen ($n = 3$)

Compound	υ_0/μM min^{-1}	Relative activity
Ebselen	5.85 ± 0.76	1
2b	Not detectable	0
3c	12.05 ± 1.82	2.1
3d	5.21 ± 0.28	0.9
3e	16.40 ± 1.02	2.8
4e	24.63 ± 2.65	4.2
5e	Not detectable	0
Salicylic acid	Not detectable	0

The high GPx-like activity of synthesized 5-seleninic acids is consistent with the more recent suggestion[3] that the seleninic acid moiety is also one of the intermediates in the GPx-like activity cycle of ebselen.

LOX inhibition

Rabbit reticulocyte 12/15-LOX inhibition. As shown in Table 3, 5-seleninic acid derivatives (**2b**, **3c–e**) and monoselenide **5e** did not inhibit 12/15-LOX effectively. In contrast, the diselenide **4e** inhibited the enzyme with an IC_{50} of about 5 μM (Table 3).

Recombinant human 5-LOX inhibition

Almost all of the 5-selenized compounds inhibited recombinant human 5-LOX at lower micromolar concentrations (Table 4).

Among them, compound **3d**, with an IC_{50} of 0.66 μM, was most effective and over 200 times more specific to 5-LOX than to 12/15-LOX. Because the specificity ratios (IC_{50}(12/15-LOX)/IC_{50}(5-LOX)) of **2b**, **3c**, **3e** and **5e** (Table 4) are based on 100 μM maximal test concentration for 12/15-LOX, the real values could be even higher than that of **3d** (Fig. 8). The non-specific property of **4e** might provide a chance to develop stronger general LOX inhibitors related to diselenides.

Zileuton (not a seleno-organic compound), the only 5-LOX inhibitor currently used in clinics, is less powerful (IC_{50} of 3.8 μM)

Table 3 12/15-LOX inhibition of synthesized compounds ($n = 3$)

Compound	Inhibition at 100 μM (%)	IC_{50}/μM
2b	6.07 ± 1.85	—
3c	16.96 ± 3.26	—
3d	45.29 ± 2.44	146
3e	1.19 ± 2.69	—
4e	96.58 ± 0.77	5.36
5e	7.31 ± 1.62	—

Table 4 5-LOX inhibition of synthesized compounds ($n = 3$) and their relative specificity

Compound	IC_{50}/μM	Ratio (12/15-LOX/5-LOX)
Ebselen	1.09	0.51
2a	1.01	7.82
2b	0.79	>127
3c	0.80	>125
3d	0.66	221
3e	2.37	>42
4e	2.09	2.56
5e	4.94	>20

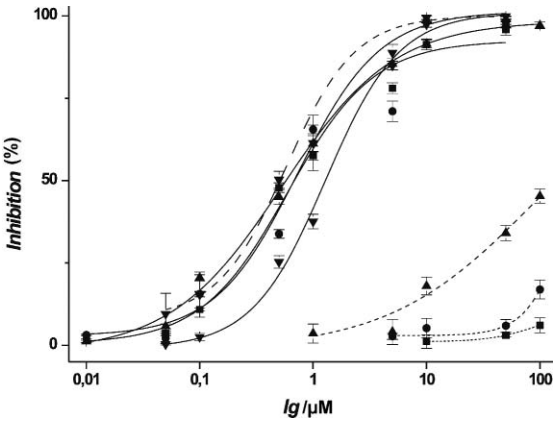
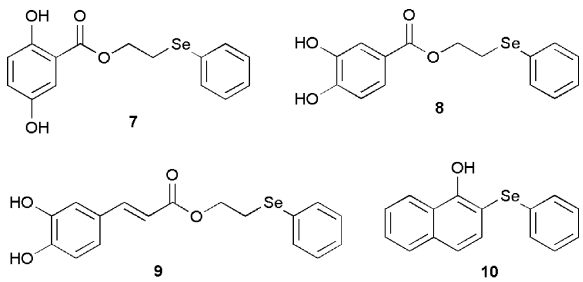


Fig. 8 Comparison of **2b** (■), **3c** (●) and **3d** (▲) with ebselen (▼) in the selectivity between 12/15 LOX (---) and 5- LOX (—) inhibition.

than compound **3d** in the model but exhibits a similar degree of isoform-specificity.[18] Although selenium-containing 5-LOX inhibitors (**7–10**) with similar or even lower IC_{50} have been reported before in different models (Fig. 9),[19,20] their isoform-specificity has not been tested yet.



No	IC_{50} (µM)
7	0.18
8	0.21
9	0.24
10	0.079

Fig. 9 Some known selenium-containing 5-LOX inhibitors.

Salicylic acid has recently been tested for the inhibition of several LOXs, where its IC_{50} for 12/15 and 5-LOXs was 49 µM and 168 µM, respectively,[21] and the specificity coefficient was, therefore, very poor (0.29).

These results indicate that 5-selenization might become a significant method for enhancing the specific inhibition of 5-LOX by salicylic acid derivatives.

The mechanism of 5-LOX inhibition of the seleno-organic compounds has not been explored in detail. However, previous investigations into the mode of action of ebselen[22] suggested that seleno-organic compounds may counteract LOX activation. In their silent form, LOXs contain ferrous non-heme iron, which is oxidized by hydroperoxide activators to its ferric form. We found that the seleno-organic compounds synthesized in this study are capable of reducing ferric iron (Fig. 10) and thus, there is the possibility that those compounds reduce the active ferric LOX to its catalytically silent ferrous form.

A necessary precondition for this mechanism is that the inhibitor is capable of penetrating the active site of the enzymes. The crystal structures of various LOX isoforms[23] suggested that the active site of LOX constitutes a deep hydrophobic U-shaped

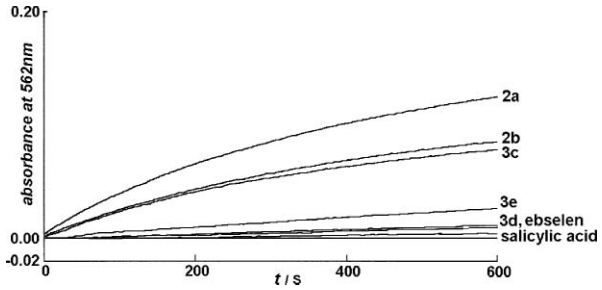


Fig. 10 Iron-reducing activity of synthesized compounds.

pocket, which buries the catalytic iron. If an inhibitor is sufficiently small and hydrophobic it can penetrate the active site to interact with the iron. Unfortunately, the crystal structure of human 5-LOX has not been solved so far. However, structural modeling on the basis of the X-ray coordinates of rabbit 12/15-LOX suggested that the active site of human 5-LOX is larger than that of 12/15-LOX and mutagenesis studies on human 5-LOX support this hypothesis,[24] but non-relationship between LOX inhibitory activity and iron-reducing activity of the tested compounds would suggest that these structural differences in active sites of the enzymes might not explain the isoform-specific inhibitory effect of them.

Instead, it could be expected that 5-seleninic acid derivatives may contribute to interaction with the active site of 5-LOX through its property of esterification or dehydration mentioned above, as aspirin (acetyl salicylic acid) irreversibly inhibits cyclooxygenase by acetylation of the active site residue.

Conclusions

Most of the novel 5-selenized salicylic acid derivatives synthesized in this study exhibit a higher GPx-like activity than ebselen. In addition, all compounds inhibit the recombinant human 5-LOX at lower micromolar concentrations and the seleninic acid derivatives (**3c–e**) show a remarkable LOX isoform-specificity. More work is needed to explore the underlying mechanistic details.

Experimental

Materials and methods

Commercial reagents salicyloylglycine, ε-aminocaproic acid, ethyl salicylate, salicylic acid and selenium tetrachloride were purchased from Merck and Sigma-Aldrich. All solvents were of extra pure grade from Roth (Germany). IR spectra were recorded on a Nicolet Avatar 360 FT-IR. Kinetic measurements were performed on a Shimadzu UV-2102 spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded on a Brucker Biospin AV 400 (^{1}H: 400 MHz; ^{13}C: 100.5 MHz) by using d_6-DMSO as a solvent. Chemical shift values (δ) are reported in ppm downfield from TMS ($\delta = 0.0$ ppm) as internal standard. ^{77}Se NMR spectra were recorded by using a Brucker Avance II 300 instrument (57.20 MHz) in d_6-DMSO (dimethylselenide as external standard). HR-MS recording was carried out on a Finnigan Thermo Scientific LTQ OrbitrapXL (ESI) in MeOH. Elemental analysis was carried out on HEKAtech EURO EA, vario EL.

General procedure for 5-selenization of salicylic acid derivatives by selenium tetrachloride

Selenium tetrachloride 1.107 g (5 mmol) was added to a solution of salicylic acid derivatives (5 mmol) in 10 ml of THF and the mixture was stirred for 1 h at room temperature. Then, 100 ml of water was added to the reaction mixture and the sample was stirred at room temperature for an additional 24 h. The white precipitate was filtered, washed with 3 × 30 ml of THF and dried in air at 30 °C. The product was purified by flash chromatography (*i*PrOH–NH_4OH–H_2O; 8 : 1 : 1). If the reaction runs under reflux for 1 h and follows the same procedure as above, one can obtain a mixture of monoselenide and diselenide as the main products, which are isolated by the same condition of flash chromatography. 6-[(2-Hydroxybenzoyl)amino]hexanoic acid as a starting material for synthesis of **2b** was synthesized by reaction of ethyl salicylate with ε-aminocaproic acid in 25% sodium methylate solution MeOH and acidification with dilute sulfuric acid. 2-[[5-[3-(carboxymethylcarbamoyl)-4-hydroxy-phenyl]seleninyloxyseleninyl-2-hydroxy-benzoyl]amino]acetic acid (**2a**) was from the previous work.

6-[(2-Hydroxybenzoyl)amino]hexanoic acid. Yield: 76%, mp: 110–112 °C (lit.,[25] 107–108 °C), Found: C, 62.21; H, 6.51; N, 5.23. Calc. for $C_{13}H_{17}NO_4$: C, 62.14; H, 6.82; N, 5.57%, δ_H 1.31 (2 H, m, CH_2), 1.53 (4 H, m, CH_2), 2.21 (2 H, t, CH_2), 3.28 (2 H, m, CH_2), 6.90 (2 H, m, Ar–H), 7.39 (H, t, Ar–H), 7.85 (H, dd, Ar–H), 8.81 (H, t, NH), 12.00 (H, s, COOH), 12.72 (H, s, OH), δ_C 24.21 (CH_2), 25.98 (CH_2), 28.58 (CH_2), 33.57 (CH_2), 38.77 (CH_2), 115.04 (C-1), 117.40 (C-3), 118.46 (C-6), 127.48 (C-4), 133.62 (C-5), 160.26 (C-2), 169.00 (C=O), 174.45 (COOH), *m/z* (ESI) 252.1232 (M^+. $C_{13}H_{17}NO_4$ requires 252.1230).

6-[[2-Hydroxy-5-[4-hydroxy-3-[(6-hydroxy-6-oxo-hexyl)carbamoyl]phenyl]seleninyloxyseleninyl-benzoyl]amino]hexanoic acid (2b). Yield: 63%, mp: 126–128 °C, Found: C, 44.24; H, 4.85; N, 3.88. Calc. for $C_{26}H_{32}N2O_{11}Se_2$: C, 44.20; H, 4.57; N, 3.97%, δ_H 1.31 (2 H, m, CH_2), 1.53 (4 H, m, CH_2), 2.21 (2 H, t, CH_2), 3.29 (2 H, m, CH_2), 7.10 (H, d, Ar–H), 7.82 (H, dd, Ar–H), 8.30 (H, d, Ar–H), 9.04 (H, t, NH), 13.16 (H, s, COOH), δ_C 24.20 (CH_2), 25.98 (CH_2), 28.51 (CH_2), 33.57 (CH_2), 38.96 (CH_2), 115.74 (C-1), 118.04 (C-3), 126.31 (C-6), 130.87 (C-4), 138.59 (C-5), 162.52 (C-2), 167.83 (C=O), 174.45 (COOH), δ_{Se} 1177, *m/z* (ESI) 709.0409 (M^+. $C_{26}H_{32}N_2O_{11}{}^{80}Se_2$ requires 709.0406).

3-Carbamoyl-4-hydroxy-benzeneseleninic acid (3c). Yield: 63%, mp: 154–156 °C, Found: C, 34.16; H, 2.86; N, 5.65. Calc. for $C_7H_7NO_4Se$: C, 33.89; H, 2.84; N, 5.65%, δ_H 7.11 (H, d, Ar–H), 7.83 (H, dd, Ar–H), 8.06 (H, s, Ar–H), 8.32 (H, d, NH), 8.60 (H, s, NH), 13.40 (H, s, OH), δ_C 115.01 (C-1), 118.12 (C-3), 126.99 (C-6), 131.17 (C-4), 138.45 (C-5), 163.38 (C-2), 170.81 (C=O), δ_{Se} 1177, *m/z* (ESI) 247.9474 (M^-. $C_7H_7NO_4{}^{80}Se$ requires 247.9468).

3-Ethoxycarbonyl-4-hydroxy-benzeneseleninic acid (3d). Yield: 17%, mp: 122–123 °C, Found: C, 39.07; H, 3.45. Calc. for $C_9H_{10}O_5Se$: C, 39.01; H, 3.64%, δ_H 1.36 (3 H, t, CH_3), 4.30 (2 H, dd, CH_2), 7.16 (H, d, Ar–H), 7.93 (H, dd, Ar–H), 8.23 (H, d, Ar–H), δ_C 14.01 (CH_3), 61.66 (CH_2), 113.75 (C-1), 118.08 (C-3), 128.12 (C-6), 132.89 (C-4), 139.37 (C-5), 162.24 (C-2), 167.88 (C=O), δ_{Se} 1176, *m/z* (ESI) 276.96209 (M^-. $C_9H_{10}O_5{}^{80}Se$ requires 276.96207).

2-Hydroxy-5-selenino-benzoic acid (3e). Yield: 15%, mp: 140–142 °C (decomp.), Found: C, 33.46; H, 2.72. Calc. for $C_7H_6O_5Se$: C, 33.75; H, 2.43%, δ_H 7.12 (H, d, Ar–H), 7.90 (H, dd, Ar–H), 8.23 (H, d, Ar–H), δ_C 113.48 (C-1), 117.84 (C-3), 128.44 (C-6), 132.94 (C-4), 139.06 (C-5), 163.40 (C-2), 171.12 (C=O), δ_{Se} 1175, *m/z* (ESI) 248.93021 (M^-. $C_7H_6O_5{}^{80}Se$ requires 248.93077).

5-(3-Carboxy-4-hydroxy-phenyl)diselanyl-2-hydroxy-benzoic acid (4e). Yield: 13%, mp: 275 °C (decomp.) (lit.,[26] 231–235 °C), Found: C, 38.86; H, 2.23. Calc. for $C_{14}H_{10}O_6Se_2$: C, 38.91; H, 2.33%, δ_H 6.81 (H, d, Ar–H), 7.54 (H, dd, Ar–H), 7.92 (H, d, Ar–H), δ_C 116.37 (C-1), 117.74 (C-3), 118.14 (C-5), 135.54 (C-6), 139.40 (C-4), 162.42 (C-2), 170.84 (C=O), δ_{Se} 502, *m/z* (ESI) 432.87284 (M^-. $C_{14}H_{10}O_6{}^{80}Se_2$ requires 432.87350).

5-(3-Carboxy-4-hydroxy-phenyl)selanyl-2-hydroxy-benzoic acid (5e). Yield: 7%, mp: 262 °C (decomp.) (lit.,[27] 272 °C), Found: C, 47.48; H, 2.82. Calc. for $C_{14}H_{10}O_6Se$: C, 47.61; H, 2.85%, δ_H 6.93 (H, d, Ar–H), 7.58 (H, dd, Ar–H), 7.85 (H, d, Ar–H), δ_C 114.35 (C-1), 118.77 (C-3), 119.25 (C-5), 134.77 (C-6), 140.26 (C-4), 160.75 (C-2), 170.90 (C=O), δ_{Se} 397, *m/z* (ESI) 352.95614 (M^-. $C_{14}H_{10}O_6{}^{80}Se$ requires 352.95698).

X-Ray structure determinations

Data collection and reduction. Crystals were mounted in inert oil on glass fibres and transferred to the cold gas stream of the diffractometer (**3c**, **3d**: Oxford Diffraction Xcalibur E with monochromated Mo-Kα radiation; **6**: Oxford Diffraction Nova A with mirror-focussed Cu-Kα radiation).

Structure refinement. The structures were refined anisotropically against F^2.[28] Hydrogen atoms of hydroxy and NH_2 groups were refined freely but with X–H distance restraints; other H atoms were included with a riding model or rigid methyl groups. For compound **3c**, the Flack parameter was refined to 0.002(8). For compound **3d**, the methoxy group was disordered over two positions (minor site occupation 19%), which were subjected to similarity restraints.

GPx-like acitivity[5]

The catalytic reaction was run at 37 °C in a 1 ml reaction mixture consisting of 100 mM Tris-HCl buffer (pH 7.4) containing 5 mM EDTA and 0.1% Triton X-100, 3 mM GSH, 0.2 mM NADPH, 1 U of glutathione reductase and 10 μM of the test compound (DMSO solution). The assay sample was equilibrated for 10 min in the absence of peroxide substrate and GPx-like reaction was initiated by addition of 0.5 mM *tert*-butyl hydroperoxide (*t*BuOOH). The time-dependent decrease in absorbance at 340 nm (10–40 s after addition of *t*BuOOH) was recorded. During the GPx-like reaction, reduced GSH is oxidized and the resulting disulfide (GSSG) is back-reduced by the glutathione reductase reaction consuming stoichiometric amounts of NADPH. Since NADPH exhibits a local absorbance maximum (A_{cat}) at 340 nm but its oxidized counterpart (NADP) does not, NADPH oxidation can be quantified by measuring the decrease in absorbance at this wavelength. A blank assay (solvent control, A_{blank}) was run in the absence of catalysts. The rate of NADPH oxidation (υ_0) was calculated using a molar absorbance coefficient for NADPH of 6.22×10^3 M^{-1} cm^{-1}.

Activity of rabbit reticulocyte 12/15-LOX[5]

The enzyme (2 µl of a 1.7 mg ml^{-1} enzyme solution) was pre-incubated with the inhibitors (DMSO solution) at 22 °C in 0.1 M phosphate buffer (pH 7.4) for 5 min and the reaction was started by the addition of linoleic acid as substrate (20 µl of a methanolic stock solution, 100 µM final concentration, assay volume 1 ml). The catalytic activity of the enzyme was assayed spectrophotometrically following the increase in absorbance at 235 nm over a time interval of 30 s.

Activity of recombinant human 5-LOX[29]

The enzyme preparation (lysate) solution (10 µl) was incubated with the inhibitors (DMSO solution) for 10 min at room temperature in 0.1 M phosphate buffer (0.5 ml final volume, pH 7.4), containing 0.2 mM arachidonic acid, 0.4 mM $CaCl_2$, 40µg mL^{-1} dipalmitoyl phosphatidylcholine, 0.1 mM ATP and 0.1 mM EDTA. The oxygenated product (5-hydroperoxyeicosateraenoic acid) was then reduced with $NaBH_4$ (50 mg ml^{-1}, 10 µl) to 5-hydroxyeicosatetraenoic acid, the mixture was acidified with acetic acid (50 µl), 0.5 ml of ice-cold methanol was added and kept in ice for 10 min. 400 µl aliquot of the clear supernatant obtained by centrifuging (10000 rpm, 10 min) was injected to RP-HPLC (Shimadzu LC10 HPLC system connected to a SPD-M19AVP diode array detector, CC 250/4 Nucleosil 120-5 C18 column). A solvent system of MeOH–H_2O–AcOH (80 : 20 : 0.1) was used at a flow rate of 1 ml min^{-1}. The peak area in the time zone between 19–20 min corresponding to a 5-HETE fraction at 235 nm was compared with that of the control without inhibitor to calculate a relative inhibitory activity.

Iron-reducing activity[30]

The reduction of Fe(III) by test compounds was monitored using ferrozine, a reagent that avidly binds Fe(II), forming a complex with a very high extinction coefficient at 562 nm. The reaction mixture comprised 50 mM γ-morpholinopropanesulfonic acid (pH 2.0) buffer, 1 mM ferrozine (buffer solution), 50 µM test compound (DMSO solution) and 100 µM $FeCl_3$ (DMSO solution) in a final volume of 1 ml. The reaction was started by addition of $FeCl_3$ and increase of absorbance at 562 nm was monitored for 10 min. As control a mixture with DMSO instead of a test compound was used.

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