

## Synthesis of intramolecularly coordinated cyclic selenenate/thioselenenate esters and their glutathione peroxidase-like activity

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The syntheses and characterization of selenenate esters, thioselenenate ester and related derivatives are described. The reactions of *n*-butyl-4-*tert*-2,6-di(formyl)phenyl selenide (**23**) or bis(2,6-di-formyl-4-*tert*-butylphenyl)diselenide (**24**) with bromine affords a new selenenate ester, 5-*tert*-butyl-7-(formyl)benzoxaselenol-3-one (**25**), stabilized by *ortho*-formyl group along with 3,3'-oxybis(5-*tert*-butyl-3H-benzo[*c*][1,2]oxoselenole-7-carbaldehyde) (**16**). Oxidation of (**25**) with H<sub>2</sub>O<sub>2</sub> gives 5-*tert*-butyl-7-(carboxylic)benzoxaselenol-3-one-Se-oxide (**26**). However, the direct oxidation of (**23**) with H<sub>2</sub>O<sub>2</sub> affords 5-*tert*-butyl-7-(butylcarboxylate)benzoxaselenol-3-one (**27**). The synthesis of 5-*tert*-butyl-7-(methylcarboxylate) benzothiaselenol-3-one (**28**) has been accomplished by the reaction of (**26**) with thionyl chloride (SOCl<sub>2</sub>) with a catalytic amount of N, N-dimethylformamide. Attempted thionation of 7-nitro-1,2-benzisoselenole(3*H*)-Se-oxide (**20**) leads to the isolation of bis(2-chloromethyl-6-nitrophenyl)diselenide (**29**). The presence of intramolecular secondary Se...O interactions in all the esters is confirmed by single crystal X-ray diffraction studies and computational studies. Glutathione peroxidase-like activity of (**16**), (**25**)-( **29**) has been determined by the coupled reductase assay.

**Keywords:** Coordination chemistry, Cyclic compounds, Selenenate esters, Thioselenenate esters, Thionation, GPx-like activity, Intramolecular interactions, Secondary interactions

Selenoenzyme, glutathione peroxidase (GPx) catalyzes the reduction of harmful peroxides and radicals and thereby, protects the cells from oxidative damage or stress at the expense of reduced glutathione (GSH)<sup>1,2</sup>. Several organoselenium compounds have been shown to mimic the GPx enzyme including the most promising drugs, ebselen (**1**)<sup>3</sup> and ALT 2074 (**2**)<sup>4</sup> (Fig. 1). In addition, several other mimics have been developed. These include ebselen analogues<sup>5</sup>, benzoselenazolinones<sup>6</sup>, selenenamides<sup>7a</sup>, related derivatives<sup>7b</sup>, diaryl diselenides<sup>8</sup> and the semisynthetic enzyme, selenosubtilisin<sup>9</sup>. More recently, heterocycles with Se—O bond/Se...O interaction have attracted immense interest as GPx mimics<sup>10</sup>. Some representative examples include, diselenides<sup>10,11</sup> (**3** and **4**), cyclic seleninate esters<sup>11,12</sup> (**5-8**), spirodioxyselenuranes<sup>11,12</sup> (**9-13**), seleninic acid anhydride<sup>13</sup> (**14**) and seleninate ester<sup>14</sup> (**15**). Recently, our group has initiated work on the synthesis and GPx-like activity of selenenate ester stabilized by intramolecularly coordinating groups<sup>15</sup>. The synthetic approach involves aromatic nucleophilic substitution (S<sub>N</sub>Ar) reactions of 2,6-disubstituted aryl bromides

with selenium nucleophiles. Ester (**16**) was isolated as a dimer from the bromination of *n*-butyl-4-*tert*-2, 6-di(formyl)phenyl selenide or bis(2,6-di-formyl-4-*tert*-butylphenyl)diselenide<sup>15</sup>. Further, the group has exploited the reactivity of 2,6-disubstituted arylselenium compounds for the synthesis of related organoselenium esters<sup>16,17</sup> (**17-22**) and evaluated their GPx-like activity. Herein, we extend our studies on the bromination of the selenide (**23**) and the diselenide (**24**) and report on the synthesis and reactivity of the novel monoester (**25**), stabilized by a formyl group.

### Materials and Methods

Solvents were purified by standard techniques and were freshly distilled prior to use<sup>18</sup>. All the reactions were performed under nitrogen or argon atmosphere by modified Schlenk techniques and monitored by using TLC techniques from time to time. Melting points were recorded with a VEEGO melting point (VMP-1) apparatus and are uncorrected. <sup>1</sup>H (399.88 MHz) and <sup>13</sup>C (100.56 MHz) were recorded on a Varian NMR-mercury plus and Buker Avance<sup>III</sup> 400 MHz spectrometer while <sup>77</sup>Se (57.26 MHz) NMR spectra

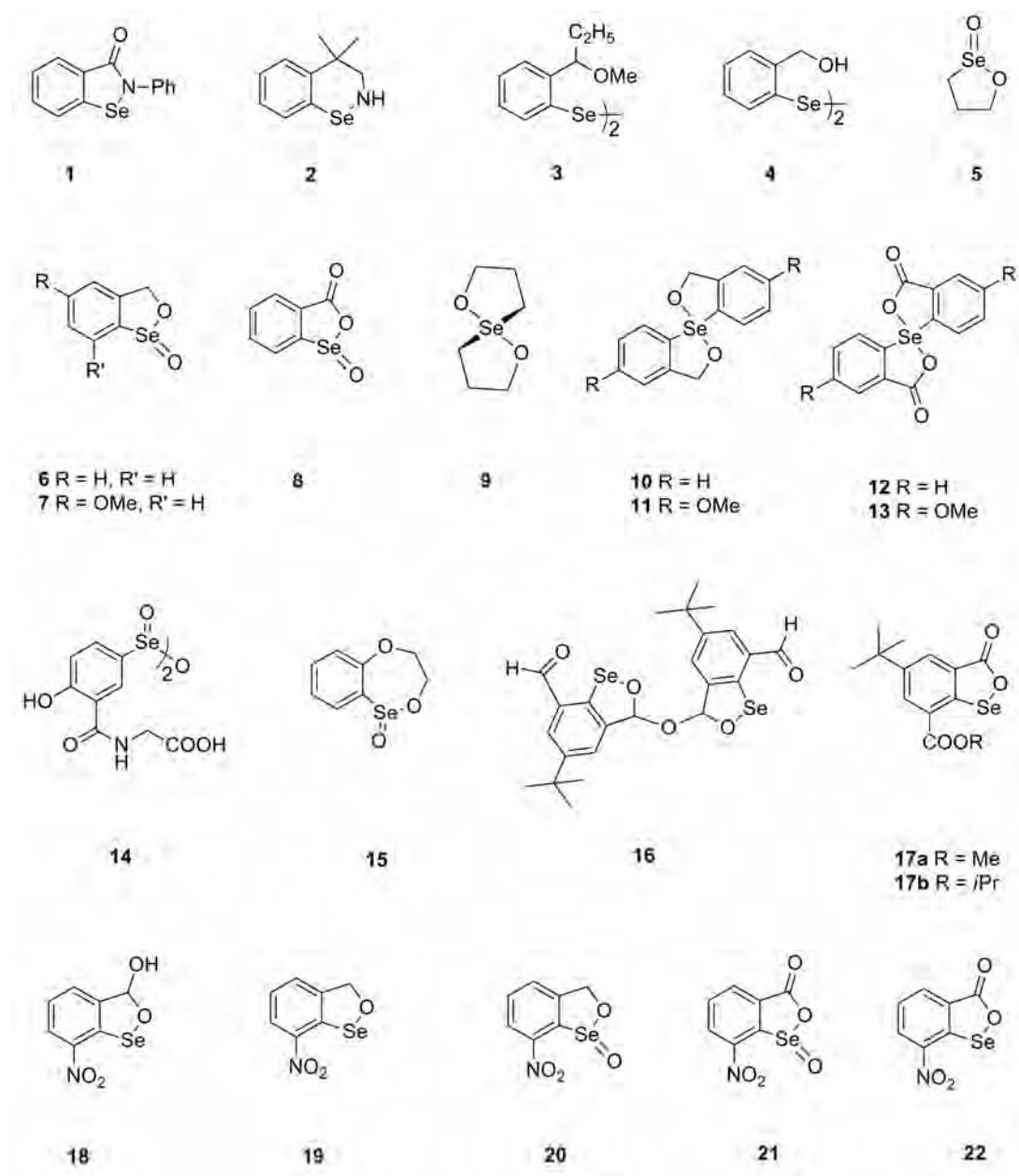


Fig. 1—Some selenium compounds with GPx-like activity.

were recorded on 300 MHz spectrometer at the indicated frequencies. Chemicals shifts ( $\delta$ ) are shown with respect to  $\text{SiMe}_4$  (TMS) as internal standard for nuclei  $^1\text{H}$  and  $^{13}\text{C}$  and with respect to  $\text{Me}_2\text{Se}$  for nuclei  $^{77}\text{Se}$  as the external standard. The mass spectra were recorded at room temperature on a micro mass Q-TOF (YA 107) mass spectrometer. Elemental analyses were analyzed on a CHN-CE instruments flash 1112 series thermoquest elemental analyzer. Infrared (IR) spectra were recorded in the range  $4000\text{--}600\text{ cm}^{-1}$  using KBr for solid samples

on a Perkin-Elmer Precisely Spectrum One FT-IR spectrometer. The UV-vis spectra in solution were recorded on a JASCO, V-570 spectrometer.

#### Synthesis of compound (25)

To a chloroform solution (3 mL) of compound (23)<sup>15b</sup> (0.48 mmol, 0.16 g), was added  $\text{Br}_2$  (0.48 mmol, 0.08 g, 0.03 mL) dissolved in chloroform (2 mL) in the presence of triethylamine (0.96 mmol, 0.10 g, 0.13 mL) at  $0^\circ\text{C}$ . After complete

addition of bromine, the reaction mixture was further stirred for 3 h at the room temperature. The reaction mixture was poured in water (10 mL) and extracted with chloroform (3×10 mL). The separated organic layer was dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated under reduced pressure. Removal of the solvent and purification of the residue by silica gel column chromatography (elution with ethyl acetate/petroleum ether) afforded the yellow compounds (**25**) (36 %) and (**16**) (8 %). Recrystallization of (**25**) from dichloromethane/petroleum ether yielded yellow crystals (0.05 g, 36 %). M. pt.: 232-235 °C. Anal. for C<sub>12</sub>H<sub>12</sub>O<sub>3</sub>Se (%): Calcd: C, 50.90; H, 4.27; Found: C, 50.54; H, 4.14. NMR spectral data (CDCl<sub>3</sub>): <sup>1</sup>H NMR δ: 1.46 (s, 9 H, *t*Bu), 8.40 (d, <sup>4</sup>*J* = 1.52 Hz, 1 H), 8.44 (d, <sup>4</sup>*J* = 1.52 Hz, 1 H), 10.45 (s, 1 H); <sup>13</sup>C {<sup>1</sup>H} δ: 31.6, 35.5, 123.7, 129.6, 133.2, 133.6, 149.0, 153.2, 169.7, 191.8; <sup>77</sup>Se {<sup>1</sup>H} δ: 1423 ppm; IR (KBr): 3086, 2961, 2863, 1703, 1607, 1542, 1345, 1323, 1238, 1226, 1066, 995, 771, 715 cm<sup>-1</sup>; ES-MS: *m/z* 285.10 (<sup>80</sup>Se, [M + H]<sup>+</sup>).

#### Synthesis of compound (26)

To a chloroform solution (2 mL) of compound (**25**) (0.12 mmol, 0.035 g), was added 30 % H<sub>2</sub>O<sub>2</sub> (0.74 mmol, 0.025 g, 0.08 mL) at room temperature. The mixture was stirred further for 2 h at the room temperature. The white precipitate obtained was filtered off and dried under vacuum (0.03 g, 83 %). M. pt. 250-253 °C. Anal. for C<sub>12</sub>H<sub>12</sub>O<sub>5</sub>Se (%): Calcd: C, 45.73; H, 3.84; Found: C, 44.85; H, 2.97. NMR spectral data (CD<sub>3</sub>OD): <sup>1</sup>H NMR δ: 1.46 (s, 9H, *t*Bu), 8.37 (s, 2H, Ar-H); <sup>13</sup>C {<sup>1</sup>H} δ: 31.4, 36.8, 128.5, 129.0, 131.8, 133.4, 152.1, 160.9, 168.5, 170.4; <sup>77</sup>Se {<sup>1</sup>H} δ: 1331 ppm; IR (KBr): 3465, 2964, 2868, 1752, 1741, 1660, 1590, 1464, 1424, 1302, 1237, 1199, 1093, 846 (Se=O), 795, 693 cm<sup>-1</sup>; ES-MS: *m/z* 316.89 (<sup>80</sup>Se, [M + H]<sup>+</sup>).

The same product was obtained in 64 % yield from a similar oxidation of diselenide (**24**) (1.49 mmol, 0.80 g) with 30 % H<sub>2</sub>O<sub>2</sub> (6 equiv) at the room temperature.

#### Synthesis of compound (27)

To a chloroform (4 mL) solution of selenide (**23**) (0.80 mmol, 0.27 g), was added 30 % H<sub>2</sub>O<sub>2</sub> (5.0 mmol, 0.17 g, 0.56 mL) at room temperature. The mixture was stirred further for 2 h at room temperature and then heated for 1 h. The colorless solution was dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and filtered and concentrated to give an oily liquid.

Recrystallization from chloroform afforded white crystals (0.20 g, 69 %). M. pt. 90-94 °C. Anal. for C<sub>16</sub>H<sub>20</sub>O<sub>4</sub>Se (%): Calcd: C, 54.09; H, 5.67; Found: C, 53.88; H, 5.26. NMR spectral data (CDCl<sub>3</sub>): <sup>1</sup>H NMR δ: 1.02 (t, <sup>3</sup>*J* = 7.41 Hz, 3 H, CH<sub>3</sub>), 1.43 (s, 9 H, *t*Bu), 1.49 (sextet, <sup>3</sup>*J* = 7.44 Hz, 2 H, C H<sub>2</sub>), 1.85 (quintet, <sup>3</sup>*J* = 6.80 Hz, 2 H, CH<sub>2</sub>), 4.52 (t, <sup>3</sup>*J* = 6.76 Hz, 2 H), 19.3, 30.8, 31.7, 35.4, 68.2, 122.8, 123.0, 130.6, 131.8, 148.8, 152.9, 169.4, 170.8; <sup>77</sup>Se {<sup>1</sup>H} δ: 1395 ppm; IR (KBr): 2962, 2873, 1713, 1695, 1639 cm<sup>-1</sup>; ES-MS: *m/z* 356.9993 ([M + H]<sup>+</sup>, <sup>80</sup>Se, 100 %).

#### Synthesis of compound (28)

To a solution of compound (**26**) (20 mmol, 0.06 g), was added SOCl<sub>2</sub> (4 mL) and DMF. The reaction mixture was refluxed for 3 h. The excess of SOCl<sub>2</sub> was removed under vacuum by applying liquid N<sub>2</sub> trap. The residue was dissolved in CHCl<sub>3</sub> (5 mL) and washed with water. The separated organic layers were dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>. Removal of the solvent and purification of the residue by silica gel column chromatography (elution with 10 % ethyl acetate/petroleum ether (60-80°) afforded the product (**28**). Recrystallization from dichloromethane/petroleum ether (1:1) gave pure yellow needle like crystals (0.05 g, 75 %). M. pt. 147 °C. Anal. for C<sub>13</sub>H<sub>14</sub>O<sub>3</sub>SSe (%): Calcd: C, 47.42; H, 4.29; S, 9.74; Found: C, 47.14; H, 3.92; S, 9.36. NMR spectral data (CDCl<sub>3</sub>): <sup>1</sup>H NMR δ: 1.41 (s, 9H), 4.08 (s, 1H, OCH<sub>3</sub>), 8.19 (d, <sup>4</sup>*J* = 2.13 Hz, 1H), 8.40 (d, <sup>4</sup>*J* = 2.13 Hz, 1H); <sup>13</sup>C {<sup>1</sup>H} δ: 31.4, 35.1, 53.5, 126.3, 130.6, 132.6, 133.6, 145.0, 151.1, 168.2, 195.5; <sup>77</sup>Se {<sup>1</sup>H} δ: 546 ppm; IR (KBr): 2957, 2865, 1676, 1654, 1558, 1474, 1438, 1425, 1363, 1328, 1310, 1257, 1194, 1165, 1002, 824, 783, 572 cm<sup>-1</sup>. ES-MS: *m/z* 331 (<sup>80</sup>Se, [M + H]<sup>+</sup>).

#### Synthesis of compound (29)

Compound (**29**) was synthesized from (**20**) (0.22 mmol, 0.05 g), SOCl<sub>2</sub> (3 mL) and DMF according to the procedure described for the preparation of (**28**). Removal of the solvent and purification of the residue by silica gel column chromatography (elution with 20 % ethyl acetate/petroleum ether (60-80°) afforded the compound (**29**). Recrystallization from dichloroform/petroleum ether (1:1) afforded pure yellow crystals (0.02 g, 33 %). M. pt. 114-116 °C. Anal. for C<sub>14</sub>H<sub>10</sub>Cl<sub>2</sub>N<sub>2</sub>O<sub>4</sub>Se<sub>2</sub> (%): Calcd: C, 33.69; H, 2.02; N, 5.61; Found: C, 33.76; H, 1.78; N, 5.95. NMR spectral data (CDCl<sub>3</sub>): <sup>1</sup>H NMR δ: 4.8 (s, 2 H, CH<sub>2</sub>), 7.54 (t, <sup>3</sup>*J* = 7.64 Hz, 1 H, Ar-H), 7.59 (dd, <sup>3,4</sup>*J* = 7.94, 1.52 Hz, 1 H, Ar-H), 7.79 (dd, <sup>3,4</sup>

$J = 6.64, 1.52$  Hz, 1 H, Ar-H);  $^{13}\text{C}$  { $^1\text{H}$ }  $\delta$ : 46.4, 123.9, 124.0, 131.4, 133.8, 144.2, 155.2;  $^{77}\text{Se}$  { $^1\text{H}$ }  $\delta$ : 445 ppm; IR (KBr): 3068, 2922, 2852, 1652, 1587, 1536, 1522, 1375, 1360, 729  $\text{cm}^{-1}$ . ES-MS:  $m/z$  249.89 ( $\text{M}^+$ ,  $^{80}\text{Se}$ , 100 %).

Compound (**29**) was also obtained in 38 % yield from (**19**) (0.22 mmol, 0.05 g) under identical conditions.

#### Coupled reductase assay

The GPx activity of the organoselenium esters (**16**) and (**25-29**) was determined by the spectrophotometric method at 340 nm described by Wilson *et al.*<sup>8a</sup>. The test mixture contained GSH (2 mM), EDTA (1 mM), GR (1.65 unit/mL) and NADPH (0.40 mM) in 0.1 M potassium phosphate buffer, pH 7.5. Selenium samples (80  $\mu\text{M}$ ) were added to the test mixture at room temperature and the reaction was started by the addition of  $\text{H}_2\text{O}_2$  (1.6 mM). The initial reduction rates were calculated from the oxidation rate of NADPH at 340 nm. The initial reduction rate was determined at least 3-4 times and calculated from the first 5-10 % of the reaction by using  $6.22 \text{ mM}^{-1}\text{cm}^{-1}$  as the extinction coefficient for NADPH.

#### X-ray crystallographic analysis

X-ray crystallographic studies were carried out on a CrysAlis CCD diffractometer using graphite-monochromatized Mo- $\text{K}_\alpha$  radiation ( $\lambda = 0.71073 \text{ \AA}$ ) and data were collected at room temperature (293 K) and 200 K. The structures were solved by direct methods and refined by a full-matrix least-squares procedure on  $F^2$  for all reflections in SHELXL-97 software<sup>19</sup>. Some details of the refinement are given in Tables S1 and S2 of the Supplementary Data.

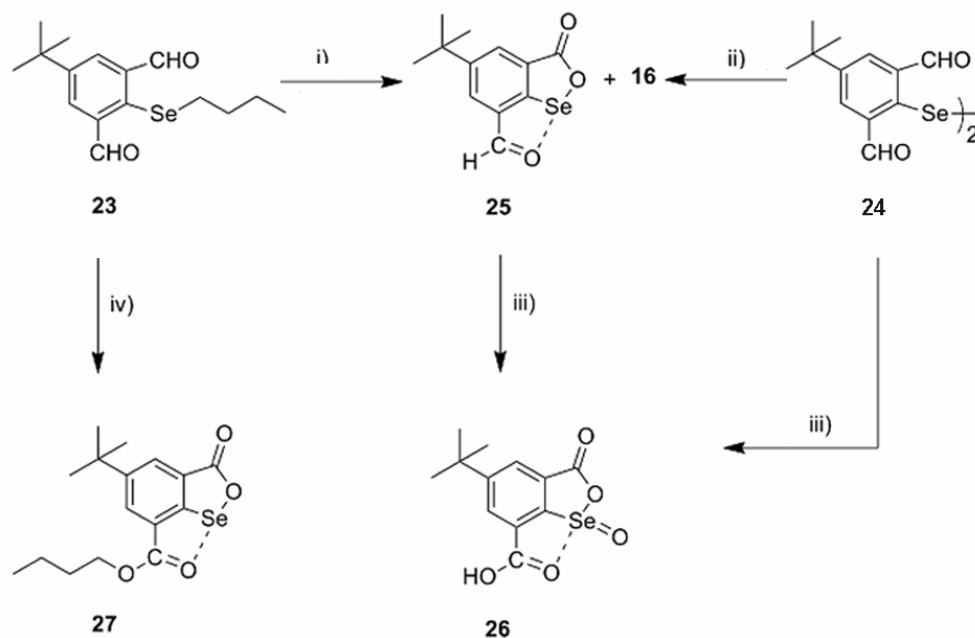
#### Computational methods

All theoretical calculations were executed by using Gaussian 03 suite of quantum chemical programs<sup>20</sup>. The geometry optimizations were carried out at the B3LYP<sup>21</sup> level of density functional theory (DFT) by using the 6-31+G (d) basis sets and all stationary points were characterized as minima by evaluating Hessian indices on respecting potential energy surfaces. The  $^{77}\text{Se}$  NMR calculations were performed at B3LYP/6-311+G(d,p) level on B3LYP/6-31+G(d)-level-optimized geometries by using the gauge-including atomic orbital (GIAO) method (referenced with respect to the peak of  $\text{Me}_2\text{Se}$ )<sup>22</sup>. The quantifications of orbital interaction were done by natural bond orbital (NBO) analysis at B3LYP/6-31+G(d)//B3LYP/6-311+G(d, p) level<sup>23</sup>. The

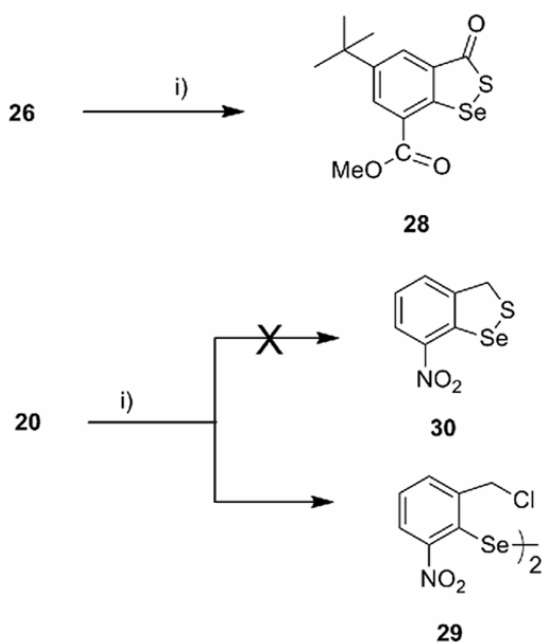
geometries were fully optimized at B3LYP level of theory by using 631+G(d) basis sets which shows a very good agreement of the optimized structural parameters with those obtained from crystal structure studies. The energies are the zero-point correction with electronic energy. AIM analysis was performed in AIM2000<sup>24</sup> at the B3LYP/6-311+G(d,p) level. Also, NICS calculations<sup>25</sup> were carried out at the B3LYP/6-311+G(d,p) level of theory optimized at the B3LYP/6-31+G(d) level.

#### Results and Discussion

In our earlier report, dimeric ester (**16**) was obtained by the bromination of (**23**) or (**24**) with  $\text{CCl}_4$  as the solvent at 0 °C and the work up was done in  $\text{CHCl}_3$ <sup>15</sup>. In the present study, when the bromination and the work up of both (**23**) and (**24**) were carried out in  $\text{CHCl}_3$ , the reaction afforded novel monoester (**25**) in good yield (36 %) along with the dimeric ester (**16**) (8 %) in low yield (Scheme 1). The selenenate esters are stabilized by the coordinating formyl group. A similar bromination of (**24**) in  $\text{CHCl}_3$  yielded the monoester (**25**) in better yield. As reported earlier, ester (**25**) also results from the common intermediate, selenenic acid<sup>15</sup>. The oxidation of (**25**) with  $\text{H}_2\text{O}_2$  afforded seleninate ester (**26**) in very good yield having  $-\text{COOH}$  as the coordinating group, *ortho* to the selenium atom. Ester (**26**) could also be obtained directly by the oxidation of diselenide (**16**) with  $\text{H}_2\text{O}_2$ , though in slightly lower yield. However, direct oxidation of (**23**) with  $\text{H}_2\text{O}_2$  afforded (**27**), where the coordinating group was an ester group (Scheme 1). Thionation of (**26**) was achieved using the "Christiaens and Lambert reaction"<sup>17</sup>. Interestingly, when the reaction of (**26**) was carried out with  $\text{SOCl}_2$  and DMF and the crude was chromatographed on silica gel (60-120 mesh) with petroleum ether (60-80°)/ethyl acetate, it afforded thioselenenate ester (**28**) (Scheme 2). The reaction has several interesting features: (i) Se(IV) is reduced to Se(II), presumably, by the *in situ* generated  $\text{SO}_2^{26a}$ , (ii) the thionation reaction is rare and the most intriguing aspect of the reaction is the esterification of  $-\text{COOH}$  group. The reaction was reproduced several times using purified  $\text{SOCl}_2$  and DMF. It always afforded product (**28**) in very good yield (Scheme 2). The structure of (**28**) was unambiguously confirmed by single-crystal X-ray diffraction studies (vide infra). Based on related reports of thionation<sup>26b</sup> and esterification<sup>26c,d</sup>, a plausible mechanism is given in Scheme 3. Compound (**28**) has been earlier reported by our

(i)  $\text{Br}_2/\text{Et}_3\text{N}/\text{CHCl}_3$ , 0 °C(ii)  $\text{Br}_2/\text{CHCl}_3$ , 0 °C(iii) 30%  $\text{H}_2\text{O}_2$  (6 equiv.)/ $\text{CHCl}_3$ , r.t.(iv) 30%  $\text{H}_2\text{O}_2$  (6 equiv.)/ $\text{CHCl}_3$ , r.t. and then heat for 1 h

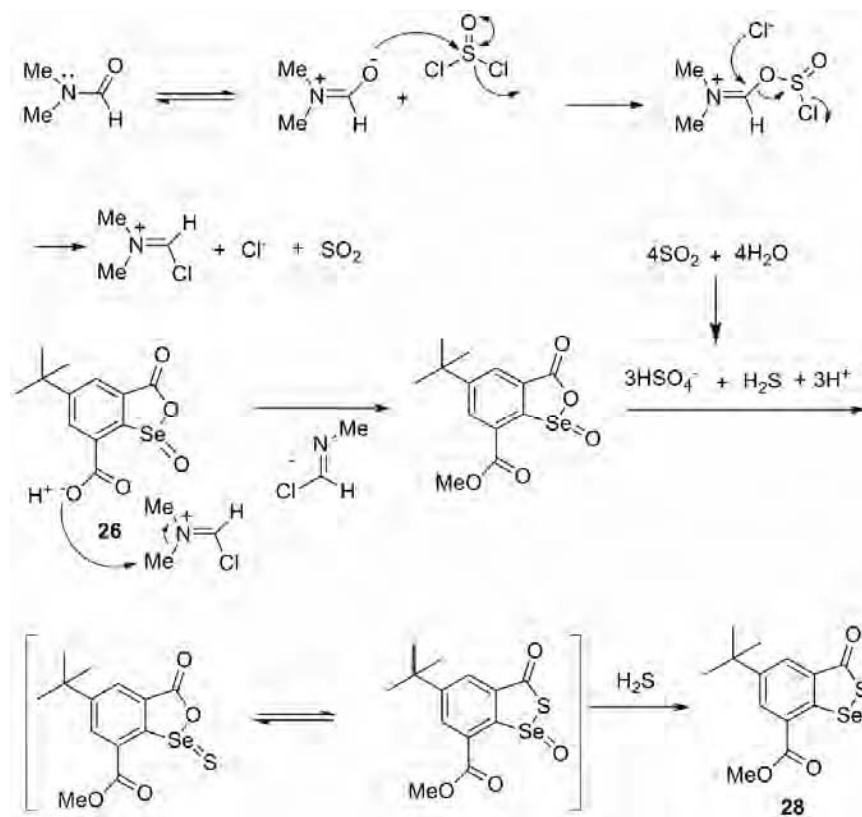
Scheme 1

(i)  $\text{SOCl}_2/\text{DMF}$ ; reflux, 3 h

Scheme 2

group by a different route, where the corresponding selenenyl bromide was treated with  $\text{H}_2\text{S}$  in  $\text{MeOH}$ <sup>16a</sup>. In order to establish the involvement of  $>\text{C}=\text{O}$  group present in the five-membered heterocycles in the thionation reaction, we carried out a similar thionation reaction<sup>16b</sup> of compound (20) (without a  $>\text{C}=\text{O}$  group) by following the same procedure as described for the preparation of compound (28). Surprisingly, the reaction afforded the diselenide (29) (33 % yield), instead of the desired product (30) (Scheme 2). Similarly, the thionation of (19) also afforded diselenide (29) in a slightly better yield (38 %).

All the newly synthesized compounds were characterized by IR, NMR ( $^1\text{H}$ ,  $^{13}\text{C}$ ,  $^{77}\text{Se}$ ) and mass spectrometry studies (see Supplementary Data). The IR spectra of (26) showed a strong peak at  $846\text{ cm}^{-1}$  for  $\nu_{\text{Se}=\text{O}}$ . In the  $^{77}\text{Se}$  NMR spectrum of (25), the signal observed at 1426 ppm is shifted slightly downfield as compared with that of (16) (1401 ppm)<sup>15</sup>. Similarly, the  $^{77}\text{Se}$  NMR spectrum of (26) showed chemical shift at 1342 ppm while Thioselenenate ester (28) showed chemical shift of 546 ppm. These chemical shifts are upfield as compared with that reported for 7-nitro-1,2-benzothiaselenol-3-one (601 ppm)<sup>27a</sup>.



Plausible mechanism for the formation of compound (28)

Scheme 3

### X-ray crystallographic studies

#### Molecular structure of (25)

The geometry around the selenium atom is nearly T-shaped with bond angle  $\text{O3}\cdots\text{Se}-\text{O1}$  of  $160.80(6)^\circ$  (Fig. 2). The  $\text{Se}\cdots\text{O3}$  distance ( $2.355(19)$  Å) is significantly shorter than the sum of the van der Waals radii<sup>28</sup> ( $3.45$  Å). The  $\text{Se}\cdots\text{O}$  distance is shorter than the average  $\text{Se}\cdots\text{O}$  distance ( $2.536$  Å) ( $\text{Se1A}\cdots\text{O1A}$   $2.604$  Å;  $\text{Se1B}\cdots\text{O1B}$   $2.465$  Å) observed for (16)<sup>15a</sup>, indicating a strong  $\text{Se}\cdots\text{O}$  interaction. However, the  $\text{Se}\cdots\text{O}$  distance is close to that reported for ester<sup>27c</sup> (22) ( $2.35(1)$  Å) and much lower than that observed in ester<sup>16b</sup> (19) ( $2.579(6)$  Å). In addition to the intramolecular interactions, some intermolecular hydrogen bonding interaction ( $2.610$  Å) between oxygen atom of carbonyl group and hydrogen atom of phenyl ring of the other molecule were observed (Fig. S33 in Supplementary Data).

#### Molecular structure of (27)

The geometry around selenium is quite similar to that observed for (25) with  $\text{O1}-\text{Se}\cdots\text{O3}$  bond angle of  $159.63(4)^\circ$  (Fig. 3). The  $\text{Se}\cdots\text{O3}$  distance ( $2.453(2)$  Å) is

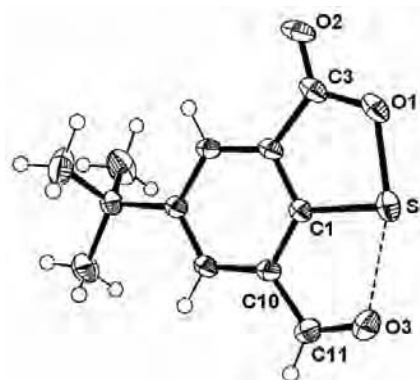


Fig. 2—The molecular structure of (25). [Selected bond lengths (Å) and angles ( $^\circ$ ):  $\text{Se}-\text{O1}$   $1.909(17)$ ;  $\text{Se}-\text{C1}$   $1.840(19)$ ;  $\text{Se}\cdots\text{O3}$   $2.355(19)$ ;  $\text{O1}\cdots\text{Se}-\text{O3}$   $160.80(6)$ ;  $\text{O1}-\text{Se}-\text{C1}$   $85.17(8)$ ;  $\text{C1}-\text{Se}\cdots\text{O3}$   $75.63(8)$ ].

slightly greater than that observed for (25), indicating a weak  $\text{Se}\cdots\text{O}$  interaction. The  $\text{Se}-\text{O1}$  distance ( $1.889(10)$  Å) is shorter than that observed for (25), suggesting that the shortening of the  $\text{Se}-\text{O1}$  bond causes weakening of the *trans*  $\text{Se}\cdots\text{O}$  bond. In the packing diagram of (27), there are intermolecular  $\text{Se}\cdots\text{O}$  interactions ( $3.019$  Å) between the selenium

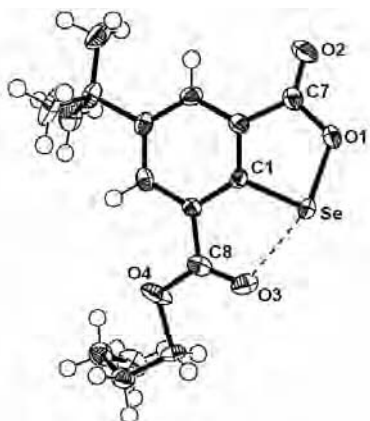


Fig. 3—The molecular structure of (27). [Selected bond lengths (Å) and angles (°): Se—O(1) 1.889(10); Se—C1 1.860(13); Se...O3 2.453(2); O1—Se...O3 159.63(4); O1—Se—C1 85.45(8); C7—O1—Se 115.07(8)].

atoms and oxygen atoms of the carbonyl group present in the five-membered ring (Fig. S34 in Supplementary Data).

#### Molecular structure of (28)

The molecular structure of (28) is shown in Fig. 4. The geometry around the selenium atom is T-shaped with S—Se...O1 bond angle of 164.52(4)°. The Se...O1 distance (2.575(9) Å) is found to be greater than that observed in (25) and (27), indicating a weak Se...O interaction. This distance is also slightly greater than that reported for 7-nitro-1,2-benzothiaselenol-3-one (Se...O 2.51(1) Å)<sup>27a</sup>. The Se—S bond distance (2.224(6) Å) is quite similar to that reported for 7-nitro-1,2-benzothiaselenol-3-one (2.222(5) Å). The bond angle, S—Se...O, is close to that observed in 7-nitro-1,2-benzothiaselenol-3-one 164.7(5)°.

#### Molecular structure of (29)

The molecular structure of compound (29) is shown in Fig. 5. The geometry around Se1 and Se2 atoms is V-shaped with C1A—Se1—Se2 and C1B—Se2—Se1 bond angles of 101.51(9)° and 100.11(10)°, respectively. The C1A—Se1—Se2—C1B dihedral angle of -105.2°, indicates a “transoid” conformation. This may be due the presence of the two *ortho* coordinating groups to the selenium atom. In contrast, diselenide (4) with one *ortho* —CH<sub>2</sub>OH coordinating group, shows a “cisoid” conformation<sup>11</sup>. Due to the *trans* dispositions of the two nitro groups, both the selenium atoms interact with both nitro group. This results in an unprecedented conformation where one oxygen of the nitro group is interacting with both the selenium atoms

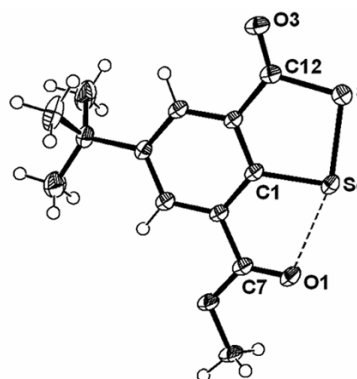


Fig. 4—The molecular structure of (28). [Selected bond lengths (Å) and angles (°): Se—S 2.224(6); Se—C1 1.883(2); Se...O1 2.575(9); S—Se...O1 164.52(4); C1—Se—S 89.87(7); C1—Se...O1 74.64(4)].

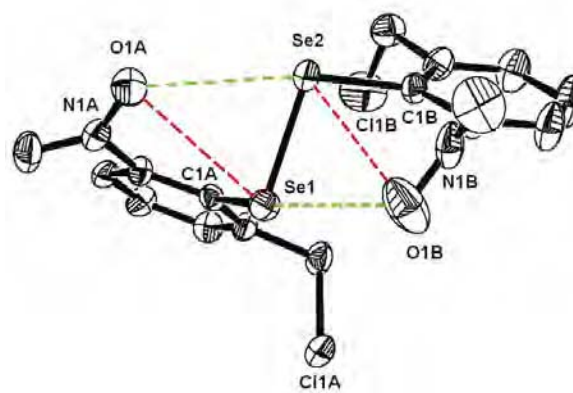


Fig. 5—The molecular structure of (29). [Hydrogen atoms are omitted for clarity. Selected bond length (Å) and angles (°): Se1—C1A 1.928(2); Se1—Se2 2.336(5); Se1...O1A 3.064(13); Se2...O1A 3.042(3), Se2...O1B 3.255(9); Se1...O1B 3.115(6), C1A—Se1—Se2 101.51(9); C1B—Se2—Se1 100.11(10); C11A...Se1...O1A 135.87(5); C1A—Se1...O1A 66.77(9); Se2—Se1...O1A 66.99(4)].

forming both five- and six-membered chelate rings. Also, each selenium atom is weakly interacting with two oxygen atoms of different nitro groups from both sides giving rise to the square of the Se<sub>2</sub>O<sub>2</sub> core. The intramolecular Se...O distances range from 3.042(3) to 3.255(9) Å. The Se...O distances are longer than those observed in (25), (27), (28) and (18–22)<sup>16b,27</sup>. The secondary bonding interactions have been further confirmed by AIM analysis (*vide infra*). There exists a distinct bond critical point (bcp) for all the four interactions ( $\rho_{\text{Se1}\cdots\text{O1A}} = 0.014$  a.u.;  $\rho_{\text{Se2}\cdots\text{O1A}} = 0.015$  a.u.;  $\rho_{\text{Se2}\cdots\text{O1B}} = 0.015$  a.u.;  $\rho_{\text{Se1}\cdots\text{O1B}} = 0.012$  a.u.) (See Fig. S38 of the Supplementary Data for the molecular graph showing *bcp*).

### Computational studies

Density functional theory (DFT) calculations have been performed to understand the nature of the strength of the secondary Se...O interactions and correlate the  $^{77}\text{Se}$  NMR chemical shifts. The geometries were fully optimized at the B3LYP level of theory by using the 6-31+G(d) basis set (for energies and coordinates see Tables S3-S8 of the Supplementary Data). The NMR and NBO calculations were performed at the B3LYP/6-311+G(d,p) level on the B3LYP/6-31+G(d)-level-optimized geometries. The calculated Se...O distances are close to the experimental values in Table 1.

The NBO second-order perturbation energy for the Se...O interaction in (**25**) is  $E_{\text{Se}\cdots\text{O}} = 24.0$  kcal/mol. For the compound (**26**), the NBO interaction energy ( $E_{\text{Se}\cdots\text{O}} = 5.9$  kcal/mol) is very small as compared to (**25**) and (**27-28**), indicating a weak intramolecular secondary Se...O interaction. The NBO interaction energy for the Se...O interaction in (**28**) is  $E_{\text{Se}\cdots\text{O}} = 11.3$  kcal/mol suggesting a stronger interaction than that in (**25**). The NBO interaction energy for the Se...O interaction in (**29**) is very small ( $E_{\text{Se}\cdots\text{O}} = 3.2$  kcal/mol) due to very weak interaction. The calculated  $^{77}\text{Se}$  NMR chemical shifts for compounds (**25-29**) are in good agreement

with the experiment value in Table 2. Compound (**25**) exhibits more positive charge on the selenium (+0.835) than in esters (**27-28**). Nucleus-independent chemical shifts (NICS) calculations for compounds (**25-28**) gave negative values (Table 2). The NICS (0) value of (**28**) in heterocyclic ring is found to be greater than that calculated for **25-27**.

We have investigated the nature of the Se...O interaction using Bader's theory of atoms in molecules (AIM)<sup>24</sup>. The values of electron density ( $\rho_{\text{Se}\cdots\text{O}}$ ) obtained for the Se...O interactions for compounds (**25-29**) range from 0.015 to 0.043  $\text{ea}_0^{-3}$  (Table 2), which are significantly lower than that of normal covalent bonds<sup>24b</sup> (e. g.  $\rho_{\text{C-C}} \approx 0.24 \text{ ea}_0^{-3}$ ) but much higher than the practical boundary of a molecule (e.g.  $\rho \approx 0.001 \text{ ea}_0^{-3}$ ). The Laplacian ( $\nabla^2$ ) of  $\rho_{\text{Se}\cdots\text{O}}$  denotes the curvature of electron density in the 3D-topological space at the BCP of two interacting atoms. Ionic bonds and hydrogen bonds involved in a closed-shell interaction result in positive  $\nabla^2\rho$ . Therefore, the sign of the Laplacian at the BCP can be used to determine the nature of the bond. The positive values obtained for Se...O interactions in (**25-29**), suggest a dominant electrostatic character (for AIM pictures see Fig. S38 of the Supplementary Data).

Table 1—The theoretical data for (**25-29**) obtained by DFT calculations at the B3LYP/6-31+G(d) level along with experimental Se...O distances. [Second-perturbation energies and NBO charges calculated at the B3LYP/6-31+G(d)//6-311+G(d,p) level]

| Comp.                     | $r_{\text{Se}\cdots\text{O}}$ (Å) (Expt.) | $r_{\text{Se}\cdots\text{O}}$ (Å) (Calcd) | $E_{\text{Se}\cdots\text{O}}$ (kcal/mol) | Charge ( $q_{\text{Se}}$ ) |
|---------------------------|-------------------------------------------|-------------------------------------------|------------------------------------------|----------------------------|
| ( <b>25</b> )             | 2.355(19)                                 | 2.395                                     | 24.0                                     | +0.835                     |
| ( <b>26</b> )             | -----                                     | 2.794                                     | 5.9                                      | +1.623                     |
| ( <b>27</b> )             | 2.453(2)                                  | 2.467                                     | 18.7                                     | +0.800                     |
| ( <b>28</b> )             | 2.575(9)                                  | 2.594                                     | 11.3                                     | +0.444                     |
| ( <b>29</b> ) (Se1...O1A) | 3.064(13)                                 | -----                                     | -----                                    | -----                      |
| (Se2...O1A)               | 3.042(3)                                  | 3.026                                     | 3.20                                     | +0.249                     |
| (Se2...O1B)               | 3.255(9)                                  | -----                                     | -----                                    | -----                      |
| (Se1...O1B)               | 3.115(6)                                  | 3.053                                     | 2.14                                     | +0.282                     |

Table 2—Summary of DFT calculations, AIM formalism, NICS values and  $^{77}\text{Se}$  NMR chemical shifts of (**25-29**) at the B3LYP/6-31+G(d)//B3LYP/6-311+G(d,p) level using the Gaussian 03 suite of quantum chemical calculations along with the experimental chemical shifts

| Comp.                     | $\rho_{\text{Se}\cdots\text{O}}$ ( $\text{ea}_0^{-3}$ ) <sup>a</sup> | $\nabla^2\rho_{\text{Se}\cdots\text{O}}$ ( $\text{ea}_0^{-5}$ ) <sup>b</sup> | $^{77}\text{Se}$ NMR (ppm) <sup>c</sup> | NICS (ppm) <sup>d</sup> |
|---------------------------|----------------------------------------------------------------------|------------------------------------------------------------------------------|-----------------------------------------|-------------------------|
| ( <b>25</b> )             | 0.043                                                                | 0.118                                                                        | 1388(1423)                              | -4.5                    |
| ( <b>26</b> )             | 0.020                                                                | 0.059                                                                        | 1331(1333)                              | -1.8                    |
| ( <b>27</b> )             | 0.036                                                                | 0.106                                                                        | 1354(1395)                              | -5.6                    |
| ( <b>28</b> )             | 0.029                                                                | 0.088                                                                        | 576(546)                                | -6.3                    |
| ( <b>29</b> ) (Se1...O1A) | 0.014                                                                | 0.049                                                                        | 512(445)                                | ---                     |
| (Se2...O1A)               | 0.015                                                                | 0.048                                                                        |                                         |                         |
| (Se2...O1B)               | 0.015                                                                | 0.044                                                                        |                                         |                         |
| (Se1...O1B)               | 0.012                                                                | 0.040                                                                        |                                         |                         |

<sup>a</sup>The electron density at the bcp. <sup>b</sup>The Laplacian of the electron density at the bcp. <sup>c</sup>The experimental values are given in parantheses and are referenced with respect to  $\text{Me}_2\text{Se}$  ( $\delta = 0$  ppm). <sup>d</sup>NICS (0) values are calculated at the centre in the five-membered heterocyclic ring in ppm.

Table 3—Initial reduction rates, ( $v_0$ ) for the reduction of  $H_2O_2$  by GSH in the presence of catalysts (**1**), (**16**) and (**25-29**)

| No.    | Catalyst             | $v_0^b$ ( $\mu M \text{ min}^{-1}$ ) |
|--------|----------------------|--------------------------------------|
| (i)    | control <sup>a</sup> | $15.2 \pm 2.0$                       |
| (ii)   | <b>1</b>             | $118.2 \pm 2.9$                      |
| (iii)  | <b>16</b>            | $258.6 \pm 0.6$                      |
| (iv)   | <b>25</b>            | $273.5 \pm 1.0$                      |
| (v)    | <b>26</b>            | $235.2 \pm 2.5$                      |
| (vi)   | <b>27</b>            | $118.7 \pm 2.8$                      |
| (vii)  | <b>28</b>            | $14.4 \pm 1.8$                       |
| (viii) | <b>29</b>            | $186.5 \pm 1.5$                      |

<sup>a</sup>Control values were obtained from the reduction of  $H_2O_2$  by GSH in absence of GPx samples. <sup>b</sup>Assay conditions: reactions were carried out with 0.1 M phosphate buffer pH 7.5, with EDTA (1 mM), GSH (2 mM), NADPH (0.4 mM), glutathione reductase (1.3 unit/ml), GPx samples (80  $\mu M$ ),  $H_2O_2$  (1.6 mM).

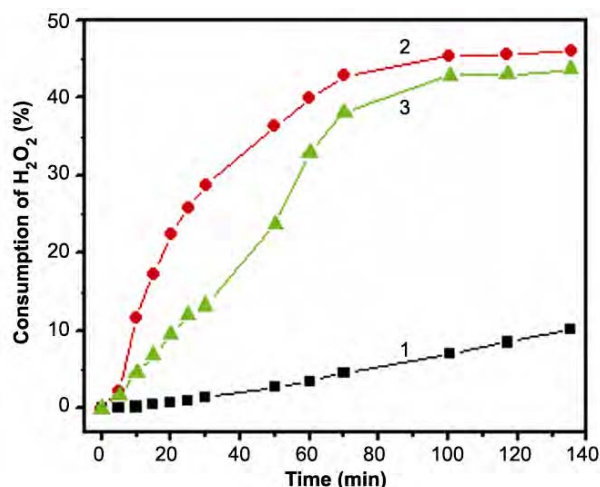


Fig. 6—The catalytic reduction of  $H_2O_2$  by GSH in the presence of selenium catalysts. [The consumption of  $H_2O_2$  was followed by micromoles of NADPH utilized per min: (1) control (no catalyst); (2) **25**; (3) **26**. Assay conditions: reactions were carried out with 0.1 M phosphate buffer pH 7.5, EDTA (1 mM), GSH (0.37 mM), NADPH (0.40 mM), GR (1.3 unit/ml), GPx samples (15  $\mu M$ ), and  $H_2O_2$  (0.30 mM).

#### Glutathione peroxidase-like activity

GPx-like activity of compounds (**25-29**) was evaluated and compared with that of ebselen (**1**) and compound (**16**) (Table 3). The initial reduction rates ( $v_0$ ) for  $H_2O_2$  by reduced glutathione (GSH) in the absence and presence of catalysts were determined from a linear fit spanning the first 5-10 % of the reaction. It was found that compounds (**16**) ( $258.6 \pm 0.6 \mu M \text{ min}^{-1}$ ), (**25**) ( $273.5 \pm 1.0 \mu M \text{ min}^{-1}$ ) and (**26**) ( $235.2 \pm 2.5 \mu M \text{ min}^{-1}$ ) were more efficient catalysts. Compound (**28**) ( $14.4 \pm 1.8 \mu M \text{ min}^{-1}$ ) showed activity close to the control value ( $15.2 \pm 2.0 \mu M \text{ min}^{-1}$ ).

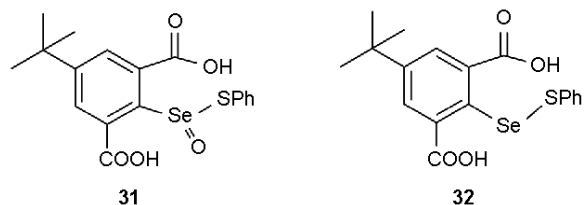


Fig. 7—Intermediates (**31**) and (**32**) observed in the catalytic mechanism of (**26**).

Further, to prove that the catalysts (**25**) and (**26**) were working as mimetics of GPx, kinetic reactions were followed for their entire duration (maximum 10000 s). Control experiment was also carried out in the presence of GSH and  $H_2O_2$  (no catalyst). The combination of catalysts (**25/26**), GSH and  $H_2O_2$  was taken in a cuvette (containing phosphate buffer, EDTA, GSH, GR and NADPH) and the decrease in the absorbance of NADPH was measured. When a graph for the consumption for  $H_2O_2$  versus time was plotted from the data obtained from Tables S22-S24 of the Supplementary Data, only 46 % and 44 % consumption of  $H_2O_2$  was observed after 135.5 min (Fig. 6).

To confirm the mechanism,  $^{77}\text{Se}$  NMR spectra were recorded for identifying the possible intermediates. Similar to the spectra of (**6**) and (**21**), in the present study peaks for only the corresponding thioseleninate ester (**31**) (1381 ppm) and selenosulfide (**32**) (621 ppm) were observed (Fig. 7). The proposed catalytic mechanism for the reduction of  $H_2O_2$  with PhSH in presence of (**26**) is depicted in Scheme S1 in the Supplementary Data.

#### Conclusions

In conclusion, novel monoester (**25**), stabilized by a formyl group has been isolated as a major product along with dimeric ester (**16**) as a minor product. This is due to the solvent chloroform used in the present study instead of carbon tetrachloride used in the previous study. The thionation reaction of (**26**) using Christiaens and Lambert reagent, affords thioselenenate ester (**28**) with several interesting features. A plausible mechanism for the unusual esterification and thionation is proposed. A similar thionation reaction of (**20**), interestingly, affords the diselenide (**29**). This observation demonstrates that the presence of  $>\text{C}=\text{O}$  group in the five-membered heterocycles is crucial for the thionation reaction. Diselenide (**29**) exhibits an unprecedented secondary bonding where one oxygen atom is interacting with two selenium atoms and one selenium atom is interacting with two oxygens at the same time.

### Supplementary Data

Crystallographic data for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre (CCDC) as supplementary publications CCDC-738428 (**25**), CCDC-807795 (**27**), CCDC-738429 (**28**) and CCDC-738430 (**24**). These data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK or via [www.ccdc.cam.ac.uk /data\\_request/cif](http://www.ccdc.cam.ac.uk/data_request/cif). All other supplementary data including Tables S1-S8, Figs S33, S34, S38, Scheme S1 and mass spectra, may be obtained from the authors on request.

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