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Combining benzo[*d*]isoselenazol-3-ones with sterically hindered alicyclic amines and nitroxides: enhanced activity as glutathione peroxidase mimics

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Benzo[*d*]isoselenazol-3-ones *N*-substituted with sterically hindered diamagnetic and paramagnetic five- or six-membered nitroxides or their precursors, including ring-opened diselenides, exhibit synergism in glutathione peroxidase (GPx) activity.

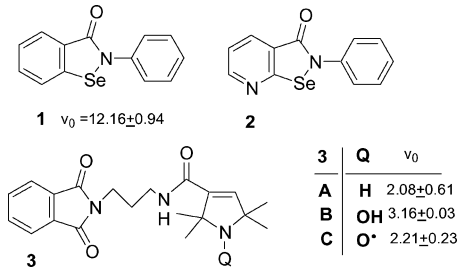
Introduction

Glutathione peroxidases (GPx) are selenoenzymes that protect various organisms from oxidative stress by catalyzing the reduction of hydroperoxides at the expense of glutathione (GSH) (eqn 1).[1,2]

$$\mathrm{ROOH} + 2\mathrm{GSH} \xrightarrow{\mathrm{GPx}} \mathrm{ROH} + \mathrm{GSSG} + \mathrm{H_2O} \qquad (1)$$

The GPx superfamily contains four types of enzymes, the classical cytosolic GPx (cGPx), phospholipid hydroperoxide GPx (PHGPx), plasma GPx (pGPx) and gastrointestinal GPx (giGPx), all of which require selenium in their active sites for catalytic activity.[3–9] The biological role of these key enzymes in the antioxidant defense system comprises not only detoxification, by reducing an overproduction of hydroperoxides, but also the regulation of intracellular signalling pathways[10] and enzyme activities, such as that of 5-lipoxygenase.[11] To overcome the intrinsic difficulties associated with the use of an enzyme as a drug, a number of low-molecular-weight organoselenium mimics[12–14] have been developed for the reduction of hydroperoxides, which include the well-known GPx mimic ebselen (**1**)[15,16] (Scheme 1.) and related compounds having direct Se–N bond,[17–22] cyclic compounds without any direct Se–N bond[23,24] α-phenylselenoketones,[25] and diaryl diselenides having Se ··· N or Se ··· O intramolecular interactions.[26–30] Ebselen (**1**) is a nontoxic compound at pharmacologically active concentrations, because its selenium is not bioavailable. It is mostly bound to proteins in the form of selenyl sulfides[15,16,31] and it is metabolized predominantly into glucuronidated species.[32] Another important feature of ebselen is its inability to oxidize GSH in the presence of oxygen which normally leads to the uncontrolled production of superoxide and other free radical species.[33] Although a number of attempts have been made to design and synthesize ebselen-related GPx mimics based on substituent effects or isosteric replacements, most of them met with limited success. For example, the replacement of the phenyl ring in ebselen by a pyridine ring (**2**) resulted in a complete loss of catalytic activity.[20] In view of the potential applications of ebselen and related derivatives, we synthesized ebselen-based compounds by incorporating five- or six-membered *N*-heterocycles (as secondary amine connected to a diselenide "**A** form" or oxidized forms: *N*-hydroxyls "**B** form", *N*-oxyls "**C** form"). A number of non-selenium compounds having these substituents have been previously shown to posses cardioprotective activity.[34] It has also been shown that pyrroline-based compounds such as **3A** exhibit markedly enhanced protection against ischemia/reperfusion-induced myocardial contractile dysfunction[35] and postischemic myocardial injury[36] probably due to their combined antioxidative and antiarrhythmic activities because the *in vivo* oxidation of **3A** to **3C**.[37]

In this work, we report the GPx activity of a series of closely related benzisoselenazolones and show, for the first time, that the synergistic effect of selenium and pyrroline substituents enhances the antioxidant activity of these compounds.



Scheme 1 Structure of ebselen **1**, pyridine analogue of ebselen **2**, cardioprotective compounds **3A**–**C** and observed initial reaction rates (v_0, μM min^{-1}) in the reaction of **1**.

Results and discussion

Compounds **12C**–**18C** were synthesized using the method described earlier,[38] *i.e.*, by treating paramagnetic amines **5**–**11** in $CHCl_3$ with 2-chloroselenyl-benzoylchloride **4**[39] at ambient temperature in the presence of 2 eq. Et_3N. Amines **5**,[34] **6**,[40] **9**,[41] **10**[42] and **11**[43] were prepared according to published procedures. Amine **7** was prepared by Suzuki coupling of paramagnetic vinylbromide **19**[44] with 3-nitrobenzene boronic acid in the presence of $Ba(OH)_2$ and $PdCl_2(PPh_3)_2$ as a catalyst in aq. dioxane followed by reduction of the resulted aromatic nitro compound **20** by Ehrenkaufer's method.[45] Alkylation of paramagnetic

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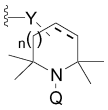
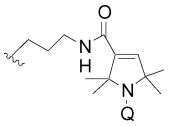
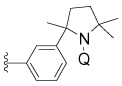
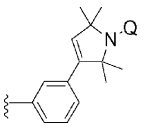
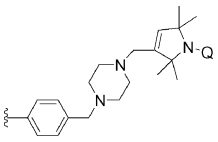
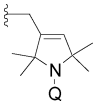
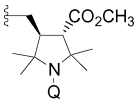
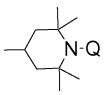
Table 1 Initial rates (v_0) for the reduction of *t*-BuOOH in the presence of catalysts[a]

Compound		Form (Q), v_0/μM min^{-1} [A: Q=H, B: Q=OH, C: Q=O•]
5, **12**		**12A** 19.18 ± 1.65 **12B** 5.10 ± 0.95 **12C** 30.94 ± 0.86
6, **13**		**13A** 25.11 ± 0.16
7, **14**		**14A** 11.94 ± 0.41
8, **15**		**15A** 30.13 ± 0.15
9, **16**		**16A** 13.82 ± 0.46 **16C** 22.11 ± 0.57
10, **17**		**17A** 17.46 ± 0.76 17C 14.05 ± 0.76 **17C** 14.05 ± 0.76
11, **18**		**11A** 2.44 ± 0.19 **18B** 23.57 ± 0.76 **18C** 21.65 ± 0.38

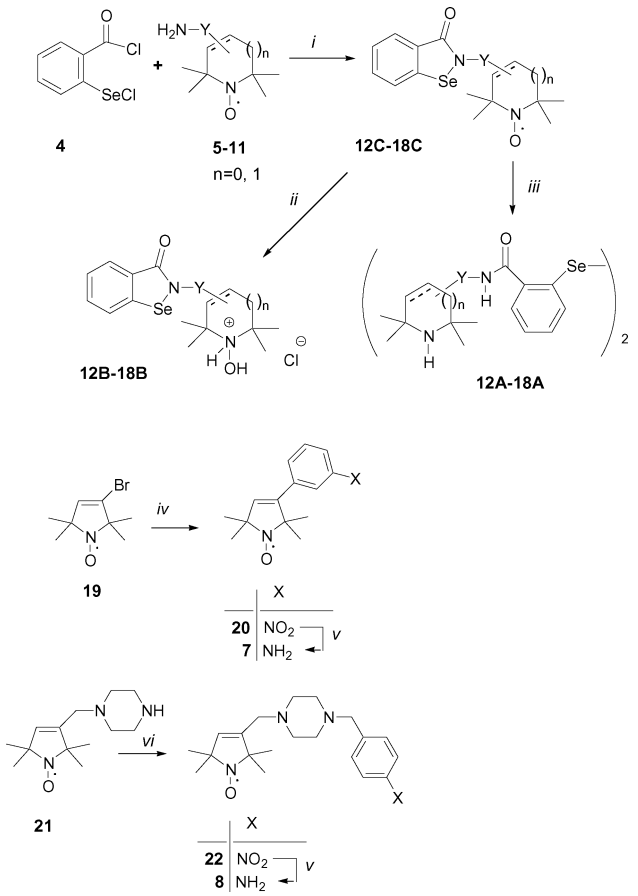
[a] Conditions: GSH: 1 mM; DTPA: 1 mM; GSSG reductase: 0.6 unit mL^{-1}; NADPH: 0.1 mM; *t*-BuOOH: 1.2 mM; selenium catalysts: 0.05 mM; in 0.1 M potassium phosphate buffer, pH 7.3, $n = 3$.

piperazine **21**[46] with 4-nitrobenzylbromide afforded compound **22** the aromatic nitro group of which was reduced to yield amine **8** with ammonium formate in the presence of palladium on charcoal. The *N*-hydroxylamines **12**–**18** "**B** form" was achieved by refluxing the corresponding nitroxides **12**–**18** "**C** form" in EtOH saturated with HCl gas.[35] Reduction of the "**C** form", a nitroxide with Fe powder in glacial acetic acid[47] yielded the corresponding secondary amine diselenides **12**–**18** "**A** form" with opening of isoselenazole ring (Scheme 2).

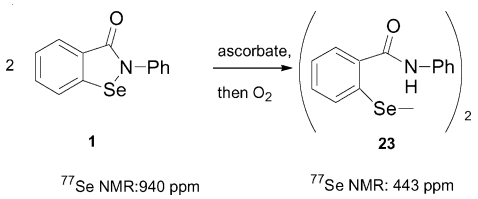
This ring opening takes place quite easily, even by reduction with ascorbic acid to selenol and oxidation during work-up or standing on air results in formation of a diselenide **23** (Scheme 3).

The ring opening followed by oxidation, *e.g.* structure of **12A**–**18A** compounds was observed by ^{77}Se NMR measurements and data are in good agreement with earlier observations.[48]

The GPx activity of new compounds for reduction of *t*-BuOOH was screened spectrophotometrically at 340 nm as described earlier[49] with minor modifications. It is evident from data in Scheme 1 and Table 1 that most of the selenium compounds used in the present study are more potent than ebselen (**1**). Interestingly, compounds **3A**, **3B** and **3C** which lack selenium in the benzene anellated five-membered ring and **11** nitroxide precursor, exhibited some GPx activity, however they are about ten-fold less active than isoselenazolone derivatives (Scheme 1). The pyrroline-substituted compounds showed remarkable GPx activity when attached to the basic benzoselenazolone unit. For example, the activity of compound **12C** ($v_0 = 30.94 \pm 0.86$ μM × min^{-1}), in which both pyrroline and isoselenazole units are present, is higher than the sum of their activities in the individual cases [v_0 (**1** + **3C**) = 14.37 ± 1.17 μM × min^{-1}], suggesting a synergestic effect. The catalytic effect was demonstrated by changing the concentration of **12C** and GSH (Table 1). The observed initial reduction rates (v_0) were directly proportional to the catalyst concentration and rate increases with increasing concentration of GSH and with excess amount of it, the expected saturation kinetics were observed (figure not shown). We can find the same case in the catalytic activity of compound **18C**, which mimics the shape of ebselen with a six-membered ring connected to the nitrogen of isoselenasolone. This compound exhibits higher activity than ebselen (**1**) itself, confirming the role of the nitroxide based substituent. In other words, these two functionalities (isoselenazole and pyrroline/pyrrolidine/piperidine) individually show moderate effects on the reduction rate, however, when they are present together, the effect is supra-additive. The substitution pattern in the pyrroline ring also affects the reduction rate as observed for compounds **12A** and **13A**. Compound **13A**, in which the pyrroline ring is attached to the phenyl ring at the 2-position, exhibits 2-fold higher activity than the 3-substituted **14A**. The best initial rates were observed in the case of compounds with



Scheme 2 . Reagents and conditions: (i) **4** (1.0 eq.), amine **5**–**11** (1.0 eq.), Et_3N (2.0 eq.), $CHCl_3$, rt, 1 h, 35–73%; (ii) EtOH–HCl, reflux, 20 min., 68–82%; (iii) Fe, AcOH, 70 °C, 1 h, then K_2CO_3, 47–59%; (iv) 3-nitrophenyl boronic acid (1 eq.), $Pd(PPh_3)_2Cl_2$ (5%), $Ba(OH)_2$ (1 eq.), dioxane–water, reflux, 3 h, 44%; (v) HCO_2NH_4 (8 eq.), Pd/C, MeOH, 40 °C, 2 h, 35–43%; (vi) 4-nitrobenzylbromide (1 eq.), K_2CO_3, $CHCl_3$, reflux, 4 h, 68%.



Scheme 3 Reduction of ebselen with ascorbic acid, followed by a spontaneous oxidation in an NMR tube.

a polar spacer group *e.g.* an amide for **12C** and an amine for **15A**. The combined catalytic effect appears to be less in the case of apolar spacers: **16A** with a methylene group and **14A** with a phenyl group as a spacer have less catalytic activity. It is interesting to note that compounds **18B** and **18C** without linking groups also show similar GPx activity as **16C**. This indicates that the ring size of nitroxide heterocycle does not affect the reaction rate. Seemingly, the oxidation state of nitroxide moiety does not effect the initial reaction rate neither the isoselenzolone/diselenide form; **12C** is more effective than **12A**, but as efficient as **15A**.

Conclusions

In conclusion, we have shown that due to the synergistic effect of selenium and amino functionalities the modification of the basic ebselen unit by introducing redox-active pyrroline groups greatly enhances the GPx activity of ebselen. The results presented here suggest a new concept that sterically hindered amino groups near the active site of GPx may act synergistically with selenium during the reduction of hydroperoxides. Compounds like **12**–**18** with broad antioxidant activity may serve as "ROS and RNS sponges" and can be promising candidates in future therapy of free radical mediated diseases.

Experimental

General

Melting points were determined with a Boetius micro melting point apparatus and are uncorrected. Elemental analyses (C, H, N, S) were performed on Carlo Erba EA 1110 CHNS elemental analyser. The IR (Specord 85) spectra were in each case consistent with the assigned structure. Mass spectra were recorded on a VG TRIO-2 instrument in the EI mode (70 eV, direct inlet), the source temperature was 210 °C, or an with Automass Multi instrument in the EI mode (70 eV, direct inlet). ESR spectra were obtained from 10^{-5} molar solutions ($CHCl_3$), using an MS200 (Magnettech GMBH, Berlin) spectrometer. All radicals exhibited three equally spaced lines with a_N = 15.1–15.5 G. ^{77}Se NMR spectra were obtained on either a Bruker AVACE400 NMR spectrometer in $CDCl_3$–MeOH (1 : 1) mixture or on a Varian INOVA 400 WB instrument and chemical shifts are reported with respect to Me_2Se. To obtain high resolution ^{77}Se NMR spectra of **12C** it was reduced with the excess of co-dissolved $(PhNH)_2$. Ebselen and ascorbic acid reaction was recorded in $CDCl_3$–DMF (1 : 1) mixture. ^{1}H and ^{13}C NMR spectra were recorded with a Varian INOVA 400 WB spectrometer at 400 MHz at 25 °C, chemical shifts are given in ppm. Preparative flash column chromatography was performed on Merck Kieselgel 60 (0.040–0.063 mm). Qualitative TLC was carried out on commercially prepared plates (20 × 20 × 0.02 cm) coated with Merck Kieselgel GF_{254}. Compounds **3A–C**,[35] **4**,[39] **5**,[35] **6**,[40] **9**,[41] **10**,[42] **13C**,[38] **19**,[44] **22**,[46] **23**[48] were prepared according to published procedures, compound **1** was purchased from Calbiochem, compound **11A** and other reagents were purchased from Aldrich. The GPx activity was followed spectrophotometrically at 340 nm on a Perkin-Elmer Lambda 35 UV-VIS Spectrophotometer. The test mixture contained GSH (1 mm), DTPA (1 mm), glutathione disulfide reductase (0.6 unit mL^{-1}), and NADPH (0.1 mm) in 0.1 M potassium phosphate buffer, pH 7.3. GPx samples were added to the test mixture at room temperature and the reaction was started by the addition of *tert*-butyl hydroperoxide (1.2 mM, final concentration). The initial reduction rates were calculated from the rate of NADPH oxidation at 340 nm. Each initial rate was measured at least three times and calculated from the first 5–10% of the reaction by using 6.22 mM^{-1} cm^{-1} as the extinction coefficient for NADPH. For the peroxidase activity, the rates were corrected for the background reaction between *t*-BuOOH and GSH.

Synthesis of 3-(3-nitrophenyl)-2,2,5,5-tetramethyl-2,5-dihydro-1*H*-pyrrol-1-yloxyl radical (20). A mixture of compound **19** (1.1 g, 5.0 mmol), $Ba(OH)_2{\cdot}8H_2O$ (1.57 g, 5.0 mmol), 3-nitrophenylboronic acid (830 mg, 5.0 mmol) and $PdCl_2(PPh_3)_2$ (140 mg, 0.2 mmol) in dioxane–water (24 mL : 6 mL) was stirred and refluxed for 3 h. After cooling the dioxane was evaporated off, the aqueous phase was extracted with CH_2Cl_2 (2 × 20 mL). The organic phase was washed with water (20 mL), separated, dried ($MgSO_4$), filtered and evaporated under reduced pressure and the residue was purified by flash column chromatography (hexane–EtOAc) on silica gel to give compound **20** as a yellow solid 574 mg (44%), mp 69–71 °C. R_f: 0.50 (hexane–EtOAc, 2 : 1). Anal. calc. for $C_{14}H_{17}N_2O_3$: C 64.35, H 6.56, N 10.72; found: C 64.18, H 6.51, N 10.64%. MS (EI): *m*/*z* (%) 261 (M^+, 28), 246 (100), 231 (53), 216 (25), 128 (37).

General procedure for Ehrenkaufer reduction (7, 8)

To a stirred solution of nitro compound **20** or **22** (5.0 mmol) and HCO_2NH_4 (2.52 g, 40 mmol) in MeOH (30 mL) at 40 °C

under N_2 atmosphere 120 mg Pd/C (10%) was added and the mixture was stirred and refluxed until consumption of starting material (*ca.* 2 h). After cooling, the mixture was filtered through Celite and washed with methanol–water (40 mL : 10 mL). The methanol was evaporated off, the aqueous layer saturated with solid K_2CO_3 and extracted with $CHCl_3$–MeOH (9 : 1) (2 × 20 mL). The organic phase was dried ($MgSO_4$), and activated MnO_2 (200 mg) was added and O_2 was bubbled through the solution at rt for 30 min. The mixture was then filtered, evaporated and the residue was purified by flash column chromatography ($CHCl_3$–MeOH) to give the title compounds.

3-(3-Aminophenyl)-2,2,5,5-tetramethyl-2,5-dihydro-1*H*-pyrrol-1-yloxyl radical (7). 404 mg (35%), mp 105–107 °C. R_f: 0.30 ($CHCl_3$–Et_2O, 2 : 1). Anal. calc. for $C_{14}H_{19}N_2O$: C 72.69, H 8.28, N 12.11; found: C 72.56, H 8.22, N 12.01%. MS (EI): *m*/*z* (%) 231 (M^+, 70), 216 (18), 201 (67), 186 (100), 158 (77).

3-[4-(4-Aminobenzyl)piperazin-1-ylmethyl]-2,2,5,5-tetramethyl-2,5-dihydro-1*H*-pyrrol-1-yloxyl radical (8). 737 mg (43%), mp 112–115 °C. R_f: 0.10 ($CHCl_3$–MeOH, 9 : 1). Anal. calc. for $C_{20}H_{31}N_4O$: C 69.92, H 9.10, N 16.32; found: C 69.90, H 9.05, N 16.19%. MS (EI): *m*/*z* (%) 343 (M^+, 22), 313 (17), 222 (26), 207 (84), 106 (100).

3-[4-(4-Nitrobenzyl)piperazin-1-ylmethyl]-2,2,5,5-tetramethyl-2,5-dihydro-1*H*-pyrrol-1-yloxyl radical (22). A solution of compound **21** (2.38 g, 10.0 mmol), 4-nitrobenzyl bromide (2.16 g, 10.0 mmol) and K_2CO_3 (1.38 g, 10.0 mmol) in $CHCl_3$ (30 mL) was stirred and refluxed for 4 h. After cooling the inorganic salt was filtered off, the organic phase was washed with water (10 mL), separated, dried ($MgSO_4$), filtered and evaporated. After flash column purification of the residue compound **22** was received as a yellow solid 2.53 g (68%), mp 107–108 °C. R_f: 0.48 ($CHCl_3$–MeOH, 9 : 1). Anal. calc. for $C_{20}H_{29}N_4O_3$: C 64.30, H 7.87, N 15.01; found: C 64.25, H 7.71, N 14.91%. MS (EI): *m*/*z* (%) 373 (M^+, 65), 343(14), 234 (100), 221 (91), 136 (33).

General procedure for synthesis of compounds 12C–18C

To a stirred solution of amine **5–11** (3.0 mmol) and Et_3N (666 mg, 6.6 mmol) in CH_2Cl_2 (20 mL) was added dropwise a solution of freshly prepared 2-(chloroseleno)benzoyl chloride **4** (762 mg, 3.0 mmol) in CH_2Cl_2 (10 mL) over 5 min at rt and the mixture was stirred for a further 1 h. The organic phase was washed with brine (10 mL), dried ($MgSO_4$), filtered, evaporated and the residue was purified by flash column chromatography ($CHCl_3$–Et_2O or $CHCl_3$–MeOH) to give compounds **12C**–**18C** as orange-yellow solids in 35–73% yield.

2-[(1-Oxyl-2,2,5,5-tetramethyl-2,5-dihydro-1*H*-pyrrol-3-carboxamidoprop)-3yl]benzo[*d*]isoselenazol-3-one radical (12C). Yellow solid 541 mg (45%), mp 126–128 °C. R_f: 0.46 ($CHCl_3$–MeOH, 9 : 1). Anal. calc. for $C_{19}H_{24}N_3O_3Se$: C 54.16, H 5.74, N 9.97; found: C 54.02, H 5.68, N 9.82%. MS (EI): *m*/*z* (%) 424/422/420/419/418/416 (M^+, 0.2/1/0.5/0.2/0.2/0.02), 394/392/390/389/388/386 (1/7/3/1/1/0.1), 186/184/182/181/180/179 (8/43/21/7/8/1), 136 (100). ^{77}Se NMR: 882 ppm.

2-[3-(1-Oxyl-2,2,5,5-tetramethyl-2,5-dihydro-1*H*-pyrrol-3-yl)phenyl]benzo[*d*]isoselenazol-3-one radical (14C). Yellow solid, 432 mg (35%), mp 148–150 °C. R_f: 0.57 ($CHCl_3$–Et_2O, 2 : 1). Anal. calc. for $C_{21}H_{21}N_2O_2Se$: C 61.17, H 5.13, N 6.79; found: C 61.10, H 5.12, N 6.67%. MS (EI): *m*/*z* (%) 415/413/411/410/409/407 (M^+, 2/9/4/1/2/0.3), 385/383/381/380/379/377 (4/20/9/3/4/0.4), 199 (100), 184 (47).

2-{4-[1-(1-Oxyl-2,2,5,5-tetramethyl-2,5-dihydro-1*H*-pyrrol-3-ylmethyl)piperazin-4-yl]phenylmethyl}benzo[*d*]isoselenazol-3-one radical (15C). Yellow solid, 880 mg 56%, mp 115–117 °C. R_f: 0.34 ($CHCl_3$–MeOH, 9 : 1). Anal. calc. for $C_{27}H_{33}N_4O_2Se$: C 54.16, H 5.74, N 9.97; found: C 54.02, H 5.68, N 9.82%. MS (EI): *m*/*z* (%) 527/525/523/522/521/519 (M^+, 0.2/1/0.5/0.2/0.2/0.02), 497/495/493/492/491/489 (0.7/4/2/0.6/0.7/0.1), 290/288/286/285/284/282 (7/40/19/6/7/1), 136 (72), 122 (100).

2-(1-Oxyl-2,2,5,5-tetramethyl-2,5-dihydro-1*H*-pyrrol-3-ylmethyl) benzo[*d*]isoselenazol-3-one radical (16C). Pale yellow solid, 854 mg (61%), mp 165–166 °C. R_f: 0.32 ($CHCl_3$–Et_2O, 2 : 1). Anal. calc. for $C_{16}H_{19}N_2O_2Se$: C 54.86, H 5.47, N 8.00; found: C 54.71, H 5.44, N 7.93%. MS (EI): *m*/*z* (%) 353/351/349/348/347/345 (M^+, 0.7/4/2/0.6/0.7/0.1), 323/321/319/318/317/315 (1.5/8/4/1/1.5/0.2), 138 (41), 122 (62), 107 (100).

2-(1-Oxyl-3-carbomethoxy-2,2,5,5-tetramethylpyrrolidine-4-ylmethyl) benzo[*d*]isoselenazol-3-one radical (17C). Orange solid, 492 mg (40%), mp 207–209 °C. R_f: 0.26 ($CHCl_3$–MeOH, 9 : 1). Anal. calc. for $C_{18}H_{23}N_2O_4Se$: C 52.42, H 5.87, N 6.80; found: C 52.22, H 5.84, N 6.61%. MS (EI): *m*/*z* (%) 413/411/409/408/407/405 (M^+, 1/5/3/0.8/1/0.1), 383/381/379/378/377/375 (0.7/4/2/0.6/0.7/0.1), 269/267/265/264/263/261 (7/40/19/6/7/1), 201/199/197/196/195/193 (18/100/47/15/18/2).

2-(1-Oxyl-2,2,6,6-tetramethylpiperidin-4-yl) benzo[*d*]isoselenazol-3-one radical (18C). Orange-pink solid, 770 mg (73%), mp 230–231 °C. R_f: 0.44 ($CHCl_3$–Et_2O, 2 : 1). Anal. calc. for $C_{16}H_{21}N_2O_2Se$: C 54.38, H 5.99, N 7.93; found: C 54.30, H 5.88, N 7.85%. MS (EI): *m*/*z* (%) 355/353/351/350/349/347 (M^+, 1/5/3/0.8/1/0.1), 186/184/182/181/180/178 (4/20/10/3/4/0.4), 140 (100).

General procedure for synthesis of compounds 12B, 13B, 15B, 18B

A solution of compound **12C**, **13C**, **15C** or **18C** (0.5 mmol) was refluxed with EtOH (15 mL) (saturated with HCl) for 20 min. After cooling, the solvent was evaporated off and the residue was crystallized with acetone or Et_2O to give title compounds as off-white or yellow solids in 68–82% yield.

2-[(1-Hydroxy-2,2,5,5-tetramethyl-2,5-dihydro-1*H*-pyrrol-3-carboxamidoprop)-3yl]benzo[*d*]isoselenazol-3-one hydrochloride (12B). Off-white solid 160 mg (70%), mp 115–117 °C. Anal. calc. for $C_{19}H_{26}ClN_3O_3Se$: C 49.74, H 5.71, N 9.16; found: C 49.72, H 5.70, N 8.99%. ^{1}H NMR (400 MHz, D_2O): δ 7.76 (m, 2H, ar CH), 7.56 (t, J = 6 Hz, 1H, ar CH), 7.39 (t, J = 6 Hz, 1H, ar CH), 6.05 (s, 1H, olephinic CH), 3.81 (t, J = 6.4 Hz, 2H, NCH_2), 3.26 (t, J = 6 Hz, 2H, NCH_2), 1.92 (m, 2H, CH_2–CH_2–CH_2), 1.45 (s, 6H, CCH_3), 1.32 (s, 6H, CCH_3). ^{13}C NMR (100.5 MHz, D_2O): δ 168.7, 164.3, 139.5, 137.1, 135.1, 132.8, 127.8, 127.1, 126.8, 125.0, 78.6, 76.0, 43.41, 37.65, 28.6, 23.3 br, 22.07 br. ^{77}Se NMR ($CDCl_3$–MeOH): δ 1043; (D_2O): δ 925 ppm.

2-[3-(1-Hydroxy-2,5,5-trimethylpirrolidin-2yl)phenyl]benzo[*d*]-isoselenazol-3-one hydrochloride (13B). White solid 148 mg (68%), mp 141–142 °C. Anal. calc. for $C_{20}H_{23}ClN_2O_2Se$: C 54.87, H 5.29, N 6.40; found: C 54.73, H 5.20, N 6.22%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.14 (d, J = 7.7 Hz, 1H, ar CH), 7.88 (d, J = 8.0 Hz, 1H, ar CH), 7.87 (s, 1H, ar CH), 7.66 (dd, J_1 = 7.7 Hz, J_2 = 7.4 Hz, 2H, ar CH), 7.56 (d, J = 7.7 Hz, 1H, ar CH), 7.51 (d, J = 7.5 Hz, 1H, ar CH), 7.47 (dd, J_1 = 7.5 Hz, J_2 = 7.4 Hz, 2H, ar CH, 2.58 (m, 1H, CH_2), 2.37 (m, 1H, CH_2), 2.21 (m, 1H, CH_2), 2.04 (m, 1H, CH_2), 1.63 (s, 3H, CCH_3), 1.41 (s, 3H, CCH_3), 1.28 (br s, 3H, CCH_3). ^{13}C NMR (100.5 MHz, D_2O): δ 168.1, 140.0, 138.3, 133.3, 130.4, 128.3, 127.4, 127.0, 126.6, 125.0, 76.6, 75.1, 35.1, 31.4 ppm. ^{77}Se NMR (76.3 MHz, DMSO-d_6): δ 891 ppm.

2-{4-[1-(1-Hydroxy-2,2,5,5-tetramethyl-2,5-dihydro-1*H*-pyrrol-3-ylmethyl)piperazin-4-yl]phenylmethyl}benzo[*d*]isoselenazol-3-one trihydrochloride (15B). White solid, 286 mg (82%), mp 195–197 °C. Anal. calc. for $C_{27}H_{37}Cl_3N_4O_2Se$: C 51.08, H 5.87,

N 8.82; found: C 51.03, H 5.79, N 8.70%. ^{1}H NMR (400 MHz, D_2O): δ 7.96 (d, $J = 7.6$ Hz, 1H, ar CH), 7.89 (d, $J = 7.2$ Hz, 1H, ar CH), 7.72 (dd, $J_1 = 7.6$ Hz, $J_2 = 7.2$ Hz, 1H, ar CH), 7.59 (br s, 4H, ar CH), 7.53 (dd, $J_1 = 7.6$ Hz, $J_2 = 7.2$ Hz, 1H, ar CH), 5.84 (s, 1H, olephinic CH), 4.41 (s, 2H, NCH_2), 4.35 (s, 2H, NCH_2), 3.51 (s, 2H, NCH_2), 3.34 (brs, 2H, NCH_2), 1.47 (s, 6H, CCH_3), 1.45 (s, 6H, CCH_3). ^{13}C NMR (100.5 MHz, D_2O): δ 167.9, 140.1, 133.3, 132.7, 127.0, 126.8, 125.0, 78.5, 76.8, 57.7, 49.6, 49.0, 24.2 br, 22.9 ppm. ^{77}Se NMR (76.3 MHz, D_2O): δ 997 ppm.

2-(1-Hydroxy-2,2,6,6-tetramethylpiperidin-4-yl) benzo[*d*]-isoselenazol-3-one radical hydrochloride (18B). Yellow solid, 144 mg (74%), mp: 205–206 °C. Anal. calc. for $C_{16}H_{23}ClN_2O_2Se$: C 49.30, H 5.95, N 7.19; found: C 49.22, H 5.92, N 7.03%. ^{1}H NMR (400 MHz, D_2O): δ 7.87 (d, $J = 7.6$ Hz, 1H, ar CH), 7.82 (d, $J = 8.0$ Hz, 1H, ar CH), 7.62 (dd, $J_1 = 8.0$ Hz, $J_2 = 7.2$ Hz, 1H, ar CH), 7.46 (dd, $J_1 = 7.6$ Hz, $J_2 = 7.2$ Hz, 1H, ar CH), 4.93 (m, 1H, NCH), 2.30 (m, 2H, C*H*H), 2.10 (n, 2H, CH*H*), 1.52 (s, 6H, CCH_3), 1.43 (s, 6H, CCH_3). ^{13}C NMR (100.5 MHz, D_2O): δ 168.7, 139.5, 132.9, 127.8, 127.4, 126.9, 125.0, 68.8, 45.0, 42.4, 27.3, 19.6 ppm. ^{77}Se NMR (76.3 MHz, D_2O): δ 890 ppm.

General procedure for synthesis of compounds 12A–18A

To a solution of nitroxide **12C–18C** (1.0 mmol) in AcOH (8 mL) Fe powder (560 mg, 10 mmol) was added and the mixture was warmed up to 70 °C until the reaction started. The mixture was stirred at room temperature for 1 h, diluted with water (15 mL), decanted and the decanted aqueous solution made alkaline with solid K_2CO_3. The mixture was extracted with $CHCl_3$ (3 × 15 mL), dried ($MgSO_4$), filtered, evaporated and after chromatographic purification ($CHCl_3$–MeOH) we got the title amines **12A–18A** in 47–59% yield.

2,2′-Diselenobis[*N*-(2,2,5,5-tetramethyl-2,5-dihydro-1*H*-pyrrol-3-carboxamido-prop-3-yl)]benzamide (12A). Beige solid, 199 mg (49%), mp 182–183 °C (2HCl salt). R_f: 0.16 (MeOH). Anal. calc. for $C_{38}H_{54}Cl_2N_6O_4Se_2$: C 51.42, H 6.13, N 9.47; found: C 51.30, H 6.00, N 9.31%. ^{1}H NMR (400 MHz, D_2O): δ 7.47 (d, $J = 7.6$ Hz, 2H), 7.48 (d, $J = 7.6$ Hz, 2H), 7.03 (dd, $J_1 = 7.6$ Hz, $J_2 = 7.2$ Hz, 2H), 6.97 (dd, $J_1 = 7.6$ Hz, $J_2 = 7.2$ Hz, 2H), 6.15 (s, 2H), 3.31 (br dd, 4H), 3.22 (br dd, 4H), 1.75 (br dd, 4H), 1.53 (s, 6H), 1.41 (s, 6H). ^{13}C NMR (100.5 MHz, D_2O): δ 170.0, 164.6, 138.1, 136.6, 133.6, 132.2, 131.3, 127.9, 127.2, 71.8, 68.8, 37.8, 37.3, 28.3, 26.5, 26.2 ppm. ^{77}Se NMR ($CDCl_3$–MeOH): δ 442 ppm; (D_2O): δ 449 ppm.

2,2′-Diselenobis[*N*-3-(2,5,5-trimethylpyrrolidin-2-yl)phenyl]-benzamide oxalate (13A). White solid, 474 mg (55%), mp 161–164 °C. R_f: 0.11 ($CHCl_3$–MeOH, 2 : 1). Anal. calc. for $C_{42}H_{48}N_4O_6Se_2$: C 58.47, H 5.61, N 6.49; found: C 58.33, H 5.87, N 6.40%. ^{1}H NMR (for base) (400 MHz, $CDCl_3$): δ 9.9 (br s, 2H, NH), 7.92 (s, 2H, ar CH), 7.80 (d, $J = 6.4$ Hz, 2H, ar CH), 7.76 (d, $J = 7.6$ Hz, 2H, ar CH), 7.22–7.28 (m, 4H, ar CH), 7.11–7.18 (m, 6H, ar CH), 2.44–2.52 (m, 2H, CH_2), 2.08–2.18 (m, 2H, CH_2), 1.95–2.02 (m, 2H, CH_2), 1.82–1.90 (m, 2H, CH_2), 1.69 (s, 6H, CCH_3), 1.21 (s, 6H, CCH_3), 1.12 (s, 6H, CCH_3). ^{13}C NMR (100.5 MHz, $CDCl_3$): δ 167.2, 143.5, 139.2, 133.9, 133.3, 132.0, 131.3, 129.4, 128.6, 126.3, 121.7, 120.8, 118.7, 70.05, 65.0, 38.3, 36.4, 29.20, 29.15, 28.6. ^{77}Se NMR (D_2O): δ 455 ppm.

2,2′-Diselenobis[*N*-3-(2,2,5,5-tetramethyl-2,5-dihydro-1*H*-pyrrol-3-yl)phenyl)]benzamide (14A). Yellow solid, 398 mg (50%), mp 217–220 °C. R_f: 0.50 ($CHCl_3$–MeOH, 2 : 1). Anal. calc. for $C_{42}H_{46}N_4O_2Se_2$: C 63.31, H 5.85, N 7.03; found: C 63.27, H 5.76, N 7.00%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.51 (d, $J = 8.0$ Hz, 1H), 7.82 (d, $J = 6.8$ Hz, 1H), 7.78 (s, 1H), 7.57 (m, 1H), 7.50 (m, 1H), 7.40 (m, 2H), 7.22 (d, $J = 7.2$ Hz, 1H), 6.07 (s, 1H), 2.90 (s, 1H), 1.65 (s, 6H), 1.54 (s, 6H). ^{13}C NMR (100.5 MHz, DMSO-d_6): δ 165.1, 142.9, 140.9, 139.9, 132.9, 131.5, 131.3, 129.9, 129.3, 127.3, 127.1, 125.7, 124.0, 123.8, 122.8, 70.5, 66.7, 27.1 ppm. ^{77}Se NMR (DMSO-d_6): δ 448 ppm.

2,2′-Diselenobis[*N*-{4-[1-(2,2,5,5-tetramethyl-2,5-dihydro-1*H*-pyrrol-3-ylmethyl)piperazin-4-yl]phenylmethyl}]benzamide (15A). Yellow solid, 479 mg (47%), mp 112–115 °C. R_f: 0.20 [MeOH–NH_4OH (aq. 25%), 40 : 1]. Anal. calc. for $C_{54}H_{70}N_8O_2Se_2$: C 63.52, H 6.91, N 10.97; found: C 63.35, H 7.02, N 10.88%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.5 (br, 2H), 7.92 (d, $J = 6.0$ Hz, 2H), 7.78 (br, 2H), 7.67 (d, $J = 6.8$ Hz, 2H), 7.39 (m, 4H), 7.26 (m, 4H), 5.36 (s, 2H), 3.49 (br, 8H), 3.41 (s, 4H), 2.83 (s, 4H), 2.35 (br, 8H) 1.13 (s, 6H), 1.10 (s, 6H). ^{13}C NMR (100.5 MHz, DMSO-d_6): δ 166.1, 142.9, 137.5 br, 133.8 br, 133.2, 131.9, 129.1, 128.5, 126.3, 120.3, 65.9, 62.6, 61.6, 54.9, 53.0, 52.6, 45.6, 30.9, 29.7, 21.7, 11.6 ppm. ^{77}Se NMR (DMSO-d_6): δ 448 ppm.

2,2′-Diselenobis[*N*-(2,2,5,5-tetramethyl-2,5-dihydro-1*H*-pyrrol-3-ylmethyl)]benzamide (16A). White solid, 349 mg (52%), mp 196–197 °C. R_f: 0.87 (MeOH–NH_4OH, 40 : 1). Anal. calc. for $C_{32}H_{42}N_4O_2Se_2$: C 57.14, H 6.29, N 8.33; found: C 57.11, H 6.15, N 8.20%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.90 (br, 2H), 7.80 (d, $J = 6.4$ Hz, 2H), 7.68 (d, $J = 6.8$ Hz, 2H), 7.31 (m, 4H), 5.40 (s, 2H), 3.93 (br s, 4H), 1.19 (s, 12H), 1.11 (s, 12H). ^{13}C NMR (100.5 MHz, DMSO-d_6): δ 167.0, 143.9, 133.1, 132.0, 131.4, 131.1, 129.8, 127.9, 126.1, 65.4, 62.6, 36.1, 31.1, 29.8 ppm. ^{77}Se NMR (DMSO-d_6): δ 447 ppm.

2,2′-Diselenobis[*N*-(3-carbomethoxy-2,2,5,5-tetramethylpyrrolidine-4-ylmethyl)]benzamide (17A). Beige solid, 443 mg (56%), mp 207–209 °C. R_f: 0.67 (MeOH–NH_4OH, 40 : 1). Anal. calc. for $C_{36}H_{50}N_4O_6Se_2$: C 54.54, H 6.36, N 7.07; found: C 54.50, H 6.20, N 6.95%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.85 (d, $J = 8.0$ Hz, 2H), 7.49 (d, $J = 7.2$ Hz, 2H), 7.20 (m, 4H), 7.04 (br s, 2H), 3.64 (s, 6H, OCH_3), 3.56–3.59 (m, 2H), 3.36 (m, 2H), 2.80 (d, 2H), 2.51 (m, 6H) 1.38 (s, 6H), 1.28 (s, 6H), 1.09 (s, 6H), 1.04 (s, 6H). ^{13}C NMR (100.5 MHz, $CDCl_3$): δ 175.0, 167.8, 133.5, 132.2, 131.6, 131.2, 126.5, 125.9, 60.1, 59.93, 59.86, 52.1, 50.5, 40.8, 31.7, 29.7, 27.4, 25.1 ppm. ^{77}Se NMR ($CDCl_3$): δ 459.6, 459.8 ppm (because of diastereomers).

2,2′-Diselenobis[*N*-(2,2,6,6-tetramethylpiperidin-4-yl)]benzamide (18A). White solid, 354 mg (49%), mp 224–226 °C. R_f: 0.35 (MeOH–NH_4OH, 40 : 1). Anal. calc. for $C_{36}H_{46}N_4O_2Se_2$: C 59.66, H 6.40, N 7.73; found: C 54.60, H 6.38, N 7.67%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.42 (br, 2H), 8.0 (d, $J = 6.4$ Hz, 2H), 7.68 (d, $J = 6.8$ Hz, 2H), 7.31 (m, 4H), 5.40 (s, 2H), 3.93 (br s, 4H), 1.19 (s, 12H), 1.11 (s, 12H). ^{13}C NMR (100.5 MHz, DMSO-d_6): δ 167.0, 143.9, 133.1, 132.0, 131.4, 131.1, 129.8, 127.9, 126.1, 65.4, 62.6, 36.1, 31.1, 29.8 ppm. ^{77}Se NMR (DMSO-d_6): δ 447 ppm.

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