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A Rapid and Efficient Synthesis of 2,2',2''-(nitrilotris(ethane-2,1-diyl))tris(benzo [d][1,2]selenazol-3(2H)-one) and its Antioxidant Activity

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ABSTRACT

In the present communication an efficient method for the preparation of stable 2,2',2''-(nitrilotris(ethane-2,1-diyl)) tris(benzo[d][1,2]selenazol-3(2H)-one) has been developed. The reported compound benzoselenazol was achieved when 2, 2'-Selenobenzoyl chloride were treated with N,N-bis(2-aminoethyl)ethane-1,2-diamine over 1h. The reported structure was systematically characterized by 1H, 13C, 77Se NMR and mass spectroscopic techniques. In addition to synthesis and characterization the glutathione peroxidase (GPx) mimetic activity of newly synthesized compound were investigated. It is observed that the reported compound shows significant antioxidant activity in contrast with reference standard ebselen.

Keywords: Ebselen; Benzoselenazol; Benzoselenazol; Selenobenzoyl chloride; Gpx Mimics; Antioxidant Activity (LOQ)

INTRODUCTION

Lipid-soluble organoselenium compound ebselen(1,2-phenyl-1,2-benzisoselenazol- 3(2H)one)(1), undergoing phase III clinical trials for a number of diseases such as stroke, hearing loss and exhibits several biological activities both in vitro and in vivo systems [1]. Ebselen is an excellent scavenger of reactive oxygen species (ROS) such as peroxynitrite (PN) and the rate of the reaction between ebselen and PN is about three orders of magnitude higher than that of naturally occurring small molecules, such as ascorbate, cysteine, and methionine [2]. Although ebselen exhibits interesting therapeutic properties, including anti- inflammatory activity and also recent evidence suggests that ebselen and its analogous are exhibits substantial antitumor and immunomodulating activities [3,4]. The catalytic mimicking activity of selenoenzymes, glutathione peroxidase (GPx, are essentially due to the ability of ebselen to catalyze the reduction of hydroperoxides by glutathione (GSH) or other thiols. In recent report it was also reported the molecular characterization of ebselen binding activity to the SARS- CoV-2 main protease [5] In contrast to this, ebselen have been associated with its ability to mimic the enzymatic properties of most of the biological activities [6-8] (Figure 1).

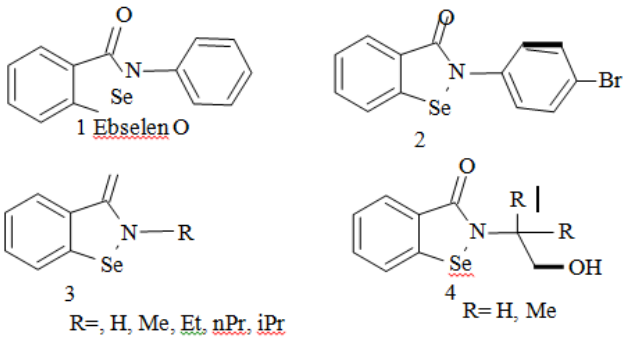
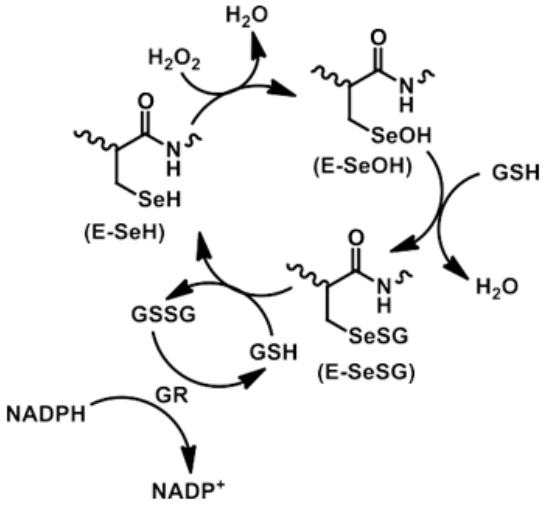


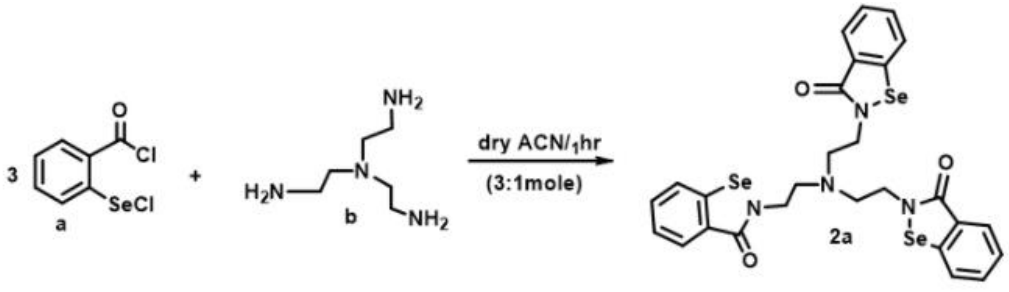
Figure 1: Proposed Ebselen analogues having different substituents on nitrogen atom

A mammalian selenoenzyme glutathione peroxidase (GPx), that protects various organisms from oxidative damage by catalyzing the reduction of harmful hydroperoxides in the presence of glutathione (GSH) (Scheme-1) or other thiol cofactor systems. Therefore, after the discovery of ebselen exhibits significant antioxidant activity by mimicking the active site of GPx. In view of this many researchers has been devoted to design and synthesis of novel analogues of ebselen [6-8].



Scheme-1: Proposed catalytic mechanism for the reduction of hydroperoxides by GPx

In view of this, several research groups have reported a number of small-molecules of selenium compounds which include the ebselen analogues, benzoselenazolinones, selenenamide as functional mimics of GPx, either by modifying the basic structure of ebselen or by substituting some structural features of the native enzyme [7-10]. The chemistry of a mammalian selenoenzyme (GPx) active site has been extensively investigated with the help of synthetic organoselenium compounds. Therefore, in order to understand the catalytic mechanism and antioxidant activity of benzoselenazol with reference to the standard ebselen (Scheme 2).



Scheme 2: Synthesis of 2,2',2"-(nitrilotris(ethane-2,1-diyl))tris(benzo[d][1,2]selenazol-3(2H)-one) (2a)

In the present communication we have synthesized benzoselenazol (2a) from 2, 2'- Selenobenzoyl chloride treated with N,N-bis(2-aminoethyl)ethane-1,2-diamine with an excellent yield. The synthesized compound was systematic characterization and checked their GPx-like antioxidant activity and compared its antioxidant activity with standard ebselen. From obtained results the reported compounds shows potential antioxidant that protects various organisms from oxidative stress by catalyzing the reduction of harmful peroxides such as H_2O_2 in the presence of glutathione (GSH).

RESULTS AND DISCUSSION

The synthesis of 2,2',2"-(nitrilotris(ethane-2,1-diyl))tris(benzo[d][1,2]selenazol-3(2H)-one) (2a): 2,2′-selenobenzoic acid with thionyl chloride afforded 2,2′-selenobenzoyl chloride, which is the key precursor for the synthesis of benzoselenazol used in this study. The reaction of 2, 2'-Selenobenzoyl chloride reacts with N,N-bis(2-aminoethyl)ethane-1,2- diamine (3:1 molar ratio) in a dry acetonitrile for 1h at room temperature afforded yellowish crystalline solid 2,2',2"-(nitrilotris(ethane-2,1-diyl))tris(benzo[d][1,2]selenazol-3(2H)- one)(2a) with very good yield.

The isolated compound was purified by column chromatography and systematically characterized by ^{1}H, ^{13}C, ^{77}Se NMR and mass spectrometric analysis. In ^{77}Se NMR shows a sharp peak at 876ppm it confirms the formation of ebselen selenazol ring and it was compared with ebselen [3,8] (Figure 2 & 3). In addition to this, reported compound was also confirmed by using IR, $^{1}H/^{13}C$-NMR and mass spectroscopic analysis with m/z= 689.9 [M^{+}].

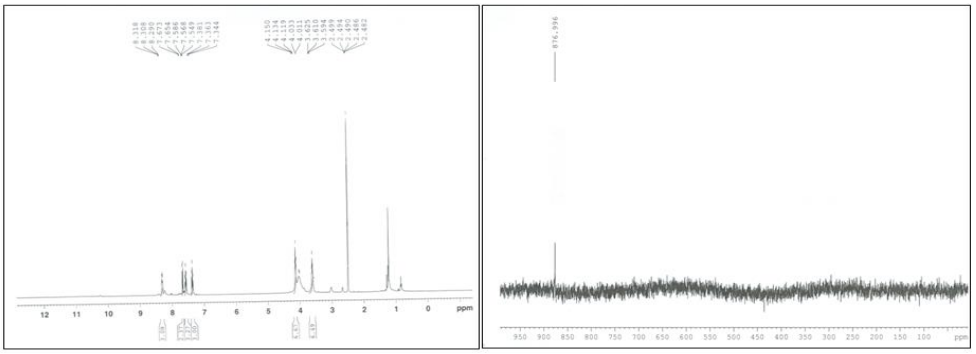


Figure 2: $^{1}H/^{77}Se$ NMR spectra of 2,2',2"-(nitrilotris(ethane-2,1-diyl))tris(benzo[d][1,2]selenazol-3(2H)-one) (2a)

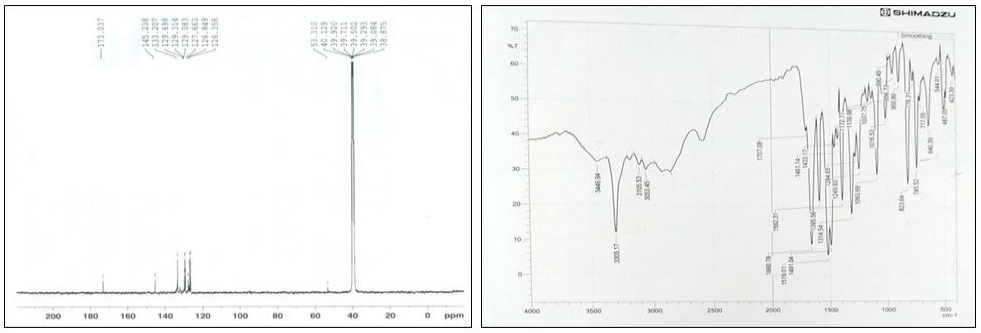
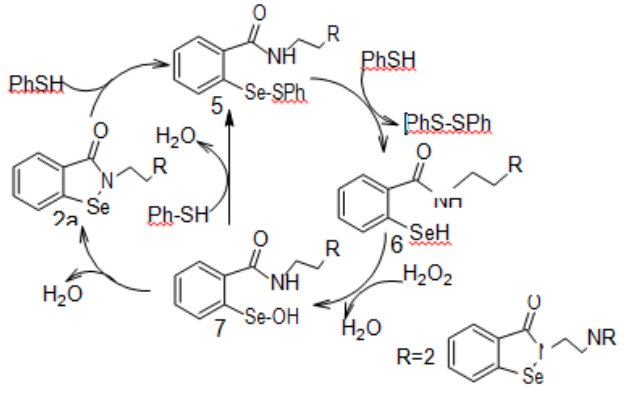


Figure 3: ^{13}CNMR and IR spectra of 2,2',2"-(nitrilotris(ethane-2,1-diyl))tris(benzo[d][1,2]selenazol-3(2H)-one) (2a)

Glutathione Peroxidase (GPx)-Like activity

In continuations of our research work on the synthesis of chalcogenuranes and antioxidant activity benzoselen zol(2a) exhibits significant antioxidant activity by mimicking glutathione peroxidase enzyme. Similarly, in our previous study also showed that some of the amino acid substituted ebselens exhibited higher antioxidant activity compared to standard ebselen [8-15] (Scheme 3).



Scheme 3: Proposed catalytic cycle of 1,2,3- benzoselenazol- 3(2H)-one)(2a) and related ebselen compounds

According to this mechanism, catalytic cycle of benzoselenazol (2a) reacts with a thiol (RSH) to produce the corresponding selenenyl sulfide5. This compound undergoes a disproportionation reaction to generate the diselenide 6 and is found to be unstable in the assay system. Consequent reaction

of 6 with peroxide produces the selenenic acid 7. Interestingly, the selenenic acid 7 having a free N-H moiety undergoes oxidative cyclization to regenerate benzoselenzol(2a) [11, 13, 16]. Therefore, one of the objectives of this study was to check the GPx-like catalytic activities of reported compound by using H_2O_2 as the substrate and glutathione (GSH) as the co-substrate. Peroxide reductions of initial rates (v0) were determined in the presence of different catalysts at 80 µM concentration. In Table-1 the initial rate values are clearly indicate that reported compound benzoselenzol (2a) show consistent activity compared to that of the ebselen. The present study ebselen used as catalysts for the comparison.

Table 1: Effect of substrate (H_2O_2) concentration on the initial rate of Ebselen and Benzoselenazol

Compound	v0 (µM.min^{-1})[a]
Ebselen	99.06 ± 3.23
Benzoselenazol	92.81 ± 2.86

[a]= Phosphate buffer (100 mM, pH 7.5) at 20ºC were carried out reactions. Catalyst: 80.0 µM; glutathione reduced 2.0 mM; NADPH: 0.4 mM; glutathione disulfide reductase 1.7 unitmL^{-1} peroxide: 1.6mM. The background reaction between peroxide and thiol were corrected with initial rates.

MATERIALS AND METHODS

Chemicals were used of highest purity. Selenium powder was purchased from Aldrich Chemical Co. Acetonitrile were dried over P_2O_5. For the synthesis Schlenk techniques and all experiments were carried out under anhydrous and anaerobic conditions. Several reactions mixtures are carryout due to unpleasant odors we used a well-ventilated fume hood. ^{1}H (400 MHz) and ^{77}Se (76.29 MHz) NMR spectra were obtained at room temperature on a 400 MHz NMR spectrometer (Bruker Optik, Ettlingen, Germany). Chemical shifts are quoted with respect to SiMe4 as internal (^{1}H), and Me2Se as external (^{77}Se) standards. UV/Vis spectrophotometer was used to measure GPx activity A Perkin–Elmer Lambda. The melting points (uncorrected) of the compounds were determined in an open capillary with a B-540 Melting Point apparatus (BOchi Labortechnik AG, Flawil, Switzerland). Mass spectral studies were carried out on a Q-TOF micro mass spectrometer (Waters Inc, Milford, MA, USA) with ESIMS mode analysis. Column chromatography was performed on glass columns loaded with silica gel or on an automated flash chromatography system (Biotage) by using preloaded silica cartridges. Thin-layer Chromatography analyses were carried out on precoated silica gel plates (Merck), and spots were visualized by UV irradiation.

GSH–GSSG Coupled Assay

GSH-GSSG coupled assay: The GPx antioxidant activity were carryout by using UV-visible spectrophotometer. The initial reduction rates and rate of NADPH oxidation at 340 nm were calculated in a GSH assay. The initial rate each sample 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 molar extinction coefficient for NADPH. For the peroxidase activity, the rates were corrected for the background reaction between peroxide and thiol.

Synthesis of 2,2',2''-(nitrilotris(ethane-2,1-diyl))tris(benzo[d][1,2]selenazol-3(2H)- one) (2a)

Disodium diselenide 0.11gm (1.40mmol) was prepared freshly according to the literature procedure and maintained under 0-5ºC. The isolated compounds treated with thionyl chloride afforded 2,2′-selenobenzoyl chloride as reported in literature [3, 8, 17]. 2,2′- selenobenzoyl chloride 1.14gm (4.50mmol) prepared separately in 25ml dry acetonitrile (ACN) to this add N,N-bis(2-aminoethyl)ethane-1,2-diamine 0.219gm(1.50mmol) drop wise and continue the reaction for additional 1h at room temperature. A yellowish solid was separate out the obtained the product was recrystallized by using acetonitrile and further purified by column chromatography. Yellowish solid 84% yield. FT-IR (νmax/cm^{-1}, KBr): 3305.17, 3053.45, 1660.78, 1519.01, 1491.04, 1314.54, 1093.69, 823.64, 745.52, 640.39, 487.05. ^{1}H-NMR (DMSO-d_6, ppm): δ 7.35–7.37 (d, J = 7.35 Hz, 2H), 7.39 (d, 2H), 7.55– 7.57 (d, J = 7.55 Hz, 2H), 7.59–7.60 (d, J = 7.58 Hz, 2H), 7.67 (d, 2H), 8.24–8.26 (d, J = 8.23 Hz, 2H), 3.59-3.62 (d, J = 3.61 Hz, 6H), 4.11–4.15 (d, J = 4.13 Hz, 6H). ^{13}C-NMR (DMSO-d_6, ppm): δ 47.70, 53.31, 116.65, 126.84, 127.66, 129.08, 129.31, 129.69, 133.20, 145.23, 173.03. ESI-MS (m/z) Calcd, $C_{27}H_{24}N_4O_3Se_3$: 690.6, found: 689.9 [M$^+$].

CONCLUSION

In conclusion we have reported efficient synthesis of 2,2',2"- (nitrilotris(ethane-2,1-diyl)) tris(benzo[d][1,2]selenazol-3(2H)-one)2a. The synthesized benzoselenazol was achieved by rapid conversion when 2, 2'-selenobenzoyl chloride mixed with N,N-bis(2-aminoethyl)ethane-1,2-diamine with quantitative yield. The reported compounds ware characterized by various spectroscopic techniques. The glutathione peroxidase (GPx) mimetic activity was discussed and it was observed that the reported compound shows powerful antioxidant activity with reference to the standard ebselen.

Supplementary Materials

Copies of the IR, ^{1}H-NMR, ^{13}C-NMR, and ^{77}SeNMR spectra for compound are available online.

Acknowledgments

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