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Synthesis and Radical Scavenging Properties of Selenophenyl Benzamide Anologs

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ABSTRACT

Free radicals that result from different kinds of oxidative stress have been concerned in a variety of human disorders, from cardiac ischemia to those affecting the central nervous system. So, there is an increasing interest in the development of antioxidant molecules that can protect cells against free radical damages. Sets of tetrasubstituted selenophene amides **4a-e** were synthesized by reaction of 2-amino-5,6,7,8-tetrahydro-4H-cyclohepta[b]selenophen-3-carbonitrile (**2)** with benzoyl chloride derivatives and the structures of the amide derivatives were characterized by MS, ^{1}H-NMR, ^{13}C-NMR and IR spectra. The synthesized compounds (**4a-e**) were evaluated in terms of *in vitro*. The antioxidant properties were determined by 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging properties and IC_{50} values of the compounds were in the range from 3.794 to 5.644 mg/mL. Compounds **4c** and **4e** showed predominant radical scavenging activity among the synthesized analogues.

Keywords: *Selenophene, Selenopheneyl benzamide, Antioxidant activity*

1. INTRODUCTION

The structures of selenaheterocyclic compounds are closely related to those of analogous sulfur compounds, but their properties often present marked difference. Reactive oxygen species such as hydroxyl radical, superoxide anion and peroxynitrite are involved in many cellular processes including the inflammatory response. The best known antiinflammatory compound is ebselen the first synthetic organoselenium therapeutic released on the market.

Ebselen (**1**) acts as glutathione peroxidase (GPx) mimic by reducing hydroperoxides to water or the corresponding alcohol. [1-4]

Having recognized the role of GPx enzymes in biochemical reactions, considerable efforts have been made to design and develop low-molecular weight organoselenium compounds, which can emulate the activity of naturally occurring selenoenzymes, GPx.[5-7]

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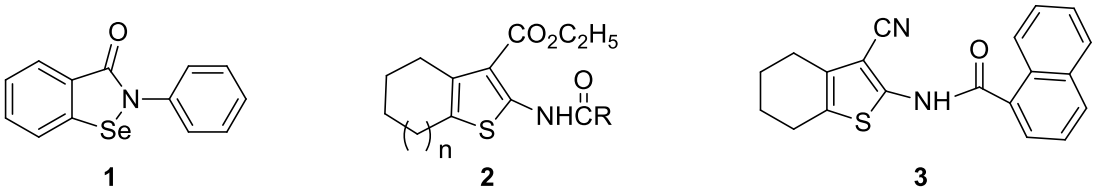


1 **2** **3**

Benzamide derivatives which are the possible metabolites of benzoxazoles show various types of biological properties such as anthelmintic, antihistaminic, antifungal, and antibacterial.[8-10]

Substituted 2-aminothiophenecarboxamides are a class of heterocycles that have attracted a great deal of research interest due their great usefulness as precursors of molecules with pharmacological properties. Thiophene amide derivatives **2** were investigated in basic pharmacological screens. It was found that most of the derivatives minör depressants and some were more potent analgesic activity than acetyl salicyclic acid.[11]

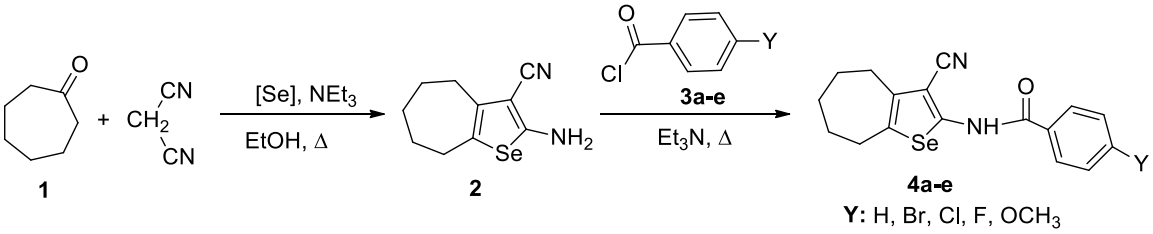
N-(3-cyano-4,5,6,7-tetrahydro-1-benzothien- 2-yl)amide derivatives **3** were identified as potent inhibitors of JNK3 with essentially equal potency against JNK2.[12]

In the literature, there are more study of the synthesis and biological evaluation of thiophene amide derivatives than its selenium anologs, selenophenyl amide, derivatives and in view of the abovementioned facts, it seemed most interesting to synthesize some condensed N-(3-cyano-5,6,7,8-tetrahydro-4H-cyclohepta[b]selenophene-2-yl)benzamide derivatives with the aim to evaluate their free radical scavenging activities.

2. RESULTS AND DISCUSSION

2.1. Chemistry

In order to explore the structure–activity relationships (SAR) at the N-(3-cyano-5,6,7,8-tetrahydro-4H-cyclohepta [b] selenophene-2-yl) benzamide derivatives which are substituted in the 4-position of aromatic ring, we embarked upon the synthesis of analogues of **4** using procedures illustrated in Scheme 1. Amine **2** was prepared from cycloheptanone, selenium and malononitrile under conditions reported by Gewald, followed by formation of amides, standard conditions.[12, 13] The compounds were purified by repeated recrystallization from ethanol and then dried. ^{1}H NMR spectra of compounds **4a–e** with the **2** has shown disappearance of signal of NH_2 (4.97 ppm) proton on the formation of amide bond and new signals of NH (9.02-9.12) appeared. The IR spectra of **4a-e** revealed the presence of C=O stretching band at 1640–1662 cm^{-1} in all the analogues.



1 **2** **4a-e**

Y: H, Br, Cl, F, OCH_3

Scheme 1. Synthesis of N-(3-cyano-5,6,7,8-tetrahydro-4H-cyclohepta[b]selenophene-2-yl) benzamide derivatives

2.2 Free radical scavenging activity

The ability to scavenging the stable free radical, 1,1-diphenyl-2-picrylhydrazyl (DPPH), was measured as a decrease in absorbance at 517 nm. IC_{50} values for compounds were determined in the range from 3.794 to 5.644 mg/mL. The best activity in the synthesized compounds has shown **4c** (IC_{50} 3.794 ± 0.041 mg/mL), having chlorine atom which conferred both electron-withdrawing inductive effects and electron-donating resonance effects at *p*-position in the aromatic ring. The positioning the chloro retained antioxidant activity because the intermediate radical was stabilized by the electron-donating resonance effect of chlorine in spite of the electron-withdrawing inductive effect of chlorine. Also the compound **4e** (IC_{50} 3.865 ± 0.164 mg/mL), bearing an electron-donating methoxy group at para position, showed the better DPPH radical scavenging activity compared to synthesized compounds (Table 1). Selenium-containing compounds showed moderate levels of DPPH radical scavenging *in vitro.* El-Sawy *et al.* synthesized a series of heterocyclic derivatives of 2-amino-4-(1-benzoylindol-3-yl)selenophene-3-carbonitrile and tested at its DPPH radical scavenging properties.[14] IC_{50} values of these compounds were measured in the range of 166.40-2844.95 µg/mL. These results are very parallel with IC_{50} values of our compounds. In summary, N-(3-cyano-5,6,7,8-tetrahydro-4H-cyclohepta[b]selenophene-2-yl)benzamide, and variants with additional substitution in the benzene ring may be represent potential radical scavenger compounds.

Table 1. DPPH radical scavenging activity (IC_{50} (mg/mL)) of novel N-(3-cyano-5,6,7,8-tetrahydro-4H-cyclohepta[b]selenophene-2-yl) benzamide compounds.

Compounds	IC_{50} (mg/mL)
4a	3.991±0.082
4b	5.644±0.246
4c	3.794±0.041
4d	4.483±0.123
4e	3.865±0.164

3. CONCLUSION

A series of tetrasubstituted selenophenyl benzamides **4a-e** were synthesized by reaction of amine **2** with benzoyl chloride derivatives. The synthesized compounds (**4a-e**) were evaluated in terms of in vitro DPPH radical scavenging properties and IC_{50} values of the compounds were in the range from 3.794 to 5.644 mg/mL.

4. EXPERIMENTAL

4.1. Chemistry

All chemicals investigated in the study were reagent grade and were purified when it was necessary. All organic solvents used in this study were purified according to standard methods. MS were obtained from Waters LCT Premier XE LTOF (TOF MS) instruments (Waters Corporation, Milferd MA, USA). ^{1}H and ^{13}C-NMR spectra were recorded with a Bruker DPX-300 MHz and 100 MHz using TMS as an internal standard and $CDCl_3$ as solvent. Mass spectra were recorded on a Micro Mass-UK Platform II mass spectrometer at Tubitak, Ankara, Turkey. IR spectra were recorded on a Mattson-5000 FT-IR instrument in KBr pellets. Melting points were determined with a Gallenkamp melting point apparatus.

The synthesis of 2-amino-5,6,7,8-tetrahydro-4H-cyclohepta[b]selenophen-3-carbonitrile (2)

Cycloheptanone (5,61 g, 0.05 mol), malononitrile (3,30 g, 0.05 mol) and selenium (3,95 g, 0.05 mol) was added into anhydrous ethanol (50 mL). Morpholine (4.36 g, 0.05 mol) was added dropwise into mixture at room temperature. The resulting mixture was refluxed for 48 hours. After this time the mixture was cooled to room temperature and poured into water-ice bath. The resulting solid was filtered off and the product was crystallized in ethanol.

Yield 71 %; m.p. 100–102 °C. IR (KBr), ν(cm^{-1}): 3425-3333 (NH_2),2192 (CN). ^{1}H NMR ($CDCl_3$) δ (ppm)= 1.63 (m, 4H, CH_2), 1.79 (m, 2H, CH_2), 2.58 (m, 2H, CH_2), 2.61 (m, 2H, CH_2), 4.97 (bs, 2H, NH_2). ^{13}C NMR ($CDCl_3$) δ (ppm)= 27.2, 28.2, 30.6, 31.1, 32.1, 93.6 (C-CN), 117.2 (CN),128.3, 138.0, 163.6 (C-NH_2).

The synthesis of N-(3-cyano-5,6,7,8-tetrahydro-4H-cyclohepta[b]selenophene-2-yl) benzamide derivatives 4a-e: General Procedure

2-Amino-5,6,7,8-tetrahydro-4H-cyclohepta[b]selenophene-3-carbonitrile (0.24 g, 0.001 mol) and triethylamine was added into dioxane (30 mL) at room temperature. Benzoyl chloride derivatives (0.0012 mol) were added dropwise into the mixture. Resulting mixture was refluxed for 7 hours. After this time the mixture cooled to room temperature and the mixture's solvent was removed by rotary evaporation. The resulting solid product was crystallized from ethanol.

The synthesis of N-(3-cyano-5,6,7,8-tetrahydro-4H-cyclohepta[b]selenophene-2-yl)-benzamide (4a)

Yield 73 %; m.p. 198-199 °C. IR (KBr), ν(cm^{-1}): 3242 (N-H), 3062 (=C-H), 2910, 2204 (CN), 1646 (C=O) . ^{1}H NMR ($CDCl_3$) δ (ppm)= 1.74 (m, 4H, CH_2), 1.88 (m, 2H, CH_2), 2.77 (m, 2H, CH_2), 2.80 (m, 2H, CH_2), 7.56 (t, *J*= 1.4 Hz, 2H, Ar-H), 7.65 (t, *J*= 1.4 Hz, 1H, Ar-H) 7.94 (d, *J*= 3.6 Hz, 2H, Ar-H), 9.08 (bs, 1H, NH). ^{13}C-APT NMR ($CDCl_3$) δ (ppm) = 27.2, 27.9, 30.1, 30.7, 32.1, 98.3, 116.3, 127.4, 129.1, 131.7, 133.1, 136.6, 138.3, 148.3, 163.7.

HRMS: m/z calculated for C17H16N2OSe $[M + H]^+$ (343,2817), found: 343,0339

The synthesis of N-(3-cyano-5,6,7,8-tetrahydro-4H-cyclohepta[b]selenophen-2-yl)-4-bromobenzamide (4b)

Yield 66%; m.p. 246-248 °C. IR (KBr), ν(cm^{-1}): 3228 (N-H), 3058 (=C-H), 2911 (C-H), 2211 (CN), 1660 (C=O). ^{1}H NMR ($CDCl_3$) δ (ppm) = 1.70 (m, 4H, CH_2), 1.87 (m, 2H, CH_2), 2.76 (m, 2H, CH_2), 2.81 (m, 2H, CH_2), 7.67 (d, *J*= 8.6 Hz, 2H, Ar-H), 7.79 (d, *J*= 8.6 Hz, 2H, Ar-H), 9.06 (bs, 1H, NH). ^{13}C-APT NMR (DMSO-d_6) δ (ppm) = 27.1, 28.2, 29.8, 30.2, 32.3, 99.8, 116.5, 126.9, 131.8, 130.9, 132.0, 137.2, 138.4, 149.0, 165.1.

HRMS: m/z calculated for C17H15BrN2OSe $[M + H]^+$ (422.1778), found: 420.9448

The synthesis of N-(3-cyano-5,6,7,8-tetrahydro-4H-cyclohepta[b]selenophen-2-yl)-4-chlorobenzamide (4c)

Yield 69%; m.p. 244-246 °C. IR (KBr), ν(cm^{-1}): 3232 (N-H), 3062 (=C-H), 2914 (C-H), 2209 (CN), 1662 (C=O) . ^{1}H NMR ($CDCl_3$) δ (ppm) = 1.72 (m, 4H, CH_2), 1.90 (m, 2H, CH_2), 2.75 (m, 2H, CH_2), 2.80 (m, 2H, CH_2), 7.52 (d, *J*= 8.5 Hz, 2H, Ar-H), 7.87 (d, *J*= 8.5 Hz, 2H, Ar-H), 9.12 (bs, 1H, NH). ^{13}C-APT NMR (DMSO-d_6) δ (ppm) = 27.2, 28.0, 29.8, 30.3, 32.1, 99.2, 116.5, 129.0, 130.8, 131.5, 137.2, 137.9, 138.4, 149.1, 164.8.

HRMS: m/z calculated for C17H15ClN2OSe $[M + H]^+$ (377.7268), found: 376,9962

The synthesis of N-(3-cyano-5,6,7,8-tetrahydro-4H-cyclohepta[b]selenophen-2-yl)-4-fluorobenzamide (4d)

Yield 67%; m.p. 223-225 °C. IR (KBr), □□(cm^{-1}): 3230 (N-H), 3076 (=C-H), 2901, 2211 (CN), 1658 (C=O). ^{1}H NMR ($CDCl_3$) □ (ppm)= 1.71 (m, 4H, CH_2), 1.87 (m, 2H, CH_2), 2.77 (m, 2H, CH_2), 2.81 (m, 2H, CH_2), 7.22 (d, *J*= 8.5 Hz, 2H, Ar-H), 7.95 (d, *J*= 8.5 Hz, 2H, Ar-H), 9.02 (bs, 1H, NH). ^{13}C-APT NMR (DMSO-d_6) □ (ppm) = 27.2, 28.0, 29.8, 30.1, 32.0, 99.1, 116.1, 116.5, 129.2, 131.8, 137.1, 138.3, 149.2, 163.4, 164.9.

HRMS: m/z calculated for C17H15FN2OSe $[M + H]^+$ (361,2722), found: 361,0244

The synthesis of N-(3-cyano-5,6,7,8-tetrahydro-4H-cyclohepta[b]selenophen-2-yl)-4-methoxybenzamide (4e)

Yield 71%; m.p. 197-199 °C. IR (KBr), □□(cm^{-1}): 3308 (N-H), 3165 (=C-H), 2930 (C-H), 2203 (CN), 1640 (C=O). ^{1}H NMR ($CDCl_3$) □ (ppm) = 1.73 (m, 4H, CH_2), 1.91 (m, 2H, CH_2), 2.78 (m, 2H, CH_2), 2.83 (m, 2H, CH_2), 3.94 (s, 3H, OCH_3), 7.05 (d, *J*= 8.5 Hz, 2H, Ar-H), 7.92 (d, *J*= 8.5 Hz, 2H, Ar-H), 9.03 (bs, 1H, NH). ^{13}C-APT NMR ($CDCl_3$) □ (ppm) = 27.2, 27.9, 30.1, 30.7, 32.2, 55.6, 97.9, 114.4, 116.3, 123.8, 129.0, 136.4, 137.9, 148.6, 163.5, 163.7

HRMS: m/z calculated for C18H18N2O2Se $[M + H]^+$ (373.3077), found: 373.0459

Free radical scavenging activity

The ability of a compound to donate a hydrogen atom was assessed on the basis of the scavenging activity of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical according to a procedure based on Shimada *et al.* (1992) with slight modifications. The reaction mixture was a total volume of 3 mL, which included 2.9 mLof DPPH ($1x10^{-4}$ M DPPH) and 0.1 mL of the corresponding sample at various concentrations. The solutions were left in the dark at room temperature for 30 min and the resulting color was measured spectrophotometrically at 520 nm against blanks. A decreasing intensity of the color purple was related to a higher radical scavenging power percentage, which was calculated using the following equation;

% radical scavenging activity = $(1-A_{sample}/A_{control})\times100$

IC_{50} which denotes the amount (mg) of compounds in 1 mL solution required to reduce initial concentration of DPPH radicals by 50% was also calculated.[15]

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CONFLICT OF INTEREST

No conflict of interest was declared by the authors.

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