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Synthesis, Characterization and Antioxidant Activity of 3-(2-Amino-1,3-Selenazol-4-yl)-2H-Chromen-2-Ones Derivatives

(Sintesis, Pencirian dan Aktiviti Antioksidan Terbitan 3-(2-Amino-1,3-selenazol-4-il)-2H-Kromen-2-On)

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ABSTRACT

Selenocompounds have been widely synthesized for their potential in pharmacology. Ebselen, a selenazole oxide, is a glutathione peroxidase mimic which is known to possess high antioxidant activity. Four derivatives of 3-(2-amino-1,3-selenazol-4-yl)-2H-chromen-2-ones were synthesized by reacting 3-(2-bromoacetyl)-chromen-2-one derivatives with selenourea through Hantzsch reaction using NaF as a catalyst in methanol-water (1:1) at room temperature. These reactions were completed in 30 min and purified using column chromatography eluted with n-hexane-ethyl acetate (7:3) to give 50-83% yields. All the compounds were successfully characterized using IR, ^{1}H and ^{13}C NMR as well as mass spectrometry. The synthesized compounds were tested with DPPH assay to determine the free radical scavenging activity and were compared to gallic and ascorbic acids as standard. Nonetheless, all compounds exhibited weak free radical scavenging activity with IC_{50} value ranging from 672.13 to 984.03 µM signifying that the derivatives may possess weak antioxidant activities.

Keywords: Coumarin; selenazole; synthesis

ABSTRAK

Sebatian seleno telah disintesis secara meluas kerana potensinya dalam bidang farmakologi. Ebselen iaitu sebatian selenazol oksida merupakan mimik glutation peroksida yang diketahui mempunyai aktiviti antipengoksida yang tinggi. Empat sebatian 3-(2-amino-1,3-selenazol-4-il)-2H-kromen-2-on disintesis dengan menindakbalaskan terbitan 3-(2-bromoasetil)-kromen-2-on dengan selenourea melalui tindak balas Hantzsch dan menggunakan NaF sebagai mangkin dalam metanol-air (1:1) pada suhu bilik. Tindak balas ini selesai dalam 30 min dan ditulenkan dengan kromatografi turus yang dielusikan dengan n-heksana-etil asetat (7:3) bagi memberikan 50-83% hasil. Kesemua sebatian dicirikan menggunakan spektroskopi IM, RMN ^{1}H dan ^{13}C serta spektrometri jisim. Sebatian yang telah disintesis diuji dengan asai DFPH bagi menentukan aktiviti penggarutan radikal bebas dan dibandingkan dengan asid galik dan askorbik sebagai piawai. Walau bagaimanapun, kesemua sebatian menunjukkan aktiviti penggarutan radikal bebas yang lemah dengan julat nilai IC_{50} diantara 672.13 ke 984.03 µM menandakan terbitan sebatian ini mungkin mempunyai aktiviti antioksida yang lemah.

Kata kunci: Koumarin; selenazol; sintesis

Introduction

The 1,3-Selenazoles were mainly synthesized by the application of the Hantzsch procedure. Various 1,3-selenazole derivatives have been prepared but so far there are only three studies reported on the synthesis of coumaryl 1,3-selenazoles. Madhav et al. (2008) have developed an efficient method to synthesize 1,3-selenazoles in good yields with high purity. The α-Bromoketones and selenourea with $CuPy_2Cl_2$ which act as a catalyst were ground in a mortar and pestle at room temperature without any solvent. The reactions were completed in 30 min with percentage yields of 87-96%. The experimental procedure was extremely simple with mild reaction conditions, environmentally friendly catalyst, no product waste or toxic solvent and a reusable catalyst. Following this study, Banothu et al. (2014) employed NaF as a catalyst in methanol-water (1:1) which decreases the reaction time from 30 min to between 1-3 min only. Recently, Ramesh et al. (2015) took a further step by applying ultrasonic irradiation and successfully shortened the reaction time from 1-3 min to only 10-60 s. All the described procedures gave excellent percentage yields of about 87-99%.

3-(2-amino-1,3-selenazol-4-yl)-*2H*-chromen-2-ones constitutes two important classes of compounds which are coumarins and 1,3-selenazoles. Both of them were known for their wide applications in pharmaceutical industry. Coumarins in both natural or synthesized exhibit wide range of bioactivities including anti-HIV, antimalarial, insecticides, antioxidant, anti-inflammatory, antitumor, hepatoprotective, anti-allergic, antiviral, antifungal, antimicrobial, antiasthmatic, antinociceptive,

antidiabetic and antidepressant activity (Anand et al. 2012; Kumar et al. 2011). Similarly, 1,3-selenazoles have been widely synthesized for their potential in pharmacology and material sciences. Selenazofurins which contain 1,3-selenazole rings are known as potent antiviral agents and 2-phenyl-1,2-benzisoselenazol-3(*2H*)-one or *Ebselen* is a known glutathione peroxidase mimics which possesses antioxidant activity (Madhu et al. 2014; Mao et al. 2013; Pizzo & Mahler 2014; Zhao et al. 2013). This study attempt to combine coumarin and 1,3-selenazole to form a new compound with wide application in bioactivities. We hypothesized that the combination of these two moieties may exhibit high potential as antiviral, antifungal, antimicrobial and antioxidants. This report will provide new insight on the potential of these compounds as free radical scavengers considering that there are no previous reports deliberating on the biological activities of these derivatives. This study aims to synthesize 3-(2-amino-1,3-selenazol-4-yl)-*2H*-chromen-2-ones and to determine the free radical scavenging activities of these derivatives.

Materials and Methods

3-(2-bromo-acetyl)-chromen-2-one, selenourea, NaF, anhydrous Na_2SO_4 and solvents (methanol, *n*-hexane, ethyl acetate) were purchased from Sigma-Aldrich and Friendemann Schmidt and used directly without further purification. Melting points were determined by using Electrothermal Melting Point Model 9100. Infrared spectra were obtained using FTIR Perkin Elmer-with spotlight 400 Imaging System in the spectral range of 4000-650 cm^{-1}. ^1H and ^{13}C were recorded by using Jeol JNM-ECP 400MHz NMR spectrometer. The gas chromatograph mass spectrometer (GC/MS) analysis were performed on an Agilent 7890A gas chromatograph (GC) directly coupled to the mass spectrometer system (MS) of an Agilent 5975C inert MSD with triple-axis detector. Electrospray ionization mass spectrometry (ESIMS) was recorded on Dionex, Bruker, MicroToF Q apparatus.

Synthesis of 3-(2-amino-1,3-selenazol-4-yl)-*2H*-chromen-2-ones

The general procedure for synthesizing 3-(2-amino-1,3-selenazol-4-yl)-*2H*-chromen-2-one (Scheme 1) was as follows: A mixture of 3-(2-Bromoacetyl)-chromen-2-one (1 mmol) and selenourea (1 mmol) were dissolved in 2 mL methanol and 2 mL water and added 0.02 g NaF. The mixture was stirred at room temperature for 30 min. After the completion of the reaction, water (5 mL) was added to the reaction mixture and extracted with ethyl acetate. The organic layer was dried over anhydrous Na_2SO_4. The solvent was evaporated under reduced pressure and the resulting product was further purified by column chromatography (*n*-hexane-ethyl acetate 7:3) (Ramesh et al. 2015; Madhav et al. 2008).

Free radical-scavenging activity (RSA): DPPH assay

The RSA of the derivatives was measured according to the method reported previously with some modification (Mahdavi et al. 2016). Briefly, 1.5 mL aliquot of each samples at 16.125, 31.25, 62.50, 125 and 250 µg/mL was added to 1 mL of 0.1 mM DPPH in methanol. The mixture was agitated vigorously for 1 min, and allowed to stand in the dark for 90 min at room temperature. The absorbance value was recorded at 517 nm. Gallic and ascorbic acids were used as reference control. All measurements were carried out in triplicate. The RSA of samples, was expressed as percentage inhibition of DPPH using the following equation:

$$I(\%) = \left[\frac{A_c - A_0}{A_c} \right] \times 100,$$

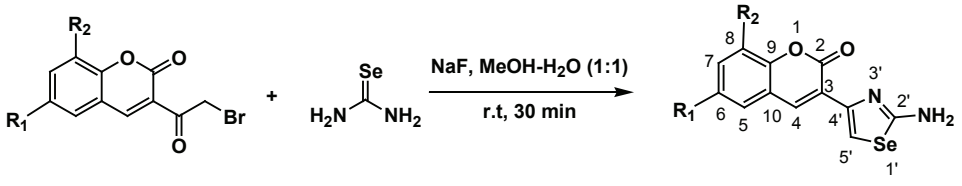
where A_c is the absorbance value of the control (DPPH solution without samples); and A_0 is the absorbance value of the compound (DPPH solution with samples).

Results and Discussion

3-(2-Amino-1,3-selenazol-4-yl)-*2H*-chromen-2-ones, b(1-4) were characterized by IR, ^1H and ^{13}C NMR and mass spectral data. In addition, radical scavenging activity for these compounds were also determine using DPPH assay presented as IC_{50} value. Overall, the reactions gave reasonable to good yields (50.1-83.2%) as summarized in Table 1. The melting points measured were similar with previous studies.

Infrared spectra

In the infrared spectrum of 3-(2-amino-1,3-selenazol-4-yl)-*2H*-chromen-2-one, b1 it was possible to observe the



b1, R_1: H; R_2: H
b2, R_1: H; R_2: CH_3
b3, R_1: Br; R_2: Br
b4, R_1: Cl; R_2: Cl

Scheme 1. Formation of 3-(2-amino-1,3-selenazol-4-yl)-*2H*-chromen-2-ones

TABLE 1. The yield (%) and melting point (°C) of 3-(2-Amino-1,3-selenazol-4-yl)-*2H*-chromen-2-one derivatives

Compounds	Yield (%)	Melting point (°C)	
		Observed	Literature review (Ref.)
b1	50.1	218-220	218-220 (Ramesh et al. 2015)
b2	81.7	212-214	166 (Madhav et al. 2008)
b3	83.2	252-253	326-328 (Ramesh et al. 2015)
b4	56.9	242-244	98 (Madhav et al. 2008)

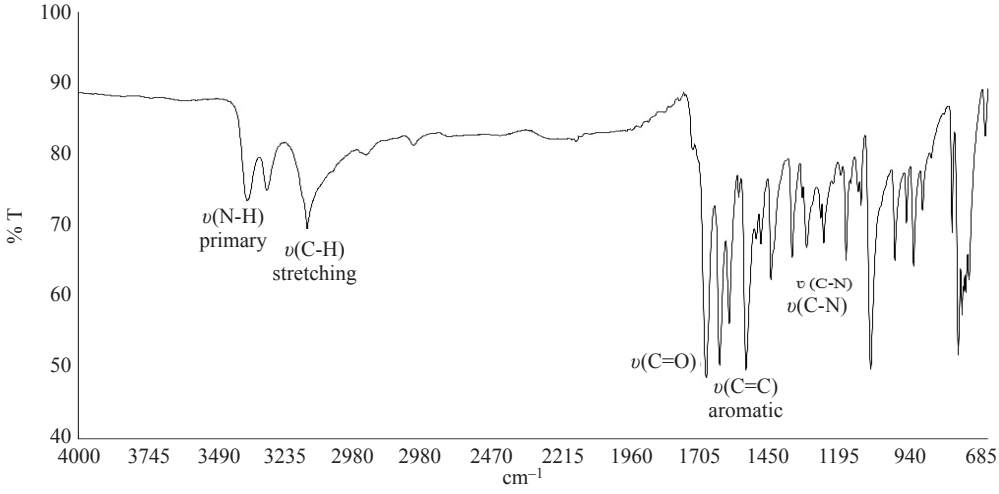


FIGURE 1. The infrared spectrum of compound 3-(2-Amino-1,3-selenazol-4-yl)-*2H*-chromen-2-one

absorptions at 3377 and 3305 cm^{-1} relating to N-H stretch for primary amine, absorption at 1687 cm^{-1} for coumarin carbonyl stretch and absorption at 1254 cm^{-1} relating to C-N stretch for amine group (Figure 1).

The characteristic functional groups for all derivatives were listed in Table 2. Two clear absorptions for primary amine were observed in the range of 3305-3397 cm^{-1} and 3377-3484 cm^{-1}. A strong absorption for C=O group were observed between 1687-1735 cm^{-1}. Compound b2, b3 and b4 showed higher absorption for C=O compared to b1 cause by the presence of CH_3, Cl and Br group which tend to draw in the electrons between carbon and oxygen atoms through its electron-withdrawing effect, so that the C=O becomes stronger.

NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY

Figure 2 shows the ^{1}H NMR spectrum for compound b1 ($C_{12}H_8N_2O_2Se$) as representative for 3-(2-amino-1,3-selenazol-4-yl)-*2H*-chromen-2-one derivatives. While the chemical shifts for ^{1}H NMR and ^{13}C NMR for b1 ($C_{12}H_8N_2O_2Se$), b2 ($C_{13}H_{10}N_2O_2Se$), b3 ($C_{12}H_6Br_2N_2O_2Se$) and b4 ($C_{12}H_6Br_2N_2O_2Se$) were listed in Tables 3 and 4, respectively. ^{1}H NMR spectrum for b1 showed clear signals for all protons except for NH protons which overlapped with H-8 signal. The spectrum also showed that H-4 is the most downfield proton at 8.54 ppm.

TABLE 2. The stretching frequencies for 3-(2-Amino-1,3-selenazol-4-yl)-*2H*-chromen-2-one derivatives

Compounds	IR Spectrum (cm^{-1})		
	υ (N-H) primary	υ (C=O)	υ (C-N)
b1	3377, 3305	1687	1254
b2	3440, 3397	1718	1252
b3	3484, 3379	1735	1297
b4	3381, 3342	1720	1250

Compound b2 showed a CH_3 signal at 2.38 ppm for a methyl group at C-8. All signals for aromatic protons were observed between 7.35 and 8.54 ppm. Signals for H-5 and H-7 were observed at 7.46-8.06 ppm and 7.35-8.06 ppm, respectively, with clear multiplicity except for b3 which only exhibit a singlet. Compound b3 and b4 exhibit higher chemical shifts for H-5 and H-7 because the presence of two halogen group and C-6 and C-8. Other than that, H-6 signals were observed at 7.58 ppm for b1 and 7.24 ppm for b2 while signal for H-8 for compound b1 was observed at 7.42 ppm. A broad NH_2 proton signal for all derivatives were observed between 7.43-7.52 ppm while H-4 and H-5′ signals showed two clear singlet signals at 8.42-8.54 ppm and 8.06-8.14 ppm, respectively.

From the ^{13}C NMR spectra, the signals for all compounds were observed between 110.2 and 169.6 ppm except for CH_3 signal for compound b2 which was observed at 15.4 ppm. Signals for C-5′ of selenazole rings were observed at the most upfield region between 110.2 and 116.1 ppm while signals for C-4′ of selenazole rings appeared between 143.7 and 145.8 ppm. Signals at 158.0-159.4 ppm belong to C=O for coumarin ring and the most

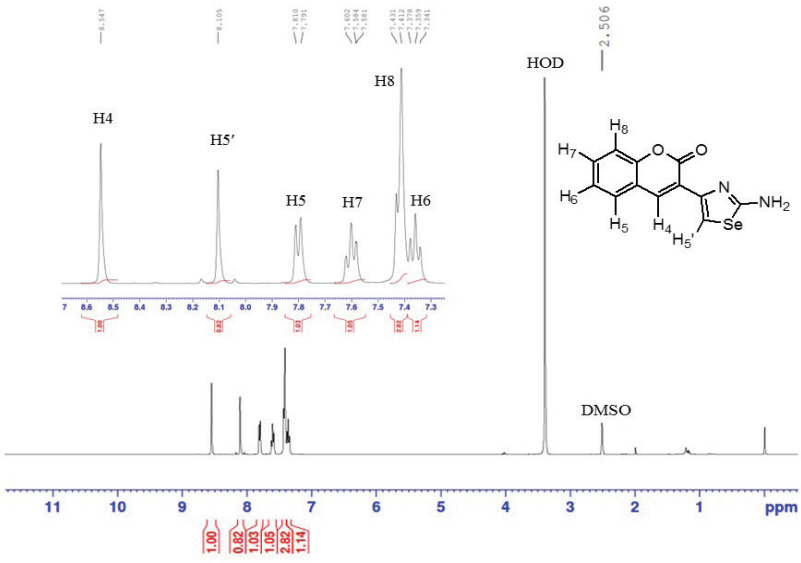


FIGURE 2. ^{1}H NMR spectrum for compound b1 in DMSO-d_6 solvent

TABLE 3. The ^{1}H NMR signals for 3-(2-amino-1,3-selenazol-4-yl)-*2H*-chromen-2-one derivatives

Compounds	δ, ppm (coupling patterns, *J-coupling*)					
	H5, H7	H6, H8	H4	H5′	NH_2	CH_3
b1 $C_{12}H_8N_2O_2Se$	7.80 (d, *J*=7.2) 7.35 (t, *J*=7.4)	7.58 (td, *J*=7.2, 1.2) 7.42 (t, *J*=8.4)	8.54 (s)	8.10 (s)	7.43 (br)	-
b2 $C_{13}H_{10}N_2O_2Se$	7.46 (d, *J*=6.4) 7.58 (d, *J*=7.2)	7.24 (t, *J*=7.2, 7.6) -	8.49 (s)	8.06 (s)	7.52 (br)	2.38 (s)
b3 $C_{12}H_6Br_2N_2O_2Se$	8.06 (s)	-	8.42 (s)	8.12 (s)	7.51 (br)	-
b4 $C_{12}H_6Cl_2N_2O_2Se$	7.86 (d, *J*=1.6), 7.92 (d, *J*=1.6)	-	8.45 (s)	8.14 (s)	7.47 (br)	-

s: singlet; d: doublet; t: triplet; td: triple doublet; br: broad

downfield signals around 169.0-169.6 ppm belong to C-2′ of selenazole rings (Koketsu & Ishihara 2008; Kurt et al. 2015).

TABLE 4. The ^{13}C NMR signals for 3-(2-amino-1,3-selenazol-4-yl)-*2H*-chromen-2-one derivatives

Carbon	δ, ppm			
	b1	b2	b3	b4
CH_3	-	15.4	-	-
C5′	114.3	114.1	110.2	116.1
C8	116.2	119.5	116.0	120.8
C10	119.9	120.8	116.9	122.4
C3	121.4	124.8	122.8	122.8
C6	125.1	125.2	126.3	128.8
C5	129.0	126.8	130.6	127.0
C7	131.8	133.0	136.0	130.6
C4	139.1	139.6	137.4	137.3
C4′	144.6	143.7	145.8	143.8
C9	152.6	150.9	148.4	146.9
C=O	159.3	159.4	158.2	158.0
C2′	169.0	169.4	169.6	169.3

MASS SPECTROMETRY

The mass spectral data for compounds b(1-4) were listed in Table 5 and the fragmentation for compound 3-(2-amino-1,3-selenazol-4-yl)-*2H*-chromen-2-one, b1 ($C_{12}H_8N_2O_2Se$) were shown in Figure 3. The molecular ion peak for compound b1 is observed at *m/z* = 292.0 and an intense fragmentation peak is observed at *m/z* = 211 after the cleavage of SeH molecule (MW = 81 g/mol).

While *m/z* for b2, b3 and b4 were recorded at 306.99, 448.80 and 360.90, respectively, which represent

TABLE 5. The mass spectral data for compounds b(1-4)

Compounds	Mol. Formula (Mol. weight)	EIMS (*m/z*)/ESIMS
b1	$C_{12}H_8N_2O_2Se$ (290.96)	EIMS: (*m/z*) = 292.0
b2	$C_{13}H_{10}N_2O_2Se$ (305.99)	ESIMS: $[M+H]^+$ = 306.99
b3	$C_{12}H_6Br_2N_2O_2Se$ (447.80)	ESIMS: $[M+H]^+$ = 448.80
b4	$C_{12}H_6Cl_2N_2O_2Se$ (359.90)	ESIMS: $[M+H]^+$ = 360.90

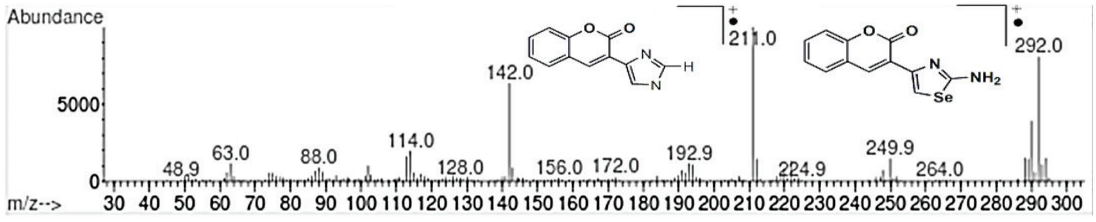


FIGURE 3. The mass-spectrum showing the molecular ion of 3-(2-amino-1,3-selenazol-4-yl)-*2H*-chromen-2-one (b1) and its fragmentations

$[C_{13}H_{10}N_2O_2Se+H]^+$, $[C_{12}H_6Br_2N_2O_2Se+H]^+$ and $[C_{12}H_6Cl_2N_2O_2Se+H]^+$.

FREE RADICAL-SCAVENGING ACTIVITY

Table 6 shows the IC_{50} (μM) values for each compound and standards. Unfortunately, the tested compounds did not exhibit a remarkable free radical scavenging activity with IC_{50} values recorded between 672.13-984.03 μM which are greater than standards (9.09 and 28.43 μM for gallic and ascorbic acids).

TABLE 6. IC_{50} (μM) values for compounds b1-b4 and standards

Compounds	IC_{50} (μM)
b1	843.59 ± 71.41
b2	639.81 ± 9.67
b3	672.13 ± 81.68
b4	984.03 ± 156.47
Gallic acid	9.09 ± 0.15
Ascorbic acid	28.43 ± 1.52

CONCLUSION

In conclusion, four derivatives of 3-(2-amino-1,3-selenazol-4-yl)-*2H*-chromen-2-one were successfully synthesized *via* Hantzsch reaction and their radical scavenging activities were evaluated. Even though the synthesized compounds exhibited weak free radical scavenging activity signifying a weak antioxidant, these derivatives may possess high potential as antiviral, antifungal, antimicrobial or other bioactivities. This report provides new insight on the free radical scavenging activity of 3-(2-amino-1,3-selenazol-4-yl)-*2H*-chromen-2-ones considering that there are no previous reports deliberating on the biological activities of these compounds.

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