Afyon Kocatepe Üniversitesi Fen ve Mühendislik Bilimleri Dergisi

Afyon Kocatepe University Journal of Science and Engineering

AKÜ FEMÜBİD **19** (2019) 021204 (317-327) AKU J. Sci. Eng. **19** (2019) 021204 (317-327)

DOI: 10.35414/akufemubid.542712

Araştırma Makalesi / Research Article

Synthesis of Indole-3-Carboxyaldehyde Thiosemicarbazone Derivatives and Investigation of Antioxidant and Anticholinesterase Properties

Murat BİNGÜL*

**Dicle Üniversitesi, Eczacılık Fakültesi, Mesleki Bilimler Bölümü, Farmasötik Kimya Anabilim Dalı, Diyarbakır.*

e-posta: muratbingul1983@gmail.com. ORCID ID: http://orcid.org/0000-0002-3909-0694.

Geliş Tarihi: 21.03.2019; Kabul Tarihi: 12.06.2019

Keywords
Indole;
Thiosemicarbazone;
Antioxidant;
Anticholinesterase

Abstract

The synthesis of four indole-3-carboxyaldehyde thiosemicarbazone compounds (**3a-d**) was achieved via the Schiff base reaction of the indole-3-carboxyaldehyde and thiosemicarbazides in high yields. The structures of thiosemicarbazones **3a** and **3b** were supported by ^{1}HNMR spectroscopy and confirmed with the literature data. To the best of our knowledge, our study is the first report for the synthesis of compound **3c** and literature search revealed that compound **3d** was also not fully characterised. The structures of compound **3c** and **3d** were fully characterised by the FT-IR, High Resolution Mass Spectrometry (HRMS), ^{1}H and ^{13}CNMR spectroscopy in this work for the first time. Moreover, antioxidant properties of synthesised compounds **3a-d** were investigated with the DPPH, ABTS and CUPRAC assays as well as the anticholinesterase properties of designated compounds were determined by the Acetylcholinesterase (ACh) and Butyrylcholinesterase (BCh) enzyme inhibition assays. The compound **3a, 3b and 3d** were determined very potent against the ABTS antioxidant assay and compound **3c** was found to be a valuable target molecule for the kinetic measurements to identify mechanism of action in the area of anticholinesterase activity assay.

İndol-3-Karboksialdehit Tiyosemikarbazon Türevlerinin Sentezi Antioksidan ve Antikolinesteraz özelliklerinin araştırılması

Anahtar kelimeler
İndol;
Tiyosemikarbazon;
Antioksidan;
Antikolinesteraz

Öz

Dört indol-3-karboksialdehit tiyosemikarbazon bileşiği indol-3-karboksialdehit ve tiyosemikarbazit bileşiklerinden Schiff bazı reaksiyonu kullanılarak yüksek verimlerde sentezlenmiştir. **3a** ve **3b** tiyosemikarbazon yapıları ^{1}HNMR spektroskopisiyle desteklenerek literatür verileriyle uyumlu olduğu kanıtlanmıştır. Bildiğimiz kadarıyla, çalışmamız **3c** bileşiğinin sentezi ve **3d** bileşiğinin tüm karakterizasyonlarının yapılması açısından ilk bilimsel çalışmadır. **3c** ve **3d** bileşiklerinin yapıları, FT-IR, yüksek çözünürlüklü kütle spektroskopisi, ^{1}H ve ^{13}CNMR spektroskopileri ile ilk olarak bu çalışmada aydınlatılmıştır. Dahası, sentezlenen **3a-d** bileşiklerinin antioksidan özellikleri üç farklı DPPH serbest radikal süpürme, ABTS katyonik radikal süpürme, CUPRAC kuprik iyonunu indirgeme kapasitesi yöntemlerinin uygulanmasıyla belirlenmiştir. Bununla beraber, belirtilen bileşiklerin antikolinesteraz özellikleri, Asetilkolinesteraz (ACh) ve Bütirilkolinesteraz (BCh) enzim inhibisyonu deneyleriyle araştırılmıştır. **3a**, **3b** ve **3d** bileşiklerinin ABTS antioksidan metodu için çok etkili olduğu belirlenmiş ve **3c** bileşiğinin antikolinesteraz aktivite alanında kinetik ölçümlerinin yapılarak etki mekanizmasının aydınlatılması konusunda değerli bir hedef molekül olduğu anlaşılmıştır.

1. Introduction

Throughout the past twenty years, thiosemicarbazones have been the subject of many structural and medicinal studies de (Oliveria *et al*. 2008, Dilovic *et al*. 2008, Yu *et al*. 2009) and these compounds have shown potent biological properties namely antiviral, antibacterial, antifungal antioxidant and anticancer activities (Hu *et al*. 2006,

Pavan *et al*. 2010). The preparation of thiosemicarbazone derivatives were achieved by the Schiff base condensation of aldehyde or ketones with the thiosemicarbazides (Zhang *et al*. 2011, Hu *et al*. 2010). It was proved that the conjugated *N,N,S* system in the thiosemicarbazone scaffolds have an important role for the interaction with biomolecules (Antholine *et al*. 1977). Due to the above mentioned reasons, an intense research was carried out on the thiosemicarbazones than the free thiosemicarbazide systems as the reaction with aldehydes or ketones help to increase their biological capacity (Shahabadi *et al*. 2010, Refat *et al*. 2012). A wide variety of heterocyclic systems have been used for the structural modifications of the targeted thiosemicarbazone systems (Hosseini-Yazdi *et al*. 2017, Rogolino *et al*. 2017, Piri *et al*. 2017, Liu *et al*. 2010). Indole heterocyclic systems are pharmacologically valuable scaffolds and biologically active natural and synthetic compounds are composed of these heterocyclic systems (Alley *et al*. 2004, Kamal *et al*. 2002). In the past century, a great interest has been given on the area of indole chemistry and different classes of these systems have been evaluated for interesting biological properties (Gözler *et al*. 1990, Havoundjian *et al*. 1987, Pchalek *et al*. 2005). While extensive studies have been performed on the cytotoxic activities of thiosemicarbazones compounds (Dilovic *et al*. 2008, Zhang *et al*. 2011, Hu *et al*. 2010, Gust *et al*. 2004) recent reports have shown that these ligands could also be useful agents for the other biological properties (Garoufis *et al*. 2009, da Silva *et al*. 2011, Salas *et al*. 2013). It was proved that these structures are quite valuable systems for antioxidant studies due to the ability of donation hydrogen or electron to the acceptors (i.e. DPPH) and reduce the production of free radicals (Hosseini-Yazdi *et al*. 2017, Piri *et al*. 2017). Moreover, a very recent report proved the ability of these systems for treatment of Alzheimer disease (AD) (Palanimuthu *et al*. 2017). It was suggested that the accumulation of bioavailable transition metals Fe, Cu and Zn would be responsible for the Alzheimer disease since the self-aggregation of amyloid-β (Aβ) via metal peptide chelation to form senile plaques, which are deposited outside of neurons (Palanimuthu *et al*. 2017). The presence of conjugated *N,N,S* tridentate systems on thiosemicarbazones would reduce the accumulation of these metals and chelation ability would be a potential therapy for the treatment of AD (Palanimuthu *et al*. 2017). In the present work, the synthetic procedures and chemical characterization of indole-3-carboxyaldehyde thiosemicarbazone systems 3a-d are reported. The targeted compounds have been subjected to the three different assays to investigate the antioxidant properties. Moreover ACh and BCh enzyme inhibition properties were also evaluated. We aimed to compare the influence of thiosemicarbazone structure over designated biological properties and make correlations between their biological activities. Three of the synthesised compounds 3a, 3b, 3d have previously been prepared by the other groups (Liu *et al*. 2010, Kakul *et al*. 2008, Haribabu *et al*. 2018) and used as a part of investigation of different biological properties such as anticancer (Haribabu *et al*. 2018) and anti-amoebic (Kakul *et al*. 2008, Liu *et al*. 2010). However, compound **3c** has been reported for the first time in our report. More importantly, to the best of our knowledge, our study is the first report for the investigation of antioxidant and anticholinesterase properties and a complementary comparison is also made between the designated properties in our study.

2. Materials and methods

2.1. Chemicals and Physical measurements

All commercially available reagents and the standards used for the biological assays were purchased from Sigma Aldrich and used without further purification. The general synthetic procedure was written for the synthesized compounds and the known compounds were reported with the appropriate references. The TLC chromatographic method was used to monitor the reactions. Merck was the supplier for the Silica gel 60 (particle size: 0.040-0.063 mm, 230-400 mesh ASTM) and the Thin Layer Chromatography plates. d^6-DMSO was the solvent for the ^1H and ^{13}C NMR spectra were recorded on a Bruker DPX 400 MHz

spectrometer at 300 K. Chemical shifts were reported as ppm and the solvent peak d^6-DMSO was given as ^{1}H d 2.50 ppm, ^{13}C d 39.52 ppm. (*J*) was given as coupling constants in Hertz (Hz) unit. m= multiplet, t= triplet, d= doublet, s= singlet, dd= doublet of doublets illustrated the standard conventions indicating multiplicity. The Thermo Scientific Nicolet IS10 FT-IR spectrometer was used for the Infrared spectroscopy data between 600 and 4000 cm^{-1}. The Mel-Temp melting point apparatus was used for the melting points measurements. The Orbitrap LTQ XL (Thermo Scientific, Waltham, MA, USA) ion trap mass spectrometer was used for the High-Resolution [ESI] mass spectra.

2.2. Biological studies

2.2.1. Antioxidant assays

DPPH Free Radical Scavenging Activity

The DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) free radical scavenge ability was determined with the method outlined in the reference (Blois 1958) with the minor modifications.

ABTS Cation Radical Decolorization Activity

The cationic radical reducing capabilities of the compounds were investigated in the presence of $ABTS^{\bullet+}$ (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)). The assay was performed with the standards reported in the reference (Re *et al*. 1999).

CUPRAC Cupric Ion Reducing Antioxidant Capacity

The antioxidant capacity of the synthesised compounds against the Cupric Ion was determined with the CUPRAC assay and the method was performed with procedure reported in the reference (Apak *et al*. 2004).

2.2.2. Anticholinesterase activity determination method

ACh and BCh enzyme inhibitory activity assays were performed with the minor modifications reported in the reference (Ellman *et al*. 1961). Galanthamine was used as standard. The samples and control was dissolved in DMSO.

$$\% \text{ Inhibition} = (A_0 - A_1) / A_0 \times 100$$

where A_0 is the value for the control absorbance and A_1 is the value for the sample absorbance

2.3. Statistical analysis

The three parallel data was obtained with the mean ± SD and Student's *t*-test was used for statistical comparisons. The *p* values < 0.05 were regarded as significant.

2.4. Chemical synthesis

2.4.1. General Procedure for the Preparation of Thiosemicarbazones

The aldehyde (1 eq.) was reacted with the appropriate thiosemicarbazides (1 eq.) in ethanol. Acetic acid (5 drops) was added as catalyst and the solution was stirred overnight at room temperature. The resulting dark yellow solution was concentrated under vacuum and the resulting yellow solid was crystallised from ethanol.

(2*Z*)-2-[(1*H*-indol-3-yl)methylidene]hydrazine-1-carbothioamide (3a)

The title compound was synthesized following the general procedure using 3-formylindole (**1**) (300mg, 2.06 mmol) and thiosemicarbazide (**2a**) (188 mg, 2.06 mmol) in EtOH (25 mL) with 5 drops of acetic acid. The compound was obtained as a white powder; yield: 72%; 217-218 °C (lit. (25) m.p. 220 °C); ^{1}H-NMR (d^6-DMSO): δ 11.6 (1H, s, indole NH), 11.2 (1H, s, NH), 8.3 (1H, s, CH), 8,2 (1H,d, H4, *J* = 8 Hz), 8.0 (1H, s, H2), 7.8 (1H, d, H7 *J* = 2.4 Hz), 7.4 (2H, d, NH2 *J* = 8 Hz), 7.1 (1H, t, H6, *J* = 7.6 Hz), 7.1 (1H, t, H5, *J* = 7.6 Hz), IR: ν_{max} 2925 C–H, 1535 C=N 1610 C=S 3138 N–H 3309 and 3444 cm^{-1} NH_2

(2*Z*)-2-[(1*H*-indol-3-yl)methylidene]-*N*-methylhydrazine-1-carbothioamide (3b)

The title compound was synthesized following the general procedure using 3-formylindole (**1**) (300mg, 2.06 mmol) and 4-methylthiosemicarbazide (**2b**) (217 mg, 2.06 mmol) in EtOH (25 mL) with 5 drops of acetic acid. The compound was obtained as a white powder; yield: 77%; 203-204 °C (lit. (14) m.p. 209-210 °C); ^{1}H-NMR (d^6-DMSO): δ 11.6 (1H, s, indole NH), 11.2 (1H, s, NH), 8.3 (1H, s, CH), 8,3 (1H,d, H4, *J* = 8 Hz), 7.9 (1H, d, NH *J* = 4,8 Hz), 7,8 (1H, s, H2), 7.8 (1H, d, H7 *J* = 8 Hz) 7.2 (1H, t, H6, *J* = 7.0 Hz), 7.1 (1H, t, H5, *J* = 7.2 Hz), 3.7 (3H, d, CH3, *J* =

3.6 Hz) IR: ν_{max} 2929 C–H, 1524 C=N, 1606 C=S, 3172 N–H and 3362 cm^{-1} N–H

(2*Z*)-2-[(1*H*-indol-3-yl)methylidene]-*N*,*N*-dimethylhydrazine-1-carbothioamide (3c)

The title compound was synthesized following the general procedure using 3-formylindole (**1**) (300mg, 2,06 mmol) and 4,4-dimethylthiosemicazrbazide (**2c**) (246 mg, 2,06 mmol) in EtOH (25 mL) with 5 drops of acetic acid. The compound was obtained as a yellow powder; yield: 67%; 215-216 °C; ^{1}H-NMR (d^6-DMSO): δ 11.5 (1H, s, indole NH), 10.6 (1H, s, NH), 8.4 (1H, s, CH), 8,3 (1H,d, H4, *J* = 8.0 Hz), 7,7 (1H, d, H2 *J* = 0,4 Hz), 7.4 (1H, d, H7 *J* = 8 Hz) 7.2 (1H, t, H6, *J* = 7.6 Hz), 7.1 (1H, t, H5, *J* = 7.6 Hz), 3.3 (6H, s, CH3); ^{13}C NMR: δ 180.4 (C=S), 142.1 (C=N), 137.5, 130.2, 124.6, 123.0, 122.6, 120.8, 112.3, 112.1 (aryl-C), 42.2 (2×CH3) (CH2), 15.3 (CH3); IR: ν_{max} 2922 C–H, 1530 C=N, 1605 C=S, 3145 cm^{-1} N–H; HRMS (ESI^+): found *m/z* 247.1012, $[M+H]^+$, $C_{12}H_{15}N_4S$ requires 247.1019.

(2*Z*)-*N*-ethyl-2-[(1*H*-indol-3-yl)methylidene]hydrazine-1-carbothioamide (3d)

The title compound was synthesized following the general procedure using 3-formylindole (**1**) (300mg, 2,06 mmol) and 4-ethylthiosemicazrbazide (**2d**) (246 mg, 2,06 mmol) in EtOH (25 mL) with 5 drops of acetic acid. The compound was obtained as a pale yellow powder; yield 82%; 220-221°C; ^{1}H-NMR (d^6-DMSO): δ 11.6 (1H, s, indole NH), 11.1 (1H, s, NH), 8.3 (1H, s, CH), 8,2 (1H,d, H4, *J* = 8 Hz), 7.9 (1H, t, NH *J* = 5.6 Hz), 7,8 (1H, s, H2), 7.4 (1H, d, H7 *J* = 8 Hz) 7.2 (1H, t, H6, *J* = 7.4 Hz), 7.1 (1H, t, H5, *J* = 7.4 Hz), 3.6 (2H, t, CH2, *J* = 6.8 Hz), 1.2 (3H, t, CH3, *J* = 7.0 Hz); ^{13}C NMR: δ 176.1 (C=S), 140.9 (C=N), 137.4, 131.3, 124.4, 123.0, 122.4, 121.0, 112.2, 111.6 (aryl-C), 38.7 (CH2), 15.3 (CH3); IR: ν_{max} 2926 C–H 1528 C=N 1606 C=S 3175 N–H and 3350 cm^{-1} N–H; HRMS (ESI^+): found *m/z* 247.1012, $[M+H]^+$, $C_{12}H_{15}N_4S$ requires 247.1010.

3. Results and Discussion

3.1. Chemistry

The desired indole-3-carboxyaldehyde thiosemicarbazones **3a-d** were obtained by Schiff base reaction of the indole-3-carboxyaldehyde **1** with appropriate thiosemicarbazides **2a-d** [14], [25], [26] using acetic acid in ethanol at room in yields of 67%–82% (Scheme 1).

The FT-IR and ^{1}H NMR spectroscopic data was given for the compounds **3a-b** to confirm the previous data. The compounds **3c-d** were fully characterised by the FT-IR, ^{1}H NMR, ^{13}C NMR and HRMS spectrometric analysis methods. The ^{1}H NMR spectra showed a characteristic singlet CH proton at 8.3-8.8 ppm, whereas the corresponding thioamide NH proton appeared at 11,1–11,7 ppm for all the indole-3-carboxyaldehyde thiosemicarbazones. The unsubstituted NH_2 protons resonated at 7.4 ppm in the case of compound **3a**. The protons for methyl groups at the thiosemicarbazone N end of the compound **3b** was found at 3.1 ppm, whereas two methyl group for the compound 3c appeared around 3.2 and 3.6 ppm. In the case of compound **3d**, the methylene and methyl groups at the thiosemicarbazone N end resonated as triplet and doublet signals around 1.2 ppm and 3.6 ppm, respectively. The characteristic NH indole peak appeared at 11.6 ppm, whereas the H2 proton resonated at 7.8 ppm as singlet. The H5 and H6 protons appeared at 7.2 and 7.1 ppm as triplet signals and two doublet of doublets signals demonstrated the H4 and H7 protons at 8.2 and 7.4 ppm, respectively. The ^{13}C NMR spectrums of compounds **3c**, **3d** confirmed the structure of the molecules, displaying the thiocarbonyl carbon signals, at 180.4 and 176.1 ppm, as a result of condensations of the aldehyde groups with thiosemicarbazides.

It was also proved with the appearance of signals at 142.1 ad 140.9 ppm corresponding to the azomethine CH carbon atoms in the products. FT-IR spectrums of the indole-3-carboxyaldehyde thiosemicarbazone derivatives showed the azomethine C–H stretching doublet absorption band at 2920–2970 cm^{-1}, whereas the absorption band at 1520–1535 cm^{-1} corresponded to the C=N moieties. Thiosemicarbazone C=S stretching

appeared at 1605-1610 cm^{-1} and a strong N–H stretching at 3140-3180 cm^{-1} appeared for all the synthesised compounds. The other N–H stretching is found around 3340-3470 cm^{-1} in the case of the mono substituted compounds 3b and 3d at the thiosemicarbazone N end. The derivative with no substitution on the NH_2 group showed broad two stretchings around 3250-3450 cm^{-1} demonstrated the NH_2 signals.

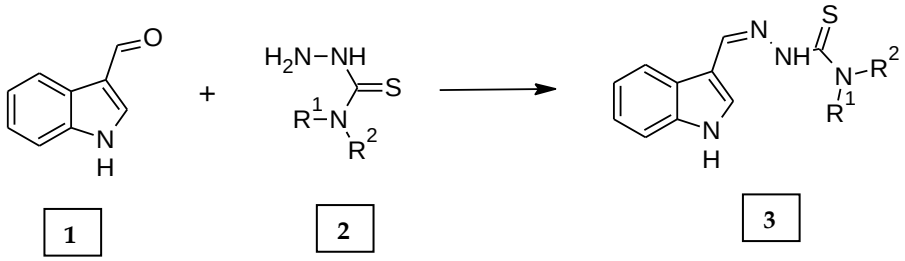
3.2. Biological studies

3.2.1. DPPH Free Radical Scavenging Assay

The Figure 1 shows the DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) radical scavenging activity of the four compounds **3a-d**, BHT, BHA and α-TOC, as standards, at the concentrations from 10 μM to 100 μM. The synthesised compounds were found to be less effective systems compared to the standards. In the case of compounds **3b** and **3d** same pattern of inhibitions were observed which the inhibition increased gradually starting from the 25 μM concentration. In the case of 100 μM concentration, the compound **3b** with methyl substitution at the thiosemicabazone N end showed the highest inhibition with the value of around 18%, whereas the ethyl substitution showed a little decrease in inhibition (around 16%). However, the compounds **3a** and **3c** demonstrated the lowest inhibitions. It was concluded that chemical structures of compounds altered at the thiosemicarbazone N end with the presence of two methyl substitutions (compound **3c**) and no substitution on NH_2 group (compound **3a**) resulted dramatic inhibition decrease for the DPPH assay.

3.2.2. ABTS Cation Radical Decolorization Assay

The initial assay was carried on the higher concentrations from 10 μM to 100 μM and



Scheme 1. Reagents and conditions: EtOH, a few drops AcOH, overnight rt

Thiosemicarbazide	R^1	R^2	Product
2a	H	H	**3a**
2b	H	Me	**3b**
2c	Me	Me	**3c**
2d	H	Et	**3d**

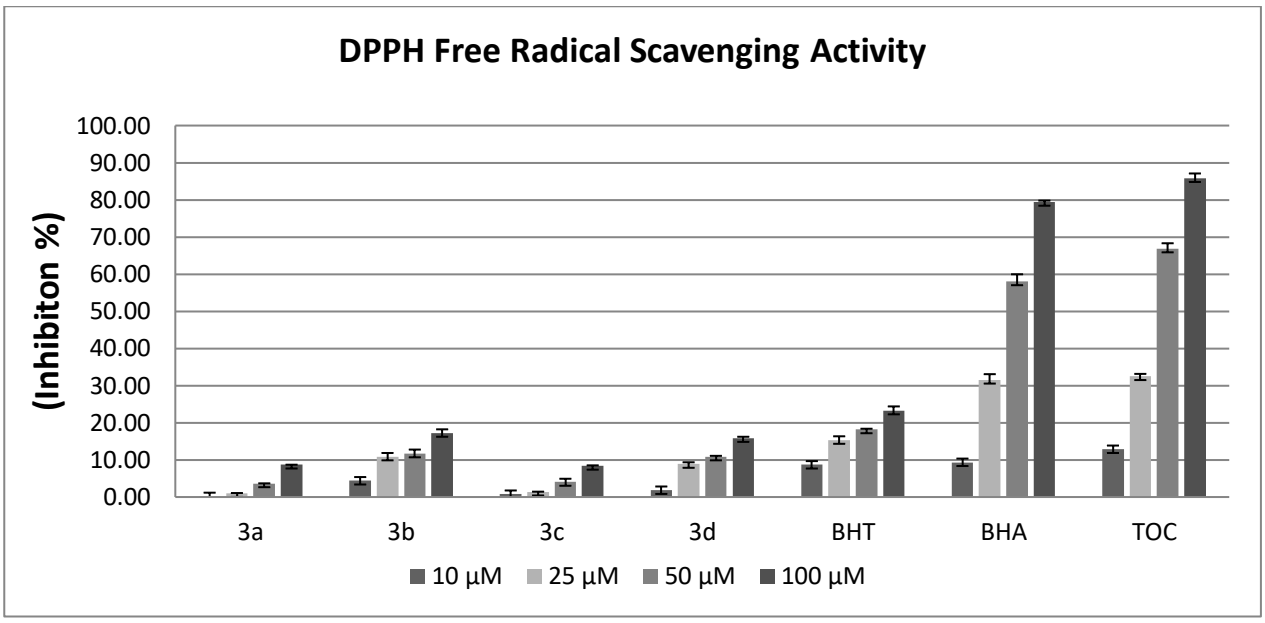


Figure 1. Inhibition (in %) values for the **3a-d** and controls in the DPPH assay. Values are means, ±SD, $n = 3$, $p < 0.05$, significantly different from each other with Student's *t*-test.

the compounds showed very potent activity. The assay was repeated with the diluted concentrations from 1 μM to 10 μM. The Figure 2 shows cationic radical reducing ability of the targeted systems. The compounds showed valuable scavenging activities by the increasing concentrations. The higher inhibition ability than the controls BHT and α-TOC was obtained in the case of compounds **3a**, **3b** and **3d** at 1 μM concentration. The 20% inhibition value was obtained in the presence of compounds **3b** and **3d** and was found to be better than all the controls at the same concentration. The similar better inhibitions pattern was obtained at the 2,5 μM concentration for the two compounds **3b** and **3d.** The highest inhibition was determined with the values of more than 65% at the 10 μM concentration which are better than the controls BHT and α-TOC in the presence of both compounds **3b** and **3d**. The compounds **3b** and **3d** are the examples of methyl and ethyl substitutions on the thiosemicarbazone N end. Similar better inhibition pattern was also observed in the case of compound **3a** with the no substitution on the thiosemicarbazone end. At the all concentrations, the compound **3a** showed higher inhibition than α-TOC and as good inhibition as control BHT. The highest inhibition was obtained at the 10μM concentrations with the value of 60% which is better than BHT and α-TOC standards. Molecular structure of compound reflected that no substitution on the N end of the thiosemicarbazone system was also responsible for the inhibition. The lowest inhibitions at all concentration were determined in the case of compound **3c** with two methyl substitutions on the thiosemicarbazone moiety and unreasonable results were not given on the figure.

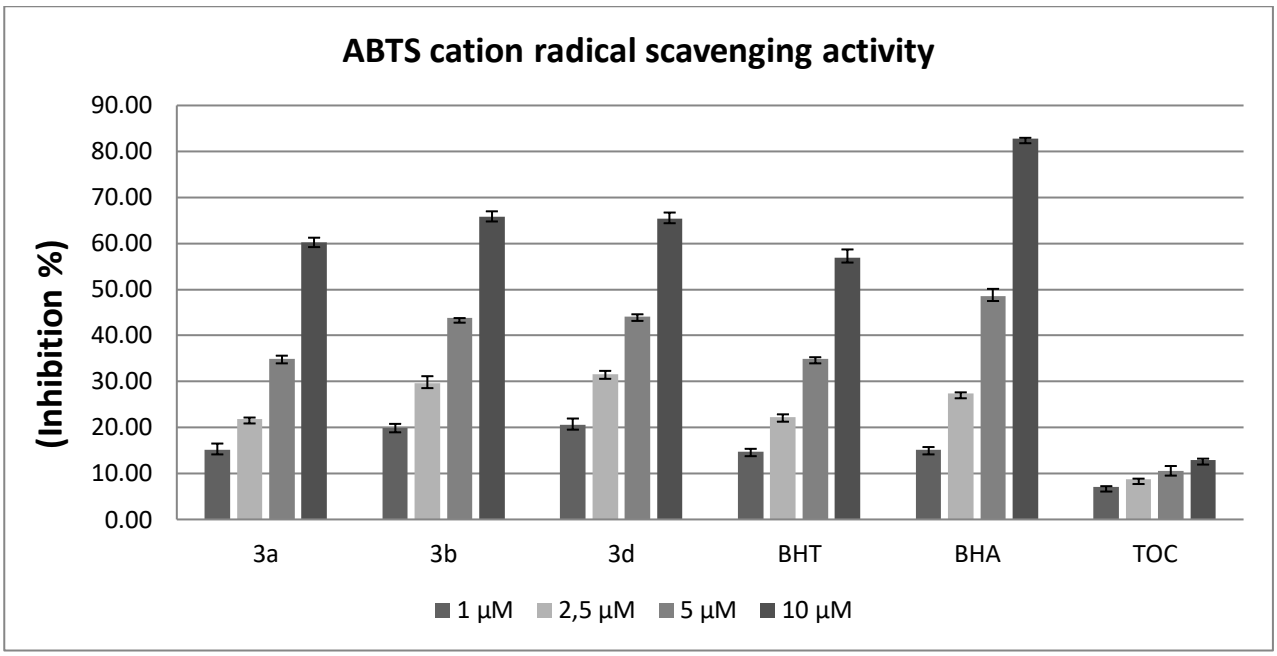


Figure 2. Inhibition (in %) values for the **3a-d** and controls in the ABTS assay. Values are means, ±SD, $n = 3$, $p < 0.05$, significantly different from each other with Student's *t*-test.

3.2.3. Cupric Reducing Antioxidant Capacity (CUPRAC)

The Figure 3 illustrates the absorbance values obtained from CUPRAC assays for the synthesised compounds **3a-d** and the standards BHT, BHA and α-TOC. The indole-3-carboxyaldehyde thiosemicarbazones **3b** and **3c** have demonstrated valuable activity pattern. Moreover, compound **3a** was found to be the best inhibitor against the all standards at all the concentrations for the CUPRAC assay. The presence of methyl (compound **3b**) or ethyl (compound **3d**) substitutions on the thiosemicarbazone N end resulted potent reducing capacity. Similar inhibition pattern was observed almost at all concentrations and concluded that these compounds showed concentration dependent behaviours for the activity. The compound **3a**, with no substitution on the thiosemicarbazone N end, was determined as the most potent derivative with the highest absorbance values at all concentrations. Even at the lowest concentration 10 µM the compound **3a** showed better activities than all the controls. The highest concentration 100 µM was the best condition for the compound **3a** with highest absorbance value. As in the DPPH and ABTS antioxidant assays, the compound **3c** showed the lowest absorbance values indicating the lowest inhibition. The presence of two methyl substituents on the thiosemicarbazone N end dramatically reduced the inhibition capacity and it was proved with the obtained results from all the antioxidant assays. The overall data revealed the importance of the NH_2 group for the valuable role in the activity.

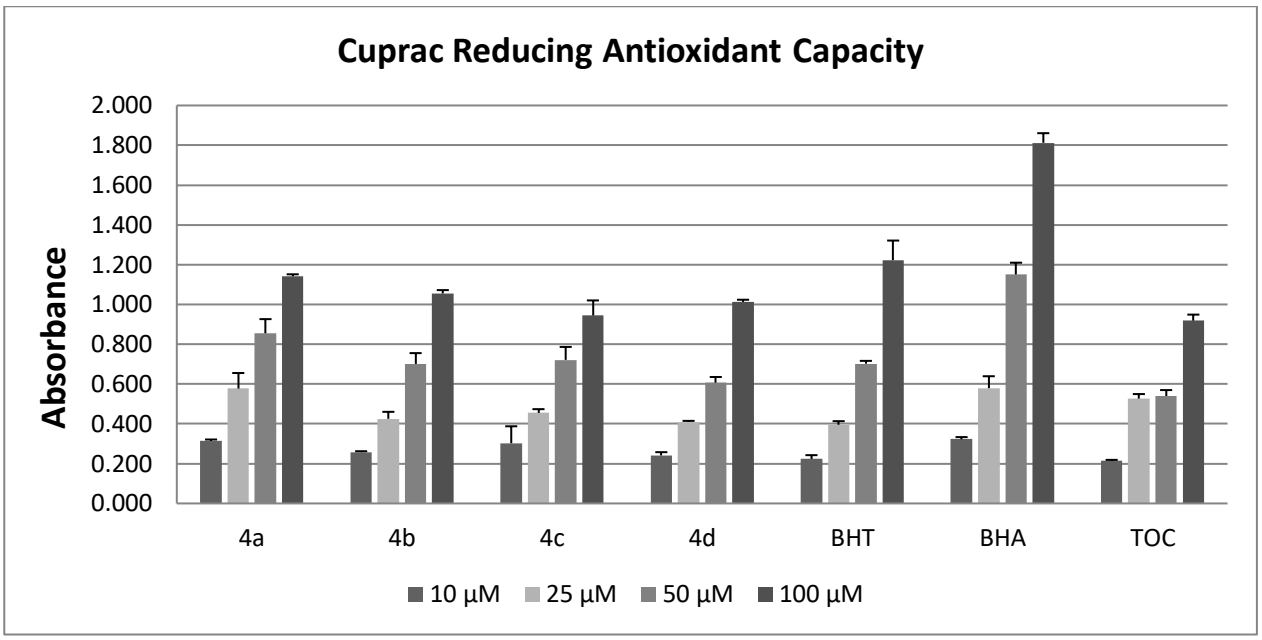


Figure 3. Absrobance values for the compounds **3a-d** and controls in the CUPRAC assay. Values are means, ±SD, $n = 3$, $p < 0.05$, significantly different from each other with Student's *t*-test.

3.2.4. Determination of Anticholinesterase Activity

The acetylcholinesterase (ACh) and Butyrylcholinesterase (BCh) enzyme inhibitory activities were evaluated for the determination of the therapeutic potential of the indole-3-carboxyaldehyde thiosemicarbazones for the treatment of AD. The inhibition of AChE by the compounds was given as percentage values at 200µg/mL concentration in Table 1 and compared with the Galanthamine as standard. As expected, the positive control Galanthamine, showed very potent inhibitions both on AChe and BChe with the values of 82.37% and 84.06%, respectively. In contrast, the compounds **3a**, **3b** and **3d** demonstrated unreasonable inhibition and determined as no enzyme inhibitory activity for ACh. In the case of BCh enzyme inhibition, the compounds **3b** and **3d** showed a slight increase with the values of 8.70% and 8.10%. More importantly, the compound **3c**, with the two methyl substituents on the thiosemicarbazone N end showed very potent enzyme inhibitory activity with the values of 90.36% and 91.06% for both ACh and BCh. This observation suggests that the additional alkyl moiety enhances the hydrophobic interactions with ACh enzyme. Hence, these structure-activity relationships provide a goal for future synthetic approaches. In addition to that compound **3c** could be a valuable target for the kinetic measurements to obtain about the mechanism of action.

4. **Conclusıon**

In conclusion, four indole-3-carboxyalde thiosemicarbazones **3a-d** were synthesised from indole-3-carboxyaldehyde and a series of thiosemicarbazides in high yields. The structures **3a** and **3b** were confirmed by the ^{1}H NMR with literature data and new compound **3c** and **3d** were fully characterised by using FT-IR, HRMS spectrometry, ^{1}H, ^{13}C NMR since the lack structural and photophysical data in the literature. The three different antioxidant (DPPH, ABTS and CUPRAC) assays and Acetylcholinesterase and Butyrylcholinesterase enzyme inhibitory activities were employed to identify the biological capacities of designated compounds. The obtained data demonstrated that the indole-3-carboxyaldehyde thiosemicarbazones systems with different substitutions on the thiosemicarbazone N end could be promising structures for the design of

Table 1. Acetylcholinesterase and Butyrylcholinesterase enzyme inhibitory activities of the indole-3-carboxyaldehyde thiosemicarbazones (**3a-d**).[a,b]

Sample	AChE (% Inhibition)	BChE (%Inhibition)
3a	NA	NA
3b	NA	8.70±0.66
3c	90.36±2.42	91.06±1.86
3d	NA	8.10±0.61
Galanthamine[c]	82.37±0.37	84.06±0.51

[a] Values expressed are means ±S.D. of three parallel measurements. (p<0.05)
[b] 200µg/mL
[c] Positive control
NA: Not Active

novel potent antioxidant systems. The DPPH was determined as the most resistant assay for the antioxidant ability of the synthesized compounds. However, the better inhibition results were obtained in the case of ABTS assay in the presence of compound **3a**, **3b** and **3d**. These three compounds showed better activity than the controls BHT and α-TOC at almost all concentrations. The best antioxidant property was obtained for compound **3a** in the case CUPRAC antioxidant assay. At all concentration, compound showed higher absorbance values than all the controls. It was concluded that the redox properties of the designated compounds acquired them to show antioxidant activity. Specifically, they could be valuable reducing systems due to the hydrogen atom donation and metal chelation behaviours eventually reduce the free or cationic radicals. In addition to this, the free NH_2 group in the targeted system is important for the Cupric ion reducing ability. The compound **3c** was found to be the best inhibitor for the anticholinesterase enzyme inhibition assay. The presence of additional alkyl moiety on the thiosemicarbazone N end was the responsible for the enhanced ACh, and BCh enzyme inhibitory activities.

References

Alley, M.C., Hollingshead, M.G., Pacula-Cox, C.M., Waud, W.R., Hartley, J.A., Howard, P.W., Gregson, S.J., Thurston, D.E. and Sausville, E.A., 2004. A Novel Rationally Designed DNA Minor Groove Interstrand Cross-Linking Agent with Potent and Broad Spectrum Antitumor Activity. *Cancer Research,* **64**, 6700-6706.

Antholine, W., Knight, J., Whelan, H. and Petering, D.H., 1977. Studies of the Reaction of 2-Formylpyridine Thiosemicarbazone and Its Iron and Copper Complexes with Biological Systems. *Molecular Pharmacology,* **13**, 89–98.

Apak, R., Güçlü, K., Özyürek, M. and Karademir, S.E.. 2004. Novel Total Antioxidant Capacity Index for Dietary Polyphenols and Vitamins C and E, Using Their Cupric Ion Reducing Capability in the Presence of Neocuproine: CUPRAC Method. *Journal of Agriculture and Food Chemistry*, **52**, 7970-81.

Blois, M.S.. 1958. Antioxidant Determinations by the Use of a Stable Free Radical. *Nature*, **181**, 1199-200.

Đilović, I., Rubčić, M., Vrdoljak, V., Pavelić, S.K., Kralj, M., Piantanida, I. and Cindric, M., 2008. Novel thiosemicarbazone derivatives as potential antitumor agents: Synthesis, physicochemical and structural properties, DNA interactions and antiproliferative activity. *Bioorganic & Medicinal Chemistry,* **16**, 5189-98.

Ellman, G.L., Courtney, K.D., Andres, V. and Featherstone, R.M., 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochemical Pharmacology*, **7,** 88-95.

Garoufis, A., Hadjikakou, S.K. and Hadjiliadis, N., 2009. Palladium coordination compounds as anti-viral, anti-fungal, anti-microbial and anti-tumor agents. *Coordination Chemistry Reviews,* **253**, 1384-97.

Gözler, B., Gözler, T., Freyer, A.J. and Shamma, M., 1988. Some Unusual Pavine and Isopavine Alkaloids from Roemeria refracta. *Journal of Natural Products,* **51**, 760-4.

Gust, R., Ott, I., Posselt, D. and Sommer, K., 2004. Development of Cobalt(3,4-diarylsalen) Complexes as Tumor Therapeutics. *Journal of Medicinal Chemistry*, **47,** 5837-46.

Haribabu, J., Tamizh, M.M., Balachandran, C., Arun, Y., Bhuvanesh, N.S.P., Endo, A. and Karvembu, R. 2018. Synthesis, structures and mechanistic pathways of anticancer activity of palladium(ii) complexes with indole-3-carbaldehyde thiosemicarbazones. *New Journal of Chemistry,* **42**, 10818-32.

Havoundjian, H., Reed, G.F., Paul, S.M. and Skolnick, P., 1987. Protection against the lethal effects of pentobarbital in mice by a benzodiazepine receptor inverse agonist, 6,7-dimethoxy-4-ethyl-3-carbomethoxy-beta-carboline. *The Journal of Clinical Investigation*, **79**, 473-7.

Hosseini-Yazdi, S.A., Mirzaahmadi, A., Khandar, A.A., Eigner, V., Dušek, M., Mahdavi, M., Soltani, S., Lotfipour, F. and White, J., 2017. Reactions of copper(II), nickel(II), and zinc(II) acetates with a new water-soluble 4-phenylthiosemicarbazone Schiff base ligand: Synthesis, characterization, unexpected cyclization, antimicrobial, antioxidant, and anticancer activities. *Polyhedron*, **124**, 156-65.

Hu, K., Yang, Z., Pan, S-S., Xu, H. and Ren, J., 2010. Synthesis and antitumor activity of liquiritigenin thiosemicarbazone derivatives. *European Journal of Medicinal Chemistry*, **45**, 3453-8.

Hu, W., Zhou, W., Xia, C. and Wen, X., 2006. Synthesis and anticancer activity of thiosemicarbazones. *Bioorganic & medicinal chemistry letters,* **16**, 2213—2218.

Husain, K., Bhat, A.R. and Azam, A., 2008. New Pd(II) complexes of the synthesized 1-N-substituted thiosemicarbazones of 3-indole carboxaldehyde: characterization and antiamoebic assessment against E. histolytica. *European journal of medicinal chemistry*, **43**, 2016-2028.

Kamal, A., Rao, M.V., Laxman, N., Ramesh. G. and Reddy, G.S.K., 2002. Recent Developments in the Design, Synthesis and Structure-Activity Relationship Studies of Pyrrolo[2,1-c][1,4]benzodiazepines as DNA-Interactive Antitumour Antibiotics. *Current Medicinal Chemistry - Anti-Cancer Agents*, **2**, 215-254.

Liu, L., Yang, J., Zhao, Z., Shi, P. and Liu, X., 2010. Solvent-free synthesis of indole-based thiosemicarbazones under microwave irradiation. *Journal of Chemical Research*, **34**, 57-60.

de Oliveira, R.B., de Souza-Fagundes, E.M. de, Soares, R.P.P., Andrade, A.A., Krettli, A.U. and Zani, C.L., 2008. Synthesis and antimalarial activity of semicarbazone and thiosemicarbazone derivatives. *European Journal of Medicinal Chemistry,* **43**, 1983-8.

Palanimuthu, D., Poon, R., Sahni, S., Anjum, R., Hibbs, D., Lin, H-Y., Bernhardt, V.P., Kalinowski, D.S. and Richardson, D.R., 2017. A novel class of thiosemicarbazones show multi-functional activity for the treatment of Alzheimer's disease. *European journal of medicinal chemistry,* **139**, 612—632.

Pavan, F.R., Maia, Pedro da, S., Leite, S.R.A., Deflon, V.M., Batista, A.A., Sato, D.N., Franzblau, G.S. and Leite, Q.F.C., 2010. Thiosemicarbazones, semicarbazones, dithiocarbazates and hydrazide/hydrazones: Anti – Mycobacterium tuberculosis activity and cytotoxicity. *European Journal of Medicinal Chemistry,* **45**, 1898-905.

Pchalek, K., Jones, A.W., Wekking, M.M.T. and Black, D.S., 2005. Synthesis of activated 3-substituted indoles: an optimised one-pot procedure. *Tetrahedron*, **61**, 77-82.

Piri, Z., Moradi-Shoeili, Z. and Assoud, A., 2017. New copper(II) complex with bioactive 2–acetylpyridine-4N-p-chlorophenyl thiosemicarbazone ligand: Synthesis, X-ray structure, and evaluation of antioxidant and antibacterial activity. *Inorganic Chemistry Communications,* **84**, 122-6.

Refat, M.S. and El-Metwaly, N.M., 2012. Spectral, thermal and biological studies of Mn(II) and Cu(II) complexes

with two thiosemicarbazide derivatives. *Spectrochimica Acta Part A, Molecular and biomolecular spectroscopy*, **92,** 336—346.

Rogolino, D., Cavazzoni, A., Gatti, A., Tegoni, M., Pelosi, G., Verdolino, V., Fumarola, C., Cretella, D., Petronini, G.P. and Carcelli, M., 2017. Anti-proliferative effects of copper(II) complexes with hydroxyquinoline-thiosemicarbazone ligands. *European Journal of Medicinal Chemistry*, **128**, 140-153.

Salas, P.F., Herrmann, C. and Orvig, C., 2013. Metalloantimalarials. *Chemical Reviews*, **113**, 3450-92.

Shahabadi, N., Kashanian, S. and Darabi, F., 2010. DNA binding and DNA cleavage studies of a water soluble cobalt(II) complex containing dinitrogen Schiff base ligand: The effect of metal on the mode of binding. *European Journal of Medicinal Chemistry*, **45**, 4239-45.

Silva, C.M., da Silva, D.L., da Modolo, L.V., Alves, R.B., Resende, M.A., de Martins, C.V.B. and de Fatima, A. 2011. Schiff bases: A short review of their antimicrobial activities. *Journal of Advanced Research,* **2(1)**, 1-8.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M. and Rice-Evans, C., 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine*, **26**, 1231-7.

Yu, Y., Kalinowski, D.S., Kovacevic, Z., Siafakas, A.R., Jansson, P.J., Stefani, C., Lovejoy, D.B., Sharpe, P.S., Bernhardt, P.V. and Richardson, D.R., 2009. Thiosemicarbazones from the Old to New: Iron Chelators That Are More Than Just Ribonucleotide Reductase Inhibitors. *Journal of Medicinal Chemistry*, **52(17)**, 5271-94.

Zhang, H-J., Qian, Y., Zhu, D-D., Yang, X-G. and Zhu, H-L., 2011. Synthesis, molecular modeling and biological evaluation of chalcone thiosemicarbazide derivatives as novel anticancer agents. *European journal of medicinal chemistry,* **46**, 4702—4708.