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Butylated hydroxy benzylidene ring: an important moiety for antioxidant synergism of semicarbazones

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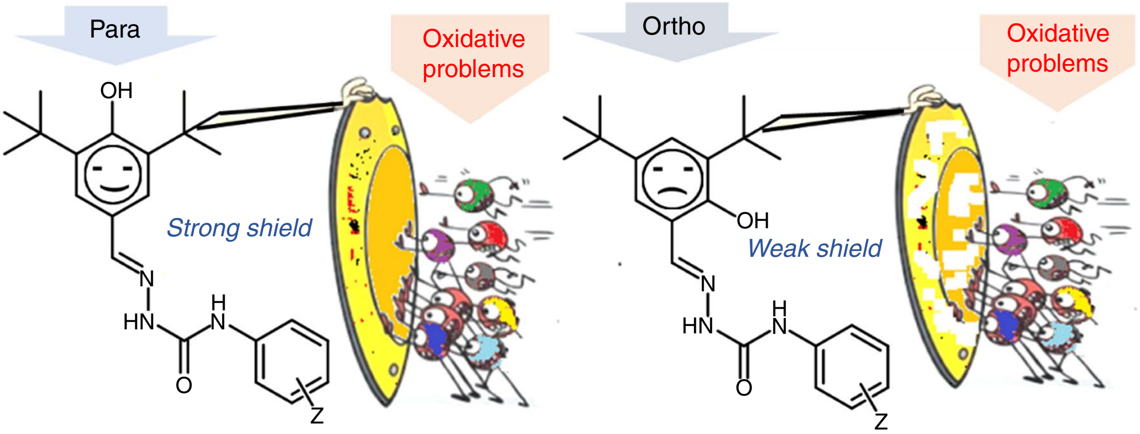
Abstract

The compounds having two or more antioxidant functions in their structure exhibit antioxidant synergism that generally increase the antioxidant activity of these compounds. In this study, two series of semicarbazone (**7a–j** and **7a′–j′**) bearing butylated ortho and para hydroxy benzylidene ring were prepared for the investigation of antioxidant synergism. This study found that intramolecular hydrogen bond can form in semicarbazones due to the inappropriate position of hydroxyl group on benzylidene ring, which adversely affects the antioxidant synergism. As consequence, butylated para hydroxyl benzylidene phenyl semicarbazone (**7a**) (IC_{50} 12.27 µM) showed ~4.3 times and ~2.7 times better antioxidant activity than compounds **7a′** (IC_{50} 53.30 µM) and BHT (IC_{50} 32.63 µM), respectively, in DPPH assay. In addition, based on the solubility in trimethylolpropane trioleate (TMPTO) as synthetic base oil and obtained IC_{50} results, oxidation stability of synthesized compounds was also evaluated by two kinds of differential scanning calorimeter (DSC) test, namely temperature ramping DSC and programmed temperature DSC. Thermogravimetric analysis is also performed for the thermal stability assessment. TMPTO incorporated with 0.25 mass% of **7c** and **7e** were found better oxidative (around 2 times) and thermal resistance than BHT. This DSC results showed another important aspect of semicarbazones that proper modification of semicarbazones can be used in the synthetic lubricant oil as a potential antioxidant. Thus, the results of this study are promising which can be taken under consideration to design and prepare more efficient multipotent semicarbazones.

Graphic abstract



Keywords Antioxidant synergism · DSC · Lubricant oil · Oxidative stability · TGA · Semicarbazones

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Extended author information available on the last page of the article

Introduction

Oxidative stress is one of the main causes of etiology and expansion of major human deteriorating disease. The imbalance of production of free radical (reactive oxygen species) and the ability of the body to counteract the free radical cause oxidative stress [1, 2]. So excessive free radicals exert toxic effects throughout the body especially responsible for cancer and other chronic diseases [3, 4]. Apart from the oxidative stress in the human body, free radicals cause auto-oxidation and premature oxidation in industrial products such as oils, rubbers, food and foodstuffs [5–7]. Premature oxidation is one of the major obstacles to develop the oxidatively and thermally stable synthetic lubricant where eco-friendly synthetic lubricants become a necessity of the modern world.

So, development of eco-friendly synthetic lubricant from sustainable resources has drawn a great interest for the last few years to replace the mineral lubricants because of the risen environmental concerns and preventive protocols [8–10]. Moreover, reliance on fossil-based resources is no longer pragmatic in perspective of feasible, eco-friendly and financial purposes and in addition, the reserve of fossil-based resources is notably diminishing with time. As a result, synthetic lubricant oils or vegetable oils have gained a great consideration because it derived from cheaper renewable resources and their better lubricant properties than the mineral oils [11]. Unfortunately, due to the lower oxidative and thermal stability it has failed to get wide acceptance. This is because the production of free radical in lubricant oil causes premature oxidative degradation that is responsible for product damage, discoloration, and reduced lifetime.

The premature oxidation or auto-oxidation process and excess free radical production in vivo or in vitro can be stopped or delayed by introducing antioxidant into the substrates. Antioxidants can inhibit the different stages of the oxidation process in industrial products by their different mood of actions or mechanisms such as scavenging free radicals or decomposing peroxides radicals and deactivating metal ions. Thus, these are divided into three main groups based on their mode of action: free radical scavenger or primary antioxidant, the other peroxide decomposer or secondary antioxidant and metal chelator or deactivator [12]. For past few years, researchers have been trying to develop efficient multipotent or multifunctional antioxidant by assembling two or more antioxidant functions in one structure, because high efficient multifunctional antioxidants would be an effective solution to get over the problems caused by free radical such as oxidative stress in the human body and low oxidative stability of industrial product, e.g., synthetic lubricant oil [11].

Butylated hydroxytoluene (BHT) is one of the widely used antioxidants, and semicarbazones are extensively reported pharmacophore which also possesses excellent antioxidant properties. Butylated hydroxytoluene (BHT) is well-known synthetic primary antioxidant. It has been extensively used in the food industry, pharmaceuticals, rubber, and lubricant due to its excellent free radical scavenging capacity [13]. It is identified as safe for use in food at low concentration as a food preservative. Thus, it has been utilized in the food industry, food packaging, fish products and so on [14, 15]. BHT is also broadly utilized in combination with other antioxidants such as butylated hydroxyanisole (BHA), propyl gallate, and citric acid for the stabilization of oils and high-fat foods [16, 17]. BHT also exhibited good synergism results with other antioxidants in the application of rubber and lubricant oil.

Again, semicarbazones, which are versatile pharmacophore agents, have earned interest in medicinal and pharmaceutical fields due to broad-spectrum biological activities such as anticonvulsant, antitumor, and anticancer properties [18–21]. Semicarbazones are also known tridentate or neutral ligand since they contain multiple donor atoms in their structure. Their metal complexes also exhibit significant activity against bacteria, fungi, and viruses which extends the versatility of the applications [22]. Moreover, substituted semicarbazones can be used as a platform chemical to prepare benzotriazepin compounds which are also an important class of drug due to their psychostimulant, antidepressant, anorexigenic, and antihypertensive properties [23]. Some researchers also revealed their antioxidant activity as they have two secondary amino in its structure [24, 25]. Sabari et al. reported some new semicarbazone derivative of curcumin for its antioxidant and antimicrobial properties. It is believed that favorable substitution of semicarbazones would affect significantly its free radical scavenging ability [26]. Example like chalcone semicarbazones, a pharmacophore model, was found to have good antioxidant activity [27].

Comparing mono-functional antioxidants such as hindered phenol and aromatic amine, inhibiting action of multifunctional antioxidants is more complicated and effective because their structures contain multiple antioxidant functions which involved in the termination of free radical chain reaction through different mechanisms [28, 29]. Multifunctional or multipotent antioxidants have been used widely into pharmaceuticals, rubber, and petroleum because of their efficacy in terminating free radicals. This type of antioxidants has drawn a tremendous attention for the treatment of several diseases [30]. For example, thiazolidinone derivatives incorporated with butylated hydroxyphenyl (BHP) exhibit antioxidant activity along with Ca^{2+} overload inhibitor and Ca^{2+} channel blocker [31].

Therefore, discovering effective multipotent antioxidants is now paramount need to effectively counteract the free radicals to reduce the risk of oxidative stress as well as to give better oxidative stability to synthetic lubricant oil. Herein, we have envisioned to make multipotent semicarbazone incorporated with two types of butylated hydroxyphenyl (BHP) as an efficient antioxidant. The purpose of this study is to develop a group of efficient multipotent semicarbazones which can exhibit excellent antioxidant activity and to study the role of the ortho and para hydroxyl group of BHP incorporated semicarbazones on the antioxidant activity. Antioxidant properties of these synthesized compounds were investigated by DPPH assay, and effects of different substituents on the antioxidant activity were also studied. Furthermore, some synthesized compounds were chosen based on easy solubility in trimethylolpropane trioleate as synthetic ester-based lubricant for the evaluation of oxidative stability by differential scanning calorimeter (DSC) and thermal stability by thermogravimetric analysis (TGA) compared with commercial antioxidant BHT.

Experimental

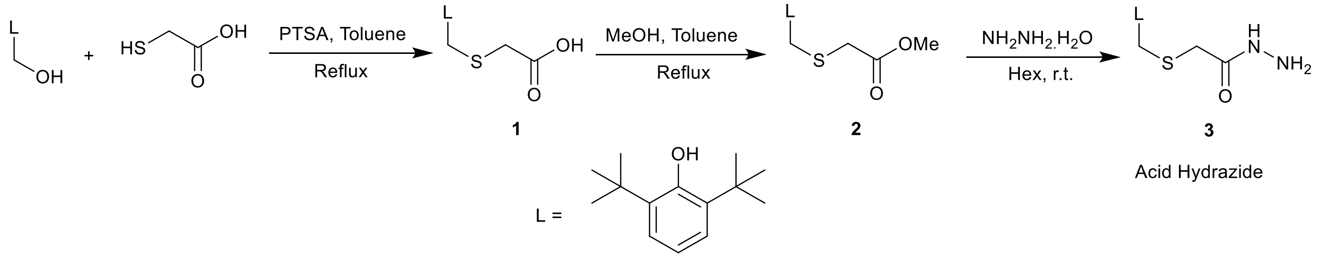
General remarks

All chemicals and reagents were of analytical grade and supplied by Sigma-Aldrich and Merck, Malaysia, and used without further purification. Double-distilled water was used throughout the experiment. Pre-coated silica gel plates (0.25 mm) were utilized for analytical thin-layer chromatography to monitor the reaction and imagined by UV light. Column chromatography was done on Silica Gel 60 (particle size: 0.040–0.063 mm). IR spectra were recorded using FTIR-ATR of the solid samples. Melting points were approximated. 600 MHz and 150 MHz NMR spectrometers were used for ^{1}H NMR and ^{13}C NMR, respectively, and tetramethylsilane was used as a reference. Chemical shifts (δ) were measured in ppm with respect to the residual solvent peak ($CHCl_3$, $\delta = 7.26$; DMSO-d_6, $\delta = 2.51$ for proton spectra; $^{13}CDCl_3$, $\delta = 77.0$; DMSO-d_6, $\delta = 40$ for carbon spectra). High-resolution mass spectra were performed on a time-of-flight Q-TOF LCMS system.

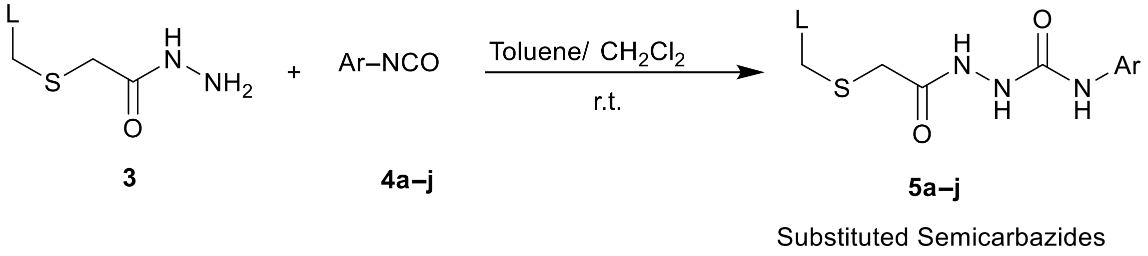
Synthesis of 2-((3,5-di-tert-butyl-4-hydroxybenzyl) thio)acetohydrazide (3) and substituted semicarbazides 5a–j

2-((3,5-di-tert-butyl-4-hydroxybenzyl)thio)acetohydrazide was synthesized according to a previously described method (Scheme 1) [32].

Acid hydrazide (1 g, 3.12 mmol) was stirred in toluene (20 mL), then an equimolar amount of aryl isocyanate was introduced, and the reaction was monitored by TLC (Scheme 2). All the substituted semicarbazides formed white precipitates during the reaction, which were filtered and washed several times with toluene. Dichloromethane (CH_2Cl_2) was used for compounds **5c**, **5d** and **5i** since 2,4-dichlorophenyl isocyanate, 4-cyanophenyl isocyanate and 4-acylphenyl isocyanate are not soluble in toluene. Compound structures were confirmed by IR, ^{13}C NMR, ^{1}H NMR and HRMS analyses. Characterization data were presented in the supporting files. We have already reported the following synthesized substituted semicarbazides



Scheme 1 Preparation of acid hydrazide (**3**)



Scheme 2 Preparation of substituted semicarbazides

5a–**5c, 5f, 5h,** and **5j** with characterization data in the literature [33]. Herein, compounds **5d**, **5e** and **5g** have been reported with characterization data (Supporting file). All the synthesized compounds were solid and stored at ambient temperature.

General procedure for the synthesis of semicarbazones (7a–j and 7a′–j′)

0.65 mmol of substituted semicarbazides (**5a**–**j**) and an equimolar quantity of aldehyde were taken into round-bottle flask with approximately 10 mL of ethanol. Then the mixture was stirred at 90 °C for 4–5 h in the presence catalytic amount of HCl (1.6 mmol) until the disappearance of the starting materials spot in the thin-layer chromatography. Afterward, the reaction mixture was allowed to warm to ambient temperature, stirred for around half an hour. The expected semicarbazones started the formation of solid precipitate during ambient temperature stirring in some of the reactions. The precipitate was filtered and washed with hexane for several times. Then the resultant products were dried and either recrystallized from hexane or column chromatography. However, the reaction in which no precipitates were found, the reaction mixture was concentrated and purified by column chromatography on silica gel with hexane/EtOAc mixture as the eluent. All characterization data have been included into supporting files.

1, 1-diphenyl-2-picrylhydrazyl (DPPH) assay

DPPH radical scavenging assay was carried out according to the literature [34] with some modifications. To a range of various concentrations 60, 40, 20, 10 µM mL^{-1} of samples, DPPH solution (50µL, 0.203 mM in MeOH) was added. Around 1 mg of synthesized compounds was dissolved in MeOH (5.0 mL, 100%) as a stock solution. This stock solution was diluted to a range of final extraction concentrations 60, 40, 20, and 10 µM. A negative control with the same DPPH concentration in MeOH without sample was used. Each assay was carried out in triplicates. The mixture was then incubated in dark for 60 min at room temperature. Absorbance at 517 nm for each sample was measured. BHT was used as positive control. The free radical scavenging activity of the compounds will be calculated as a percentage of radical inhibition by using the formula:

$$\text{Percentage of Inhibition (\%)} = \left[(\text{Ac} - \text{As})/\text{Ac}\right] \times 100,$$

where As = Absorbance of the compounds/positive control, and Ac = Absorbance of control (MeOH solution).

To determine the concentration required to achieve 50% inhibition (IC_{50}) of DPPH radical, the percentage of DPPH inhibition for each compound will be plotted against extract concentration.

Differential scanning calorimetry (DSC)

Temperature ramping DSC

Differential scanning calorimetry was conducted for the evaluation of oxidation induction time and oxidation onset temperature by utilizing TA DSC Q2000. For the assessment of OIT, 5 mg of sample oil was placed in an open aluminum pan under 2 bar pressures of nitrogen. Metal indium was used for the temperature calibration at 20 °C heating rate. The sample was heated in a nitrogen atmosphere until reached the isothermal temperature 150 °C and then switched to oxygen gas at 20 mL min^{-1} rate.

Programmed temperature DSC

This experiment was also carried out by the same instrument. 3 mg of oil sample was placed in an open aluminum pan for the maximum interaction between oil and reactant gas in order to avoid gas diffusions limitations. Temperature scanning rate of 20 °C min^{-1} was kept in the temperature ramping experiments, and 99.99% pure oxygen flow was maintained at 20 mL min^{-1}.

Thermogravimetric analysis (TGA)

The thermal stability of TMPTO and TMPTO with 0.25 mass% of BHT, **7c** and **7e** in air was investigated by examining the thermal decomposition temperature by the way of thermogravimetric analysis (TGA) over a temperature limit from ambient to approximately 600 °C, at scanning rate of 10 °C min^{-1} using a thermal analyzer.

Results and discussion

Synthesis of BHA incorporated semicarbazones

Recently, we disclosed a robust reaction protocol for the synthesis of semicarbazones to skip the limitations of previously reported methods such as functional group incompatibility and lower yields [33]. In brief, this reaction protocol involved the synthesis of acid hydrazide that was reacted with aryl isocyanate to obtain substituted semicarbazides;

it was then treated with benzaldehyde to get expected semicarbazones.

Accordingly, 10 substituted semicarbazides were prepared by the reaction of a pre-prepared acid hydrazide and 10 different aryl isocyanate at room temperature using two different solvents: toluene and dichloromethane based on the solubility of the reactants. All the substituted semicarbazides (**4a**–**j**) were obtained with notable yields but at different reaction completion period. The results are summarized in Table 1.

Then the synthesized substituted semicarbazides were taken into the reaction with 3,5-di-tert-butyl-4-hydroxy benzaldehyde in the presence of acidic ethanol at 90 °C. The results are outlined in Table 2. *N*^{1}-(3,5-di-tert-butyl-4-hydroxy benzylidene)-*N*^{4}-(substituted phenyl)-semicarbazones (**7a**–**j**) were obtained successfully with notable yields. Semicarbazones containing 4-fluoro phenyl **7b**, 2,4-di-chloro phenyl **7c**, 4-cyano phenyl **7d**, 3-methoxy phenyl **7e**, m-tolyl **7f**, naphthalene **7j** attached at N-4 were isolated with around 90% more product yields. Again, acetyl **7h**, ethyl ester **7i** substituted phenyl semicarbazones provided more than 80% yields.

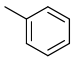
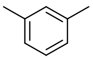
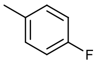
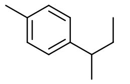
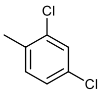
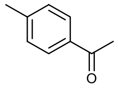
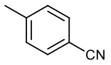
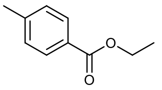
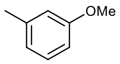
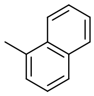
After obtaining the substituted semicarbazones (**7a**–**j**) containing 3,5-di-tert-butyl-4-hydroxyphenyl, we prepared another series of same substituted semicarbazones attaching with 3,5-di-tert-butyl-2-hydroxyphenyl to investigate antioxidant activity of both series. Thus, pre-prepared substituted semicarbazides (**5a**–**j**) were reacted with 3,5-di-tert-butyl-2-hydroxy benzaldehyde **6′** upon heating with acidic ethanol. Interestingly, 3,5-di-tert-butyl-2-hydroxy benzaldehyde also went into reaction with all the synthesized substituted semicarbazides (**5a**–**j**) and also provided the noticeable yields. The results are shown in Table 3. All *N*^{1}-(3,5-di-tert-butyl-2-hydroxy benzylidene)-*N*^{4}-(substituted phenyl)-semicarbazones (**7a′**–**j′**) were obtained with around 90% more products yields.

Antioxidant activity evaluation by DPPH assay

The free radical scavenging ability of series *N*^{1}-(3,5-di-tert-butyl-4-hydroxy benzylidene)-*N*^{4}-(substituted phenyl)-semicarbazones (**7a**–**j**) and *N*^{1}-(3,5-di-tert-butyl-2-hydroxy benzylidene)-*N*^{4}-(substituted phenyl)-semicarbazones (**7a′**–**j′**) were evaluated by a non-enzymatic method DPPH assay. Reduction of DPPH by antioxidant causes the color changes from purple to yellow and it is also observed by the decreasing absorption at 517 nm in the spectrophotometer. The free radical scavenging properties of the synthesized semicarbazones were investigated by the interaction between experimental compounds and 0.2 mM 1,1-diphenyl-2-picrylhydrazine (DPPH) for 30 min. The antioxidant activities of the series 1 of *N*^{1}-(3,5-di-tert-butyl-4-hydroxy benzylidene)-*N*^{4}-(substituted phenyl)-semicarbazones (**7a**–**j**) obtained from DPPH assay are summarized in Fig. 1.

Although all the compounds (**7a**–**j**) possessed the reasonable free radical scavenging properties, most of them exhibited promising oxidation inhibition in comparison with standard BHT. The compound **7a** showed remarkable antioxidant activity in comparison with BHT as it is shown in Fig. 1 that IC$_{50}$ value of **7a** was found 12.27 µM, whereas

Table 1 Evaluation of the reaction of substituted semicarbazides formation **5a**–**j**$^{a–c}$

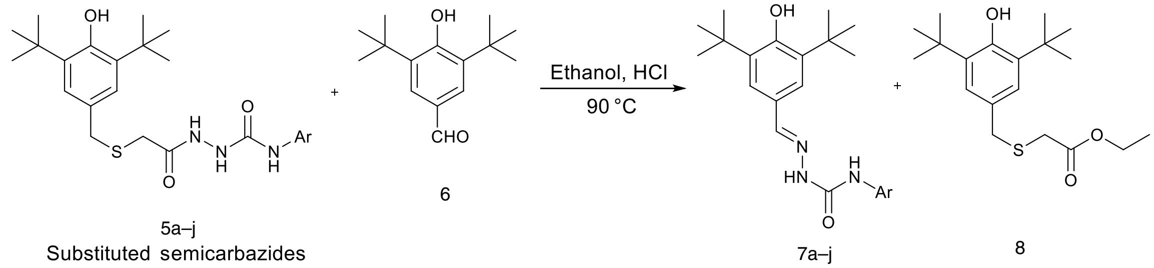
Substituted semicarbazide	Ar	Yields %	Substituted semicarbazide	Ar	Yields %
5a		99^b	5f		90^b
5b		98^b	5g		90^b
5c		98^c	5h		95^b
5d		95^c	5i		90^c
5e		95^b	5j		90^b

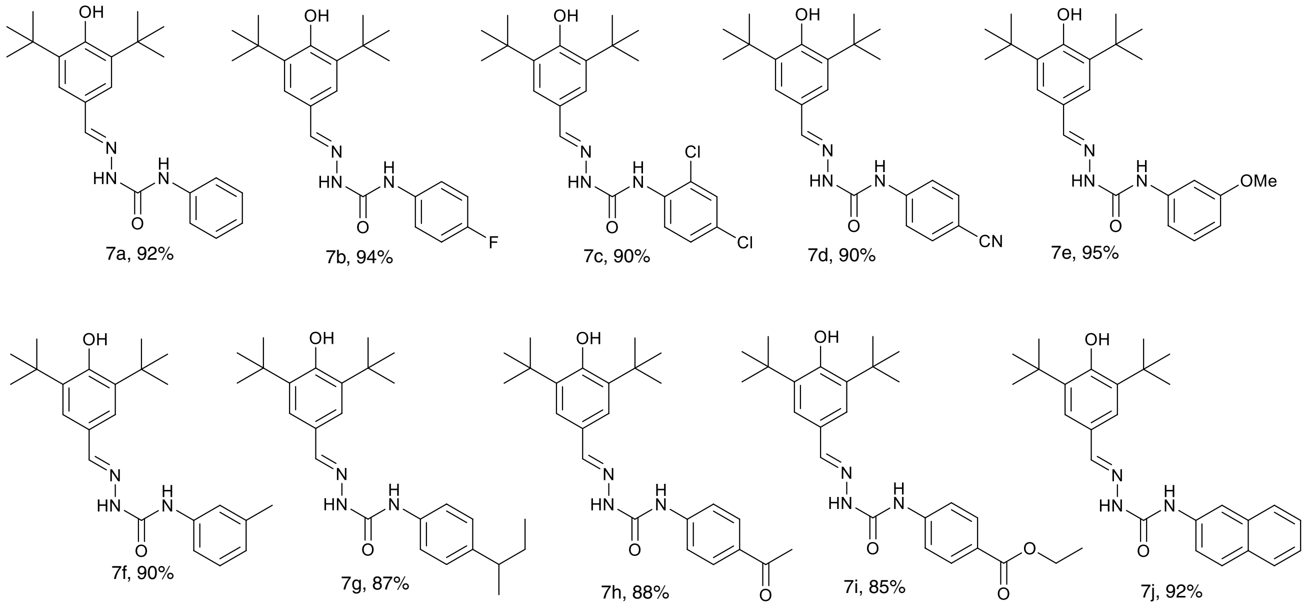
^{a}Reagents and conditions: 3 (3.12 mmol), **4a**–**j** (3.12 mmol), room temperature

^{b}Toluene was used as a solvent

^{c}Dichloromethane was used as a solvent

Table 2 Evaluation of N^1-(3,5-di-tert-butyl-4-hydroxy benzylidene)-N^4-(substituted phenyl)-semicarbazones (**7a–j**)[a,b]





7a, 92% 7b, 94% 7c, 90% 7d, 90% 7e, 95%

7f, 90% 7g, 87% 7h, 88% 7i, 85% 7j, 92%

[a]Reagents and conditions: **5a–g** (0.65 mmol), **6** (0.65 mmol), HCl (1.6 mmol), ethanol (10 mL), 90 °C

[b]Isolated yield

IC_{50} 32.63 μM value was obtained for BHT. The lower value of IC_{50} of compounds indicates the better antioxidant activity. Compounds (**7a–e**, **7g**, **7h**) showed the lower IC_{50} values than standard BHT. The increasing order of IC_{50} can be made from results in Fig. 1 as **7a** > **7b** > **7h** > **7e** > **7c** > **7g** > **7d** > **7i** > BHT > **7j** > **7f**.

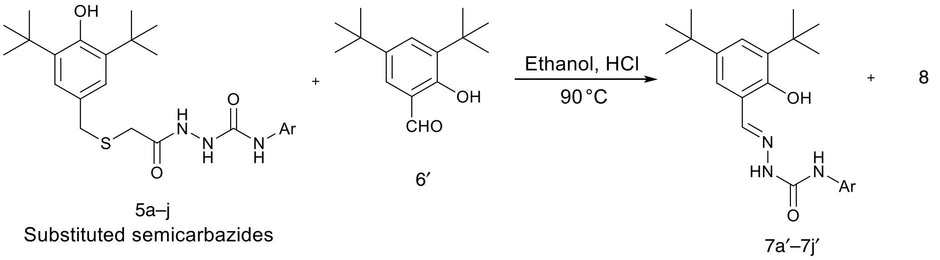
The series of N^1-(3,5-di-tert-butyl-2-hydroxy benzylidene)-N^4-(substituted phenyl)-semicarbazones (**7a′–j′**) were also conducted with DPPH assay, and results are shown in Fig. 2. Free radical scavenging properties of these compounds can be ordered from the obtained results as follows: **7e′** > **7i′** > **7h′** > BHT > **7b′** > **7g′** > **7c′** > **7d′** > **7a′** > **7f′** > **7j′**.

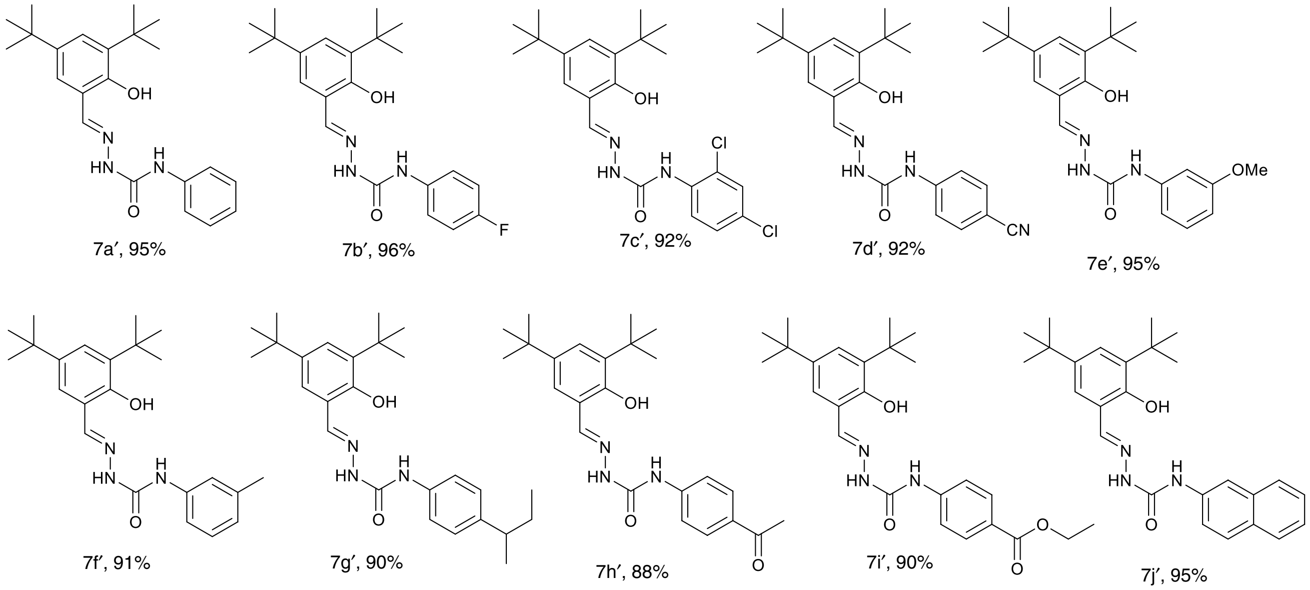
In this series, 3-methoxyphenyl semicarbazones, 3-ethoxy carbonyl phenyl semicarbazone and 4- acetyl phenyl semicarbazones showed better radical scavenging properties than standard BHT, though the rest of the compounds exhibited reasonably antioxidant properties.

Generally, it is observed in DPPH assay results (Figs. 1, 2) that N^1-(3,5-di-tert-butyl-4-hydroxy benzylidene)-N^4-(substituted phenyl)-semicarbazones (**7a–j**) offer the better antioxidant activity than N^1-(3,5-di-tert-butyl-2-hydroxy benzylidene)-N^4-(substituted phenyl)-semicarbazones (**7a′–j′**). This may primarily be attributed to the position of hydroxyl radical (-OH) on the benzylidene ring at N-1 of semicarbazones and the substituents effects of the N-4 ring.

Higher stability of phenoxy radical and lower bond dissociation enthalpies BDE values of O–H bonds are the prerequisite of a hindered phenol to be an antioxidant. It is well known that antioxidant activity of hindered phenol such as BHT varies with the values of BDE of O–H bond and stability of phenoxy radical [35]. The BDE values of O–H in BHT depend on the OH group position on the benzene ring and its substituents [13]. The stability of phenoxy radical depends on the steric hindrance. So, in butylated hydroxytoluene, the

Table 3 Evaluation of N^1-(3,5-di-tert-butyl-2-hydroxy benzylidene)-N^4-(substituted phenyl)-semicarbazones (**7a′–j′**)[a, b]




7a′, 95%
7b′, 96%
7c′, 92%
7d′, 92%
7e′, 95%
7f′, 91%
7g′, 90%
7h′, 88%
7i′, 90%
7j′, 95%

[a] Reagents and conditions: **5a–g** (0.65 mmol), **6′** (0.65 mmol), HCl (1.6 mmol), ethanol (10 mL), 90 °C

[b] Isolated yield

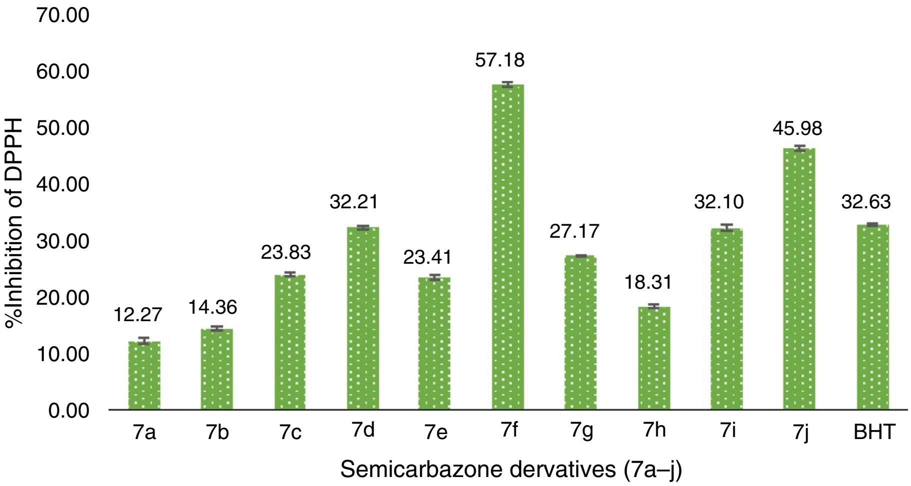
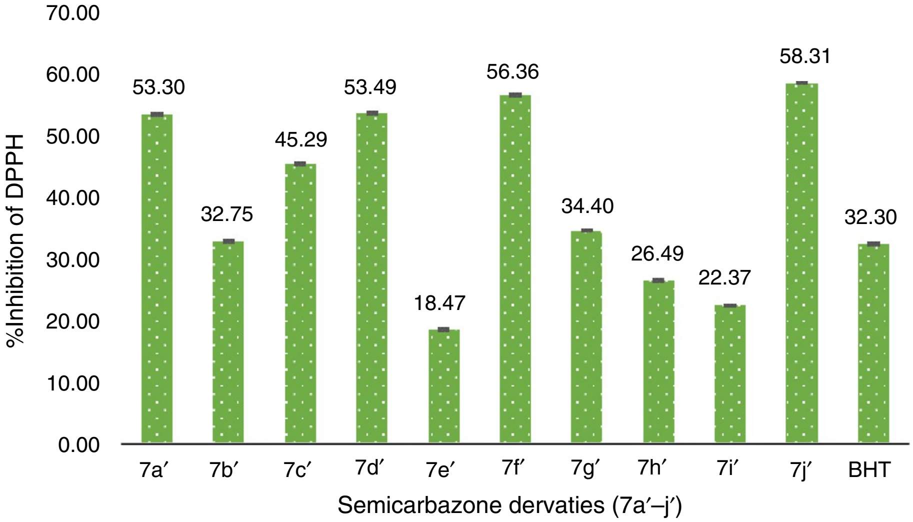
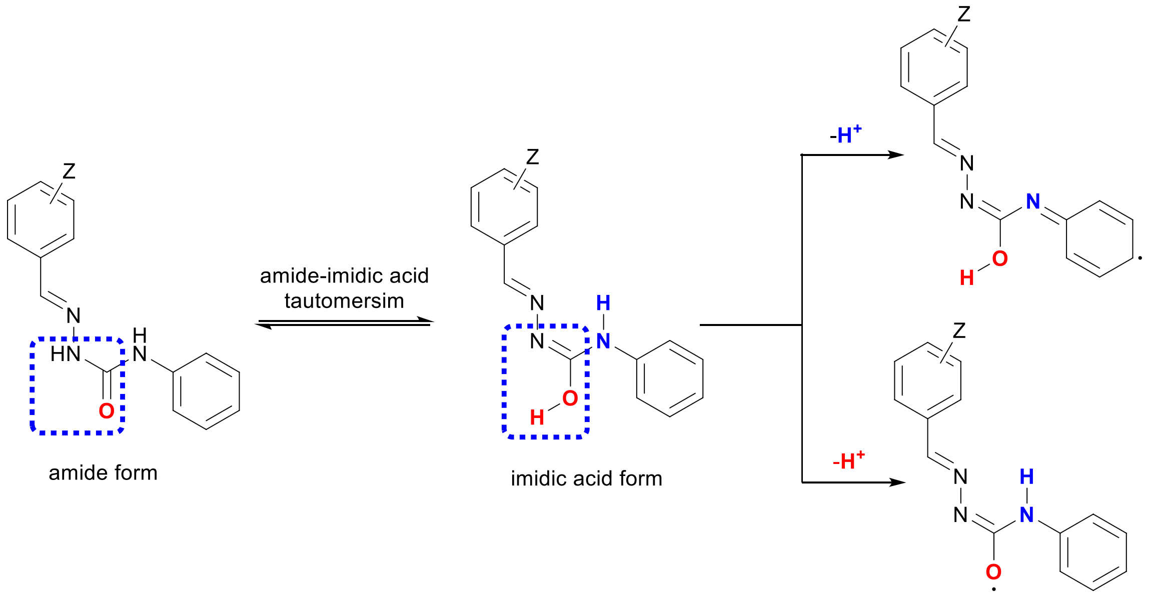


Fig. 1 Antioxidant activity of synthesized compounds (**7a–j**) by DPPH assay

sterically hindered hydroxyl group is obtained by flanking two bulky groups like tertiary butyl groups to attain more stable phenoxy radical. Thus, the phenoxy radical, formed by donating a proton to reduce free radical, is not affected by any electrophiles readily.

Fig. 2 Antioxidant activity of synthesized semicarbazones (**7a′**–**j′**) by DPPH assay





Scheme 3 Amide-imidic acid form of semicarbazone

In case of semicarbazones, amide-imidic acid tautomerism might play an important role on their antioxidant properties shown in Scheme 3. Hydroxyl group of imidic acid form of semicarbazones and/or secondary aromatic amine might involve in the H-atom transfer mechanism (or single electron transfer) to reduce the free radicals.

In the structure of first series 1 (**7a**–**j**), the para hydroxyl group of butylated hydroxyphenyl (BHP) is flanked by two tertiary butyl groups, so the antioxidant activity of butylated hydroxyphenyl moiety in **7a**–**j** may remain same as BHT due to the favorable position (ortho) of O–H group but may be affected by the substituents. Consequently, phenyl semicarbazones bearing BHP **7a** showed promising antioxidant activity (IC_{50}12.27 µM or 4.51 µg/L) in DPPH assay which is around 2.7 time better than BHT (IC_{50} 32.63 µM). The oxidation inhibition of phenyl semicarbazone bearing benzylidene ring (*N*^{1}-benzylidene-*N*^{4}-phenyl-semicarbazones) was reported 33.29 µg/L (IC_{50} value) in 0.1 mM DPPH concentration by Jafri et al. [24]. While phenyl semicarbazones incorporated with BHP (**7a**) showed significant antioxidant activity (IC_{50} 4.51 µg/L) in 0.2 mM DPPH concentration in this study. This is may be due to the combination of two antioxidant functions in one structure that able to exhibit the antioxidant synergistic effect. However, this antioxidant activity varies with the presence of different substituents at N-4 ring of semicarbazones. BHA incorporated semicarbazones containing 4-fluoro **7b**, 4-acetyl **7h**, 3-methoxy **7e**, 4-chloro **7c**, 4-Cyano **7d**, 4-ester **7i** and 4-sec-butyl **7g** at N-4 benzene ring of semicarbazone exhibited lower IC_{50} values than BHT, but 3-methyl **7f** and naphthyl **7j** substituted semicarbazones showed higher IC_{50} values.

In the structure of the second series, the hydroxyl group of BHP is not flanked by two tertiary butyl groups in 3,5-di-tert-butyl-2-hydroxy benzylidene ring. This may increase the BDE value of O–H group, resulting in the lower antioxidant activity of the compounds of this series 2 in comparison with series 1. BDE value of O–H group of this series might be affected by the hydrogen bond which would occur between the hydrogen of OH group and nitrogen of imine group. Due to the formation of a hydrogen bond between H and N, it becomes difficult to abstract the proton from –OH group due to the high BDE values. It can be displayed in Fig. 3 that describes the probable formation of hydrogen bond in series 2 structure (**7a′**–**j′**).

This hydrogen bond might decrease the normal antioxidant activity of butylated hydroxyphenyl which results are the lower antioxidant activity of series 2 compounds. This series may not be able to provide any synergistic effect between BHP and semicarbazones functional group because of the formation of intramolecular H-bond. This may be attributed to the higher IC_{50} value of the compound **7a′** (53.30 µM), resulting in the lower antioxidant activity which is around 4.3 times lower than the antioxidant activity of **7a** (IC_{50}12.27 µM). Thus, the antioxidant activity of series 2 might be obtained only from semicarbazones functions which have been described earlier. Interestingly, 3-methoxyphenyl **7e′**, 4-ethyl carbonyl phenyl **7i′** and 4-acetyl phenyl **7h′** semicarbazones incorporated with 3,5-di-tert-butyl-2-hydroxyphenyl showed better antioxidant activity than standard BHT in DPPH assay. However, it can be concluded from the above discussion that semicarbazone bearing 3,5-di-tert-butyl-4-hydroxyphenyl can be effective antioxidant compounds than the semicarbazones incorporated with 3,5-di-tert-butyl-2-hydroxyphenyl.

Oxidation stability assessment of trimethylolpropane trioleate (TMPTO)

After the careful evaluation of antioxidant properties of the series 1 and series 2 compounds, we proceeded to investigate the oxidation stability of synthesized compounds in TMPTO as ester-based lubricant oil based on the solubility and obtained IC_{50} results. Compounds **7a**, **7b**, **7c**, **7e**, **7g** and **7a′** were easily soluble in TMPTO and we carried out two types of differential scanning calorimeter (DSC) test, namely temperature ramping DSC and programmed temperature DSC. Generally, lower mass% of antioxidant is preferred to be used in the industrial lubricant composition which could be generally 0.1–2 mass% [36]. The use of 0.25 mass% of our experimented samples showed significant difference in oxidative stability evaluation in the DSC experiment. Thus, 0.25 mass% were chosen to be blended with TMPTO base oil for the evaluation of oxidative stability. The samples were blended in TMPTO with very less amount (0.25 mass%) and conducted temperature ramping DSC with a constant scanning rate of 20 °C min^{-1} to assess their oxidation decomposition temperature. The obtained results from temperature ramping DSC analysis are outlined in Fig. 4. Base oil TMPTO exhibited the oxidation onset temperature (OOT) at 173.93 °C, OOT of TMPTO incorporated with 0.25% of BHT, compounds **7a** and **7a′** did not show any significant difference. However, TMPTO incorporated with N^1-(3,5-di-tert-butyl-4-hydroxy benzylidene)-N^4-(3-methoxyphenyl)-semicarbazones (**7e**) showed higher OOT at 185.4 °C.

Afterward, all experimental samples were carried out for programmed temperature DSC at 150 °C isothermal temperature for the evaluation of oxidation induction time (OIT). OIT of the experimental samples is outlined in Fig. 5.

OIT of TMPTO base oil was observed 1.07 min, though incorporation of 0.25 mass% of **7a** and **7a′** didn't show any significant increase in OIT, whereas BHT showed 5.48 min OIT. But TMPTO blended with 0.25 mass% of **7e** and **7c**

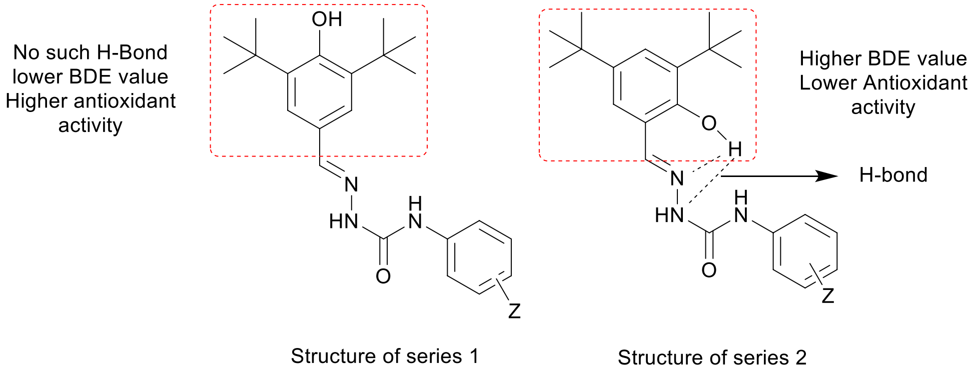


Fig. 3 Probable structure for the formation of hydrogen bond in series 2

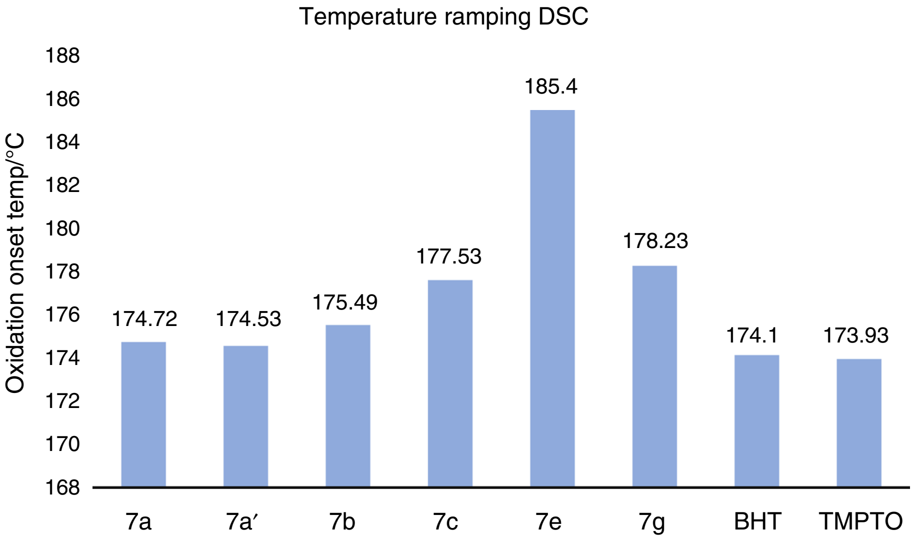


Fig. 4 Temperature ramping DSC analysis for the TMPTO with 0.25 mass% antioxidants

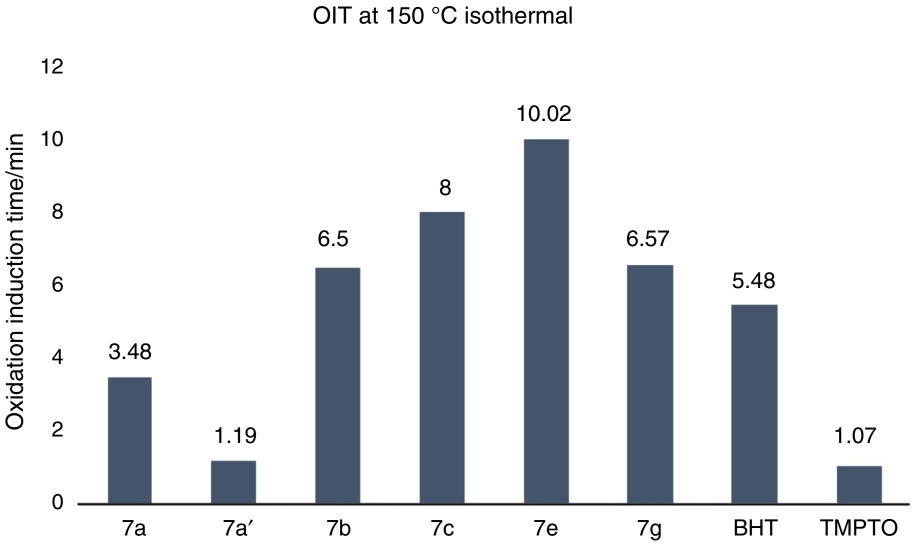


Fig. 5 Oxidation induction time of TMPTO incorporated with 0.25% experimental sample at 150 °C isothermal

showed better OIT values (10.02 min and 8 min, respectively) shown in Fig. 6. Again, TMPTO incorporated 4-fluorophenyl semicarbazones (**7b**) and 4-sec-butyl-phenyl semicarbazones (**7g**) showed almost identical OIT values (6.5 and 6.57 min, respectively).

This experiment was again carried out at 125 °C isothermal temperature with the same blended TMPTO oil to observe the change of OIT. The obtained results are displayed in Table 4.

It was found that 0.25 mass% **7e** and **7c** inhibited oxidation stability (OIT) of TMPTO 42.23 min and 41.52 min, respectively, at 125 °C isothermal (Fig. 7), while Blank TMPTO showed 2.25 min OIT.

Afterward, for the further assessment of antioxidant blended oil, we carried out the conventional thermogravimetric analysis (TGA) with TMPTO base oil and TMPTO with 0.25 mass% of **7c**, **7e** and BHT in order to evaluate the thermal stability.

It was seen from Fig. 8 that TMPTO with 0.25 mass% of compound **7e** and **7c** started decomposition at 373.93 °C and 372.77 °C, respectively, whereas only TMPTO decomposed at 341.07 °C. On the other hand, 341.86 °C decomposition temperatures were found for the TMPTO with 0.25 mass% of BHT which indicates that synthesized compounds **7c** and **7e** showed the higher thermal stability in contrast with the base oil TMPTO and standard antioxidant BHT.

It was found in the DSC and TGA results that oxidation stability of TMPTO increased significantly in the combination of compounds **7e** and **7c** compared to BHT. It was reported that the compounds having two or more antioxidant functions in one structure can able to exert auto-synergism in lubricant oil [11]. Due to the presence of two antioxidant functions (BHA and semicarbazone function) in the

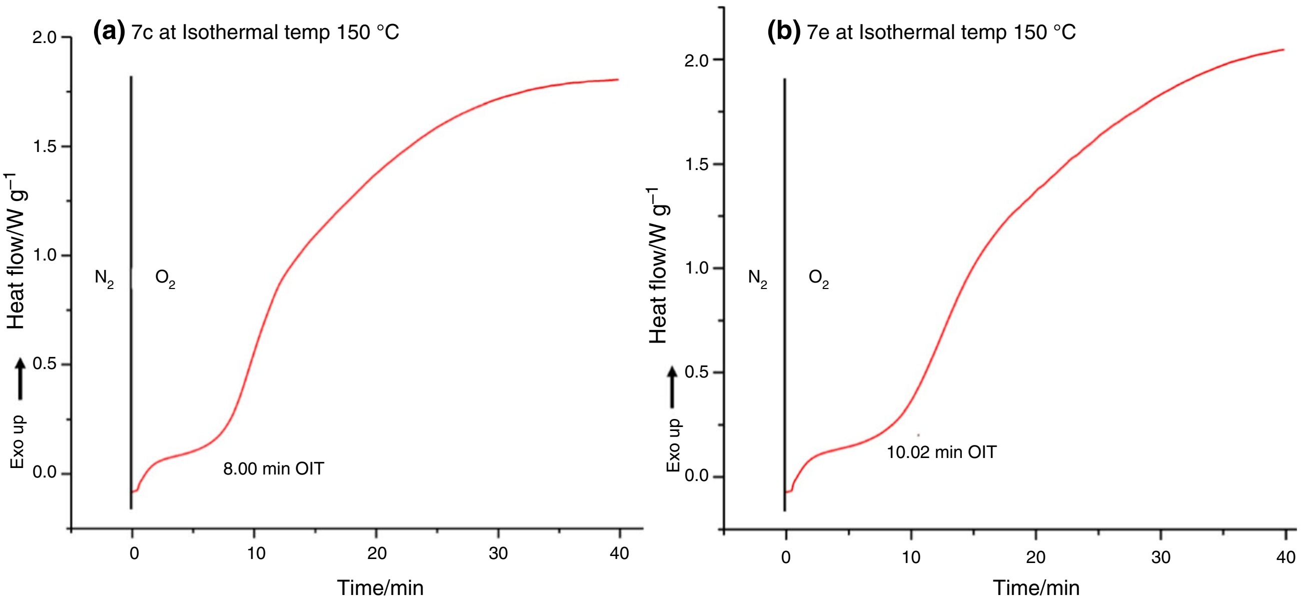


Fig. 6 OIT at isothermal temperature 150 °C measured by the heat flow vs time

Table 4 Oxidation induction time of TMPTO incorporated with 0.25% experimental sample at 125 °C isothermal

Compounds no.	0.25 mass% **7a**	0.25 mass% **7b**	0.25 mass% **7c**	0.25 mass% **7e**	0.25 mass% **7g**	0.25 mass% **7a′**	0.25 mass% BHT	TMPTO
OIT min	8.26	–	41.52	42.23	–	7.42	15.2	2.25

'–' No OIT was observed for the **7c** and **7g**

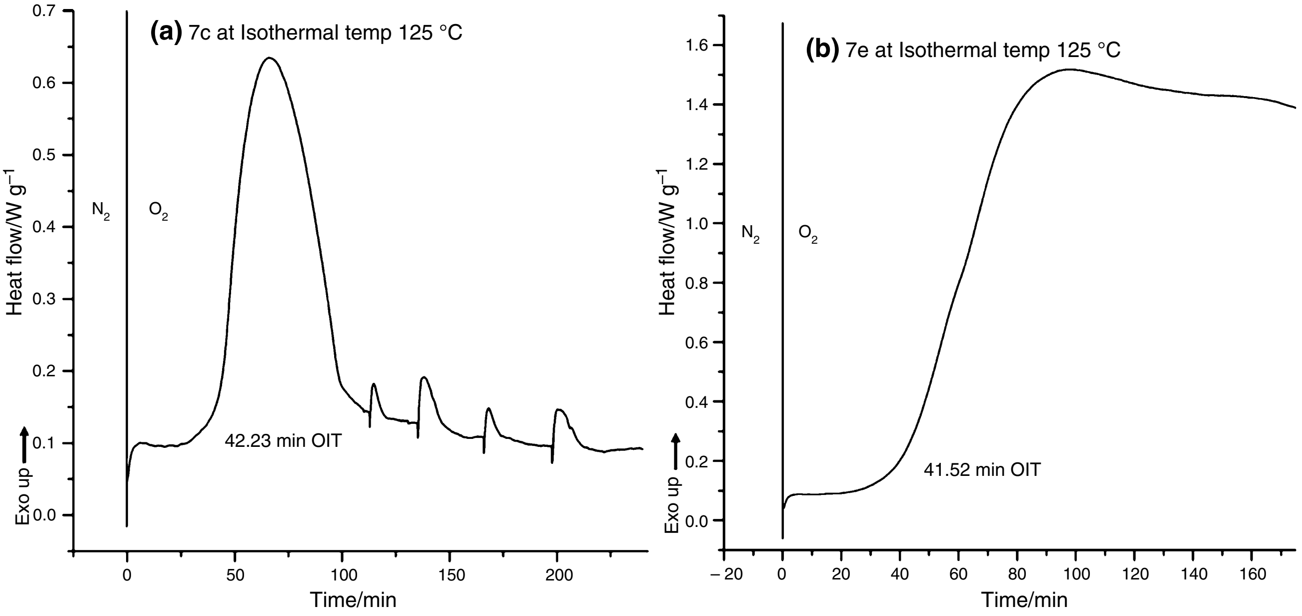


Fig. 7 OIT at isothermal temperature 125 °C measured by the heat flow versus time

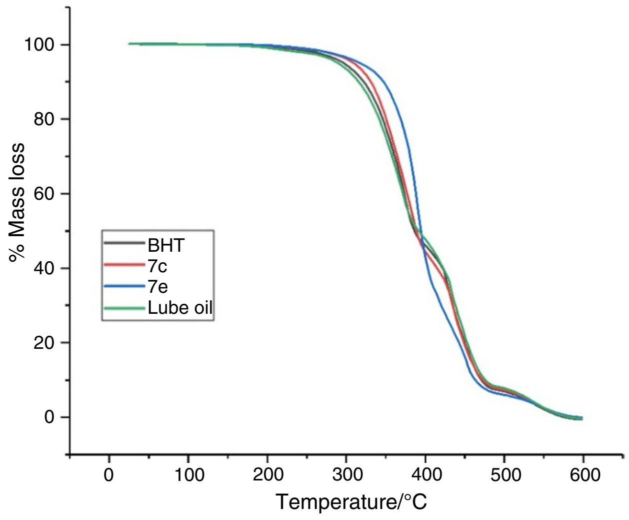
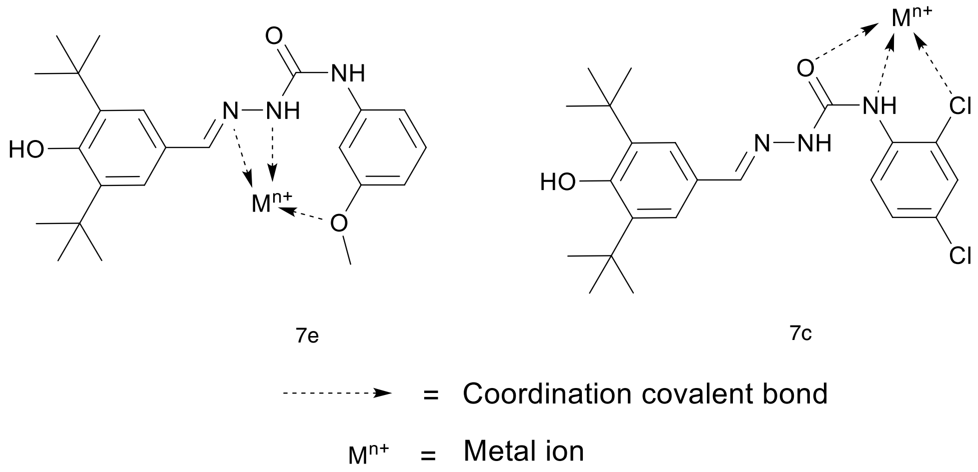


Fig. 8 Thermal decomposition temperature of TMPTO base oil and TMPTO with 0.25 mass% antioxidants obtained by thermogravimetric analysis

one structure, the synthesized semicarbazone may able to exhibit auto-synergistic effect to protect the oil from premature oxidation. But the OIT of compounds **7a** and **7a′** obtained by programmed temperature DSC didn't show any significant effect in the oxidation stability of lubricant oil. So, no antioxidant synergism was observed in the oil oxidation stability test for the compound **7a** and **7a′**, though it showed the highest antioxidant activity in non-enzymatic free radical scavenger test DPPH assay (Fig. 1). It was found in the literature [37] that the combination of two primary antioxidants (phenolic antioxidant and secondary aromatic amino antioxidant) exhibits negative synergistic effect in the oxidation of lubricant oil. Unfortunately, both butylated hydroxyphenyl (hindered phenol) and semicarbazone function (probably aromatic secondary amine) in structure of synthesized semicarbazones act as primary antioxidant. This may be a probable reason for the lower oxidation stability of the synthesized compounds in oil. Interestingly, OIT results of compounds **7e**, **7c**, **7b**, and **7g** show the better oxidation stability in comparison with BHT.

Generally, semicarbazones were reported as neutral tridentate ligand due to the presence of azomethine N and carbamoyl O, which can provide the stronger binding ability to metal ions. So several binding modes can be observed if additional coordinating functional group is present in the semicarbazone derivatives [38, 39]. Thus, the metal ions can easily be deactivated by appropriate semicarbazones through the formation of several coordination bond between donor atoms and metal ions. Deactivating metal ions in the lubricant oil is prerequisite to accelerate the oxidation stability since metal ions are very susceptible to convert the acid and hydroperoxide into free radicals. Therefore, metal deactivators or chelators are one of the important antioxidant classes in lubricant additives [40]. Generally metal deactivators or chelators deactivated the metal ions in lubricant oil which are responsible for accelerating oxidation reaction by the production of free radicals [41]. Therefore, all chelating metal deactivators contain multiple donor atoms which can able to deactivate the metal ions by the formation of complex compounds. Thus, semicarbazones can act as metal deactivator because of having multiple donor atoms. Due to the presence of additional donor atoms as substituents at N-4 ring of semicarbazones, several binding modes of semicarbazones might be observed and would be effective metal deactivator. Compounds **7e** and **7c** was found better oxidative stability in TMPTO in DSC experiment, and this may be due to the presence of 3-methoxy (**7e**) and 2,4-di-chlorine (**7c**) substituents at N-4 ring of semicarbazones. Thus, methoxy and chlorine groups might act as donor atom and their favorable position at N-4 ring of semicarbazone structure enhanced the metal deactivator properties by the increase in the metal binding mode [42, 43] which can be shown in Fig. 9.

Fig. 9 Metal binding mode of compound **7e** and **7c**



Therefore, incorporation of butylated hydroxyphenyl into semicarbazones provided significant free radical scavenging properties shown in DPPH results. However, it was not observed in differential scanning calorimeter test (oxidation stability) of lubricant oil. However, the presence of electron withdrawing substituents or donor atom at N-4 ring of semicarbazones may increase the metal binding mode which would help to efficiently deactivate the metal ions in the lubricant oil. Moreover, insertion of suitable functional groups at the appropriate position of the N-4 ring of semicarbazones helps to obtain several metal binding modes which are very useful to prepare a variety of metal complexes with important chemical properties.

Conclusions

In conclusion, two series of semicarbazones bearing 3,5-di-tert-butyl-4-hydroxyphenyl (**7a–j**) and 3,5-di-tert-butyl-2-hydroxyphenyl (**7a′–j′**) were accessed easily by the reaction of substituted semicarbazides with 3,5-di-tert-butyl-4-hydroxybenzaldehyde and 3,5-di-tert-butyl-2-hydroxybenzaldehyde, respectively. Antioxidant evaluation test DPPH assay revealed that series 1 (**7a–j**) exhibited promising free radical scavenging properties than series 2 (**7a′–j′**). It was discussed that better antioxidant activity of series 1 was found for the synergistic effect of butylated hydroxyphenyl and semicarbazones functions, while the formation of hydrogen bond lowers antioxidant activity of series 2 (**7a′–j′**). Compounds **7a**, **7b**, **7c**, **7e**, **7g**, **7a′** and BHT were separately blended with trimethylolpropane trioleate in 0.25 mass% to carry out the oxidation stability test by differential scanning calorimeter. And compounds **7e**, **7b**, **7c** showed the better oxidative stability of synthetic lubricant oil. The probable reason was concluded that incorporation of electron withdrawing group (3-methoxy and 2,4-di-chloro) at the N-4 position of semicarbazones (series 1) would make efficient metal deactivator or chelator since they can act as polydentate ligand because of having multiple donor atom in their structure. This DSC results revealed another important aspect of semicarbazones that proper modification of it can be used in the synthetic lubricant oil as a potential antioxidant. Again, it can also be anticipated that the synthesized compounds in this study would provide significant pharmacological properties since semicarbazone derivatives possess important pharmacophore properties as anticonvulsant, anticancer, etc.

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