Chapter - 2

Synthesis, characterization and evaluation of antioxidant activities of 5H-Dibenz[b,f]azepine and its analogues

There are two ways to see life:
One is as though everything is a miracle.
The other as though nothing is a miracle.
- Albert Einstein
INTRODUCTION

An extensive review on the physical, chemical, spectral and biological aspects of 5H-dibenz[b,f]azepine and its analogues was discussed in the chapter 1. There are virtually limitless series of structurally novel N-substituted 5H-dibenz[b,f]azepines with a wide range of physical, chemical and biological properties. In the following paragraph the updated literature and some of the patents work on 5H-dibenz[b,f]azepines are highlighted in the sequential order.

The investigation relates to a new class of organic compounds and more particularly to basic derivatives of iminodibenzyl to their salts and quaternary ammonium compounds were reported\(^1\). Morcol et al reports on new derivatives of iminodibenzyl, and their anti-epileptic activity\(^2\). Iminodibenzyl (10,11-dihydro-5H-dibenz[b,f]azepine) can be converted by suitable substitution at the nitrogen atom into pharmacologically valuable substances which have different effects. For example, substance with antiallergic, spasmolytic and psychoplectic activity are obtained by the introduction of dialkylamine-alkyl radicals and the introduction of dialkylamine - alcanoyl radicals produce substances with local anesthetic activity\(^3\). 3-Substituted 10,11-dihydro-5H-dibenz[b,f]azepine and 3-substituted 5H-dibenz[b,f]azepine of general formula.

Where X is ethylene or vinylene group, CH\(_2\)-CH\(_2\)- or –CH=CH–,
Y represents bromine or chlorine atom\(^4\).
Schindler *et al*\(^5\) reports on 3-Alkyl- or- 3- Alkanoyl-10,11-dihydro-5H-dibenz[b,f]azepine and 3-Alkyl-5H-dibenz[b,f]azepine and its derivatives having general formulas

Where R\(_1\) = Alkanoyl radical with 2-4 carbon atom R\(_2\) = Alkyl radical with 2-4 carbon atom. 10-Lower alkoxy-5H-dibenz[b,f]azepine and their derivatives were synthesized and reported\(^6\). 5-Dialkylaminoalkyl-5H-dibenz[b,f]azepine having general formula

Where R\(_1\) and R\(_2\) = hydrogen, halogen, trifluromethyl, alkyl or alkoxy, A = divalent, stight or branched lower alkylene chain and Z= amino, monoalkylamino, dialkylamino having useful therapeutic activity, specially as general central nervous system depressants and particularly as antiemetics, tranquilizers, calmatives, antishock agents was reported\(^7\). 10-Aminoalkyl-5H-dibenz[b,f]azepines and the corresponding 10,11-dihydro compounds, optionally bearing an alkyl substituent in the 5-position, possess pharmacological properties particularly antidepressant with the following general formula are reported.
Wherein, Am is a monoalkyl amino or dialkylamino, monoalkenylamino or dialkenylamino radical, Z is straight or branched chain alkylene or residue with maximum six carbon atom

and preferably 2 to 6 carbon atoms of which at least one is located between the carbon atom in 10 or 11-position. 5-Lower alkyl-4H-5,6,7,8,14,15-hexahydro-5,9-diazocino[1,2,3-de]-dibenzoazepines which are pharmacologically useful as antidepressants were also reported.

Plethora of information available in the literature unequivocally suggest that 5H-dibenz[b,f]azepine and its analogues are of various pharmacological importance. A view of recently published work has been highlighted in the following paragraph. Photochemical cyclodimerization and rearrangement of 5H-dibenz[b,f]azepine derivatives and photochemical reactivity of N-acyl dibenz[b,f]azepine analogues were recently reported. H. Blattner have proposed the process for the manufacture of substituted 5H-dibenz[b,f]azepines which are used for treating epilepsy. Restricted conformational processes in 10,11-dihydro-5H-dibenz[b,f]azepine derivatives by DNMR study has been reported. In vitro and in vivo microdialysis calibration for the measurement of carbamazepine and its metabolites in rat brain tissue using the internal reference technique were investigated by Van Belle et al. The reaction procedure for dehydrogenation of iminodibenzyl (10,11-dihydro-5H-dibenz[b,f]azepine) to iminostilbene (5H-dibenz[b,f]azepine) was investigated by comparison of potassium-promoted manganese, iron and cobalt oxide catalysts has been reported by Knell et al. Jens Querner et al systematically studied the conformational isomers in the photocyclodimerization of N-acylated dibenz[b,f]azepine derivatives were studied and
reported\textsuperscript{16}. Structures and vibrational spectra of 5H-dibenz[b,f]azepine and 5H-dibenz[a,d]cycloheptene-5-ol on the basis of quantum mechanical calculations were also studied and reported\textsuperscript{17}. Carbamazepine, one of the derivative of 5H-dibenz[b,f]azepine involving multiple-component crystalline solids studied from crystal engineering of the composition of pharmaceutical phases were reported\textsuperscript{18}. Structural studies of the polymorphs of carbamazepine, its dihydrate, and two solvates were also reported\textsuperscript{19}. Recently structural derivatives of basic analogues of 5H-dibenz[b,f]azepine such as 10-dethoxy-5H-dibenz[b,f]azepine, 1-(10,11-dihydrodibenz[b,f]azepine-5-yl)ethanone, 5-chlorocarbonyl - 10,11-dihydro-5H-dibenz[b,f]azepine, 10-oxo-10,11-dihydro-5H-dibenz[b,f]azepine-5-carboxamide has been studied by X-ray crystallography\textsuperscript{20-23}. 
Present Investigation

Literature on 5H-dibenz[b,f]azepine and their analogues reveals that these analogues are biologically important by having antiallergic activity, specially antihistaminic activity, spasmylytic, serotonin antagonistic, anticonvulsive, antiemetic, antiepileptic, anti-inflammatory, sedative, and fungicidal action\textsuperscript{24}. Even though enormous work has been reported on biological activities the studies on the antioxidant activities have not yet been reported. Thus, in order to explore the antioxidant activities and structure-activity-relationship of 5H-dibenz[b,f]azepine and its basic analogues, the present work has been carried out. Initially, we have synthesized the following compounds presented in \textbf{table 2.1}, by following the literature method\textsuperscript{24}. 
Table 2.1. List of synthesized compounds.

<table>
<thead>
<tr>
<th>Name</th>
<th>Compound number</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>5H-Dibenz[b,f]azepine</td>
<td>I</td>
<td><img src="image" alt="Structure of 5H-Dibenz[b,f]azepine I" /></td>
</tr>
<tr>
<td>5H-Dibenz[b,f]azepine-5-carboxamide</td>
<td>II</td>
<td><img src="image" alt="Structure of 5H-Dibenz[b,f]azepine-5-carboxamide II" /></td>
</tr>
<tr>
<td>1-(5H-Dibenz[b,f]azepine-5-yl) ethanone</td>
<td>III</td>
<td><img src="image" alt="Structure of 1-(5H-Dibenz[b,f]azepine-5-yl) ethanone III" /></td>
</tr>
<tr>
<td>10-Methoxy-5H-dibenz[b,f]azepine</td>
<td>IV</td>
<td><img src="image" alt="Structure of 10-Methoxy-5H-dibenz[b,f]azepine IV" /></td>
</tr>
<tr>
<td>10,11-Dihydro-5H-dibenz[b,f]azepine-5-carbonyl chloride</td>
<td>V</td>
<td><img src="image" alt="Structure of 10,11-Dihydro-5H-dibenz[b,f]azepine-5-carbonyl chloride V" /></td>
</tr>
<tr>
<td>1-(10,11-Dihydro-5H-dibenz[b,f]azepine-5-yl) ethanone</td>
<td>VI</td>
<td><img src="image" alt="Structure of 1-(10,11-Dihydro-5H-dibenz[b,f]azepine-5-yl) ethanone VI" /></td>
</tr>
</tbody>
</table>
Synthesis of 5H-dibenz[b,f]azepine and its derivatives

5H-Dibenz[b,f]azepine

5H-Dibenz[b,f]azepine (I) was prepared by coupling of \( o \)-nitro toluene (2 mM) in methanol in presence of basic catalyst sodium formate and KOH in methanol (1 mM) by refluxing for 4 hr to form bibenzyl (\( o, o' \)-dinitroazepine). This is reduced to give 10,11-Dihydro-dibenz[b,f]azepine (a) upon refluxing with phosphoric acid, a cyclization agent for 3 hr, further upon dehydrogenation with CaO in dimethyl aniline solution upon reflux for 2 hr to obtain 5H-dibenz[b,f]azepine.

5H-Dibenz[b,f]azepine-5-carboxamide

5H-Dibenz[b,f]azepine (1.93 g, 10 mM) was refluxed in the presence of COCl\(_2\) with strong base (NaN\(_2\)) for 4 hr to get chloro carbonyl dibenz[b,f]azepine (0.253 g, 10 mM), which upon further reflux with concentrated ammonia (25 mL) to give 5H-Dibenz[b,f]azepine-5-carboxamide (II).
1-Dibenz[b,f]azepine-5-yl)ethanone

5H-Dibenz[b,f]azepine-5-yl)ethanone (III) was prepared by refluxing 5H-dibenz[b,f]azepine (1.93 g, 10 mM) in acetic anhydride (25 mL) for 6 hr.

10-Methoxy-5H-dibenz[b,f]azepine

10-Methoxy-5H-dibenz[b,f]azepine (IV) was prepared by brominating N-acetyl-5H-dibenz[b,f]azepine (2.35 g, 10 mM) using bromine (3.2 g, 20 mM) in dichloromethane (25 mL) to obtain dibromo derivative, which was further refluxed with KOH (1.12 g, 20 mM) in CH₃OH (25 mL) for 6 hr to obtain the product. It was further purified by crystallization using dichloromethane and ethanol (1:1 v/v)

5-Chlorocarbonyl-10,11-dihydro-5H-dibenz[b,f]azepine

5-Chlorocarbonyl-10,11-dihydro-5H-dibenz[b,f]azepine (V) obtained by refluxing 10,11-Dihydro-5H-dibenz[b,f]azepine (1.95 g, 10 mM) with concentrated triphosgene (25 mL) in presence of di base (NaNH₂) for 4 hr.
1-(10,11-Dihydrobenz[b,f]azepine-5-yl)ethanone

1-(10,11-Dihydrobenz[b,f]azepine-5-yl)ethanone (VI) was prepared by refluxing 10,11-Dihydro-5H-dibenz[b,f]azepine (1.95 g, 10 mM) in acetic anhydride (25 mL) for 6 hr. It was purified by crystallization using methanol.

**Materials and Methods**

The organic solvents such as methanol (E-Merck), chloroform (E-Merck), n-hexane (E-Merck), benzene (Qulegens), tetrahydrofuran (THF) grade (S.d.fine.chem), diethyl ether (Ranbaxy), ethyl acetate (E-Merck), acetic acid (E-Merck) were of analytical reagent grade. Distilled water-double distilled water by quartz distillation unit. All the chemical reagents were obtained from the standard commercial sources unless otherwise indicated. Sodium bicarbonate, anhydrous sodium sulphate (Ranbaxy), phoszene, ethyl formate, o,o’ ethylene diamine, phosphoric acid, dimethyl aniline, sodium amide, acetyl chloride, bromine, acetic anhydride (s.d.fine. chem). tlc aluminium sheets-Silica gel 60 F254 was also purchased from Merck.

The reagents and solvents used in the present investigation were purified and dried according to standard procedure$^{25-28}$. Anhydrous sodium sulphate was activated by
heating over naked flame for 3-4 hr, cooled in desiccator in fused calcium chloride atmosphere and stored in air-tight bottle.

Melting point of the compounds is determined using SELACO-650 and Veego VMP-III model hot stage melting point apparatus and is uncorrected. The pH of the solution was measured using pH meter, model APX 175 (control Dynamic Instrumentation Pvt. Ltd). The pH meter was standardized using buffer tablets of 9.2, 7.0 and 4.0 at 25°C.

Identification and structure elucidation of newly synthesized compounds under study was carried out by using various spectroscopic techniques such as IR, ¹H NMR, mass and elemental analysis.

A Nicolet 5700 FTIR instrument was used for recording IR spectra for the synthesized compounds. About 2.0 to 3.0 mg of compound was prepared as KBr pellet and the IR spectra were recorded. A Bruker DRX-500MHz spectrometer operating at 500.13 MHz was used. ¹H NMR spectra in CDCl₃ or DMSO-d₆ with 40 mg of the sample dissolved in 0.5 ml of solvent with tetramethylsilane(TMS) as internal standard for measuring the chemical shift values to within±0.001 ppm. Mass spectra of the synthesized compound were obtained using a Q-TOF Waters Ultima instrument (No-Q-Tof GAA 082, Water Corporation, Manchester, UK) fitted with an Electron spray ionization(ESI) source. The data acquisition software used was Version 4.0.

The synthesized compounds were further purified by column chromatography using activated silica gel [60-120 mesh] packed on to the glass column [450×40 mm] with methanol as solvent. The crude product was loaded and eluted using mixture of n-hexane : ethyl acetate(90:10). The fractions were collected separately and the active fraction was concentrated by using the rotary evaporator. The pure compound was monitored for single
spot through thin layer chromatography (tlc). The plates were developed using n-hexane : ethyl acetate (90 : 10) as mobile solvent. The spot was located by exposing the tlc plates to iodine vapours.

All the synthesized compounds were characterized and the data were presented in the table 2.2-2.7. The IR, $^1$H NMR, and mass spectra of compound I and IV are presented in the figure 2.1-2.6.
Table 2.2: Physico-chemical and spectral data of 5H-dibenz[b,f]azepine(I)

<table>
<thead>
<tr>
<th>IUPAC Name</th>
<th>5H-Dibenz[b,f]azepine</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Molecular formula</strong></td>
<td>C₁₄H₁₁N</td>
</tr>
<tr>
<td><strong>Nature</strong></td>
<td>Orange yellow solid</td>
</tr>
<tr>
<td><strong>Melting point</strong></td>
<td>196-199 (197-201°C)²⁴</td>
</tr>
<tr>
<td><strong>Yield</strong></td>
<td>72 %</td>
</tr>
<tr>
<td><strong>Mass</strong></td>
<td>M⁺ 194.16</td>
</tr>
<tr>
<td><strong>IR</strong></td>
<td>3360.0 (N-H), 3046.3 (Ar-H) cm⁻¹</td>
</tr>
<tr>
<td><strong>¹H NMR</strong></td>
<td>3.3 (s, 1H, N-H), 6.7-8.1 (m, 8H, Ar-H), 6.2 (m, 2H, seven membered Ar-H)</td>
</tr>
</tbody>
</table>
| **Elemental Analysis** | Anal.Calcd: C, 87.01; H, 5.74; N, 7.25 %
               | Found: C, 87.00; H, 5.75; N, 7.25 % |
Table 2.3: Physico-chemical and spectral data of 5H-dibenzo[b,f]azepine-5-carboxamide(II)

<table>
<thead>
<tr>
<th>IUPAC Name</th>
<th>5H-Dibenzo[b,f]azepine-5-carboxamide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular formula</td>
<td>C_{15}H_{12}N_{2}O</td>
</tr>
<tr>
<td>Nature</td>
<td>White solid</td>
</tr>
<tr>
<td>Melting point</td>
<td>191-193 (190-193 °C)\textsuperscript{24}</td>
</tr>
<tr>
<td>Yield</td>
<td>81 %</td>
</tr>
<tr>
<td>Mass</td>
<td>M\textsuperscript{+} 236.15</td>
</tr>
<tr>
<td>IR</td>
<td>3421.0-3465.4 (NH\textsubscript{2}), 3163.4 (Ar-H), 1681 (C=O) cm\textsuperscript{-1}</td>
</tr>
<tr>
<td>\textsuperscript{1}H NMR</td>
<td>\textsuperscript{1}H NMR (\delta, CDCl\textsubscript{3}): 6.9 (s, 2H, NH\textsubscript{2}), 7.3-7.5 (m, 8H, Ar-H), 7.0 (m, 2H, seven membered Ar-H).</td>
</tr>
<tr>
<td>Elemental Analysis</td>
<td>Anal.Calcd: C, 76.25; H, 5.12; N, 11.86%</td>
</tr>
<tr>
<td></td>
<td>Found: C, 76.22; H, 5.11; N, 11.85%</td>
</tr>
</tbody>
</table>
Table 2.4: Physico-chemical and spectral data of 1-(5H-dibenz[b,f]azepine-5-yl)ethanone (III)

<table>
<thead>
<tr>
<th>IUPAC Name</th>
<th>1-(5H-Dibenz[b,f]azepine-5-yl)ethanone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular formula</td>
<td>C_{16}H_{13}NO</td>
</tr>
<tr>
<td>Nature</td>
<td>Brown solid</td>
</tr>
<tr>
<td>Melting point</td>
<td>158-161 (159-162 °C)^{24}</td>
</tr>
<tr>
<td>Yield</td>
<td>79%</td>
</tr>
<tr>
<td>Mass</td>
<td>M^+ 235.18</td>
</tr>
<tr>
<td>IR</td>
<td>3069.0 (Ar-H); 1668.9 (C=O)</td>
</tr>
<tr>
<td>^1H NMR</td>
<td>7.2-7.5 (m, 8H, Ar-H), 7.0 (d, 2H, seven membered Ar-H), 2.0 (s, 3H, CH₃) cm⁻¹</td>
</tr>
<tr>
<td>Elemental Analysis</td>
<td>Anal.Calcd: C, 81.68; H, 5.57; N, 5.95%</td>
</tr>
<tr>
<td></td>
<td>Found: C, 81.66; H, 5.57; N, 5.98%</td>
</tr>
</tbody>
</table>
Table 2.5: Physico-chemical and spectral data of 10-methoxy-5H-dibenz[b,f]azepine(IV)

<table>
<thead>
<tr>
<th>IUPAC Name</th>
<th>10-Methoxy-5H-dibenz[b,f]azepine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular formula</td>
<td>C_{13}H_{13}NO</td>
</tr>
<tr>
<td>Nature</td>
<td>Yellow solid</td>
</tr>
<tr>
<td>Melting point</td>
<td>180-182 (181-183 0C)²⁴</td>
</tr>
<tr>
<td>Yield</td>
<td>76 %</td>
</tr>
<tr>
<td>Mass</td>
<td>M⁺ 224.17</td>
</tr>
<tr>
<td>IR</td>
<td>3360.0 (N-H), 3163.4 (Ar-H)</td>
</tr>
<tr>
<td>^{1}H NMR</td>
<td>3.3 (s, 1H, N-H), 6.8-7.9 (m, 8H, Ar-H), 7.0 (m, 1H, seven membered Ar-H), 3.8 (s, 3H, OCH₃).</td>
</tr>
<tr>
<td>Elemental Analysis</td>
<td>Anal.Calcd: C, 80.69; H, 5.87; N, 6.27%</td>
</tr>
<tr>
<td></td>
<td>Found: C, 80.68; H, 5.87; N, 6.28%</td>
</tr>
</tbody>
</table>
Table 2.6: Physico-chemical and spectral data of 10,11-dihydro-5H-dibenz[b,f]azepine-5-carbonyl chloride (V)

<table>
<thead>
<tr>
<th>IUPAC Name</th>
<th>10,11-Dihydro-5H-dibenz[b,f]azepine -5-carbonyl chloride</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular formula</td>
<td>C_{15}H_{10}NOCl</td>
</tr>
<tr>
<td>Nature</td>
<td>White solid</td>
</tr>
<tr>
<td>Melting point</td>
<td>148-150 (149-151 °C)</td>
</tr>
<tr>
<td>Yield</td>
<td>73 %</td>
</tr>
<tr>
<td>Mass</td>
<td>M^+ 257.77</td>
</tr>
<tr>
<td>IR</td>
<td>3163.4 (Ar-H), 1726.7 (C=O)</td>
</tr>
<tr>
<td>^1H NMR</td>
<td>^1H NMR (δ, CDCl₃): 7.2-7.6 (m, 8H, Ar-H), 2.5 (s, 2H, seven membered Ar-H) cm⁻¹</td>
</tr>
<tr>
<td>Elemental Analysis</td>
<td>Anal. Calcd: C, 69.91; H, 4.69; N, 5.43%</td>
</tr>
<tr>
<td></td>
<td>Found: C, 69.90; H, 4.66; N, 5.45%</td>
</tr>
</tbody>
</table>
Table 2.7: Physico-chemical and spectral data of 1-(10,11-dihydro-5H-dibenz[b,f]azepine-5-yl)ethanone (VI)

<table>
<thead>
<tr>
<th>IUPAC Name</th>
<th>1-(10,11-Dihydro-5H-dibenz[b,f]azepine-5-yl)ethanone</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Molecular formula</strong></td>
<td>C_{16}H_{15}NO</td>
</tr>
<tr>
<td><strong>Nature</strong></td>
<td>Light yellow solid</td>
</tr>
<tr>
<td><strong>Melting point</strong></td>
<td>153-155 (153-156 °C)²⁴</td>
</tr>
<tr>
<td><strong>Yield</strong></td>
<td>71 %</td>
</tr>
<tr>
<td><strong>Mass</strong></td>
<td>M⁺ 237.33</td>
</tr>
<tr>
<td><strong>IR</strong></td>
<td>3163.4 (Ar-H), 1667.6 (C=O) cm⁻¹</td>
</tr>
<tr>
<td><strong>¹H NMR</strong></td>
<td>¹H NMR (δ, CDCl₃): 7.3-7.6 (m, 8H, Ar-H), 2.5 (s, 2H, seven membered Ar-H), 1.9 (s, 3H, CH₃) 2.5 (d, 2H, 2 CH₂) cm⁻¹</td>
</tr>
<tr>
<td><strong>Elemental Analysis</strong></td>
<td>Anal. Calcd: C, 80.98; H, 6.37; N, 5.90%</td>
</tr>
<tr>
<td></td>
<td>Found: C, 80.98; H, 6.37; N, 5.95%</td>
</tr>
</tbody>
</table>
Figure 2.1. IR spectra for 5H-dibenz[b,f]azepine(I)
Figure 2.2. $^1$H NMR spectra for 5H-dibenz[b,f]azepine(I)
Figure 2.3. Mass spectra for 5H-dibenz[b,f]azepine(I)
Figure 2.4. I.R spectra for 10-methoxy-5H-dibenzo[b,f]azepine(IV)
Figure. 2.5. $^1$H NMR spectra for 10-methoxy-5H-dibenzo[b,f]azepine(IV)
Figure 2.6. Mass spectra for 10-methoxy-5H-dibenz[b,f]azepine (IV)
Evaluation of Antioxidant Activities

Background

Oxidation of organic compounds is one of the efficient methods of organic synthesis. On the other side, the autooxidation of organic compounds, their mixtures, and products promotes their rapid deterioration due to the action of atmospheric oxygen. Products such as rubber, polymers, hydrocarbon fuels, lubricants, organic solvents, semi products, drugs etc. are spoiled due to oxidation by oxygen. Antioxidant prevents the rapid development of these undesirable processes. They were the object of intensive study during the last 40 years. The practical use of antioxidants began in the end of the 19th century.

It is also necessary in case of foods to determine the efficacy of natural antioxidants for food preservation or protection, against oxidative damage, to avoid deleterious changes and loss of commercial and nutritional value. It is also necessary to develop a rapid method for determining the potential antioxidants capacity.

The antioxidant effectiveness is measured by monitoring the inhibition of oxidation of a suitable substrate. After the substrate is oxidized under standard conditions, the extent of oxidation (as end point) is measured by chemical, instrumental or sensory methods. Hence essential features of any test are a suitable substrate, an oxidation initiator and an appropriate measure of end point.

The antioxidant test in biological systems is classified into two groups. Those assays used to evaluate lipid peroxidation - in this method the lipid or lipoprotein substrate under
standard conditions is used and the degree of oxidation inhibition is measured\(^{33}\) and those assays used to measure free radical scavenging ability\(^ {34}\).

In general, two types of approaches have been taken (i) inhibition assays, for which the extent of the scavenging of a free radical by hydrogen atom or electron donation is the marker of antioxidant activity. These inhibition tests are indirect tests of total antioxidant power\(^ {35}\). (ii) assays involving the presence of antioxidant systems during the generation of the radical, for which the activity is measured on the rate of oxidation of a target molecule\(^ {36}\). The antioxidant activity measurement system can also be classified into six categories and are reported by various author\(^ {37-41}\).

Numerous methods based on colorimetry\(^ {42}\), Spectrophotometry\(^ {43}\), fluorimetry\(^ {44}\), voltammetry\(^ {45}\), polarography\(^ {46}\), thin-layer chromatography\(^ {47}\), paper chromatography\(^ {48}\), gel permeation chromatography\(^ {49}\), gass-liquid chromatography\(^ {50}\) and high performance liquid chromatography(HPLC)\(^ {51}\).

Numerous *in vivo* and *in vitro* assays are applied for the evaluation of antioxidants. Some of the important assays among them are

1. **In vitro methods**
   
   a. DPPH(2,2-diphenyl-1-picyrylhydrazyl) assay.
   
   b. TEAC(Trolox equivalent Antioxidant Capacity) /ABTS(2,2-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid) assay.
   
   c. ORAC(Oxygen Radical Absorbance Capacity) assay.
   
   d. FRAP(Ferric Reducing Ability of Plasma) assay.
   
   e. TRAP(Total Radical Trapping Antioxidant Parameter) assay.
   
   f. TBARS(Thiobarbituric Acid Reactive Substances) assay.
g. Inhibition of lipid peroxidation using β-Carotene linoleate assay.

h. Phosphomolyndate assay.

i. Inhibition of Human low-density lipoprotein(LDL) oxidation assay.

j. Lipid peroxidation activity in Egg Liposome model system.

k. Reducing power assay.

2. *In vivo* methods

a. Superoxide dismutase and glutathione peroxidase determination.

b. TOSC(Total Oxygen Scavenging Capacity) assay

c. Method to measure lipid peroxidation

   i. Conjugated diene assay.

   ii. Lipid peroxide PD(peroxide detecting) assay.

   iii. Linoleyl hydroperoxide(L-OOH) and Linoleyl hydroxide(L-OH) assay.

d. Crocin based assay.

In the present investigation, evaluation of antioxidant activities of newly synthesized compounds was carried out by the following *in vitro* methods.

A. DPPH(2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay.

B. Inhibition of lipid peroxidation using β-Carotene linoleate assay.

C. Reducing power assay.

D. Inhibition of Human low-density lipoprotein(LDL) oxidation assay.
DPPH free radical scavenging assay\textsuperscript{52}

**Principle**

There is an increasing interest in antioxidants, particularly in those intended to prevent the presumed deleterious effects of free radicals in the human body, and to prevent the deterioration of fats and other constituents of foodstuffs. In both the cases, there is a preference for antioxidants from natural rather than from synthetic sources\textsuperscript{53}. There is therefore a parallel increase in the use of methods for estimating the efficiency of such substances as antioxidants\textsuperscript{54-55}. One such method that is currently popular is based upon the use of the stable free radical 2,2-diphenyl-1-picrylhydrazyl(DPPH).

The DPPH is a stable free radical (C\textsubscript{12}H\textsubscript{12}N\textsubscript{5}O\textsubscript{6}, M = 394.33). The assay method is based on the measurement of scavenging capacity of antioxidants towards it. The odd electron of nitrogen atom in DPPH is reduced by receiving a hydrogen atom from the antioxidants to the corresponding hydrazine\textsuperscript{56}.

\[ \begin{align*}
\text{\textit{a- a- Diphenyl-\textbeta- picrly hydrazyl}} & \quad \text{\textit{a- a- Diphenyl-\textbeta- picrly hydrazine}} \\
\end{align*} \]
The ability is evaluated using electron spin resonance spectroscopy on the basis that the DPPH signal intensity is inversely proportional to the test antioxidant concentration and to the reaction time \(^{57-60}\). However, the more frequently used technique is the decolouration assay, which evaluates the absorbance decrease at 515-528 nm produced by the addition of the antioxidant to a DPPH solution in ethanol or methanol. DPPH has been used to evaluate the antioxidant activity of phenolic compounds by measuring the change in absorbance at 515-517 nm \(^{61-62}\).

When a solution of \(\alpha-\alpha\)-Diphenyl-\(\beta\)-picryl hydrazyl mixed with that of a substance that can donate a hydrogen atom, then this gives rise to the reduced form \(\alpha-\alpha\)-Diphenyl-\(\beta\)-picryl hydrazine with the loss of this violet colour (although there would be expected to be a residual pale yellow colour from the picryl group still present). The primary reaction is

\[
Z \cdot + AH = Z-H + A \cdot \quad [1]
\]

Where, \(Z \cdot\) represents DPPH radical, AH is the donor molecule, ZH is the reduced form and \(A \cdot\) is the free radical. Then \(A \cdot\) undergo further reactions which control the overall stoichiometry that is, the number of molecules of DPPH reduced (decolorized) by one molecule of the reductant. The reaction [1] is therefore intended to provide the link with the reactions taking place in an oxidizing system, such as the autooxidation of lipid or other unsaturated substances; the DPPH molecule \(Z \cdot\) is thus intended to represent the
free radicals formed in the system whose activity is to be suppressed by the substance AH.

The parameter EC\textsubscript{50} or IC\textsubscript{50} (efficient concentration or inhibitory concentration)

One parameter that has been introduced recently for the interpretation of the results from DPPH method, is the “efficient concentration or inhibitory concentration” (EC\textsubscript{50} or IC\textsubscript{50}). This is defined as the concentration of substrate that cause 50\% loss of the DPPH activity (colour). This parameter was apparently introduced by Brand-Williams and his colleagues\textsuperscript{63-64}.

**Material required:**

- DPPH
- Distilled ethyl alcohol.
- Stoppard test tube.
- Spectrophotometer.
- Aluminum foil.
- Standard flask (50 and 25 mL)
- Micro pipette (10 mL)
- Cuvet.
- Ascorbic acid(AA).
- Butylated hydroxyl anisole(BHA).
Test solution:

The experimental compounds were dissolved in distilled ethyl alcohol (50 mL) to prepare 1000 µM solution. Solutions of different concentrations (10, 25, 50, 100, 200 and 500 µM) were prepared by serial dilution.

Test procedure:

The DPPH radical scavenging effect was carried out according to the method first employed by Blois. Compounds of different concentrations were prepared in distilled ethanol, 1 mL of each compound solutions having different concentrations (10, 25, 50, 100, 200 and 500 µM) were taken in different test tubes, 4 mL of 0.1 mM ethanol solution of DPPH was added and shaken vigorously. The tubes were then incubated in the dark room at RT for 20 min. A DPPH blank was prepared without compound, and ethanol was used for the baseline correction. Changes (decrease) in the absorbance at 517 nm were measured using a UV–visible spectrophotometer (Shimadzu 160A). The radical scavenging activities were expressed as the inhibition percentage and were calculated using the formula:

Radical scavenging activity (%) = \[ \left( \frac{A_0 - A_1}{A_0} \right) \times 100 \]

Where \( A_0 \) is absorbance of the control (blank, without compound) and \( A_1 \) is absorbance of the compound. The radical scavenging activity of BHA and ascorbic acid was also measured and compared with that of the different synthesized compound. The compound concentration providing 50% inhibition (IC\(_{50}\)) was calculated from the graph of radical scavenging activity (RSA) percentage against compound concentrations.
Inhibition of lipid peroxidation using β-Carotene linoleate assay\textsuperscript{65}

Principle

Numerous techniques have been developed for the evaluation of antioxidants, some of which have been reviewed recently\textsuperscript{66}. The method developed by Marco provides a rapid, reliable system for the analyses that alleviates many of the shortcomings encountered with other techniques. A procedure is described for rapid evaluation of antioxidants. The oxidative destruction of carotene in the emulsion was observed directly with a colorimeter. The antioxidants were then evaluated according to their effect on the rate of carotene decolorization. This procedure is simple and gives quick results. The experimental setup is uncomplicated, and 10 or more samples can be evaluated simultaneously without difficulty. Heat-induced oxidation of an aqueous emulsion system of β-carotene-linolic acid was employed as an antioxidant test reaction. The test is based on the fact that β-carotene undergoes rapid discoloration in the absence of antioxidant\textsuperscript{67}. The mechanism of lipid peroxidation is shown in (figure 2.7). During oxidation, the initiator abstracts hydrogen atom from the allylic position of the fatty acid (the most labile position), and this is followed by a rapid isomarization of one of the double bonds to yield a trans configuration, thereby forming two conjugated double bonds and a new radical in either 13 or 9 position\textsuperscript{68} (in Figure. 2.7, position 13 is shown). The pentadienyl free radical so formed then attacks highly unsaturated β-carotene molecules in an effort to reacquire a hydrogen atom. As the β-carotene molecules lose their conjugation, the carotenoids lose their characteristic orange colour. This process can be monitored spectrophotometrically.
The presence of antioxidants can hinder the extent of β-carotene degradation by “neutralizing” the linoleate free radical and any other free radicals formed within the system. This method is based on the determination of the coupled oxidation of carotene and linoleic acid. The basic principle is that “Linoleic acid which is unsaturated fatty acid gets oxidized by Reactive Oxygen Species(ROS) produced by oxygenated water. The products formed initiate the β-carotene oxidation, which leads to discoloration. The absorbance is measured at 470 nm. This is prevented by antioxidants, hence extent of decrease in discoloration indicates the activity i.e., more prevention of discoloration indicates higher antioxidant activity”. This assay is simple, reproducible and time efficient for rapid evaluation of antioxidant properties.

**Material required:**

- β-Carotene.
- Linoleic acid.
- Oxygenated water.
- Double distilled water.
- Chloroform.
- Tween-40 (polyoxyethylene sorbitan mono palmitate).
- Distilled ethyl alcohol.
- Stoppered test tube.
- Water bath with temperature regulator.
- Spectrophotometer.
- Aluminium foil.
- Standard flask (50, 25 and 10 mL).
- Micro pipette (10 mL).
- Cuvet.
- Ascorbic acid (AA).
- Butylated hydroxyl anisole (BHA).

**Test solution:**

The experimental compounds were dissolved in distilled ethyl alcohol (50 mL) to prepare 1000 µM solution. Solutions of different concentrations (10 and 25 µM) were prepared by serial dilution.

**Test procedure:**

Each compound at the final concentrations of 10 and 25 µM/mL were incorporated into β-carotene-linoleic acid model system independently and the activity was monitored spectrophotometrically at 470 nm.

**Preparation of the suspension**

The substrate suspension was prepared by addition of β-carotene (4 mg dissolved in 5 mL chloroform) into a covered round bottomed flask containing Tween-40 (600 mg).
followed by the addition of linoleic acid (60 µL). The chloroform was removed completely under vaccum using rotavapour at 40°C. The resulting solution was diluted with triple distilled water (30 mL) and the emulsion was mixed well and diluted with oxygenated water (120 mL). The aliquots (4 mL) was transferred to different stopper test tubes containing compound (50 and 100 µM/mL) in distilled ethanol. Control was prepared with distilled ethanol (1 mL) and emulsion (4 mL). BHA and ascorbic acid solution as internal standards of the same concentration were also analyzed for comparison. Zero adjustment was done using distilled water. Absorbance of the samples was measured at a wavelength of 470 nm, immediately (t=0), and subsequently after every 30 min for 3 hr (t=180). The tubes were placed in a water bath at 50°C between the readings. Antioxidant activities (AA) of each compound were evaluated in triplicates in terms of photooxidation of β-carotene using the following formula:

\[
\% \text{ AA} = 100 \left[1 - \frac{(A_0 - A_t)}{(A_0^o - A_0^t)}\right]
\]

Where, AA = Antioxidant Activity.

\[
A_0 = \text{Initial absorbance of the sample.}
\]

\[
A_t = \text{Absorbance of the sample after time'\(t'\).}
\]

\[
A_0^o = \text{Initial absorbance of the control.}
\]

\[
A_0^t = \text{Absorbance of control after time'\(t'\).}
\]
Reducing power assay (Iron reducing activity)\textsuperscript{69}

**Principle**

The reducing power is an assay determined using a modified iron(III) to iron(II) reduction assay. In this assay, the yellow colour of the test solution change to various shades of green and blue depending upon the reducing power of each compound. The presence of reducers (i.e., antioxidants) causes the reaction of the ferricyanide(Fe\textsuperscript{3+}) complex to the ferrous(Fe\textsuperscript{2+}) form giving, after the addition of trichloroacetic acid and ferric chloride. Therefore, the Fe\textsuperscript{2+} can be monitored by measurement of the formation of Perl’s Prussian blue at 700 nm. This is one of the simplest methods for determining antioxidant activity.

**Material required:**

- Phosphate buffer.
- 1% Potassium ferric cyanide.
- 10% Trichloroacetic acid.
- Double distilled water.
- 0.1% Ferric chloride.
- Distilled ethyl alcohol.
- Stoppard test tube.
- Water bath.
- Spectrophotometer.
- Standard flask (50, 25 and 10 mL).
- Micro pipette (10 mL).
- Cuvet.
- Ascorbic acid (AA).
- Butylated hydroxyl anisole (BHA).

**Test solution:**

The experimental compounds were dissolved in distilled ethyl alcohol (50 mL) to prepare 1000 µM solution. Solutions of different concentrations (10, 25, 50, 100, 200 and 500 µM) were prepared by serial dilution.

**Test procedure:**

The reducing power of synthesized compounds was determined according to the method of Jayaprakasha *et al.*\(^{69}\). The compounds having 50 and 100 µM were mixed with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of 1% potassium ferric cyanide, and then incubated at 50\(^\circ\)C for 20 min. To this mixture 2.5 mL of 10% trichloroacetic acid was added and the mixture was centrifuged at 3000 rpm for 20 min. The upper layer (2.5 mL) was mixed with 2.5 mL of distilled water and 0.5 mL of 0.1% ferric chloride and the absorbance was taken at 700 nm. Increased absorbance of the reaction mixture indicates the increased reducing power.
Inhibition of Human low-density lipoprotein(LDL) oxidation assay\textsuperscript{70}

Principle

Oxidation of low density lipoprotein(LDL) has been suggested to play a key role in the development of atherosclerosis and as a consequence an important role in the prevention of LDL oxidation has been attributed to the antioxidants contained within LDL and in plasma\textsuperscript{71}. Oxidation of polyunsaturated lipid components of LDL by active oxygen species may explain apparent abnormalities observed in attempts to relate heart disease exclusively to fat intakes. It is believed that the oxidation of LDL plays a key role in the pathogenesis of atherosclerosis (figure 2.8a-2.8b)\textsuperscript{72-75}.

Figure 2.8a. Deposition of fatty material in artery vessel wall containing Low-density Lipoprotein(LDL).
Figure 2.8b. Blockage of artery by blood clot due to the presence of fatty material Low-density Lipoprotein (LDL) leads to atherosclerosis.

Oxidation reactions are propagated by peroxyl radicals and therefore, the capacity of an individual LDL particle to scavenge these oxidants may be an important indicator of its atherosclerotic potential. It has been suggested that oxidative modification of LDL may play a role in the development of atherosclerosis. Once initiated, oxidation of LDL is a free-radical-driven lipid peroxidation chain reaction. Lipid peroxidation is initiated by free radical attack on a double bond associated with a polyunsaturated fatty acid (PUFA). This results in the removal of a hydrogen atom from allylic position, the rate of which determines the rate of initiation, a key step. Molecular rearrangement of the resulting unstable carbon radical results in a more stable configuration, a conjugated diene. The conjugated diene reacts very quickly with molecular oxygen, and the peroxyl radical thus formed is a crucial intermediate (figure 2.9).
A PUFA peroxyl radical in LDL may abstract a hydrogen atom from an adjacent PUFA to form a hydroperoxide and another lipid radical, a reaction which results in chain propagation. Removal of hydrogen atoms by the peroxyl radical from other lipids, including cholesterol, eventually yields oxysterols. Lipid hydroperoxides fragment to shorter-chain aldehydes, including malondialdehyde(MDA) and 4-hydroxynonenal. Such modification of LDL can be inhibited by antioxidants can stop chain reaction by donating...
an electron or hydrogen to the peroxyl radical of the fatty acid, and thus stops the propagation steps\textsuperscript{78-79}.

**Material required:**

- Fresh blood was obtained from fasting adult human volunteers.
- Isolated LDL.
- Phosphate buffered saline (PBS) pH 7.4
- Copper sulphate (2 mM)
- Phosphate buffer (50 mM, pH 7.4)
- Thiobarbutaric acid (TBA, 1 % in 50 mM NaOH)
- Trichloro acetic acid (TCA, 2.8 %).
- Double distilled water.
- Preparative ultra centrifugation using a Beckman L8-55 ultra centrifuge.
- Distilled ethyl alcohol.
- Stoppered test tube.
- Water bath.
- Spectrophotometer.
- Standard flask (50, 25 and 10 mL).
- Micro pipette (10 mL).
- Cuvet.
- Ascorbic acid (AA).
- Butylated hydroxyl anisole (BHA).
Test solution:

The experimental compounds were dissolved in distilled ethyl alcohol (50 mL) to prepare 1000 µM solution. Solutions of different concentrations (10, 25, 50, 100, 200 and 500 µM) were prepared by serial dilution.

Test procedure:

Fresh blood was obtained from fasting adult human volunteers and plasma was immediately separated by centrifugation at 1500 rpm for 10 min at 4 °C. LDL [0.1 mg LDL protein/mL] was isolated from freshly separated plasma by preparative ultra centrifugation using a Beckman L8-55 ultra centrifuge. The LDL was prepared from the plasma according to the method of Princen, et al \(^7\) using a differential ultra centrifugation method. Protein was estimated in compounds by using the method of Lowry, et al \(^8\). The isolated LDL was extensively dialyzed against phosphate buffered saline (PBS) pH 7.4 sterilized by filtration (0.2 µm Millipore membrane system, USA) and stored at 4 °C under nitrogen. Plasma was separated from blood drawn from human volunteers and stored at 4 °C until used. In brief, various concentrations (50, and 100 µM) of compounds were taken in test tubes 40 µL of copper sulphate (2 mM) was added and the volume was made up to 1.5 mL with phosphate buffer (50 mM, pH 7.4). A tube without compound and copper sulphate served as a negative control, and another tube without copper sulphate served as a positive control. All of the tubes were incubated at 37 °C for 45 min. To the aliquots of 1mL drawn at 2, 4 and 6 hr intervals, from each tube were added 0.25 mL of thiobarbutaric acid (TBA, 1 % in 50 mM NaOH) and 0.25 mL of trichloro acetic acid (TCA, 2.8 %). The tubes were incubated again at 95 °C for 45 min. Cooled to room temperature and centrifuged at 2500 rpm for 15 min. A pink chromogen was extracted after the mixture was cooled to room temperature by
spectrophotometer against an appropriate blank. The amount of oxidation and the results were expressed as protection per unit of protein concentration (0.1 mg LDL protein/mL). The percentage protection was calculated using the formula:

\[(\text{Oxidation in control} - \text{Oxidation in experimental} / \text{Oxidation in control}) \times 100.\]

**Results and Discussion**

In the present study the synthesis of 5H-dibenz[b,f]azepine and some of its analogues was carried out. The experimental protocol was simple, efficient and proceeds to get quantitative yield. The structural studies of synthesized compounds were carried out by using various spectroscopic techniques namely infrared (IR) spectroscopy, nuclear magnetic resonance (NMR) spectroscopy, mass spectroscopy, and elemental analyzer. The presence of required peak and absorbance of extraneous peaks confirms the synthesis. The data are reported in the table 2.2-2.7. The typical IR, \(^1\)HMR, and mass spectra of compound I and IV is showed in the figure 2.1-2.6.

The synthesized compounds were further evaluated for their ability towards antioxidant activities by using various *in vitro* assays namely,

1. DPPH free radical scavenging activity.
2. Inhibition of lipid peroxidation in \(\beta\)-carotene linoleate system.
3. Reducing power assay and
4. Inhibition of human low-density lipoprotein (LDL) oxidation.
DPPH free radical scavenging activity

The scavenging effects of all of the synthesized compounds on the DPPH free radical were evaluated. The radical scavenging activities of the synthesized compounds are summarized in figure 2.10.

![Figure 2.10](image)

Figure 2.10. Percentage DPPH radical scavenging activity of 5H-dibenz[b,f]azepine and its analogues at different concentration. Each value represents means ± SD (n=3)

Among the synthesized analogues compounds I and IV showed appreciable radical scavenging activity. The presence of N-H group which can donate hydrogen atom in compound I may contribute to the radical scavenging activity. The presence of the electron donating -OCH₃ group at the 11th position of the seven membered ring IV with N-H group may also contribute for better activity than 5H-dibenz[b,f]azepine(I) whereas, the presence of carbonyl group in the other compounds II, III, V and VI may hinder the scavenging ability and shows negligible scavenging activity over DPPH. The presence of methoxy group in the seven membered ring may enhance the stability of the nitrogen centered
radical due to electron conjugation effect. All the six synthesized compounds scavenged DPPH radical significantly in a concentration-dependent manner.

50% Inhibition concentration (IC$_{50}$) for the synthesized compounds and the standards were calculated and showed in the table 2.8.

Table 2.8. IC$_{50}$ values for DPPH radical scavenging activity (%) of 5H-dibenz[b,f]azepine and its analogues. Each values represents mean ±SD (n=3)

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC$_{50}$ (µM/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compound I</td>
<td>4.46±0.21</td>
</tr>
<tr>
<td>Compound II</td>
<td>270.10±0.11</td>
</tr>
<tr>
<td>Compound III</td>
<td>187.26±0.43</td>
</tr>
<tr>
<td>Compound IV</td>
<td>2.10±0.34</td>
</tr>
<tr>
<td>Compound V</td>
<td>197.32±0.22</td>
</tr>
<tr>
<td>Compound VI</td>
<td>187.26±0.21</td>
</tr>
<tr>
<td>AA</td>
<td>4.94±0.33</td>
</tr>
<tr>
<td>BHA</td>
<td>5.26±0.10</td>
</tr>
</tbody>
</table>

The comparative studies on DPPH activity of synthesized compounds and the standards (AA and BHA) was done. From the table compound I and IV displayed two fold better DPPH activity than the standards.
Inhibition of lipid peroxidation in β-carotene linoleate system

Bleaching of β-carotene with respect to time of synthesized compounds at different concentrations (50 and 100 µM) are represented in figure 2.11. The figure indicates that due to the oxidation of linoleic acid which eventually attacks the highly unsaturated β-Carotene molecule undergo rapid discoloration, compound II, III, V, VI loses its chromophore when it allowed for oxidation during different time interval (0 min-180 min). Initially the absorbance of the compounds was more and decreased drastically within the time allowed for oxidation, but in case of compound I and IV there is negligible decrease in the absorbance was observed.
Figure 2.11. Absorbance change of β-carotene at 470 nm in the presence of synthesized compounds, positive control and references (BHA, AA) at different concentrations (50 and 100 µM). Each value represents means ± SD (n=3).

The control (no additive) is decolorized within 150 min, indicating that rapid oxidation occurred.

The addition of compound I and IV and references (AA and BHA) at different concentrations inhibit the bleaching time of β-Carotene showing better activity. The presence of antioxidant compounds I and IV, binds the extent of β-Carotene bleaching by
neutralizing the linoleate free radical; hence extent of decrease in discoloration indicates higher antioxidant activity.

The antioxidant activity for 5H-dibenzo[b,f]azepine and its five derivatives along with the standards (AA and BHA) was calculated and the percentage activities are showed in figure 2.12.

![Antioxidant activity (%) of the 5H-dibenzo[b,f]azepine and its derivatives at 50 µM and 100 µM concentration in β-Carotene-Linoleic acid system. Values are means of triplicate determination ± standard deviation.](image)

**Figure 2.12.** Antioxidant activity (%) of the 5H-dibenzo[b,f]azepine and its derivatives at 50 µM and 100 µM concentration in β-Carotene-Linoleic acid system. Values are means of triplicate determination ± standard deviation.

The figure indicates that there is significant difference in the activity (%) at different concentrations. It was observed that the antioxidant activities (%) of all the compounds increase with increase in concentrations. This effect of concentration on antioxidant activity becomes more pronounced as the time allowed for oxidation increases. The antioxidant activities of compounds were compared to the standards (BHA, ascorbic acid) and the compounds II, III, V, and VI possess less antioxidant activity but compound I and IV showed promising antioxidants. Also the antioxidant activity of I and IV remained
stable even with the time increase allowed for oxidation, where as II, III, V, and VI decreased drastically as the time of oxidation increased. This was particularly noticeable at lower concentration (50 µM). This is because compound I contains free amino group (N-H bond) which can quenches the radical. The introducing of electron-with drawing group (-OCH₃) on the seven membered ring of the compound I i.e., compound IV leads considerable increase in the scavenging activity. Where as in the case of compound II, III, V and VI the presence of carbonyl group may hinder the antioxidant capacity. Hence in this assay compound I and IV increases the stability there by increases the antioxidant activity compared to other compounds.

Reducing power assay

Reducing power of the 5H-dibenz[b,f]azepine, its derivatives and standards (BHA and ascorbic acid) using the potassium ferricyanide reduction method were depicted in figure 2.13. Compound I and IV possess good reducing power ability compared to the other compounds. The figure indicates that the compound I having free N-H group reduces free radicals but compound IV having methoxy group in addition to N-H group reduces considerably more than compound I. These two compounds may react with free radicals to convert them to more stable products and terminate radical chain reaction. The presence of carbonyl group in the compound II, III, V, and VI may hinder the reduction of Fe³⁺ to Fe²⁺ and shows the negligible reducing ability. In general, the reducing power observed in the present study was in the following order compound IV > compound I > BHA > AA > compound VI > compound III> compound II > compound V. The data presented here indicate that the
marked reducing power of compound I and IV seems to be the result of their antioxidant activity.

![Graph showing antioxidant activity](image)

**Figure 2.13.** Antioxidant activity of the 5H-dibenz[b,f]azepine and its derivatives at different concentrations using reducing power assay. Each value represents means ± SD (n=3). High absorbance at 700 nm indicates high reducing power.

The reducing power of the internal standards (BHA, ascorbic acid) was also carried out and comparative study over the synthesized compounds was done. Compound I and IV shows promising activity than the internal standards.

Inhibition of human low-density lipoprotein (LDL) oxidation

In general, oxidation of LDL follows a radical chain reaction that generates conjugated diene hydroperoxide as its initial product. It has been reported that inhibition of human LDL oxidation may arise due to free radical scavenging and/or metal ion chelation. The polyunsaturated fatty acids (PUFA) of human...
LDL were oxidized, and the MDA formed have been estimated using by thiobarbutharic acid(TBA) method. Fresh blood was obtained from fasting adult human volunteers and plasma was immediately separated by centrifugation at 1500 rpm for 10 min at 4 °C. LDL [0.1 mg LDL protein/mL] was isolated from freshly separated plasma by preparative ultra centrifugation (figure 2.14).

![Fig 2.14](image)

Figure 2.14. Isolation of plasma containing LDL from fresh blood.

The antioxidant activity of compounds against human LDL oxidation at different concentrations is showed in the figure 2.15.

![Fig 2.15](image)

Figure 2.15. Antioxidant activity (%) of 5H-dibenz[b,f]azepine and its five analogues on human LDL oxidation in different concentrations (5, 10, and 15 µM/mL of LDL). Values represent means±SD.
Compound I and IV showed 85.44 and 92.13 % protections at 5 µM level of compound at the end of 6 hr after the induction of oxidation. Whereas, 92.94 and 94.76 % protection at 10 µM level of compound and 94.69 and 96.39 % protection at 15 µM level of compound used at the end of 6 hr. The results indicate a dose dependent effect of compound against LDL oxidation. More effects on human LDL oxidation by the compounds I and IV and less effect by the compounds II, III, V and VI. This is because compound (I) contains free amino group (N-H bond) which can quenches the radical may inhibit the LDL oxidation. Introducing of electron-with drawing group (-OCH₃) on the seven member ring of the compound I leads considerable increase in the antioxidant activity of compound II. Whereas in the case of compound II, III, V and VI the presence of carbonyl group may hinder the antioxidant capacity on human LDL oxidation. Hence in this assay compound I and IV increases and stabilizes the antioxidant activity compared to other compounds in different time interval. Percentage inhibition of LDL oxidation for the standards like BHA, ascorbic acid was also determined and compared. The antioxidant activity of BHA and ascorbic acid was still lower than that of the compound I and II. In general, antioxidant activity on human LDL oxidation observed in the present study was in the following order compound IV > compound I > compound AA > compound BHA > compound II > compound III > compound VI > compound V. These investigation can predict that the antioxidant activity of 5H-dibenz[b,f]azepine and its derivatives on human LDL oxidation and lipid peroxidation activity in the liposome system could be related to its direct radical scavenging properties.

The compound I and IV showed high antioxidant activity in all the four assays tested. Even they are more potent than the standards like AA and BHA.
Since compound I and compound IV showed good antioxidant activities, we have selected these two as model compounds for the further investigation on the synthesis and antioxidant properties of their various analogues.
References


