Chapter 1

*(E)-2-Benzylidene-7-chloro-9-phenyl-3,4-dihydroacridin-1(2H)-ones: Synthesis and antioxidant activity*

1.1 INTRODUCTION

1.1.1 HETEROCYCLIC CHEMISTRY

The study and synthesis of nitrogen heterocycles have attracted much importance among the researchers, because of their excellent chemotherapeutic characters and natural occurrence in living system (Gilchrist, 2007). Heterocyclic compounds are organic compounds containing at least one atom of carbon and at least one element other than carbon, such as sulfur, oxygen or nitrogen within a ring structure (Alexandru et al., 2004). In recent years heterocyclic chemistry recognized as general significance that almost all aspects of modern organic chemistry, medicinal chemistry and biochemistry (Rajiv et al., 2011). Amazingly, replacement of just one carbon atom of carbocyclic ring with a heteroatom which changes physical as well as chemical properties of the heterocyclic molecule (Kunied et al., 2002). The heterocyclic compounds having lesser common atoms such phosphorus, tin, boron, silicon, bromine, etc., have been a subject of much investigation in recent years (Ingle et al., 2011). Heterocycles are inextricably woven into the life processes. The vital interest of the pharmaceutical and agrochemical industries in heterocycles is often connected with their natural occurrence. More than 90 % of drugs contain heterocycles and the interface between chemistry and biology, at which so much new scientific insight, discovery and application is taking place is crossed by heterocyclic compounds. The most active heterocycles that have shown considerable biological actions such as antibiotic, antifungal, antiinflammatory, antiviral, anticancer, anticonvulsant, anthelmintic, antihistamine, antidepressant activities (Mukhtyar et al., 2013).

In short, heterocyclic chemistry is the branch of chemistry dealing with synthesis, properties and applications of heterocycles.
Nitrogen heterocyclic rings are much important because of its numerous biological activities (Nekrasov, 2001). Among these, acridine scaffolds are known to associate with several biological activities. Graebe and Caro in 1870 discovered the heterocyclic compounds which was named acridine due to its acrid smell and irritating action on skin (Acheson et al., 1973).

Acridone derivatives are also found in plant sources, which are proving worthwhile in various disorders Fig 1.1 (Giridhar et al., 2010). Acridine itself is highly water insoluble but development of water soluble derivatives made them useful as raw materials for the production of dyes, antitumor, anti-parasitic, antibacterial, antifungal, antiviral agents and multi-drug resistance modulators (Denny et al., 2004).

The tricyclic, planar acridine moiety is responsible for intercalation between base pairs of double-stranded DNA through $\pi-\pi$ interactions and, therefore, causes alteration in the cellular machinery (Lerman et al., 1963). Acridine analogues posses anticancer, antimicrobial, antiviral, antimalarial and anti-inflammatory activities (kumar et al., 2012; Kaur and Singh., 2011). Fig 1.2 evidence for synthetic and biologically important acridine analogous (Grzegorz et al., 2011).
1.1.3 CHALCONE CHEMISTRY

The chemistry of chalcones generated intensive scientific studies throughout the world. Chalcones are one of the subclasses of flavonoid family (En-Hui et al., 2013). The coloured nature of chalcones is due to the presence of the chromophore and auxochromes groups. Chalcones are natural or synthetic chemical compounds having various biological activities, such as antioxidant (John et al., 1995), antimalarial (Chen et al., 1997), antileishmanial (Nielsen et al., 1998), antiinflammatory (Hsin et al.,1998), antitumor (Kumar et al., 2003) and antibacterial (Prasad et al., 2008). Activity of these chalcones depends on various substitutions like -OH, -Me, -OMe, -OC₄H₉, -N(CH₃)₂. Fig 1.3 demonstrates the chalcone contain different structures and its reported activities (Zdzislawa et al., 2007).
Fig 1.3 Demonstrates the chalcone contain different structures and its reported activities, 1.13-1.18

Furthermore, chalcones are valuable intermediate to prepare pyranones, pyrimidines, thiazines, imino-2-oxime, cyano pyridines, cyano pyrans, pyrazolines, indazoles and isoxazoles derivatives in organic synthesis (Hassan et al., 2011; Soleiman et al., 2005; Wentao et al., 2013; Simerpeet and Damanjit., 2013). In chemical point of view, chalcones and their hetero analogues is the ability to act as activated unsaturated system in conjugated addition reaction of carbanions in the presence of basic catalyst (Yu-Ting et al., 2013; Patel et al., 2012). More commonly Michael addition used to involve for build many heterocyclic on chalcone intermediates (Raghunath et al., 2010).

Because of the above reports on acridine and chalcone analogues, our interest is to construct the heterocyclic molecule containing acridine core uncles.

1.2 REPORTED SYNTHETIC APPROACH

A lot of work had been carried out on the acridone nucleus due to its variety of pharmacological activities (kumar et al., 2012; Kumar and Kumari., 2011). Acridone and its derivatives can be synthesized by a number of methods such as Ullmann condensation (Mann and Saunders., 2007), benzyne mechanism (Ho and Jou., 2001),
and by radical reaction of quinines (Chuang and Wang., 1999) etc. A special emphasis is laid on the recent literature of acridone nucleus.

**Ullmann synthesis**

Ullmann synthesis involves the condensation of \( o \)-halobenzoic acids, 1.19 with substituted aniline, 1.20 in the presence of copper powder and potassium carbonate to give N-(substitutedphenyl)anthranillic acids, 1.21. The N-(substitutedphenyl)anthranillic acids cycle to acridone/substituted acridones, 1.22 under the influence of strong acids (Mann and Saunders., 2007).

![Chemical structure](image)

**Reagents and conditions:** (i) Copper powder, K\(_2\)CO\(_3\), reflux for 1.5 h (ii) Conc. H\(_2\)SO\(_4\), Polyphosphoric acid, \( \Delta \)

**Benzyne mechanism**

Diazotisation of anthralic acid, 1.23 reacted with benzyne, 1.24 in presence of butynitrite gives an intermediate, 1.25, further 1.25 on refluxing THF give rise to acridinone, 1.27 (Ho and Jou., 2001).
**Reagents and conditions:** (iii) n-BuONO, reflux (iv) benzyne, THF, reflux

**Radical reaction of quinones**

Quinone derivatives, 1.28 on treatment with 1-nitrile–ethylacetate, 1.29 in the presence of MeCN, Mn(OAc)$_3$ resulted in the formation of 2-chloroacridone derivatives, 1.30 by radical reaction mechanism (Chuang and Wang., 1999).

**Reagents and conditions:** (v) MeCN, Mn(OAc)$_2$, 80 °C, 36 h

Mohammad et al., 2007 was reported, synthesis of 7-chloro-9-phenyl-3,4-dihydroacridin-1(2H)-one, 1.31 via Friedländer condensation. Zr(NO$_3$)$_4$ and Zr(HSO$_4$)$_4$ were found to be more efficient than other investigated Lewis acids in the catalyzed condensation of o-aminoaryl ketones, 1.31 or o-amino benzonitrile with ketones or β-diketones, 1.32 in water under reflux conditions. Some general Lewis acids were found to catalyze the Friedlander quinoline synthesis in water under reflux conditions. Zr(NO$_3$)$_4$ and Zr(HSO$_4$)$_4$ were superior to other investigated catalysts. Theis methods enjoy the advantages that water endowed to the green organic synthesis. The catalysts are reusable, safe, and cheap, without any need for the application of chromatographic methods in the work-up procedure.
**Reagents and conditions:** (vi) Lewis acid (10 mol %), H_2O, reflux

Chalcone is an α,β-unsaturated ketone. Chemically, they are open-chained molecules bearing two aromatic rings linked by a three-carbon enone. These chalcone generally synthesized by aldol or claisen schmidt condensation reactions.

**Aldol synthesis of chalcones**

Benzaldehyde and acetophenone derivative are treated with base to form a chalcone. This crossed-aldol condensation does not form mixtures because the substituted benzaldehydes have no hydrogens, and therefore cannot form an enolate. The benzophenone derivatives can form enolates, and they will react with the aldehyde more rapidly than with themselves because aldehydes are more electrophilic than ketones. Most of the aldol condensations involving aromatic aldehydes, the aldol addition products readily undergo dehydration to give unsaturated carbonyl compounds. The double bond formed is conjugated with both the carbonyl group and the aromatic ring. This step is irreversible under the reaction conditions and serves to drive the equilibrium toward the formation of the products.

Kadutskii et al., 2006 reported, crotonization in the benzo[a]acridine series. The reaction of ketone, 1.34 with aromatic aldehyde, 1.35 was carried out by heating equimolar amount of reactants in ethanol, in the presence of aqueous potassium hydroxide. As expected corresponding classical crotonization product, 10-arylmethylidene-8,9,10,11-tetrahydrobenzo[a]acridine-11-ones, 1.36 was isolated.

**Reagents and conditions:** (vii) KOH/EtOH, 15-20 min, reflux
Detsi et al., 2009 reported, naturally occurring and synthetic flavonoids belonging to the 2’-hydroxy-chalcone, 1.39 and aurone families have been synthesized and screened for their antioxidant and soybean lipoxygenase inhibitory activity.

Reagents and conditions: (viii) 20 % KOH/EtOH, rt, 24-36 h, stirred

Maheswari et al., 2012 reported, a facile regio and stereoselective synthesis of dispirooxindolyl-[acridine-2',3-pyrrrolidine/thiapyrrolizidine]-1’-ones, 1.42 via 1,3-dipolar cycloaddition of azomethine ylides. This cycloaddition was regioselective with the electron rich carbon of the dipole adding to the β-carbon of the α-β-unsaturated system, 1.42 and stereoselective affording only one diastereomer exclusively, despite the presence of three/four stereocenters in the product.

Reagents and conditions: (ix) H$_2$O, EtOH, 10-20 min, rt
1.3 PRESENT WORK: RESULTS AND DISCUSSION

**Scheme 1.1** Synthesis of 7-chloro-3,4-dihydro-9-phenylacridin-1(2H)-one, 1.33

![Synthesis of 7-chloro-3,4-dihydro-9-phenylacridin-1(2H)-one](image)

**Scheme 1.2** Synthesis of (E)-2-(benzylidine-7-chloro-9-phenyl3,4-dihydro acridin-1(2H)-ones, 1.43a-f

![Synthesis of (E)-2-(benzylidine-7-chloro-9-phenyl3,4-dihydro acridin-1(2H)-ones](image)

All the synthesized derivatives, 1.44a-f were examined for antioxidant activity. As presented in Table 1.2, (E)-2-(2,5-dimethoxybenzylidene)-7-chloro-3,4-dihydro-9-phenylacridin-1-(2H)-one, 1.44b showed good radical-scavenging activities in comparison to ascorbic acid, at 0.04 to 0.06 mM concentration. This was attributed to presence of two methoxy aryl moiety of 1.44b (that can donate hydrogen atoms). After donating a hydrogen atom, compounds exist in its radical form, and the electron conjugation effect in the structure stabilizes the radical of DPPH. The difference in activity among the compounds, 1.44a-f was due to the difference in the substitution of these compounds. Among them, compounds, 1.44a and 1.44b with having two methoxy substituent’s showed higher hydrogen donor ability to DPPH radical (IC$_{50}$ values were 8.98 and 7.04 respectively). Since the corresponding IC$_{50}$ values for all synthesized compounds were higher than compound, 1.44b (Table 1.2). DPPH antiradical scavenging activity was also time-dependent Fig 1.4.

Absorbance was taken at different time periods 30, 45, 60, 90 and 120 min and it was plotted against UV absorption values Fig 1.4. Percentage inhibition was plotted against various concentrations 0.01, 0.02, 0.04, 0.06, 0.08, and 0.1 mM (Fig 1.5), the linear regression analysis equation was used to obtain the IC$_{50}$ value.
Table 1.1 Summary of Synthesized (E)-2-(benzylidene-7-chloro-9-phenyl3,4-dihydroacridin-1(2H)-ones, 1.44a-f

<table>
<thead>
<tr>
<th>S.NO</th>
<th>Compounds</th>
<th>R</th>
<th>M.P.</th>
<th>Yield %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.44a</td>
<td>0CH₃O</td>
<td>190-192</td>
<td>88</td>
</tr>
<tr>
<td>2</td>
<td>1.44b</td>
<td>0CH₃O</td>
<td>174-176</td>
<td>87</td>
</tr>
<tr>
<td>3</td>
<td>1.44c</td>
<td>0CH₃O</td>
<td>148-150</td>
<td>89</td>
</tr>
<tr>
<td>4</td>
<td>1.44d</td>
<td>Cl</td>
<td>152-154</td>
<td>85</td>
</tr>
<tr>
<td>5</td>
<td>1.44e</td>
<td>Cl</td>
<td>192-194</td>
<td>87</td>
</tr>
<tr>
<td>6</td>
<td>1.44f</td>
<td></td>
<td>193-195</td>
<td>83</td>
</tr>
</tbody>
</table>

A lower IC₅₀ value of compounds, 1.44a and 1.44b indicates high in antioxidant activity Table 1.2. The entire synthesized compounds, 1.33 and 1.44a-f scavenged DPPH radical, significantly in a concentration dependent manner. Their comparable scavenging activities were expressed in IC₅₀ (concentration required for 50 % inhibition of 1 mM DPPH) value. In compound, 1.44b having two methoxy functional groups are para to each other.
Fig 1.4 Time dependent DPPH antiradical scavenging activity.

Fig 1.5 Percentage inhibition DPPH radical scavenging activity
Table 1.2 50 % inhibition of free radical scavenging activity.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>( \text{aIC}_{50} \times 10^{-3} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ascorbic acid</td>
<td>6.84</td>
</tr>
<tr>
<td>1.33</td>
<td>20.05</td>
</tr>
<tr>
<td>1.44a</td>
<td>8.68</td>
</tr>
<tr>
<td>1.44b</td>
<td>7.04</td>
</tr>
<tr>
<td>1.44c</td>
<td>8.94</td>
</tr>
<tr>
<td>1.44d</td>
<td>9.81</td>
</tr>
<tr>
<td>1.44e</td>
<td>9.52</td>
</tr>
<tr>
<td>1.44f</td>
<td>10.61</td>
</tr>
<tr>
<td>9-amino-acridine-propranol</td>
<td>13.6</td>
</tr>
</tbody>
</table>

\( \text{aIC}_{50} \) values were determined by linear regression analysis using different concentrations in triplicate.

Therefore, radical ion resulted from the abstraction of -H atom by DPPH would stabilize by other hydroxy group. We compared this result with literature report Dickens et al., 2002) \( \text{IC}_{50} \) value of 9-amino-acridine-propranol [\( \text{IC}_{50} \) value of 13.6]. While in compound, 1.44a both the methoxy groups are meta and para position, therefore the radical ion resulted from the abstraction of -H atom not that much stabilized from methoxy group compare with compound, 1.44b. Thus, compound, 1.44b shows better scavenging effect as compare to compound, 1.44a. Compound, 1.44c is less active compared to compounds, 1.44a and 1.44b due to the presence of single methoxy group. Also it showed better antioxidant activity compared to compounds, 1.44d, 1.44e & 1.44f due to presence of methoxy group (i.e., the presence of the electron donating -OCH\(_3\) group at the meta position enhanced the stabilization of the resulting oxygen entered radical as the number of conjugating structure is more than that without the -OCH\(_3\) group) which is absent in the later case. Compounds, 1.44d and 1.44e are less active compared to compounds, 1.44a-d. Because of the absence of electron releasing group. But it showed higher activity
compared to compound, 1.44f due to the presence of electron withdrawing chloro groups in them which would destabilize the ring.

1.4 CONCLUSION

A series of new (E)-2-benzylidene-7-chloro-9-phenyl-3,4-dihydroacridin-1(2H)-ones, 1.44a-f were synthesized. All the compounds were evaluated for antioxidant activity using DPPH free radical scavenging method. The results indicated that compounds, 1.44a and 1.44b have good antioxidant activity. Compound, 1.44b shown better activity compare with standard ascorbic acid at the concentration of 0.04 to 0.06 mM. The antioxidant activity of these compounds is strongly associated with the type of substitution on the benzene ring. The results of this study provide useful information for further structural optimization of these compounds and rapid detection of the activity of the compounds.

1.5 EXPERIMENTAL SECTION

1.5.1 SYNTHESIS OF 7-CHLORO-3,4-DIHYDRO-9-PHENYLACRIDINE-1(2H)-ONE, 1.33

7-Chloro-3,4-dihydro-9-phenylacridin-1(2H)-one, 1.33 was synthesized (Bharathi et al., 2013) a mixture of 2-amino-5-chlorobenzophenone (0.01 mol), 1.31 1,3-hexanediene (0.01 mol), 1.32 and glacial acetic acid (10 mL) in presence of Conc. H$_2$SO$_4$ (3-4 drops) were heated at 150 °C for 6 h. The completion of reaction was monitored by TLC. The reaction mixture was then cooled to room temperature and the solution was poured into crushed ice and the precipitate was neutralized with dilute NaOH solution. The precipitate was recrystallized from ethanol.

1.5.2 SYNTHESIS OF (E)-2-(BENZYLIDINE-7-CHLORO-9-PHENYL-3,4-DIHYDROACRIDINE-(2H)-ONES, 1.44a-f

Compounds, 1.44a–f were synthesized by Claisen–Schmidt condensation. The reaction involves condensation of equimolar quantities of 7-chloro-3,4-dihydro-9-phenylacridin-1(2H)-one, 1.33 with a substituted aromatic aldehydes, 1.43a-f in the presence of alcoholic potassium hydroxide (KOH) solution, resulting in the formation of α, β- unsaturated ketones. The substituted α, β- unsaturated ketones, 1.44a-f were prepared by stirring a solution of 2.75 g KOH in 20 mL ethanol. The round bottom
flask was immersed in crushed ice and 1 mmol (0.307 g) of 7-chloro-3,4-dihydro-9-phenylacridin-1(2H)-one, 1.33 were added. Exactly 1 mmol of substituted aromatic aldehydes, 1.43a–f were added with stirring at below 25 °C for 7–8 h (Bharathi et al., 2013). The reaction mixture was then cooled to room temperature and the solution was poured into crushed ice and the precipitate was neutralized with dilute HCl solution. The precipitate was isolated by filtration and left to dry. The crude product was separated by column chromatography with ethyl acetate–petroleum ether (1:9) as eluent. The synthetic pathway is depicted in Scheme 1.2. Yields and melting points of compounds, 1.44a–f are summarized in Table 1.1.

The spectral details are as follows,

7-chloro-3,4-dihydro-9-phenylacridin-1(2H)-one, 1.33

Yellow solid; M.F: C_{19}H_{14}ClNO; Yield 81 %; M.P: 185 °C; I.R. (KBr pellet) ν_{max}/(Cm^{-1}): 1670 (-C=O); ^1H NMR (CDCl3): δ (ppm), 2.23–2.26 (t, J = 6.2 Hz, 2H, –CH₂), 2.68-2.71 (t, J = 6.2 Hz, 2H, –CH₂), 3.34-3.36 (t, J = 5.6 Hz, 2H, –CH₂), 7.15–7.16 (d, J = 4.4 Hz, 2H), 7.41 (s, 1H), 7.50 (m, 3H), 7.67–7.69 (d, J = 8.8 Hz, 1H), 7.98–8.00 (d, J = 9.2 Hz, 1H); ^13C NMR (CDCl3): δ (ppm) 21.39, 34.66, 40.71, 124.58, 126.85, 128.03, 128.12, 128.41, 128.45, 3x130.31, 132.54, 132.70, 136.97, 147.18, 150.63, 162.65, 197.81; Exact Mass: 307.08; Found ESI-MS m/z: 308.19 [M+1].

(E)-7-chloro-2-(3,4-dimethoxybenzylidene)-9-phenyl-3,4-dihydroacridin-1(2H)-one, 1.44a

Pale yellow solid; M.F: C_{28}H_{22}ClNO_{3}; Yield: 88 %; M.P: 190-192 °C; FT-IR (KBr pellet) ν_{max}/(Cm^{-1}): 1670 cm^{-1} (-C=O), 2843 cm^{-1} (-OCH₃); ^1H NMR (400 MHz, CDCl3): δ (ppm), 3.28-3.30 (d, J = 8.8 Hz, 4H, 2-CH₂), 3.89 (s, 3H, -OCH₃), 3.92 (s, 3H, -OCH₃), 6.90-6.92 (d, J = 8.4 Hz, 1H), 6.96 (s, 1H), 7.08-7.10 (d, J = 8.4 Hz, 1H), 7.24-7.26 (d, J = 6.8 Hz, 2H), 7.49 (s, 1H), 7.52 (m, 3H), 7.68-7.70 (d, J = 8 Hz, 1H), 8.01-8.04 (d, J = 8.8 Hz, 2H); ^13C NMR (400 MHz, CDCl3): δ (ppm), 25.98, 33.64, 56.08, 56.10, 111.13, 113.49, 123.88, 125.87, 2x126.81, 128.16, 128.39, 128.43, 128.55, 128.78, 2x130.42, 132.44, 132.56, 133.70, 136.80, 138.07, 147.04, 148.94,
150.18, 150.48, 161.39, 187.40; Exact Mass: 455.13; Found ESI-MS: m/z 456.54 [M+1].

**1.44b**

Yellow solid; M.F: C_{28}H_{22}ClNO_3; Yield: 87 %; M.P: 174-176 °C; FT-IR (KBr pellet) \( v_{\text{max}} / (\text{Cm}^{-1}) \): 1672 cm\(^{-1}\) (-C=O), 2831 cm\(^{-1}\) (-OCH\(_3\)); \(^1\)H-NMR (400 MHz, CDCl\(_3\)): \( \delta \) (ppm), 3.17 (m, 2H, -CH\(_2\)), 3.29-3.30 (m, 2H, -CH\(_2\)), 3.76 (s, 3H, -OCH\(_3\)), 3.78 (s, 3H, -OCH\(_3\)), 6.82-6.87 (m, 3H), 7.24-7.25 (d, \( J = 5.6 \) Hz, 2H), 7.47 (s, 1H), 7.52 (s, 1H), 7.67-7.70 (d, \( J = 8.4 \) Hz, 2H), 7.94 (s, 1H), 8.00-8.03 (d, \( J = 8.8 \) Hz, 2H); \(^{13}\)C NMR (400 MHz, CDCl\(_3\)): \( \delta \) (ppm), 25.93, 33.62, 55.41, 114.54, 115.69, 122.57, 125.58, 126.81, 2x128.45, 128.47, 128.72, 2x129.68, 130.41, 132.55, 132.58, 135.64, 136.75, 136.83, 137.80, 147.01, 150.92, 152.93, 153.03, 161.73, 187.07; Exact Mass: 455.13; Found ESI-MS: m/z 456.80 [M+1].

**1.44c**

Pale yellow solid; M.F: C_{27}H_{20}ClNO_2; Yield: 89 %; M.P: 148-150 °C; FT-IR (KBr pellet) \( v_{\text{max}} / (\text{Cm}^{-1}) \): 1670 cm\(^{-1}\) (-C=O), 2829 cm\(^{-1}\) (-OCH\(_3\)); \(^1\)H NMR (400 MHz, CDCl\(_3\)): \( \delta \) (ppm), 3.26-3.31 (m, 4H, 2-CH\(_2\)), 3.82 (s, 3H, -OCH\(_3\)), 6.89-6.92 (d, \( J = 8.4 \) Hz, 1H), 6.94 (s, 1H), 7.01-7.03 (d, \( J = 7.6 \) Hz, 1H), 7.24-7.26 (d, \( J = 6 \) Hz, 2H), 7.30-7.34 (t, \( J = 7.8 \) Hz, 1H), 7.49 (s, 1H), 7.53 (m, 1H), 7.68-7.72 (m, 3H), 8.01-8.03 (d, \( J = 8.8 \) Hz, 2H); \(^{13}\)C NMR (400 MHz, CDCl\(_3\)): \( \delta \) (ppm), 25.93, 33.62, 55.41, 114.54, 115.69, 122.57, 125.58, 126.81, 2x128.45, 128.47, 128.72, 2x129.68, 130.41, 132.55, 132.58, 135.64, 136.75, 136.83, 137.80, 147.01, 150.79, 159.65, 161.43, 187.38; Exact Mass: 425.12; Found ESI-MS: m/z 426.98 [M+1].

**1.44d**

Light green solid; M.F: C_{26}H_{17}ClNO; Yield: 85 %; M.P: 152-154 °C; FT-IR (KBr pellet) \( v_{\text{max}} / (\text{Cm}^{-1}) \):
1678 cm\(^{-1}\) (-C=O); \(^1\)H NMR (400 MHz, CDCl\(_3\)): δ (ppm), 3.09-3.10 (t, \(J = 6\) Hz, 2H, -CH\(_2\)), 3.29-3.32 (t, \(J = 6\) Hz, 2H, -CH\(_2\)), 7.23-7.31 (m, 3H), 7.41-7.42 (d, \(J = 3.6\) Hz, 2H), 7.46 (s, 1H), 7.53-7.55 (d, \(J = 5.2\) Hz, 2H), 7.68-7.70 (d, \(J = 8.8\) Hz, 2H), 7.88 (s, 1H), 8.00-8.02 (d, \(J = 8.8\) Hz, 2H); \(^13\)C NMR (400 MHz, CDCl\(_3\)): δ (ppm), 26.04, 33.84, 125.13, 126.54, 2x126.88, 128.20, 128.34, 128.52, 2x128.76, 130.01, 130.05, 130.37, 130.40, 132.66, 132.72, 134.11, 134.91, 135.21, 136.86, 136.90, 147.12, 151.32, 161.54, 186.83; Exact Mass: 429.07; Found ESI-MS: m/z 430.4 [M+1].

\((E)-7\)-chboro-2-(4-chlorobenzylidene)-9-phenyl-3,4-dihydroacridin-1(2H)-one, \textbf{1.44e}

Brown solid; M.F: C\(_{26}\)H\(_{17}\)Cl\(_2\)NO; Yield: 87%; M.P: 192-194 °C; FT-IR (KBr pellet) \(\nu_{\text{max}}/\text{(Cm}^{-1})\): 1670 cm\(^{-1}\) (-C=O); \(^1\)H NMR (400 MHz, CDCl\(_3\)): δ (ppm), 3.21-3.22 (t, \(J = 7.2\) Hz, 2H, -CH\(_2\)), 3.30-3.31 (t, \(J = 5.6\) Hz, 2H, -CH\(_2\)), 6.90-6.92 (d, \(J = 8.4\) Hz, 1H), 6.96 (s, 1H), 7.24-7.25 (m, 2H), 7.34-7.37 (d, \(J = 7.8\) Hz, 2H), 7.49 (s, 1H), 7.53-7.55 (d, \(J = 6\) Hz, 2H), 7.69-7.71 (s, 1H), 8.02-8.04 (d, \(J = 8.6\) Hz, 2H); \(^13\)C NMR (400 MHz, CDCl\(_3\)): δ (ppm), 25.89, 33.52, 125.46, 2x126.83, 2x128.23, 128.46, 128.48, 128.73, 2x128.97, 2x130.44, 131.39, 132.64, 132.67, 133.93, 135.10, 135.83, 136.50, 136.69, 147.12, 150.89, 161.24, 187.17; Exact Mass: 429.07; Found ESI-MS: m/z 430.4 [M+1].

\((E)-2\)-benzylidene-7-chloro-9-phenyl-3,4-dihydroacridin-1(2H)-one, \textbf{1.44f}

Green solid; M.F: C\(_{26}\)H\(_{18}\)ClNO; Yield: 83%; M.P: 193-195 °C; FT-IR (KBr pellet) \(\nu_{\text{max}}/\text{(Cm}^{-1})\): 1670 cm\(^{-1}\) (-C=O); \(^1\)H NMR (400 MHz, CDCl\(_3\)): δ (ppm), 3.33-3.36 (t, \(J = 6\) Hz, 2H, -CH\(_2\)), 3.71-3.73 (t, \(J = 6.4\) Hz, 2H, -CH\(_2\)), 7.20-7.26 (m, 4H), 7.35-7.37 (d, \(J = 7.6\) Hz, 1H), 7.49 (s, 1H), 7.53-7.54 (d, \(J = 4.4\) Hz, 2H), 7.61 (s, 1H), 7.69-7.71 (m, 1H), 8.02-8.05 (d, \(J = 8.8\) Hz, 2H), 8.70-8.71 (d, \(J = 4\) Hz, 2H); \(^13\)C NMR (400 MHz, CDCl\(_3\)): δ (ppm), 25.56, 33.39, 123.08, 125.62, 126.81, 127.62, 128.22, 128.50, 128.54, 2x128.68, 3x130.47, 132.56, 132.60, 134.44, 136.53, 136.78, 139.27, 147.12, 149.70, 150.88, 155.18, 162.05, 188.09; Exact Mass: 395.11; Found ESI-MS: m/z 397.95 [M+2].
1.6 DPPH FREE RADICAL SCAVENGING ASSAY

Antioxidant assay (Roopan et al., 2008) was based on the measurements of scavenging ability of compounds towards the stable 2,2-diphenyl-1-picrylhydrazyl radical (DPPH). The disappearance of this commercially available radical is measured spectrophotometrically at 517 nm in ethanolic solution. About 1 mL of the sample solutions containing different concentrations were mixed with 3 mL of 0.1 mM solution of DPPH. The mixture was kept in dark for 30 minutes. The absorbance was measured after incubation at 517 nm, against a blank of ethanol without DPPH. The control solution consist a mixture of 1 mL ethanol and DPPH. Ascorbic acid was used as a standard. Results were expressed as percentage of inhibition of the DPPH radical. Percentage of inhibition of the DPPH radical was calculated according to the following equation (1)

\[
% \text{Inhibition} = \left[ \frac{A_C - A_S}{A_C} \right] \times 100 \% \quad \text{------------------ (1)}
\]

Where, \( A_C \) - Absorbance of Control, 
\( A_S \) - Absorbance of Sample

The antioxidant activity was expressed in 50 % inhibitory concentration (IC\(_{50}\)) based on the amount of compound required for 50 % decrease of the initial DPPH radical concentration.