Evalauation of Biological Activities of Carbanionic Sigma Complexes

Anticonvulsant Activity

As the synthesized carbanionic sigma complexes are the derivatives of barbituric acid (barbiturates), the anticonvulsant activities of them have been measured employing Maximal Electro Shock (MES) method (Fig. 66). The results of MES method are presented in Table 64. Many conventional antiepileptic drugs act on ion channels like sodium (Fig. 67) calcium (Fig. 68) chloride (Fig. 69) and receptors like GABA receptors and enzymes like GABA transaminase. It has been pointed out that most of the barbiturates affect GABA response by binding to an allosteric site on the GABA_A receptor. Through this site barbiturate increases the open time of chloride channel activated by GABA. Furthermore it has also been specified that at high anesthetic doses, the barbiturates directly activate the chloride channel.

For a long period it has been thought that a single drug may be able to treat all forms of epilepsy, but the current research findings insisted that it is quite unlikely that a wide variety of epilepsies can be managed with one drug. Drugs used in the treatment of two major seizure types, partial [impaired consciousness lasting 30 sec to 2 mins often associated with purposeless movements such as lip smacking or hand wringing, confusion, amnesia etc.] and generalized [loss of consciousness and sustained contractions (tonic) of muscles throughout the body followed by periods of muscle contraction alternating with periods of relaxation (clonic), typically lasting 1 to 2 mins resulting in increased heart rate, increased blood pressure, loss of bladder and bowel control], are quite distinct in their clinical profiles. Different structurally distinct barbiturates influence different brain regions with different potencies. For example, at anticonvulsant doses, phenobarbital 51 is less sedative than pentobarbital 52.
Pentobarbital 52 enhances GABA binding in all brain regions, whereas phenobarbital enhances GABA binding only in some regions.\textsuperscript{217} The conventional drugs\textsuperscript{1,217} used for the treatment of tonic – clonic seizures (grand mal type) includes (i) carbamazepine 53 (ii) phenobarbital 51 (iii) phenytoin 54 (iv) primidone 55 (v) valproate 56 (vi) ethosuximide 57 and (vii) lamotrigine 58.
Eventhough phenobarbital has been in use in the treatment of grand mal type epilepsy for the past hundred years, it has many undesirable properties and side effects. It has long half-life period (90 days). It has low safety margin and hence should be handled scrupulously. The barbiturates synthesized in the present work are extremely stable at room temperature as they are molecular salts. The ionic nature of synthesized barbiturates facilitates their dissolution in water. They are soluble in n-octanol also. The log p values (Table 65) were calculated using XLOGP2 and ALOGPS 2.1 programs. As the log p values are less than 5, they are eligible to become drugs.

In MES method, the reduction or abolition of various phases of convulsion (flexor, extensor, clonus and stupor) is taken as the measure of anticonvulsant activity. In the present investigation, among the carbanionic sigma complexes derived using aliphatic tertiary amines, the complexes $36b$ and $36c$ have quite appreciable anticonvulsant activity as they abolish clonus phase of convulsion even at low concentration (25 mg/kg: Table 64). This implies that not only the barbiturate anion part but also the cation part of synthesized barbiturates plays an important role in modulating the sub unit of chloride ion channel, resulting in enhanced anticonvulsant activity. Among the carbanionic σ complexes derived from aromatic heterocyclic amines ($39a$ – $39d$), the complex $39b$ possesses good potency and efficacy which is reflected from the reduction in two phases of convulsion (flexor and extensor) even at low dosage (25 mg/kg). As this complex fully abolishes the extensor phase of convulsion, it may presumably be a good biologically active molecule for curing
grand mal type epilepsy. It appears that methyl group ortho with respect to nitrogen atom of pyridinium ion in the cationic part of complex 39b plays a significant role in modulating the ion channel. The enhanced activity is also noticed in other complexes 39a, 39c and 39d, which have substituted pyridinium cation. The enhanced activity of these complexes may be attributed to the presence of pyrimidine (from barbiturate residue) and pyridine moiety within the same molecule and thus enhances the biological profile remarkably. A similar enhanced biological profiles have been reported in the literature of the molecules containing pyridine and pyrimidine rings. The carbanionic σ complexes 36e, 44a and 44b moderately reduce the time spent by the animal in each of the phases only at high concentration (100 mg/kg). The carbanionic σ complex 45 abolishes the tonic flexor, which is the first phase of convulsion, even at low concentration (25 mg/kg). This may be attributed to the presence of 1,4-diazabicyclo[2.2.2] octane moiety.

The hypnotic activity results of the synthesized barbiturates (Table 66) indicate that the complexes with substituted pyridinium cation moiety (36a – 36d) induce hypnosis in albino mice for a longer time than the other carbanionic σ complexes. This indicates that substituted pyridinium ions also play an important role in inducing hypnosis.

**Acute toxicity studies on carbanionic sigma complexes**

Acute toxicity studies are required for all biologically active molecules to understand the safer dose concentrations for clinical trials. LD$_{50}$ of the synthesized barbiturates were done as per OECD guidelines (revised draft 423). Ten weeks old female mice (nulliparous and non-pregnant) were subjected for the LD$_{50}$ studies. Care has been taken in such a way that the weight of the animal to be tested should fall in an interval within ±20% of the mean weight of any previously dosed animals. The animals were chosen and kept in their cages five days prior to dosing to allow for acclimatization to the laboratory conditions. The volume of aqueous solution of the carbanionic σ complexes was 2 mL/100 g body weight of the animal. Doses were prepared shortly prior to administration. Animals were fasted prior to dosing. After the dose was administered, food was with held for 1-2 hours. The dose level was
selected from one of the fixed levels 5, 50, 300 and 2000 mg/kg. The flow charts of the Table 67 describe the procedure which was followed. Animals were observed individually for 30 minutes and after dosing periodically for 24 hours. The observations were then made daily for a total of 14 days. The carbanionic σ complexes fall under class 4 (LD₅₀ greater than 1500 mg/kg). Carbanionic σ complexes 36a, 39a and 39d were also subjected to behavioural studies. The animals did not show any signs of acute toxicity and behavioural changes (Table 68).

**Antimicrobial activity of the carbanionic sigma complexes**

As the carbanionic σ complexes derived from DCDNB, BA and bipyridine crystallize differentially in the solvents DMSO and ethanol, their anticonvulsant activity (Table 69), hypnotic activity (Table 70) and antimicrobial activity (Table 71) (Disc Diffusion Method) have also been examined. The synthesized molecules are screened for their antimicrobial activity towards bacterial pathogens such as *Staphylococcus aureus*, *E.coli*, *Bacillus subtilis* and *Pseudomonas aeruginosa* and fungal pathogens such as *Candida albicans* and *Aspergillus niger*. These two molecules differ in all three activities slightly.

**Cytotoxicity study (MTT assay)**

The results of MTT assay are listed in Table 72. The results revealed that the complexes exhibit low range of cytotoxicity (IC₅₀ > 430 µM).

**Antimalarial and antituberculosis activity**

The results of antimalarial and antituberculosis activities of the some of the complexes are presented in Tables 73 and 74 respectively. The results imply that the carbanionic sigma complexes do not possess promising antimalarial and antituberculosis activities.
Table 64

Anticonvulsant activity of carbanionic sigma complexes (barbiturates) against maximal electroshock induced convulsion in albino rats

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Treatment</th>
<th>Time (sec) in various phases of convulsion</th>
<th>Recovery / Death</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Flexor</td>
<td>Extensor</td>
</tr>
<tr>
<td>1.</td>
<td>Control</td>
<td>12 ± 1</td>
<td>21 ± 1.5</td>
</tr>
<tr>
<td></td>
<td>Normal Saline (5 ml/kg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>Barbiturate 36a</td>
<td>6.0±1.2</td>
<td>2.0±0.3</td>
</tr>
<tr>
<td></td>
<td>i. 25 mg/kg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>Barbiturate 36b</td>
<td>2.0±0.50</td>
<td>4.0±0.2</td>
</tr>
<tr>
<td></td>
<td>i. 25 mg/kg</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ii. 50 mg/kg</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>iii. 100 mg/kg</td>
<td>3.4±0.06</td>
<td>15.5±0.24</td>
</tr>
<tr>
<td>4.</td>
<td>Barbiturate 36c</td>
<td>8±1.1</td>
<td>7±1.4</td>
</tr>
<tr>
<td></td>
<td>i. 25 mg/kg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>Barbiturate 36d</td>
<td>3±0.5</td>
<td>2.0±0.7</td>
</tr>
<tr>
<td></td>
<td>i. 25 mg/kg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td>Barbiturate 36e</td>
<td>3.0±0.73</td>
<td>6.0±0.42</td>
</tr>
<tr>
<td></td>
<td>i. 100 mg/kg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7.</td>
<td>Barbiturate 39a</td>
<td>5.9±0.11</td>
<td>8.3±0.13</td>
</tr>
<tr>
<td></td>
<td>i. 50 mg/kg</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ii. 100 mg/kg</td>
<td>5.4±0.21</td>
<td>7.1±0.22</td>
</tr>
<tr>
<td>8.</td>
<td>Barbiturate 39b</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td>i. 25 mg/kg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9.</td>
<td>Barbiturate 39c</td>
<td>2.0±0.6</td>
<td>7.0±0.5</td>
</tr>
<tr>
<td></td>
<td>i. 25 mg/kg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10.</td>
<td>Barbiturate 39d</td>
<td>5.1±0.17</td>
<td>5.7±0.47</td>
</tr>
<tr>
<td></td>
<td>i. 50 mg/kg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11.</td>
<td>Barbiturate 44a</td>
<td>3.0±0.41</td>
<td>4.0±1.17</td>
</tr>
<tr>
<td></td>
<td>i. 100 mg/kg</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 65
Log P values of carbanionic $\sigma$ complexes (barbiturates)

<table>
<thead>
<tr>
<th>Complex</th>
<th>M. wt</th>
<th>MF</th>
<th>ALOG PS</th>
<th>XLOG P2</th>
<th>AVE LOG P</th>
</tr>
</thead>
<tbody>
<tr>
<td>36a</td>
<td>387.77</td>
<td>C$<em>{13}$H$</em>{14}$N$_5$O$_7$Cl</td>
<td>0.54</td>
<td>1.41</td>
<td>0.97</td>
</tr>
<tr>
<td>36b</td>
<td>429.86</td>
<td>C$<em>{16}$H$</em>{20}$N$_5$O$_7$Cl</td>
<td>1.37</td>
<td>2.18</td>
<td>1.78</td>
</tr>
<tr>
<td>36c</td>
<td>514.04</td>
<td>C$<em>{22}$H$</em>{32}$N$_5$O$_7$Cl</td>
<td>2.99</td>
<td>4.96</td>
<td>3.98</td>
</tr>
<tr>
<td>36d</td>
<td>445.86</td>
<td>C$<em>{16}$H$</em>{20}$N$_5$O$_8$Cl</td>
<td>0.81</td>
<td>1.05</td>
<td>0.93</td>
</tr>
<tr>
<td>36e</td>
<td>477.90</td>
<td>C$<em>{20}$H$</em>{20}$N$_5$O$_7$Cl</td>
<td>2.14</td>
<td>3.49</td>
<td>2.82</td>
</tr>
<tr>
<td>39a</td>
<td>422.77</td>
<td>C$<em>{15}$H$</em>{14}$N$_5$O$_7$Cl</td>
<td>2.21</td>
<td>2.18</td>
<td>2.19</td>
</tr>
<tr>
<td>39b</td>
<td>421.78</td>
<td>C$<em>{16}$H$</em>{12}$N$_5$O$_7$Cl</td>
<td>2.71</td>
<td>2.58</td>
<td>2.65</td>
</tr>
<tr>
<td>39c</td>
<td>421.78</td>
<td>C$<em>{16}$H$</em>{12}$N$_5$O$_7$Cl</td>
<td>2.78</td>
<td>2.49</td>
<td>2.64</td>
</tr>
<tr>
<td>39d</td>
<td>421.78</td>
<td>C$<em>{16}$H$</em>{12}$N$_5$O$_7$Cl</td>
<td>2.79</td>
<td>2.70</td>
<td>2.75</td>
</tr>
<tr>
<td>44a</td>
<td>457.81</td>
<td>C$<em>{19}$H$</em>{12}$N$_5$O$_7$Cl</td>
<td>3.53</td>
<td>3.62</td>
<td>3.58</td>
</tr>
<tr>
<td>44d</td>
<td>473.81</td>
<td>C$<em>{19}$H$</em>{12}$N$_5$O$_8$Cl</td>
<td>3.54</td>
<td>3.22</td>
<td>3.38</td>
</tr>
<tr>
<td>45</td>
<td>440.84</td>
<td>C$<em>{16}$H$</em>{17}$N$_6$O$_7$Cl</td>
<td>-0.25</td>
<td>0.34</td>
<td>0.05</td>
</tr>
</tbody>
</table>

Mean ± S.E.M.  N = 6,  P < 0.001 Vs standard
### Table 66
Hypnotic action of carbanionic sigma complexes (barbiturates)

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Sample</th>
<th>Dose (mg/kg)</th>
<th>Time of Administration (mins) (a)</th>
<th>Time of loss of reflex (mins) (b)</th>
<th>Time of Recovery (mins) (c)</th>
<th>On set of action (mins) (b-a)</th>
<th>Duration of action (mins) (c-b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control (Saline)</td>
<td>0</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>2</td>
<td>36a</td>
<td>100</td>
<td>0</td>
<td>36.8 ± 7.2</td>
<td>124.4 ± 8.7</td>
<td>36.80 ± 7.2</td>
<td>87.60</td>
</tr>
<tr>
<td>3</td>
<td>36b</td>
<td>100</td>
<td>0</td>
<td>44.20 ± 5.8</td>
<td>96.40 ± 4.2</td>
<td>44.20 ± 5.8</td>
<td>55.20</td>
</tr>
<tr>
<td>4</td>
<td>36c</td>
<td>100</td>
<td>0</td>
<td>34.60 ± 6.4</td>
<td>118.70 ± 4.6</td>
<td>34.60 ± 6.4</td>
<td>84.1</td>
</tr>
<tr>
<td>5</td>
<td>36d</td>
<td>100</td>
<td>0</td>
<td>37.90 ± 4.4</td>
<td>116.8 ± 4.6</td>
<td>37.90 ± 4.4</td>
<td>78.9</td>
</tr>
<tr>
<td>6</td>
<td>36e</td>
<td>100</td>
<td>0</td>
<td>42.30 ± 5.4</td>
<td>76.30 ± 8.4</td>
<td>42.30 ± 5.4</td>
<td>34.0</td>
</tr>
<tr>
<td>7</td>
<td>39a</td>
<td>100</td>
<td>0</td>
<td>36.40 ± 3.1</td>
<td>108.30 ± 1.3</td>
<td>36.40 ± 3.1</td>
<td>71.8</td>
</tr>
<tr>
<td>8</td>
<td>39b</td>
<td>100</td>
<td>0</td>
<td>36.40 ± 7.1</td>
<td>104.90 ± 4.8</td>
<td>36.40 ± 7.1</td>
<td>68.5</td>
</tr>
<tr>
<td>9</td>
<td>39c</td>
<td>100</td>
<td>0</td>
<td>31.60 ± 6.4</td>
<td>96.2</td>
<td>31.60 ± 6.4</td>
<td>64.6</td>
</tr>
<tr>
<td>10</td>
<td>44a</td>
<td>100</td>
<td>0</td>
<td>33.40 ± 6.9</td>
<td>64.70 ± 4.9</td>
<td>33.40 ± 6.9</td>
<td>31.3</td>
</tr>
<tr>
<td>11</td>
<td>44b</td>
<td>100</td>
<td>0</td>
<td>48.30 ± 8.4</td>
<td>90.50 ± 11.2</td>
<td>48.30 ± 8.4</td>
<td>42.2</td>
</tr>
<tr>
<td>12</td>
<td>45</td>
<td>100</td>
<td>0</td>
<td>48.20 ± 9.0</td>
<td>108.40 ± 3.6</td>
<td>48.20 ± 9.0</td>
<td>60.2</td>
</tr>
<tr>
<td>13</td>
<td>Pentobarbitone</td>
<td>20</td>
<td>0</td>
<td>26.8 ± 1.42</td>
<td>168.36 ± 6.4</td>
<td>26.8 ± 1.42</td>
<td>131.56</td>
</tr>
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</table>

Mean ± S.E.M,  N = 6,  P < 0.001 Vs standard
ANNEX 7a: TEST PROCEDURE WITH A STARTING DOSE OF 5 MG/KG BODY WEIGHT

Table 67
OECD/OCDE
Table 68
Data showing effect of carbanionic \( \sigma \) complexes on behavioural profiles of mice

<table>
<thead>
<tr>
<th>Responses</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Score (Normal Saline)</td>
</tr>
<tr>
<td>Muscle Tone</td>
<td></td>
</tr>
<tr>
<td>Limp tone</td>
<td>4</td>
</tr>
<tr>
<td>Grip tone</td>
<td>4</td>
</tr>
<tr>
<td>Body tone</td>
<td>4</td>
</tr>
<tr>
<td>Abdominal tone</td>
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<tr>
<td>Reflexes</td>
<td></td>
</tr>
<tr>
<td>Pinna</td>
<td>4</td>
</tr>
<tr>
<td>Corneal</td>
<td>4</td>
</tr>
<tr>
<td>Writhing</td>
<td>0</td>
</tr>
<tr>
<td>Autonomic Profile</td>
<td></td>
</tr>
<tr>
<td>Urination</td>
<td>0</td>
</tr>
<tr>
<td>Salivation</td>
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<tr>
<td>Respiratory rate</td>
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<td>Awareness</td>
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<td>Alertness</td>
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<td>Passivity</td>
<td>0</td>
</tr>
<tr>
<td>Aggression</td>
<td>0</td>
</tr>
<tr>
<td>Mood</td>
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</tr>
<tr>
<td>Restlessness</td>
<td>0</td>
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<tr>
<td>Fearfulness</td>
<td>0</td>
</tr>
<tr>
<td>Touch response</td>
<td>4</td>
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<tr>
<td>Motor Activity</td>
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<tr>
<td>Pain response</td>
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<tr>
<td>Twitches</td>
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<td>CNS excitation</td>
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</tr>
<tr>
<td>Tremors</td>
<td>0</td>
</tr>
<tr>
<td>Convulsions</td>
<td>0</td>
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<td>Posture</td>
<td></td>
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<tr>
<td>Limb posture</td>
<td>4</td>
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### Table 69
Anticonvulsant activity of the carbanionic sigma complexes (with disordered 2,2'-bipyridinium cation moiety and co-crystal)

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Treatment</th>
<th>Time (sec) in various phases of convulsion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Flexor</td>
</tr>
<tr>
<td>1.</td>
<td>Control</td>
<td>Normal Saline (5 ml/kg)</td>
</tr>
<tr>
<td>2.</td>
<td>Disordered 2,2'-bipyridinium cation moiety</td>
<td>i. 25 mg/kg</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ii. 50 mg/kg</td>
</tr>
<tr>
<td></td>
<td></td>
<td>iii. 100 mg/kg</td>
</tr>
<tr>
<td>3.</td>
<td>Co-crystal</td>
<td>i. 25 mg/kg</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ii. 50 mg/kg</td>
</tr>
<tr>
<td></td>
<td></td>
<td>iii. 100 mg/kg</td>
</tr>
<tr>
<td>4.</td>
<td>Phenobarbitone</td>
<td>i. 20mg/kg</td>
</tr>
</tbody>
</table>

Mean ± S.E.M, N = 6, P < 0.001 Vs standard

### Table 70
Hypnotic action of carbanionic sigma complexes (with disordered 2,2'-bipyridinium cation moiety and co-crystal)

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Sample</th>
<th>Dose (mg/kg)</th>
<th>Time of Administration (mins) (a)</th>
<th>Time of loss of reflex (mins) (b)</th>
<th>Time of Recovery (mins) (c)</th>
<th>On set of action (mins) (b-a)</th>
<th>Duration of action (mins) (c-b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control (Saline)</td>
<td>0</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>2</td>
<td>Disordered 2,2'-bipyridinium cation moiety</td>
<td>150</td>
<td>0</td>
<td>57.60 ± 8.6</td>
<td>120.8 ± 10.8</td>
<td>57.6 ± 6.6</td>
<td>63.2 ± 7.2</td>
</tr>
<tr>
<td>3</td>
<td>Co-crystal</td>
<td>150</td>
<td>0</td>
<td>45.7 ± 7.7</td>
<td>98.6 ± 9.7</td>
<td>45.7 ± 7.7</td>
<td>52.9 ± 4.8</td>
</tr>
<tr>
<td>4</td>
<td>Phenobarbitone</td>
<td>20</td>
<td>0</td>
<td>26.8 ± 1.42</td>
<td>168.36 ± 6.4</td>
<td>26.8 ± 1.42</td>
<td>131.56</td>
</tr>
</tbody>
</table>

Mean ± S.E.M, N = 6, P < 0.001 Vs standard
Table 71
Antimicrobial activity of the carbanionic sigma complex with disordered 2,2′-bipyridinium cation moiety

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Name of the Microorganisms</th>
<th>Zone of inhibition in mm</th>
<th>Solvent control</th>
<th>Std</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>20 ppm</td>
<td>50 ppm</td>
<td>100 ppm</td>
</tr>
<tr>
<td>1</td>
<td><em>Staphylococcus aureus</em> (NCIM 2079)</td>
<td>12</td>
<td>15</td>
<td>18</td>
</tr>
<tr>
<td>2</td>
<td><em>E. coli</em> (NCIM 2065)</td>
<td>13</td>
<td>14</td>
<td>16</td>
</tr>
<tr>
<td>3</td>
<td><em>Bacillus subtilis</em> (NCIM 2063)</td>
<td>12</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>4</td>
<td><em>Pseudomonas aeruginosa</em> (NCIM 2036)</td>
<td>10</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>5</td>
<td><em>Candida albicans</em> (NCIM 3102)</td>
<td>12</td>
<td>12</td>
<td>14</td>
</tr>
<tr>
<td>6</td>
<td><em>Aspergillus niger</em> (NCIM 105)</td>
<td>10</td>
<td>12</td>
<td>14</td>
</tr>
</tbody>
</table>

Standard-Ciprofloxacin 5 μg/disc for bacteria; Nystatin 100 units / disc for fungi.

Table 71a
Antimicrobial activity of the carbanionic sigma complex (co-crystal)

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Name of the Microorganisms</th>
<th>Zone of inhibition in mm</th>
<th>Solvent control</th>
<th>Std</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>20 ppm</td>
<td>50 ppm</td>
<td>100 ppm</td>
</tr>
<tr>
<td>1</td>
<td><em>Staphylococcus aureus</em> (NCIM 2079)</td>
<td>15</td>
<td>17</td>
<td>20</td>
</tr>
<tr>
<td>2</td>
<td><em>E. coli</em> (NCIM 2065)</td>
<td>14</td>
<td>20</td>
<td>22</td>
</tr>
<tr>
<td>3</td>
<td><em>Pseudomonas aeruginosa</em> (NCIM 2036)</td>
<td>14</td>
<td>18</td>
<td>21</td>
</tr>
<tr>
<td>4</td>
<td><em>Bacillus subtilis</em> (NCIM 2063)</td>
<td>14</td>
<td>16</td>
<td>18</td>
</tr>
<tr>
<td>5</td>
<td><em>Aspergillus niger</em> (NCIM 105)</td>
<td>12</td>
<td>14</td>
<td>20</td>
</tr>
<tr>
<td>6</td>
<td><em>Candida albicans</em> (NCIM 3102)</td>
<td>12</td>
<td>16</td>
<td>18</td>
</tr>
</tbody>
</table>

Standard-Ciprofloxacin 5 μg/disc for bacteria; Nystatin 100 units / disc for fungi.
### Table 72
Cytotoxicity results of carbanionic sigma complexes

<table>
<thead>
<tr>
<th>Complex</th>
<th>Inhibitory Concentration (IC$_{50}$) (micromol / mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>36a</td>
<td>453.45 ± 12.22</td>
</tr>
<tr>
<td>36b</td>
<td>542.63 ± 10.17</td>
</tr>
<tr>
<td>36c</td>
<td>593.45 ± 15.86</td>
</tr>
<tr>
<td>36d</td>
<td>441.26 ± 14.60</td>
</tr>
<tr>
<td>36e</td>
<td>580</td>
</tr>
<tr>
<td>39a</td>
<td>672.65 ± 17.63</td>
</tr>
<tr>
<td>39b</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>44a</td>
<td>480</td>
</tr>
<tr>
<td>44b</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>45</td>
<td>564.16 ± 16.75</td>
</tr>
</tbody>
</table>

### Table 73
Antimalarial activity of carbanionic sigma complexes

<table>
<thead>
<tr>
<th>Complex</th>
<th>IC$_{50}$ (µM)</th>
<th>CC$_{50}$ (µM)</th>
<th>SI CC$<em>{50}$ / IC$</em>{50}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>36a</td>
<td>&gt;1.0</td>
<td>196.19</td>
<td>&lt;196.19</td>
</tr>
<tr>
<td>39a</td>
<td>&gt;1.0</td>
<td>132.29</td>
<td>&lt;132.29</td>
</tr>
<tr>
<td>Standard</td>
<td>0.004</td>
<td>&gt;200</td>
<td>&gt;50000</td>
</tr>
</tbody>
</table>
Table 74

Antituberculosis activity of carbanionic sigma complexes

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Complexes</th>
<th>Activity</th>
<th>Cytotoxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>MABA</td>
<td>BACTEC</td>
</tr>
<tr>
<td>1.</td>
<td>36a</td>
<td>&gt;50 µM</td>
<td>IA</td>
</tr>
<tr>
<td>2.</td>
<td>36b</td>
<td>&gt;50 µM</td>
<td>IA</td>
</tr>
<tr>
<td>3.</td>
<td>36d</td>
<td>&gt;50 µM</td>
<td>IA</td>
</tr>
<tr>
<td>4.</td>
<td>39a</td>
<td>&gt;50 µM</td>
<td>IA</td>
</tr>
<tr>
<td>5.</td>
<td>39b</td>
<td>&gt;50 uM</td>
<td>IA</td>
</tr>
<tr>
<td>6.</td>
<td>36e</td>
<td>&gt;50 µM</td>
<td>IA</td>
</tr>
</tbody>
</table>
Fig. 66
Animal House
Periyar College of Pharmaceutical Sciences
CPCSEA approved institution (Reg No. 265/CPCSEA)

Electroconvulsometer

Continued…
Oral administration

Corneal shock
Fig. 67  Sodium ion channel

Fig. 68  Calcium ion channel
Fig. 69

Chloride ion channel-barbiturate site
Fig. 70

Antibacterial activity of carbanionic sigma complex with disordered 2,2’-bipyridinium cation moiety against *Staphylococcus aureus* (gram positive bacteria)
Fig. 71

Antibacterial activity of carbanionic sigma complex with disordered 2,2’-bipyridinium cation moiety against *E. coli* (gram negative bacteria)
Fig. 72
Antibacterial activity of carbanionic sigma complex with disordered 2,2’-bipyridinium cation moiety against *Bacillus subtilis* (gram positive bacteria)
Fig. 73

Antibacterial activity of carbanionic sigma complex with disordered 2,2’-bipyridinium cation moiety against

*Pseudomonas aeruginosa* (gram negative bacteria)
Fig. 74
Antifungal activity of carbanionic sigma complex with disordered 2,2’-bipyridinium cation moiety against *Candida albicans*
Fig. 75

Antifungal activity of carbanionic sigma complex with disordered 2,2'-bipyridinium cation moiety against

*Aspergillus niger*
Fig. 76

Antibacterial activity of carbanionic sigma complex (co-crystal) against

*Staphylococcus aureus* (gram positive bacteria)
Fig. 77

Antibacterial activity of carbanionic sigma complex (co-crystal) against

*E.coli* (gram negative bacteria)
Fig. 78

Antibacterial activity of carbanionic sigma complex (co-crystal) against

_Pseudomonas aeruginosa_ (gram negative bacteria)
Fig. 79

Antibacterial activity of carbanionic sigma complex (co-crystal) against

*Bacillus subtilis* (gram positive bacteria)
Fig. 80

Antifungal activity of carbanionic sigma complex (co-crystal) against

Aspergillus niger
Fig. 81

Antifungal activity of carbanionic sigma complex (co-crystal) against

*Candida albicans*