Synthesis of Novel 4-substituted Arylsemicarbazones of Menthone and Evaluation of its Anticonvulsant Activity

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ABSTRACT: A series of 4-N-arylsubstituted semicarbazones of menthone was designed and synthesized to meet the structural requirements essential for anticonvulsant activity. All the compounds were evaluated for anticonvulsant activity. Anticonvulsant activity was determined after intraperitoneal (i p) administration to mice by maximal electroshock (MES) and minimal motor impairment was determined by convulsometer (Medicraft convulsometer). All the compounds were tested for anticonvulsant activity employing Maximal Electroshock method. As compared to standard phenytoin, the test compounds exhibited significant anticonvulsant activity ranging from 72.06%-184.33%. The order of potency of the test compounds was as follows 2 > 4 > 6 > 5 > 1 > 3. The effects of all these drugs were well comparable with standard drug phenytoin. However, the compound 2 was the most potent.

KEY WORDS: Substituted semicarbazones, Anticonvulsant, MES, Menthone

Introduction

Epilepsy is a common disorder of the central nervous system that causes people to have recurring seizures. Approximately 0.4%-1% of the population worldwide suffers from this disorder. Many options are available, from different chemical classes such as hydantoins, barbiturates, benzodiazepines, gamma-aminobutyric acid (GABA) analogs, dibenzepines and carbamates to treat the disorder. But the conventional antiepileptic drugs suffer from a range of side effects. Furthermore, the convulsions of 25% of epileptics are inadequately controlled by currently available medications. In the recent years, much efforts has been devoted to explore the novel approaches by elucidating the cellular and molecular mechanisms of the hyperexcitability to provide specific target for novel therapies and as a result several new drugs such as vigabatrin, lamotrigine, gabapentin, tiagabine, fosphenytoin, eslicarbazepine have appeared in the market. However none of the available antiepileptic drugs is ideal as they can be associated with chronic and adverse side effects. Thus the search for new anticonvulsant drugs continues to be an active area of investigation in medicinal chemistry. Aryl semicarbazones have recently acquired an important place as anticonvulsants and can be considered a new class of compounds with anticonvulsant activity.

Furthermore recently Pandeya et al. and Yogeeswari et al. have suggested a new pharmacophore model for semicarbazones displaying an anticonvulsant activity. They proposed that the terminal amino function of semicarbazones was not essential for activity and could be substituted with a lipophilic aryl ring. Proposed pharmacophore model contain four binding sites for interaction with a macromolecular complex in vivo. These binding sites include: An aryl hydrophobic binding site (A) with halo substituent preferably at para position, a hydrogen bonding domain (HBD), an electron donar group (D) and another hydrophobic–hydrophilic site controlling the pharmacokinetic properties of the anticonvulsant (C).

A new series of p-nitro phenyl substituted semicarbazones of acetone and phenoxy/p-bromophenoxy acetyl hydrazones and evaluated for anticonvulsant activity. Moreover some substituted semicarbazones of carvone, citral, pyridine-4-carbaldehyde, methyl pyridyl ketone and levulinic acid were evaluated which showed good anticonvulsant activity. The cyclic terpene carvone and acyclic terpene citral were selected with the hope that they may modify lipophilicity of molecule, which may improve CNS activity. Based on the above model a number of active semicarbazones have been synthesized in our laboratory. In the present study menthone was selected as carbonyl group. It will increase lipophilicity of the molecule, which may result in improved anticonvulsant activity.
Materials and Methods

Chemistry

The melting points were determined on Thermoink precision melting point apparatus by open capillary method and are uncorrected. Thin layer chromatography (TLC) was performed using silica gel G as stationary phase and iodine vapours were used for visualization. IR spectrum were recorded on Shimadzu Fourier Transform Infrared Spectrophotometer (FTIR-8400s), using KBr discs and UV \( \lambda_{\text{max}} \) were taken on Shimadzu UV visible spectrophotometer (UV-1700s) using methanol as solvent. All compounds have been sent to CDRL, Lucknow, for mass and elemental analysis. Mass spectra were recorded on a JEOL SX 102/DA-600 mass spectrophotometer/Data system using Argon/Xenon (6kV, 10mA) as the FAB gas. The Accelerating voltage was 10 kV and the spectra were recorded at room temperature. m-Nitrobenzyl alcohol (NBA) was used as the matrix by EI (electron impact) method. Elemental analysis (CHN) was carried out on Carlo Erba 1108 elemental analyzer.

Different parasubstituted arylsemicarbazides were prepared by the method of Pandeya et al. (1999) as scheme shown in fig 1. In 100 mL round bottom flask, 5.25g of menthone was placed and dissolved in sufficient quantity of rectified spirit. Equimolar quantity of substituted semicarbazide (0.01 mole) was added and warmed to effect solution. The pH of the reaction mixture was adjusted between 4-5 by adding glacial acetic acid. The reaction mixture was refluxed for a period of 3-4 hrs. Then the reaction mixture was cooled to room temperature. In some cases the precipitate was obtained while in others the reaction mixture was poured on ice, stirring with glass rod. The precipitated product thus obtained was filtered on Buckner funnel, washed with water and recrystallised from 90% ethanol. Physical characterization and elemental analysis of synthesized compounds are given in Tables 1 and 2.

The carbonyl group, C=O, governs the chemistry of the menthone. It does in two ways: (a) by providing a site for nucleophilic addition and (b) by increasing the acidity of the hydrogen atoms attached to alpha carbon. Both these effects are due to the ability of oxygen to accommodate a negative charge.

The menthone reacts with nucleophiles such as semicarbazide to form imine like derivatives. This conversion of aldehydes and ketones into imine like derivatives is mildly exothermic and proceeds at rate, which reaches maximum when the pH of the reaction mixture is about 5-6. This means that the rate-determining step of these reactions involves a bonding of the nucleophilic nitrogen reagent to the conjugate acid of the carbonyl reactant. The reaction mechanism of semicarbazone formation can be represented as in Fig 2.

\[ \text{R} \rightarrow \text{R} + \text{H, Cl, Br, OCH}_3, \text{NO}_2, \text{CH}_3 \]

\[ \text{HN} \rightarrow \text{CO-NH}_3 \]

\[ \text{HN}\rightarrow \text{CO-NH-NH}_2 \]

\[ \text{R} + \text{H, Cl, Br, OCH}_3, \text{NO}_2, \text{CH}_3 \]

Fig. 1 Scheme for synthesis of substituted semicarbazone.
If the acid is present, hydrogen ion becomes attached to carbonyl oxygen. This prior protonation lowers the $E_{ac}$ for nucleophilic attack, since it permits oxygen to acquire the electrons without having to accept a negative charge and making the carbonyl carbon atom more electrophilic.

The spectral data of the synthesized compounds were as follows:

**Compound 1 (2-isopropyl-5-ethylcyclohexanone-N4-phenylsemicarbazone)**

UV ($\lambda_{max}, \text{nm}$) 240; IR (KBr, $\nu \text{cm}^{-1}$), 3421(secondary NH stretch), 3314 (amide NH stretch), 1595(C=N stretch), 1653 (C=O stretch), 825 (phenyl stretch); $^1$HNMR (DMSOd6, $\delta$); 7.2-7.5 (m, 4H, p-chlorophenyl), 3.5(s, 1H, CONH), 9.7(s, 1H, =NNH), 1.52-2.02 (m, 9H, 3CH3), 2.08-2.5 (m, 3H, CH), 0.76-0.99(m, 6H, 3CH2); MS (m/z): Calculated Molecular weight is 300.44 and Molecular weight from Mass spectra 300.

**Compound 2 (2-isopropyl-5-methylcyclohexanone-N4-chlorophenylsemicarbazone)**

UV ($\lambda_{max}, \text{nm}$) 260-262 ; IR (KBr, $\nu \text{cm}^{-1}$), 3432 (secondary NH stretch), 3312(amide NH stretch), 1598(C=N stretch), 1658 (C=O stretch), 805 (phenyl stretch); $^1$HNMR (DMSOd6, $\delta$); 7.7-7.6(m, 4H, p-bromophenyl), 3.5(s, 1H, CONH), 9.7(s, 1H, =NNH), 1.52-2.02(m, 9H, 3CH3), 2.08-2.5 (m, 3H, CH), 0.76-0.99(m, 6H, 3CH2); MS (m/z): Calculated Molecular weight is 319.83 and Molecular weight from Mass spectra 320.

**Compound 3 (2-isopropyl-5-methylcyclohexanone-N4-bromophenylsemicarbazone)**

UV ($\lambda_{max}, \text{nm}$) 260-262; IR (KBr, $\nu \text{cm}^{-1}$), 3432 (secondary NH stretch), 3312(amide NH stretch), 1598(C=N stretch), 1658 (C=O stretch), 805 (phenyl stretch); $^1$HNMR (DMSOd6, $\delta$); 7.7-7.6(m, 4H, p-bromophenyl), 3.5(s, 1H, CONH), 9.7(s, 1H, =NNH), 1.52-2.02(m, 9H, 3CH3), 2.08-2.5 (m, 3H, CH), 0.76-0.99(m, 6H, 3CH2); MS (m/z): Calculated Molecular weight is 379.29 and Molecular weight from Mass spectra 380.

**Compound 4 (2-isopropyl-5-methylcyclohexanone-N4-methoxyphenylsemicarbazone)**

UV ($\lambda_{max}, \text{nm}$) 165-168; IR (KBr, $\nu \text{cm}^{-1}$), 3421(secondary NH stretch), 3318 (amide NH stretch), 1597(C=N stretch), 1658 (C=O stretch), 823 (phenyl stretch), 2810 (O-CH3 stretch); $^1$HNMR (DMSOd6, $\delta$); 7.2-7.5 (m, 4H, p-chlorophenyl), 3.5(s, 1H, CONH), 9.7(s, 1H, =NNH), 1.52-2.02(m, 9H, 3CH3), 2.08-2.5 (m, 3H, CH), 0.76-0.99(m, 6H, 3CH2); MS (m/z): Calculated Molecular weight is 330.39 and Molecular weight from Mass spectra 330.

**Compound 5 (2-isopropyl-5-methylcyclohexanone-N4-nitrophenylsemicarbazone)**

UV ($\lambda_{max}, \text{nm}$) 148-150; IR (KBr, $\nu \text{cm}^{-1}$), 3421(secondary NH stretch), 3314 (amide NH stretch), 1597(C=N stretch), 1658 (C=O stretch), 820 (phenyl stretch), 2606 (NO2 stretch); $^1$HNMR (DMSOd6, $\delta$); 7.2-7.5 (m, 4H, p-chlorophenyl), 3.5(s, 1H, CONH), 9.7(s, 1H, =NNH), 1.52-2.02 (m, 9H, 3CH3), 2.08-2.5 (m, 3CH, CH), 0.76-0.99(m, 6H, 3CH2); MS (m/z): Calculated Molecular weight is 345.39 and Molecular weight from Mass spectra 345.
Compound 6 (2-isopropyl-5-methylcyclohexanone-N4-methylphenylsemicarbazone)

UV (λ<sub>max</sub>, nm) 180-182; IR (KBr, υ cm<sup>-1</sup>) (KBr, υ cm<sup>-1</sup>), 3431 (secondary NH stretch), 3311 (amide NH stretch), 1598 (C=N stretch), 1658 (C=O stretch), 812 (phenyl stretch); 1HNMR (DMSOd<sub>6</sub>, δ); 7.2-7.5 (m, 4H, p-chlorophenyl), 3.5 (s, 1H, CONH), 9.7 (s, 1H, =NNH), 1.52-2.02 (m, 9H, 3CH<sub>3</sub>), 2.08-2.5 (m, 3H, CH), 0.76-0.99 (m, 6H, 3CH<sub>2</sub>); MS (m/z): Calculated Molecular weight is 314.31 and Molecular weight from Mass spectra 314.

<table>
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<tr>
<th>Compound no</th>
<th>R</th>
<th>Molecular formula</th>
<th>M.P. (°C)</th>
<th>Yield (%)</th>
<th>R&lt;sup&gt;+&lt;/sup&gt;</th>
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<tr>
<td>1</td>
<td>H</td>
<td>C&lt;sub&gt;10&lt;/sub&gt;H&lt;sub&gt;12&lt;/sub&gt;N&lt;sub&gt;2&lt;/sub&gt;O&lt;sub&gt;2&lt;/sub&gt;</td>
<td>120-122</td>
<td>74.17</td>
<td>0.56</td>
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<tr>
<td>2</td>
<td>CI</td>
<td>C&lt;sub&gt;10&lt;/sub&gt;H&lt;sub&gt;12&lt;/sub&gt;N&lt;sub&gt;2&lt;/sub&gt;OCl</td>
<td>178-180</td>
<td>70.01</td>
<td>0.64</td>
</tr>
<tr>
<td>3</td>
<td>Br</td>
<td>C&lt;sub&gt;10&lt;/sub&gt;H&lt;sub&gt;12&lt;/sub&gt;N&lt;sub&gt;2&lt;/sub&gt;OBr</td>
<td>280-292</td>
<td>84.34</td>
<td>0.47</td>
</tr>
<tr>
<td>4</td>
<td>OCH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>C&lt;sub&gt;10&lt;/sub&gt;H&lt;sub&gt;12&lt;/sub&gt;N&lt;sub&gt;2&lt;/sub&gt;O&lt;sub&gt;2&lt;/sub&gt;OCH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>208-210</td>
<td>68.2</td>
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<td>5</td>
<td>NO&lt;sub&gt;2&lt;/sub&gt;</td>
<td>C&lt;sub&gt;10&lt;/sub&gt;H&lt;sub&gt;12&lt;/sub&gt;N&lt;sub&gt;2&lt;/sub&gt;O&lt;sub&gt;2&lt;/sub&gt;NO&lt;sub&gt;2&lt;/sub&gt;</td>
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<td>69.09</td>
<td>0.71</td>
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<td>6</td>
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<td>C&lt;sub&gt;10&lt;/sub&gt;H&lt;sub&gt;12&lt;/sub&gt;N&lt;sub&gt;2&lt;/sub&gt;O&lt;sub&gt;2&lt;/sub&gt;OCH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>145-147</td>
<td>56.2</td>
<td>0.48</td>
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</table>

Table 2 The elemental analysis of the semicarbazones (1-6).

<table>
<thead>
<tr>
<th>Compound No</th>
<th>Molecular formula</th>
<th>Calculated %</th>
<th>Obtained %</th>
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<tr>
<td></td>
<td>C</td>
<td>H</td>
<td>N</td>
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<tr>
<td>1</td>
<td>71.64</td>
<td>8.78</td>
<td>14.62</td>
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<td>2</td>
<td>63.44</td>
<td>7.51</td>
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<td>3</td>
<td>55.73</td>
<td>6.6</td>
<td>11.47</td>
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<td>4</td>
<td>68.1</td>
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<td>5</td>
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<td>6</td>
<td>71.72</td>
<td>9.02</td>
<td>13.24</td>
</tr>
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</table>

Anticonvulsant Screening

Anticonvulsant evaluation of semicarbazones was undertaken by following the National Institute of Health (NIH) Anticonvulsant Drug Development (ADD) Program protocol.\textsuperscript{18}

The albino mice of either sex weighing 18-30 g were used as experimental animals. They were housed in perspex cages under the standard conditions of temperature (25 ± 2°C) and light (12:12 h light:dark, light turned on at 07.00 h). Animals were allowed to acclimatize to lab conditions with free access to food and water for 24 h period before testing. 40% propylene glycol in distilled water was used as vehicle Phenytoin was used as standard.

Drug Solution

All the test compounds and standard phenytoin were suspended in 40% propylene glycol so as to represent their concentration equimolar to 25-mg/kg-body weight dose of phenytoin.

Procedure

The animals were divided into eight groups of five animals in each and received the treatments as shown in Table 3.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>mg/kg</th>
<th>Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>Vehicle</td>
<td>-</td>
<td>All the animals received drug equivalent to 25 mg/kg i.p. phenytoin.</td>
</tr>
<tr>
<td>Group 2</td>
<td>Phenytoin</td>
<td>25.00</td>
<td></td>
</tr>
<tr>
<td>Group 3</td>
<td>01</td>
<td>28.28</td>
<td></td>
</tr>
<tr>
<td>Group 4</td>
<td>02</td>
<td>31.69</td>
<td></td>
</tr>
<tr>
<td>Group 5</td>
<td>03</td>
<td>36.10</td>
<td></td>
</tr>
<tr>
<td>Group 6</td>
<td>04</td>
<td>31.25</td>
<td></td>
</tr>
<tr>
<td>Group 7</td>
<td>05</td>
<td>32.74</td>
<td></td>
</tr>
<tr>
<td>Group 8</td>
<td>06</td>
<td>29.67</td>
<td></td>
</tr>
</tbody>
</table>

After 30 min of drug or vehicle injections, animals were subjected to electroshock with corneal electrodes using convulsometer (Medicraft convulsometer). The stimulus parameters were 42 mA, AC in pulse of 60 Hz, for 0.2 sec. The duration of hind leg extension was recorded for each animal. The absence of tonus or significant delay in hind leg extension was considered as drug having anticonvulsant activity.

The percentage of animals protected were recorded and the percent potency of test drug was calculated using formula:

\[
\% \text{potency of anticonvulsant drug} = 100 \times \left(1 - \frac{\text{Mean value of compound} - \text{Mean value of standard}}{\text{Mean value of standard}}\right)
\]

Results and Discussion

The anticonvulsant activities of tested compounds is reported in Table 4. All the compounds except vehicle have shown 100% protection in animals. All the new compounds tested here showed profound anticonvulsant activity as evident from marked reduction in duration of hind leg extension as compared to vehicle control. However percentage of animals protected varied from drug to drug. The order of potency in protecting the animals was: compound 2 > compound 4 > compound 6 > compound 5 > compound 1 > compound 3. The effects of all these drugs were well comparable with standard drug phenytoin. However, the compound compound 2 in Fig 3 was most potent. Most of the compounds exhibited anticonvulsant activity at 30 min rather than 4h. Therefore it can be said that the onset of action for the compounds is rapid.

Since the aryl substituted semicarbazones shows good anticonvulsant activity we synthesised p-substituted phenyl semicarbazones and selected menthone as carbonyl group which have resulted in improved anticonvulsant activity. This leads to conclusion that substitution at the terminal amino groups with menthone moiety increases the lipophilicity of the molecules and improves pharmacokinetic properties of the molecules.
The results of the present study validated the pharmacophore model proposed by Pandeya et al. with four binding sites essential for anticonvulsant activity. Thus 4-aryl substituted menthone semicarbazones has emerged as an anticonvulsants with broad spectrum of anticonvulsant activity.

References


