INTRODUCTION

Several quinazolinone alkaloids are known to elicit a wide variety of biological response. The substituent present on C-2 and N-3 of the quinazoline molecule plays a critical role in promoting several biological activities.

7-Azaindole (1H-pyrrolo (2, 3-b) pyridine) nucleus is present only in a few natural products such as alkaloids from the variolin family. Nevertheless, 7-Azaindole derivatives have attracted much attention due to their physicochemical and pharmacological properties.

It is evident from the literature that the presence of the 4(3H)-quinazolinone nucleus found to have various pharmacological activities like antibacterial (3), analgesic and anti-inflammatory (4), antifungal (5) anticonvulsant (6), anticancer (7), and antihypertensive (8) activities.

It is also evident from the literature that azaisatins (9) are also biologically active and found to have various pharmacological activities like antiproliferative (10), antimalarial, antihistaminic (11), antiserotonin, antibacterial (12), analgesic (13), anti-inflammatory (12), antifungal (12), antihypertensive, CNS depressant (14), tranquilizers, neuroleptics, anticonvulsant (10) and antiobesity. So, keeping this in view the present work is to synthesize the title compounds to obtain derivatives of azaisatin.
MATERIALS AND METHODS

Melting points were taken in open capillary tubes on Sigma-Aldrich melting point apparatus and are uncorrected. All the synthesized compounds were purified by thin layer chromatogram on silica gel G using toluene: ethyl acetate (7:3) and visualized with UV light. IR spectra were recorded on PERKIN-ELMER BX Series FTIR spectrometer using KBr pellets. $^1\text{HNMR}$ spectra were recorded in a CDCl$_3$ as a solvent and tetra methyl silane (TMS) as an interval standard. MASS Spectra of the compounds were recorded on a Agilent Mass spectroscopy 1100 series using ESI technique.

The physical constants of different synthesized azaisatins derivatives are shown in Table No.1

The spectral data of different synthesized azaisatins derivatives are shown in Table No.2

SCHEME – I

![Scheme I](image)

Figure 1

Anupama Devi et al; Synthesis and screening of different Azaisatins derivatives containing 4-(3H)-quinazolinones for their CNS activity (Sedative & Hypnotic)

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GENERAL PROCEDURE

SCHEME-I

A) Synthesis of Methyl-2-acetamidobenzoate (II)

In 100ml of round bottomed flask, a solution of Methyl-2-aminobenzoate (I) (0.016 mole) in acetic anhydride (0.0127 mole) were taken and refluxed for 8-12 hours and the reaction was monitored by TLC for completion. The solution was cooled, poured into cold water (50ml) containing a drop of pyridine and stirred until the oil was solidified. The product was filtered, washed with cold water (4x50) and dried. The solid product was recrystallized from ethanol (6ml/g).

Yield: 80 %, m.p: 98 – 100 °C

B) Synthesis of 2-Phenyl-4(H)-3,1-benoxazin-4-one

For the synthesis of 2-phenyl-4(H)-3,1-benoxazin-4-one, a solution of benzoyl chloride and pyridine was added to 2-aminobenzoic acid. The reaction mixture was refluxed for 8-12 hours and the reaction was monitored by TLC for completion. The solution was cooled, poured into cold water (50ml) containing a drop of pyridine and stirred until the oil was solidified. The product was filtered, washed with cold water (4x50) and dried. The solid product was recrystallized from ethanol (6ml/g).

Yield: 80 %, m.p: 98 – 100 °C

C) Synthesis of 3-Amino-2-phenylquinazolin-4(3H)-one

For the synthesis of 3-amino-2-phenylquinazolin-4(3H)-one, a solution of hydrazine hydrate and methanol was added to a solution of 2-aminobenzoic acid. The reaction mixture was refluxed for 8-12 hours and the reaction was monitored by TLC for completion. The solution was cooled, poured into cold water (50ml) containing a drop of pyridine and stirred until the oil was solidified. The product was filtered, washed with cold water (4x50) and dried. The solid product was recrystallized from ethanol (6ml/g).

Yield: 80 %, m.p: 98 – 100 °C

D) Synthesis of 3-(1,2-Dihydro-1-substituted-2-oxopyrrolo[2,3-b]pyridin-3-ylideneamino)-2-phenylquinazolin-4(3H)-ones

For the synthesis of 3-(1,2-Dihydro-1-substituted-2-oxopyrrolo[2,3-b]pyridin-3-ylideneamino)-2-phenylquinazolin-4(3H)-ones, a solution of 3-aminobenzoic acid and a substituted 1,2-dihydro-2-oxopyrrolo[2,3-b]pyridin-3-carboxylic acid was added to a solution of pyridine and methanol. The reaction mixture was refluxed for 8-12 hours and the reaction was monitored by TLC for completion. The solution was cooled, poured into cold water (50ml) containing a drop of pyridine and stirred until the oil was solidified. The product was filtered, washed with cold water (4x50) and dried. The solid product was recrystallized from ethanol (6ml/g).

Yield: 80 %, m.p: 98 – 100 °C
B) Synthesis of 3-Amino-2-methylquinazolin-4(3H)-one (III)

In 100ml of round bottomed flask, a solution of hydrazine hydrate (10ml) and Methyl-2-acetoamidobenzoate (II, 0.01 moles) in ethanol were taken and refluxed for 8-12 hours and the reaction was monitored by TLC for completion. The solution was cooled, poured into cold water and the product was filtered, washed with cold water and dried. The solid product was recrystallized from ethanol.

Yield: 84 %, m.p: 151 – 152 °C

C) Synthesis of 3-(1,2-Dihydro-1-substituted-2-oxopyrrolo[2,3-b]pyridin-3-ylideneamino)-2-methylquinazolin-4(3H)-ones (IV) a-d

A mixture of 3-Amino-2-methylquinazolin-4(3H)-one (III, 0.001 mole) and substituted azaisatin (0.01 mole) in 10ml of glacial acetic acid were refluxed for 10-15 minutes at 140 watt in Catalyst Systems Scientific microwave System and the reaction was monitored by TLC for completion. The resultant solution was poured into cold water. The product was filtered, washed with cold water and dried. The solid product was recrystallized from absolute alcohol.

Yield: 82 %, m.p: 168-172 °C

TABLE 1: PHYSICAL CONSTANTS OF DIFFERENT AZAISATINS DERIVATIVES CONTAINING 4-(3H)-QUINAZOLINONES

C) Synthesis of 3-(1, 2-Dihydro-1-substituted-2-oxopyrrolo [2, 3-b] pyridin-3-ylideneamino)-2-phenylquinazolin-4(3H)-ones (VIII) a-d

A mixture of 3-Phenyl-2-methylquinazolin-4(3H)-one (VII, 0.001 mole) and substituted azaisatin (0.01 mole) in 10ml of glacial acetic acid were refluxed for 10-15 minutes at 140 watt in Catalyst Systems Scientific microwave System and the reaction was monitored by TLC for completion. The resultant solution was poured into cold water. The product was filtered, washed with cold water and dried. The solid product was recrystallized from absolute ethanol.
<table>
<thead>
<tr>
<th>Compound</th>
<th>R</th>
<th>R₁</th>
<th>Molecular weight (gms)</th>
<th>Molecular formula</th>
<th>m.p (°C)</th>
<th>Yield %</th>
<th>Recrystallization solvent</th>
</tr>
</thead>
<tbody>
<tr>
<td>IV a</td>
<td>–CH₃</td>
<td>–CH₃</td>
<td>319</td>
<td>C₁₇H₁₆N₂O₂</td>
<td>207°C-209°C</td>
<td>75</td>
<td>Absolute Ethanol</td>
</tr>
<tr>
<td>IV b</td>
<td>–CH₃</td>
<td>–C₆H₅</td>
<td>333</td>
<td>C₁₈H₁₇N₂O₂</td>
<td>200°C-202°C</td>
<td>78</td>
<td>Absolute Ethanol</td>
</tr>
<tr>
<td>IV c</td>
<td>–CH₃</td>
<td></td>
<td>347</td>
<td>C₁₅H₁₇N₃O₂</td>
<td>192°C-194°C</td>
<td>70</td>
<td>Absolute Ethanol</td>
</tr>
<tr>
<td>IV d</td>
<td>–CH₃</td>
<td>–C₆H₅</td>
<td>389</td>
<td>C₂₂H₂₃N₆O₂</td>
<td>197°C-198°C</td>
<td>79</td>
<td>Absolute Ethanol</td>
</tr>
<tr>
<td>VIII a</td>
<td>–C₆H₅</td>
<td>–CH₃</td>
<td>381</td>
<td>C₂₂H₂₃N₆O₂</td>
<td>216°C-218°C</td>
<td>72</td>
<td>Absolute Ethanol</td>
</tr>
<tr>
<td>VIII b</td>
<td>–C₆H₅</td>
<td>–C₆H₅</td>
<td>395</td>
<td>C₂₃H₁₇N₃O₂</td>
<td>204°C-206°C</td>
<td>75</td>
<td>Absolute Ethanol</td>
</tr>
<tr>
<td>VIII c</td>
<td>–CH₃</td>
<td></td>
<td>409</td>
<td>C₂₄H₂₃N₆O₂</td>
<td>213°C-215°C</td>
<td>74</td>
<td>Absolute Ethanol</td>
</tr>
<tr>
<td>VIII d</td>
<td>–C₆H₅</td>
<td>–C₆H₅</td>
<td>451</td>
<td>C₂₇H₂₃N₆O₂</td>
<td>208°C-210°C</td>
<td>73</td>
<td>Absolute Ethanol</td>
</tr>
</tbody>
</table>

**TABLE 2: PHYSICAL CONSTANTS OF DIFFERENT AZAISATINS DERIVATIVES CONTAINING 4-(3H)-QUINAZOLINONES**

![Diagram of molecule](image)

<table>
<thead>
<tr>
<th>COMPOUND</th>
<th>IR (KBr) cm⁻¹</th>
<th>¹H NMR (400 MHz, CDCl₃)</th>
<th>MASS SPECTRUM m/z</th>
</tr>
</thead>
<tbody>
<tr>
<td>Synthesis of Methyl-2-acetamido benzoate (II)</td>
<td>3273.63 (N-H, stretch), 1692.23 (C = O, stretch), 1591.75 (C=O, stretch),</td>
<td>δ (ppm): 8.223 (d, 1H, Ar-H), 7.734 (t, 1H, Ar-H), 7.652 (d, 1H, Ar-H), 7.448 (d, 1H, Ar-H), 4.907 (s, 2H, NH₂), 2.713 (s, 3H, CH₃),</td>
<td>The molecular ion was observed at 194 (M + H)⁺, 216 (M + Na)⁺</td>
</tr>
<tr>
<td>Synthesis of 3-Amino-2-methylquinazolin-4(3H)-one (III)</td>
<td>3537.96, 3302.76 (d, N-H, stretch), 3198.21 (C-H, stretch), 1718.19 (C = O, stretch),</td>
<td>δ (ppm): 8.306 (d, 1H, Ar-H), 7.800 (m, 4H, Ar-H), 7.520 (m, 4H, Ar-H), 5.015 (s, 2H, NH₂),</td>
<td>The molecular ion was observed at 176.3 (M + H)⁺</td>
</tr>
<tr>
<td>Synthesis of 3-Amino-2-phenylquinazolin-4(3H)-one (VII)</td>
<td>3568.18, 3309.03 (d, N-H, stretch), 1718.24 (C = O, stretch), 1662.94 (C= N, stretch),</td>
<td>δ (ppm): 8.334 (d, 1H, Ar-H), 8.095 (d, 1H, Ar-H), 6.948 (t, 1H, Ar-H), 7.855 (d, 1H, Ar-H), 7.752 (t, 1H, Ar-H), 7.554 (m, 2H, Ar-H), 2.320 (s, 3H, CH₃), 3.914 (q, 2H, CH₂), 1.331 (t, 3H, CH₃),</td>
<td>The molecular ion was observed at 333 (M⁺), 372 (M⁺ + K)⁺, and 689 (2M⁺ + Na)⁺</td>
</tr>
<tr>
<td>3-(1,2-Dihydro-1-ethyl-2-oxopyrrolo(2,3-b)pyrind-3-ylideneamino)-2-methylquinazolin-4(3H)-one (IV b)</td>
<td>1718.25 (C = O, stretch), 1700.21 (C=O, stretch), 1654.23 (C= N, stretch),</td>
<td>δ (ppm): 8.334 (d, 1H, Ar-H), 8.095 (d, 1H, Ar-H), 6.948 (t, 1H, Ar-H), 7.855 (d, 1H, Ar-H), 7.752 (t, 1H, Ar-H), 7.554 (m, 2H, Ar-H), 2.320 (s, 3H, CH₃), 3.914 (q, 2H, CH₂), 1.331 (t, 3H, CH₃),</td>
<td>The molecular ion was observed at 333 (M⁺) and 418 (M⁺ + Na)⁺</td>
</tr>
<tr>
<td>3-(1,2-Dihydro-1-ethyl-2-oxopyrrolo(2,3-b)pyrind-3-ylideneamino)-2-phenylquinazolin-4(3H)-one (VIII b)</td>
<td>1718.17 (C = O, stretch), 1699.75 (C=O, stretch), 1654.24 (C= N, stretch),</td>
<td>δ (ppm): 8.334 (d, 1H, Ar-H), 8.095 (d, 1H, Ar-H), 7.104 (t, 1H, Ar-H), 7.844 (d, 3H, Ar-H), 7.670 (m, 3H, Ar-H), 7.542 (m, 3H, Ar-H), 3.987 (q, 2H, CH₂), 1.393 (t, 3H, CH₃),</td>
<td>The molecular ion was observed at 396 (M⁺ + H)⁺</td>
</tr>
<tr>
<td>3-(1,2-Dihydro-1-isopropyl-2-oxopyrrolo(2,3-b)pyrind-3-ylideneamino)-2-phenylquinazolin-4(3H)-one</td>
<td>1718.20 (C = O, stretch), 1677.36 (C=O, stretch), 1654.24 (C= N, stretch),</td>
<td>δ (ppm): 8.305 (d, 1H, Ar-H), 8.095 (d, 1H, Ar-H), 7.078 (t, 1H, Ar-H), 7.843 (d, 3H, Ar-H), 7.669 (m, 3H, Ar-H), 7.540 (m, 3H, Ar-H), 4.839 (m, 1H, CH), 1.615 (d, 6H, CH₃),</td>
<td>The molecular ion was observed at 410 (M⁺ + H)⁺ and 432 (M⁺ + Na)⁺</td>
</tr>
</tbody>
</table>
RESULTS

SEDATIVE AND HYPNOTIC ACTIVITY

EXPERIMENT METHOD

Acute Toxicity

Healthy and adult Swiss albino mice of either sex weighing between 20-25g were used in the present investigation. Animals were fasted for 24 hours. These animals are used for each step. The dose level to be used as the starting dose is selected from one of four fixed levels 5, 50, 300 and 2000 mg/kg body weight. The flow charts of drawn below describe the procedure that should be followed for each of starting doses.

Chart: Test procedure with a starting dose of 5 mg/kg body weight.

In this method, mice of either sex were randomly taken and divided into control, standard and different test groups, each group contain six animals. Group I served as control and treated with normal saline (10 ml/kg, i.p.), group II (standard) treated with standard drug Diazepam hydrochloride (3mg/kg, i.p.) 30 minutes before the administration of pentobarbitone (50mg/kg, i.p.). Test groups III-VI were treated with test compounds (50 and 100 mg/kg). Pentobarbitone (50mg/kg, i.p.) was administered 30 min later. Onset of sleep and duration of sleep measured for all the groups. Onset of action was recorded by noting the time of loss of reflex, duration of sleep recorded by time difference between loss of righting reflex and recovery time.

ACUTE TOXICITY STUDIES:

This study has been done with the doses 5, 50, 300 and 2000mg/kg (b.w). Mortality of the mice was observed with the dose of 2000 mg/kg (b.w). Again two more test doses that is 500 and 1000 mg/kg (b.w) were administered. Mortality observed with 1000 mg/kg (b.w) and the test animals were safe at 500 mg/kg (b.w), intraperitonealy.

SEDATIVE AND HYPNOTIC ACTIVITY

Pentobarbitone induced sleeping time (18)

Treatments

Sleeping Time (min)

Normal

Diazepam 30mg/kg

IV a 50mg/kg

IV b 100mg/kg

IV c 150mg/kg

IV d 200mg/kg

Treatments

Sleep latency (sec)

Normal

Diazepam 30mg/kg

IV a 50mg/kg

IV b 100mg/kg

IV c 150mg/kg

IV d 200mg/kg
and after 30 min of administration mice are placed again in actophotometer for 10 min and the activity was monitored. Percentage decrease in activities were calculated for each group using the formula:

\[
\text{Percentage decrease in activity} = \frac{\text{Initial} - \text{Final}}{\text{Initial}} \times 100
\]

**Traction test**

The screening of the animals was done by placing the forepaws of the mice in a small wire (60 cm long and 0.15 cm diameter) rigidly supported above a bench top. Normally the mice grasp the wire with the forepaws, and placed at least one hind foot on the wire. The test was conducted on six group of animals (n=6). The
basal activities score for mice noted before administration of drug. Subsequently 30 min after the injection of the test compounds 50mg/kg, 100mg/kg, vehicle (10 ml/kg, normal saline) and diazepam (4mg/kg) respectively. The complete reestablishment time of hind paws on to the wire is recorded.

**Discussion**

**Pentobarbitone induced sleeping time**

Loss of righting reflex was observed by the administration of the VIII b 50mg/kg, VIII c 50mg/kg, IV b 100mg/kg and IV d 50mg/kg doses implies that Loss of righting reflex induced by phenobarbital is potentiated by GABA agonist and inhibited by GABA antagonist; the activation of GABA receptor partially mediates the sleep response. It is thus plausible to assert that the sedative effect of the test compounds is due to the facilitation of GABAergic transmission.

**Locomotor activity**

The reduction in locomotor activity following the administration of the VIII b 100mg/kg, VIII c 50mg/kg, IV c 100mg/kg and IV d 50mg/kg, 100mg/kg doses implies that they exerted a depressive effect on the CNS. It has been established that increase in the concentration of gamma-amino butyric acid (GABA) may lead to CNS depressant effect. This led to further exploration of the effect of the test compounds on activities responsible for increase in GABA concentration, such as potentiation of Pentobarbitone induced sleeping time, motor coordination.

Test compounds activity was potentiated by pentobarbitone in induced hypnosis suggest a GABA-mediated effect on the CNS since CNS depressants extend barbiturate sleeping time. It is known that sedative-hypnotic drugs induce their effect on the Gabaergic system in the brain and inhibition of neuronal output could be facilitated by GABA (an inhibitory neurotransmitter) release.

**Traction Test**

The traction test brings more precisely into play equilibration muscle strength and tonus. IV d 100mg/kg and VIII c 100mg/kg doses showed significant activity compared with normal control. CCK2 receptor-deficient mice did not grasp the bar with at least one hind paw in less than 5 sec’s, showing a significant impairment of performance in the traction test.
impairment is reproducible since the same results were obtained using other batches of mice. The compounds with phenyl substitution at C2 in quinazolinone nucleus were found to be more active than the compounds with methyl substitution and the compounds with ethyl, isopropyl and hexyl substitutions at position 1 in azaisatin moiety were found to have good sedative and hypnotic activity.

**Conclusion**

1. All the synthesized compounds have been found to have good sedative and hypnotic activity.
2. Compounds VIII b 100mg/kg, VIII c 50mg/kg, IV c 100mg/kg and IV d 50mg/kg, 100mg/kg doses reduced locomotor activity. It has been established that increase in the concentration of gamma-amino butyric acid (GABA) may lead to CNS depressant effect.
3. The test compounds VIII b 50mg/kg, VIII c 50mg/kg, IV b 100mg/kg and IV d 50mg/kg doses potentiated the pentobarbitone induced hypnosis suggest a GABA-mediated effect on the CNS since CNS depressants extend barbiturate sleeping time.
4. IV d 100mg/kg and VIII c 100mg/kg doses showed significant activity in traction test. CCK2 receptor-deficient mice did not grasp the bar with at least one hind paw in less than 5 s, showing a significant impairment of performance in the traction test.
5. It is known that sedative-hypnotic drugs induce their effect on the Gabaergic system in the brain and inhibition of neuronal output could be facilitated by GABA (an inhibitory neurotransmitter) release, so the synthesized compounds found to have sedative and hypnotic activity.

**Acknowledgement**

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**Conflict of Interest Statement**

We declare that we have no conflict of interest.

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