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Synthesis and screening of 3-(1, 2-Dihydro-1-Substituted-2-Oxopyrrolo (2, 3-b) Pyridin-3-Ylideneamino)-2-(Substituted)-Quinazolin-4(3H)-Ones

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Abstract:
Quinazolinones are building blocks for approximately 150 naturally occurring alkaloids isolated from a number of families of the plant kingdom, from animals and microorganisms. Quinazolinones are also a class of drugs which function as hypnotic/sedative that contain a 4-quinazolinone core. In recent years, 7-azaisatins have received considerable interest in view of their potential relationship to pharmacologically important indoles and purine nucleus, and also because of their interesting biological activities.
Hence a new azaisatin derivatives containing 4(3H)-quinazolinones has been designed and synthesized. Their structures have been elucidated on the basis of elemental analysis and spectral studies. All the compounds synthesized were screened for their CNS activity (Sedatives & Hypnotics) which exhibited some authentic results towards testing organism invitro and invivo studies.

Keywords: Quinazolinone, Azaindole, Azaisatin, CNS activity.

Introduction

Several quinazolinone [(1)] 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 in critical role in promoting several biological activities.

7-Azaindole [(2)] (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.

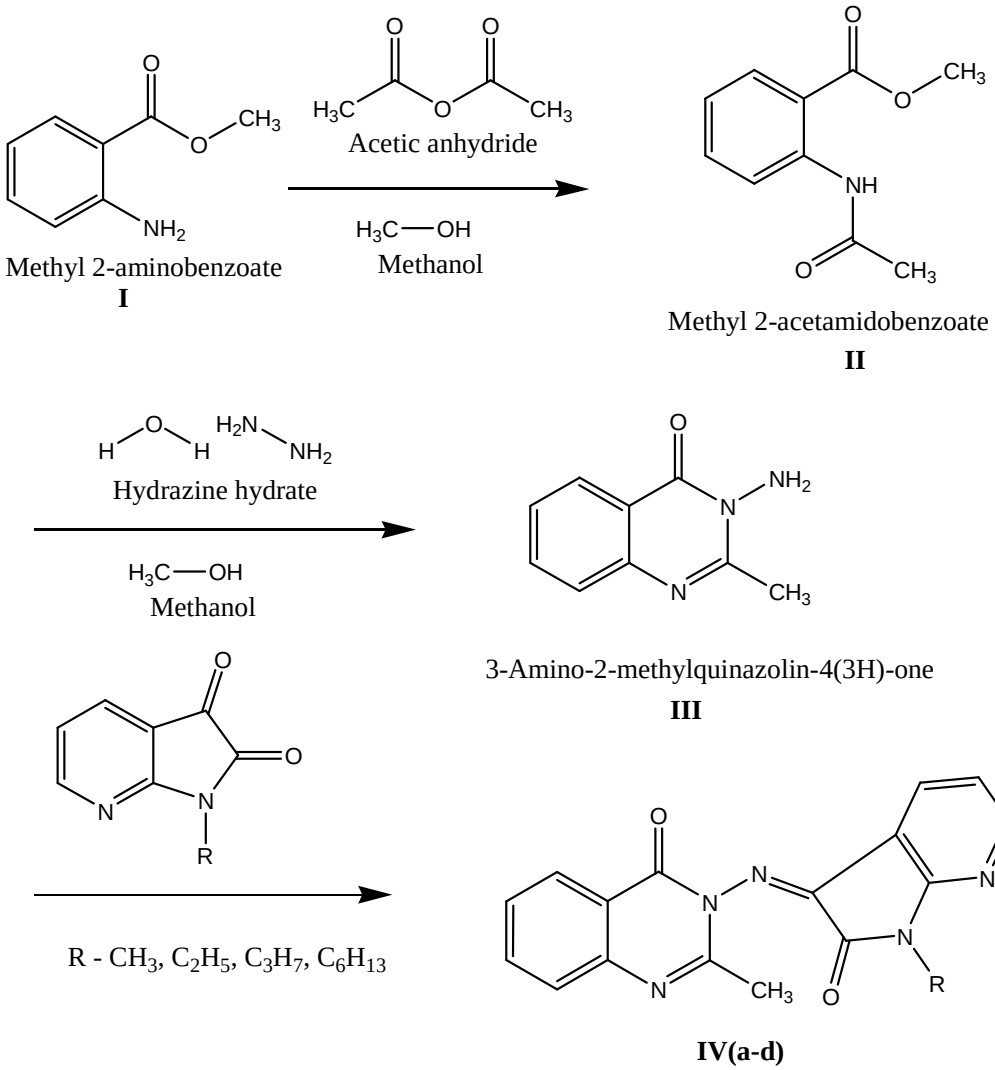
MATERIALS AND METHODS

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}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

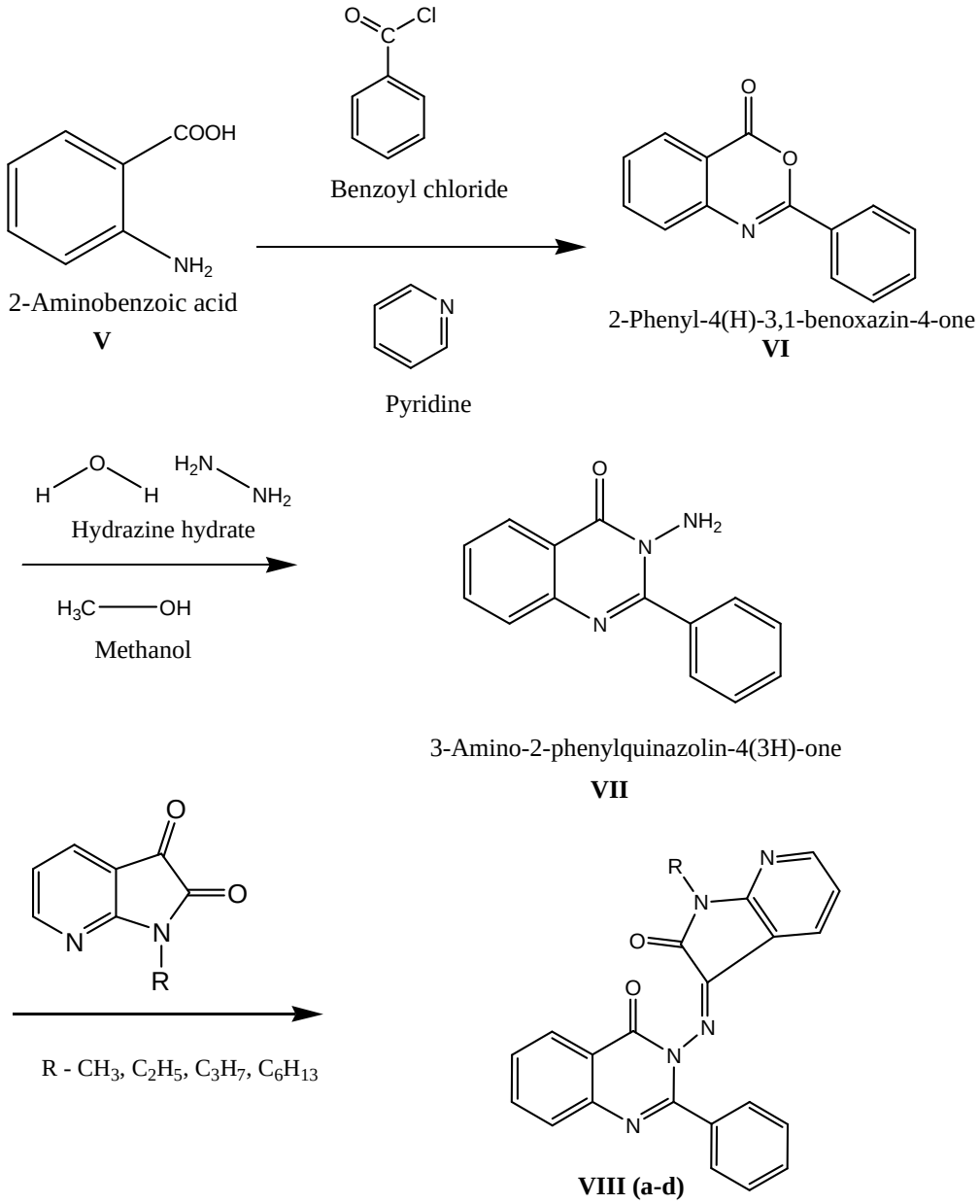
SCHEME – I



3-(1,2-Dihydro-1-substituted-2-oxopyrrolo[2,3-*b*] pyridin-3-ylideneamino)-2-methylquinazolin-4(3*H*)-ones

Figure 1

SCHEME – II



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

Figure 2

GENERAL PROCEDURE

SCHEME-I

A) Synthesis of Methyl-2-acetamidobenzoate [15] (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 ^{0} C

B) Synthesis of 3-Amino-2-methylquinazolin-4(3H)-one [15] (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 ^{0} C

C) Synthesis of 3-(1,2-Dihydro-1-substituted-2-oxopyrrolo(2,3-b) pyridin-3-ylideneamino)-2-methylquinazolin-4(3H)-ones [16] (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 10-15minutes 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.

SCHEME-II

A) Synthesis of 2-Phenyl-(4H)-3, 1-benzoxazin-4-one [17] (VI)

To a stirred solution of 2-aminobenzoic acid (I) (0.01 mole) in pyridine (6ml), benzoyl chloride (0.01 mole) was added drop wise maintaining the temperature near 8^{0}C for about 1.5 hours. Reaction mixture was stirred for another 3 hours at room temperature, while stirring a solid product separated out. Whole reaction mixture was neutralized with 5% $NaHCO_3$ solution. A pale yellow deposited product was filtered, washed with cold water and dried. The solid product was recrystallized from ethanol.

Yield: 86 %, m.p: 114 – 116 ^{0} C

B) Synthesis of 3-Amino-2-phenylquinazolin-4(3H)-one [17] (VII)

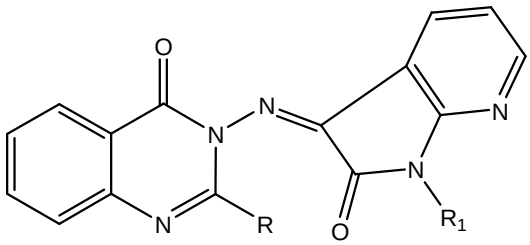
In 100ml of round bottomed flask, a solution of hydrazine hydrate (0.10 mole) and 2-phenyl-4Hbenzo(d) (3, 1) oxazin-4-one (II, 0.05 mole) in methanol were taken and refluxed for 3-6 hours and the reaction was monitored by TLC. The solution was cooled, poured into cold water and the product was filtered, washed with cold water and dried. The solid product was recrystallized from ethanol.

Yield: 82 %, m.p: 168-172 ^{0} C

C) Synthesis of 3-(1, 2-Dihydro-1-substituted-2-oxopyrrolo (2, 3-b) pyridin-3-ylideneamino)-2-phenylquinazolin-4(3H)-ones [16] (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 10-15minutes 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 1: PHYSICAL CONSTANTS OF DIFFERENT AZAISATINS DERIVATIVES CONTAINING 4-(3H)-QUINAZOLINONES



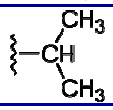
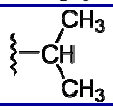
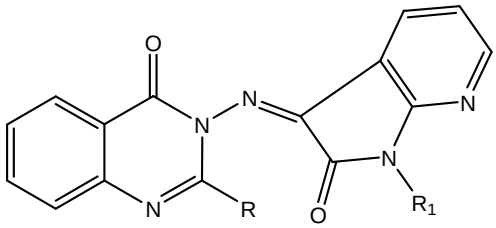
Compound	R	R_1	Molecular weight (gms)	Molecular formula	m.p (^{0}C)	Yield %	Recrystallization solvent
IV a	$-CH_3$	$-CH_3$	319	$C_{17}H_{13}N_5O_2$	207^0C-209^0C	75	Absolute Ethanol
IV b	$-CH_3$	$-C_2H_5$	333	$C_{18}H_{15}N_5O_2$	200^0C-202^0C	78	Absolute Ethanol
IV c	$-CH_3$		347	$C_{19}H_{17}N_5O_2$	192^0C-194^0C	70	Absolute Ethanol
IV d	$-CH_3$	$-C_6H_{13}$	389	$C_{22}H_{23}N_5O_2$	197^0C-198^0C	79	Absolute Ethanol
VIII a	$-C_6H_5$	$-CH_3$	381	$C_{22}H_{15}N_5O_2$	216^0C-218^0C	72	Absolute Ethanol
VIII b	$-C_6H_5$	$-C_2H_5$	395	$C_{23}H_{17}N_5O_2$	204^0C-206^0C	75	Absolute Ethanol
VIII c	$-C_6H_5$		409	$C_{24}H_{19}N_5O_2$	213^0C-215^0C	74	Absolute Ethanol
VIII d	$-C_6H_5$	$-C_6H_{13}$	451	$C_{27}H_{25}N_5O_2$	208^0C-210^0C	73	Absolute Ethanol

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



COMPOUND	IR (KBr) cm^{-1}	^{1}H NMR (400 MHz, $CDCl_3$)	MASS SPECTRUM m/z
Synthesis of Methyl-2-acetamidobenzoate (II)	3273.63 (N-H, stretch 1692.23 (C = O, stretch), 1591.75 (C=O, stretch),	--------	The molecular ion was observed at 194 $(M + H)^+$ 216 $(M + Na)^+$
Synthesis of 3-Amino-2-methylquinazolin-4(3H)-one (III)	3537.96,3302.76 (d,N-H, stretch), 3198.21 (C-H, stretch), 1718.19 (C = O, stretch)	δ (ppm): 8.223 (d, 1H, Ar-H), 7.734 (t,1H,Ar-H), 7.652 (d, 1H,Ar-H), 7.448 (t,1H,Ar-H), 4.907 (s, 2H,NH_2), 2.713 (s, 3H, CH_3)	The molecular ion was observed at 176.3 $(M + H)^+$
Synthesis of 3-Amino-2-phenylquinazolin-4(3H)-one (VII)	3568.18,3309.03 (d,N-H, stretch), 1718.24 (C = O, stretch), 1662.94 (C=N, stretch)	δ (ppm): 8.306 (d, 1H, Ar-H), 7.800 (m,4H,Ar-H), 7.520 (m, 4H,Ar-H), 5.015 (s, 2H,NH_2),	The molecular ion was observed at 238 $(M + H)^+$ 260 $(M + Na)^+$ and 497 $(2M + Na)^+$
(IV b) 3-(1,2-Dihydro-1-ethyl-2-oxopyrrolo(2,3-b)pyridin-3-ylideneamino)-2-methylquinazolin-4(3H)-one	1718.25 (C = O, stretch), 1700.21 (C=O, stretch), 1654.23 (C=N, stretch)	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, CH_3) ,3.914 (q, 2H, CH_2), 1.331 (t, 3H,CH_3)	The molecular ion was observed at 333 (M^+) 372 $(M + K)^+$ and 689 $(2M + Na)^+$
(VIII b) 3-(1,2-Dihydro-1-ethyl-2-oxopyrrolo(2,3-b)pyridin-3-ylideneamino)-2-phenylquinazolin-4(3H)-one	1718.17 (C = O, stretch), 1699.75 (C=O, stretch), 1654.24 (C=N, stretch)	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, CH_2), 1.393 (t, 3H,CH_3)	The molecular ion was observed at 396 $(M + H)^+$ and 418 $(M + Na)^+$
(VIII c) 3-(1,2-Dihydro-1-isopropyl-2-oxopyrrolo(2,3-b)pyridin-3-ylideneamino)-2-phenylquinazolin-4(3H)-one	1718.20 (C = O, stretch), 1677.36 (C=O, stretch), 1654.24 (C=N, stretch)	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,CH_3)	The molecular ion was observed at 410 $(M + H)^+$ and 432 $(M + Na)^+$

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RESULTS

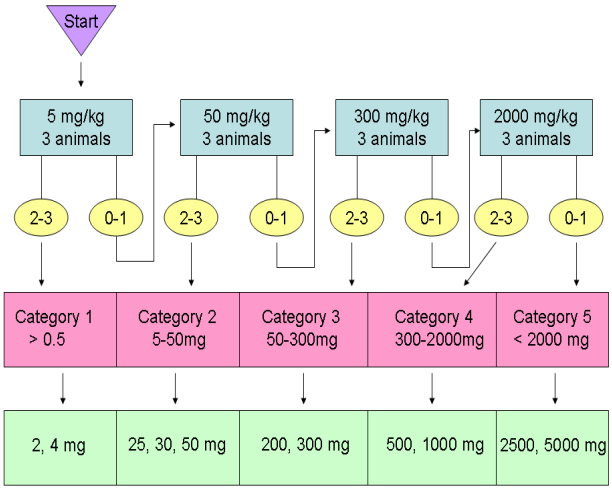
SEDATIVE AND HYPNOTIC ACTIVITY

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 24hours. 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.



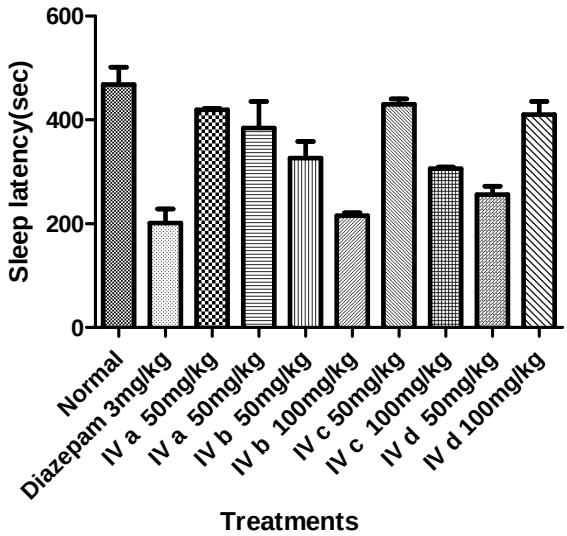
ACUTE TOXICITY STUDIES:

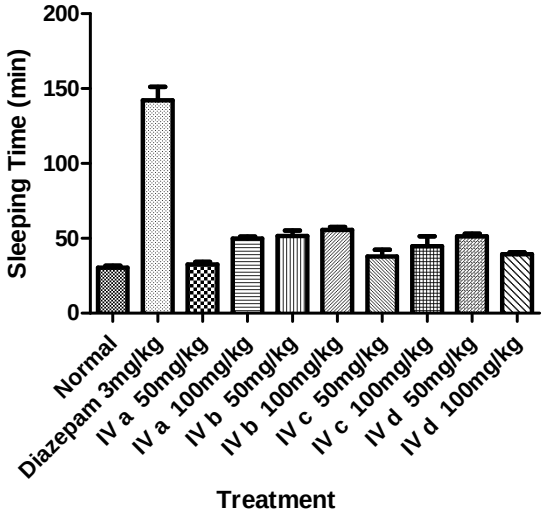
This study has been done with the doses 5, 50, 300 and 2000mg/kg (b.w). Mortality of the mice was observed with the dose of 2000 mg/kg (b.w). Again two more test doses that is 500 and 1000 mg/kg (b.w) were administered. Mortality observed with 1000 mg/kg (b.w) and the test animals were safe at 500 mg/kg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

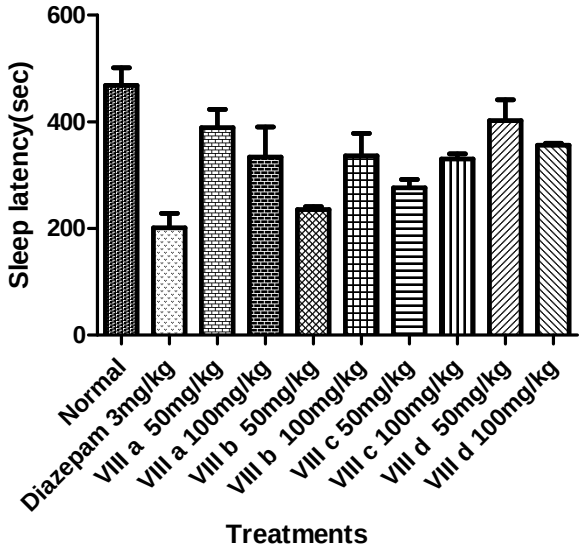
SEDATIVE AND HYPNOTIC ACTIVITY

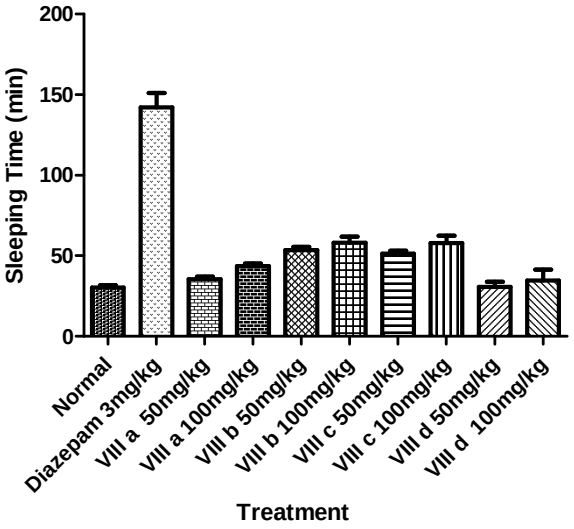
Pentobarbitone induced sleeping time [18]

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.







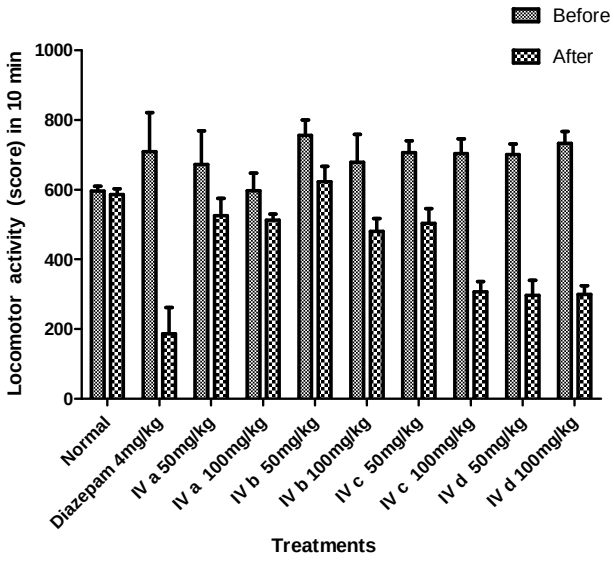


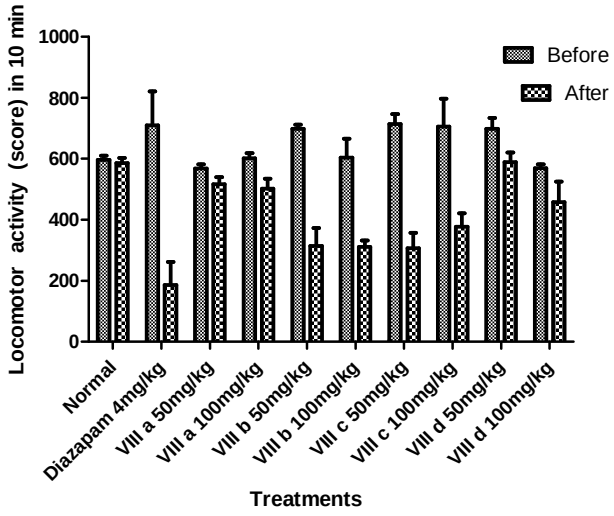
Locomotor activity

Procedure:

The CNS locomotor activity of given test compounds was evaluated in mice using actophotometer. Briefly, Albino mice of either sex (20 - 25 g) were randomly divided into six groups of six animals in each group. The mice were placed individually inside the chamber of actophotometer for 10 min and basal activity score was noted. Group I was treated with vehicle (10ml/kg i.p, normal saline) and standard drug Diazepam (4 mg/kg, i.p.) administered to group II. The animals of the group III-VI were treated with test compounds (50 and 100 mg/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,

Percentage decrease in activity = (Initial-final)/Initial X 100

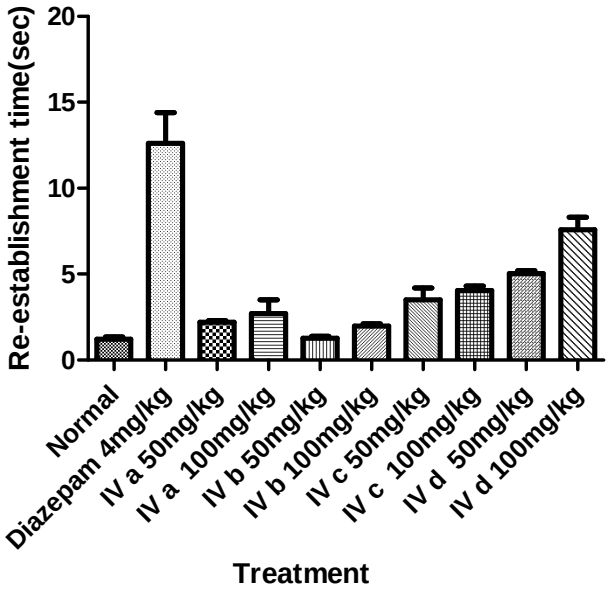


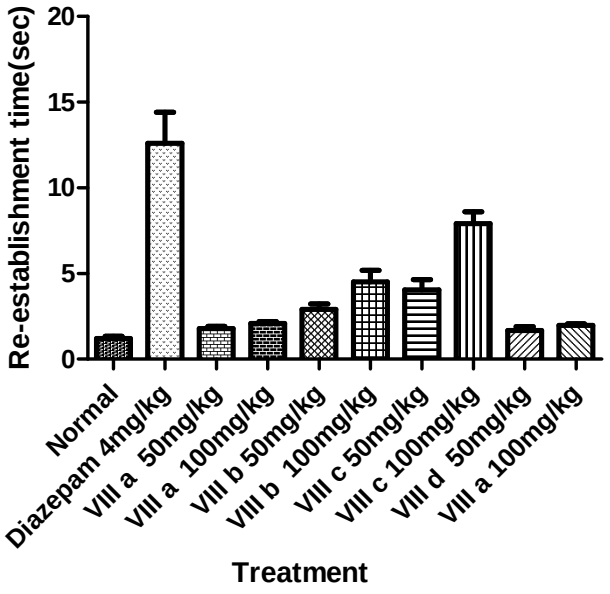


Traction test

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. This

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.

6. 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

Acknowledgement

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Conflict of Interest Statement

We declare that we have no conflict of interest.

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