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Synthesis, characterisation and evaluation of some 1,5-benzodiazepine quinolin-2-one derivatives as possible hypnotic agents

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A series of novel 3-(2-(phenyl/substituted phenyl)-1*H*-benzo[*b*] [1,5]-diazepin-4-yl)-4-methoxy-1-phenyl/methylquinolin-2(1*H*)-one **[IV-a(1-6)/IV-b(1-6)]** derivatives are synthesised by condensation of 3-substituted cinnamoyl-4-methoxy-1-phenyl/methylquinolin-2(1*H*)-one **[III-a(1-6)/III-b(1-6)]** with *o*-phenylenediamine. The results of the docking studies reveal that the synthesised compounds exhibited well conserved hydrogen bonding with one or more amino acid residues in the active pocket of alpha1beta3gamma2L GABA(A) Receptor (PDB ID: 6HUO) using Molegro Virtual Docker Software (MVD-2013, 6.0). The title compounds exhibit Mol Dock Score in the range of -124.502 to -149.448 with score more or comparable to the standard ligand score of -127.4127 and better than the standard drug -92.3878. All the synthesized compounds were satisfactorily characterized by spectral analysis and were tested for *in vivo* hypnotic activity based on the potentiation of barbiturate (phenobarbitone) sleeping time in miceusing diazepam as reference standard. All the compounds, except 4-methoxy-1-methyl-3-(2-(3-nitrophenyl)-1*H*-benzo[*b*][1,5]-diazepin-4-yl) quinolin-2(1*H*)-one **(IV-b4)**, significantly decrease the sleep latency, prolonged the duration of sleep and also showed good muscle relaxation property. Among the synthesised compounds, 4-methoxy-3-(2-(3-methoxyphenyl)-1*H*-benzo[*b*][1,5]-diazepin-4-yl)-1-phenylquinolin-2(1*H*)-one (**IV-a3**) is found to be the most potenthypnotic agentwith sleep latency of 26.67 ± 2.629 min and sleeping time of 111.00 ± 6.028 minutesand matches with pharmacophore mapping of the designed molecule with the MolDock score.

Keywords: 1,5-Benzodiazepine, Docking, Hypnotic, Insomnia, Quinolin-2-one

Insomnia is currently the most common sleep disorder affecting millions of people and has become a prevalent and disruptive problem in modern society[1-4]. Insomnia or sleeplessness is a sleep disorder with an experience of inadequate or poor-quality sleep characterized by long sleep latency or difficulty in falling asleep[5-7]. The prevalence of chronic insomnia increases with age and is more common in women. An estimated 6-10% of the adult population suffers from chronic insomnia.While the prevalence of sleep disorders is 20–40% for the population in general, this rate rises to 50% in people with the age of 65 years and older[8, 9]. Studies conducted on general populations report that about one third of adults suffer from insomnia symptoms, of which about 10-15% reported accompanying daytime impairments and 6-10% experience symptoms of insomnia disorder, which is the most common of all sleep disorders[10]. The drugs currently used are effective, but there is an ample need for the development of newer hypnotics with lesser side effects[11].

Among various heterocyclic compounds, quinolones predominately occur in the nature and they exhibit various activities on Central Nervous System (CNS) viz. antidepressant[12], antianxiety activity[13], etc. Research on quinolin-2-one led to a discovery of 4-hydroxyquinolin-2(1*H*)-ones, found to be the fundamental ring system of large number of alkaloids of *Rutaceae* family. Quinolin-2-one alkaloids such as integriquinolone, haplamineare known to exhibit cytotoxic activity, flindersine as an antibacterial agent, waltherione A,C and D as anti-HIV agent[14,15]. The synthetic analogues like carteolol as a β-blocker in the ophthalmic preparation, rebamipideas an antacid and aripiprazole, a new atypical antipsychotic drug approved by USFDA in November 2002 for the

treatment of schizophrenia and acute maniac episodes of bipolar disorders [16].

Benzodiazepines enhance the effect of neurotransmitter Gamma aminobutyric acid (GABA-A) resulting in sedative, hypnotic (sleep inducing), anxiolytic (antianxiety), anticonvulsant and muscle relaxant properties and these properties make them useful in treating anxiety, insomnia, agitation, seizures, muscle spasms, alcohol withdrawal and as a premedication for medical or dental procedures [17].

The biological relevance of the quinolone and benzodiazepine family and the need for ideal sleep-promoting drugs promoted our interest in the synthesis of derivatives with substituted 1,5-benzodiazepine moiety at 3rd position of quinolin-2(1*H*)-one framework. The study aims at evaluation of *in-vivo* hypnotic activity of synthesised 1,5-benzodiazepinylquinoline-2-one derivatives by potentiation of barbiturate induced sleeping time method in swiss albino mice.

Experimental Details

General Information

All the chemicals and solvents used were of synthetic grade and were purchased from SD Fine-Chem Limited, Mumbai and Molychem, Mumbai. Completion of the reaction was monitored by analytical thin layer chromatography (TLC) using E-Merck make 0.25 mm silica gel plates and the purity of the compounds was checked by a single spot in TLC and solvent system for TLC was determined on trial-and-error basis. Visualization of Thin layer chromatography plate was accomplished with ultraviolet (UV) light (256 nm) and iodine chamber. Synthesized compounds were purified by the recrystallization process and their melting points determined by Thiels melting point apparatus and were uncorrected. Fourier Transform Infrared (FTIR) spectra were recorded on Shimadzu IRAffinity-1 Spectrophotometer by using KBr pellets. The ^{1}H NMR and ^{13}C NMR was recorded on Bruker Avance II 400 NMR Spectrometer by using deuterated chloroform ($CDCl_3$) or deuterated Dimethyl sulfoxide (DMSO- d_6) as solvents and Tetramethylsilane (TMS) as an internal standard. Chemical shifts are expressed as δ values (ppm). The Mass spectra were recorded on Waters, Q-TOF Micromass (LC-MS). The pharmacophore mapping and docking studies of was carried out using Molegro Virtual Docker (MVD-2013, 6.0).

Synthesis of 3-acetyl-4-methoxy-1-phenyl/methyl quinolin-2(1*H*)-one {II-a/II-b}

To a solution of 3-acetyl-4-hydroxy-1-phenyl/methyl quinolin-2(1*H*)-one **{Ia/Ib}**(10 mmol) and dimethyl sulphate (1.051 mL, 11.1 mmol) in acetone (100 mL),potassium carbonate (1.531 g, 11.1 mmol) was added and the mixture was refluxed for 48-56 h and the completion of the reaction was monitored by Thin Layer Chromatography (TLC) using ethyl acetate and chloroform as a mobile phase in the ratio 3:7. The reaction was filtered, the residue was washed with water and orange coloured solid collected. Thus obtained solid was further purified by suspending the compound in 20% sodium carbonate solution, filtered, dried and recrystallized using suitable solvent.

Spectral data of 3-acetyl-4-methoxy-1-phenylquinolin-2(1*H*)-one (II-a)

IR (KBr, cm^{-1}): 3074.53, 3016.67 (aromatic -C-H); 2997.38, 2949.16 (aliphatic -C-H str.); 1705.07 (-C=O acetyl); 1639.49 (-C=O amide); 1080.14 (-C-O-C); ^{1}H NMR (DMSO-d_6, δ ppm): 8.0-6.0 (m, 9H, Ar-H); 4.0 (s, 3H, OCH_3); 2.6 (s, 3H, $COCH_3$); ^{13}C NMR ($CDCl_3$, δ ppm): 206.59 (1C, C=O acetyl); 173.71(1C, C-4 of quinolin-2-one); 161.64 (1C, C=O amide); 141.40-113.94 (12C, aromatic carbon); 105.77 (1C, C-3 of quinolin-2-one); 61.48 (1C, -OCH_3); 29.04 (1C, -CH_3).

Synthesis of 3-substituted cinnamoyl-4-methoxy-1-phenyl quinolin-2(1*H*)-one {III-a (1-6)}

A mixture of 3-acetyl-4-methoxy-1-phenyl quinolin-2(1*H*)-one **{II-a}** (0.01mol) and substituted aromatic aldehyde (0.012 mol) were dissolved in n-butanol (30 mL) with heating. Then 0.3 mL of piperidine were added to the above reaction mixture, refluxed for 4-6 h and the completion of the reaction was monitored by TLC using mobile phase containing ethyl acetate and chloroform in the ratio 1:1 and then the solvent was removed by using IKA rota evaporator. The residue was triturated with 20-30 mL of ethanol until a precipitate was formed, filtrated and recrystallized using suitable solvent.

Spectral data of 3-cinnamoyl-4-methoxy-1-phenylquinolin-2(1*H*)-one (III-a1)

IR (KBr, cm^{-1}): 3101.54, 3064.89 (aromatic -C-H); 2929.87, 2845.00 (aliphatic -C-H str.); 1697.36 (-C=O); 1622.13 (-C=O amide); 1122.57 (-C-O-C);

^{1}H NMR (DMSO-d_6, δ ppm): 8.1-7.1 (m, 14H, Ar-H); 7.7 (d, 1H, CHβ); 7.6 (d, 1H, CHα); 3.4 (s, 3H, O-CH_3).

Synthesis of 3-substituted cinnamoyl-4-methoxy-1-methyl quinolin-2(1*H*)-one {III-b(1-6)}

A mixture of 3-acetyl-4-methoxy-1-methyl quinolin-2(1*H*)-one **{II-b}** (0.05 mol) and the substituted aromatic aldehyde (0.05 mol) was dissolved in a solution of 10% ethanolic KOH (2.5 mL). Then the mixture was stirred for 2 h and the completion of the reaction was monitored by TLC using mobile phase containing ethyl acetate and chloroform in the ratio 1:1. The solid compound obtained was separated by filtration and washed with water and further recrystallized using suitable solvent.

Spectral data of 3-[3-(4-chlorophenyl) acryloyl]-4-methoxy-1-methylquinolin-2(1*H*)-one (III-b2)

IR (KBr, cm^{-1}): 3080.32, 3064.89, 3018.16 (aromatic -C-H); 2951.09, 2883.58 (aliphatic -C-H str.); 1664.57 (-C=O); 1615.56 (-C=O amide); 1070.59 (-C-O-C); 817.82 (-C-Cl); ^{1}H NMR (DMSO-d_6, δ ppm): 8.0-7.6 (m, 8H, Ar-H); 7.2 (d, 1H, CHα); 7.1 (d, 1H, CHβ); 3.9 (s, 3H, O-CH_3); 3.6 (s, 3H, N-CH_3).

Spectral data of 3-[3-(4-fluorophenyl) acryloyl]-4-methoxy-1-methylquinolin-2(1*H*)-one (III-b5)

IR (KBr, cm^{-1}): 3059.10, 3008.95 (aromatic -C-H); 2954.95, 2860.43(aliphatic -C-H str.); 1678.07 (-C=O); 1639.49 (-C=O amide); 1159.22 (-C-O-C); 758.02 (-C-F); ^{1}H NMR (DMSO-d_6, δ ppm): 7.9-7.2 (m, 8H, Ar-H);7.1 (d, 1H, CHα); 7.0 (d, 1H, CHβ); 3.9 (s, 3H, O-CH_3); 3.6 (s, 3H, N-CH_3).

Synthesis of 4-methoxy-1-phenyl/methyl-3-(2-phenyl /substituted phenyl-1*H*-benzo[*b*][1,5]-diazepin-4-yl)quinolin-2(1*H*)-one {IV-a(1-6)/IV-b(1-6)}

A mixture of *o*-phenylenediamine (0.015 mol), 3/4-substituted cinnamoyl/unsubstituted cinnamoyl-4-methoxy-1-phenyl/methyl quinolin-2(1*H*)-one **{III-a(1-6)/III-b(1-6)}** (0.01 mol) were dissolved in methanol (15 ml) and few drops of glacial acetic acid were added. Reaction mixture was refluxed for 6 h. The progress of the reaction was monitored by TLC using mobile phase containing ethyl acetate and cyclohexane in the ratio 3:7. The excess solvent was removed by distillation and then poured into crushed ice. The crude solid product obtained was filtered, washed with water and recrystallized using suitable solvent (Scheme 1).

Spectral data of 4-methoxy-1-phenyl-3-(2-phenyl-1*H*-benzo[*b*][1,5]-diazepin-4-yl) quinolin-2(1*H*)-one (IV-a1)

IR (KBr, cm^{-1}): 3317.56 (-NH); 3074.53, 3016.67 (aromatic -C-H); 2997.38, 2949.16, 2856.58 (aliphatic -C-H str.); 1631.78 (-C=O amide), 1168.86 (-C-O-C); ^{1}H NMR ($CDCl_3$, δ ppm): 8.3-6.5 (m, 18H, Ar-H); 6.2 (s, 1H, -C-H of benzodiazepine); 5.7 (s, 1H, -NH); 3.9 (s, 3H, O-CH_3).

Spectral data of 4-methoxy-3-(2-(3-methoxyphenyl)-1H-benzo[*b*][1,5]-diazepin-4-yl)-1-phenylquinolin-2(1*H*)-one (IV-a3)

IR (KBr, cm^{-1}): 3288.63 (-NH); 3074.53, 3012.81 (aromatic –C-H); 2956.87, 2927.94, 2872.01 (aliphatic -C-H str.); 1612.49 (-C=O amide); 1105.21, 1089.78 (-C-O-C); ^{1}H NMR ($CDCl_3$, δ ppm): 8.1-6.6 (m, 17H, Ar-H); 6.5 (s, 1H, -C-H of benzodiazepine); 5.7(s, 1H, NH); 3.7 (s, 3H, O-CH_3 of quinolone); 3.5 (s, 3H, O-CH_3 of phenyl); Mass spectrum of 4-methoxy-3-(2-(3-methoxyphenyl)-1*H*-benzo[*b*][1,5]-diazepin-4-yl)-1-phenylquinolin-2(1*H*)-one; (m/z) = 501 [M^{+1}].

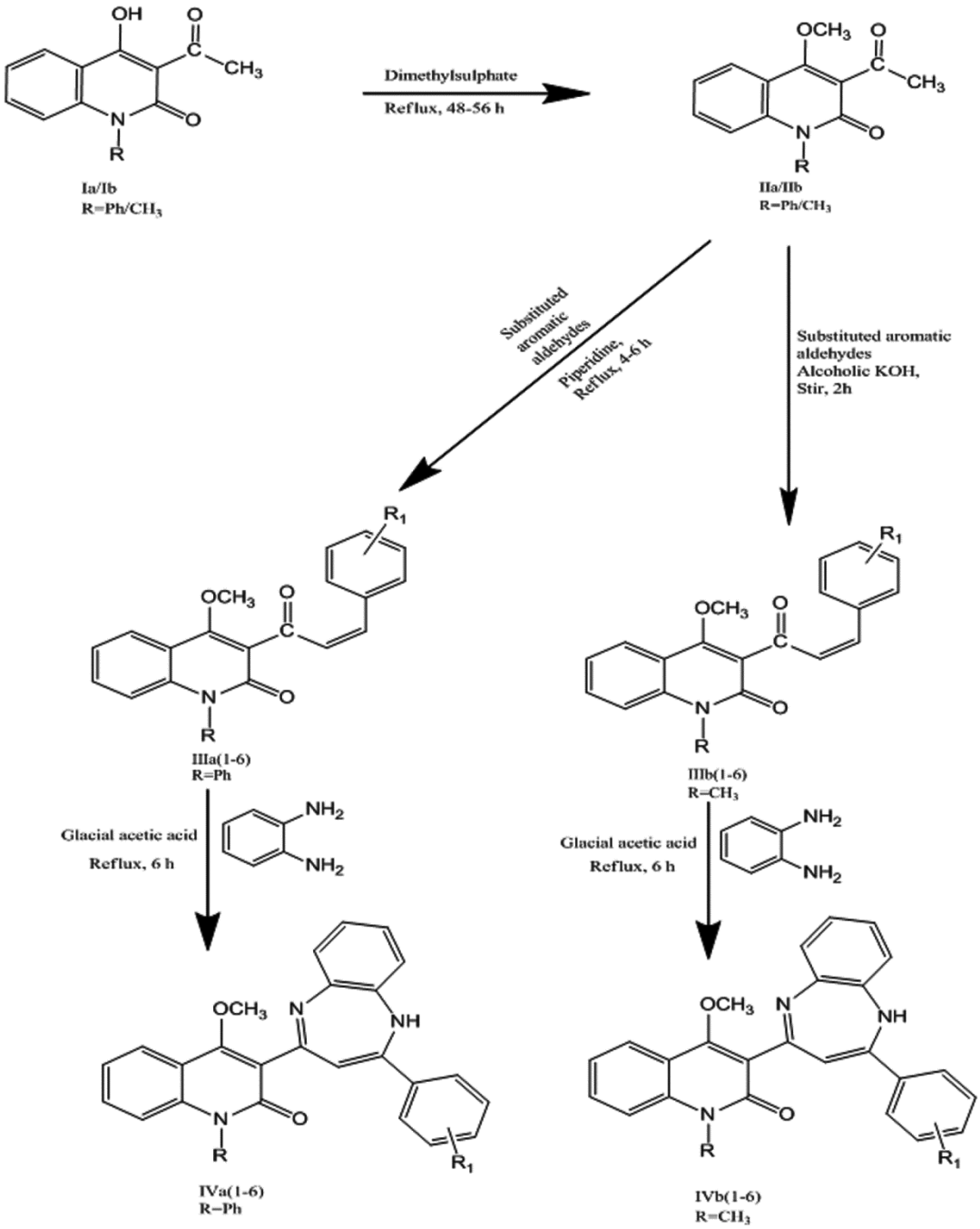
Spectral data of 4-methoxy-1-methyl-3-(2-(4-nitrophenyl)-1*H*-benzo[*b*][1,5]-diazepin-4-yl) quinolin-2(1*H*)-one (IV-b3)

IR (KBr, cm^{-1}): 3344.57 (-NH); 3062.93 (aromatic -C-H); 2929.87, 2848.86 (aliphatic -C-H str.); 1616.35 (-C=O amide); 1564.27 and 1342.46 (-NO_2); 1089.78 (-C-O-C); ^{1}H NMR ($CDCl_3$, δ ppm): 8.2-6.9 (m, 12H, Ar-H); 6.7 (s, 1H, -C-H of benzodiazepine); 5.7 (s, 1H, NH); 3.8 (s, 3H, O-CH_3); 3.4 (s, 3H, N-CH_3).

^{13}C NMR ($CDCl_3$, δ ppm): 169.26 (1C, C-4 of quinolin-2-one); 167.26 (1C, C=O amide); 163.08 (1C, C=N); 152.41-112.57 (18C of aromatic carbon); 102.11 (1C, C-3 of quinolin-2-one); 95.28 (1C, C-3 of benzodiazepine); 60.08 (1C, O-CH_3); 52.88 (1C,C-N); 29.10 (1C, N-CH_3); Mass spectrum of 4-methoxy-1-methyl-3-(2-(4-nitrophenyl)-1*H*-benzo[*b*][1,5]-diazepin-4-yl) quinolin-2(1*H*)-one; (m/z) = 454 [M^{+1}].

Spectral data of 3-(2-(4-bromophenyl)-1*H*-benzo[*b*][1,5]-diazepin-4-yl)-4-methoxy-1-methylquinolin-2(1*H*)-one (IV-b6)

IR (KBr, cm^{-1}): 3350.37 (-NH); 3064.89 (aromatic -C-H); 2954.95, 2916.37, 2848.86 (aliphatic -C-H



Scheme 1 — Synthesis of 4-methoxy-1-phenyl/methyl-3-(2-phenyl/substituted phenyl-1*H*-benzo[*b*][1,5]-diazepin-4-yl)quinolin-2(1*H*)-one**[IV-a(1-6)/IV-b(1-6)]**

str.); 1635.64 (-C=O amide); 1091.78(-C-O-C); 676.28 (-C-Br).; ^{1}H NMR ($CDCl_3$, δ ppm): 8.0-6.9 (m, 12H, Ar-H); 6.7 (s, 1H, -C-H of benzodiazepine); 5.6 (s, 1H, NH); 3.9 (s, 3H, O-CH_3); 3.6 (s, 3H, N-CH_3)**.**

The physical data of the title compounds prepared in above manner are tabulated in Table 1

Molecular docking studies

Molecular docking studies of title compounds were carried out using Molegro Virtual Docker (MVD-2013, version 6.0) software. The selected molecules were built using Chemdraw 12.0.2. The 2D structures were then converted into energy minimized 3D structures, which were saved as MDL

Table 1 — The physical data of 3-(2-(phenyl/substituted phenyl)-1*H*-benzo[*b*][1,5]-diazepin-4-yl)-4-methoxy-1-phenyl/methylquinolin-2(1*H*)-one {IV-a(1-6)/IV-b(1-6)}

Compound	R	R_1	Molecular formula	Molecular Weight	M. P.(ºC)	% Yield	R_f Value
IV-a1	C_6H_5	H	$C_{32}H_{23}N_3O_2$	481.37	140-2	67	0.81
IV-a2	C_6H_5	4-Cl	$C_{31}H_{22}N_3O_2Cl$	503.97	161-3	70	0.69
IV-a3	C_6H_5	3-OCH_3	$C_{32}H_{25}N_3O_3$	499.54	182-4	57	0.61
IV-a4	C_6H_5	4-OH	$C_{31}H_{23}N_3O_3$	484.51	172-4	45	0.89
IV-a5	C_6H_5	4-F	$C_{31}H_{22}N_3O_2F$	487.52	180-2	47	0.86
IV-a6	C_6H_5	4-Br	$C_{31}H_{22}N_3O_2Br$	548.42	155-7	75	0.79
IV-b1	CH_3	H	$C_{26}H_{21}N_3O_2$	407.45	150-2	49	0.88
IV-b2	CH_3	4-Cl	$C_{26}H_{20}N_3O_2Cl$	441.90	163-5	68	0.72
IV-b3	CH_3	4-NO_2	$C_{26}H_{20}N_4O_4$	452.43	127-9	58	0.73
IV-b4	CH_3	3-NO_2	$C_{26}H_{20}N_4O_4$	452.43	140-2	49	0.58
IV-b5	CH_3	4-F	$C_{26}H_{20}N_3O_2F$	425.44	175-7	63	0.89
IV-b6	CH_3	4-Br	$C_{26}H_{20}N_3O_2Br$	486.35	145-7	65	0.88

MolFile(.mol2).[18,19] The title compounds exhibited well conserved hydrogen bonding with one or more amino acid residues in the active pocket of alpha1beta3gamma2L GABA(A) receptor (PDB ID: 6HUO). MolDock scores and hydrogen bonding of the test compounds were compared with diazepam as standard. MolDock scores aregiven in Table 2. The MolDock score of compound 4-methoxy-1-methyl-3-(2-(4-nitrophenyl)-1H-benzo[*b*][1,5]-diazepin-4-yl) quinolin-2(1H)-one **(**IVa-3) binding with amino acid binding site of 6HUO.The -O from -OCH_3 at 4th position of the quinolin-2-one moiety forms H bond with –NH of His 102. The –N at 5th position from 1,5-benzodiazepin-4-yl forms –H bond with –OH of Ser 205 and MolDock score is -149.448 was higher than standard drug diazepam with docking score -92.3878.

Table 2 — MolDock scores of 4-methoxy-1-phenyl/methyl-3-(2-phenyl/substituted phenyl-1*H*-benzo[*b*][1,5]-diazepin-4-yl)quinolin-2(1*H*)-one[IV-a(1-6)/IV-b(1-6)].

Name	MolDockScore	Rerank Score	H Bond
IVa-1	-138.897	-109.493	-5.17051
IVa-2	-133.467	-105.899	-2.5
IVa-3	-149.448	-106.083	-5
IVa-4	-136.522	-105.728	-0.341898
IVa-5	-137.494	-106.339	-0.327493
IVa-6	-136.803	-105.977	-0.432223
IVb-1	-124.502	-107.38	-4.46517
IVb-2	-128.369	-100.718	-5.81894
IVb-3	-125.979	-101.658	-0.0145817
IVb-4	-134.092	-117.359	-8.17761
IVb-5	-136.448	-95.2612	-6.06568
IVb-6	-128.495	-101.018	-5.78862
08H_501 [D]	-127.412	-101.898	-14.5763
Diazepam	-92.3878	-76.4405	-4.40504

Evaluation of hypnotic activity

All the synthesized compounds were evaluated for their *in vivo* hypnotic activity in mice. The *in vivo* hypnotic test was based on the potentiation of barbiturate (phenobarbitone) sleeping time in swiss albino mice and the test was performed after obtaining the necessary permission for the conduct of experiment on mice from Institutional Animal Ethics Committee (IAEC) vide letter No.CPCSEA.REG.NO.1659/PO/a/CPCSEA dtd.12.11.2013.

Experimental animals

Male Swiss Albino mice weighing around 20-30 g were housed at standard laboratory conditions in a 12 h light/dark cycle. Food and water were available *ad libitum*. Tests were performed only after the mice had acclimated to the above environment. Numbers of animals in each group were six. Each animal was used for only one experimental condition. The study was approved and conducted as per the norms of the Institutional Animal Ethics Committee.

Determination of acute toxicity {Median lethal dose (LD_{50})}

The acute toxicity of synthesized compounds was determined by using swiss albino mice of either sex (20-30 g), maintained under standard husbandry conditions. The animals were fasted overnight prior to experiment and fixed dose by Organisation for Economic Co-operation and Development [(OECD) Guideline No. 425]method of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). The synthesized compounds

were subjected to the *in vivo* hypnotic activity as per literature[20].

Standard drug[20]

Diazepam (3 mg/kg) is used for the *in vivo* hypnotic activity as standard.

Preparation of synthesised derivatives

All the synthesised compounds to be tested and phenobarbitone were dissolved in 0.5% Tween 80 solution[20].

Experimental design; In vivo hypnotic activity: Barbiturate induced sleeping time test

Experimental mice randomly divided into fourteen groups and each group contains six mice (male). Group I (control was orally given 0.5 % Tween 80 solution. Group II (Reference) was given Diazepam (3 mg/kg) orally. The remaining groups received the test compounds **IV-a(1-6)/IV-b(1-6)** at a dose of 50 mg/kg orally. All groups were given phenobarbitone sodium (60 mg/kg) subcutaneously, 60 min after oral administration of vehicle (Blank), standard drug and synthesized compounds. The animals were observed for the latent period (time between phenobarbitone administration to loss of righting reflex) and duration of sleep (time between the loss and recovery of righting reflex). Another parameter, muscle relaxation time in mice was also observed [20].

Statistical analysis

All the data are expressed in mean ± Standard Error of Mean (SEM). The significance of differences in means between control and treated animals for different parameters was determined by one-way analysis ofvariance (ANOVA) followed by Dunnett's multiple comparison test. Significant analysis was performed using the prism 5.0 (GraphPad SoftwareInc.)[21]. The hypnotic activity of the synthesized compounds was studied by using phenobarbitone induced sleep test. With the exception of **IV-b4**, all the compounds significantly decreased the sleep latency, prolonged the duration of sleep and also showed good muscle relaxation property. Among all, **IV-a1, IV-a2, IV-a3** and I**V-b6** were found to be the most potent hypnotic agents. The results are tabulated in Table 3

Results and Discussion

The starting material, 3-aetyl-4-hydroxy-1-phenyl/methyl quinolin-2(*1H*)-one **{Ia/Ib},** was synthesised as per the literature[21]. Methylation of compound **Ia/Ib** with strong methylating agent, dimethyl sulphate, by replacing hydrogen of hydroxyl group to yield 3-acetyl-4-methoxy-1-phenyl/ methylquinolin-2(1*H*)-one **[II-a/II-b].** Compounds III-a(1-6) and III-b(1-6) were synthesised following Claisen-Schmidt reaction.Synthesis of 3-(2-(phenyl/ substitutedphenyl)-1*H*-benzo[*b*][1,5]-diazepin-4-yl)-4-methoxy-1-phenyl/methylquinolin-2(1*H*)-one **[IV-a(1-6)/IV-b(1-6)]** involves Michael addition reaction mechanism.

The pharmacophore mapping of title compounds showed better binding to the target protein alpha1beta3gamma2L GABA(A) receptor with

Table 3 — *In vivo* hypnotic activity of 3-(2-(phenyl/substituted phenyl)-1*H*-benzo[*b*][1,5]-diazepin-4-yl)-4-methoxy-1-phenyl/methylquinolin-2(1*H*)-one derivatives.

Sr. No.	Treatment	Sleep latency (min)	Duration of sleep (min)	Muscle relaxation time (min)
1	IV-a1	37.67±1.406***	92.67±3.816***	148.00±5.604***
2	IV-a2	36.50±1.522***	104.80±6.279***	176.70±2.692***
3	IV-a3	26.67±2.629***	111.00±6.028***	169.20±3.754***
4	IV-a4	50.33±1.282***	51.00±5.298***	93.00±4.539
5	IV-a5	54.00±3.376***	36.17±2.372	119.7±3.471***
6	IV-a6	60.17±1.138	32.00±2.145	76.17±3.497
7	IV-b1	56.67±3.040*	34.67±2.777	75.00±3.000
8	IV-b2	55.00±3.512**	86.33±5.333***	103.80±5.333**
9	IV-b3	47.00±1.238***	80.33±2.486***	119.8±6.369***
10	IV-b4	73±0.5774	30.83±3.092	63.17±4.571
11	IV-b5	52.00±1.789***	45.17±3.572*	102.5±4.904***
12	IV-b6	38.67±2.060***	110.70±2.140***	168.30±1.542***
13	Control(vehicle)	67.17±1.621	27.67 ± 1.687	69.17±6.204
14	Diazepam	37.17±3.070***	121.80±3.816***	143.50±9.677***

Values are mean ± SEM (n=6), *p< 0.05, **p< 0.01, ***p<0.001 as comparison to control {one-way analysis of variance (ANOVA) followed by multiple comparison Dunnett's test}.

MolDock score ranged from -124.502 to -149.448. Diazepam was used as reference standard for comparing the efficiency. Diazepam exhibited MolDock score of -92.3878. The MolDock score of all the twelve synthesised compounds was found to be higher than the standard ligand diazepam; with compound 4-methoxy-3-(2-(3-methoxyphenyl)-1H-benzo[*b*][1,5]-diazepin-4-yl)-1-phenylquinolin-2(1*H*)-one **(IVa-3)** having the highest MolDock score of -149.448 and also exhibited highest hypnotic activity with sleep latency of 26.67±2.629 and sleep duration of 111.00±6.028. The site of binding of ligand, standard drug and selected title compounds are shown in Fig.1-5.

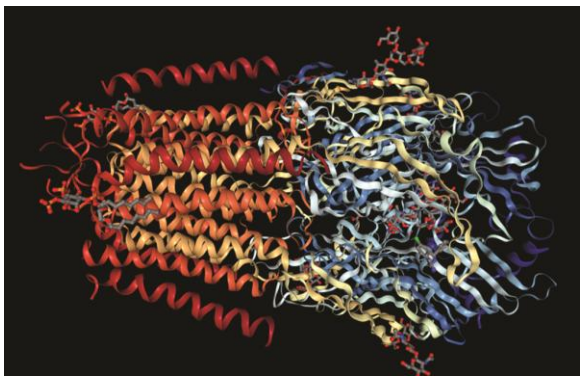


Fig. 1 — Structure of alpha1beta3gamma2L GABA(A) Receptor in complex with Alprazolam(Xanax), GABA and megabody Mb38 (PDB ID: 6HUO)

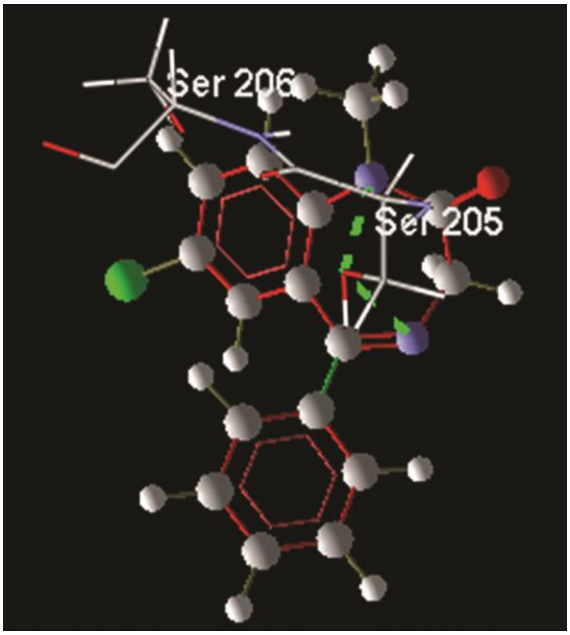


Fig. 2 — Diazepam (standard) docked in its best of its conformation into the binding site of 6HUO. The –N at 1st position forms H bond with –OH of Ser 205 and –N at 4thposition forms H bond with –OH of Ser 205

The pharmacological activity for the evaluation of *in vivo* hypnotic activity is based on the potentiation of sleeping time induced by the barbiturates. Mice are used for biological activity, since metabolic elimination of barbiturates is rapid in these species. The loss of righting reflex is measured as the criterion for the duration of barbiturate induced sleeping time.

The compounds at a dose of 50 mg/kg body weight showed potentiation of barbiturate induced sleeping time and significantly induced sleep at an earlier stage (i.e. decreased sleep latency) and also prolonged the duration of sleep in the test animals as compared to the control. With the exception of compound **IV-b4**, all the compounds significantly decreased the sleep

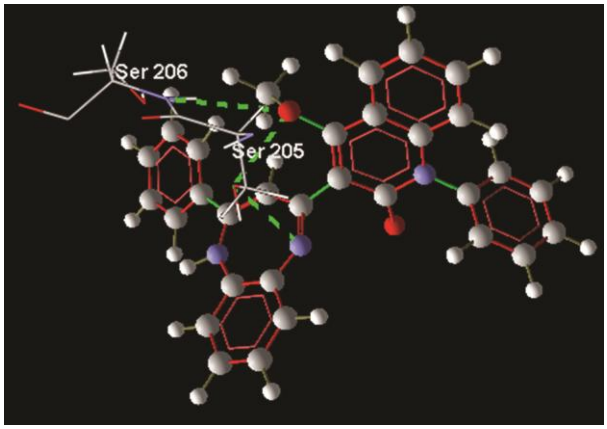


Fig. 3 — Compound 4-methoxy-1-phenyl-3-(2-phenyl-1*H*-benzo[*b*][1,5]-diazepin-4-yl)quinolin-2(1*H*)-one (IV-a1) docked in its best of its conformation into the binding site of 6HUO. The -O from -OCH_3 at 4th position of the quinolin-2-one moiety forms H bond with –NH of Ser 206 and –NH of Ser 205. The –N at 5thpositionfrom 1,5-benzodiazepin-4-yl forms H bond with –OH of Ser 205

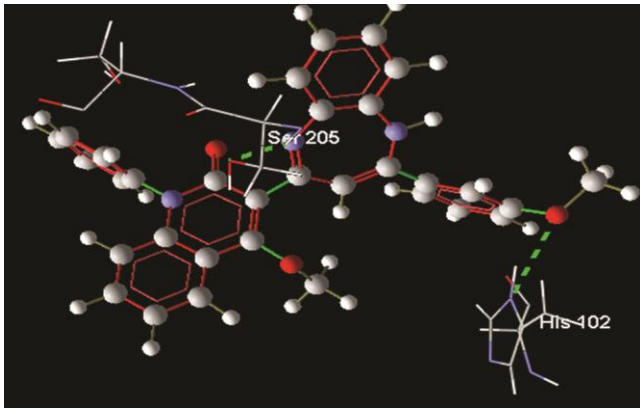


Fig. 4 — Compound4-methoxy-3-(2-(3-methoxyphenyl)-1*H*-benzo[*b*][1,5]-diazepin-4-yl)-1-phenylquinolin-2(1*H*)-one (IV-a3)docked in its best of its conformation into the binding site of 6HUO.The -O from -OCH_3 at 4th position of the quinolin-2-one moiety forms H bond with –NH of His 102. The –N at 5thposition from1,5-benzodiazepin-4-yl forms –H bond with –OH of Ser 205

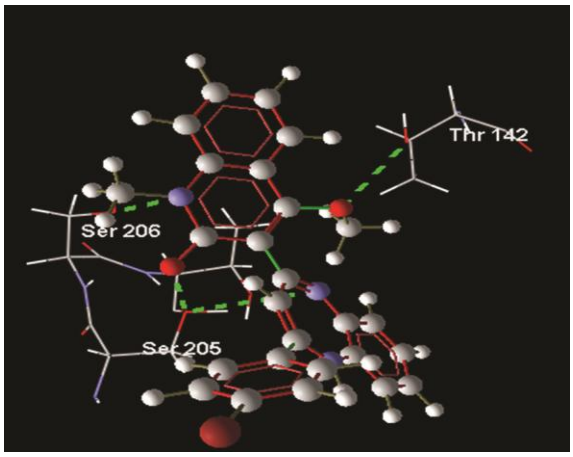


Fig. 5 — Compound3-(2-(4-bromophenyl)-1*H*-benzo[*b*][1,5]-diazepin-4-yl)-4-methoxy-1-methylquinolin-2(1*H*)-one (IV-b6)docked in its best of its conformation into the binding site of 6HUO.The –N at 5th position of the 1,5-benzodiazepin-4-yl forms –H bond with –OH of Ser 205.The -O from -OCH_3 at 4th position of the quinolin-2-one moiety forms H bond with –OH of Thr 142. The C=O at 2nd position of quinolin-2-one moiety forms H bond with –OH of Ser 205. The –N at 1st position of the quinolin-2-one moiety forms H bond with-OH of Ser 206

latency, prolonged the duration of sleep and also showed good muscle relaxation property. Among all,4-methoxy-3-(2-(3-methoxyphenyl)-1H-benzo[*b*][1,5]-diazepin-4-yl)-1-phenylquinolin-2(1H)-one (**IV-a3)** was found to be the most potent hypnotic agentsand matches docking studies with a dock score of -149.448 with expected binding to the receptor.

Compound4-methoxy-3-(2-(4-chlorophenyl)-1H-benzo[*b*][1,5]-diazepin-4-yl)-1-phenylquinolin-2(1H)-one **(IV-a2)** showed sleep latency of 36.50 ± 1.522 min and sleeping time of 104.80 ± 6.279 min; compound 4-methoxy-3-(2-(3-methoxyphenyl)-1H-benzo[*b*][1,5]-diazepin-4-yl)-1-phenylquinolin-2(1H)-one (**IV-a3**) showed sleep latency of 26.67 ± 2.629 min and sleeping time of 111.00 ± 6.028 min; and compound 3-(2-(4-bromophenyl)-1H-benzo[*b*][1,5]-diazepin-4-yl)-4-methoxy-1-methylquinolin-2(1H)-one **(IV-b6)** showed sleep latency of 38.67 ± 2.060 min and sleeping time of 110.70 ± 2.140 min. The control group showed sleep latency of 67.17 ± 1.621 min and sleeping time of 27.67 ± 1.678 min. Thus, on comparison with the control group it is observed that compound **IV-a2**, **IV-a3** and **IV-b6** are potent hypnotic agents.

Conclusion

Twelve derivatives of 2,4-disubstituted-1,5-benzodiazepine i.e. {3-(2-(substituted phenyl/phenyl)-1*H*-benzo[*b*][1,5]-diazepin-4-yl)-4-methoxy-1-phenyl/methylquinolin-2(1*H*)-one} **[IV-a(1-6)/IV-b(1-6)]**} were synthesized and confirmed by IR, NMR and Mass spectral data. The proposed method for synthesis of novel 1,5-benzodiazepine quinolin-2-one derivatives was found to be convenient and gave a good yield. Synthesised compounds with R= C_6H_5, R_1=Cl **[IV-a2]**; R=C_6H_5, $R_{1=}$ OCH_3**[V-a3]**; and R= CH_3, R_1=Br **[IV-b6]** potentiated phenobarbitone induced sleeping time in mice significantly, showed suppression in sleep latency and increased the duration of sleep when compared with the control group, thus showed excellent hypnotic activity at a dose of 50 mg/kg given orally. The results of molecular docking studies revealed that the synthesised compounds exhibit higher docking score as compared to standard ligand diazepam. Compound IVa-3 showed the highest MolDock score of -149.488. Hence the compound **IVa-3** was considered as most potent hypnotic drug.

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