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p-TSA catalyzed synthesis of 4-aryl-2,7,7-trimethyl-5-oxo-N-phenyl-1,4,5,6,7,8-hexahydroquinoline-3-carboxamides derivatives as CNS active agents and molecular docking studies

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ABSTRACT

A facile and efficient synthetic route to 4-aryl-2,7,7-trimethyl-5-oxo-N-phenyl-1,4,5,6,7,8-hexahydroquinoline-3-carboxamides has been developed via four-component condensation reactions of aldehydes, dimedone, acetoacetanilide and ammonium acetate in the presence of p-toluenesulfonic acid (p-TSA) catalyst in ethanol at ambient temperature through grinding. Simple work-up procedure, environmentally friendly, inexpensive and non-toxic catalyst, rapid reaction along with excellent product yields is the significant features of this practical method. Some hexahydroquinolines derivatives have been designed (7a-f) based on the in silico docking studies using the crystal structure of A_1 adenosine receptor (PDB ID: 3QAK) employing GLIDE v5 standard docking program (Schrodinger Inc.). All the designed compounds showed binding affinities as well as interactions with all the crucial amino acid residues on par with the reference standard (Fluoxetine). Compounds which were predicted to have good binding affinity (docking score) were considered for further MDA and histological studies

Keywords*: p*-toluenesulfonic acid, multi-component reactions, aryl-aldehyde, acetoacetanilide, ammonium acetate, dimedone, grindstone chemistry, Malondialdehyde (MDA), molecular docking.

INTRODUCTION

Multi component reactions allow the creation of several bonds in a single reaction are attracting increasing attention as one of the most powerful emerging synthetic tools for the creation of molecular diversity and complexity. They also have considerable advantages in terms of user and environmental benign ness because of the step reduction and atom economy associated to their use [1]. Consequently, the design of novel MCRs has attracted great attention from research groups working in medicinal chemistry and drug discovery.

Quinoline and their derivatives performing as a nucleus in several natural products and drugs attributing to their diverse applications in the pharmaceutical industries uphold a remarkable place among the heterocyclic compounds[2]. Quinolines having 1,4-DHP (1,4-dihydro pyridine) nucleus have been reported as significant

compounds due to their therapeutic and pharmacological properties such as vasodilator, antitumor, geroprotective, bronchodilator, antimalarial, anti-inflammatory, antiasthematic, and antibacterial activities[3],[4]

In particular Hexahydro-quinolines are a class of hetrocycles that possess anxiolytic property[5], antidepressant [6] and antihistaminic activity[7], antispermatogenic agents[8] calcium antagonistic activity[9] and anticancer activity[10]. Hantzsch and Liebigs reported the synthesis of 1,4-dihyropyridine by traditional method which involved cyclocondensation of aldehyde with ethyl acetoacetate and ammonia reflux in acetic acid or in alcohol or for a prolonged period of time[2].

A brief review on the literature reveals that the synthesis of quinoline derivatives can be achieved by using Melamine trisulfonic acid (MTSA)[1], Triton X-100 in water at room temperature[11], ammonium acetate[12], $Bi(NO_3)_3.5H_2O$[13], and Water dispersed magnetic nanoparticles (DMNPs) of γ-Fe_2O_3[14].

In order to develop simple and environmentally friendly experimental procedures using readily available reagents and catalysts for the synthesis of biologically active molecules, we became interested in the possibility of developing a one-pot synthesis of 1,4,5,6,7,8-hexahydroquinoline-3-carboxamide derivatives catalyzed by p-toluene sulphonic acid (*p*-TSA) a inexpensive and readily available catalyst. We present our results using grindstone technique utilizing p-TSA in solvent free conditions. To the best of our knowledge, 1,4,5,6,7,8-hexahydroquinoline-3-carboxamide derivatives catalyzed by p-TSA in solvent free conditions have not been reported.

MATERIALS AND METHODS

2.1 Material and methods

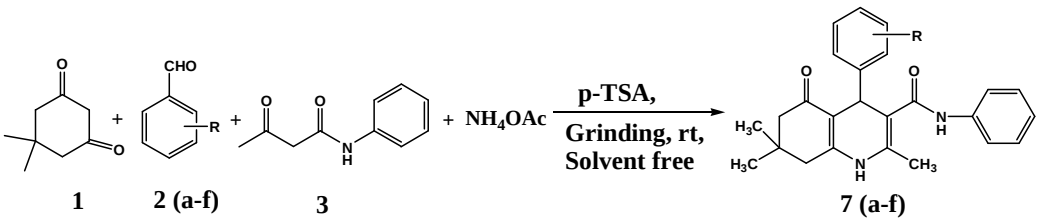
The chemicals and reagents used from various chemical companies like Alfa-Aesar, HiMedia, Merck India and CDH. The progress of the reaction was monitored by thin layer chromatography (TLC- ethyl acetate: n-hexane- 1:3) using pre-coated silica gel G plates using UV chamber for visualization of TLC spots. IR spectra were recorded on Shimadzu FT-IR spectrophotometer using KBr pellets. ^{1}H NMR spectra were obtained using on Bruker's AVANCE-III 400MHz FT NMR spectrometers by using $CDCl_3$ as solvent and TMS as internal standard. The chemical shift was expressed in δ ppm. Mass spectra were determined on Applied Biosystems 3200 Q Trap LC/MS/MS instrument.

2.2. Chemistry

2.3 General experimental procedure for the synthesis of hexahydroquinoline-3-carboxamide

A mixture of dimedone **4** (2 mmol), aryl-aldehyde **2** (2 mmol), acetoacetanilide **5** (2mmol) and ammonium acetate (3 mmol) in the presence of a catalytic amount (10 mol%) of p-toluenesulfonic acid (p-TSA) and ethanol (1.0 mL) was thoroughly ground using a mortar and pestle of appropriate size till the completion of reaction as indicated by TLC.

The resulting yellow colored product was washed with water to remove any unreacted ammonium acetate and kept in a dessicator for drying. The crude product was extracted by ethyl acetate. The organic layer was dried over anhydrous sodium sulphate and evaporated to dryness to give crude product. The pure product was obtained from recrystallization from ethanol.



Scheme 1. Synthetic route to hexahydroquinoline-3-carboxamide

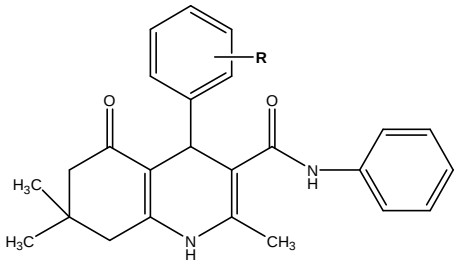


Table No. 1 The Synthesis of 1,4,5,6,7,8-hexahydroquinoline-3-carboxamide derivatives (7a-k)

Entry	R	Product
1	H	7a
2	m-Cl	7c
3	3,4-Cl_2	7e
4	o-OH	7f
5	p-OH	7g
6	o-NO_2	7k

2.4 The spectroscopic and analytical data for the synthesized compounds are presented below

2.4.1. 2,7,7-trimethyl-5-oxo-N,4-diphenyl-1,4,5,6,7,8-hexahydroquinoline-3-carboxamide (7a)

IR:(KBr, cm^{-1}): 3251, 1678, 1632, 698; ^{1}H-NMR (400 MHz , $CDCl_3$): δ: 0.844 (s, 3H, CH_3), 1.034 (s, 3H, CH_3), 2.183-2.266 (m, 4H, 2x CH_2), 4.905 (s, 1H, CH), 6.973-7.003 (m, 1H, ArH), 7.170-7.206 (m, 4H, ArH), 7.244 (s, 1H, ArH), 7.288-7.326 (t, 2H, ArH, J= 7.6 Hz), 7.439-j7.457 (d, 2H, ArH, J= 7.2 Hz); M.S (ESI): m/z = 386 (M^+); Anal. Calcd. for $C_{25}H_{26}N_2O_2$: C, 77.69; H, 6.78; N, 7.25%. Found:, C, 77.51; H, 6.74; N, 7.31%.

2.4.2. 4-(3-chlorophenyl)-2,7,7-trimethyl-5-oxo-N-phenyl-1,4,5,6,7,8-hexahydroquinoline-3-carboxamide (7b)

IR: (KBr, cm^{-1}): 3259, 1680, 1592, 753, 689; ^{1}H-NMR (400 MHz , $CDCl_3$): δ: 0.912, 0.951 (2s, 6H, 2x CH_3), 1.857 (s, 3H, CH_3), 2.227-2.443 (m, 4H, 2x CH_2), 5.054 (s, 1H, CH), 7.261-7.418 (m, 9H, ArH); M.S (ESI): m/z = 420.9 (M^+); Anal. Calcd. for $C_{25}H_{25}ClN_2O_2$: C, 71.33; H, 5.99; H, 8.42%. Found: C, 71.28; H, 6.06; N, 8.48%.

2.4.3. 4-(3,4-dichlorophenyl)-2,7,7-trimethyl-5-oxo-N-phenyl-1,4,5,6,7,8-hexahydroquinoline-3-carboxamide (7c)

IR: (KBr, cm^{-1}): 3261, 1682, 1600, 751, 690 ; ^{1}H-NMR (400 MHz , $CDCl_3$): δ: 0.904 (s, 3H, CH_3), 1.075 (s, 3H, CH_3), 2.186-2.300 (m, 4H, 2x CH_2), 2.384 (s, 3H, CH_3), 4.935 (s,1H, CH), 7.044-7.087 (m, 2H, ArH), 7.244 (s, 2H, ArH), 7.266-7.275 (d, 2H, ArH, J= 3.6 Hz), 7.294 (s, 1H, ArH), 7.316-7.320 (d, 1H, ArH, J= 1.2 Hz); M.S (ESI): m/z = 431.9 (M^+); Anal. Calcd. for $C_{25}H_{24}Cl_2N_2O_2$: C, 65.94; H, 5.31; N, 6.15%. Found: C, 65.99; H, 5.25; N, 6.21%.

2.4.4. 4-(2-hydroxyphenyl)-2,7,7-trimethyl-5-oxo-N-phenyl-1,4,5,6,7,8-hexahydroquinoline-3-carboxamide (7d)

IR:(KBr, cm^{-1}): 3340, 3250, 2956, 1680, 1634, 1190, 692 ; ^{1}H-NMR (400 MHz , $CDCl_3$): δ: 0.891 (s, 3H, CH_3), 0.969 (s, 3H, CH_3), 1.903-2.152 (m, 4H, 2x CH_2), 2.330 (s, 3H, CH_3), 4.978 (s, 1H, CH), 6.767-6.805 (m, 2H, ArH), 6.942-7.013 (m, 3H, ArH), 7.107 (s, 1H, ArH), 7.149 (s, 1H, ArH), 7.234-7.251 (d, 2H, ArH, J= 6.8 Hz), 8.782 (s, 1H, OH); Anal. Calcd. for $C_{25}H_{26}N_2O_3$: C, 74.60; H, 6.51; N, 6.96%. Found, C, 74.94; H, 6.55; N, 6.92%.

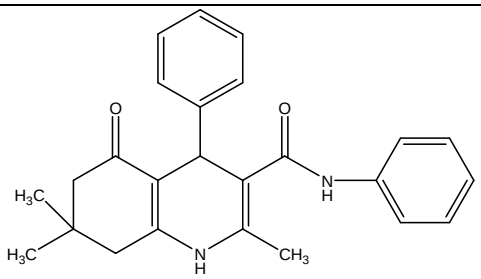
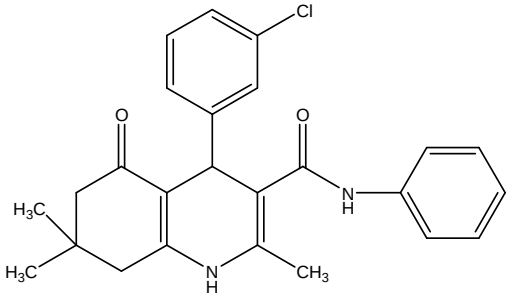
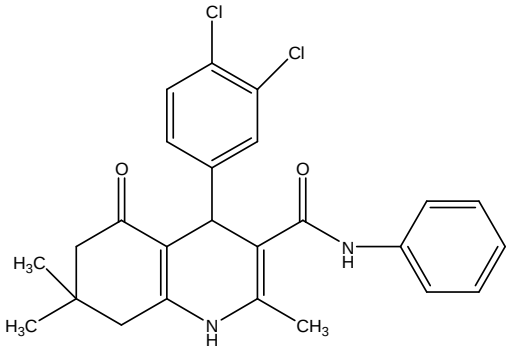
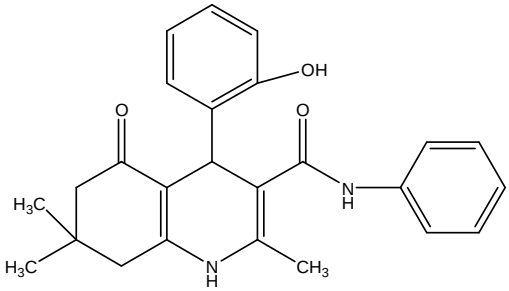
2.4.5. 4-(4-hydroxyphenyl)-2,7,7-trimethyl-5-oxo-N-phenyl-1,4,5,6,7,8-hexahydroquinoline-3-carboxamide (7e)

IR: (KBr, cm^{-1}): 3330, 3254, 2954, 1686, 1632, 1200, 686 ; ^{1}H-NMR (400 MHz , $CDCl_3$): δ: 0.918 (s, 3H, CH_3), 0.999 (s, 3H, CH_3), 1.974-2.201 (m. 4H, 2x CH2), 2.247 (s, 3H, CH_3), 5.016 (s, 1H, CH), 7.020-7.054 (t, 1H, ArH, J= 6.8 Hz), 7.214-7.255 (m, 2H, ArH), 7.355-7.397 (t, 2H, ArH, J= 8.4 Hz), 7.438-7.522 (m, 4H, ArH), 8.903 (s, 1H, OH); Anal. Calcd. for $C_{25}H_{26}N_2O_3$: C, 74.60; H, 6.51; N, 6.96%. Found: C, 74.67; H, 6.48; N, 6.35%.

2.4.6. 2,7,7-trimethyl-4-(2-nitrophenyl)-5-oxo-N-phenyl-1,4,5,6,7,8-hexahydroquinoline-3-carboxamide [7f]

IR: (KBr, cm^{-1}): 3184, 2960, 1684, 1630, 1350, 663 ; ^{1}H-NMR (400 MHz , $CDCl_3$): δ: 0.957 (s, 3H, CH_3), 1.026 (s, 3H, CH_3), 2.111-2.212 (m. 4H, 2x CH_2), 2.531 (s, 3H, CH_3), 5.216 (s, 1H, CH), 7.055-7.092 (t, 1H, ArH, J= 7.6 Hz), 7.222-7.293 (m, 3H, ArH), 7.371-7.403 (t, 2H, ArH, J=6.4 Hz), 7.450-7.581 (m, 2H, ArH), 7.652-7.692 (t, 1H, ArH, J= 8 Hz); Anal. Calcd. for $C_{25}H_{25}N_3O_4$: C, 69.59; H, 5.84; N, 9.74%. Found: C, 69.81, H, 5.91; N, 9.55%.

Table No. 2 The experimental details of 1,4,5,6,7,8-hexahydroquinoline-3-carboxamide derivatives (7a-k)

S. No	Product		Time (min)	Yield %	m. p. (^{0}C)
1	7a	 2,7,7-trimethyl-5-oxo-N,4-diphenyl-1,4,5,6,7,8-hexahydroquinoline-3-carboxamide	15	78	245-247
2	7c	 4-(3-chlorophenyl)-2,7,7-trimethyl-5-oxo-N-phenyl-1,4,5,6,7,8-hexahydroquinoline-3-carboxamide	15	74	238-240
3	7e	 4-(3,4-dichlorophenyl)-2,7,7-trimethyl-5-oxo-N-phenyl-1,4,5,6,7,8-hexahydroquinoline-3-carboxamide	10	78	274-276
4	7f	 4-(2-hydroxyphenyl)-2,7,7-trimethyl-5-oxo-N-phenyl-1,4,5,6,7,8-hexahydroquinoline-3-carboxamide	20	72	290-292

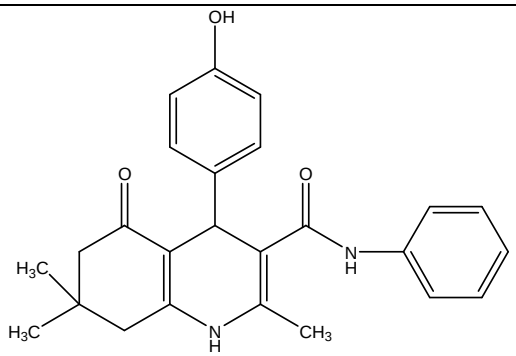
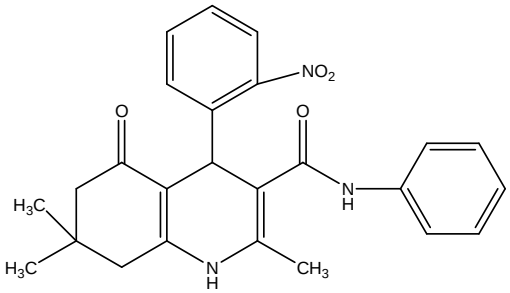
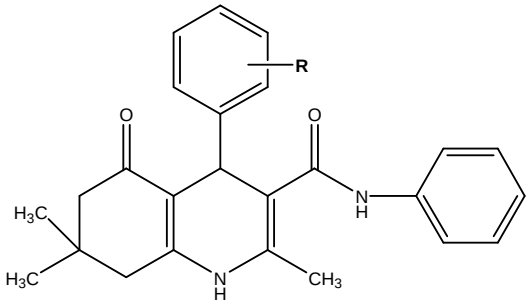
5	7g	 4-(4-hydroxyphenyl)-2,7,7-trimethyl-5-oxo-N-phenyl-1,4,5,6,7,8-hexahydroquinoline-3-carboxamide	25	76	>300
6	7k	 2,7,7-trimethyl-4-(2-nitrophenyl)-5-oxo-N-phenyl-1,4,5,6,7,8-hexahydroquinoline-3-carboxamide	10	84	255-257

Table No. 3 IR Absorption frequencies of synthesized derivatives (7a-k) in cm^{-1}

Entry	N-H str	C-H Str (CH_3)	C=O str	C=O-N	O-H str	C-O str	N=O str	C-Cl str	C-H def (Ar)
7a	3251	-	1632	1678	-	-	-	-	698
7c	3259	-	1592	1680	-	-	-	753	689
7e	3261	-	1600	1682	-	-	-	751	690
7f	3250	2956	1634	1680	3340	1190	-	-	692
7g	3254	2954	1632	1686	3330	1200	-	-	686
7k	3184	2960	1630	1684	-	-	1350	-	663

Table No. 4 Glide score of series 7 synthesized derivatives (7a-f)



Entry	R	Product	Glide Score (3QAK)
1	H	7a	-6.187364
2	m-Cl	7b	-6.402567
3	3,4-Cl_2	7c	-6.011966
4	o-OH	7d	-6.176105
5	p-OH	7e	-6.792379
6	o-NO_2	7f	-6.292634
7	Flouxetine	Standard	-6.661533

2.5. Molecular Docking

Computer Aided Drug Design (CADD) is a specialized discipline that utilizes computational methods to simulate drug-receptor interactions. CADD methods are mainly dependent on bioinformatics tools, applications and databases[15]

The computational studies were carried out using HP Desktop PC, (Core 2 Duo Processor; 1GB RAM) running on Windows XP using Maestro 9.0. The synthesized molecules were evaluated in silico (docking) using the homology models of A_1 adenosine receptor (3QAK). Various parameters including docking score, and glide score were calculated. Similar data emphasizing the degree of interaction between the test compounds and receptor were deduced additionally.

2.5.1 Protein preparation

PDB structure (www.rcsb.org) A_1 adenosine receptor (3QAK) was downloaded, refined, and prepared using Schrodinger protein preparation wizard tool (Glide), which performs the following steps: assigning of bond orders, addition of hydrogen, optimization of hydrogen bonds by flipping amino side chains, correction of charges, and minimization of the protein complex. All the bound water molecules, ligands and cofactors were removed (preprocess) from the proteins which were taken in .mae format. The tool neutralized the side chains that are not close to the binding cavity and do not participate in salt bridges. This step is then followed by restrained minimization of co-crystallized complex, which reorients side chain hydroxyl groups and alleviates potential steric clashes. The complex obtained was minimized using OPLS_2005 force field with Polack-Ribiere Conjugate Gradient (PRCG) algorithm. The minimization was terminated either completion of 5,000 steps (or) after the energy gradient converged below 0.05 kcal/mol.

2.5.2. Preparation of Ligands

Structures of the ligands (7a-f) were sketched using built panel of Maestro and taken in .mae format. LigPrep is a utility of Schrodinger software suit that combines tools for generating 3D structures from 1D (Smiles) and 2D (SDF) representation, searching for tautomers, steric isomers and perform a geometry minimization of the ligands. Molecular Mechanics Force Fields (OPLS_2005) with default settings were employed for the ligand minimization.

2.5.3. Docking Studies

Various conformations of the ligands generated by LigPrep were docked employing Ligand Docking tool under the Glide menu. The receptor grid and ligands were defined by browsing the respective files. Docking was performed using standard precision (SP) mode with generation of at most 10 poses per ligand. Besides, per residue interaction scores were also calculated during the run. Docking efficiency was evaluated on the basis of various parameters including Docking score, GScore, Glide emodel, potential energy, binding energy and complex energy[16].

2.6. Neurochemical analysis

2.6.1 Malondialdehyde (MDA) determination

Lipid peroxidation was assayed by measuring the level of malondialdehyde (MDA). The biochemical estimations of MDA from brain homogenate were performed to study their antioxidant property. Malondialdehyde forms a 1:2 adduct with thiobarbituric acid which could be measured by spectrophotometry. A high level of MDA is a marker of lipid peroxidation.

The Extent of malondialdehyde (MDA) formation was measured in tissue homogenates; equal volumes (2 ml) of the tissue homogenate and trichloroacetic acid (10% w/v) were mixed. The mixture was then cooled for 15 min and centrifuged. To the supernatant (0.5 ml), 3 ml of (0.67%) thiobarbituric acid was added, the reaction mixture was then kept in boiling water for 10 minutes, cooled and thereafter absorbance was measured against blank at 532 nm on Shimadzu 1700 UV spectrophotometer. The amount of MDA formed was expressed as nM of MDA/g of wet tissue.

2.7. Histological study Experimental

Swiss albino mice (20-25 g) of both sexes were randomly assigned into each group. The mice were maintained in the Animal holdings room of the Sapiens Bio-Analytical Research Lab, Bhopal. Animals were housed under standard normal laboratory conditions (12 h light-dark cycles) in groups of six in cages in the laboratory three days prior to the experiments. They were kept at an ambient temperature of 25 ± 2°C and allowed free access to food and

water except at the time they were brought out of the cage. Experimental protocols and procedures were approved by the Animal Experimentation Ethics Committee (CEPA), approval no 1413/PO/a/11/CPCSEA.

Suspensions of test samples were prepared in 0.5% CMC solution and finally prepared suspension was stored in separate glass bottle at 2-8^{0}C. Concentration of all prepared suspension was 10mg/ml. The mice in the treatment groups received 40mg/kg body weight of test compounds suspended in 0.5% CMC and the control group received equal volume of 0.5% CMC. The treated mice in groups were sacrificed by cervical dislocation on the 4th (7a, 7c and 7f), 8th (7e and 7g) and 14th (control) day of the experiment respectively, while that of the control group was sacrificed at the end of the experiment. Their brains were isolated and stored in 10% formalin solution and histological slides were prepared.

The tissue were dehydrated in an ascending grade of alcohol (ethanol), cleared in xylene and embedded in paraffin wax. Serial sections of 7 microns thick were obtained using a rotatory microtome. Some of the deparaffinised sections were stained routinely with haematoxyline and eosin (H&E) method. The photographes of the desired sections were taken for further observations.

RESULTS AND DISCUSSION

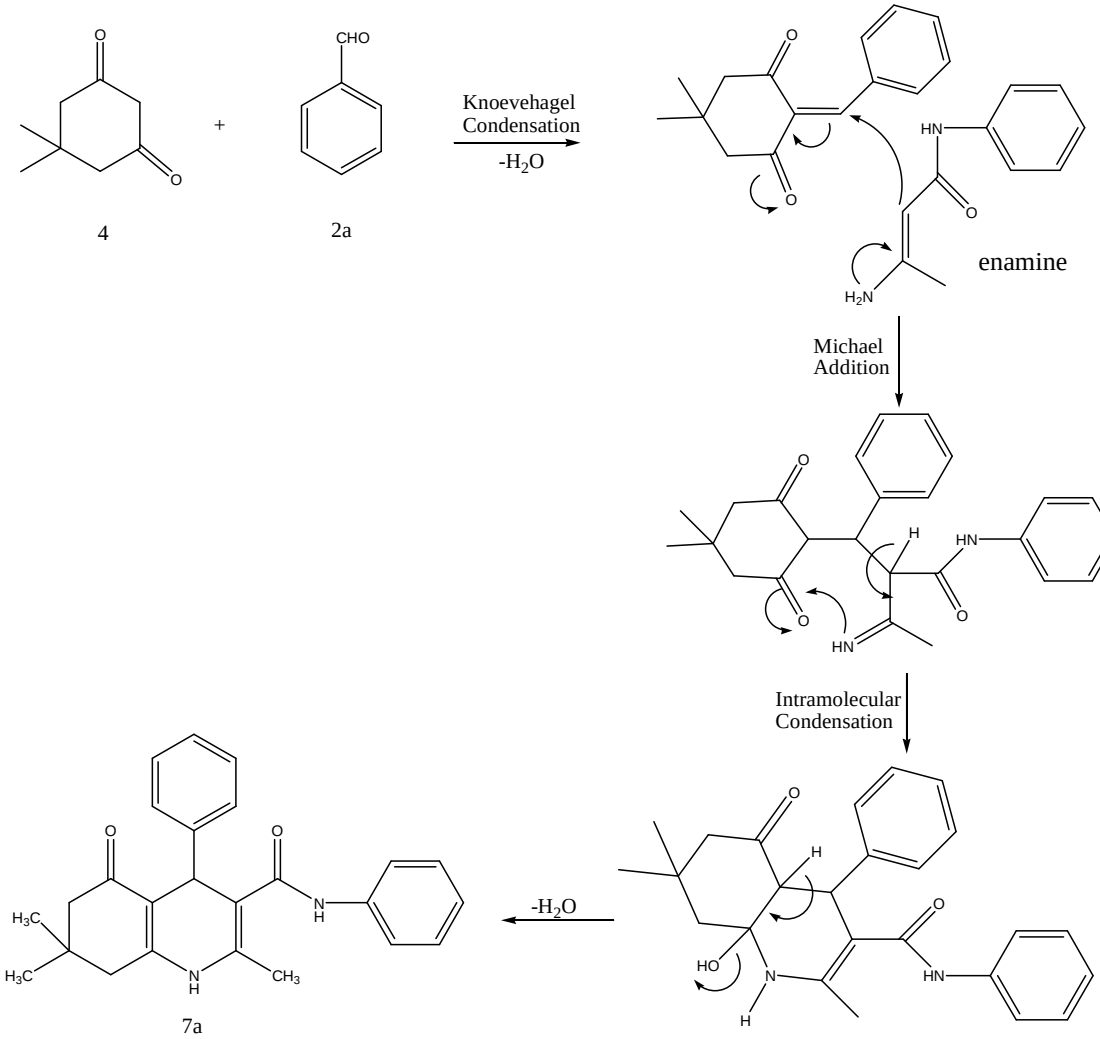
3.1 Chemistry

Recently the progress of solvent free synthesis has attracted much attention of chemists as they are environmentally benign processes. There are many reactions (Grignard, Aldol condensation, Reformatsky reaction, Dieckmann condensation, Knoevenagel condensation and polyhydroquinolines synthesis), which have been reported under solvent-free condition at room temperature using grinding technique.

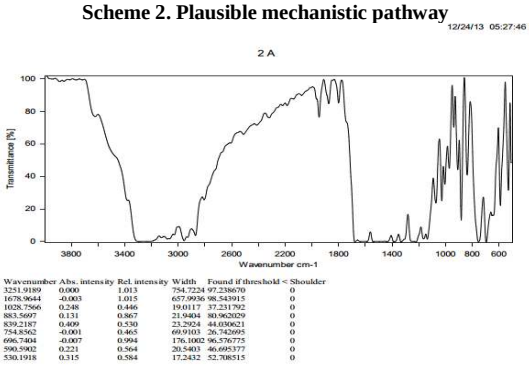
The scheme 1 shows a typical synthetic strategy employed to obtain the title compounds (7a-f). A mechanistic rationale exhibiting a probable sequence of events for the indeno-fused heterocycles is given in Scheme 2. The present reaction proceeds via initial Knoevenagel condensation of dimedone (4) with aryl-aldehyde (2a-f). Condensation of acetoacetanilide (5) and ammonium acetate in the presence of p-TSA forms ester enamine which further undergoes *in situ* Michael addition reaction with Knoevenagel product to yield products (7a-f). The structures of all the synthesized compounds were established on the basis of spectral analysis. Table No. 2 shows the reaction time, percentage yield and melting point range of series synthesized derivatives (7a-f).

All the products were characterized by IR, ^{1}H NMR, Mass and elemental analysis. All the synthesized derivatives (7a-k) showed a weak N-H str absorption peaks around 3250 cm^{-1}, shown in Table No 3. An IR spectrum of 7a is shown in Fig. 1. Derivatives 7d, 7e and 7f showed aliphatic C-H str absorption around 2955 cm^{-1}. The IR spectrum of all derivatives showed a sharp characteristic C=O str around 1632 cm^{-1} and C=O-N str around 1680 cm^{-1}. All derivatives (7a-f) showed aromatic C-H def around 690 cm^{-1} confirming the aromatic skeleton in the structure. Derivatives 7b and 7c showed C-Cl str around 750 cm^{-1}. Derivatives 7d and 7e showed a weak O-H str around 3340 cm^{-1} and C-O str around 1200 cm^{-1} due to aromatic phenolic group. IR spectrum of derivative 7f showed N=O str at 1350 cm^{-1} due to NO_2 group.

^{1}HNMR spectrum of 7a showed two singlets for three protons each at δ 0.884 and 1.034 for 7-CH_3 protons, a singlet for the three protons of the 2-CH_3 group at δ 2.353 ppm (Fig. 2). A multiplet between δ 2.183-2.266 ppm due to four CH_2 protons is present. A one proton singlet resonates at δ 4.905 ppm due to CH group. Further the spectra of 7a showed a multiplet at δ 6.973-7.457 for 10 aromatic protons. The mass spectrum of compound 7a shows the presence of a molecular ion peak $(M)^+$ with m/z 386 and fragment ion peaks for $(M\text{-}Ph)^+$ and $(M\text{-}PhNH)^+$ confirming the structure (Fig 3).



Scheme 2. Plausible mechanistic pathway



Wavenumber	Abs. intensity	Rel. intensity	Width	Found if threshold < Shoulder	
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1678.9644	-0.003	1.015	657.9936	98.543915	0
1028.7566	0.248	0.446	19.0117	37.231792	0
883.5697	0.131	0.867	21.9404	80.962029	0
839.2187	0.409	0.530	23.2924	44.030621	0
754.8562	-0.001	0.465	69.9103	26.742695	0
696.7404	-0.007	0.994	176.1002	96.576775	0
590.5902	0.221	0.564	20.5403	46.695377	0
530.1918	0.315	0.584	17.2432	52.708515	0

Experiment GOHAR.xpm	Path of File C:\OPUS_7.0.129
Operator Name Default	Date of Measurement 12/24/2013
Instrument Type Tensor 37	Sample Form SOLID
Resolution 4	Sample Scans 24

Fig. 1 IR Spectrum of 7a derivative

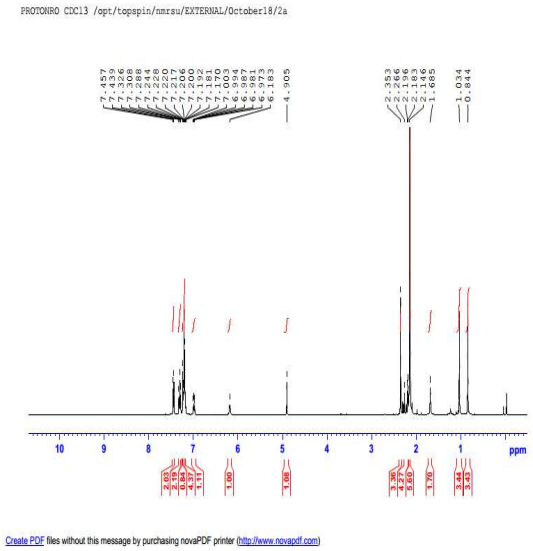

Fig. 2 ^{1}HNMR spectrum of derivative 7a

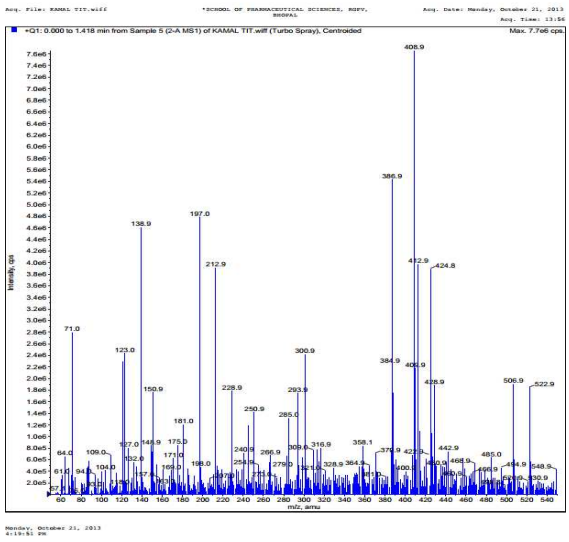

Fig. 3 Mass spectrum of derivative 7a

The melting point of 7e is highest (>300^{0}C) within the synthesized derivatives (7a-f), followed by 7d (290-292^{0}C), both derivatives containing hydroxyl (OH) functional groups at 2 and 4 position respectively. The 3,4-Cl_2 (7c) derivative has higher melting point than 3-Cl (7b) derivative. Further derivative 7b has the lowest melting point among the synthesized series (7a-f).

3.2 Docking analysis

In the present investigation, in silico docking studies were performed using the crystal structure of A_1 adenosine receptor (3QAK) to recognize the hypothetical binding mode of the ligands with the receptor in order to study a series of hexahydroquinoline-3-carboxamide (7a-f) as possible CNS (central nervous system) active agents. To investigate the ability of molecular docking to reproduce an experimentally observed ligand binding mode, the fluoxetine has been used as reference ligand and docked back into its binding site of the crystal structure of the using Glide standard docking program (Schrodinger Inc). A molecular docking technique was performed by using Glide, in order to investigate the intermolecular interaction between ligands and the receptor. Flexible docking simulations

were performed for understanding the binding mode of the ligands on A_1 adenosine receptor as well as to obtain information for further structural development. All the docking results are shown in Table No. 4

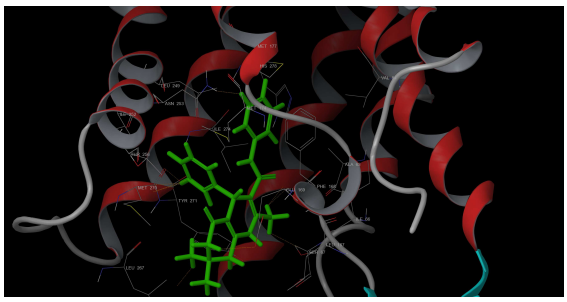

Fig. 4 Docking interaction of 7a with 3QAK

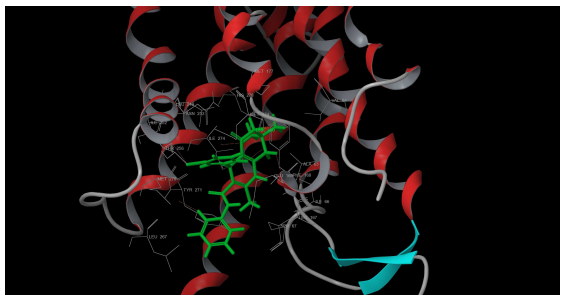

Fig. 5 Docking interaction of 7b with 3QAK

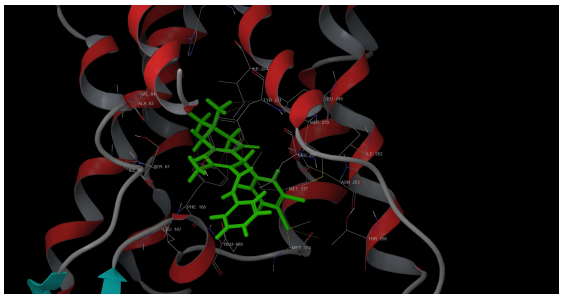

Fig 6. Docking interaction of 7c with 3QAK

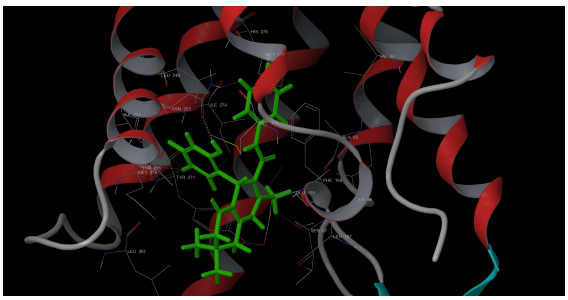

Fig. 7 Docking interaction of 7d with 3QAK

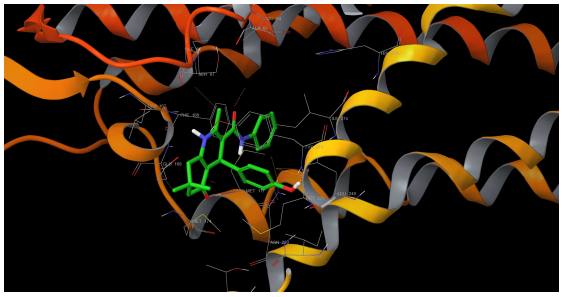

Fig. 8 Docking interaction of 7e with 3QAK

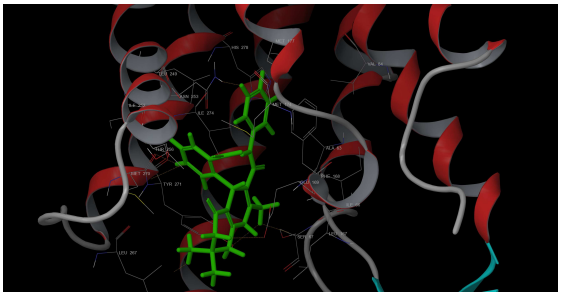

Fig. 9 Docking interaction of 7f with 3QAK

All of the hexahydroquinoline-3-carboxamide (7a-f) derivatives were docked into the active site of A2a adenosine receptor to study the possible mode of their interaction. Docking of these compounds into inhibitor binding cavity of A2a confirms that these compounds dock in a similar binding modus like native co-crystallized ligand, UKA. The designed compounds were found to accommodate the binding pocket of the receptor showing the important interactions with all the crucial amino acid residues. Inhibitor binding cavity of A2a is outlined by residues Ser 67, Phe 168 Glu 169, Asn 253, Met 270, Ala 273. Analysis of the receptor/ligand complex models generated after successful docking of the 5H-indeno[1,2-b]quinoline derivatives was based on parameters such as Hydrogen bond interactions, π-π stacking/hydrophobic interactions, binding energy, RMSD of active site residues and orientation of the docked compound within the active site. The glide score varies from -6.012 (7c) to -6.792 (7e) and are very close to standard anxiolytic drug fluoxetine -6.661.

3.3. Malondialdehyde (MDA) Determination

Table No. 5 Neurochemical estimation of the synthesize compounds in MDA tests

Groups	MDA (nmol/mg protein)
Control	28.75 ± 6.2
Phenytoin	10.00 ± 1.10**
7b	25.00 ± 0.08ns
7e	18.00 ± 0.75*

*Values represent means ±S.E.M. (n =3). *P < 0.05, **P< 0.01, ***P < 0.001 compared with vehicle (One-way ANOVA followed by Dunnett's post hoc test).*

The levels of MDA in all experimental groups (measured by UV spectrophotometer) in comparison with their corresponding control show that administered derivatives (7b, 7e) caused a decrease in MDA level compared with control. The results are shown in Table No. 5.

3.4. Histological Study

The cerebral cortex is the key structures of memory formation. It also integrates higher mental functions, general movement, visceral functions, and behavioral reactions. In present study, sections of the cerebral cortex were prepared from control and drug treated albino mice and the effects of drug treated on the cerebral cortex were histologically examined in order to describe any observed changes. A variety of histological changes were observed in the cerebral cortex of drug treated groups when compared with the control. Table No. 6 showed details of histological studies.

Table No 6. Histological study of synthesized derivatives (7a-g)

S. No	Group	Day of sacrifice	Observation
1	7a	4th	Normal histological feature without vacuoles
2	7c	4th	Normal histological feature without vacuoles
3	7e	8th	Lesions and vacuoles were present locally, necrosis absent
4	7f	4th	Normal histological feature without vacuoles
5	7g	8th	Lesions and vacuoles were present locally, necrosis absent
6	Control	14th	Normal histological features with the neurons appearing distinct and the glial cells normal without vacuolation in the stroma.

The sections of mid brain from the control group showed normal histological features with the neurons appearing distinct and the glial cells normal without vacuolation in the stroma.

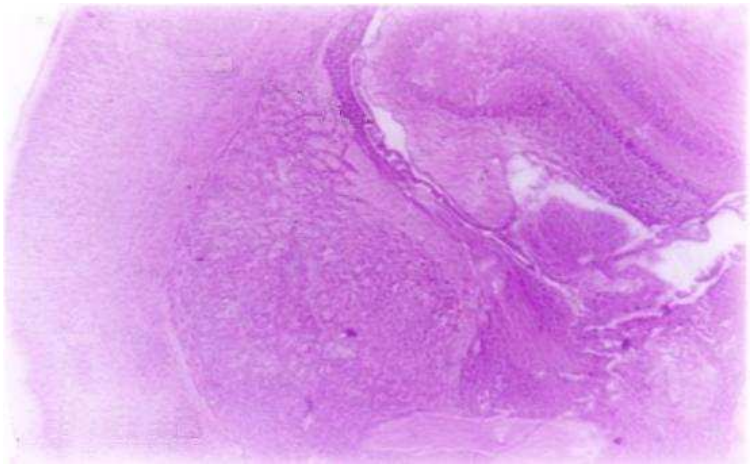

Fig.10 Normal mice brain- neocortex, ependymal layer (EP) and ventricle (Control). Cortex was free from any inflammatory or degenerative changes

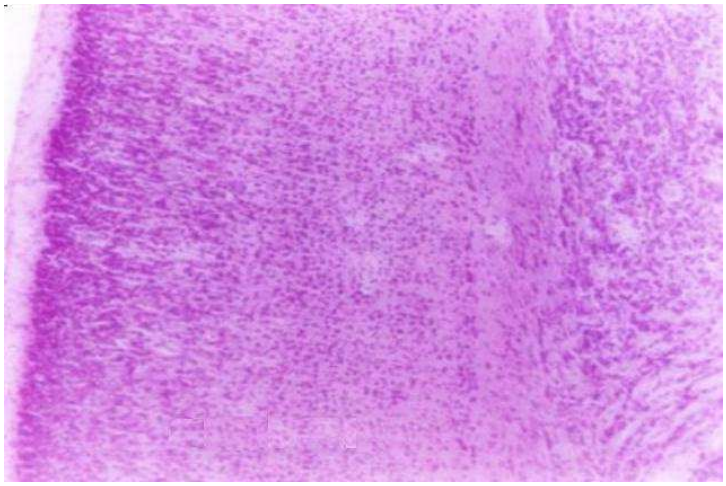

Fig.11 Drug treated (7g) brain, 30X magnification. Showing vacuolated cortical layers

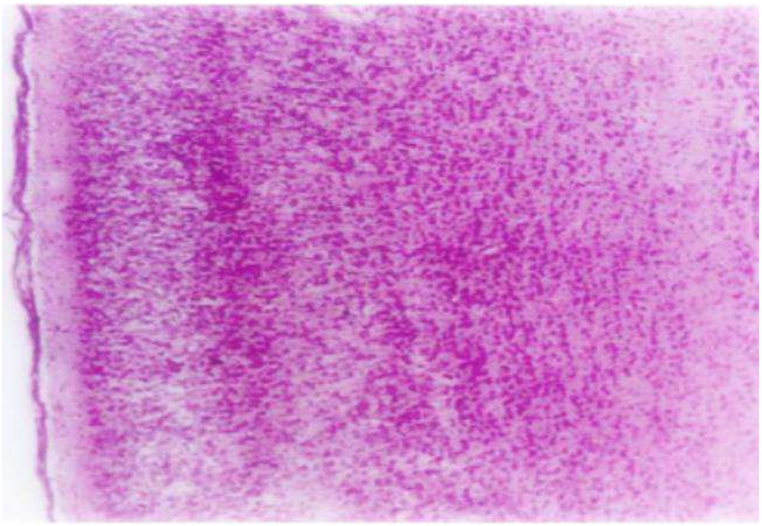

Fig.12 Drug treated (7c) brain, 30X magnification. Showing vacuolated cortical layers

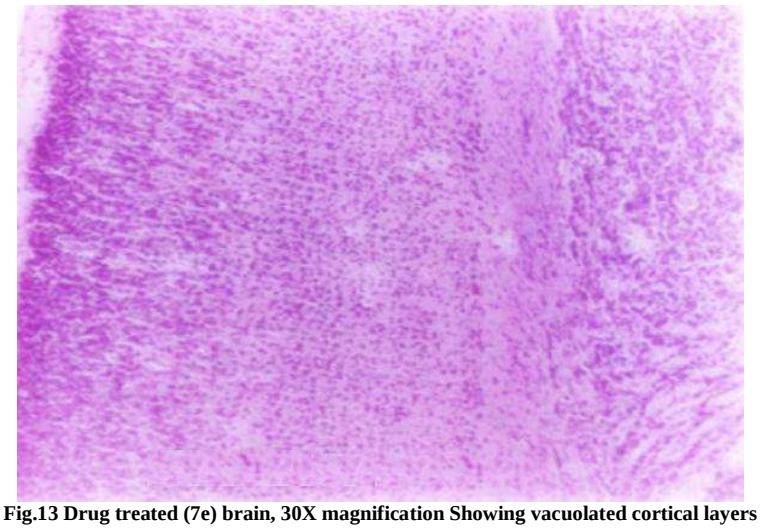

Fig.13 Drug treated (7e) brain, 30X magnification Showing vacuolated cortical layers

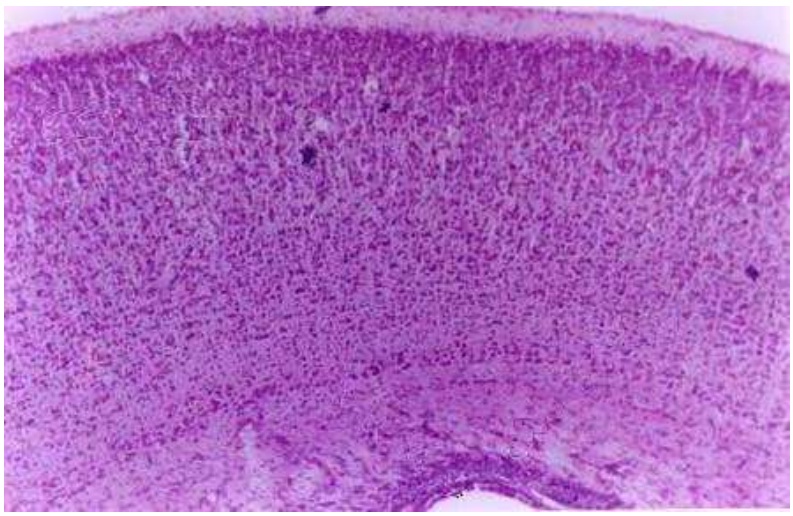

Fig.14 Drug treated (7a) brain showing altered cortical layers. 30X magnification

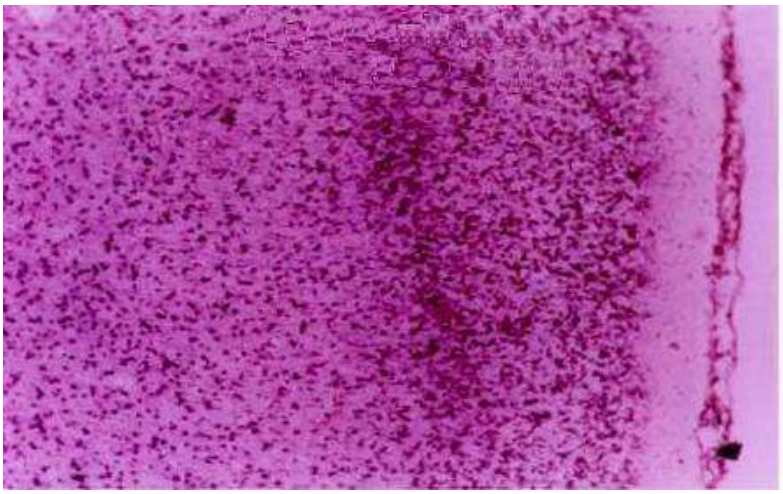

Fig.15 Drug treated (7f) brain showing altered cortical layers. 30X magnification

In animals that were exposed to test compounds and were sacrificed on 4th day, lesions were not seen in all animals of that group (7a, 7c and 7f). However in the group 7e, and 7g which were sacrificed on 8th day, lesions were present but doesn't transform into vacuole and neuro-degeneration. Moreover these lesions are limited to a particular area of brain e.g. cortex and doesn't extend to the ventricles

CONCLUSION

In conclusion, we have developed a facile and efficient method for the synthesis of a variety of hexahydroquinolines derivatives via an improved grindstone reaction catalyzed by p-TSA. The reaction conditions are mild and the reaction gives excellent yields of the products at room temperature. This method does not involve the use of toxic solvents thus it is an environmentally friendly process. The milder conditions, shorter reaction times, low costs, easy workup and high yields make this process attractive over the other existing methods.

Moreover, a structure based docking studies were carried out using the crystal structure of A_1 adenosine receptor (3QAK) to gain insight into the structural requirement for effective binding with the receptor as possible CNS active agents. All the designed compounds showed binding affinities as well as interactions with all the crucial amino acid residues on par with the reference standard. Further in this study we have attempted a straightforward

synthesis of hexahydroquinolines derivatives that would act to reduce neuro-inflammation and oxidative stress in brain, using multi-component reactions.

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