ETHYL-4-(DIMETHYLAMINO)-2-(4-METHOXYPHENYL) PROPANOTE AS ANXIOLYTIC, ANTI-INFLAMMATORY AND ANTI-BACTERIAL AGENT

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ABSTRACT

An improved process for the synthesis of Ethyl-4-(dimethylamino)-2-(4-methoxyphenyl)propanote was carried out by hydrolysis of 2-(4-methoxyphenyl)acetonitrile using sodium hydroxide to obtain 2-(4-methoxyphenyl)acetic acid. 2-(4-methoxyphenyl)acetic acid was then esterified with ethanol to obtain 2-(4-methoxyphenyl)acetate. 2-(4-methoxyphenyl)acetate then undergoes Mannich reaction with paraformaldehyde and dimethylamine in presence of catalytic amount of tertiary butyl ammonium bromide. The synthesized compounds were characterized by IR, ^{1}H NMR and ^{13}C NMR. The final compound was then subjected to purity analysis by HPLC. Also, the final compound was then subjected to pharmacological screening for anxiolytic activity by Elevated plus maze test, Open field test and Motor co-ordination test by Rota rod. The compound synthesized was also screened for *in-vitro* anti-inflammatory activity by protein denaturation method and antibacterial activity by serial dilution method.

KEYWORDS: Mannich reaction, Anxiolytic, Anti-inflammatory, Anti-bacterial, Serial dilution method.

INTRODUCTION

Depression is the most common mental health condition in the general population, characterised by sadness, loss of interest or pleasure, feelings of guilt or low self-worth, disturbed sleep or appetite, feelings of tiredness, and poor concentration.[1] In its most severe form, depression can lead to suicide and increased risk of mortality.[2] Depression is a disorder of major public health importance, in terms of its prevalence and the suffering, dysfunction, morbidity, and economic burden. Depression is more common in women than in men.[3] Globally, the burden of depression has been rising and major depressive disorder (DD) was the third leading cause of disability in 2015. Estimated global prevalence of depressive episode/DD varies from 3.2% to 4.7%.[4] The report on Global Burden of Disease estimates the point prevalence of unipolar depressive episodes to be 1.9% for men and 3.2% for women.[5] The global pooled period prevalence of mood disorders was 5.4%, and its prevalence in WHO-World Mental Health Survey ranged from 0.8% to 9.6% across countries. By 2030, unipolar depression is predicted to be the second leading contributor to the global burden of disease.[6] Burden of depression is further amplified by its 'cause and consequence' relationship with many non-communicable diseases (NCDs) and thus has a huge impact on individuals, families and societies. Depression is one of the most commonly diagnosed mental disorders in primary care settings. In India, it is estimated that nearly one-third of patients seeking help from healthcare facilities could have symptoms related to depression, and the crude prevalence rate of mood disorder was estimated to vary from as low as 0.5 to as high as 78 per 1000 population.[7]

Evidence suggests that the antidepressant venlafaxine hydrochloride selectively inhibits serotonin (5-HT) uptake at low doses, whereas at high doses, it h=inhibits both 5-HT and norepinephrine (NE) uptake[8] and is weak inhibitor of dopamine reuptake. Venlafaxine has no significant affinity for muscarinic, histaminergic, or adrenergic receptors in vitro.[9] It inhibits the serotonin transporter at 30 fold lower concentrations than norepinephrine transporter. It display differential effects on norepinephrine reuptake in healthy versus depressed patients.[10] (Development and optimization of venlafaxine hydrochloride floating microspheres using response surface plots)

Chemistry of venlafaxine is explained based of structure activity relationship. (SAR).[11]

- Venlafaxine and Desvenlafaxine, both include cycloalkanol ethylamine scaffold.

- Increasing the electron withdrawing character of aromatic ring provides additional potent inhibitory effect on NE uptake in addition to improved selectivity for NE over serotonin transport.

- Presence of electron withdrawing group ($-CF_3$) at meta-position resulted in increased potent inhibitory effect of NE and most selectivity above serotonin uptake.

- Presence of piperazine ring shows NE and DA reuptake nhibition.

In the present investigation, the modification in the synthesis of the title compound has been carried out using mannich reaction (a three component condensation reaction), in which a compounds having active hydrogen atom is made to react with formaldehyde and an NH-amine derivative. This reaction is a established method for the preparation of β-aminoketones and aldehydes (mannich bases) and is one of the vital basic reaction types in organic chemistry.[12]

MATERIALS AND METHOD

Chemical: 2-(4-methoxyphenyl)acetonitrile, NaOH, Water, IPA, conc. H_2SO_4, EtOH, Paraformaldehyde, TBAB, K_2CO_3, Toluene, Dimethyl amine hydrochloride, EtOH, conc. HCl. All the chemicals were obtained from R&D laboratory of R L Fine Chemicals, Yelhanka New Town, Bengaluru-560064 Karnataka.

Completion of the reaction was checked on aluminium coated TLC plates using 50% ethyl acetate: n-hexane and acetone: chloroform (1:9) as mobile phase and visualized under UV light. Infrared (IR) spectra were recorded using Shimadzu IR Affinity-1 FTIR spectrometer. ^{1}H and ^{13}C NMR spectra were recorded using Nanalysis 60 MHz spectrometer using $CDCl_3$ as solvent and tetramethylsilane (TMS) as internal standard.
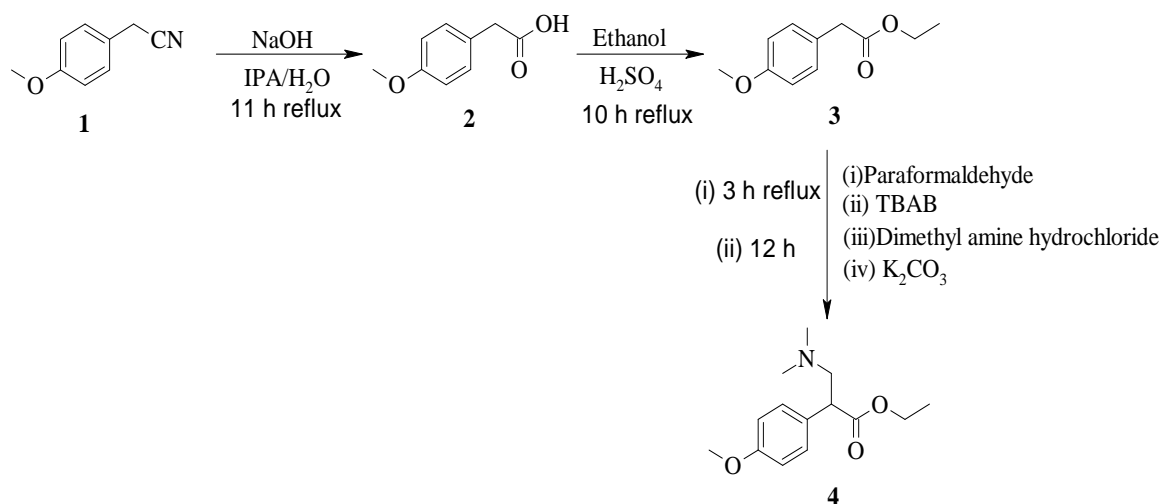
Fig. 1: Synthetic pathway for Ethyl-4-(dimethylamino)-2-(4-methoxyphenyl)propanote.

Synthesis of 2-(4-methoxyphenyl)acetic acid: Nucleophillic hydrolysis reaction was carried out in round bottom flask taking 2-(4-methoxyphenyl)acetonitrile **1** (0.101 mol) and IPA (30 mL). NaOH (2% $^w/_w$) was dissolved in water (75 mL) and added to above solution and refluxed. The improvement in the reaction was monitored by TLC using elution system of 50% ethyl acetate: n Hexane. After completion of reaction, (11 h) reaction mixture was distilled. Residue obtained was acidified with HCl. Water was added and extracted with dichloromethane. Dichloromethane layer was separated and distilled under reduced pressure to obtain 2-(4-methoxyphenyl)acetic acid **2.**

Yellow solid; Percentage yield: 86.84%; IR (Neat, cm^{-1}): 3116 (OH), 2968 (Ar-CH$_2$-), 1026-1244 (Ar-O-CH$_3$), 1691 (C=O); ^{1}H NMR (60 MHz, δ ppm, CDCl$_3$): 3.57 (s, 2H, -CH$_2$-), 3.72-3.85 (s, 3H, CH$_3$-O-), 6.79-7.29 (dd, 4H, Ar-H), 11.37 (s, 1H, OH); ^{13}C NMR (15 MHz, δ ppm, CDCl$_3$): 40.3 (-CH$_2$-), 55.2 (CH$_3$-O-), 114.1-158.8 (Ar-CH), 178 (C=O).

Synthesis of ethyl 2-(4-methoxyphenyl)acetate: Esterification reaction was carried out in round bottom flask taking 2-(4-methoxyphenyl)acetic acid **2** (0.0876 mol), EtOH (43.71 mL) and conc. H$_2$SO$_4$ (2 mL) and refluxed. Improvement in the reaction was monitored by TLC using elution system of 50% ethyl acetate: n-Hexane. After completion of reaction, (10 h) reaction mixture was distilled. Residue obtained was added with water and extracted with dichloromethane. Dichloromethane layer was separated and distilled under reduced pressure. The product obtained was purified by Flash chromatography using silica gel as stationary phase and 2% ethyl acetate: n-Hexane mobile phase to obtain ethyl 2-(4-methoxyphenyl)acetate **3**.

Light green liqid; Percentage yield: 76.84%; IR (Neat, cm^{-1}): 3116 (Ar-CH), 1722 (ester), 1583-1463 (Ar C=C), 1244-1026 (Ar-O-R); ^{1}H NMR (60 MHz, δ ppm, CDCl$_3$): 1.09-1.36 (t, 3H, CH$_3$-of ester), 3.54 (s, 2H, Ar-CH$_2$-O), 3.7 (s, 3H, CH$_3$-O-), 6.74-7.28 (dd, 4H, Ar -CH-), 7.5-7.88 (q, 2H, -CH$_2$- of ester); ^{13}C NMR (15 MHz, δ ppm, CDCl$_3$): 14.1 (CH$_3$-of ester), 40.5 (Ar-CH$_2$-O), 55.1 (CH$_3$-O-), 60.7 (-CH$_2$- of ester), 114-158.6 (Ar carbons), 171.9 (>C=O).

Synthesis of ethyl-4-(dimethylamino)-2-(4-methoxyphenyl)propanote: Mannich reaction was carried out in round bottom flask taking ethyl 2-(4-methoxyphenyl)acetate **3** (0.0672 mol), paraformaldehyde (0.1006 mol), TBAB (1% $^w/_w$) and toluene (52.2 mL). Temperature of reaction mixture was increased to 80-85˚C. After 3hr, reaction mixture was transferred to ice bath and temperature of 0-5˚C was maintained. Dimethyl amine hydrochloride (0.1344 mol), K$_2$CO$_3$ (0.1344 mol) were added. The improvement in the reaction was monitored by Acetone: CHCl$_3$ (1:9). After completion of reaction, (12 h) reaction mixture was added with water and extracted with ethyl acetate. Ethyl acetate layer was separated and distilled out under reduced pressure. The product obtained was purified by flash chromatography by using stationary phase silica gel and mobile phase dichloromethane to obtain ethyl-4-(dimethylamino)-2-(4-methoxyphenyl)propanote **4**.

Light green liquid; Percentage yield: 38%; IR (Neat, cm^{-1}): 1732 (ester), 3062 (Ar C-H), 2974-2854 (Aliphatic C-H), 1462-1610 (Ar C=C), 1249 (Aliphatic C-N); ^{1}H NMR (60 MHz, δ ppm, CDCl$_3$): 1-04-1.30 (t, 3H, -CH$_3$ of ester), 2.22 (s, 6H, N(-CH$_3$)$_2$), 2.87-3.24 (t, 1H, Ar-CH-), 3.75(s, 3H, CH$_3$-O-), 4.03-4.17 (d, 2H, -CH$_2$-N<), 6.72-7.31 (dd, 4H, Ar C-H); ^{13}C MNR (15 MHz, δ ppm, CDCl$_3$): 13.9 (-CH$_3$ of ester), 45.5 (Ar-CH<), 49.4 (N-(CH$_3$)$_2$), 55 (CH$_3$-O-), 60.6 (-CH$_2$-N<), 62.8 (-CH$_2$- of ester), 114-158.9 (Ar carbons), 173.3 (>C=O); HPLC: 92.2% Pure.

PHARMACOLOGICAL SCREENING

The synthesized compound was screened for anxiolytic activity by elevated plus maze test, open field test and motor co-ordination test by rota-rod. The synthesized compound was also tested for *in-vitro* anti-inflammatory activity using inhibition of protein denaturation and antibacterial activity using serial dilution method.

Animals: Healthy **s**wiss albino mice (20-30gm) of either sex were used for the experiment. The Institutional Animal Ethics Committee (IAEC) approved the experimental protocol

(BLDE/DPC/644/2018-19) dated 15/12/2018. All the procedures were performed in accordance with IAEC. All the animals were procured from animal house of H.S.K. College of Pharmacy, Bagalkot, Karnataka. The animals were acclimatized by keeping them in propylene cages (29x22x14) containing husk as bedding material and maintained under controlled of temperature (25±2˚C), humidity (55±5%) and 12hr light and 12hr dark cycles. The animals were fed with standard pellet diet and water *al* libitum.

Elevated plus maze test: The EPMT apparatus consisted of four arms elevated 30 cm above the floor. Two of the arms were covered with high walls and other arms were linked via central area to form plus sign. Healthy swiss albino mice weighing 20-30gm were used for the experiment. Animals were sorted into 4 groups, viz., control, standard (diazepam 1mg/kg), test group-I (T1) (5 mg/kg) and test group-II (T2) (10 mg/kg), each containing 6 animals. The animals were treated with test drug and diazepam 30min prior to the test. Each mouse was placed separately on central platform facing towards an open arm. The frequency and duration of entries into the open and closed arms were observed for 300sec. total number of entries in open arm and closed arm as well as time spent in open arm and closed arm were recorded.[13]

Open field test: The arena of open field was divided into 16 squares; 4 squares in the centre and 12 squares in the periphery. The experimental area was illuminated with 40-W lamp. Animals were sorted into 4 groups, viz., control, standard (diazepam 1mg/kg), test group-I (T1) (5 mg/kg) and test group-II (T2) (10 mg/kg), each containing 6 animals. The animals were treated with test drug and diazepam 30min prior to the test. The animals were placed individually in the centre and latency, number of squares crossed and number of assisted rearings were observed for 300sec.[13]

Motor co-ordination test by rota-rod: The apparatus consisted of a base platform and an iron rod of 3cm diameter and 30cm length, with non-slippery surface. The training for the mice was given 20 times at 5-15 min interval. The interval between mounting of animal on the rod and falling off of it were recorded as performance time. Animals were sorted into 4 groups, viz., control, standard (diazepam 1mg/kg), test group-I (T1) (5 mg/kg) and test group-II (T2) (10 mg/kg), each containing 6 animals. The animals were treated with test dose and diazepam 30min prior to the test. The performance time was measured at 15min interval for 90min for 300sec.[13]

Statistical analysis: All the values were expressed as mean ± SEM. The results were statistically evaluated using one-way analysis of variance (ANOVA) followed by Dunnet's multiple comparison test using Graphpad Prism software. A *P* value of <0.05 was considered as the level of significance.

***In-Vitro* Anti-inflammatory activity by inhibition of protein denaturation:** The reaction mixture consisted of bovine serum albumin (0.45mL) and test compound (0.05ml) (25, 50, 100, 200μgm/ml). pH was adjusted using small amount of 1N HCl. The samples were incubated at 37°C for 20 min and then heated at 57°C for 3 min. After cooling, 2.5mL of buffer solution was added into each test tube. Turbidity was measured spectrophotometrically at 600nm. The experiment was performed in triplicate. The percentage inhibition of protein denaturation was calculated as follows[14]:

$$Percentage\ Inhibition = \frac{(Abs\ Standard - Abs\ sample)}{Abs\ Contol} \times 100$$

Antibacterial activity by serial dilution method: Serial dilution is sequential dilution method to reduce an intense culture of cells to a more utilizable concentration. This method is used to determine the minimum inhibitory concentration (MIC) of antimicrobial agents and are the reference methods for antimicrobial susceptibility testing. 9mL of nutrient broth was taken in 5 test tubes each for cefotaxime and test compound. 0.1mL of bacterial culture of different strains (*E.coli, S.aureus, P.aeruginosa* and *K.pneumoniae*) was added to each test tube. 1m of test compound (1000 μgm/mL) was added in 1st tube. 1mL of solution was pipetted from 1st tube and added to 2nd tube (100 μgm/mL). The method was repeated to attain concentrations of 10 μgm/mL, 1 μgm/mL and 0.1 μgm/mL. The same method was followed for the cefotaxime drug. The tubes were then incubated for 24hrs and the turbidity of the solution was recorded using Nephlo-Turbidometer against McFarland standard at 100NTU. Blank contained nutrient broth and different bacterial cultures without drug.

$$Turbidity = \frac{1}{Inhibition\ of\ microbial\ growth}$$

RESULTS AND DISCUSSION

We have modified and improved the process for the synthesis of ethyl-4-(dimethylamino)-2-(4-methoxyphenyl)propanote. The synthetic pathway has been illustrated in **fig. 1**. Compound **1** on reaction with sodium hydroxide in presence of isopropyl alcohol gave 2-(4-methoxyphenyl)acetic acid **2**. Esterification of compound **2** with ethanol and H_2SO_4 resulted

in 2-(4-methoxyphenyl)acetate **3**. This was further converted to ethyl-4-(dimethylamino)-2-(4-methoxyphenyl)propanote **4** by Mannich reaction with paraformaldehyde, TBAB, dimethyl amine hydrochloride and K_2CO_3.

IR spectrum of the final compound **4** gave stretching vibrations at 1732 cm^{-1} corresponding to ester, 3062 cm^{-1} corresponding to Ar C-H, 2974-2854 cm^{-1} corresponding to aliphatic C-H and 1249 cm^{-1} corresponding to aliphatic C-N **(fig. 2)**.

^{1}H NMR spectra of the final compound **4** showed the protons of methyl group of ester as triplet at δ=1-04-1.30 ppm, methyl groups of dimethyl amine as singlet at δ=2.22ppm, methylne group (Ar-CH<) as triplet at δ=2.87-3.24pm, methoxy group as singlet at δ= 3.75ppm, methylene group (-CH$_2$-N<) as doublet at δ=4.03-4.17ppm and benzene as doublet of doublet at δ=6.72-7.31ppm. While ^{13}C NMR spectra showed the carbon of methyl group of ester (C_{14}) at δ=13.9ppm, methylene group (C_9) at δ=45.5ppm, methyl groups of dimethyl amine ($C_{17,18}$) at δ=49.4ppm, methoxy group (C_8) at δ=55ppm, methylne group (C_{15}) at δ=60.6ppm, methylene group of ester (C_{13}) at δ=62.8ppm, benzene ($C_{1,3}$, C_5, $C_{4,6}$ and C_2) at δ=114ppm, 128.9ppm, 129.6ppm and 158.9ppm, respectively. The carbon of carbonyl group (C_{10}) appeared at δ=173.3ppm **(fig. 2)**. Additionally the HPLC purity was found to be 92.2%.
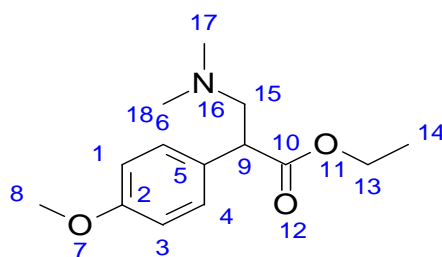

Fig. 2: Atomic enumeration of compound Ethyl-4-(dimethylamino)-2-(4-methoxyphenyl)propanote.

The outcome of elevated plus maze test shown that the compound possessed anxiolytic activity, since time spent in open arms is representative of anxiolytic activity. From the data **(Table 1)**, standard (diazepam treated mice 1 mg/kg) didn't show any significant increase in number of open arm entries, time spent in open arms, decrease in number of closed arm entries, time spent in closed arms as compared to control. T1 (5 mg/kg) didn't show any significant changes in open arm and closed arm entries, but showed non-significant increase in time spent in open arms and non-significant decrease in time spent in closed arm as compared to standard. While, T2 (10mg/kg) showed significant reduction (P < 0.05) in

number of closed arm entries, but non-significant increase in time spent in open arm and non-significant decrease in closed arm as compared to standard. The EPMT is used to evaluate psychomotor performance **(fig. 3)**.

Table 1: Effect of test drug on behaviour of mice in elevated plus maze test.

Treatment (mg/kg)	No. of entries Open arm	Time spent (sec) Open arm	No. of entries Closed arm	Time spent (sec) Closed arm
Normal	4.00 ± 0.51	56.33 ± 3.20	5.66 ± 0.61	225.2 ± 4.11
Daizepam (1)	4.00 ± 0.51^{ns}	54.33 ± 5.04^{ns}	7.33 ± 0.84^{ns}	227.2 ± 7.85^{ns}
T1 (5)	4.00 ± 0.51^{ns}	91.00 ± 19.30^{ns}	5.66 ± 0.80^{ns}	182.8 ± 23.40^{ns}
T2 (10)	3.16 ± 0.54^{ns}	106.7 ± 41.68^{ns}	3.66 ± 0.95^{a}	174.7 ± 39.37^{ns}

Values represent Mean $\pm$ SEM (n=6);$^{a}P<0.05,^{b}P<0.01,$ $^{c}P<0.001$, v/s Daizepam Control group; One-way ANOVA followed by multiple comparison Dennett's test and unpaired t-test;*P<0.05, **P<0.01, ***P<0.001.
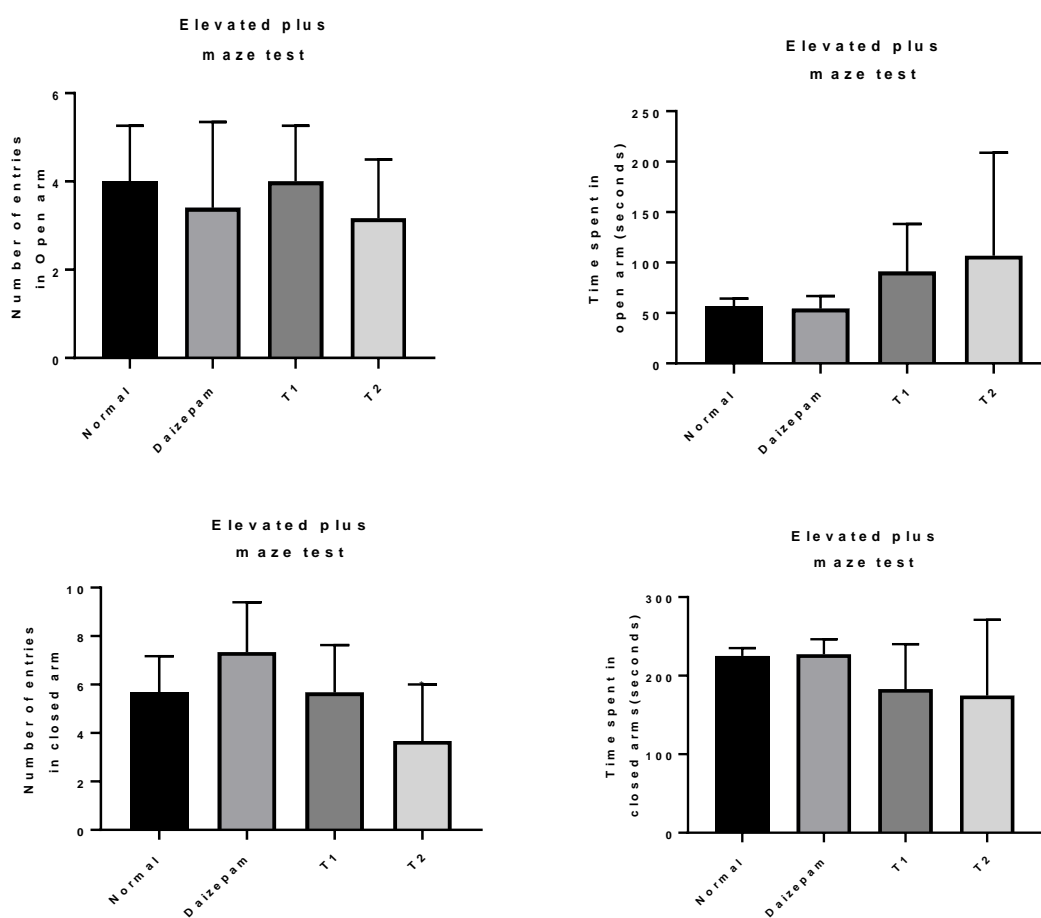

Fig. 3: Effect of test drug on behaviour of mice in elevated plus maze test.

The outcome of open field test shown that the compound possessed anxiolytic activity. From the data **(Table 2)**, standard (diazepam treated mice 1 mg/kg) showed non-significant

decrease in ambulatory movement as compared to control. T1 (5 mg/kg) showed non-significant increase in latency, but significant decrease in ambulatory movement (P<0.001) and significant decrease in number of assisted rearings (P<0.05) when compared to standard. T_2 (10 mg/kg) showed non-significant decrease in latency, but significant decrease in ambulatory movement (P<0.001) and non-significant increase in number of assisted rearings as compared to standard. The OFT is used to examine anxiety related behavior characterized by the normal aversion of animal to an open, bright area **(fig. 3).**

Table 2: Effect of test drug on behaviour of mice in open field test.

Treatment (mg/kg)	Latency (sec)	No. of squares croessed	No. of assisted rearings
Normal	4.167±0.7491	159.2±33.97	0.6667±0.3333
Daizepam (1)	4.167±0.9458[ns]	153.5±28.83 [ns]	9.833±1.922[c]
T1 (5)	5.333±1.406 [ns]	7.167±1.046[c]	4.000±0.5164[a]
T2 (10)	2.667±0.4944 [ns]	7.833±0.8333[c]	13.50±2.078 [ns]

Values represent Mean ± SEM (n=6);[a]P<0.05,[b]P<0.01, [c]P<0.001, v/s Daizepam Control group; One-way ANOVA followed by multiple comparison Dennett's test and unpaired t-test;*P<0.05, **P<0.01, ***P<0.001.
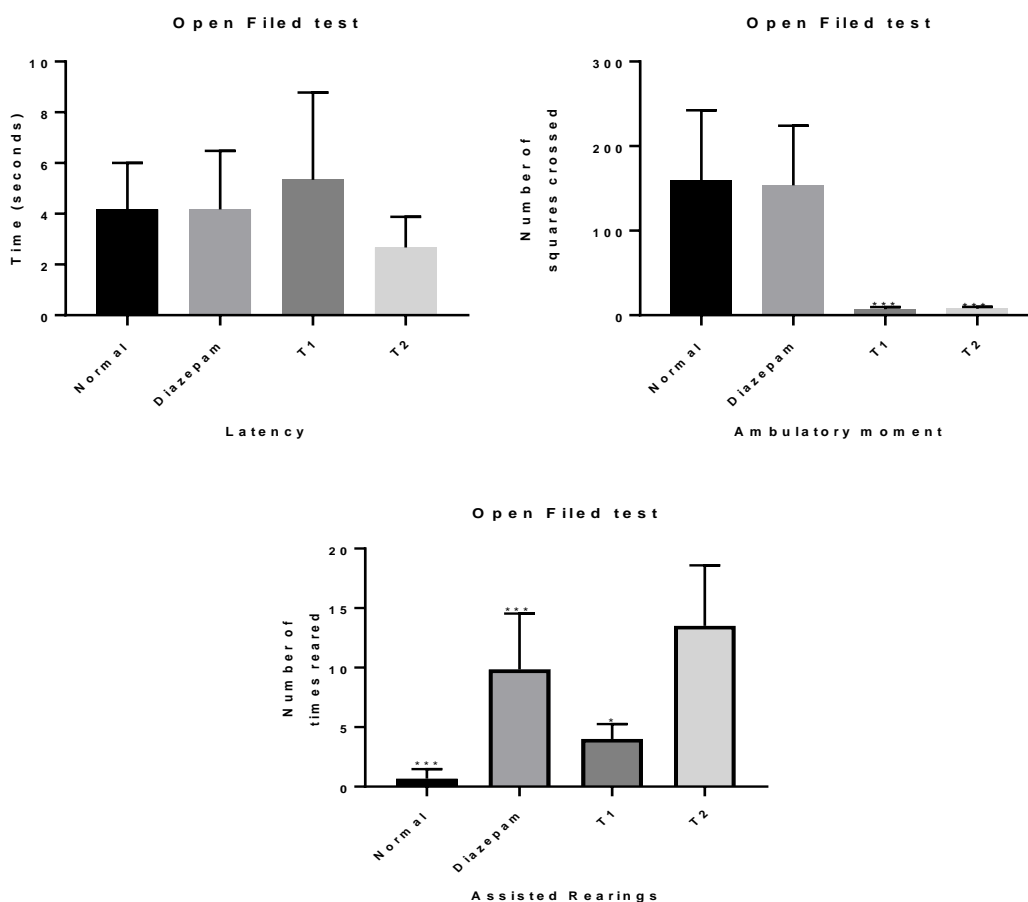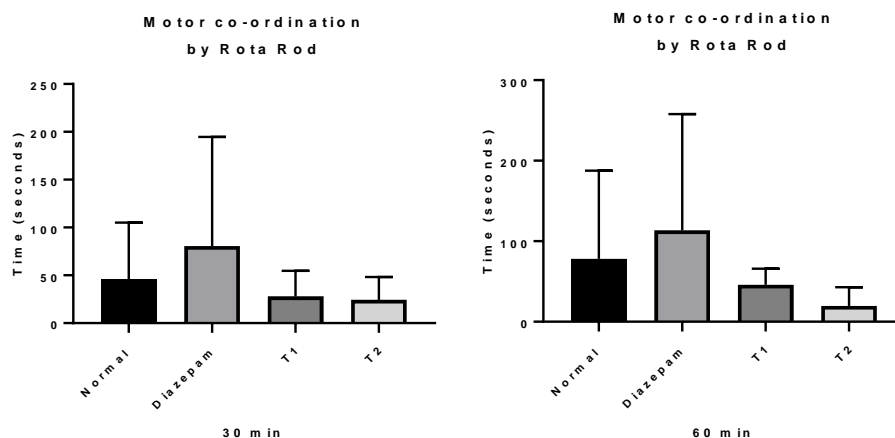

Fig. 3: Effect of test drug on behaviour of mice in open field test.

The outcome of motor co-ordination test by rota rod shown non-significant reduction in the fall of time from rotating rod, indicating muscle relaxation activity relating to anxiolytic behavior. From the data **(Table 3)**, standard (diazepam treated mice 1 mg/kg) showed non-significant decrease in time of animal remained without falling from rotating rod as compared to control. T1 (5 mg/kg) and T2 (10 mg/kg) also showed non-significant reduction in the time animal remained without falling from rotating rod as compared to standard. The RRT was reported to predict motor dysfunction produced by centrally acting drugs to determine possible alterations in the motor co-ordination of the animal. Skeletal muscle relaxation together with calming effect reduces anxiety and tension **(fig. 4).**

Table 3: Effect of test drug on behaviour of mice rota rod.

Treatment (mg/kg)	Time (sec) of animals remained without falling from rotating rod		
	30	60	90
Normal	46.00 ± 24.10	78.00 ± 44.76	104.0 ± 49.40
Daizepam (1)	80.50 ± 46.58^{ns}	113.3 ± 59.04^{ns}	123.2 ± 57.01^{ns}
T1 (5)	27.83 ± 10.93^{ns}	45.83 ± 8.167^{ns}	32.00 ± 12.39^{ns}
T2 (10)	24.33 ± 9.746^{ns}	19.67 ± 19.67^{ns}	10.83 ± 5.332^{ns}

Values represent Mean $\pm$ SEM (n=6);$^{a}P<0.05,^{b}P<0.01,$ $^{c}P<0.001,$ v/s Daizepam Control group; One-way ANOVA followed by multiple comparison Dennett's test and unpaired t-test;*P<0.05, **P<0.01, ***P<0.001.
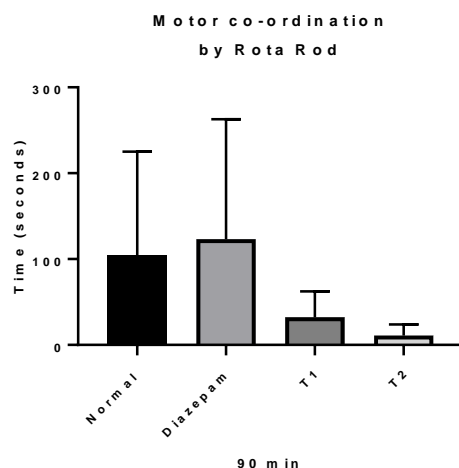
Fig. 4: Effect of test drug on behaviour of mice rota rod.

The results obtained from *in-vitro* anti-inflammatory test showed that the test drug can be used as anti-inflammatory agent, since it produced effective inhibition of protein denaturation. From the data **(Table 4)**, test drug has shown maximum of 31.94% inhibition of protein denaturation at 200 µgm/ml, as compared with that of diclofenac sodium which has shown maximum of 41.02% inhibition at 25 µgm/ml. Denaturation of protein is well documented cause of inflammation **(fig. 5)**.

Table 4: Effect of test drug on inhibition of protein denaturation.

Treatment	Concentration (µgm/mL)	Absorbance 600 nm	Inhibition of protein denaturation (%)
Control	--	0.046±0.00057	--
Diclofenac sodium	25	0.077±0.00033****	41.02
	50	0.077±0.00057****	39.47
	100	0.073±0.00088****	37.83
	200	0.073±0.00088****	36.11
Test	25	0.067±0.00088^c	14.11
	50	0.067±0.00088^c	9.21
	100	0.055±0.00088^c	25.67
	200	0.052±0.00023^c	31.94

Values represent Mean ± SEM (n=3);^{a}P<0.05,^{b}P<0.01, ^{c}P<0.001, v/s Diclofenac sodium control group; One-way ANOVA followed by multiple comparison Dennett's test and unpaired t-test;*P<0.05, **P<0.01, ***P<0.001.
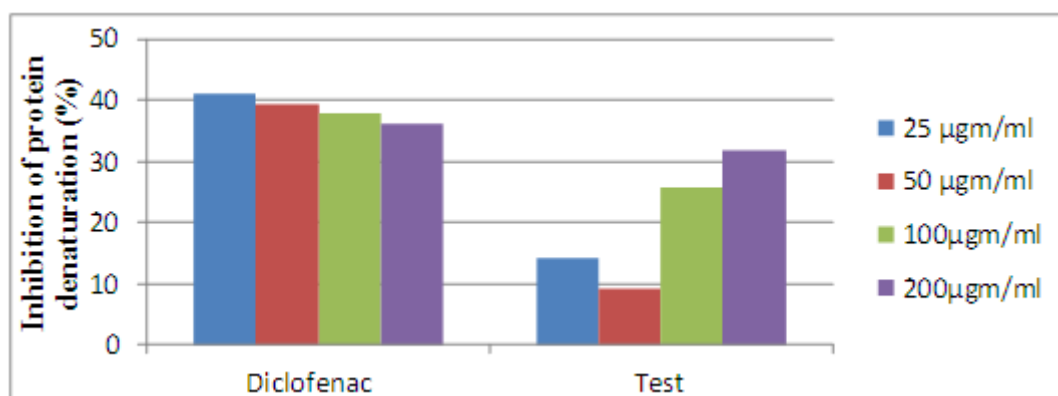
Fig. 5: Effect of test drug on inhibition of protein denaturation.

The outcome of antibacterial test by serial dilution method demonstrates that the test drug possesses noteworthy inhibition of bacterial growth. The test was carried out on *E.coli*, *S.aureus*, *P.aureginosa* and *K.pneumoniae* strains of bacteria. From the data **(Table 5)**, Cefotaxime showed 28.27% (100μgm/ml), 15.05% (1000μgm/ml), 54.60% (1000μgm/ml) and 97.35% (10μgm/ml) of growth of *E.coli*, *Staph.aureus*, *P.aureginosa* and *K.pneumoniae*, respectively. Test drug showed 18.06% (1000μgm/ml) and 65.42% (1000μgm/ml) of growth against *P.aureginosa* and *K.pneumoniae*, repectively. While, the test drug didn't show any inhibition of growth against *E.coli* and *S.aureus*. This method is used to determine the minimum inhibitory concentration (MIC) of antimicrobial agents and are the reference methods for antimicrobial susceptibility testing **(fig. 6)**.

Table 5: Effect of test drug on inhibition of microbial growth.

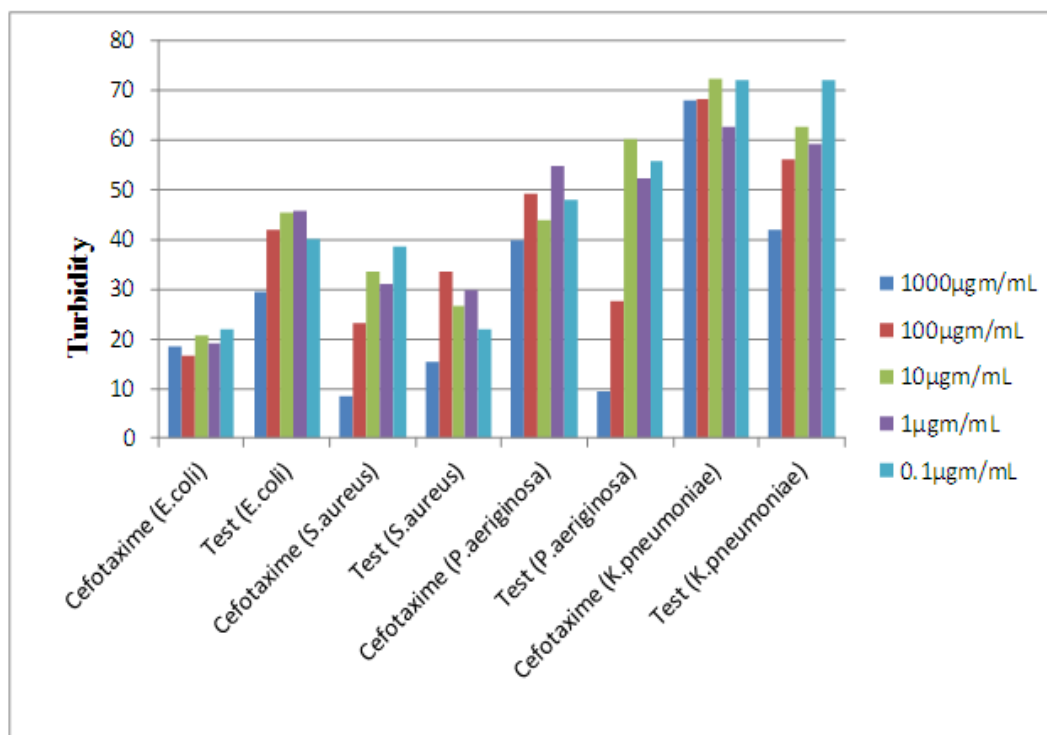| Treatment	Concentration (μgm/mL)	Microbial growth (Turbidity)			
		E.coli	*S.aureus*	*P.aeriginosa*	*K.pneumoniae*
Normal	--	**58.7**	**55.8**	**72.7**	**64.2**
Cefotaxime	1000	18.3	8.4	39.7	67.6
	100	16.6	23	49	68.2
	10	20.5	33.3	43.7	72.2
	1	19	31.1	54.7	62.5
	0.1	21.8	38.5	47.7	72
Test	1000	29.3	15.2	9.5	42
	100	42	33.5	27.4	55.9
	10	45.4	26.5	59.9	62.4
	1	45.6	29.8	52.1	59
	0.1	40	21.9	55.8	71.8

Fig. 6: Effect of test drug on inhibition of microbial growth.

CONCLUSION

Derivative of venlafaxine was synthesised. All the compounds were characterized by IR, ^{1}H NMR and ^{13}C NMR and purity of final compound was determined by HPLC.

The synthesized compound was screened for *in-vivo* antipsychotic, *in-vivo* antidepressant, *in-vitro* anti-inflammatory and antimicrobial activities and the activities were compared with standard.

Compound T2 (10 mg/kg) showed significant (P < 0.05) decrease in anxiolytic behaviour as compared to T1 when tested for anti-anxiolytic effect by Elevated Plus Maze Test.

Compounds T1 (5 mg/kg) and T2 (10 mg/kg) showed significant (P < 0.01) decrease in ambulatory movement that corresponds to decrease in anxiolytic behavior when tested with Open Field Test.

Compound T1 (5 mg/kg) and T2 (10 mg/kg) showed non-significant decrease in falling threshold, which corresponds to the muscle relaxation activity relating to anxiolytic behavior when tested with Motor co-ordination test by Rota rod.

Test compound showed effective inhibition of protein denaturation when tested for anti-inflammatory activity using inhibition of protein denaturation method.

Test compound showed noteworthy antimicrobial activity against *P.aureginosa* and *K.pneumoniae,* whereas, it didn't show any effect on *E.coli* and *S.aureus* bacteria.

Further, a detailed study can be done with reference to the synthesized compoundand could be studied as molecular level for determination of active groups required for the drug-receptor interaction and modifying the structure based on the interactions.

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