Chapter 2

Biological Applications of Pyrimidine based Diazepine derivatives

2.1. Introduction

2.1.1. History of Anxiolytic, Sedative and Hypnotic Activity

It was clear that cannabis was trusted that it could cure all mental and physical sickness however numerous doctors were still in issue to acknowledge the full estimation of cannabis.

2.1.2. Therapeutic Applications of Cannabis

Truth be told, there is no adequate proof to clarify a considerable lot of these cases. The most recent few decades have seen awesome change blast of research investigating the potential helpful uses of cannabis and cannabis determined meds. As an incredible change today, more confirmation exists in association with the intense remedial uses of cannabis than is accessible for probably the most generally utilized regular medicines and treatments [Sulak, et al., 2011].

It is broadly comprehended, in spite of the fact that not as generally perceived by the restorative group, that cannabis was both capable and safe and can cure the anguish of many serious ceaseless and intense medicinal conditions. How is it conceivable that one plant could do as so much. The appropriate response lies in the decently as of late found physiological
framework known as the endo cannabinoid framework, which assumes an essential part in tutoring and controlling about each real capacity in all vertebrates [de Fonseca, et al., 2005, Gieringer, Rosenthal, Carter, et al., 2008, Grotenhermen, et al., 2006]. For the most part Our bodies create five known concoction mixes called endogenous, cannabinoids or endocannabinoids [de Fonseca, Grotenhermen, et al., 2006], comparable in structure and activity to the substance mixes discovered greatest in cannabis, called phytocannabinoids, of which no less than 85 have been distinguished and disconnected from the plant [El-Alfy, et al., 2010].

2.1.3. Various Definitions of Anxiety

Anxiety is characterized as sentiment stress, surprise, vulnerability and dread that originate from contemplating some risk or threat [Glanze, et al., 1987]. The American Psychological Association characterizes uneasiness as a feeling described by sentiments of strain, stressed contemplations and physical changes like expanded pulse. Furthermore, other medical problems now days, tension is gradually perceived as an autonomous element prompting coronary illness and mortality [Roest, Martens, de Jonge, Denollet, 2010, Shen, et al., 2008]. And therefore, interventions that reduce anxiety are allies and should be implemented.

Taber's Cyclopedic Medical Dictionary characterizes an anxiolytic as a medication that eases uneasiness [Venes, et al., 2001]. Dorland 1903, in The
American Illustrated Medical Dictionary, characterizes soothing as an operator bringing on or creating significant rest, mesmerizing as a medication that produces rest a relaxant as a specialist that reduces strain, a nerve as mollifying apprehensive energy a solution for anxious issue, and a calming as a cure that relieves fervor additionally characterizes a cerebral soothing as one which especially influences the cerebrum. To this class have place cannabis, camphor, the bromids, hyoscin, and the mesmerizing and revulsive specialists [Dorland, et al., 1903]. Also includes cannabis in a list of the nervetrunk sedatives.

Opiate was initially simply one more medium of medication delivered rest or daze in this way, and it is ploned that cannabis [Hoerr, Osol, et al., 1952]. Be that as it may, in many parts of the restorative world the term opiate has come to be related only with medications more intense than hypnotics [Taber, et al., 1952] such as opiates and other drugs that produce physical withdrawal symptoms [Glanze, et al., 1987]. Since cannabis withdrawal side effects have verifiably been depicted as mellow [Marihuana, et al., 1950, Schifferes, et al., 1963], and additionally affirmed to be so by late government surveys of all accessible confirmation [Nolin, Kenny, Banks, Maheu & Rossiter, et al., 2002] opiate as connected to cannabis is do not fitting anymore and is currently a sedative particular term. A portion of the main recorded advantages of cannabis utilize were to treat stress and uneasiness, where cannabis was known as the plant of overlooking worrie [Ratsch, et al.,
1998/2001, Russo, et al., 2007] additionally indicates the work of Thompson, who put in 50 years disentangling therapeutic writings from the Bronze Age human advancement of Assyria, whose interpretation of a cuneiform word contended to be for cannabis, likens to the weaving opiate.

2.1.4. Anxiety drugs used in 17th century

In the popular Book of a Thousand Nights and a Night, also called Arabian Nights, an accumulation of stories dating from the Islamic Golden Age, bhang is said ordinarily in connection to delivering rest. For example there is the tale of King Omar bringing about Princess Abrizah to fall into a profound rest by utilizing a bit of concentrated Bhang [Burton, et al., 2003]. A few hundred years after the fact, in 1563, Garcia Da Orta 1563/1895, doctor, pioneer of tropical solution, and naturalist, composed that bangue, an Indian drink made with cannabis, could raise a man most importantly considerations and tensions and at around a similar time, in China, Li Shih-Chen included cannabis sativa in his popular and far reaching 1578 (first distributed 1593) home grown Pen-ts'ao kang mu as a solution for apprehensive issue (Li Shih-Chen, 1593/1911, Moreover, in the Rajvallabha, a seventeenth century Materia. Medica managing drugs utilized as a part of India, ganja is said to be a craving satisfying medication was gotten by men on earth, through longing for the welfare surprisingly. To the individuals who routinely utilize it conceives bliss and devastates each nervousness [Grierson, et al., 1894].
Beginning in the late 1850, hashish confection for therapeutic intentions was frequently publicized in daily papers and magazines. For instance, in the October sixteenth, 1858 release of Harper's Weekly, a little notice for Gunjah Wallah's Hasheesh Candy guarantees the client. A most pleasurable and innocuous stimulant – Cures Nervousness, Weakness, Melancholy Inspires all classes with new life and vitality [Ludlow, et al., 1857/1975]. Other examples include similar advertisements in Vanity Fair Antique cannabis medicines [Hasheesh candy, et al., 2010].

2.1.5. Cannabis Used as Hypnotic and Sedative Drugs


States Indian hemp is utilized to mitigate torment for prompting, while Dock's 1908 Textbook of Materia Medica for Nurses calls cannabis an entrancing and clarifies that it can prompt a rest [Robinson, et al., 1946]
points out that cannabis is sometimes used as a hypnotic in those cases where opium, because of long-continued use, has lost its potential. Furthermore, he dispensatory of the United States of America of 1947 states that cannabis is accustomed to prompting rest and to remember eagerness [Osol, Farrar, et al., 1947]. A couple of years after the fact, solomon again avowed cannabis produces rest in Pharmacology and Therapeutics. In 1959, Fishbein attested that marihuana makes one be casual and after that to experience sluggishness in the advanced family wellbeing guide.

2.1.6. Effects of cannabis

The low dosages by and large create the impacts that make individuals like smoking pot. They incorporate mellow elation, unwinding, expanded friendliness and a non-particular reduction in tension [Nolin, et al., 2002]. Every nation that has charged an expertly directed investigation of the impacts of cannabis utilize has reached a similar conclusion Cannabis, when utilized at appropriate measurements has anxiolytic, trancelike, relaxant, calming and soothing properties.

Humans brought cannabis with them wherever they migrated [Robinson, et al., 1996]. Chopra and Chopra 1957 describe the indigenous use of cannabis in India and among the uses are to relieve pain, to help sleeping, to remove fatigue and to calm restlessness. According to African people interviewed by ethnobotanists, dagga helps them to forget all their troubles

2.1.7. Modern Uses of Tetrahydrocannabinols

The list included Analgesic, hypnotic, Antidepressant, tranquilizer. But other research helps a role for cannabis in inducing anxiety in users however, cannabis appears to increase anxiety in those already highly psychosis-prone in experience sampling studies [Holland, et al., 2010], but not in a nonclinical population [Tournier, Sorbara, Gindre, Swendsen, Verdoux, 2003]. Other proof focuses to THC and especially the measurement of THC, being for the most part in charge of tension reactions experienced by a few clients for an audit of this writing [Zuardi, Shirakawa, Finkelfarb, Karniol, et al., 1982, Degroot, et al., 2008].

[Hayatbakhsh, et al., 2007] have found a connection between age of onset, level of use and later anxiety and it was the most grounded for the individuals who started use before age 15. Likewise, a current meta-investigation of 83 studies found that for cannabis clients, age at onset of first psychosis was more youthful than for nonusers [Large, Sharma, Compton, Slade, Nielssen, 2011]. In any case, it is imperative to note that no less than one longitudinal review completing members from preschool age 18 found
that the individuals who explored different avenues regarding drug use amid their puberty best balanced, both mentally and socially, contrasted with both continuous clients and teetotalers [Shedler, Block, et al., 1990]. It is likewise imperative to note that as a top priority connection does not equivalent causation. Truth be told, genuine confirmation proposes that representing youth injuries, for example, sexual mishandle may disengage the affiliation here and there found between cannabis utilize psychosis and in this way, scientists examining the relationship between cannabis utilize and psychiatric clutters ought to modify their investigations to represent this conceivably jumbling variable [Houston, Murphy, Shevlin, Adamson, et al., 2011].

Also, audits and Meta examination of the current writing toss question on the charged causal part cannabis plays in the advancement of uneasiness issue. For instance, in a current survey of the confirmation for the capable part cannabis plays in the advancement of psychiatric issue, the creators compress that, taken together, the epidemiological verification does not stand a causative or accelerating part for cannabis in perpetual wretchedness or tension [Fride, Russo, et al., 2006]. Besides, a current meta-investigation of planned accomplice considers, which controlled for potential perplexing factors, likewise inferred that the proof does not bolster a causal part for cannabis in tension or other full of feeling issue [Moore, et al., 2007].
2.1.8. Development of Medicine in Pharmaceuticals

Curiously, pharmaceutical organizations indicate dynamic intensity of included in creating medications gotten from cannabis, confirmation is mounting for the endless remedial capability of cannabis and the cannabinoids. For instance, GW Pharmaceuticals examine with their protected entire plant remove buccal splash, Sativex, likewise demonstrates the sleep inducing capability of cannabinoids and cannabis.

This is likely because of the proportions of the dynamic mixes, as Sativex contains a 1:1 proportion of tetrahydrocannabinol (THC) to cannabidiol (CBD), THC being the essential psychoactive fixing in cannabis and CBD being another critical yet nonpsychoactive cannabinoid that counters a portion of the undesirable impacts of THC alone [McPartland, Russo, et al., 2006, 2007, 2011]. Truth be told, regardless of some examination that CBD alone enhances rest, it gives the idea that CBD overwhelming concentrates are somewhat enacting while THC predominant concentrates leave remaining narcotic impacts and along these lines, the two mixes act synergistically, in the best possible mix, to give most extreme advantages the minimum unfavorable outcomes [Russo, et al., 2007].

The specialists have found that the cannabinoids THC, CBD and cannabichromene (CBC) as the primary wellsprings of cannabis' soothing [McPartland, Russo, et al., 2006, 2007 and Ratsch, et al., 1998/2001]
anxiolytic and antipsychotic effects [Degroot, et al., 2008, Zuardi, Crippa, Hallak, Moreira, Guimaraes, et al., 2006, Zuardi, Guimaraes, et al., 1997]. For example, an investigation of sound ordinary subjects with actuated uneasiness found that tension was lessened by CBD alone [Zuardi, Cosme, Graeff, Guimaraes, et al., 1993]. In fact [Degroot, et al., 2008] gives the 12 preclinical and clinical reviews, all demonstrating that CBD produces anxiolytic impacts. Moreover, notwithstanding settling THC instigated tension, CBD likewise mitigates a considerable lot of the other negative impacts related with THC, for example, intellectual disability [Zuardi, et al., 1982, Russo, et al., 2007].

2.1.9. Physiological Disorders

Further evidence comes from studies with people suffering from psychosis and schizophrenia. This is important for this discussion because of the very high levels of anxiety, agitation and stress that are often caused by the more dramatic symptoms of psychosis, schizophrenia and therefore, a reduction in psychotic symptoms also leads to a reduction in anxiety and stress. For instance, a study of 79 psychotics found that those who used cannabis recreationally reported less anxiety, depression, insomnia, and physical discomfort than those who did not use cannabis [Warner, et al., 1994] and a stage II clinical trial on the impacts of CBD in schizophrenia and schizophreniform psychosis uncovered that CBD was as powerful as amisulpride, a standard antipsychotic in Europe, Australia and accessible in Canada through the Special Access Program however not accessible in the

Validating backing for the anxiolytic and trancelike, soothing and narcotic impacts of cannabis and the cannabinoids originates from a shocking source. In 2007, rimonabant, a CB1 receptor blocker created as a hostile to weight sedate by French organization Sanofi-Aventis under the exchange name Acomplia, was denied food and drug administration (FDA) endorsement by the U.S. since it was connected to expanded sorrow, self-destructive considerations, suicide endeavors, suicide occasions, nervousness [Gieringer, et al., 2008].

In any case, in spite of the proof and both government and state programs taking into consideration the utilization of cannabis for tension, it is recognized that treating uneasiness with cannabis might be trouble some as a result of contrasts in individual cosmetics, requirement for consistent measurement levels, cost, accessibility and wrongdoing [Mikuriya, et al., 1998].

2.1.10. Comparisons with Synthetic Medicines

Lester Grinspoon, resigned Harvard Medical educator and incredibly famous cannabis master who had some expertise in the review and treatment of schizophrenia, announced that in clinical practice cannabis and
cannabinoids think about positively in both adequacy and wellbeing to numerous pharmaceutical antianxiety drugs [Grinspoon, Bakalar, et al., 1993]. Additionally, various autonomous appraisals of the wellbeing, viability and reliance capability of cannabis unmistakably demonstrate that cannabis and cannabis based meds are all around endured, non-dangerous, can't prompt passing by overdose, and are probably not going to prompt reliance in by far most of patients. For example, the Institute of Medicine's 1999 report, Marijuana and Medicine Assessing the Science Base, in connection to the wellbeing of cannabis, noticed the reactions of cannabinoid medications are inside the adequate dangers related with affirmed solutions [Joy, Watson, Benson, et al., 1999]. A smoker would hypothetically need to expend almost 1,500 pounds of maryjane inside around 15 minutes to actuate a deadly reaction. In down to earth terms, cannabis can't instigate a deadly reaction thus of medication related poisonous quality. In strict restorative terms cannabis is far more secure than numerous sustenances we ordinarily devour. For instance, eating 10 crude potatoes can bring about a dangerous reaction. By examination, it is physically difficult to eat enough pot to prompt passing. Weed in its normal shape is one of the most secure remedially dynamic substances known to man. By any measure of normal investigation cannabis can be securely utilized inside the administered routine of therapeutic care [Young, et al., 1988].
It is important to note that emerging evidence demonstrates that many patients are turning to cannabis to safely and effectively reduce and replace synthetic antianxiety, hypnotic, soporific and sedative medications after having grown tired of the negative side effects associated with their use. For example, many cannabis buyers club members say they use cannabis as a substitute for prescription narcotics [Gieringer, et al., 1996] and in examinations of 2,480 California patients, Dr. Mikuriya found that 27% reported using cannabis for mood disorders and another 5% used cannabis as a substitute for more toxic drugs [Gieringer, et al., 2002]. It is essential to note that rising proof shows that numerous patients are swinging to cannabis to securely and adequately decrease and supplant manufactured antianxiety, trancelike, soothing and narcotic prescriptions subsequent to having become sick of the negative symptoms related with their utilization. For instance, numerous cannabis purchasers club individuals say they utilize cannabis as a substitute for medicine opiates [Holland, et al., 2010]. As of now, the most complete review at any point led in Canada examining the hindrances medicinal cannabis clients experience while attempting to access their pharmaceutical of decision is in progress. The overview incorporates a few inquiries concerning cannabis as a substitute for both unlawful medications and endorsed pharmaceuticals.

2.1.11. Medicines are used in cultural basis.

American medication utilize regularly happens without social tenets and in an unsupervised setting. It is in fact that Jamaican ladies we examined
had been instructed in a social convention of utilizing maryjane as a medication. What's more, it has been in tea drain and different species and as a remedial substance [Brady, Rätsch et al., 1998].

Note that one can likewise induce the preventive prescription properties of cannabis by making a stride back and rather than concentrating on maybe a couple limit therapeutic conditions for which cannabis might be utilized, perceiving the sheer volume of and generally different constant and intense medicinal conditions and indications for which cannabis has as of now been demonstrated powerful in treating, for which gathering proof emphatically proposes cannabis is viable in treating and for which preparatory research demonstrates cannabis is conceivably successful in treating. These varied and serious medical conditions and symptoms include, but are not limited to, nausea and vomiting, wasting syndromes associated with AIDS and cancer, multiple sclerosis, amyotrophic lateral sclerosis, spinal cord diseases and injuries, epilepsy and other seizure disorders, a variety of chronic pain conditions a variety of movement disorders, glaucoma, a variety of psychiatric disorders, a variety of inflammatory diseases, and various cancers [Fride, Russo, et al., 2006, Grinspoon, Bakalar, Russo, et al., 2005, Guzmán, et al., 2003, Health Canada, et al., 2010, Russo, et al., 2001]. A rational analysis of the body of evidence demonstrating the ability of a single substance, cannabis, to treat or potentially treat such a wide variety of medical conditions and symptoms through its actions on the endo cannabinoid system,
which acts as a bodily homeostatic regulator, very strongly suggests that cannabis likely plays a role in delaying the progression and preventing many illnesses when used properly.

### 2.1.12. Use of Cannabis Medicine

Cannabis has paradoxical effects, having for instance both relaxing and stimulating effects. However, these effects are based upon many factors, including the strain of cannabis, the quality, potency and purity of the strain, number of types and ratios of cannabinoids, number of types and ratios of terpenes, dose and the health, setting, mindset and diet of the user, as well as the user’s experience with and tolerance to the various cannabinoids [Gieringer, et al., 2008, Russo, et al., 2006, 2011].

We comprehend that numerous human services experts are hesitant to prescribe cannabis to their patients because of the potential aspiratory harm from smoking entire cannabis. Or more all smoking cannabis will prompt any arrangement wellbeing dangerous like malignancy and other savage ailment [Guzmán, et al., 2003].

### 2.2. Anxiolytic, Sedative and Hypnotic Activity of Pyrimidinodiazepines

#### 2.2.1. Material and methods

**2. 2.1.1. Animals**

Male Swiss albino mice (20–25 g) were used throughout the study. They were provided standard pellet diet and tap water adlibitum and were
exposed to 12 hours light and 12 hours dark cycle. The animals were acclimatized to the laboratory conditions before experiments. Care of the animals was taken as per guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forests, Government of India.

2. 2.1.2. Drugs

Diazepam hydrochloride (Calmpose injection, Ranbaxy Laboratories, Gurgaon, India) was procured and used as a standard drug and it was diluted with physiological saline to the required strength before use. The test compounds were suspended in 0.3% w/v of sodium carboxymethyl cellulose (CMC) and were administered through oral gavage.

2. 2.1.3. Acute Toxicity Studies

Acute Oral Toxicity (AOT 423) guideline was followed for the study of acute toxicity of the synthesized compounds [Yemitan, O. K. et al., 2005]. Briefly, overnight fasted animals were treated with various concentrations of the test compounds (5, 50, 300 and 2000 mg/kg, bw) by oral route. The animals were observed for any behavioral changes or mortality for first 24 hours. The animals were maintained for another 14 days to check the abnormal changes and mortality. Based on the results, the dose was fixed for pharmacological studies.
2. 2.1.4. Anxiolytic activity-Elevated plus Maze (EPM) Test

To assess the anti-anxiety activity of test compounds, elevated plus maze (EPM) was used [Roy, R. et al., 1986]. The plus-maze apparatus consisting of two open arms (16 × 5 cm) and two closed arms (16 × 5 × 12 cm) having an open roof, with the plus-maze elevated (25 cm) from the floor. Each mouse was placed at the centre of the elevated plus maze with its head facing the open arms. During this 5 minutes experiment, the behavior of the mouse was recorded as: (a) the number of entries into the open arms, (b) average time spent by the mouse in the open arms (average time = total time spent in open arms/number of entries in arms).

Procedure

Animals were divided in to 11 groups, each 6 in a group. The Group I served as control served with vehicle, Group II served as standard served with diazepam at the dose of 0.5mg/kg. Group III - XI the animals were treated with the test compounds at the dose of (5 mg/kg, bw, p.o). The compounds were administered orally using a tuberculin syringe fitted with oral cannula. The dose administration schedule was so adjusted that each mouse was having its turn on the elevated plus-maze apparatus 45 minutes after the administration of the dose. During the entire experiment, the animals were allowed to socialize. Every precaution was taken to ensure that no external stimuli, other than the height of plus-maze could invoke anxiety in the animals.The parameters observed is (a). Time spent in open arm and (b).
Number of arm entries in order to assess the anxiolytic activity. Figure 1

Information of atoms is given in the form Carbon labeled with Arabian numerals, hydrogen labeled with Roman numerals and nitrogen shown in blue colours.

2. 2.1.5. Sedative and Hypnotic Activity-Hole - Board Test

To evaluate the sedative and hypnotic activity of the synthesized compounds, Hole-Board Test was performed [Costa-Campos, L. et al., 2004, Kalueff, A. V. et al., 2004]. The hole-board apparatus comprised a grey Perspex panel (40×40cm, 2.2 cm thick) with 16 equidistant holes (3 cm diameter) in the floor. Photocells below the surface of the holes provided the measure of the number of head dips. The board was positioned 15 cm above the table and was divided with black water-resistant marker into 9 squares of 10×10 cm. Thirty minutes after the administration of the test drug, each mouse was individually placed in the centre of the board (facing away from the observer). During 5 minutes test period, number of head dips was noted.

Procedure

Animals were divided into 11 groups, each 6 in a group. The Group I served as control served with vehicle, Group II served as standard served with diazepam at the dose of 3mg/kg. Group III - XI the animals were treated with the test compounds at the dose of (5 mg/kg, bw, p.o). The test compounds were administered orally using a tuberculin syringe fitted with oral canola.
After 45 minutes of oral administration of the test compounds, the animals were placed in to the center of the perforated board and observed for the period of 5 minutes and the number of head exploration was counted in order to find out the sedative and hypnotic activity.

2. 2.1.6. Locomotor activity

For the purpose of evaluating the spontaneous locomotor activity of the synthesized compounds, actophotometer was used. Actophotometer (24×22×10 cm) (Popular Traders, Ambala) with automatic counting of animal movements in the floor of activity cage was utilized for the present study. Mice were placed individually in activity cage for 5 minutes test period, 45 minutes after administration of test drugs. Locomotor activity was observed in terms of the activity scores.

Procedure

Animals were divided into 11 groups; each group consists of 6 animals. The Group I served as control served with vehicle, Group II animals served as standard which receives diazepam at the dose of 3mg/kg, through intra-peritoneal route. Group III - XI the animals were treated with the test compounds at the dose of (5 mg/kg, bw, p.o). The test compounds were administered orally using a tuberculin syringe fitted with oral cannula. After 45 minutes of oral administration of the various fractions the animals were placed in to the actophotometer. Movement of the animal is recorded by the
light beams present inside the actophotometer for the evaluation of the locomotor activity.

2.3. Results and Discussion
2.3.1. Anxiolytic Activity

The time spent in the open arms by the animals treated with standard drug diazepam and test compounds was significantly increased when compared to the control group which was treated with the same volume of vehicle. Among the test compounds, all the three substituted benzodiazepine series showed significant activity, which shows mild to moderate activity. These compounds do not show any significant difference. When compared to the standard drug which indicates that the compounds are equipotent to the standard drug used i.e., diazepam. The results are shown in the figure 16 and figure 17.

The time spend in the open arm is greatly increased and the number arm entry also gradually increased based on the potency of the test compounds. Since, increases in open arm entry parameters are the most representative indices of anxiolytic activity. The time spent on the central platform appears to be related to decision making or risk assessment, and the total arm entries is a measure reflecting changes in anxiety or in general activity. The interconvulsant, anxiolytic and sedative effects of benzodiazepines and their derivatives are good candidates for enhancing the action of Gama Amino Butyric Acid (GABAA) [Xiao-Liang, Y. et al., 2010].
Benzodiazepines bind to the gamma subunit of GABA<sub>A</sub> receptor, due to which a desired structural modification of the modification of the receptor results, which leads to an increase in GABAA receptor activity [Muhammed, N. et al., 2013].

**Table 6: Antianxiety Activity of Pyrimidine based diazepines**

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Group</th>
<th>Time Spend in Open arms (Sec)</th>
<th>Entries in open arm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>44.33±6.8</td>
<td>4.33±0.86</td>
</tr>
<tr>
<td>2</td>
<td>STD (Diazepam 0.5mg/kg).</td>
<td>163.33±7.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13.5±1.25&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>3</td>
<td>Ben-Me</td>
<td>121.16±11.19&lt;sup&gt;a,e&lt;/sup&gt;</td>
<td>9.2+0.89</td>
</tr>
<tr>
<td>4</td>
<td>Ben</td>
<td>129.16±3.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.16±0.98</td>
</tr>
<tr>
<td>5</td>
<td>Ben-F</td>
<td>128±8.69&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.83±0.75</td>
</tr>
</tbody>
</table>

* N=6; Values are expressed as Mean ± SEM; <sup>a</sup>P<0.001, <sup>b</sup>P<0.01 Vs Control group; <sup>c</sup>P<0.001, <sup>d</sup>P<0.01, <sup>e</sup>P<0.05 Vs Standard group; Data were analyzed by using One way ANOVA followed by Tukey Kramer Multiple comparison Test.

- Ben - 3-phenyl-2, 5, 7, 9-tetraaza-bicyclo [4.3.1] deca-1(9), 2, 4, 6(10), 7-pentaene compound with toluene.
- Ben-F-3-(4-Fluoro-phenyl)-2,5,7,9-tetraazabicyclo[4:3:1]deca- 1(9),2,4,6(10),7-pentaene compound with 1-fluro-4-methyl-benzene.
Figure 16. Anxiolytic activity of Pyrimidine based diazepine derivatives in Time spent in open arms

[Values are expressed as Mean ± SEM; \(^a\)P<0.001, \(^b\)P<0.01 Vs Control group; \(^c\)P<0.001, \(^d\)P<0.01, \(^e\)P<0.05 Vs Standard group; Data were analyzed by using One way ANOVA followed by Tukey Kramer Multiple comparison Test.]

- Ben - 3-phenyl-2, 5, 7, 9-tetraaza-bicyclo [4.3.1] deca-1(9), 2, 4, 6(10), 7-pentaene compound with toluene.
- Ben-F - 3-(4-Fluoro-phenyl)-2,5,7,9-tetraazabicyclo[4:3:1]deca-1(9),2,4,6(10),7-pentaene compound with 1-fluro-4-methyl-benzene
Figure 17. Anxiolytic activity of Pyrimidine based diazepine derivatives
in Number of entries in open arm.

Values are expressed as Mean ± SEM; \(^{a}P<0.001, \(^{b}P<0.01\) Vs Control group; \(^{c}P<0.001, \(^{d}P<0.01, \(^{e}P<0.05\) Vs Standard group; Data were analyzed by using One way ANOVA followed by Tukey Kramer Multiple comparison Test.

2. 3.2. Sedative and Hypnotic Activity

The hole-board test used to assess the sedative and hypnotic potential of the test compounds. This test confirmed the CNS calming nature of the test compounds. The results are shown in the figure 3. The number of hole explore was counted for the Period of 5 minutes and the results revealed that
the control animal was active whereas the head exploration has been largely reduced in the animals treated with the standard diazepam drug at the dose of 4 mg/kg. The test compounds possess various degree of hole exploration values. Diazepam is CNS depressant used in the management of sleep disorders such as insomnia [Brambilla, P. et al., 2003]; these compounds have a binding site on GABA receptor type-A ionophore complex (GABA-A). It decreases activity, moderates excitement and calms the recipient. Since GABAergic transmission can produce profound sedation in mice. The inhibitory action of GABA consists in the opening of chloride channels to allow hyperpolarizing the membrane, leading to CNS depression and resulting in sedative and hypnotic activity. Glutamate and GABA are quantitatively the most important excitatory and inhibitory neurotransmitters, respectively, in the mammalian brain [Brambilla, P. et al., 2003]. Thus, receptors for these two neurotransmitters are regarded as important targets for psychotropic drugs. The activity of the test compound may be due to binding with these receptors.

**Table 7: Sedative and hypnotic activity of Pyrimidine based diazepines**

<table>
<thead>
<tr>
<th>S.NO</th>
<th>Group</th>
<th>Explored Holes During 5 Minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>15.16±1.16</td>
</tr>
<tr>
<td>2</td>
<td>STD (Diazepam 3mg/kg)</td>
<td>3.1±0.015&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>3</td>
<td>Ben</td>
<td>4.5±1.04&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>4</td>
<td>Ben-Me</td>
<td>4.66±0.56&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>5</td>
<td>Ben-F</td>
<td>3.66±0.81&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>
N=6; Values are expressed as Mean ± SEM; $^aP<0.001$, $^bP<0.01$ Vs Control group; $^cP<0.001$, $^dP<0.01$ Vs Standard group; Data were analyzed by using One way ANOVA followed by Tukey Kramer Multiple comparison Test.

- Ben - 3-phenyl-2, 5, 7, 9-tetraaza-bicyclo [4.3.1] deca-1(9), 2, 4, 6(10), 7-Pentaene compound with toluene.
- Ben-F-3-(4-Fluoro-phenyl)-2,5,7,9-tetraazabicyclo[4:3:1]deca-1(9),2,4,6(10),7-pentaene compound with 1-fluro-4-methyl-benzene.

![Bar chart showing explored holes during 5 minutes for different compounds](image)

**Figure 18. Sedative and Hypnotic activity of Pyrimidine based diazepine derivatives**
Values are expressed as Mean ± SEM; \(^{a}P<0.001, \(^{b}P<0.01\) Vs Control group; \(^{c}P<0.001, \(^{d}P<0.01\) Vs Standard group; Data were analyzed by using One way ANOVA followed by Tukey Kramer Multiple comparison Test.

There is evidence for the derivatives of annulated Pyrrolo (1,4) benzodiazepines to exhibit sleep inducing activity and anxiolytic activity almost equivalent to that of standard Diazepam [Jing-Jing, C. et al., 2008]. But our test compounds exhibits better sedative property than standard Diazepamines. The order of sedative activity was, BEN-Me> BEN> BEN-F> standard Diazepam (figure 18). The reason for BEN-Me exhibiting the superior activity could be the presence of + mesomeric functional groups. In this Sedative activity aspect, tetracyclic benzodiazepines like Bretazenil are inferior, when compared with our tricyclic Pyrimidinobenzodiazepines [Kumarasamy, S. et al., 2014].

2.3.3. Locomotor Activity

In the locomotor activity, the mean score before and after treatment were measured and are shown in the figure 19. There is significant difference observed in the mean score before and after treatment with standard and test compounds. After the standard/test drug treatment, the mean score for the diazepam treated group and test compounds treated, shows significant activity when compared to control group and slightly better statistical significance was observed when compared to the standard diazepam. There was very slight
variation among the test compounds and standard and the order of mean score after the treatment was found to be BEN-F>BEN>BEN-Me>Diazepam. The results observed revealed that the compounds significantly reduce the locomotor activity. Locomotor activity is considered as an index of alertness and a reduction is indicative of sedative activity. GABA is the major inhibitory neurotransmitter in CNS and different anxiolytic, muscle relaxant and sedative.

Table 8: Locomotor Activity of Pyrimidine based dizepines

<table>
<thead>
<tr>
<th>S.No</th>
<th>Group</th>
<th>Mean Score Before Treatment</th>
<th>Mean Score After Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>294±6.95</td>
<td>257±15.41</td>
</tr>
<tr>
<td>2</td>
<td>STD (Diazepam 3mg/kg)</td>
<td>277.6±7.78</td>
<td>93.33±7.03&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>3</td>
<td>Ben-Me</td>
<td>250.33±5.68</td>
<td>116.83±10.75&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>4</td>
<td>Ben</td>
<td>252.16±11.95</td>
<td>120.5±11.14&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>5</td>
<td>Ben-F</td>
<td>255.33±14.93</td>
<td>123.66±10.6&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

N=6; Values are expressed as Mean ± SEM; <sup>a</sup>P<0.001, <sup>b</sup>P<0.05 Vs Control group; <sup>c</sup>P<0.001 Vs Standard group; Data were analyzed by using One way ANOVA followed by Tukey Kramer Multiple comparison Test.

- Ben - 3-phenyl-2, 5, 7, 9-tetraaza-bicyclo [4.3.1] deca-1(9), 2, 4, 6(10), 7-pentaene compound with toluene.

Ben-F-3-(4-Fluoro-phenyl)-2,5,7,9-tetraazabicyclo[4:3:1]deca- 1(9),2,4,6(10),7-pentaene compound with 1-fluro-4-methyl-benzene.

![Graph showing Locomotor activity of Pyrimidine based diazepine derivatives]

**Figure 19. Locomotor activity of Pyrimidine based diazepine derivatives**

Values are expressed as Mean ± SEM; \(^{a}P<0.001, \(^{b}P<0.05\) Vs Control group; \(^{c}P<0.001\) Vs Standard group; Data were analyzed by using One way ANOVA followed by Tukey Kramer Multiple comparison Test.]

Hypnotic drugs exhibit their action via GABA [Garcia, D. A. et al., 2006]. Therefore, it is possible that test compounds may act by potentiating GABAergic inhibition in the CNS via membrane hyper-polarization leading to a reduction in the firing rate of critical neurons in the brain or it may be due
to direct activation of GABA receptors by the test compounds. The increase in efficiency of the natural brain chemical, GABAA, by the Benzodiazepines is achieved along with the decrease in excitability of neurons [Garcia, D. A. et al., 2006].

2.4. Conclusion

The pyrimidino diazepines as a very good option for nontoxic drug possessing better anxiolytic, sedative and hypnotic properties than a few of the reported tricyclic compounds. When compared with Diazepam, this Pyrimidine based drugs shows a little lower effective as an anxiolytic drug and better effect as a sedative and hypnotic. The improvement in locomotor activity when compared with diazepam shows this drug is better than Diazepam.
2.5. Reference


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