SYNTHESIS OF BIOACTIVE MOLECULES USING NOVEL METHODS AND EVALUATION OF THEIR BIOLOGICAL ACTIVITIES

Thesis

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By

Jyoti Pandey

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This list is obviously incomplete but allow me to submit that the omission are inadvertent and I once again record my deep felt gratitude to all those who cooperated me in this endeavour.

Pantnagar
August, 2010

(Jyoti Pandey)
Authoress
This is to certify that the thesis entitled “SYNTHESIS OF BIOACTIVE MOLECULES USING NOVEL METHODS AND EVALUATION OF THEIR BIOLOGICAL ACTIVITIES” submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy with major in Agricultural Chemicals and minor in Biochemistry of the College of Post-Graduate Studies, G.B. Pant University of Agriculture and Technology, Pantnagar, is a record of bona-fide research carried out by Ms. Jyoti Pandey, Id. No. 35443, under my supervision and no part of the thesis has been submitted for any other degree or diploma.

The assistance and help received during the course of this investigation have been duly acknowledged.

Pantnagar
August, 2010

(Virendra Kumar)
Chairman
Advisory Committee
CERTIFICATE

We, the undersigned, members of the Advisory Committee of Ms. Jyoti Pandey, Id. No. 35443, a candidate for the degree of Doctor of Philosophy with major in Agricultural Chemicals and minor in Biochemistry, agree that the thesis entitled “SYNTHESIS OF BIOACTIVE MOLECULES USING NOVEL METHODS AND EVALUATION OF THEIR BIOLOGICAL ACTIVITIES” may be submitted in partial fulfilment of the requirements for the degree.

(Virendra Kumar)
Chairman
Advisory Committee

(Anjana Srivastava)
Member

(S. P. Singh)
Member

(Rakesh Mall)
Member

Ex-Officio Member
Head of the Department
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**Abbreviations**

µl  Microliter
BHT  Butylated Hydroxy Toluene
DMSO  Dimethylsulfoxide
DPPH  2,2-diphenyl-1-picryl hydrazyl
EAA  Ethyl Aceto Acetate
FRAP  Ferric Reducing/Antioxidant Power
FT-IR  Fourier Transform Infrared
M.P.  Melting Point
Mwi  Microwave irradiation
NA  Nutrient Agar
NB  Nutrient Broth
NMR  Nuclear Magnetic Resonance
ppm  parts per million
r.t.  Room temperature
RSA  Radical Scavenging Action
SET  Single Electron Transfer
SGI  Seed germination inhibition
TLC  Thin Layer Chromatography
TMS  Tetra Methyl Silane
UV-vis  Ultraviolet visible
Introduction
These days the discipline of natural product synthesis, both total and partial (semisynthesis) is an important field of investigation whose dividends range from new scientific knowledge to practical applications. Theme of natural product synthesis is being utilized for various purposes like synthesis of challenging molecular architecture, finding a cost effective synthesis, development of new synthetic technologies and moreover modifying the structure of a natural product by synthesizing its analogues to enhance its physical and chemical properties in order to improve its potency and selectivity for superior pharmacological or pesticidal properties. These analogues are devoid of problems which are generally associated with the compounds picked up and synthesized randomly. So the classes which have been undertaken in the present work are such that occur in nature. In all the five classes of compounds have been undertaken for synthesis in order to develop novel techniques and catalysts that help make the synthesis of these classes of compounds facile and environmental friendly. The classes taken up for studies are as follows.

**Cinnamic acids amide derivatives**

Substituted cinnamic acids are widely distributed in nature and are found in coffee beans, olives, propolis, fruits and vegetables. They are found usually as simple derivatives including amide, esters, sugar esters and glycosides (Kavitha et al., 2000) or in rather more complex forms such as rosmarinic acid (dimer), chlorogenic acid (quinic ester of
caffeic acid), lithospermic acid (trimer), verbascoside (heterosidic ester and glycoside of dihydroxyphenethylethanol and caffeic acid) and the flavonoid linked derivatives (*Macheix et al., 1990*).

Cinnamic acid analogues are found to exhibit antibacterial, antiviral, anti-inflammatory, antiatherosclerotic, antioxidative, antiproliferative, immunostimulatory, neuroprotective properties and allelochemical effects (*Jia et al., 2006*). Cinnamic acid derivatives have not been studied systematically for their herbicidal activity, the first report of systematic study of cinnamic acid derivatives for herbicidal activity was successfully published from our laboratory (*Vishnoi et al., 2009*).

**β-Benzoylacrylic acids and their ester analogues**

Aryl and heteroaryl substituted (E)-4-oxo-4-phenylbut-2-enoic acid derivatives represent an important class of compounds with diverse pharmacologic activities including antiulcer, cytoprotective, kynurenine-3-hydroxylase and human cytomegalovirus protease inhibiting activity (*Berkes et al., 2007*). 4-oxo-4-phenylbut-2-enoates exhibit a wide spectrum of pharmacological activities including anticancer, antimicrobial, antifungal and antagonistic (*Trifonov et al., 2007*). These compounds have not been studied for herbicidal activity.

**Benzodiazepines derivatives**

Benzodiazepines are important compounds because of their pharmacological properties (*Smalley, 1979*). 1, 5-Benzodiazepines have been extensively used as anticonvulsant, antianxiety, analgesic, sedative, antidepressive, hypnotic and anti-inflammatory agents.
Benzodiazepine derivatives are also commercially used such as dyes for acrylic fibers. In the last decade, the area of biological interest of 1, 5-benzodiazepines has been extended to several diseases such as cancer viral infection and cardiovascular disorders (Di Braccio et al., 2001).

In particular, 1, 5-benzodiazepines are useful precursors for the synthesis of fused ring benzodiazepine derivatives such as triazolo, oxadiazolo, oxazino, and furano benzodiazepines (Aversa et al., 1986). Due to their wide range of applications, these compounds have received a great deal of attention in connection with their synthesis. Their utilities were fully realized in the process of drug discovery and the total synthesis of complex natural products. Although a great number of such useful reactions have been reported, the development of a novel multi-component reaction is still important in the fields of medicinal and organic synthesis. Despite their importance from a pharmacological, industrial and synthetic point of view, comparatively few methods for their preparation are reported in the literature.

In view of the pharmaceutical importance and diverse biological activities displayed by benzodiazepine derivatives, there is need for developing new methods for the synthesis of this class of compounds.

**Benzimidazole derivatives**

Benzimidazoles represent one of the biggest groups of heterocyclic compounds and have attracted a great deal of interest over the decades due to their wide and potent biological activities (Alamgir et al., 2007). Benzimidazole moiety is a core structure in various synthetic
pharmaceuticals displaying a broad spectrum of biological activities including antiulcer, antitumor, antiviral, anti-inflammatory, analgesic, anti-histamine, antioxidant, anti-proliferative, anti-allergic, anti-kinase and anti-cancer activities. They are also inhibitors of photosynthesis, aldose reductase and antagonist of neurotransmitter receptors. They also are found to exhibit appreciable herbicidal activity.

There are many drugs based on benzimidazoles currently in the market such as omeprazole (proton pump inhibitor), pimobendan (ionodilator), and mebendazole (anthelmintic) *(Alamgir et al., 2007).*

Most of the described methods for the synthesis of benzimidazoles make use of volatile organic solvents and involve solid-phase synthesis via o-nitroanilines or the condensation of o-phenylenediamines with carboxylic acid derivatives, aldehydes and aryl halides. More recently, cleaner protocols have been described, including solvent-free conditions and the use of water and ionic liquid as green solvents *(Dudd et al.,* 2007).
However, most of these protocols use expensive and toxic reagents and/or long reaction times. Thus the development of clean, general, and selective routes to synthesize benzimidazole, including the use of new catalysts, alternative or non-solvents, and non-classical energy sources continues to be an important field of research.

**β-acetamido keto esters**

Acetamido carbonyl compounds are important as these exhibit diverse biological and pharmaceutical properties \((\text{Casimir et al., 1995})\) and are used in the preparation of important compounds examples being the use in preparation of 1, 3-aminoalcohols, antibiotic nikkomycin or neopolyoximes \((\text{Kobinata et al., 1980; Dahn et al., 1976}).\)

![Nikkomycin B](image)

Therefore, the synthesis of β-acetamido carbonyl compounds continues to be a challenging endeavor. Several strategies have been developed for the preparation of β-acetamido ketones and the best known method for the synthesis of these compounds is the Dakin-West reaction \((\text{Dakin and West, 1928})\) discovered in 1928, which is exactly the condensation between amino acid and acetic anhydride in the presence of a base providing the acetamido ketones.
Various catalyst as CoCl$_2$, montmorillonite K-10 clay, silica sulfuric acid, ZrOCl$_2$.8H$_2$O etc for the synthesis of $\beta$-acetamido carbonyl compounds by have been used for reports are in literature (Bhatia et al., 1994). The use of organo catalyst and other environmentally benign catalyst is required to make the synthesis of this class of compounds facile and economic.

Keeping in view the diverse biological activities of afore mentioned classes of compounds, the primary goal of the work presented in this thesis is to develop new techniques/catalysts for the synthesis of benzodiazepines, benzimidazoles and $\beta$-acetamido esters as well as to study the biological activities of the synthesized compounds. Besides cinnamic acid amide derivatives and $\beta$-Benzoylacrylic acids ester analogues have synthesized by known methods in view of their potential and less studied herbicidal activity.

1. To develop new techniques/catalysts for the synthesis of 1,5 benzodiazepines, benzimidazoles and $\beta$-acetamido esters.
2. To synthesize cinnamic acid amide derivatives and 4-oxo-4-phenyl-2-butenoic ester analogues.
3. Spectral analysis of the synthesized compounds using UV, IR and NMR spectroscopic techniques to elucidate their structure.
4. Screening of synthesized cinnamic acid amide derivatives and 4-oxo-4-phenyl-2-butenoic ester analogues for herbicidal activity.
5. Screening of synthesized benzodiazepine derivatives for antioxidant, antibacterial and pharmacological activity.
Review of Literature
2.1 Cinnamic acid amide derivatives

Cinnamic acid amide derivatives have been prepared from substituted cinnamic acids and aryl/alkyl amines. Various methods for the synthesis of cinnamic acids are reported in literature. Few methods for amide formation are available in literature. The review of methods for preparation of substituted cinnamic acids and amides is given.

Cinnamic acid can be prepared by the reduction of phenyl propiolic acid with zinc and acetic acid or heating benzal malonic acid, by the condensation of ethyl acetate with benzaldehyde in the presence of sodium ethylate or by the so-called “Perkin reaction”, the latter being the method commonly employed (www.encyclopedia.jrank.org).

The microwave irradiation shortened the reaction time of the Perkin reaction by 60-fold over classical heating. Cesium salts (acetate, carbonate, fluoride) with a small amount of pyridine were found to be the best catalysts under all conditions tested (Veverkova et al., 1999).

Cinnamic acids have been prepared in moderate to high yields by a new direct synthesis using aromatic aldehydes and aliphatic carboxylic acids, in the presence of boron tribromide as reagent, 4-dimethylaminopyridine (4-DMAP) and pyridine (Py) as bases and N-methyl-2-pyrolidinone (NMP) as solvent, at reflux (180-190°C) for 8-12 hours (Chiriac et al., 2005).
A simple, mild and environment-friendly procedure has been developed for Knoevenagel condensation between aromatic aldehydes or ketones and malonic acid in the presence of tetrabutylammoniumbromide and K$_2$CO$_3$ under microwave irradiation in water. The products are obtained in excellent yields and are in a state of high purity (Gupta and Wakhloo, 2007).

A convenient, inexpensive and efficient synthesis of cinnamic acids by reacting benzaldehyes and malonic acid in acetic acid and piperidine is reported under microwave irradiation which utilizes short reaction time ranging 5-7 min. depending upon the nature of groups present at the phenyl ring (Sharma et al., 2003).

A procedure for Doebner condensation to synthesize α, β-unsaturated carboxylic acid using ionic liquids as environmentally benign media has been developed by Jiang et al. (2009) Ionic liquids [Bmim]BF$_4$ and [Bpy]BF$_4$ were employed as environmentally benign media in Doebner condensation. The good result showed that [Bmim]BF$_4$ and [Bpy]BF$_4$ were efficient media for Doebner condensation, which could be recycled easily. The highest yield could reach 93% and 90% in [Bmim]BF$_4$ and [Bpy]BF$_4$, respectively.

Trans cinnamic acids were obtained in near quantitative yield when various aromatic aldehydes were treated with malonic acid along with 1, 8-bis(dimethyl amino) naphthalene called proton sponge on the surface of graphite by microwave irradiation. This method has some advantages in terms of simplicity of performance, avoids the need for
extensive purification, solvent free workup, inexpensive starting materials and good yields of the trans cinnamic acids (Khabazzadeh et al., 2007)

Polystyrene-IIDQ, (N-isobutoxycarbonyl-2-isobutoxy-1, 2-dihydroquinolone) a polymer-supported coupling reagent, was synthesized in the three steps from Merrifield resin in 86% overall conversion. This reagent efficiently coupled carboxylic acids to amines in good yields and high purities, required no pre-activation step, and was tolerant of the order of reagent addition. PS-IIDQ was observed to be more efficient than polymer supported carbodiimides (PSEDC and PS-DCC) and gave higher yields than HATU (O-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate) for general amide bond formation, including the coupling of anilines and hindered substrates. When evaluated with five carboxalic acids and nine amines (including anilines and secondary amines) PS-IIDQ gave an average isolated yields of 73% (Valeuruy and Bradley 2007).

Maki et al., (2007) reported that N-alkyl-4-boronpyridinium halides are effective not only for amide condensation between equimolar mixtures of carboxylic acids and amines but also for the esterification of carboxylic acids in alcohol solvents. Furthermore, perchlorocatecholborane is more effective than areneboronic acid. In addition, Lewis acid assisted Bronsted acid (LBA), which is prepared from a 1:2 mixture of boric acid and tetrachlorocatechol, is effective for the Ritter reaction from alcohols and nitriles to amides.
Decarboxylation of substituted cinnamic acids having a hydroxyl group at the *para* position gave predominantly the corresponding styrene derivatives in the presence of base with microwave heating. The reaction was conducted either under solvent-free conditions or using a solvent. When a primary amine was used as a base, the yield of the styrene or amide depended on the substituent of the cinnamic acid (Nomura *et al.*, 2005).

### 2.1.1 Biological activities of cinnamic acid amide derivatives

Cinnamic acid amides have been found to exhibit diverse biological activities. Important one are reviewed.

Thirty five derivatives of cinnamic acids and related compounds were treated for inhibition against phenylalanine ammonia lyase (PAL) derived from sweet potato, pea and yeast. Caffic and gallic acids showed inhibition against PAL originating from higher plants, but not against PAL. In contrast, yeast PAL was specifically inhibited by p-hydroxycinnamic acid and p-hydroxybenzoic acids. The result suggest that caffeic and gallic acid may act as regulatory substances in phenylprpanoid metabolism in higher plants. Inhibition experiments with synthetic cinnamic acid derivatives have related that the presence of hydrophobic aromatic ring, α, β-double bond and carboxyl group is essential for inhibitory activity. 2-Napthoic acid which fulfils these structural requirements showed a strong inhibition. O-chloro cinnamic acid, one of the strongest inhibitors found in this study, showed an inhibitory effect on the growth of the roots of rice seedings (Sato *et al.*, 1982)
A series of amides of caffeic acid were synthesized and evaluated for their antiplatelet and anti-oxidative activities by Hung et al. (2005). N-(2-Bromo-phenyl)-3-(3, 4-hydroxy–phenyl)-acrylamide (Figure. 2.1) exhibited potent activity against arachidonic acid induced (AA) platelet aggregation, comparable with invalid caffeic acid. It was also found that caffeic anilides displayed more potent antioxidative activity in the radical scavenging activity assay than trolox and vitamin E.

Figure 2.1: Structure of caffeic anilides

Heureux et al. (2005) synthesized Cinnamic acid amide derivatives, (S, E)-n-1-[3-(6-fluopyridi-3-yl)phenyl]ethyl-3-(2-fluorophenyl) acrylamide as a potent KCNO₂ potassium channel opener. Among this series some of derivatives were found to exhibits balanced potency and efficacy.

A series of 3-(3, 4-dichlorophenyl)acrylamide derivatives were synthesized, and their inhibitory activities towards hLGPα were evaluated (Onda et al., 2008). Among the derivatives, (2E, 2OE)-N,NO-pentane-1, 5-diylbis[3-(3, 4- dichlorophenyl)acrylamide inhibited hLGPα with an IC₅₀ value of 0.023 IM. An X-ray crystallographic study of the enzyme -6c complex showed that the inhibitor is bound at the dimer interface site, where the 3, 4dichlorophenyl moiety interacts hydrophobically with the enzyme.
Figure 2.2: KCNO₂ Potassium channel opener cinnamic acid amide derivatives

Figure 2.3: 3-(3, 4-dichlorophenyl)acrylamide derivatives
Cinnamic acid derivatives (thioesters and amides) (Figure. 2.3) with 4-hydroxy and 4-alkoxy groups were synthesized and investigated for in vitro activities of these compounds. Among them some displayed good in vitro antibacterial activity, such as (E)-N-(2-acetamidoethyl)-3-[4-(E)-3, 7-dimethylocta-2, 6-ienyloxy]phenyl]acrylamide that showed a minimum inhibitory concentration of 0.1 g/mL (0.26 1M) against mycobacterium tuberculosis H37Rv (Yoya et al., 2009).

2.1.2 Allelochemicals as natural herbicides: An approach towards weed management

Austrian plant physiologist, Hans Mollish (1937), coined the term allelopathy in 1937. Rice (1984) defined allelopathy as the effects of one plant (including microorganisms) on another plants through the release of a chemical compounds into the environment. This definition includes both stimulatory and inhibitory effects, depending on the concentration of the compounds Rice (1995) discussed the applied aspect of allelopathy in natural weed management. Cinnamic acid derivatives were found in wheat (Lam et al., 1992) and Oxalis pescaprae (Greca et al., 2009) as potent allelochemicals. Taking cues from these investigation in the present study cinnamic acid amide derivatives have been tested for herbicidal activity against barnyard grass (Echinochola colona). Allelochemical effects of various class of compounds revealed by different crops/plant species their applications and limitations in weed management have been briefly reviewed.
Putnam (1983, 1988) termed allelochemicals as nature’s own herbicides. Duke et al. (2000) discussed that natural compounds have several benefits over synthetic compounds. For example, natural compounds may have novel structure due to diversity of molecular structure. Unlike a high proportion of synthetic pesticides, natural compounds are mostly water soluble and non-halogenated molecules. Natural products relatively have short half-life and therefore considered safe of environmental toxicology standpoint (Duke et al., 2002). There is a need to discover new herbicides since the number of herbicides resistant weed is increasing and conventional synthetic herbicides are becoming less and less effective against the resistant weed biotypes (Heap, 1997; Itoh et al., 1999; Bhowmik 2000; Fischer et al., 2000 and Duke et al., 2002).

Duke et al. (2000) discussed the approaches needed to select sources of natural products for the discovery of potential herbicides. These are: (1) obtain pure compounds from other laboratories, (ii) obtain previously unexploited biological material and (iii) employ ethnobiological and/or chemical ecology data to select material. Understanding the ecological and physiological aspects of a compound has two main approaches: (i) allelopathy, e.g. sorgoleon, and (ii) sequestering of a compound by a species to avoid autotoxicity, e.g. artemisinin (Duke et al., 2000).

Rotational or smoother crops such as rye (Secale cereale L.), Wheat (Triticum aestivum L.), buckwheat (Fagopyrum esculentum
Moench.), black mustard (*Brassica nigra* (L.) Koch), sorghum-sudangrass hybrid (*Sorghum bicolor* (L.) Moench x *S. sudanense* (Piper) Stapf) are used in weed management. Allelopathic compounds reported to play role in weed management are: allyl isothiocynate (black mustard), fatty acids (buck wheat), isoflavonoids and phenolics (clovers, *Trifolium* spp.; sweet clover, *Melilotus* spp.), phenolic acids and scopolectin (oat, *Avena sativa* L.), hydroxamic acids (cereals), phenolic acids, dhurrin, sorgoleone (sorghum, sudangrass) Crop residue can provide selective weed control through their physical presence on the soil surface through the release of allelochemicals (*Inderjit and Keating, 1999*).

*Inderjit et al. (2001)* studied the interaction between wheat and *Lolium perenne* L. (perennial ryegrass). These authors observed root inhibition of perennial ryegrass dependant on the density of wheat seeds. Lemerle *et al.* (2001) investigated the competitive advantages of 12 wheat varities against *Lolium rigidum* Gaudin (rigid ryegrass). It was found that variation in crop grain yield was mainly due to variety and environmental effects. These authors stressed the need of introducing greater genetic variability into wheat to enhance competitiveness. Hydroxamic acids (4-hydroxy-1, 4-benzoxazin-3-ones) are present in wild and cultivated members of the family Poaceae (ribe Triticeae) (*Niemeyer et al., 1992*).

Significant amount of literature is available on the differential production of hydroxamic acids in cereals. The main hydroxamic acids...
reported from cereals are DIBOA and DIMBOA (2, 4-digydroxy-7-methoxy-1, 4-benzoxazin-3-one); their distribution with cultivated Poaceae, however, is uneven (Niemeyer, 1988). While wheat has both DIMBOA and DIBOA, rye contains only DIBOA.

These are certain limitation associated allelochemicals as natural herbicides. High cost, limited activity and selectivity restrict the use of natural herbicides e.g. maize gluten (Bhowmik, 1992) and pelargonic acid (Duke et al., 2001). Natural herbicide may also be toxic to ontarget organism. For example, a natural compounds, alpha-terthienyl, isolated from the roots of the common marigold (Inderjit and Bhowmike, 2002). In addition to its herbicidal activity, alpha-terthienyl was also toxic to non-target species. Toxicity to non-target species is one of the main reasons which limit the use of natural compounds as herbicides.

Duke et al. (2000) discussed some shortcomings of employing natural products as herbicides. These are: (i) many natural compounds are extremely expensive to synthesize, e.g. in spite of the excellent herbicidal activity of tentoxin, it is too expensive to manufacture , (ii) natural products have generally short environmental half lives, and (iii) some natural compounds have potential mammalian toxicity and are carcinogenic; for example, AAL-toxin and fumonisin are toxic to mammalian cells. Some natural products may have other problems, like allergy. Sorgoleone, for example, is reported to cause dermatitis (Inderjit and Bhowmike, 2002).
2.2 4-oxo-4-phenyl-2-butenoic esters

There are few reports in literature for the synthesis of substituted benzoacrylic esters or 4-oxo-4-phenyl-2-butenoic esters. The compounds 4-oxo-4-phenyl-2-butenoic acids have been prepared by Friedel-Craft’s acylation of substituted benzene with maleic anhydride (Grummitt et al., 1955). Carbons of substituted benzene tend to become highly nucleophilic, thereby facilitating an attack by an incipient electrophile (Tarakeshwar et al., 1998). Substitued phenacyl bromides have been prepared by bromination of substituted acetophenones (Furniss et al., 1980). Esters have been prepared by using substituted benzoylacrylic acids and substituted phenacyl bromides (Katritzky et al., 2001).

2.2.1 Biological activity of 4-oxo-4-phenyl-2-butenoic esters

The literature available regarding biological activities of 4-oxo-4-phenyl-2-butenoic esters is scanty. Biological activities of close derivatives of 4-oxo-4-phenyl-2-butenoic acids have been reviewed. Antonio et al. (1997) have reported 4-phenyl-4-oxo-butenoic acid derivatives as inhibitors of kynurenine-3-hydroxylase for the prevention and treatment of a neurodegenerative disease wherein the inhibition of such an enzyme is needed.

Butenoic acid esters exhibit antagonistic activity against phospholipase A2, inhibition activity against snake venom PLA2 and activity against neoplastic He La cells (Nuhn et al., 1999 and Juranic et al., 1999). The esters of substituted 4-oxo-2-butenoic acids and more particularly certain moieties of these novel compounds and their
close derivatives have been found as bacteriostatic agents for humans or non-humans against *Mycobacterium tuberculosis* ([Miles et al., 2004]). Butenoates exhibit a wide spectrum of pharmacological activities including anticancer, antimicrobial, antifungal, antagonistic etc ([Trifonov et al., 2007]). The esters of 2- isopropyl-3-methyl-3-butenoic acid showed insecticidal activity to brown plant hopper and green rice leafhopper ([Furuhata et al., 1987]).

2.3 Benzodiazepines

The benzodiazepines (BZDs), are the most frequently prescribed drugs for the pharmacotherapy ([Sternbach et al., 1968; Sternbach, 1971; Martin, 1987]). The first benzodiazepine, chlordiazepoxide (Librium), was discovered accidentally by Leo Sternbach in 1955, and made available in 1960 by Hoffmann-La Roche, which has also marketed diazepam (Valium) first introduce benzodiazepines as a drugs ([Shorter, 2005; Sternbach 1971]). The term benzodiazepine, is the chemical name for the heterocyclic ring system. It is a fusion between the benzene and diazepine ring systems ([IUPAC, 1993; Moss, 1998]). According to Hantzsch–Widman nomenclature, a diazepine is a heterocycle compound with two nitrogen atoms, five carbon atom and the maximum possible number of cumulative double bonds. The "benzo" prefix indicates the benzene ring fused onto the diazepine ring ([IUPAC, 1993, Moss, 1998]).

Benzodiazepines are bicyclic heterocyclic compounds having a benzene nucleus fused to a seven-membered ring containing two
nitrogen atoms (Essaber et al., 1999), due to this benzodiazepine skeleton is known in medicinal chemistry as a “privileged structure” (Horton et al., 2003).

![Chemical structures](image)

**Figure 2.4**: Some pharmacologically important benzodiazepines

In addition, 1, 5-benzodiazepines are used as starting materials for the synthesis of various heterocyclic ring compounds (Essaber et al., 1999; El-Sayed et al., 1999). Due to the widespread applications of benzodiazepines, the synthesis involving inexpensive and environmentally safe catalytic systems is the great challenge. Several methods were documented for the synthesis of 1, 5-benzodiazepines which include the condensation of o-phenylenediamines (OPD) with α, β-unsaturated compounds (Ried and Stahlofen, 1957) α-haloketones or with ketones (Ried and Torinus 1959), catalyzed by NaBH₄ (Morales, 1986), Polyphosphoric acid on SiO₂ (Jung et al., 1999), MgO-POCl₃ (Balakrishna and Kaboudin, 2001), Yb(OTf)₃ (Curini, et al., 2001), SmI₂ (Chen et al., 2001), Al₂O₃/P₂O₅ under microwave
(Kaboudin and Navace, 2001), acetic acid-MWI (Pozarentzi et al., 2002), ionic liquids such as Amberlyst- 15®/ [bmim]PF6 (Yadav et al., 2002), [bbim]Br (Jarikote et al., 2003), solid super acid sulfated zirconia (Reddy et al., 2003), Ag₃PW₁₂O₄₀ (Yadav et al., 2004), Sc(OTf)₃ (De and Gibbs 2005), In-Br₃ (Yadav et al., 2005), Molecular Iodine (Chen and Lu, 2005), Zirconia solid acid (Benjaram, et al., 2005), Zeolite (Hegedus et al., 2005), ceric ammonium nitrate (CAN) (Varala et al., 2006), NBS (Kuo et al., 2006), (CH₃)₂S/Br₂ (Das et al., 2006), p-toluenesulfonic acid (Pasha and Jayashankara, 2006), ZrCl₄ (Reddy, et al., 2007), Ga(OTf)₃ (Pan et al., 2008), And boric acid (Gholap and Tambe, 2008),

However, many of these methods are associated with several disadvantages such as long reaction time, drastic reaction conditions, very expensive reagents, low yield, tedious work-up procedures and occurrence of several side products. The main disadvantage is that the catalysts are destroyed during the work-up procedure and cannot be recovered or reused. Therefore, it is important to find a simple, cheap, recoverable and/or reusable and selective catalyst for the synthesis of 1, 5-benzodiazepines.

The use of microwave irradiation (MWI) is well known for the synthesis of a variety of compounds (Kobayashi et al., 1967; Kobayashi and Matsuda, 1968) where the chemical reactions are accelerated because of selective absorption of microwaves by polar molecules. The coupling of MWI together with solid-supported reagents
under solvent-free conditions (Varma and Saini 1997; Kidwai 2001; Kidwai et al., 2002) provides unique chemical processes with special attributes such as enhanced reaction rate, higher yield, greater selectivity, and ease of manipulation (Kidwai and Venkataramanan, 2001). The limitations of microwave-assisted reactions in solution, namely the development of high pressure and the need for specialized vessels, are circumvented via this solid state strategy which enables organic reactions to occur rapidly in open vessels at atmospheric pressure (Varma and Dahiya, 1998).

2.3.2 Biological activities of benzodiazepines

2.3.2.1 Antioxidant Activity

Oxidation involves the transfer of electrons from one atom to another. The oxidized molecule loses an electron while the receiving molecule is reduced. Oxidation reactions are an essential part of aerobic metabolism, since oxygen is an electron acceptor in the electron flow system that produces energy (Lee et al., 2003). Oxidation becomes a problem when reactions become uncoupled and free radicals are formed. Free radicals are molecules that are highly reactive and unstable because they contain an unpaired electron. Electrons are most stable in pairs, hence the free radicals tend to attach to or receive hydrogen ions from molecules with lower bond dissociation energy like unsaturated fatty acids or phenolic antioxidants. Reactive oxygen species (ROS) are oxygen-centered free radicals. Examples of the ROS...
species are superoxides \((O_2^{•-})\), peroxyls \((ROO•)\), alkoxyls \((RO•)\), hydroxyls \((HO•)\) and nitric oxides \((NO•)\) (Pietta, 2000).

ROS species have been linked to cell membrane damage, protein and enzyme modifications, and DNA damage within the body (Ramarathnam et al., 1995). These types of reactions are also thought to accelerate aging and to play a causative role in a variety of degenerative diseases such as cancer and heart disease. ROS are not only involved in reactions in the body, but also take part in autoxidation occurring in food materials. Autoxidation of lipids is of concern to the food industry because it leads to the development of off flavors, which shorten the acceptable shelf life or reduce the sensory quality of food. Multiple factors affect the rate at which lipid oxidation occurs in foods including fatty acid composition, oxygen concentration, temperature, surface area, moisture, pro- and anti-oxidants available, and radiant energy in the system (Nawar, 1985).

Synthetic antioxidants are used in commercial processing of foods and other materials to delay oxidation processes. There are two types of antioxidants, preventative and chain-breaking. Preventative antioxidants can chelate metals and/or decompose peroxides in order to stall the initiation of free radicals before oxidation (Nawar, 1985). Polyphosphates, citric acid, citrate esters, and ethylenediaminetetraacetic acid (EDTA) are common antioxidant synergists and are generally used to chelate metal ions, which are possible catalysts of oxidation. Eliminating trace metals and peroxides is difficult in foods and in the body; therefore, focus has
been on the action of free radical acceptors. The mechanism of antioxidants in the autoxidation cycle is molecule dependant. A free radical acceptor, like hydroquinone or resveratrol, reacts with the ROO• species during propagation instead of the R• species during initiation. Hydroquinone is a phenolic compound and has the ability to donate both hydrogen ions from the two hydroxyl groups attached to the phenolic ring structure. (Equation 1.0) shows how hydroquinone reacts with peroxy radicals to form stable semiquinone resonance hybrids (Nawar, 1985). Semiquinone radicals may react with each other to produce more quinone species (Equation 1.1), or react with more peroxy radicals (ROO•) to further inhibit free radical propagation. Flavonoids and phenolic compounds have the same ability to donate hydrogen ions while maintaining a stable structure.

**Equation 1.0.**

\[
\text{ROO}^\cdot + \text{Hydroquinone} \rightarrow \text{ROOH} + \text{Semiquinone Radicals}
\]

**Equation 1.1-**

\[
\text{Semiquinone Radicals}
\]
The example of hydroquinone as an antioxidant describes the first pathway of antioxidant activity, the hydrogen atom transfer. Simplistically displayed in equation 2.0, a peroxy radical (ROO•), or radical formed during oxidation, is quenched by the donation of a hydrogen atom by the aromatic ring antioxidant (ArOH).

**Hydrogen Atom Transfer**

\[
\text{ROO}^\bullet + \text{ArOH} \rightarrow \text{ArO}^\bullet + \text{ROOH} \quad \text{(Equation 2.0)}
\]

The aromatic radical (ArO•) remain stable by aromatic ring stabilization and hydroperoxide is produced. As an antioxidant is applied to a system, it will inhibit propagation of the peroxy radical (ROO•) by hydrogen atom transfer, and will also follow a single electron transfer pathway for antioxidant activity.

**Single Electron Transfer**

\[
\text{ROO}^\bullet + \text{ArOH} \rightarrow \text{ROO}^- + \text{ArOH}^+\bullet \quad \text{(Equation 3.0)}
\]
\[
\text{ROO}^- + \text{ArOH}^+\bullet \rightarrow \text{ArO}^\bullet + \text{ROOH} \quad \text{(Equation 3.1)}
\]

This mechanism involves the antioxidant molecule (ArOH) donating an electron to stabilize the peroxy radical (ROO•) and create a peroxy radical with a negative charge (ROO-) due to an over abundance of electrons on the oxygen. With a positively charged aromatic free radical available, the opposite charges attract, and a proton (H+) is donated to quench the charged hydroperoxide. Logically, this pathway plays a minor role in physiological conditions, yet the hydrogen atom transfer pathway will dominate in most cases (Wright, 2001). The single electron transfer
model has been shown to have strong solvent dependence because of stabilization of charged species within certain solvents.

**DPPH (2, 2-diphenyl-1-picrylhydrazyl) Method**

The hydrogen atom transfer predominates as the mechanism for antioxidants to quench free radicals and inhibit peroxyl radical oxidation. Quenching and preventing radical propagation is mimicked in the 2,2- diphenyl-1-picrylhydrazyl (DPPH) assay *(Blois, 1958).* The DPPH method allows a direct investigation of the ability for the extract or antioxidant to donate hydrogen and/or electrons to quench the DPPH radical. As the radical is quenched, the color of the solution changes from a deep purple to a light yellow and the absorbance at 515 nm decreases. *Brand-Williams et al. (1995)* found that certain antioxidant compounds elicited different reaction kinetics with DPPH•. In the study, antioxidants such as BHT and protocatechuic acid did not reach steady state, or the reaction endpoint, until three and two hours respectively, whereas compounds like ascorbic acid, isoascorbic acid, and isoeugenol achieved steady state within one minute. At steady state, the DPPH• reaction has been shown to have a stoichiometric correlation with the quantity of antioxidant present. Caffeic acid, gentisic acid and gallic acid exhibited the highest antiradical activity with stoichiometry of 4.54, 5.6, and 6.25 reduced DPPH• molecules per molecule of antioxidant respectively while one molecule of phenol, ascorbic acid, α-tocopherol, and BHT reduced <1, 1.85, 2, and 2.63 molecules of DPPH• respectively *(Brand-Williams et al., 1995).*
According to Brand-Williams, there are three ways to explain the different efficiencies of monophenolic compounds in reducing one DPPH•. One mechanism involves the delocalization of an electron onto the parasubstituted group of the molecule prior to the donation of second hydrogen to reduce DPPH•. Another pathway involves the dimerization between two phenoxy radicals in which two hydroxyl groups would be regenerated through an intramolecular transfer of H•, consequently reacting further with DPPH•.

![Figure 2.5: Optimized structure of DPPH radical (C, dark grey; H, light grey; N, blue; O, red) (Velkova et al., 2007).](image)

Generally there exist two mechanism for antioxidant to scavenge DPPH. The first is a direct H atom-abstraction process (eq 1), and the second is a proton concerted electron-transfer process (eq 2) (Wang and Zhang, 2003).
DPPH• + RXH $\rightarrow$ DPPHH + RX• \hspace{1cm} \text{(Equation 4.0)}

DPPH• + RXH $\rightarrow$ DPPH⁻ + RXH•+
$\rightarrow$ DPPHH + RX• \hspace{1cm} \text{(Equation 4.1)}

In which, X represent O, N, S or C. The first pathway is governed to large extent by X-H bond dissociation enthalpies (BDEs) of RXH and DPPHH. Only if the BDE of the former is lower than that of the latter, the reaction is permitted. While, the second pathway is determined by ionization potentials (IPs) of RXH and DPPH•. The prerequisite for the reaction is that the IP of RXH is lower than that of DPPH•.

The purple coloured DPPH has a strong characteristic absorption at 517 nm and can undergo reaction with hydrogen donating antioxidant compounds to yield the stable yellow DPPH-H molecule easily monitored with UV spectroscopy (Prior et al., 2005).

2.3.2.2 Pharmacology properties of benzodiazepines

**Benzodiazepine receptor (BDZ-R)**

In 1977 experiments suggested that the BDZs are bound to specific receptors in the membranes of rat brain cells (Mohler and Okada, 1977; Squires and Braestrup, 1977) which are closely related (allosterically) to a GABA receptor and to a chloride ionophore channel. In contribute to discrete localization of opioid receptors, benzodiazepine receptors are distributed mainly in CNS. This is evidenced from behavioral and electrophysiological studies that benzodiazepines interact at many levels of the brain to elicit their effect (Tallman et al.,...
Gamma-aminobutyric acid (GABA) is the most abundant neurotransmitter in the central nervous system (CNS) and GABA receptors are widely distributed throughout the brain with high concentrations in the cortex and limbic system. Benzodiazepines bind to the GABA$_A$ receptor, reducing the quantity of GABA required to open the chloride channel, hyperpolarise the neuron and inhibit neurotransmission. In contrast to drugs such as barbiturates, that bind at other sites on the GABA receptor, benzodiazepines cannot directly open the chloride channel, rendering them safer in overdose (Nutt and Malizia, 2001).

Tallman Tallman et al (1980) supported the idea that this binding site may be the means through which the BDZs produce their pharmacological response. The benzodiazepine receptor (BZR) is a cell-surface GABA receptor/chloride ion channel complex. Which can bind either classical benzodiazepines or several other compounds with very different structure. The interaction of these compounds can produce an intrinsic activity ranging from full agonists as sedative-hypnotic, anxiolytic, anticonvulsant and mayorelaxant to antagonists devoid of pharmacological efficacy or to inverse agonist with proconvulsant and anxiogenic properties. Considerable efforts have been directed towards interaction assuming that agonist, antagonist and inverse agonists bind to the same binding domain of the BDZs.

**Mechanism of action:** Benzodiazepines exert their effect by interacting with inhibitory neurotransmitter receptor directly activated by GABA.
GABA receptors are membrane bound protein that can be divided into into GABA_A and GABA_B (Admin and Weiss, 1993). Inotropic GABA_A receptor composed of 5 subunits, that coassemble to form an integral chloride channel. GABA_B receptor (Metabotropic) are made up of single peptide with 7 transmembrane domain coupled with G-protein for their signal transduction. Benzodiazepines acts at GABA_A receptor but not at GABA_B i.e. binding to a specific site is distinct. Benzodiazepines increase the amount of chloride current generated by GABA_A receptor activation. GABA_A receptor consist of a pentamer of homologous subunit so far 16 different subunits have been identified. Studies of cloned GABA_A receptor have shown that the coassemble of γ with α and β subunit confers benzodiazepines sensitivity to GABA_A receptor (Pritchett et al., 1989, Schofield et al., 1987). Benzodiazepines bind at the interface between α and β subunit and both subunit determine the pharmacology of benzodiazepines receptors (McKernan et al., 1995).

Inhibiting effect of benzodiazepines on muscle hypertonia or the spread of seizure activity can be rationalized by potentiation of inhibitory GABA-ergic circuits at various levels of neuraxis and decrease spontaneous or evoked activity of major neurons in brain and spinal cord.

GABA increases chloride conduction and can prevent neuronal discharge by shunting of electrical current that would have depolarise the membrane of initial segment. Benzodiazepines, thus markedly
prolong the period of brief activation of GABA ergic pathway leading to spontaneous or inhibiting of neuronal discharge to produce their hypnotic, sedative and anxeolytic action. (Twyman et al., 1989).

**Therapeutic uses**

Benzodiazepine has been used widely for various ailments arising due to neurobehavioral changes. These drugs are used as hypnotics, effective in the relieving sleeponset insomania (estazolam, flurazepam) and for the treatment of other neurological disorder such as anxiety disorders, agrophobia management of alcohol withdrawn (alprazolam, chlordiazepoxide, clorazepate) seizure disorder (clonazepam, diazepam) adjunctive treatment in acute mania and certain movement disorder (clonazepam) and preanesthetic medication (diazepam, lorazepam) (Charney, et al., 2001)

**2.4 Benzimidazoles**

According to literature the first benzimidazole was prepared in 1872 by Hoebrecker (Hobreckerf, 1872), who obtained 2, 5 (or 2, 6) dimethylbenzimidazole (II) by the reduction of 2-nitro-4-methylacetanilide (III)

\[
\begin{align*}
\text{III} & \xrightarrow{\text{Sn, HCl}} \text{H}_3\text{C} \text{NHCOCH}_3 \\
\text{III} & \xrightarrow{-\text{H}_2\text{O}} \text{H}_3\text{C} \text{NHCOCH}_3 \\
\end{align*}
\]

Or

\[
\begin{align*}
\text{II} & \xrightarrow{\text{H}_3\text{C}} \text{H}_3\text{C} \text{N} \text{CH}_3 \\
\text{II} & \xrightarrow{\text{H}_3\text{C}} \text{H}_3\text{C} \text{N} \text{CH}_3 \\
\end{align*}
\]
After several years later Ladenburg (Ladenburag, 1875) obtained the same compound by refluxing 3, 4-diaminotoluene with acetic acid.

\[
\text{H}_3\text{C} \quad \text{NH}_2 \quad + \quad \text{CH}_3\text{COOH} \quad -\text{H}_2\text{O} \quad \rightarrow \quad \text{H}_3\text{C} \quad \text{NH}_2 \quad \text{NHCOCH}_3
\]

The benzimidazoles are known also as benziminazoles or benzoglyoxalines. Mostly in the previous literature, they have been named also as derivatives of o-phenylenediamine. Benzimidazole according to this nomenclature would be called methenyl-o-phenylenediamine and 2-methylbenzimidazole would be called ethenyl-o-phenylenediamine. Also, they have been named as derivatives of the grouping composing the imidazole portion of the ring. Thus, for example, benzimidazole has also been called o-phenyleneformamidie) and 2 (3H)-benzimidazolone (IV) and 2(3H)-benzimidazolethione (V) are known also as o-phenyleneurea and o-phenylenethiourea, respectively.

\[
\begin{align*}
\text{IV} & : \quad \text{NH} & \quad \text{NH} \\
\text{V} & : \quad \text{NH} & \quad \text{C} = \text{S}
\end{align*}
\]

The numbering system for the benzimidazoles is as follows:
Benzimidazoles which contain a hydrogen atom attached to nitrogen in the 1-position readily tautomerize. This may be depicted as follows (DAY, 1950).

![Benzimidazole tautomerization](image)

Benzimidazole-derived alkaloids are rare in nature, and only a few examples of these natural products can be found in the literature. On the other hand, the occurrence of the imidazole skeleton in various natural sources is quite common (Lewis, 1992; He et al., 1992 and Faulkner, 1992) The benzimidazole alkaloid kealiiquinone (Figure 2.6) has been isolated from a yellow button-like Micronesian sponge species of Leucetta (Faulkner, 1992)

![Kealiiquinone](image)

**Figure: 2.6.** Benzimidazole obtained from natural product

Synthesis of benzimidazoles can be summerised by the following types (Preston, 1974).
1. From reactions of o-arylene diamines with carbonyl-containing compounds, imidates, and miscellaneous compounds
2. From o-nitroarylamines and o-dinitroarenes
3. From o-(N-acylamino and aroylamino)arylamines and nitrobenzene
4. From N-benzylidene-2-nitro- and 2-azidoanilines
5. From amidines and related compounds
6. From quinone derivatives
5. From heterocyclic compounds

Benzimidazoles mostly have been prepared from the reaction of 1, 2-diaminobenzenes with carboxylic acids under harsh dehydrating reaction conditions, utilizing strong acids such as polyphosphoric acid, hydrochloric acid, boric acid, or p-toluenesulfonic acid (Jing et al., 2006). Mild reagents, particularly mineral acids (Rastogi and Sharma, 1983), inorganic clays (Bougrin et al., 2001), or Lewis acids (Tondon and Kumar, 2004) has improved both the yield and purity of this reaction [VanVliet et al., 2005]. Synthesis of benzimidazoles via the condensation of 1, 2-diaminobenzenes with aldehydes needs an oxidative reagent to generate the benzimidazole nucleus. Various oxidative reagents, such as nitrobenzene, benzoquinone, sodium metabisulfite, mercuric oxide, lead tetraacetate, iodine, copper(II) acetate, indium perfluorooctane sulfonates, ytterbium perfluorooctane sulfonates, and even air, have been employed for this purpose (Hegedus et al., 2006). A variety of benzimidazoles could also be produced via coupling of 1, 2-diaminobenzenes with carboxylic acid derivatives such as nitriles, imidates, orthoesters, anhydrides or lactones (Lin et al., 2006).
The use of microwave irradiation as a source of heat in synthetic chemistry has been achieved a promising method of increasing productivity and quality and reducing reaction time. *Gedye et al.* (1986) in 1986 first use microwave for the synthesis of benzimidazoles. It has become a focal point in chemical synthesis in recent years in terms of sustainable and green chemistry for improved resource management. Since 1986, various substituted benzimidazole derivatives have been synthesized through microwave heating (*Bougrin and Soufiaoui, 1995*).

Recently, 2-alkyl- and 2-aryl-substituted benzimidazole derivatives (c) have been synthesized from 1, 2-diaminobenzene dihydrochloride (a) and its corresponding acids (b) in the presence of polyphosphoric acid using microwave-assisted methods (Scheme: 2.1)

\[
\begin{align*}
\text{(a)} & \quad \text{NH}_2\text{HCl} \quad \text{NH}_2\text{HCl} \\
\text{(b)} & \quad \text{O} \quad \text{OH} \\
\text{(c)} & \quad \text{MW} 
\end{align*}
\]

R=H, Me, Ph, 4-NH₂C₆H₄, 4-ClC₆H₄

**Scheme 2.1:** Synthesis of alkyl and aryl benzimidazoles under microwave conditions

The reaction time required for the synthesis of benzimidazole derivatives (c) was reduced to minutes by this method compared to conventional synthesis, which required up to four hours of heating to complete the reaction. Furthermore, it was found that the application of microwave irradiation increased yields by 10–50% (Table 2.1).
Table 2.1: Yield and reaction time for benzimidazole synthesis using microwave irradiation.

<table>
<thead>
<tr>
<th>R</th>
<th>Conventional heating</th>
<th>Yield (%)</th>
<th>Microwave irradiation</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H</td>
<td>2 h</td>
<td>80</td>
<td>1 min 20 s</td>
<td>92</td>
</tr>
<tr>
<td>Me</td>
<td>45 min</td>
<td>48</td>
<td>1 min 20 s</td>
<td>89</td>
</tr>
<tr>
<td>pH</td>
<td>4 h</td>
<td>34</td>
<td>4 min 30 s</td>
<td>84</td>
</tr>
<tr>
<td>4-NH₂C₆H₄</td>
<td>4 h</td>
<td>57</td>
<td>5 min</td>
<td>95</td>
</tr>
<tr>
<td>4-ClNH₂</td>
<td>4 h</td>
<td>43</td>
<td>4 min 30 s</td>
<td>89</td>
</tr>
</tbody>
</table>

It has been proposed that microwave heating easily provides the energy of activation required for the chemical reaction (Dubey and Moorthy, 2007). A similar one-pot high-yield procedure for the generation of 2-substituted benzimidazoles from the esters using ethane-1, 2-diol as a solvent has been described (Jing et al., 2006). The single-step synthesis of benzimidazoles from a range of other diamines and carboxylic acids under microwave irradiation conditions has been developed, which provided a practical and efficient method for the high-throughput synthesis of 2-substituted benzimidazoles (Lin et al., 2006).

In addition, benzimidazoles containing furyl and aryl substituents at the C-2 position have been synthesized from 1, 2-diaminobenzene and the corresponding carboxylic acids under microwave irradiation in the presence of artificial zeolites and catalytic amounts of DMF, used as the catalyst and energy transfer medium respectively. With this microwave
technique, the reaction time was greatly shortened and the products were obtained in higher yields with easier workup than conventional heating methods. Conventional condensation of 1, 2-diaminobenzene (d) with 6-fluoro-3, 4-dihydro-2H-chroman-2-carboxylic acid (e) under ‘Phillips’ conditions or using Eaton’s reagent (1:10 mixture of phosphorus pentoxide/methanesulfonic acid) yielded 2-(6-fluorochroman-2-yl)-1H-benzimidazole (f) (Scheme: 2.2) (Kumar et al. 2006). However, irradiating the reaction mixture containing polyphosphoric acid as a catalyst with microwaves afforded the compound (f) in comparable yields in a matter of three minutes (Kumar et al. 2006).

\[
\begin{align*}
\text{(d)} & \quad \text{(e)} \\
\text{(f)} & \\
\end{align*}
\]

**Scheme 2.2:** Reagent and conditions: a. 4N HCl, reflux, 6h, 85%; b. MW, PPA, 100w, 170°C, 3min, 85%; c. Eaton’s reagent, 100°C, 5h, 80%;

Recently, microwave-assisted synthesis of eighteen 2-(alkyloxyaryl)-1Hbenzimidazole derivatives (h) related to the natural stilbenoid family has been reported (Scheme: 2.3) (Navarrete-Vazquez et al. 2006). These bioisosteric benzimidazole analogs (h) have been synthesized in high yields through a rapid three-component reaction starting from commercially available aldehydes (g) and 1, 2-diaminobenzene (d), and
sodium metabisulfite in the absence of solvent. The in vitro spasmolytic activity of these compounds on the spontaneous contractions of the rat ileum suggests that bioactivity of these compounds depends upon the presence of oxygenated groups attached at C-2 and/or C-4 of the phenyl ring respectively (Navarrete-Vazquez et al. 2006). Recently, a facile, rapid one-pot procedure for the generation of 2-substituted benzimidazoles (k) directly from 2-nitroanilines (i) using a microwave procedure has been demonstrated (Scheme 6). An advantage of this approach is that the intermediate N-acyl derivatives (j) need not be isolated prior to cyclization (VanVliet et al., 2005)

\[ \text{R1} = \text{H, 4, 5-dimethyl, 5-OH, 5-OMe, 5-COOH, 5-CN, 5-CF}_3, 4, 6-\text{dichloro} \]

\[ \text{R2} = \text{H, Me, CF}_3 \]

**Scheme 2.3:** Synthesis of 2-(alkyloxyaryl)-benzimidazoles.

**Scheme 2.4:** Synthesis of 2-substitued benzimidazoles from 2-nitroanilines
Classical condensation-cyclization reactions using 1, 2-diaminobenzenes (l), 2-mercaptopacetic acid (m) and appropriately substituted aromatic aldehydes (n) in dry benzene under reflux required a long reaction time to afford the thiazobenzimidazoles (o), which are potent anti-HIV agents, by Scheme: 2.5. On the other hand, the microwave-assisted synthesis of 1$H$, 3$H$-thiazolo[3, 4- a]benzimidazoles (o) was completed in toluene within 12 minutes (Rao et al., 2004). Furthermore, a versatile and efficient microwave-promoted combinatorial library synthesis of two long alkyl chain benzimidazoles from o-substituted amines and fatty acids employing either bentonite, alumina or silica gel as solid supports has been developed (Martinez-Palou et al., 2005) Bismuth chloride (Su and Sun, 2005) montmorillonite clay K-10 (Perumal et al., 2004) and silica impregnated with sulfuric acid (Montazeri and Rad-Moghadam 2006) have also been reported to act as inorganic catalysts for the benzimidazole ring closure reaction under microwave irradiation conditions.

Scheme 2.5: Synthesis of thiazobenzimidazoles
Biological Activities of the Benzimidazole Analogues: Benzimidazole compounds show various biological activities (Spasov et al., 1999). These are:

Antibacterial and Antifungal Agents: The search for compounds with antibacterial activity has gained increasing importance in recent times, due to growing worldwide concern over the alarming increase in the rate of infection by antibiotic-resistant microorganisms (Zinner, 2005). 2-Mercaptobenzimidazole derivatives are known to possess varied biological activities (Narkhede et al., 2008). Recently, an efficient and rapid synthesis of novel benzimidazole azetidin-2-ones has been established (Desai and Desai, 2006) and antibacterial screening revealed that all newly synthesized azetidin-2-ones exhibited potent antibacterial activity against *Bacillus subtilis*, *Staphylococcus aureus* and *Escherichia coli*. Benzimidazole benzyl ethers have exhibited good antibacterial activity against *S. aureus* and antifungal activity against *Candida albicans* and *Candida krusei*. N-alkylated or acylated derivatives of benzimidazole also exhibited good antibacterial activities (Kumar et al. 2006). Numerous other reports of benzimidazole derivatives with antimicrobial activities have been published (Goker et al., 2002; Ayhan and Altanlar, 2003; Kus and Altanlar 2003; Ayhan and Altanlar, 2006; Ates-Alagoz et al., 2007 and Leonard et al., 2007).
Anthelmintic Agents: Anthelmintic resistance is almost cosmopolitan in distribution and it has been reported in almost all species of domestic animals and even in some parasites of human beings. All of the major groups of anthelmintics have encountered variable degrees of resistance from different species of gastrointestinal nematodes (Jabbar et al., 2006) Bearing in mind previous benzimidazole anthelmintics (e.g., albendazole, mebendazole), the search for new anthelmintic drugs is being actively pursued. Synthetic benzimidazole piperazine derivatives exhibited 50% anthelmintic activity in mice infected with Syphacia obvelata (Anichina et al., 2006) Furthermore, piperazine derivatives of 5(6)-substituted-(1H-benzimidazol-2-ylthio) acetic acids (Mavrova et al., 2006) and benzimidazolyl crotonic acid anilide (Figure: 2.8) have shown good anthelmintic activity (Gaur et al., 2000).
Anti-inflammatory and Antiulcer Agents: Pyrimidobenzimidazole (Sondhi et al., 2002) and dioxinobenzimidazothiazol-9-ones exhibited anti-inflammatory and analgesic activity, as evaluated by carrageenan-induced rat paw edema and phenylquinone-induced writhing tests. In addition, N-benzoyl and N-tosyl benzimidazole compounds showed significant anti-inflammatory activity, as indicated by ear swelling induced by xylene in mice,
and their ulcer indices were all lower than those of aspirin (Puratchikody et al., 2002). Furthermore, N-morpholinomethylbenzimidazole and its derivatives have been recently reported to show significant anti-inflammatory activity (Leonard et al., 2007).

![Pyrimidobenzimidazole](image1.png)

![Dioxinobenzimidazothiazol-9-ones](image2.png)

R = 4-F-C₆H₄, 2-furyl, 3-pyridyl

**Figure 2.9:** Some Anti-inflammatory and Antiulcer benzimidazole derivatives.

Despite the success of several commercial benzimidazole proton pump inhibitors for the treatment of ulcer disease, work is still in progress to discover new benzimidazole-derived antiulcer drugs. Cinitapride related benzimidazole derivatives have been prepared and studied for their antiulcerative activity (Srinivasulu et al., 2005). In addition, 1,3-disubstituted 3,4-dihydropyrimido[1,6-a]benzimidazoles and 3-substituted 3,4-dihydropyrimido[1,6-a]benzimidazol-1(2H)-thiones exhibited good gastric antisecretory activity (> 50% inhibition)
Enzyme and Receptor Agonists/Antagonists:

Several benzimidazole derivatives have been reported to act on various enzymes and receptors. Some examples of benzimidazoles acting as agonists or antagonists of various receptors and enzymes are listed in (Table: 2.2).

β-Acetamido esters: β-Acetamido carbonyl compounds can be used as the precursor of 1, 3-amino alcohols (Barluenga et al., 1993) β-amino acids (Mukhopadhyay et al., 1997) and γ-lactams (Rao et al., 2003). They are the building blocks of numerous pharmaceutical and biological compounds (Casimir et al., 1995). Dakin et al. first reported the preparation of this kind of compound by Dakin–West reaction in 1928, which is exactly the condensation between an amino acid and acetic anhydride in the presence of a base providing the acetamido ketones (Dakin and West 1928). Iqbal et al. (1997) developed the Cobalt-catalyzed one-pot multicomponent coupling route involving ketones, aldehydes, acetonitrile as well as acetyl chloride to prepare this class of compound (Mukhopadhyay et al., 1997) Later on, a number of catalyst systems were reported for the synthesis of β-acetamido carbonyl compounds via the same reaction.
Table 2.2: Benzimidazole derivatives that act on enzymes/receptors

<table>
<thead>
<tr>
<th>Compound</th>
<th>Enzyme/receptor</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="Image1" alt="Chemical Structure 1" /></td>
<td>Androgen receptor</td>
<td>Antagonist</td>
</tr>
<tr>
<td><img src="Image2" alt="Chemical Structure 2" /></td>
<td>Cannabinoid 2 (CB2) receptor</td>
<td>Agonist</td>
</tr>
<tr>
<td><img src="Image3" alt="Chemical Structure 3" /></td>
<td>Cholecystokinin B receptor</td>
<td>Antagonist</td>
</tr>
<tr>
<td><img src="Image4" alt="Chemical Structure 4" /></td>
<td>Cyclin-dependent kinase 1 (CDK1)</td>
<td>Inhibitory</td>
</tr>
<tr>
<td><img src="Image5" alt="Chemical Structure 5" /></td>
<td>Enkephalinase B (DPP III)</td>
<td>Antagonist</td>
</tr>
<tr>
<td><img src="Image6" alt="Chemical Structure 6" /></td>
<td>Gelatinase B</td>
<td>Inhibitory</td>
</tr>
<tr>
<td><img src="Image7" alt="Chemical Structure 7" /></td>
<td>Lymphocyte specific kinase</td>
<td>Inhibitory</td>
</tr>
</tbody>
</table>
Recently, other synthetic methods have been used for the formation of β-amido ketones through the multi-component condensation of aryl aldehydes, enolizable ketones and acetyl chlorides in acetonitrile in the presence of Lewis or Brønsted acid catalysts such as CoCl$_2$ (Bhatia et al., 1994) montmorillonite K-10 clay (Bahulayan et al., 2003) silica sulfuric acid (Khodaei et al., 2005), BiCl$_3$ generated from BiOCl (Ghosh et al., 2005), sulfuric acid absorbed on silica gel (Yakaiah et al., 2005), (Sc(OTf)$_3$ and Cu(OTf)$_2$ (Pandey et al., 2005), silica supported H$_3$PW$_{12}$O$_{40}$ (Rafiee et al., 2005), ZrOCl$_2$.8H$_2$O (Ghosh et al., 2006), heteropoly acid (Rafiee et al., 2006), CeCl$_3$.7H$_2$O (Khan et al., 2006), Amberlyst-15 (Das and Reddy 2006) iodine (Das et al., 2006), K$_5$CoW$_{12}$O$_{40}$.3H$_2$O (Nagarapu et al., 2007), ZnO (Maghsoodlou et al., 2007), FeCl$_3$.6H$_2$O (Khan et al., 2007) Ferric salts and recently developed Ce(SO$_4$)$_3$ (Selvam and Perumal, 2009) have applied for the formation of carbon-nitrogen and carbon-oxygen bonds in organic synthesis (Salehi et al., 1998; Salehi, et al., 2000; and Khodaei et al., 2001).

![Figure 2.16: Ce(SO$_4$)$_3$ catalysed synthesis of β-acetamido ketones](image)

Figure 2.16: Ce(SO$_4$)$_3$ catalysed synthesis of β-acetamido ketones
Materials and Methods
Chapter 3  MATERIALS AND METHODS

3.1 Instruments, Chemicals and Glasswares

3.1.1 Instruments

Melting points were determined on a Buchi B-540 apparatus and are uncorrected. $^1$H NMR spectra were recorded on a Varian 600 MHz, Brucker Avance II 500MHz, Brucker avance 400 MHz and JEOL AL 300 MHz NMR spectrophotometers, and IR spectra were recorded on a Brucker IR spectrophotometer. Absorbances were measured by UV spectrophotometer (Visiscan-167). Microwave irradiations were carried out in Electrolux/Nutrytion and Bajaj 2800 ET-B microwave oven. All irradiation were carried out at 25 and 40 percent energy level.

3.1.2 Chemicals and Glasswares

The chemicals used were of Laboratory Reagent grade and Analytical Reagent grade and were purchased from Sigma-Aldrich and E. Merck Ltd. India. The glass wares used during the study were of Borosil made. The solvents were distilled prior to their use. All reactions were monitored with silica gel thin layer chromatography (TLC) plates and using hexane and ethyl acetate as solvent system. The seeds of weed (Echinochola colona and phalaris minor) were obtained from Crop Research Centre, G. B. Pant University of Agriculture and Technology, Pantnagar, India.
3.2 Synthesis of substituted Cinnamic Acids Amide derivatives

3.2.1 Synthesis of substituted cinnamic acids.

Pulverization of malonic acid to remove moisture was done by heating it for two to three hours at 90-100°C. Moisture from pyridine was removed by allowing it to stand over KOH pellets and shaking occasionally.

A 500 mL round bottom flask was charged with (60 mmole) of substituted benzaldehyde and (100 mmole) malonic acid, dissolved in 20 mL of pyridine. Trace amount of piperidine (0.8 mL) was also added. The round bottom flask was stoppered by a guard tube filled with dry calcium chloride and kept on the magnetic stirrer.

The reaction progress was monitored from time to time by running reaction mixture on silica gel TLC plates using hexane and ethyl acetate (80: 20) solvent system. The reaction took four days to complete. After completion of reaction, the reaction mixture was poured in excess of dil. hydrochloric acid. Precipitates obtained were filtered, washed with cold water repeatedly and dried (Kavitha et al., 2000).

3.2.2. Synthesis of amide derivatives of substituted cinnamic acids

To a solution of substituted cinnamic acid (1.0 mmole) in chloroform containing triethylamine (0.1 mL) and amine (1.0 mmole), POCl₃ (2 mmole) was added dropwise at 0°C with constant stirring. Triethylamine (0.2 mL) was then added in one
portion. The reaction mixture was stirred for another 30 min. at low temperature. The reaction progress was monitored over silica gel TLC plate. After completion of reaction, the reaction mixture was poured into crushed ice, the organic phase separated out. The aqueous phase was extracted with DCM and combined layers were washed successively with dil. hydrochloric acid, aqueous sodium bicarbonate, water and dried over anhydrous calcium chloride. Evaporation of solvent gave a brown residue, which was recrystallized from different solvents i.e. chloroform, dichloromethane, ethanol and water (Vig et al., 1979).

![Synthesis of substituted cinnamic acids](image)

Substituents-

a. \( R = H \)  
b. \( R = 4-\text{OH} \)

c. \( R = 3-\text{NO}_2 \)  
d. \( R = 4-\text{NO}_2 \)

e. \( R = 4-\text{OMe} \)  
f. \( R = 2-\text{Cl} \)

**Scheme 3.1:** Synthesis of substituted cinnamic acids

The amides of substituted cinnamic acids were prepared using six different types of amines: piperidine, cyclohexylamine, aniline, N-methylaniline, morpholine and 2-chloro aniline.
Materials and Methods

Figure 3.1: Structure of different amines used for synthesis of substituted cinnamic acid amide derivatives

Scheme 3.2: Synthesis of substituted cinnamic acid amide derivatives
3.3 Biological activity of cinnamic acid amide derivatives

3.3.1 Herbicidal Activity

3.3.1.1 Preparation of test solution for screening of herbicidal activity

A stock solution of 500 ppm of each compound was prepared in distilled water. First the compound was dissolved in minimum amount of ethanol, the one drop of surfactant Tween-20 (10% solution in water) was added and made up with distilled water. Further dilutions of the stock solution were made to get solutions of 200 ppm, 100 ppm and 50 ppm concentrations. The test solution of standard pendimethalin and butachlor were also prepared in the same way in distilled water.

3.3.1.2 Bioassay

The seeds were surface sterilized in 95% ethanol for 15 seconds and sown in Petri plates of 90 mm diameter. Twenty seeds were taken in each Petriplate. The Petriplates were layered with germination papers, to each plate 7 mL of test solutions of different compounds of varying concentrations (50 ppm, 100 ppm and 200 ppm) were poured. A mixture of distilled water: ethanol: Tween-20 (98:1.5:0.5) amounting 7 mL was taken as control. Three replicates of each concentration were taken.

The *Echinochola colona* were allowed to germinate at 30-35 °C with 24 hours of photoperiod. After 120 hours, the numbers of seeds germinated in each Petri plate were counted and percent seed germination inhibition values were calculated *(Feo et al., 2003).*
3.3.1.3 Statistical Analysis

The percent seed germination inhibition values were determined and subjected to analysis variance (ANOVA). Critical differences (CDs) were calculated at P=0.05.

3.4 Synthesis of 4-oxo-4-phenyl-butenoic esters

3.4.1 Synthesis of Substituted β-Benzoylacrylic acid or 4-oxo-4-phenyl-2-butenoic acid

Maleic anhydride (24.5g, 0.25mol) and 150 ml of substituted benzene were charged in a 500 ml flask equipped with a magnetic stirring bar, thermometer and condenser. To the mixture was added portion wise (72g, 0.54mol) aluminium chloride at room temperature. The mixture was stirred at 80°C for 30 minutes. Then the contents of flask were poured onto 300 ml of ice water and 75ml of concentrated hydrochloric acid. The solution was extracted with 2X350 ml of ethyl acetate. The organic layer was dried over 20g of anhydrous magnesium sulphate and filtered and the filtrate was concentrated under reduced pressure to give β-Benzoylacrylic acid (Aslan, 2007).

\[
\begin{align*}
\text{Substitued benzene} & \quad \text{Maleic anhydride} \quad \text{AlCl}_3 \\
\text{4-oxo-4-phenyl-2-butenoic acid} & \\
R = & \text{H, p-CH}_3, \text{p-C}_2\text{H}_5, \text{p-Cl, p-Br, p-OH, p-OCH}_3
\end{align*}
\]

Scheme 3.3: Synthesis of β-Benzoylacrylic acid
The different substituted 4-oxo-4-phenyl-2-butenoic acids synthesized by the scheme are:

1. (E)-4-oxo-4-phenylbut-2-enoic acid
2. (E)-4-(4-methoxyphenyl)-4-oxobut-2-enoic acid
3. (E)-4-oxo-4-p-tolylbut-2-enoic acid
4. (E)-4-(4-chlorophenyl)-4-oxobut-2-enoic acid
5. (E)-4-(4-bromophenyl)-4-oxobut-2-enoic acid
6. (E)-4-(4-ethylphenyl)-4-oxobut-2-enoic acid
7. (E)-4-(4-hydroxyphenyl)-4-oxobut-2-enoic acid
8. (E)-4-(4-formylphenyl)-4-oxobut-2-enoic acid

### 3.4.2 Synthesis of substituted phenacylbromides

Phenacylbromides were prepared by bromination of substituted acetophenones. To a round bottom flask solution of 5g (0.025 mole) of substituted acetophenone in 10 ml of glacial acetic acid was added. To this 4g (1.25ml, 0.025mole) bromine was added from a dropping funnel, the mixture was stirred vigorously during the addition at the temperature below 20°C. Phenacylbromide commences to separate as needle after about half of the bromine has been introduced. When the addition is completed, the mixture was cooled in ice water. Crude product was filtered and washed with 50% alcohol until colorless. Crude product obtained was crystallized from ethyl alcohol (Furniss et al., 1980).
The different substituted phenacylbromides synthesized by the scheme are:

1. Phenacylbromide
2. Chlorophenacylbromide
3. Methylphenacylbromide
4. Methoxyphenacylbromide
5. Nitrophenacylbromide

**Figure 3.2:** Structure of different substituted phenacylbromides
3.4.3 Synthesis of 4-oxo-4-phenyl-2-butenoic esters

Esters were prepared by using substituted benzoylacrylic acids and substituted phenacyl bromides by the method given in literature (Katrizky et al., 2001). To a round bottom flask, substituted benzoylacrylic acid (1.25 mmol) and substituted phenacyl bromide (1.375 mmol) were added. To the mixture 3.7 ml of DMF and 0.10g (1.25 mmol) of sodium bicarbonate were added. The reaction mixture was heated on water bath at 80°C and cooled to room temperature. Then it was extracted with ethyl acetate. The organic layer was separated and dried over MgSO₄. The solvent was evaporated in the rotavapour. Crude mixture was crystallized from ethyl alcohol.

\[
\begin{align*}
\text{Substitued 4-oxo-4-phenyl-2-butenoic acid} & \quad + \quad \text{Substituted phenacylbromide} \\
\text{NaHCO}_3 & \\
\text{Substitued 4-oxo-4-phenyl-2-butenoic esters.}
\end{align*}
\]

R = H, p-CH₃, p-C₂H₅, p-Cl, p-Br, p-OH, p-OCH₃

R’=H, p-CH₃, p-OCH₃, p-Cl, p-NO₂

Scheme 3.5: Synthesis of 4-oxo-4-phenyl-2-butenoic esters
3.5 Biological activity of 4-oxo-4-phenyl-2-butenolic esters

3.5.1 Herbicidal Activity

3.5.1.1 Preparation of test solution for screening of herbicidal activity

A stock solution of 500 ppm of each compound was prepared in water. The compound was first dissolved in minimum amount of DMSO, then one drop of surfactant Tween-20 (10% solution in water) was added and made up with distilled water. Further dilutions of the stock solution were made to get solutions of 200 ppm, 100 ppm and 50 ppm concentration. The test solution of standard pendimethalin were also prepared by serial dilution in distilled water.

3.5.1.2 Bioassay

The seeds were surface sterilized in 95% ethanol for 15 seconds and sown in Petri plates of 90 mm diameter. Twenty seeds were taken in each Petri plate. The Petri plates were layered with germination papers, to each plate 7 mL of test solutions of different compounds of varying concentrations (50 ppm, 100 ppm and 200 ppm) were poured. A mixture of distilled water: DMSO: Tween-20 (98:1.5:0.5) was taken as control. Three replicates of each concentration were taken.

The Phalaris minor were allowed to germinate at 20-25°C with 24 hours of photoperiod. After 120 hours, the numbers of seeds germinated in each Petri plate were counted and percent seed germination inhibition values were calculated (Feo et al., 2003).
3.5.1.3 Statistical Analysis

The percent seed germination inhibition values were determined and subjected to analysis variance (ANOVA). Critical differences (CDs) were calculated at $P=0.05$.

3.6 Synthesis of 1, 5 benzodiazepines

Trichloroacetic acid (0.02 mmol) was added to the mixture of 1, 2 phenylenediamine (1.0 mmol), β-ketoesters (1.3 mmol) and aldehydes (1.0 mmol) (Scheme: 3.6) The mixture was placed in microwave under irradiation for a period of 2-3 minutes. The reaction progress was monitored over silica gel TLC plates using hexane and ethyl acetate (80: 20) solvent system. After completion of the reaction, the mixture was cooled to room temperature and was extracted with ethyl acetate. The solvent was evaporated in vacuo. The obtained product were identified by their spectral (NMR and IR) data. (Murai et al., 2008)

\[
\begin{align*}
\text{NH}_2 & \quad \text{NH}_2 \\
& + \\
\text{OR} & \quad \text{CHO} \\
& \xrightarrow{\text{Microwave}} \\
\text{Cl}_3\text{CCOOH (0.02eq)} & \quad \text{HN} \quad \text{HN} \\
& \quad \text{OR}_1
\end{align*}
\]

$R_1= -\text{OMe, -CH}_2\text{C}_6\text{H}_5$

$X= 2-\text{NO}_2, 3-\text{NO}_2, 4-\text{NO}_2, 2-\text{OH}, 3-\text{OH}, 4-\text{OH}, 4-\text{OMe, 4-Cl}$

**Scheme 3.6:** Synthesis of 1, 5 benzodiazepines
3.7 Biological activity of benzodiazepines

3.7.1. Antioxidant Activity of benzodiazepines

Antioxidant potential of the synthetic compounds was evaluated in terms of 2, 2’-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging ability and the reducing power in comparison with the synthetic and natural antioxidants. Butylated hydroxy toluene (BHT) is taken as synthetic and gallic acid and catechin were used as the natural antioxidants. All determinations were performed in quartet.

3.7.1.1 Reducing Power Activity

The reducing power of the compounds was determined by the methods reported earlier (Benzie and Strain, 1996; Yen and Chen, 1995 and Singh, et al., 2005). Various amounts of ethanolic solutions of Benzodiazepine derivatives i.e., 5 μL, 10 μL, 15 μL, 20 μL and 25 μL were mixed with 2.5 mL of the phosphate buffer (200 mM, pH 6.6) and 2.5 mL of 1% potassium ferricyanide. The mixtures were incubated at 50°C. After incubation, 2.5 mL of 10% trichloroacetic acid was added to the mixtures, followed by centrifugation at 650 rpm for 10 minutes. The upper layer (5mL) was decanted and mixed with 5 mL of distilled water and 1 mL of 0.1% ferric chloride and the absorbance of the resultant solution was measured at 700 nm using UV-vis-spectrophotometer. The control and standard were subjected to the same procedure except for the control, where there was no addition of the sample and for standard 5 μL, 10 μL, 15 μL, 20 μL and 25 μL of the sample were replaced with 5 μL, 10 μL, 15 μL, 20 μL and 25 μL ethanolic solutions (1000 ppm) of the
BHT and gallic acid. Absorbance at 700 nm was plotted against the different amounts of the compounds. Increase in absorbance indicates increase in reducing power.

3.7.1.2 DPPH (2, 2’-Diphenyl picryl hydrazyl) Radical Scavenging Activity

This experimental procedure was adapted from Chen and Ho (1995). Various amounts (5 µL, 10µL, 15 µL, 20 µL, and 25 µL) of ethanolic solutions of benzodiazepine derivatives were mixed with 5ml of 0.004% methanolic solution of DPPH. The reaction mixtures were shaken vigorously and then kept in the dark for the 30 min. The absorbances of the resulting solutions were measured in 1 cm cuvettes at 517 nm. The DPPH solution was freshly prepared daily, stored in a flask, covered and kept in dark at 4°C during the measurements. The control and standard were subjected to the same procedure except for the control, where there was no addition of the sample and for the standard 5 µL, 10 µL, 15 µL, 20 µL and 25 µL of the sample were replaced with 5 µL, 10 µL, 15 µL, 20 µL and 25 µL ethanolic solutions (1000 ppm) of the BHT, catechin and gallic acid. A lower absorbance indicates a higher radical scavenging power.

DPPH scavenging activity was calculated according to the following equation-

$$\text{DPPH scavenging activity (\%)} = \left[1 - \left(\frac{A_t}{A_o}\right)\right] \times 100$$

(where $A_t$ is the absorbance of the sample at 517 nm and $A_o$ is the absorbance of the control at 517 nm)
3.7.1.3. **Effect of the chelating activity of Fe\(^{+2}\)**

The chelating activity of synthetic compounds to ferrous ions (Fe\(^{+2}\)) was measured with slight modification of the method reported by **Decker and Welch (1990)**. The Fe\(^{+2}\) ion were monitored by measuring the formation of ferrous-ferrozine complex. Different amounts (5 µL, 10 µL, 15 µL, 20 µL and 25 µL) of ethanolic solution of benzodiazepine derivatives were first mixed in 1mL of methanol then FeCl\(_2\) (2mM, 0.1mL) and ferrozine (5mM, 0.2mL) were added to the mixture. The mixture was shaken and left at room temperature for 10 minutes. A lower absorbance indicates a higher chelating power. The chelating activity on Fe\(^{+2}\) of the sample were compared with that of EDTA at a level of 0.01mM and citric acid at a level of 0.025 M. Chelating activity was calculated according to equation

\[
\text{Chelating activity} \, (\%) = [1-(A_t/A_0)] \times 100
\]

(where \(A_t\) is the absorbance of the sample at 562 nm and \(A_0\) is the absorbance of the control at 562 nm).

3.7.2 **Antibacterial Activity**

3.7.2.1 **Antibacterial assay**

Test Micro-organisms (four) bacterial strains *Bacillus cereus*, *Pseudomonas aeruginosa*, *Gordonia terrae* and *Acinetobactor junii* were obtained from the Department of Microbiology, CBSH, G.B. Pant university of Agriculture and Technology, Pantnagar, India. Chloramphenicol, Ampicillin, Streptomycin were taken as a standard antibiotics.
3.7.2.2 **Culture media**

Nutrient agar (NA) and nutrient broth (NB) were obtained from Hi Media Ltd, Mumbai and stored at 4°C.

3.7.2.3 **Preparation of media**

For the preparation of different media, NA and NB were weighed and poured in the distilled water. After proper plugging it was autoclaved at 120°C at 15-20 lbs pressure for 20 minutes.

3.7.2.4 **Preparation of agar plates**

Autoclaved nutrient agar cooled to 45°C was poured (around 20 ml) into each Petri plate sterilized in laminar flow and kept undisturbed as such till it solidified. After solidification of agar medium, these Petri plates were incubated at 30°C overnight for sterile testing.

3.7.2.5 **Preparation of stock inoculum**

Pure cultures of test bacteria were prepared by emulsifying 5 colonies in 5 mL of sterilized nutrient broth. Tubes with nutrient broth and inoculated bacterial cultures were incubated overnight at 30°C. Next day cultures showing marked turbidity in the tubes were used.

3.7.2.6 **Disc diffusion method**

This is the most common method to evaluate the antimicrobial activity *(NCCLS, 1997)*. Bacterial suspension of 0.1 mL of (10 times diluted) was added to the previously prepared nutrient agar plates and bacterial strain was thoroughly spread on the agar surface, using bent
rod. The sterilized Whatman filter paper No. 1 disc (5mm in diameter) was thoroughly soaked with the solution of compounds (50 µL of 30 ppm solution) and placed in the inoculated plates.

3.7.2.7 Placement of the disc

Fine pointed forceps were used to place the discs on the previously inoculated plates with the maximum possible aseptic precautions. The discs were firmly pressed against the nutrient agar medium so that those were in complete contact with the agar surface. The discs were placed equidistantly from each other on the seeded plates.

3.7.2.8 Recording observations

The plates were incubated at 30°C. After incubation, relative susceptibility of each organism was determined by a clear zone of inhibition around the disc impregnated with the compounds as well as the antibiotics. Zone of inhibition (mm) was measured with the help of scale.

3.7.3 Evaluation of pharmacological activity of 1, 5 Benzodiazepine derivatives on smooth muscles of rats

Six healthy adult male wistar rats, weighing about 150-200g were procured from Experimental Animal House of College of Veterinary Sciences of this university for the present study on smooth muscles. Rats were maintained in the laboratory animal house of Department of Pharmacology and Toxicology, College of Veterinary and Animal Sciences at Pantnagar.
3.7.3.1 Drug

For the present study derivatives of benzodiazepines (Table 4.16), acetylcholine, adrenaline and atropine were freshly prepared and used as drugs. Serial dilution of benzodiazepines derivatives was prepared in Tyrode solution and mixed for 10 min, with the help of an Vortexer, GeNei™ mixture immediately before the use in experiments.

3.7.3.2 Collection, preparation and mounting of tissues

After 15 days acclimation, female rats were anaesthized with chloform and sacrified for collecting duodenum (Ghosh, 2005). The duodenal portion of intestine was collected in a Petri dish containing aerated physiological saline solution and cleaned by removing the connective tissue, pancreas and fat attached to it without causing damage to the smooth muscles. A piece of 15 mm length of duodenum was cut and mounted in an organ bath of 20 ml capacity containing tyrode solution (ph 7.40) which was continuously bubbled with atmospheric air and maintained at a temperature of 37±0.5°C. The tissue was allowed to equilibrate under a constant resting tension of 0.5 g for a period of 30 min. During equilibrium period, the bathing fluid was regularly changed at every 15 min interval.

3.7.3.3 Calibration of physiograph and recording of responses

Isomeric contraction were recorded using a force transducer (0-50 g) connected to 4 channel polygraph (Biopac, USA). The scale of the tension was adjusted to 0.5 g. After equilibration period of about 30 min, the
Table 3.1: Composition of Tyrode solution (Ghose, 2005)

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Molecular weight</th>
<th>Amount in g/l</th>
<th>Concentration (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>58.45</td>
<td>8.0</td>
<td>13.7</td>
</tr>
<tr>
<td>KCl</td>
<td>74.56</td>
<td>0.2</td>
<td>2.7</td>
</tr>
<tr>
<td>CaCl₂</td>
<td>110.99</td>
<td>0.2</td>
<td>1.8</td>
</tr>
<tr>
<td>MgCl₂.6H₂O</td>
<td>95.23</td>
<td>0.05</td>
<td>0.1-1.0</td>
</tr>
<tr>
<td>NaHCO₃</td>
<td>84.00</td>
<td>1.0</td>
<td>11.9</td>
</tr>
<tr>
<td>NaH₂PO₄</td>
<td>119.97</td>
<td>0.05</td>
<td>0.4</td>
</tr>
<tr>
<td>Glucose</td>
<td>180.16</td>
<td>1.0</td>
<td>5.55</td>
</tr>
<tr>
<td>pH adjusted to</td>
<td></td>
<td></td>
<td>7.4</td>
</tr>
</tbody>
</table>

Drugs were added into the bath fluid and the isometric responses were recorded on 4 channel polygraph. To record the subsequent response(s) in the same tissue, an interval of 5 min with 2-3 washing was provided so that the tissues came to its base line after eliciting the previous response.

To avoid the development of fatigue in the preparation and evade anomaly in the responses only one set of experiment was carried out on each tissue. After the experiment was over, the tissue was taken out of the bath and placed in a fold of filter paper to remove fluid and moisture and weighed to examine any loss or gain in its weight. The absolute tension generated by the tissues was converted to the response in g per g of tissue and per cent maximum response in term of concentration.

3.7.3.4 Recording of normal contractility of isolated duodenum tissue

Normal motility peristaltic movements were obtained in isolated duodenal tissues of rats exhibited in tyrode solution. After equilibration period of 30 min and before adding any drug. The normal motility was recorded for a period of 15 min.
3.7.3.5 **Effect on acetylcholine-induced responses**

After providing sufficient rest of 5-10 min to the tissue response of acetylcholine recorded with repeated intervening washings for the previous response. Acetylcholine (0.1 ml, 10^{-3} M) was added to the organ bath in an increasing order to record the concentration-dependent responses. The tissue were allowed to show maximum responses to each concentration of the acetylcholine which took about 0.5-1.0 min duration.

3.7.3.6 **Effect of test compounds on smooth muscles**

After giving dose of acetylcholine, tissue was washed so that the tissue comes to its base line after  eliciting the previous response. Test compounds (0.1 and 0.5 ml) were added to the organ bath in an increasing order to record concentration dependent responses, which took about 1-1.5 min time.

3.7.3.7 **Effect on atropine-induced responses**

Atropine 100 µg (0.5 ml, 0.1 mg/ml) was added to the organ bath. The tissue were allowed to show maximum response of the atropine. After the dose of atropine, acetylcholine, test compounds were repeated in same order.

3.7.3.8 **Effect on adreline-induced responses**

Response of adrenaline (0.1 ml, 20 mg/ml) was recorded after providing sufficient rest of 5-10 min to the tissue with repeated intervening washing so that the tissue comes to its base line after...
eliciting the previous response. Adrenaline (2 µg, 0.5 µl) was added to the organ bath. The tissues were allowed to show maximum response of the adrenaline, which took about 1-1.5 min time. Then rest compounds as used in previous concentration were repeated to compare the effect with adrenaline.

3.7.3.9 Effect after α₁-receptor blocked by prazosine

The responses of prazosine (0.1 ml, 10mg/ml) and α₁ adrenergic antagonist was reported after providing sufficient rest of 5-10 min. to the tissue till its come to its base line. The tissue was allowed to maximum response of prazosine which to come out 1.5 min. but test compound were tested as used in the previous concentration giving repeated intervening washing as given earlier.

3.8 Synthesis of benzimidazoles

3.8.1 Microwave assisted synthesis of benzimidazole derivatives using Oxalic acid as a catalyst

Aldehyde (1.0 mmol) and o-phenylenediamine (1.0 mmol) were thoroughly mixed in THF (2 mL), then oxalic acid as a catalyst (0.2 mmol) was added, and the mixture was placed under microwave irradiation for a period of 2-3 minutes. The reaction progress was monitored over silica gel TLC plates using hexane and ethyl acetate (80:20) solvent system. After completion of reaction, the mixture was cooled to room temperature. The reaction mixture was added dropwise with vigorous stirring into a solution of Na₂CO₃ (0.2 mmol) in H₂O (20 mL). Solid product was collected by filtration, washed with H₂O and dried.
cases where gummy material precipitated, the product was extracted into EtOAc, the organic phase was washed with H$_2$O, brine and the dried over Na$_2$SO$_4$. Removal of solvent afforded product. The obtained product were identified by their spectral (NMR and IR) data (Xiangming et al., 2007).

\[
\text{NH}_2 + \text{RCHO} \xrightarrow{\text{Oxalic Acid 20 mole % Microwave}} \text{N} \text{H} \text{R}
\]

R= C$_6$H$_5$, 2-ClC$_6$H$_4$, 4-ClC$_6$H$_4$, 4-NO$_2$C$_6$H$_4$, 3-NO$_2$C$_6$H$_4$, 4-CH$_3$OC$_6$H$_4$, 3-CH$_3$OC$_6$H$_4$, 4-FC$_6$H$_5$, 4-CH$_3$CHO, 4-OHC$_6$H$_5$, 3-OHC$_6$H$_5$.

**Scheme 3.7:** Synthesis of benzimidazole derivatives using oxalic acid as a catalyst

### 3.8.2 Microwave assisted synthesis of benzimidazoles and its derivatives using TiCl$_4$ as a catalyst.

Aldehyde (1.0 mmole) and o-phenylenediamine (1.0 mmole) were thoroughly mixed in THF (2 mL), then TiCl$_4$ as a catalyst (0.2 mmole) was added, and the mixture was placed under microwave irradiation for a period of 2-3 minutes. The reaction progress was monitored from time to time by running reaction mixture on a silica gel TLC plates using hexane and ethyl acetate (80: 20) solvent system. After completion of reaction, the reaction mixture was cooled to room temperature. The reaction mixture was added dropwise with vigorous stirring into a mixture of Na$_2$CO$_3$ (0.2 mmole) and H$_2$O (20 mL). The solid product was collected by filtration, washed with H$_2$O and dried. In cases where gummy material precipitated, the product was
extracted with EtOAc, the organic phase was washed with H₂O, brine and dried over Na₂SO₄. Evaporation of solvent gave the crude product, which was purified by column chromatography over silica gel (hexane: ethyl acetate, 3:1) to afford the corresponding benzimidazole. The obtained product were identified by their spectral (NMR and IR) data (Xiangming et al., 2007).

\[
\begin{align*}
\text{NH}_2 \quad \text{NH}_2 \\
\begin{array}{c}
\text{RCHO} \\
\end{array}
\xrightarrow{\text{TiCl}_4 \quad 20 \text{ mole %}}
\begin{array}{c}
\begin{array}{c}
\text{R} \\
\end{array}
\end{array}
\end{align*}
\]

\[\text{R}= 2-\text{ClC}_6\text{H}_4, 4-\text{ClC}_6\text{H}_4, 4-\text{NO}_2\text{C}_6\text{H}_4, 3-\text{NO}_2\text{C}_6\text{H}_4, 4-\text{CH}_3\text{OC}_6\text{H}_4, 2-\text{OHC}_6\text{H}_5.\]

**Scheme 3.8:** Synthesis of benzimidazole derivatives using TiCl₄ as a catalyst

### 3.8.3 Microwave assisted synthesis of benzimidazole derivatives using ferric chloride as a catalyst

Aldehyde (1.0 mmol) and o-phenylenediamine (1.0 mmol) were thoroughly mixed in THF (2 mL), then ferric chloride as a catalyst (0.2 mmol) was added, and the mixture was placed under microwave irradiation for a period of 2-3 minutes in a microwave oven. The reaction progress was monitored over silica gel TLC plates using hexane and ethyl acetate (80: 20) solvent system. When the reaction was completed, the reaction mixture was cooled to room temperature. The reaction mixture was added dropwise with vigorous stirring into a mixture of Na₂CO₃ (0.2 mmol) in H₂O (20 mL). Solid product was collected by filtration, washed with H₂O and dried. In cases where
gummy material precipitated, the product was extracted with EtOAc, the organic phase was washed with H₂O, brine and dried over Na₂SO₄. Evaporation of solvent gave the crude product, which was purified by column chromatography over silica gel (hexane: ethyl acetate, 3:1) to afford the corresponding benzimidazole. The products were characterized by their spectral (NMR and IR) data (Xiangming et al., 2007).

\[
\begin{align*}
\text{NH}_2 & \quad \text{NH}_2 \quad + \quad \text{RCHO} \quad \text{FeCl}_3 \quad 20 \text{ mole} \% \\
\text{Microwave} & \quad \rightarrow \\
\text{R=C}_6\text{H}_5, 3-\text{NO}_2\text{C}_6\text{H}_4, 4-\text{CH}_3\text{OC}_6\text{H}_4, 4-\text{CH}_3\text{C}_6\text{H}_5, 2-\text{OHC}_6\text{H}_5
\end{align*}
\]

**Scheme 3.9:** Synthesis of benzimidazole derivatives using ferric chloride as a catalyst

### 3.8.4 Synthesis of 1, 2 disubstitued benzimidazoles using SiO₂/CaCl₂.2H₂O as a catalyst.

#### 3.8.4.1 Formation of Solid supported Catalyst: To a 100-mL beaker, silica gel- 60 (7.5 g), CaCl₂.2H₂O (2.5 g) and water (3.0 mL) were added. The suspension was stirred for 15 min at room temperature, dried at 80°C for 3h and for additional 15h at 150°C in an oven and then cooled in a desiccator.

#### 3.8.4.2 Procedure for the synthesis of 1, 2 disubstitued benzimidazoles

**Method A:** To a mixture of aldehyde (2 mmol) and o-phenylenediamine (1 mmol) was added 0.120 g of SiO₂/ CaCl₂.2H₂O, and the mixture was stirred at room temperature. The reaction progress was monitored over
TLC. After stirring for 5.0 min to 8 hours, ethyl acetate (10 mL) was added, and the organic solution was separated from SiO\textsubscript{2}/CaCl\textsubscript{2}.2H\textsubscript{2}O by filtration. The solvent was evaporated under reduced pressure, and the residue was purified by column chromatography over silica gel eluting with hexanes:AcOEt 9:1 mixture, yielding the products \textit{(Jacob et al., 2009)}.

**Method B:** SiO\textsubscript{2}/CaCl\textsubscript{2}.2H\textsubscript{2}O (0.120 g) was added to the mixture of 1, 2 phenylenediamine (1 mmol), and aldehydes (2 mmol). The mixture was placed under microwave irradiation for a period of 2-4 minutes. After the completion of the reaction (monitored over silica gel TLC) the reaction mixture was cooled to room temperature and was extracted with ethyl acetate. The solvent was evaporated in vacuo, and the residue was purified by column chromatography (silica gel) eluting with hexanes:AcOEt 9:1 mixture, yielding the products \textit{(Jacob et al., 2009)}.

\[
\begin{array}{c}
\text{NH}_2 \\
\text{NH}_2 \\
\text{SiO}_2/\text{CaCl}_2\cdot2\text{H}_2\text{O}
\end{array}
\xrightarrow{\text{r.t or MW}}
\begin{array}{c}
\text{NH} \\
\text{RCHO}
\end{array}
\xrightarrow{\text{SiO}_2/\text{CaCl}_2\cdot2\text{H}_2\text{O}}
\begin{array}{c}
\text{N} \\
\text{R} = \text{C}_6\text{H}_5\text{CHO, 3-NO}_2, 4-\text{NO}_2, 4-\text{CH}_3, 4-\text{F}, 4-\text{Cl}, 2-\text{Cl}, 4-\text{MeO}, 3-\text{MeO}, 4-\text{OH}, 3-\text{OH}
\end{array}
\]

**Scheme 3.10:** Synthesis of 1, 2 disubstitued benzimidazole derivatives using SiO\textsubscript{2}/CaCl\textsubscript{2}.2H\textsubscript{2}O as a catalyst
3.9 Synthesis of β-acetamido esters

3.9.1 Synthesis of β-acetamido keto esters via direct Mannich-type reaction using CaCl2.2H2O as a Catalyst.

3.9.1.1 Conventional Method

CaCl2.2H2O (1.0 mmol) was added to a solution of aldehyde (10.0 mmol), acetamide (11.0 mmol), and acetylacetone/ethylacetoacetate/acetoephone (11.0 mmol). The reaction was stirred at reflux temperature until the reaction was completed (monitored over silica gel TLC). Then the mixture was extracted with ethyl acetate (3×25 ml), washed with water (3×30 ml), dried over Na2SO4. After the removal of solvent the crude mixture was recrystallized from hexane-ethyl acetate (Mao et al., 2009)

3.9.1.2 Microwave assisted Method

CaCl2.2H2O (1.0 mmol) was added to a solution of aldehyde (10.0 mmol), acetamide (11.0 mmol), and acetylacetone/ethylacetoacetate/acetoephone (11.0 mmol). The reaction was placed under microwave under irradiation for a period of 2-4 minute. After the completion of the reaction (monitored over silica gel TLC) the reaction mixture cooled to room temperature. Then the mixture was extracted with ethyl acetate (3×25 ml), washed with water (3×30 ml), dried over Na2SO4. After the removal of the Solvent, the crude mixture was recrystallized from hexane-ethyl acetate.
R₁= C₆H₅CHO, 4-Cl, 4-MeO, 4-F, 4-CH₃, 2-Cl.

**Scheme 3.11:** Synthesis of β–acetamido carbonyl compounds catalysed by CaCl₂.2H₂O

### 3.9.2 Synthesis of β-acetamido keto esters via direct Mannich-type reaction using Oxalic Acid as a Catalyst

#### 3.9.2.1 Conventional Method

Oxalic acid (1.0mmol) was added to a solution of aldehyde (10.0mmol), acetamide (11.0mmol), and acetylacetone/ethylacetoacetate /acetophenone (11.0mmol). The reaction mixture was stirred at reflux temperature. The reaction progress was monitored over silica gel TLC plate. After the completion of reaction the mixture was extracted with ethyl acetate (3×25 ml), washed with water (3×30 ml), dried over Na₂SO₄. After the removal of solvent, the crude mixture was obtained which was recrystallized from hexane-ethylacetate (Mao et al., 2009).
3.9.2.2 Microwave Irridation Method

Oxalic acid (1.0mmol) was added to a solution of aldehyde (10.0mmol), acetamide (11.0mmol), and acetylacetone /ethylacetoacetate/ acetophenone (11.0mmol). The reaction mixture was placed under microwave irradiation for a period of 3-4 minute. After the completion of the reaction (monitored over silica gel TLC) the reaction mixture was cooled to room temperature. Then the mixture was extracted with ethyl acetate (3×25 ml), washed with water (3×30 ml), dried over Na₂SO₄. After the removal of solvent crude mixture was recrystallized from hexane-ethyl acetate.

R1= C₆H₅CHO, 4Cl, 4MeO, 4F, 4CH₃, 2Cl.

**Scheme 3.12:** Synthesis of β-acetamido carbonyl compounds catalysed by Oxalic Acid
3.10 Characterization Techniques and Instrumentation

The following techniques and instrumentation were involved in the synthesis and characterization of above synthesized compounds.

3.10.1 Melting Points

Melting points were determined in open capillaries on a Buchi B-540 melting point apparatus and are uncorrected.

3.10.2 Thin Layer Chromatography

3.10.2.1 Preparation of TLC plates

The TLC plates were washed properly and then cleaned by acetone and dried in oven at 120°C. The slurry of silica gel G was made in water. It was then spread on the plates with the help of applicator. The plates were air dried and then placed in oven for drying. Prior to use, these plates were activated at 120°C in oven.

3.10.2.2 Loading of the compounds

The compounds were loaded on the TLC plates by the capillaries. A small spot was made on plates. After air drying of spots, these plates were placed in TLC chamber for development with appropriate solvent system. The solvent system used was hexane: ethyl acetate in varying composition depending on the polarity of the compounds.

3.10.2.3 Detection of the compounds

The ascending chromatography method was used in TLC. When the solvent reached upto 2/3rd, then plates were taken out and were
kept for air drying. After air drying these were viewed under UV and then were placed in iodine chamber for the clear visualization of spots.

3.10.3 UV-vis spectra

UV-vis spectra were recorded over spectrophotometer model Visiscan-167 (Williard et al., 1986).

3.10.4 FT-IR Spectra

FT-IR spectra were recorded on Bruker FT-IR Spectrophotometer utilizing KBr pellets (Silverstein and Webster, 1998).

3.10.5 \(^1\)H NMR Spectra

\(^1\)H NMR spectra were recorded on Varian Inova 600 MHz, Brucker Avance II 500MHz, Brucker Avance 400 MHz and JEOL AL 300 MHz NMR spectrophotometer. Chemical shifts are recorded in \(\delta\) (ppm) using TMS as internal standard. For all the synthetic compounds CDCl\(_3\) or DMSO were used as solvent (Veverková et al., 1999; Maheswara et al., 2006).
Results and Discussion
RESULTS AND DISCUSSION

4.1. Synthesis of cinnamic acid amide derivatives

4.1.1. Synthesis of substitute cinnamic acids

Substituted cinnamic acids were synthesized by the reaction of substituted benzaldehydes with malonic acid in presence of pyridine and piperidine. The per cent yield, colour and physical properties are displayed in the (Table 4.1).

Table 4.1: Substituted cinnamic acids.

<table>
<thead>
<tr>
<th>Compounds No.</th>
<th>Reactant</th>
<th>Product</th>
<th>Yield (%)</th>
<th>Physical properties of products</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>benzaldehyde</td>
<td>cinnamic acid</td>
<td>80.5</td>
<td>M.P. (°C) 134 Colour White</td>
</tr>
<tr>
<td>2.</td>
<td>4-hydroxy benzaldehyde</td>
<td>4-hydroxy cinnamic acid</td>
<td>83.2</td>
<td>M.P. (°C) 182 Colour White</td>
</tr>
<tr>
<td>3.</td>
<td>3-nitro benzaldehyde</td>
<td>3-nitro cinnamic acid</td>
<td>97.7</td>
<td>M.P. (°C) 194 Colour Yellowish-white</td>
</tr>
<tr>
<td>4.</td>
<td>4-nitro benzaldehyde</td>
<td>4-nitro cinnamic acid</td>
<td>69.2</td>
<td>M.P. (°C) 280 Colour Yellowish-white</td>
</tr>
<tr>
<td>5.</td>
<td>2-chloro benzaldehyde</td>
<td>2-chloro cinnamic acid</td>
<td>95.2</td>
<td>M.P. (°C) 191 Colour White</td>
</tr>
<tr>
<td>6.</td>
<td>4-methoxy benzaldehyde</td>
<td>4-methoxy cinnamic acid</td>
<td>78.4</td>
<td>M.P. (°C) 174 Colour White</td>
</tr>
</tbody>
</table>
4.1.2 Synthesis of substituted cinnamic acid amides

The % yield, colour and physical properties of the amides are described in these (Tables 4.2-4.7).

Table: 4.2. Amides of substituted cinnamic acids with piperidine.

<table>
<thead>
<tr>
<th>Compounds No.</th>
<th>Reactant</th>
<th>Product</th>
<th>Yield (%)</th>
<th>Physical properties of products</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>cinnamic acid</td>
<td>Cinnamoyl piperidine</td>
<td>70.0</td>
<td>M.P. (0°C) 142  Colour Brown</td>
</tr>
<tr>
<td>8</td>
<td>4-hydroxy cinnamic acid</td>
<td>4-hydroxy cinnamoyl piperidine</td>
<td>65.0</td>
<td>M.P. (0°C) 165  Colour Off white</td>
</tr>
<tr>
<td>9</td>
<td>3-nitro cinnamic acid</td>
<td>3-nitro cinnamoyl piperidine</td>
<td>78.0</td>
<td>M.P. (0°C) 120  Colour Off white</td>
</tr>
<tr>
<td>10</td>
<td>4-nitro cinnamic acid</td>
<td>4-nitro cinnamoyl piperidine</td>
<td>78.0</td>
<td>M.P. (0°C) 175  Colour Off white</td>
</tr>
<tr>
<td>11</td>
<td>2-chloro cinnamic acid</td>
<td>2-chlorocinnamoyl piperidine</td>
<td>71.0</td>
<td>M.P. (0°C) 102  Colour White</td>
</tr>
<tr>
<td>12</td>
<td>4-methoxy cinnamic acid</td>
<td>4-methoxy cinnamoyl piperidine</td>
<td>72.0</td>
<td>M.P. (0°C) 172  Colour White</td>
</tr>
</tbody>
</table>
Results and Discussion

Figure 4.2: Amides of substituted cinnamic acids with piperidine

Table 4.3: Amides of substituted cinnamic acids with cyclohexyl amine.

<table>
<thead>
<tr>
<th>Compounds No.</th>
<th>Reactant</th>
<th>Product</th>
<th>Yield (%)</th>
<th>Physical properties of products</th>
</tr>
</thead>
<tbody>
<tr>
<td>13.</td>
<td>cinnamic acid</td>
<td>N-cyclohexyl cinnamide</td>
<td>72.0</td>
<td>M.P. (°C)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>176</td>
</tr>
<tr>
<td>14.</td>
<td>3-nitro cinnamic acid</td>
<td>N-cyclohexyl-1-(3′-nitro) cinnamide</td>
<td>79.0</td>
<td>182</td>
</tr>
<tr>
<td>15.</td>
<td>4-nitro cinnamic acid</td>
<td>N-cyclohexyl-1-(4′-nitro) cinnamide</td>
<td>78.0</td>
<td>175</td>
</tr>
<tr>
<td>16.</td>
<td>2-chloro cinnamic acid</td>
<td>N-cyclohexyl-1-(2′chloro) cinnamide</td>
<td>75.0</td>
<td>160</td>
</tr>
<tr>
<td>17.</td>
<td>4-methoxy cinnamic acid</td>
<td>N-cyclohexyl-1-(4′-methoxy) cinnamide</td>
<td>74.0</td>
<td>170</td>
</tr>
</tbody>
</table>
Figure 4.3: Amides of substituted cinnamic acids with cyclohexyl amine.

Table 4.4: Amides of substituted cinnamic acids with aniline.

<table>
<thead>
<tr>
<th>Compounds No.</th>
<th>Reactant</th>
<th>Product</th>
<th>Yield (%)</th>
<th>Physical properties of products</th>
</tr>
</thead>
<tbody>
<tr>
<td>18</td>
<td>cinnamic acid</td>
<td>cinnamanilide</td>
<td>75.0</td>
<td>M.P. 176</td>
</tr>
<tr>
<td>19</td>
<td>4-hydroxy cinnamic acid</td>
<td>4-hydroxy cinnamanilide</td>
<td>76.0</td>
<td>25</td>
</tr>
<tr>
<td>20</td>
<td>3-nitro cinnamic acid</td>
<td>3-nitro cinnamanilide</td>
<td>70.0</td>
<td>163</td>
</tr>
<tr>
<td>21</td>
<td>4-nitro cinnamic acid</td>
<td>4-nitro cinnamanilide</td>
<td>79.5</td>
<td>182</td>
</tr>
<tr>
<td>22</td>
<td>2-chloro cinnamic acid</td>
<td>2-chloro cinnamanilide</td>
<td>76.0</td>
<td>146</td>
</tr>
<tr>
<td>23</td>
<td>4-methoxy cinnamic acid</td>
<td>4-methoxy cinnamanilide</td>
<td>68.4</td>
<td>135</td>
</tr>
</tbody>
</table>
Results and Discussion

Figure 4.4: Amides of substituted cinnamic acids with aniline.

Table 4.5: Amides of substituted cinnamic acids with N-methyl aniline.

<table>
<thead>
<tr>
<th>Compounds No.</th>
<th>Reactant</th>
<th>Product</th>
<th>Yield (%)</th>
<th>M.P. (°C)</th>
<th>Colour</th>
</tr>
</thead>
<tbody>
<tr>
<td>24.</td>
<td>cinnamic acid</td>
<td>N- methyl cinnamicilide</td>
<td>70.0</td>
<td>170</td>
<td>Light grey</td>
</tr>
<tr>
<td>25.</td>
<td>3-nitro cinnamic acid</td>
<td>N- methyl 3-nitro cinnamicilide</td>
<td>75.7</td>
<td>62</td>
<td>Brown</td>
</tr>
<tr>
<td>26.</td>
<td>4-nitro cinnamic acid</td>
<td>N- methyl 4-nitro cinnamicilide</td>
<td>73.0</td>
<td>78</td>
<td>Off white</td>
</tr>
<tr>
<td>27.</td>
<td>2-chloro cinnamic acid</td>
<td>N- methyl 2-chloro cinnamicilide</td>
<td>77.0</td>
<td>22</td>
<td>Grey</td>
</tr>
<tr>
<td>28.</td>
<td>4-methoxy cinnamic acid</td>
<td>N- methyl 4-methoxy cinnamicilide</td>
<td>75.0</td>
<td>136</td>
<td>Black</td>
</tr>
</tbody>
</table>
Figure 4.5: Amides of substituted cinnamic acids with N-methyl aniline.

Table 4.6: Amides of substituted cinnamic acids with morpholine

<table>
<thead>
<tr>
<th>Compounds No.</th>
<th>Reactant</th>
<th>Product</th>
<th>Yield (%)</th>
<th>Physical properties of products</th>
</tr>
</thead>
<tbody>
<tr>
<td>29.</td>
<td>cinnamic acid</td>
<td>Cinnamoyl morpholine</td>
<td>75.0</td>
<td>170 White</td>
</tr>
<tr>
<td>30.</td>
<td>3-nitro cinnamic acid</td>
<td>3-nitrocinnamoyl morpholine</td>
<td>76.0</td>
<td>135 Light brown</td>
</tr>
<tr>
<td>31.</td>
<td>4-nitro cinnamic acid</td>
<td>4-nitrocinnamoyl morpholine</td>
<td>74.0</td>
<td>141 Off white</td>
</tr>
<tr>
<td>32.</td>
<td>2-chloro cinnamic acid</td>
<td>2-chlorocinnamoyl morpholine</td>
<td>77.0</td>
<td>137 White</td>
</tr>
<tr>
<td>33.</td>
<td>4-methoxy cinnamic acid</td>
<td>4-methoxycinnamoyl morpholine</td>
<td>73.0</td>
<td>142 Light pistachio</td>
</tr>
</tbody>
</table>
Figure 4.6: Amides of substituted cinnamic acids with morpholine.

Table 4.7: Amides of substituted cinnamic acids with o-chloro aniline.

| Compounds No. | Reactant                  | Product                              | Yield (%) | Physical properties of products |
|---------------|---------------------------|                                     |           | M.P. (°C) | Colour         |
| 34.           | cinnamic acid             | 2-chloro cinnamanilide              | 80.0      | 180       | White         |
| 35.           | 4-hydroxy cinnamic acid   | 2-chloro-(4΄-hydroxy) cinnamanilide | 89.2      | 167       | Light brown   |
| 36.           | 3-nitro cinnamic acid     | 2-chloro-(3΄-nitro) cinnamanilide   | 82.4      | 210       | Brown         |
| 37.           | 4-nitro cinnamic acid     | 2-chloro-(4΄-nitro) cinnamanilide   | 65.0      | 158       | Off white     |
| 38.           | 2-chloro cinnamic acid    | 2-chloro-(2΄-chloro) cinnamanilide  | 75.0      | 162       | White         |
| 39.           | 4-methoxy cinnamic acid   | 2-chloro-(4΄-methoxy) cinnamanilide | 71.0      | 130       | Brown         |
4.1.3 Spectral Characterization

4.1.3.1 FTIR spectral analysis

The FTIR spectra of all the synthetic compounds were recorded in the KBr pellets. The FTIR spectra of substituted cinnamic acid were characterized by the presence of absorption band at 2940-2560 cm\(^{-1}\) which was due to aliphatic C-H str. vibration. The absorption band at 1700-1650 cm\(^{-1}\) was due to the C=O groups in the compounds. The absorption band from 1622-1444 cm\(^{-1}\) corresponds to the str. vibrations of aromatic ring double bonds.
The FTIR spectra of substituted cinnamic acid amides were characterized by the presence of absorption band at 3425-3270 cm\(^{-1}\) which was due to –NH group, absorption band at 3125-2880 cm\(^{-1}\) was due to C-H groups and absorption band at 1700-1662 due to the C=O groups in the compounds.

### 4.1.3.2 \(^1\)H NMR spectal analysis

The \(^1\)H NMR spectra of all compounds were taken in the deuterated chloroform with Bruker Avance II 300 MHz and Bruker Avance II 400 MHz Spectrometer.

#### 4.1.3.2.1 \(^1\)H NMR specta of Substitued cinnamic acids

In the spectrum of different substituted cinnamic acid the chemical shift values of the proton of –CH=CH- are approximately at the \(\delta\) 6 and 7.7 ppm. Both proton show coupling and split in to doublet which is equal to 16 Hz.

#### 4.1.3.2.2 \(^1\)H NMR spectral of Substitued cinnamic acid amide derivatives

In the NMR spectrum of different substituted cinnamic acid amide derivatives, two doublets of two protons of –CH=CH– at 6.6 and 7.7 ppm and J =16 Hz exhibit that they are strongly coupled and trans to each other. The –NH proton gives a broad peak at 6.18-6.20 ppm.

1. **4-methoxy cinnamic acid:** IR (KBR) 2937.3, 2842.7, 2559.3, 1947.9 1888.6, 1686.1, 1622.7-1444.4 Cm\(^{-1}\), \(^1\)H NMR (CDCl\(_3\), 400
 Results and Discussion

2. **3-Nitro cinnamic acid**: IR (KBR) 3427, 1688, 1625, 1600, 1535, 1348, 845 Cm\(^{-1}\). \(^1\)H NMR (CDCl\(_3\), 300 MHz) \(\delta\) 6.19 (1H, d, J= 16.2 Hz, trans –CH=CH-): 7.28 (1H, d, J=16.2 Hz, trans-CH=CH-): 7.83-7.35 (4H, m, Ar-H).

3. **4-Hydroxic cinnamic acid**: IR (KBR) 3376, 1674, 1630,1598, 1448,1214 Cm\(^{-1}\). \(^1\)H NMR (CDCl\(_3\), 300 MHz) \(\delta\) 6.24 (1H, d, J=16 Hz, –CH=CH-): 6.84 (2H, d, J=8.5 Hz, H-6 Ar-H): 7.35 (2H, d, J=8.4 Hz, Ar-H): 7.6 (1H, d, J=15.9 Hz, trans –CH=CH-).

4. **2-Chloro cinnamic acid**: IR (KBR) 3419, 3075, 2946, 2880, 1789, 1761, 1653, 1598 Cm\(^{-1}\). \(^1\)H NMR (CDCl\(_3\), 300 MHz) \(\delta\) 6.43 (1H, d, J=16 Hz, trans -CH=CH-): 7.291-7.688 (4H, m, Ar-H): 8.191 (1H, d, J = 16 Hz, trans –CH=CH-).

5. **Cinnamoyl piperidine**: IR (KBR) 3450, 3029, 2858.8, 1642, 1590, 1501, 1441.3 Cm\(^{-1}\). \(^1\)H NMR (CDCl\(_3\), 300 MHz) \(\delta\) 1.61- 1.68 (6H, m, -CH\(_2\)): 3.34 (4H, m, -CH\(_2\)): 6.87 (1H, d, J=15.8 Hz, trans –CH=CH-): 7.26-7.50 (5H, m, Ar-H):

4.1.4. **Herbicidal Activity of cinnamic acid amide derivatives**

Mean percent seed germination inhibition values for different substituted cinnamic acids amides are presented in **Tables (4.8-4.13)**.
Table 4.8: Mean Percent Seed Germination Inhibition Values of Substituted Amides of piperidine

<table>
<thead>
<tr>
<th>Name of the Compound</th>
<th>Mean 50ppm</th>
<th>Percent 100ppm</th>
<th>Inhibition 200ppm</th>
<th>Value CD at 5%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Butachlor (standard)</td>
<td>53.33</td>
<td>60.00</td>
<td>65.00</td>
<td>8.79</td>
</tr>
<tr>
<td>pendimethalin (standard)</td>
<td>56.67</td>
<td>61.67</td>
<td>68.33</td>
<td>14.10</td>
</tr>
<tr>
<td>Cinnamoyl piperidine</td>
<td>66.66</td>
<td>71.66</td>
<td>75.00</td>
<td>9.40</td>
</tr>
<tr>
<td>1-(2′-chlorocinnamoyl) piperidine</td>
<td>58.33</td>
<td>63.33</td>
<td>71.66</td>
<td>16.29</td>
</tr>
<tr>
<td>1-(4′-methoxycinnamoyl) piperidine</td>
<td>55.00</td>
<td>61.67</td>
<td>70.00</td>
<td>8.79</td>
</tr>
<tr>
<td>1-(3′-nitrocinnamoyl) piperidine</td>
<td>55.00</td>
<td>58.33</td>
<td>63.33</td>
<td>14.87</td>
</tr>
<tr>
<td>1-(4′-nitrocinnamoyl) piperidine</td>
<td>56.66</td>
<td>58.33</td>
<td>61.66</td>
<td>11.51</td>
</tr>
<tr>
<td>1-(4′-hydroxycinnamoyl) piperidine</td>
<td>53.33</td>
<td>58.33</td>
<td>63.33</td>
<td>5.75</td>
</tr>
<tr>
<td>CD at 5%</td>
<td>12.98</td>
<td>9.34</td>
<td>7.28</td>
<td></td>
</tr>
</tbody>
</table>

Perusal of Table-4.8 clearly indicates that there is no significant increase in mean percent seed germination inhibition (SGI) activity with increase in concentration except for 1-(4′-methoxycinnamoyl) piperidine. Thus in most of the cases activities are at par for different concentration. At 50 ppm conc. highest SGI activity is exhibited by cinnamoyl piperidine. Inhibition values of other compounds except 1-(4′-hydroxy cinnamoyl) piperidine and butachlor (standard) are at par with that of cinnamoyl piperidine. Lower activity for 1-(4′-hydroxy
cinnamoyl) piperidine may be attributed to the presence of electron donating -OH substituent group present on benzene ring of acid moiety of amide. At 100 ppm concentration highest SGI activity is observed again for cinnamoyl piperidine and the value is at par with 1-(2′-chlorocinnamoyl)piperidine. Inhibition values of other compounds even including standard butachlor and pendimethalin are significantly lower than cinnamoyl piperidine. Higher values of mean percent seed germination inhibition of electron withdrawing chlorine group present on benzene ring on acid moiety. One more factor seems responsible for affecting the activity less bulky group are more effective in increasing or decreasing activity. Amongst electron attracting group less bulky chlorine group tends to increase the activity more in comparison to more bulky group (\(-\text{NO}_2\)) likewise less bulky electron donating group (\(-\text{OH}\)) is more effective in comparison to more bulky \((-\text{OCH}_3)\) group in decreasing the activity. At 200 ppm concentration the same trend is observed.

Perusal of Table-4.9 reveals that for most of the compounds mean percent SGI activity does not increase significantly with increase in concentration. At 50 ppm concentration maximum value for mean percent SGI activity is exhibited by N-cyclohexyl-1-(4′-nitro)cinnamamide which is at par with N-cyclohexylcinnamamide. Other compounds N-cyclohexyl-1-(2′-chlo)cinnamamide, N-cyclohexyl-1-(4′-methoxy)cinnamamide and N-cyclohexyl-1-(3′-nitro)cinnamamide
including standards butachlor and pendimethalin exhibit lower SGI activity. Higher value in case of N-cyclohexyl-1-(4′-nitro)cinnamamide may be attributed to the presence of electron attracting -NO₂ group though effect of substituent group is not contrasting in case of other compounds. At 100 ppm same trend is observed. At 200 ppm concentration, highest value was exhibited again by N-cyclohexyl-1-(4′-nitro) cinnamamide and values of all other compounds are at par with it that of except N-cyclohexyl-1-(3′-nitro) cinnamamide.

**Table 4.9: Mean Percent Seed Germination Inhibition Values of Substituted Amides of cyclohexyl amine.**

<table>
<thead>
<tr>
<th>Name of the Compound</th>
<th>Mean 50ppm</th>
<th>Percent 100ppm</th>
<th>Inhibition 200ppm</th>
<th>Value CD at 5%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Butachlor (standard)</td>
<td>53.33</td>
<td>60.00</td>
<td>65.00</td>
<td>8.79</td>
</tr>
<tr>
<td>pendimethalin (standard)</td>
<td>56.67</td>
<td>61.67</td>
<td>68.33</td>
<td>14.10</td>
</tr>
<tr>
<td>N-cyclohexyl cinnamamide</td>
<td>65.00</td>
<td>66.67</td>
<td>70.00</td>
<td>6.65</td>
</tr>
<tr>
<td>N-cyclohexyl 1-(2′-chloro) cinnamamide</td>
<td>53.33</td>
<td>55.00</td>
<td>68.33</td>
<td>11.02</td>
</tr>
<tr>
<td>N-cyclohexyl 1-(4′-methoxy) cinnamamide</td>
<td>53.33</td>
<td>56.67</td>
<td>65.00</td>
<td>7.43</td>
</tr>
<tr>
<td>N-cyclohexyl-1-(3′-nitro) cinnamamide</td>
<td>53.33</td>
<td>58.33</td>
<td>58.33</td>
<td>15.23</td>
</tr>
<tr>
<td>CD at 5%</td>
<td>12.38</td>
<td>8.54</td>
<td>10.10</td>
<td></td>
</tr>
</tbody>
</table>

Perusal of table-4.10 indicates that for all the compounds mean percent SGI activity does not increase significantly with increase in
concentration from 100 ppm to 200 ppm. For all compounds, values at 100 ppm concentration are at par with value of 200 ppm.

At 50 ppm concentration maximum value for mean percent SGI activity is exhibited by 4-Nitrocinnamanilide which is at par with cinnamanilide and 4-hydroxy-cinnamanilide. Other compounds 2-chlorocinnamanilide, 4-methoxycinnamanilide, 3-nitrocinnamanilide including butachlor and pendimethalin (standard) exhibit lower mean percent SGI activity. Higher value in case of 4-nitrocinnamanilide may be attributed to presence of electron attracting nitro group though effect of substituent groups for other compounds could not be correlated with electron donating or electron attracting tendencies of substituents groups. At 100 ppm concentration highest mean percent SGI activity is observed again for 4-nitrocinnamanilide and cinnamanilide which is at par with all other compounds including standards. The case of 3-nitrocinnamanilide is exceptional as it exhibit lower mean percent SGI activity inspite of having electron withdrawing substituent group. At 200 ppm concentration highest mean percent SGI activity was obtained for cinnamanilide and 4-nitrocinnamanilide. The compound 2-chlorocinnamanilide, 4-methoxycinnamanilide, and 4-hydroxycinnamanilide were found to be at par with cinnamanilide and 4-nitrocinnamanilide. While 3-nitro cinnamanilide and both standards were to found to exhibit significantly lower activity then cinnamanilide and 4-nitro cinnamanilide.
Table 4.10: Mean Percent Seed Germination Inhibition Values of Substituted Amides of aniline

<table>
<thead>
<tr>
<th>Name of the Compound</th>
<th>Mean 50ppm</th>
<th>Percent 100ppm</th>
<th>Inhibition 200ppm</th>
<th>Value CD at 5%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Butachlor (standard)</td>
<td>53.33</td>
<td>60.00</td>
<td>65.00</td>
<td>8.79</td>
</tr>
<tr>
<td>pendimethalin (standard)</td>
<td>56.67</td>
<td>61.67</td>
<td>68.33</td>
<td>14.10</td>
</tr>
<tr>
<td>cinnamanilide</td>
<td>61.67</td>
<td>71.67</td>
<td>76.67</td>
<td>5.75</td>
</tr>
<tr>
<td>2 chlorocinnamanilide</td>
<td>55.00</td>
<td>60.00</td>
<td>70.00</td>
<td>12.87</td>
</tr>
<tr>
<td>4-methoxy cinnamanilide</td>
<td>55.00</td>
<td>68.33</td>
<td>73.33</td>
<td>13.71</td>
</tr>
<tr>
<td>3-nitro cinnamanilide</td>
<td>58.33</td>
<td>58.33</td>
<td>68.33</td>
<td>15.23</td>
</tr>
<tr>
<td>4-nitro cinnamanilide</td>
<td>71.67</td>
<td>71.67</td>
<td>76.67</td>
<td>5.75</td>
</tr>
<tr>
<td>4-hydroxy cinnamanilide</td>
<td>60.00</td>
<td>66.67</td>
<td>71.67</td>
<td>9.40</td>
</tr>
<tr>
<td>CD at 5%</td>
<td>12.11</td>
<td>9.17</td>
<td>7.49</td>
<td></td>
</tr>
</tbody>
</table>

Persual of Table-4.11 indicates that there is no significant increase in Percent SGI values with increase in concentration for compounds N-methyl cinnamanilide, N-methyl-(3’-nitro)cinnamanilide and N-methyl-(4’-nitro)cinnamanilide. For compounds N-methyl-1(2’-chloro)cinnamanilide and N-methyl-(4’-methoxy)cinnamanilide there is significant increase in mean percent SGI values with increase in concentration. At 50 ppm concentration highest mean percent SGI activity is exhibited by N-methylcinnamanilide. Activities of other compounds including pendimethalin & butachlor are significantly lower than that of N-methylcinnamanilide. At 100 ppm concentration again highest mean percent SGI activity is exhibited by N-methylcinnamanilide, other
compounds including standards butachlor and pendimethalin exhibit significantly lower mean percent SGI activity than N-methylcinnamanilide. At 200 ppm concentration same trend is observed. It is observed that any substituent whether electron withdrawing or electron donating present on benzene ring of acid moiety tends of decrease the activity.

**Table 4.11: Mean Percent Seed Germination Inhibition Values of Substituted Amides of N-methyl aniline**

<table>
<thead>
<tr>
<th>Name of the Compound</th>
<th>Mean 50ppm</th>
<th>Percent 100ppm</th>
<th>Inhibition 200ppm</th>
<th>Value CD at 5%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Butachlor (standard)</td>
<td>53.33</td>
<td>60.00</td>
<td>65.00</td>
<td>8.79</td>
</tr>
<tr>
<td>pendimethalin (standard)</td>
<td>56.67</td>
<td>61.67</td>
<td>68.33</td>
<td>14.10</td>
</tr>
<tr>
<td>N- methyl cinnamanilide</td>
<td>70.00</td>
<td>75.00</td>
<td>85.00</td>
<td>11.51</td>
</tr>
<tr>
<td>N- methyl-1(2′-chlro)cinnamanilide</td>
<td>53.33</td>
<td>56.67</td>
<td>68.33</td>
<td>8.14</td>
</tr>
<tr>
<td>N- methyl-(4′-methoxy)cinnamanilide</td>
<td>51.67</td>
<td>56.67</td>
<td>68.33</td>
<td>8.14</td>
</tr>
<tr>
<td>N- methyl-(3′-nitro)cinnamanilide</td>
<td>53.33</td>
<td>53.33</td>
<td>56.67</td>
<td>5.75</td>
</tr>
<tr>
<td>N- methyl-(4′-nitro)cinnamanilide</td>
<td>51.67</td>
<td>51.67</td>
<td>55.00</td>
<td>9.40</td>
</tr>
<tr>
<td>CD at 5%</td>
<td>11.47</td>
<td>7.87</td>
<td>5.05</td>
<td></td>
</tr>
</tbody>
</table>

Table-4.12 indicates that for compounds Cinnamoylmorpholine, 1-(3′-nitrocinnamoyl)morpholine, 1-(4′-nitrocinnamoyl)morpholine and 1-(4′-methoxycinnamoyl)morpholine there is no significant increase in mean percent SGI activity with increase in concentration whereas for compound 1-(2′-chlorocinnamoyl)morpholine there is significant increase in mean percent SGI value with increase in concentration. At 50 ppm
concentration 1-(3′-nitrocinnamoyl)morpholine and cinnamoylmorpholine exhibit highest mean percent SGI activity. All other compounds including butachlor and pendimethalin (standards) are at par with cinnamoylmorpholine and 1-(3′-nitrocinnamoyl)morpholine. At 100 ppm concentration compound cinnamoylmorpholine exhibit highest mean percent (SGI) activity followed by 1-(4′-methoxycinnamoyl)morpholine. All other compounds including butachlor and pendimethalin (standard) are at par with cinnamoylmorpholine and 1-(4′-methoxycinnamoyl)morpholine. At 200 ppm same trend is observed.

**Table 4.12: Mean Percent Seed Germination Inhibition Values of Substituted Amides of morpholine**

<table>
<thead>
<tr>
<th>Name of the Compound</th>
<th>Mean 50ppm</th>
<th>Percent 100ppm</th>
<th>Inhibition 200ppm</th>
<th>Value CD at 5%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Butachlor (standard)</td>
<td>53.33</td>
<td>60.00</td>
<td>65.00</td>
<td>8.79</td>
</tr>
<tr>
<td>pendimethalin (standard)</td>
<td>56.67</td>
<td>61.67</td>
<td>68.33</td>
<td>14.10</td>
</tr>
<tr>
<td>Cinnamoyl morpholine</td>
<td>63.33</td>
<td>66.67</td>
<td>73.33</td>
<td>16.29</td>
</tr>
<tr>
<td>1-(2′-chlorocinnamoyl) morpholine</td>
<td>55.00</td>
<td>58.33</td>
<td>70.00</td>
<td>14.49</td>
</tr>
<tr>
<td>1-(4′-methoxycinnamoyl) morpholine</td>
<td>56.67</td>
<td>66.67</td>
<td>76.67</td>
<td>16.29</td>
</tr>
<tr>
<td>1-(3′-nitrocinnamoyl) morpholine</td>
<td>63.33</td>
<td>63.33</td>
<td>66.67</td>
<td>11.51</td>
</tr>
<tr>
<td>1-(4′-nitrocinnamoyl) morpholine</td>
<td>56.67</td>
<td>61.67</td>
<td>61.67</td>
<td>25.10</td>
</tr>
<tr>
<td>CD at 5%</td>
<td>15.16</td>
<td>13.23</td>
<td>11.93</td>
<td></td>
</tr>
</tbody>
</table>

Table-4.13 indicates that there is no significant increase in mean percent SGI activity with increase in concentration except for
2-chloro(2′-chloro)cinnamanilide. At 50 ppm concentration 2-chloro-(4′-nitro)cinnamanilide exhibit highest activity. Other compounds including butachlor and pendimethalin are at par with it. 2-Chloro(2′-chloro)cinnamanilide and 2-chloro-(3′-nitro)cinnamanilide exhibit significantly lower activity than 2-chloro-(4′-nitro)cinnamanilide. At 100 ppm same trend is observed. At 200 ppm concentration again 2-chloro-(4′-nitro)cinnamanilide exhibit highest activity. Compounds 2-chlorocinnamanilide, 2-chloro-(2′-chloro)cinnamanilide are at par with it. whereas compounds 2-chloro(4′-methoxy)cinnamanilide, 2-chloro-(3′-nitro)cinnamanilide exhibit significantly lower mean percent SGI activity than 2-chloro-(4′-nitro)cinnamanilide.

Table 4.13: Mean Percent Seed Germination Inhibition Values of Substituted Amides of 2-chloro aniline

<table>
<thead>
<tr>
<th>Name of the Compound</th>
<th>Mean 50ppm</th>
<th>Percent 100ppm</th>
<th>Inhibition 200ppm</th>
<th>Value CD at 5%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Butachlor (standard)</td>
<td>53.33</td>
<td>60.00</td>
<td>65.00</td>
<td>8.79</td>
</tr>
<tr>
<td>pendimethalin (standard)</td>
<td>56.67</td>
<td>61.67</td>
<td>68.33</td>
<td>14.10</td>
</tr>
<tr>
<td>2-chlorocinnamanilide</td>
<td>60.00</td>
<td>68.33</td>
<td>73.33</td>
<td>14.87</td>
</tr>
<tr>
<td>2-chloro-(2′-chloro)cinnamanilide</td>
<td>50.00</td>
<td>53.33</td>
<td>75.00</td>
<td>11.98</td>
</tr>
<tr>
<td>2-chloro(4′-methoxy)cinnamanilide</td>
<td>56.67</td>
<td>60.00</td>
<td>68.33</td>
<td>13.71</td>
</tr>
<tr>
<td>2-chloro-(3′-nitro)cinnamanilide</td>
<td>50.00</td>
<td>53.33</td>
<td>65.00</td>
<td>15.59</td>
</tr>
<tr>
<td>2-chloro-(4′-nitro)cinnamanilide</td>
<td>63.33</td>
<td>71.67</td>
<td>78.33</td>
<td>17.27</td>
</tr>
<tr>
<td>2-chloro-(4′-hydroxy)cinnamanilide</td>
<td>56.67</td>
<td>63.33</td>
<td>68.33</td>
<td>15.23</td>
</tr>
<tr>
<td>CD at 5%</td>
<td>12.11</td>
<td>14.88</td>
<td>9.00</td>
<td></td>
</tr>
</tbody>
</table>
4.2 Synthesis of 4-oxo-4-phenyl-2-butenoic esters analogues

Substituted 4-oxo-4-phenyl-2-butenoic ester were prepared by the reaction of substituted 4-oxo-4-phenyl-2-butenoic acids and substituted phenacylbromides in presence of base and the solvent DMF. The % yield, colour and physical properties are displayed in the (Table 4.14).

4.2 Spectral Characterization

4.2.1 UV-vis spectal analysis

The UV-Vis spectra of all the synthesized butenoates were taken in chloroform. The UV-Vis spectra of substituted 4-oxo-4-phenylbut-2-enoates are shown in appendix. The UV-Vis spectra of substituted 4-oxo-4-phenylbut-2-enoates exhibit λmax at 235-260 nm due to n-π* (>C=O group) transition.

4.2.2 FTIR spectal analysis

The FT-IR spectra of all the synthetic compounds were recorded as KBr pellets. The FTIR spectra of all synthesized butenoates were characterized by the presence of absorption band for aromatic C-H str. appearing at 3070-3083.6 cm⁻¹ and aliphatic at 2930-2942.2 cm⁻¹. Peaks for three >C=O str. appeared at 1725.2-1662.8 cm⁻¹. The C=C str. for aromatic ring appears at 1624.7 cm⁻¹. The C-O str. vibration gives absorption band at 1176.9 cm⁻¹.

4.2.3 ¹H NMR spectal analysis

The NMR spectra of the synthesized butenoates were taken in the deuterated chloroform with Bruker Avance II 400 NMR spectrometer.
Table 4.14: Physical properties of 4-oxo-4-phenyl-2-butenolic ester analogues

<table>
<thead>
<tr>
<th>Serial No.</th>
<th>Ester</th>
<th>Code No.</th>
<th>Yield (%)</th>
<th>M.P. (°C)</th>
<th>Colour</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>2-(4-methylphenyl)-2-oxoethyl (2E)-4-oxo-4-phenylbut-2-enoate</td>
<td>B1</td>
<td>72.0</td>
<td>155</td>
<td>white</td>
</tr>
<tr>
<td>2.</td>
<td>2-(4-chlorophenyl)-2-oxoethyl (2E)-4-oxo-4-phenylbut-2-enoate</td>
<td>B2</td>
<td>63.0</td>
<td>137</td>
<td>white</td>
</tr>
<tr>
<td>3.</td>
<td>2-oxo-2-phenylethyl (2E)-4-oxo-4-phenylbut-2-enoate</td>
<td>B3</td>
<td>57.0</td>
<td>123</td>
<td>Light yellow</td>
</tr>
<tr>
<td>4.</td>
<td>2-(4-methoxyphenyl)-2-oxoethyl (2E)-4-oxo-4-phenylbut-2-enoate</td>
<td>B4</td>
<td>63.0</td>
<td>80</td>
<td>Brown</td>
</tr>
<tr>
<td>5.</td>
<td>2-(4-nitrophenyl)-2-oxoethyl (2E)-4-oxo-4-phenylbut-2-enoate</td>
<td>B5</td>
<td>63.0</td>
<td>81</td>
<td>Light yellow</td>
</tr>
<tr>
<td>6.</td>
<td>2-(4-methylphenyl)-2-oxoethyl (2E)-4-(4-chlorophenyl)-4-oxobut-2-enoate</td>
<td>C1</td>
<td>53.0</td>
<td>151</td>
<td>Light yellow</td>
</tr>
<tr>
<td>7.</td>
<td>2-(4-chlorophenyl)-2-oxoethyl (2E)-4-(4-chlorophenyl)-4-oxobut-2-enoate</td>
<td>C2</td>
<td>55.0</td>
<td>143</td>
<td>white</td>
</tr>
<tr>
<td>8.</td>
<td>2-oxo-2-phenylethyl (2E)-4-(4-chlorophenyl)-4-oxobut-2-enoate</td>
<td>C3</td>
<td>56.0</td>
<td>161</td>
<td>Light yellow</td>
</tr>
<tr>
<td>9.</td>
<td>2-(4-methylphenyl)-2-oxoethyl (2E)-4-(4-ethylphenyl)-4-oxobut-2-enoate</td>
<td>E1</td>
<td>52.0</td>
<td>86</td>
<td>Light yellow</td>
</tr>
<tr>
<td>10.</td>
<td>2-(4-chlorophenyl)-2-oxoethyl (2E)-4-(4-ethylphenyl)-4-oxobut-2-enoate</td>
<td>E2</td>
<td>57.0</td>
<td>66</td>
<td>brown</td>
</tr>
<tr>
<td>11.</td>
<td>2-(4-methylphenyl)-2-oxoethyl (2E)-4-(4-methylphenyl)-4-oxobut-2-enoate</td>
<td>M1</td>
<td>62.0</td>
<td>132</td>
<td>Light yellow</td>
</tr>
<tr>
<td>12.</td>
<td>2-(4-chlorophenyl)-2-oxoethyl (2E)-4-(4-methylphenyl)-4-oxobut-2-enoate</td>
<td>M2</td>
<td>53.0</td>
<td>153</td>
<td>Yellow</td>
</tr>
<tr>
<td>13.</td>
<td>2-oxo-2-phenylethyl (2E)-4-(4-methylphenyl)-4-oxobut-2-enoate</td>
<td>M3</td>
<td>78.0</td>
<td>73</td>
<td>Light brown</td>
</tr>
<tr>
<td>14.</td>
<td>2-(4-nitrophenyl)-2-oxoethyl (2E)-4-(4-methylphenyl)-4-oxobut-2-enoate</td>
<td>M4</td>
<td>56.0</td>
<td>90</td>
<td>Light brown</td>
</tr>
<tr>
<td>15.</td>
<td>2-oxo-2-phenylethyl (2E)-4-(4-bromophenyl)-4-oxobut-2-enoate</td>
<td>Br1</td>
<td>45.0</td>
<td>143</td>
<td>white</td>
</tr>
<tr>
<td>16.</td>
<td>2-(4-chlorophenyl)-2-oxoethyl (2E)-4-(4-bromophenyl)-4-oxobut-2-enoate</td>
<td>Br2</td>
<td>61.0</td>
<td>157</td>
<td>Light yellow</td>
</tr>
<tr>
<td>17.</td>
<td>2-(4-methoxyphenyl)-2-oxoethyl (2E)-4-(4-bromophenyl)-4-oxobut-2-enoate</td>
<td>Br3</td>
<td>73.0</td>
<td>103</td>
<td>Light yellow</td>
</tr>
<tr>
<td>18.</td>
<td>2-(4-nitrophenyl)-2-oxoethyl (2E)-4-(4-bromophenyl)-4-oxobut-2-enoate</td>
<td>Br4</td>
<td>55.0</td>
<td>92</td>
<td>Light brown</td>
</tr>
</tbody>
</table>
Results and Discussion

2-(4-methylphenyl)-2-oxoethyl (2E)-4-oxo-4-phenylbut-2-enoate

2-(4-chlorophenyl)-2-oxoethyl (2E)-4-oxo-4-phenylbut-2-enoate

2-oxo-2-phenylethyl (2E)-4-oxo-4-phenylbut-2-enoate

2-(4-methoxyphenyl)-2-oxoethyl (2E)-4-oxo-4-phenylbut-2-enoate

2-(4-nitrophenyl)-2-oxoethyl (2E)-4-oxo-4-phenylbut-2-enoate

2-(4-methylphenyl)-2-oxoethyl (2E)-4-(4-chlorophenyl)-4-oxobut-2-enoate
Results and Discussion

2-(4-chlorophenyl)-2-oxoethyl \((2E)-4\)-(4-chlorophenyl)-4-oxobut-2-enoate

2-oxo-2-phenylethyl \((2E)-4\)-(4-chlorophenyl)-4-oxobut-2-enoate

2-(4-methylphenyl)-2-oxoethyl \((2E)-4\)-(4-ethylphenyl)-4-oxobut-2-enoate

2-(4-chlorophenyl)-2-oxoethyl \((2E)-4\)-(4-ethylphenyl)-4-oxobut-2-enoate

2-(4-chlorophenyl)-2-oxoethyl \((2E)-4\)-(4-methylphenyl)-4-oxobut-2-enoate

2-(4-chlorophenyl)-2-oxoethyl \((2E)-4\)-(4-methylphenyl)-4-oxobut-2-enoate

2-(4-chlorophenyl)-2-oxoethyl \((2E)-4\)-(4-chlorophenyl)-4-oxobut-2-enoate

2-(4-methylphenyl)-2-oxoethyl \((2E)-4\)-(4-methylphenyl)-4-oxobut-2-enoate
Results and Discussion

Figure 4.8: Structure of 4-oxo-4-phenyl-2-butenoic ester analogues.
NMR spectrum of above compounds gives the chemical shift values of the \(-\text{CH}═\text{CH}−\) at \(\delta 7.00\) and \(8.00\). Both the protons show coupling and split into doublet with equal \(J\approx15.6\) Hz. The high value of \(J\) indicates trans structure. The two protons of CH2 give signal at \(\delta 5.40\) as a singlet. The protons of two benzene rings give overlapping signals between \(\delta 7.40\)-8.09.

4.2.4 Spectral characterization data:

1. **2-(4-chlorophenyl)-2-oxoethyl (2E)-4-oxo-4-phenylbut-2-enoate (B2):** \(\lambda_{\text{max}}\) (nm)/DCM, 248. IR \(\nu_{\text{max}}\) (cm\(^{-1}\), KBr): 3073.6, 2942.2, 1725.2, 1702.9, 1662.8, 1624.7, 1176.9. \(\text{\textsuperscript{1}H NMR}\) (400 MHz, CDCl\(_3\)): 7.04 (1H, d, \(J=15.8\), -CH=CH-), 8.02 (1H, d, -CH=CH-), 5.46 (2H, s, CH\(_2\)), 7.48-8.04 (m, benzene ring).

2. **2-(4-nitrophenyl)-2-oxoethyl (2E)-4-oxo-4-phenylbut-2-enoate (B5):** \(\lambda_{\text{max}}\) (nm)/DCM, 240. IR \(\nu_{\text{max}}\) (cm\(^{-1}\), KBr): 3079.0, 2938.2, 1734.7, 1707.6, 1671.4, 1614.4, 1166.1. \(\text{\textsuperscript{1}H NMR}\) (400 MHz, CDCl\(_3\)): 7.04 (1H, d, \(J=15.7\), -CH=CH-), 8.04 (1H, d, -CH=CH-), 5.53 (2H, s, CH\(_2\)), 7.51-8.78 (m, benzene ring).

3. **2-(4-methylphenyl)-2-oxoethyl (2E)-4-(4-chlorophenyl)-4-oxobut-2-enoate (C1):** \(\lambda_{\text{max}}\) (nm)/DCM, 250. IR \(\nu_{\text{max}}\) (cm\(^{-1}\), KBr): 3076.5, 2928.1, 1727.3, 1696.7, 1666.8, 1625.7, 1171.5. \(\text{\textsuperscript{1}H NMR}\) (400 MHz, CDCl\(_3\)): 7.05 (1H, d, \(J=15.9\), -CH=CH-), 7.97 (1H, d, -CH=CH-), 2.43 (3H, s, CH\(_3\)), 5.49 (2H, s, CH\(_2\)), 7.26-7.99 (m, benzene ring).
4. **2-oxo-2-phenylethyl (2E)-4-(4-chlorophenyl)-4-oxobut-2-enoate (C3):** $\lambda_{\text{max}}$ (nm)/DCM, 244. $^1$H NMR (400 MHz, CDCl$_3$): 7.05 (1H, d, J=15.8, -CH=CH-), 7.97 (1H, d, -CH=CH-), 5.41 (2H, s, CH$_2$), 7.49-7.97 (m, benzene ring).

5. **2-(4-methylphenyl)-2-oxoethyl (2E)-4-(4-methylphenyl)-4-oxobut-2-enoate (M1):** $\lambda_{\text{max}}$ (nm)/DCM, 254. $^1$H NMR (400 MHz, CDCl$_3$): 7.02 (1H, d, J=15.9, -CH=CH-), 8.02 (1H, d, -CH=CH-), 2.44 (3H, s, CH$_3$), 5.46 (2H, s, CH$_2$), 7.30-7.93 (m, benzene ring).

6. **2-(4-chlorophenyl)-2-oxoethyl (2E)-4-(4-methylphenyl)-4-oxobut-2-enoate (M2):** $\lambda_{\text{max}}$ (nm)/DCM, 258. IR $\nu_{\text{max}}$ (cm$^{-1}$, KBr): 3068.2, 2941.9, 1729.0, 1705.7, 1661.6, 1621.5, 1171.5. $^1$H NMR (400 MHz, CDCl$_3$): 7.02 (1H, d, J=15.7, -CH=CH-), 8.02 (1H, d, -CH=CH-), 2.44 (3H, s, CH$_3$), 5.46 (2H, s, CH$_2$), 7.30-7.93 (m, benzene ring).

7. **2-(4-chlorophenyl)-2-oxoethyl (2E)-4-(4-bromophenyl)-4-oxobut-2-enoate (Br2):** $\lambda_{\text{max}}$ (nm)/DCM, 260. $^1$H NMR (400 MHz, CDCl$_3$): 7.04 (1H, d, J=15.9, -CH=CH-), 7.96 (1H, d, -CH=CH-), 5.47 (2H, s, CH$_2$), 3.89, 6.99-8.15 (m, benzene ring).

8. **2-(4-methylphenyl)-2-oxoethyl (2E)-4-(4-ethylphenyl)-4-oxobut-2-enoate (E1):** $\lambda_{\text{max}}$ (nm)/DCM, 256. $^1$H NMR (400 MHz, CDCl$_3$): 7.03 (1H, d, J=15.8, -CH=CH-), 8.02 (1H, d, -CH=CH-), 5.48 (2H, s, CH$_2$), 2.73(2H, q), 1.27(3H, t), 7.29-7.96 (m, benzene ring).
4.2.5. Herbicidal Activity

Mean percent seed germination inhibition values for different substituted 4-Oxo-4-phenyl-2-butanoic esters analogues presented in

Table 4.15.

Table 4.15: Mean Percent Seed Germination Inhibition Values of 4-oxo-4-phenyl-2-butenoic esters

<table>
<thead>
<tr>
<th>Name of the Compound</th>
<th>Mean 50ppm</th>
<th>Percent 100ppm</th>
<th>Inhibition 200ppm</th>
<th>Value CD at 5%</th>
</tr>
</thead>
<tbody>
<tr>
<td>pendimethalin (standard)</td>
<td>80.00</td>
<td>93.00</td>
<td>100.00</td>
<td>3.32</td>
</tr>
<tr>
<td>B1</td>
<td>78.33</td>
<td>83.33</td>
<td>88.33</td>
<td>8.14</td>
</tr>
<tr>
<td>B2</td>
<td>76.66</td>
<td>85.00</td>
<td>91.66</td>
<td>7.43</td>
</tr>
<tr>
<td>B3</td>
<td>83.33</td>
<td>90.00</td>
<td>93.33</td>
<td>4.70</td>
</tr>
<tr>
<td>B4</td>
<td>78.33</td>
<td>86.66</td>
<td>95.00</td>
<td>4.70</td>
</tr>
<tr>
<td>B5</td>
<td>83.33</td>
<td>88.33</td>
<td>95.00</td>
<td>4.70</td>
</tr>
<tr>
<td>C1</td>
<td>80.00</td>
<td>90.00</td>
<td>93.33</td>
<td>6.65</td>
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<tr>
<td>C2</td>
<td>63.33</td>
<td>76.66</td>
<td>91.66</td>
<td>8.14</td>
</tr>
<tr>
<td>C3</td>
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<td>70.00</td>
<td>85.00</td>
<td>8.79</td>
</tr>
<tr>
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<td>91.66</td>
<td>95.00</td>
<td>4.70</td>
</tr>
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<td>85.00</td>
<td>91.66</td>
<td>9.40</td>
</tr>
<tr>
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<td>85.00</td>
<td>88.33</td>
<td>7.43</td>
</tr>
<tr>
<td>M4</td>
<td>81.66</td>
<td>88.33</td>
<td>93.33</td>
<td>5.75</td>
</tr>
<tr>
<td>Br1</td>
<td>86.66</td>
<td>91.66</td>
<td>95.00</td>
<td>4.70</td>
</tr>
<tr>
<td>Br2</td>
<td>78.33</td>
<td>85.00</td>
<td>91.66</td>
<td>9.40</td>
</tr>
<tr>
<td>Br3</td>
<td>78.33</td>
<td>85.00</td>
<td>88.33</td>
<td>7.43</td>
</tr>
<tr>
<td>Br4</td>
<td>81.67</td>
<td>88.33</td>
<td>93.33</td>
<td>5.75</td>
</tr>
<tr>
<td>CD at 5%</td>
<td>6.47</td>
<td>6.38</td>
<td>4.09</td>
<td></td>
</tr>
</tbody>
</table>
Perusal of Table-4.15 clearly reveals that compounds (B4), (B5), (C2), (E2), (M4), (Br4)) shows significant increase in mean percent inhibition value with increase in concentration.

At 50 ppm concentration compounds (M1) and (Br1) exhibit highest mean percent inhibition values being at par with each other. Values of compounds (B3), (B5), (M4), including pendimethalin (standard) are at par with those of (M1) and (Br1). Remaining compounds exhibit significantly lower activity than (M1) and (Br1).

At 100 ppm concentration again compounds (M1) and (Br1) shows highest mean percent inhibition values. Values of compounds (B3), (B4), (B5), (C1), (E1), (M4), and (Br4) including pendimethalin (standard) are at par with those of (M1) and (Br1). Compounds (B1), (B2), (C2), (C3), (E2), (M2), (M3), (Br2) And (Br3) exhibit significantly lower activity than (M1) and (Br1).

At 200 ppm concentration compounds (B4), (B5), (M1) and (Br1) show highest mean percent inhibition values being at par with each other. Percent inhibition value of compounds (B3), (C1), (E1), (M4) and (Br4) including pendimethalin (standard) are at par with those of compounds (B4), (B5), (M1), and (Br1). Compounds (B1), (B2), (C2), (C3), (E2), (M2), (M3), (Br2) and (Br3) exhibit significantly lower activity than compounds (B4), (B5), (M1) and (Br1).

Basic structure of the ester molecule may be depicted as

![Basic Structure of the Ester Molecule](image-url)
The structure is made up of two moieties, consisting of two benzene rings, ring A and ring B. Benzene ring A is of acid moiety and B that of alcohol moiety. Henceforth, in the discussion ring A and ring B will be stated on ‘benzene ring of acid moiety’ and ‘benzene ring of alcohol moiety’ respectively. Different effects have been obtained by varying the substituents R and R’ on benzene rings.

In all, the six substituents three electron releasing (-CH₃, -C₂H₅, and –OCH₃) and three electron withdrawing (-Cl, -Br and -NO₂) and two sites (benzene ring of acid moiety and benzene ring of alcohol moiety) for substitution have been used to obtain eighteen analogues. Perusal of Table-1 clearly indicate that activity is independent of nature of specific substituent’s particular site.

Though in our previous investigations carried out on cinnamic acid amide derivatives for seed germination inhibition activity, we established structural activity relationships and concluded that activity is effected by electronic effects also, as electron withdrawing group on benzene ring of acid moiety and electron donating group on benzene ring of amine moiety were found to increase the activity (Vishnoi et al., 2009). Omokawa et al., (Omokawa et al., 1985) also studied the effects of substituents on benzene ring of α-phenyl sulphonyl propanamide in which they found electron withdrawing groups to increase the activity remarkably. In this investigation it is clear that role of substituents on benzene ring to increase or decrease the activity of
the compound does not depend upon the nature of substituent group exclusively, but this is the structure of compound which makes substituents as activator or deactivator. In the present investigation we conclude that presence of two extra carbony groups in the compounds as compared to cinnamic acid amide derivatives is overriding factor in deciding the activity which is less effected by the nature of substituents and site of substitution.

4.3. Organo catalysed microwave assisted three component reaction to produce 1, 5- benzodiazepine derivatives

The compounds were prepared in good yield by microwave assisted three component reaction of aromatic aldehyde, methyl acetoacetate/ benzylacetoacetate and 1, 2 phenylenediamine using trichloroacetic acid as catalyst without solvent. The compounds synthesized and their yield presented in Table 4.16.

Table 4.16: Synthesis of various 1, 5- benzodiazepine derivatives

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Ester</th>
<th>Aldehyde</th>
<th>Product</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>MAA</td>
<td>CHO</td>
<td>(2)-methyl 2-(2,3-dihydro-2-phenyl-1H-benzo [b][1,4]diazepin-4(5H)-ylidene)acetate bdap1</td>
<td>92%</td>
</tr>
</tbody>
</table>
Results and Discussion

2. MAA CHO NO2 NH NH OMe NO2 (Z)-methyl 2-(2,3-dihydro-2-(2-nitrophenyl)-1H-benzo[b][1,4]diazepin-4(5H)-ylidene)acetate bdap2 80%

3. MAA CHO NO2 NH NH OMe NO2 (Z)-methyl 2-(2,3-dihydro-2-(3-nitrophenyl)-1H-benzo[b][1,4]diazepin-4(5H)-ylidene)acetate bdap3 81%

4. MAA CHO NO2 NH NH OMe NO2 (Z)-methyl 2-(2,3-dihydro-2-(4-nitrophenyl)-1H-benzo[b][1,4]diazepin-4(5H)-ylidene)acetate bdap4 82%
5. MAA \( \text{CHO} \) \( \text{OH} \) 
\[(Z)-\text{methyl 2-(2,3-dihydro-2-}
\text{(2-hydroxyphenyl)-1}\text{H-benzo[b}]
\[1,4\text{]diazepin-4(5}\text{H}\text{-ylidene})\text{acetate} \text{bdap5} \quad 88\%

6. MAA \( \text{CHO} \) \( \text{OH} \) 
\[(Z)-\text{methyl 2-(2,3-dihydro-2-}
\text{(3-hydroxyphenyl)-1}\text{H-benzo[b}]
\[1,4\text{]diazepin-4(5}\text{H}\text{-ylidene})\text{acetate} \text{bdap6} \quad 80\%

7. MAA \( \text{CHO} \) \( \text{OH} \) 
\[(Z)-\text{methyl 2-(2,3-dihydro-2-}
\text{(4-hydroxyphenyl)-1}\text{H-benzo[b}]
\[1,4\text{]diazepin-4(5}\text{H}\text{-ylidene})\text{acetate} \text{bdap7} \quad 80\%
8. MAA

\[(\mathcal{Z})\text{-methyl 2-(2,3-dihydro-2-(4-methoxyphenyl)-1H-benzo}[b]\n\[1,4]\text{diazepin-4(5H)-ylidene})acetate bda8 93\%

9. MAA

\[(\mathcal{Z})\text{-methyl 2-(2-(2-chlorophenyl)-2,3-dihydro-1H-benzo}[b]\n\[1,4]\text{diazepin-4(5H)-ylidene})acetate bda9 92\%

10. BAA

\[(\mathcal{Z})\text{-benzyl 2-(2,3-dihydro-2-phenyl-1H-benzo}[b]\n\[1,4]\text{diazepin-4(5H)-ylidene})acetate bda10 95\%
The reaction was examined in details for the formation of intermediate (1) (Murai et al., 2008). In case phenylenediamine and aldehydes are mixed first in presence of acid catalysts, intermediate (2) is formed which gives intermediate (1) on addition of methyl acetoacetate. The step involving the formation of (1) from (2) is slow (scheme 4.1).

Scheme 4.1: 1, 2 Phenylenediamine reacts with aldehyde and then with methyl acetoacetate
In case 1, 2 phenylenediamine and methyl acetoacetate are reacted first in presence of acid catalysts, intermediate (3) is formed which gives intermediate (1) on addition of aldehydes. This step is fast. It is obvious that mixing all there components 1, 2 phenylenediamine, aldehydes and methyl acetoacetate will lead to the formation of same final product following Scheme 4.2.

Scheme 4.2: 1, 2 phenylenediamine reacts with methyl acetoacetate then with aldehyde

The reaction was also tried in the absence of catalyst and it was observed that the reaction did not take place in case aldehydes were solid while preceded very slowly in case aldehydes were liquids at room temperature. Reaction was optimized for the amount of catalyst and it was found that reaction could be carried well taking 0.02 equivalent of
catalyst. Minimum amount of catalyst used by conventional methods was 0.1 equivalents (Chen and Lu, 2005) We got success in reducing the amount of catalyst significantly. Conclusion is that this is the one pot, solvent less, very easy, rapid, high yielding and eco friendly procedure for the synthesis of 1, 5-benzodiazepine derivatives.

4.3.2 Spectral Characterization

4.3.2.1 FTIR spectral analysis

The FTIR spectra of all the benzodiazepine derivatives were recorded in KBr pellets.

The FTIR spectra of benzodiazepine derivatives showed bands at 3000-3100 cm\(^{-1}\) for aromatic \(-\text{CH} \text{ str.}\) Aromatic alkenic \(\text{str}\) shows at 1600 cm\(^{-1}\). The absorption at 1350-1500 cm\(^{-1}\) shows \(-\text{NH} \text{ str.}\)

4.3.2.2 \(^1\text{H NMR spectral analysis}\)

The NMR spectra of all compounds were taken in the CDCl\(_3\) with JEOL AL300 FTNMR.

In the \(^1\text{HNMR}\) spectra of benzodiazepine derivatives the protons of benzene ring 7.6-7.85 while the protons of benzene ring of benzodiazepine moiety comparatively upfield \(\delta\) values at 7.1-7.62. Proton of NH shows double doublet at 3.89-4.69 for coupling constant J=8.0 and 4.0-4.5 Hz.

4.3.2.3. Spectral Characterisation Data

1. \textbf{bdap1}. IR (KBr) 3060, 1662, 1623, 1593, 1494, 1252, 1111.52 cm\(^{-1}\). \(^1\text{HNMR}\) (CDCl\(_3\), 300MHz) \(\delta\) 2.39 (m, 2H, CH\(_2\)): 3.47 (s, 3H,
Results and Discussion

2. **bdap 8.** IR (KBr) 3360, 2953, 2840, 1600, 1512, 1436, 1258, 1174 cm$^{-1}$. $^1$HNMR (CDCl$_3$, 300MHz) δ 2.4-2.27 (m, 2H, CH$_2$): 3.74 (s, 3H, OCH$_3$): 3.85 (s, 2H, CH): 3.86 (s, 3H, OCH$_3$): 3.84 (s, 1H, NH): 3.89 (dd, 1H, NH): 7.62 (dd, 4H, Ph): 7.85-7.51 (m, 4H, Ph):

3. **bdap 10.** $^1$HNMR (CDCl$_3$, 300MHz) δ 2.39 (s, 2H, CH$_2$): 2.23 (m, 2H, CH$_2$): 3.49 (s, 1H, CH): 3.49 (s, 1H, NH): 4.69 (dd, J= 9.1, 4.1 Hz, NH): 7.43-7.24 (m, 5H, Ph): 7.56-7.45 (m, 5H, Ph): 8.12-7.59 (m, 4H, Ph):

4.3.3 **Antioxidant activity studies of 1, 5 benzodiazepines derivatives**

Free radical scavenging is one of the best mechanism by which antioxidants inhibit lipid oxidation. DPPH radical scavenging activity evalution is standard assay in antioxidant activity studies and gives rapid and efficient method for screening of the compounds. Reducing power activity and chelating activity also are good techniques to support the antioxidant evaluation activity studies. DPPH radical scavenging, activity reducing power activity and chelating activity of all the compounds and standard were estimated using these three methods.

4.3.3.1 **Reducing Power Activity**

During the course of reducing power assay, the presence of reductants (antioxidants) in the tested samples would result in reducing Fe$^{3+}$/ferricyanide complex to ferrous form (Fe$^{2+}$). The Fe$^{2+}$ can therefore
be monitored by measuring the formation of Perl’s Prussian blue at 700 nm (Chung et al., 2002). Mechanistically, ferric reducing/antioxidant power (FRAP) is based on single electron transfer reaction (SET) as described in equation (1). The relevant chemical reaction of the FRAP method involves a single electron reaction between Fe (III) and a single donor ArOH (equation (2)) (Ou et al., 2002).

\[
\begin{align*}
M(n) + A-H & \rightarrow M(n-1) + A-H^+ \\
Fe(III) + ArOH & \rightarrow Fe(II) + ArOH^+ \cdot
\end{align*}
\]

(Equation- 1)

(Equation- 2)

Table 4.17: Reducing power activity of the 1, 5 benzodiazepines derivatives

<table>
<thead>
<tr>
<th>Compounds</th>
<th>5µl</th>
<th>10 µl</th>
<th>15 µl</th>
<th>20 µl</th>
<th>25 µl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bdap1</td>
<td>0.49</td>
<td>0.62</td>
<td>0.71</td>
<td>0.86</td>
<td>0.99</td>
</tr>
<tr>
<td>Bdap2</td>
<td>0.44</td>
<td>0.57</td>
<td>0.61</td>
<td>0.73</td>
<td>0.81</td>
</tr>
<tr>
<td>Bdap3</td>
<td>0.45</td>
<td>0.58</td>
<td>0.63</td>
<td>0.75</td>
<td>0.86</td>
</tr>
<tr>
<td>Bdap4</td>
<td>0.47</td>
<td>0.59</td>
<td>0.64</td>
<td>0.78</td>
<td>0.88</td>
</tr>
<tr>
<td>Bdap5</td>
<td>0.59</td>
<td>0.68</td>
<td>0.79</td>
<td>0.95</td>
<td>1.1</td>
</tr>
<tr>
<td>Bdap6</td>
<td>0.62</td>
<td>0.72</td>
<td>0.85</td>
<td>0.99</td>
<td>1.19</td>
</tr>
<tr>
<td>Bdap7</td>
<td>0.63</td>
<td>0.73</td>
<td>0.86</td>
<td>1.0</td>
<td>1.2</td>
</tr>
<tr>
<td>Bdap8</td>
<td>0.71</td>
<td>0.86</td>
<td>0.95</td>
<td>1.15</td>
<td>1.31</td>
</tr>
<tr>
<td>Bdap9</td>
<td>0.44</td>
<td>0.58</td>
<td>0.60</td>
<td>0.74</td>
<td>0.81</td>
</tr>
<tr>
<td>Bdap10</td>
<td>0.50</td>
<td>0.63</td>
<td>0.72</td>
<td>0.88</td>
<td>0.99</td>
</tr>
<tr>
<td>BHT</td>
<td>2.30</td>
<td>2.41</td>
<td>2.45</td>
<td>2.49</td>
<td>2.50</td>
</tr>
<tr>
<td>Catechin</td>
<td>4.0</td>
<td>4.0</td>
<td>4.0</td>
<td>4.0</td>
<td>4.0</td>
</tr>
<tr>
<td>Citric acid</td>
<td>4.0</td>
<td>4.0</td>
<td>4.0</td>
<td>4.0</td>
<td>4.0</td>
</tr>
</tbody>
</table>
Figure 4.9: Reducing power of 1, 5 benzodiazepine derivatives

Perusal of Table 4.17 and Figure 4.9 indicates that reducing power of all the compounds is less as compared to the standard BHT, catechin and citric acid.

Table 4.17 clearly indicate that activity increases with increase in concentration. At concentration 25 µl the compounds bdap8 (1.31) exhibited the highest reducing power activity followed by bdap6 (1.19), bdap1 (0.99), bdap10 (0.99). All compounds shows same trend in all concentration.

4.3.3.2. DPPH Radical Scavenging Activity

A freshly prepared DPPH solution exhibits a deep purple color with an absorption maximum at 517 nm. When an antioxidant is present in medium, the color intensity decreases which gives less absorbance. The antioxidant molecule quench DPPH free radicals by
providing hydrogen atoms or electron via a free radical attack on DPPH molecule and convert them to colorless product (Amararowicz et al., 2004; Sidduraju and Becker 2007).

Antioxidants are known to interrupt the free radical chain of oxidation and to donate hydrogen from phenolic hydroxyl groups, thereby, forming stable free radicals, which do not initiate or propagate further oxidation of lipids (Sherwin, 1978).

The model of scavenging stable DPPH free radicals can be used to evaluate the antioxidative activities in a relatively short time (Brand-Williams et al., 1995) compared to other methods. The effect of antioxidants on DPPH radical scavenging was thought to be due to their hydrogen donating ability (Blois, 1958).

The determination of scavenging stable DPPH radical is a very fast method to evaluate the antioxidant activity. With this method it is possible to determine the antiradical power of an antioxidant activity by measurement of the decrease in absorbance of the DPPH radical at 517 nm. The radical form of this molecule absorbs at 517 nm which disappears after acceptance of an electron or hydrogen radical from an antioxidant compound to become a stable diamagnetic molecule (Matthaus, 2002).

DPPH radical scavenging activity was observed for all the tested compounds at selected dose levels, although these compounds differed in their activity to react with and quench DPPH radicals. The compounds having the hydroxyl groups have shown the radical scavenging activity because they can donate hydrogen to DPPH radical.
Figure 4.10: Radical Scavenging Action (RSA) of Antioxidants (ArOH).

Persual of table 4.18 reveals that the at 25 µl concentration maximum radical scavenging activity has been exhibited by bdap8 (90.58), followed by bdap7 (90.25), bdap6 (89.89), and bdap5 (88.46). With increase in the amount of test solution an increase in scavenging activity was also observed for all compounds. The radical scavenging activity of all the compounds were compared with standard BHT, gallic acid and catechin.

Table 4.18: Percent DPPH radical scavenging activity of the 1, 5 benzodiazepines derivatives

<table>
<thead>
<tr>
<th>Compounds</th>
<th>5µl</th>
<th>10 µl</th>
<th>15 µl</th>
<th>20 µl</th>
<th>25 µl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bdap1</td>
<td>66.36</td>
<td>67.12</td>
<td>68.13</td>
<td>69.23</td>
<td>70.22</td>
</tr>
<tr>
<td>Bdap2</td>
<td>59.80</td>
<td>61.93</td>
<td>63.25</td>
<td>64.37</td>
<td>66.03</td>
</tr>
<tr>
<td>Bdap3</td>
<td>62.59</td>
<td>63.21</td>
<td>64.67</td>
<td>65.98</td>
<td>66.74</td>
</tr>
<tr>
<td>Bdap4</td>
<td>55.67</td>
<td>56.98</td>
<td>57.75</td>
<td>59.13</td>
<td>59.82</td>
</tr>
<tr>
<td>Bdap5</td>
<td>75.81</td>
<td>78.54</td>
<td>81.22</td>
<td>85.12</td>
<td>88.46</td>
</tr>
<tr>
<td>Bdap6</td>
<td>71.24</td>
<td>74.32</td>
<td>80.01</td>
<td>86.28</td>
<td>89.89</td>
</tr>
<tr>
<td>Bdap7</td>
<td>73.04</td>
<td>78.97</td>
<td>83.61</td>
<td>87.64</td>
<td>90.25</td>
</tr>
<tr>
<td>Bdap8</td>
<td>71.93</td>
<td>76.86</td>
<td>81.55</td>
<td>86.93</td>
<td>90.58</td>
</tr>
<tr>
<td>Bdap9</td>
<td>68.94</td>
<td>69.89</td>
<td>70.98</td>
<td>72.31</td>
<td>73.04</td>
</tr>
<tr>
<td>Bdap10</td>
<td>66.83</td>
<td>67.61</td>
<td>68.63</td>
<td>69.56</td>
<td>70.88</td>
</tr>
<tr>
<td>BHT</td>
<td>117.92</td>
<td>118.54</td>
<td>119.65</td>
<td>119.84</td>
<td>141.45</td>
</tr>
<tr>
<td>Catechin</td>
<td>116.40</td>
<td>116.31</td>
<td>120.69</td>
<td>121.38</td>
<td>121.45</td>
</tr>
<tr>
<td>Gallic Acid</td>
<td>84.84</td>
<td>85.64</td>
<td>85.91</td>
<td>85.93</td>
<td>86.85</td>
</tr>
</tbody>
</table>
4.3.3.3 Chelating activity

In this investigation, measurement of chelating activity of benzodiazepine derivatives on Fe$^{2+}$ was estimated using various amounts of the compounds (5 µl, 10 µl, 15 µl, 20 µl and 25 µl). The variation in the chelating activity with increase in concentration of the benzodiazepines derivatives is shown in Table 4.19.

EDTA and citric acid were taken as a standard chelating agent. All compounds exhibited good chelating activity. Compound bdap8 (73.21) exhibited highest chelating activity, followed by bdap7 (71.13), bdap6 (69.09) and badp5 (68.91). With increase in the amount of test solution an increase in percent chelating activity was observed for all compounds.
Table 4.19: Percent chelating activity of the benzodiazepines derivatives on Fe$^{2+}$ ion

<table>
<thead>
<tr>
<th>Compounds</th>
<th>5µl</th>
<th>10 µl</th>
<th>15 µl</th>
<th>20 µl</th>
<th>25 µl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bdap1</td>
<td>53.87</td>
<td>56.519</td>
<td>59.85</td>
<td>61.26</td>
<td>64.07</td>
</tr>
<tr>
<td>Bdap2</td>
<td>47.84</td>
<td>49.62</td>
<td>51.58</td>
<td>53.07</td>
<td>55.45</td>
</tr>
<tr>
<td>Bdap3</td>
<td>51.25</td>
<td>52.48</td>
<td>54.74</td>
<td>56.87</td>
<td>58.94</td>
</tr>
<tr>
<td>Bdap4</td>
<td>49.27</td>
<td>51.29</td>
<td>52.38</td>
<td>54.57</td>
<td>56.17</td>
</tr>
<tr>
<td>Bdap5</td>
<td>52.03</td>
<td>55.04</td>
<td>58.22</td>
<td>61.67</td>
<td>68.91</td>
</tr>
<tr>
<td>Bdap6</td>
<td>49.96</td>
<td>54.25</td>
<td>57.21</td>
<td>63.78</td>
<td>69.06</td>
</tr>
<tr>
<td>Bdap7</td>
<td>55.63</td>
<td>58.96</td>
<td>62.74</td>
<td>66.45</td>
<td>71.13</td>
</tr>
<tr>
<td>Bdap8</td>
<td>61.95</td>
<td>63.67</td>
<td>65.75</td>
<td>69.94</td>
<td>73.21</td>
</tr>
<tr>
<td>Bdap9</td>
<td>48.58</td>
<td>51.42</td>
<td>53.41</td>
<td>55.83</td>
<td>58.54</td>
</tr>
<tr>
<td>Bdap10</td>
<td>52.83</td>
<td>57.21</td>
<td>60.48</td>
<td>62.52</td>
<td>64.95</td>
</tr>
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<td>87.62</td>
<td>88.04</td>
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<td>89.20</td>
</tr>
<tr>
<td>Citric acid</td>
<td>86.19</td>
<td>87.03</td>
<td>89.37</td>
<td>89.55</td>
<td>92.55</td>
</tr>
</tbody>
</table>

Figure 4.12: Chelating activity of Benzodiazepine derivatives
4.3.4 Antibacterial activity of benzodiazepines derivatives:

Antibacterial screening of all the synthetic compounds was carried out by disc diffusion method and shown in (Fig. 4.13- 4.16). The results were compared with standard antibiotic Streptomycin, Chloramphenicol and Ampicillin.

Table 4.20: Antibacterial activities of Benzodiazepine derivatives against standard microworganism.

<table>
<thead>
<tr>
<th>Compounds (30ppm)</th>
<th>Standard Bacterial strain</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bacillus Cereus</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>+</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>-</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>+</td>
</tr>
<tr>
<td>bdap1</td>
<td>+</td>
</tr>
<tr>
<td>bdap2</td>
<td>-</td>
</tr>
<tr>
<td>bdap3</td>
<td>-</td>
</tr>
<tr>
<td>bdap4</td>
<td>-</td>
</tr>
<tr>
<td>bdap5</td>
<td>-</td>
</tr>
<tr>
<td>bdap6</td>
<td>-</td>
</tr>
<tr>
<td>bdap7</td>
<td>+</td>
</tr>
<tr>
<td>bdap8</td>
<td>+</td>
</tr>
<tr>
<td>bdap9</td>
<td>+</td>
</tr>
<tr>
<td>bdap10</td>
<td>+</td>
</tr>
<tr>
<td>bdap11</td>
<td>+</td>
</tr>
</tbody>
</table>

Marked activity has been observed to mostly benzodiazepine derivatives against Gordonia terrae and Bacillus Cereus while Acinetobactor junii and Pseudomonas aeruginosa no significant activity has been recorded. bdap10 has shown good activity against all bacterial strain.
Figure 4.13. Antibacterial activity of Compounds against Acinetobactor sp

Figure 4.14. Antibacterial activity of compounds against Pseudomonas sp.

Figure 4.15. Antibacterial activity of Compounds against Bacillus sp.

Figure 4.16. Antibacterial activity of Compounds against Gordonia sp.
All the compounds except bdap5 and bdap7 exhibited marked activity against *Gordonia terrae*. The compounds bdap1, bdap7, bdap8, bdap9, bdap10 and bdap11 exhibited good activity against *Bacillus Cereus* while compounds bdap2, bdap3, bdap4, bdap5 and bdap6 did not exhibit activity against this bacteria. Against *acinetobactor junii*, the compounds bdap1, bdap3, bdap4, bdap5 and bdap10 exhibited good activity while against *Pseudomonas aeruginosa* only bdap8, bdap10 and bdap11 exhibited good activity. The activity of the compounds could not be correlated with the structure of the compounds or the electronic effects of the substituents on the benzene ring of benzaldehyde.

### 4.3.5. Pharmacological activity of 1, 5 Benzodiazepine derivatives on smooth muscles of rats

Isolated duodenal smooth muscles of the rat exhibited normal mortality. Acetylcoline (0.1 ml, $10^{-3}$ M) produced contraction in the duodenal smooth muscle in a dose dependent manner. Adrenaline (2.0 µg, 0.1 ml, 20mg/ml) caused mild relaxation in the smooth muscles which persisted for about 1.5-2.0 minutes (Fig. 4.17).

The test compounds were given after acetylcoline and adrenaline. Test sample of the benzodiazepine bdap1, bdap2, bdap3 and bdap4 (0.1ml, 20%) produced smooth muscle relaxation which persisted for 2-minutes and the tissue revealed the relaxation effect in dose dependent manner when exposed to higher dose (0.5 ml\(\times\)20%) (Fig. 4.18). Test samples bdap5, bdap6, bdap7, bdap8 and bdap9 also produced (0.1ml, 20%) relaxation in the smooth muscle activity in dose dependent manner (Fig. 4.19).
Figure 4.17: Effect of acetylcoline ((0.1 ml, 10^{-3}M), Epinephrin (0.1 ml \times 10^{-4} M), bdap1, bdap2, bdap3, bdap4, bdap5, bdap6 and bdap9 (0.1 ml, 20%) on duodenal smooth muscle of rat.
Figure 4.18: Effect of acetylcoline (0.1 ml, 10^{-3} M), Epinephrin (0.1 ml\times10^{-4} M), bdap7, bdap8, bdap9 and bdap10 (0.5 ml, 20%)
**Figure 4.19:** Effect of Prazosine (0.1 ml, 100 mg/ml), bdap1, bdap2, bdap3, bdap4, bdap5, bdap6, bdap7, bdap8 and bdap9 (0.5 ml, 20%)
Atropine (0.1ml, 100 µg/ml) relaxed the tissues slightly. After atropine the effect of acetylcoline was not observed, however, adrenaline caused relaxation to the extent as produced before atropine. The effect of the test compound was not altered and produced the similar effect as produced prior to atropine. Prazosin (0.1 ml, 100 mg/ml) slightly increased the contraction of the duodenal smooth muscles. Epinephrine (2.0 µg) also produced slight relaxation after prazosin. The effect of test compound bdap5 was blocked by prazosin where as other test compounds produced relaxation as produced before the prazosin exposure.

Isolated duodenal smooth muscle exhibited normal mortality acetylcoline produced contraction in dose dependent manner due to the stimulation of muscarinic receptors in the duodenal smooth muscle. This effect of acetylcoline was abolished by muscarinic antagonistic atropine (Hoffman and Taylor, 2001). Epinephrine caused mild relaxation in the duodenal muscles as a result of stimulation of adrenergic α and β receptors. This response was, however, reduced following pretreatment with prazosin because it blocked of α1 receptor in the smooth muscle cell of the intestine. The blockade by the prazosin and atropine did not affect the benzodiazepine compounds mediated relaxation in this study. The muscle relaxation by benzodiazepine compounds might have occurred due to their direct effect on the chloride conductance in the smooth muscle cell as the compound increase the conductance of chloride channel in CNS (Tallman et al., 1980; Nutt and Malizia, 2001).
The effect of benzodiazepine is mediated mainly through activation of GABA<sub>A</sub> receptors distributed in the brain tissue. As the response of benzodiazepine compound is not abolished by muscarinic antagonist atropine and adrenergic antagonist prazosine, the effect is not mediated through involvement of muscarinic and adrenergic receptors. Prazosin might have increased the threshold of smooth muscle cells bdap5 did not elicit relaxation after prazosin. It is concluded from this study that the benzodiazepine compounds produced mild smooth relaxation which might have occurred as result of an increase in conductance of the chloride channels and muscarinic and adrenergic receptors are not involved in smooth muscle relaxation. Thus, these compounds may clinically be beneficial in the treatment of the condition like diarrhoea occurring as a result of enhanced intestinal mortality which, however, needs further investigation.

4.4 Synthesis of Benzimidazoles

4.4.1 Microwave assisted synthesis of benzimidazoles and its derivatives using Oxalic acid as a catalyst.

The compounds were prepared in good yield by microwave assisted synthesis of benzimidazole by aromatic aldehyde and 1, 2 phenylenediamine using oxalic acid as catalyst in Dimethylformamide (DMF). Oxalic acid containing two carboxylic groups joined directly is a strong organic acid. Better yields are obtained using oxalic acid catalyst and time duration of reaction is also less. Work up of reaction is also easy. The compounds synthesized and their yields are presented in Table 4.21.
Table 4.21: Synthesis of benzimidazole derivatives catalysed by Oxalic acid

<table>
<thead>
<tr>
<th>Entry</th>
<th>Aldehyde</th>
<th>Product</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>CHO</td>
<td><img src="image" alt="2-phenyl-1H-benzo[d]imidazole" /></td>
<td>a1 78%</td>
</tr>
<tr>
<td>2.</td>
<td>CHO</td>
<td><img src="image" alt="2-(3-nitrophenyl)-1H-benzo[d]imidazole" /></td>
<td>a2 83%</td>
</tr>
<tr>
<td>3.</td>
<td>CHO</td>
<td><img src="image" alt="2-(4-nitrophenyl)-1H-benzo[d]imidazole" /></td>
<td>a3 84%</td>
</tr>
<tr>
<td>4.</td>
<td>CHO</td>
<td><img src="image" alt="2-p-tolyl-1H-benzo[d]imidazole" /></td>
<td>a4 82%</td>
</tr>
<tr>
<td>5.</td>
<td>CHO</td>
<td><img src="image" alt="2-(4-fluorophenyl)-1H-benzo[d]imidazole" /></td>
<td>a5 83%</td>
</tr>
</tbody>
</table>
6. \( \text{CHO} \) \( \text{Cl} \) 2-(4-chlorophenyl)-1\( H \)-benzo\( d \)imidazole \( \text{a6} \) 84%

7. \( \text{CHO} \) \( \text{Cl} \) 2-(2-chlorophenyl)-1\( H \)-benzo\( d \)imidazole \( \text{a7} \) 80%

8. \( \text{OCH}_3 \) \( \text{CHO} \) 2-(4-methoxyphenyl)-1\( H \)-benzo\( d \)imidazole \( \text{a8} \) 83%

9. \( \text{OCH}_3 \) \( \text{CHO} \) 2-(3-methoxyphenyl)-1\( H \)-benzo\( d \)imidazole \( \text{a9} \) 83%

10. \( \text{OH} \) \( \text{CHO} \) 4-(1\( H \)-benzo\( d \)imidazol-2-yl)phenol \( \text{a10} \) 81%

11. \( \text{OH} \) \( \text{CHO} \) 3-(1\( H \)-benzo\( d \)imidazol-2-yl)phenol \( \text{a11} \) 81%
Benzimidazole product from aromatic aldehyde, 1, 2 phenylenediamine and oxalic acid used as a catalyst formed according to mechanism (Kokare et al., 2007).

\[
\begin{align*}
\text{NH}_2 & \quad \text{ArCHO} \quad (\text{CO}_2\text{H})_2 \quad \text{N} \quad \text{NH}_2 \\
\text{NH}_2 & \quad \text{Ar} \quad \text{H} \quad \text{N} \quad \text{H} \\
\text{N} & \quad \text{Ar} \quad \text{H} \\
\end{align*}
\]

Scheme 4.3: Proposed mechanism for the formation of disubstituted benzimidazoles catalysed by Oxlic acid (Kokare et al., 2007).

In conclusion we have reported here a facile method for the synthesis of benzimidazole derivatives by the condensation of 1, 2 phenylenediamine and aldehyde using oxalic acid as a catalyst. Moreover catalyst works well under microwave irradiation. Conventional methodology involves simply stirring of reaction mixture. The reaction time (25-60 minutes) for conventional method is reduced to minutes (2-4 minute) under microwave irradiation.

4.4.2 Microwave assisted synthesis of benzimidazoles and their derivatives using TiCl₄ as a catalyst.

The compounds were prepared in good yield by microwave assisted synthesis of benzimidazole by aromatic aldehyde and 1, 2
phenylenediamine using TiCl₄ as catalyst in Tetrahydrofuraun (THF). Titanium (IV) chloride is moderately strong Lewis acid with many application evidenced in conversion of ketones to N-alkylimines, in Aldol condensation of aryl ketones with aryl aldehyde, in Michael addition of silyl enol ethers to α, β-enones etc. Conventional method for synthesis of benzimidazole derivatives earlier have been carried out by using TiCl₄ (Nagawade and Shinde, 2007). Better yield are obtained using TiCl₄ catalyst and time duration of reaction is also less under microwave irradiation. Workup of the reaction is also very easy. The compounds synthesized and their yields are presented in Table 4.22

Table 4.22: Synthesis of benzimidazole derivatives catalysed by TiCl₄

<table>
<thead>
<tr>
<th>Entry</th>
<th>Aldehyde</th>
<th>Product</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>CHO</td>
<td><img src="image1" alt="Imidazole 1" /></td>
<td>b₁</td>
</tr>
<tr>
<td>2.</td>
<td>CHO</td>
<td><img src="image2" alt="Imidazole 2" /></td>
<td>b₂</td>
</tr>
</tbody>
</table>
4.4.3 Microwave assisted synthesis of benzimidazoles and its derivatives using FeCl₃ as a catalyst

The compounds were prepared in good yield by microwave assisted synthesis of benzimidazole by aromatic aldehyde and 1, 2 phenylenediamine using FeCl₃ as catalyst in Tetrahydrofuran (THF). The compounds synthesized and their yields are presented in Table 4.3
Table 4.23: Synthesis of benzimidazole derivatives catalysed by FeCl₃

<table>
<thead>
<tr>
<th>Entry</th>
<th>Aldehyde</th>
<th>Product</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>CHO</td>
<td>2-phenyl-1H-benzo[d]imidazole</td>
<td>c1 78%</td>
</tr>
<tr>
<td>2.</td>
<td>CHO</td>
<td>2-(3-nitrophenyl)-1H-benzo[d]imidazole</td>
<td>c2 78%</td>
</tr>
<tr>
<td>3.</td>
<td>CHO</td>
<td>2-(4-methoxyphenyl)-1H-benzo[d]imidazole</td>
<td>c3 80%</td>
</tr>
<tr>
<td>4.</td>
<td>CHO</td>
<td>2-p-tolyl-1H-benzo[d]imidazole</td>
<td>c4 79%</td>
</tr>
<tr>
<td>5.</td>
<td>CHO</td>
<td>4-(1H-benzo[d]imidazol-2-yl)phenol</td>
<td>c5 80%</td>
</tr>
</tbody>
</table>
4.4.4 Spectral Characterization

4.4.4.1 FTIR spectral analysis

The FTIR spectra of all the benzimidazole derivatives were recorded in KBr pellets.

The FTIR spectra of benzimidazole derivatives showed bands at 3000-3100 cm\(^{-1}\) for aromatic –CH str. Aromatic alkenic str shows at 1600 cm\(^{-1}\). The absorption at 1350-1500 cm\(^{-1}\) shows –NH str and the absorption at 900-960 cm\(^{-1}\) for cis aromatic alkenic protons.

4.4.4.2 \(^1\)H NMR spectral analysis

The \(^1\)HNMR spectra of all compounds were taken in the CDCl\(_3\) with Varian 600 MHz and JEOL AL 300 MHz.

In the \(^1\)HNMR spectra of benzimidazole derivatives the protons of benzene ring of imidazole moiety shows downfield δ value at 7.8-8.5 while protons of benzene ring attached with basic imidazole ring comparatively upfield δ values at 7.0-7.85.

4.4.4.3 Spectral Characterisation Data

1. a2: IR (KBr) 3181, 1520, 1436, 1348, 975, 740 cm\(^{-1}\). \(^1\)HNMR (CDCl\(_3\), 300MHz) δ 7.9- 7.4 (m, 4H, Ph): 8.30 (t, 1H, Ph): 8.5 (d, 1H, Ph): 8.62 (d, 1H, J=7.7 Hz, PH): 9.0 (1H, s, PH): 13.29 (s, 1H, NH).

2. a4: IR (KBr) 3021, 1611, 1444, 1275, 963, 747 cm\(^{-1}\). \(^1\)HNMR (CDCl\(_3\), 300MHz) δ 2.39 (s, 3H, CH\(_3\)): 7.22 (d, 2H, J=7.9 Hz): 7.28 (d, 2H): 7.5-7.9 (m, 4H, Ph):
3.  **a7:** IR (KBr) 2851, 1647, 1442, 1398, 1297, 974, 946 cm\(^{-1}\). \(^1\)HNMR (CDCl\(_3\), 600MHz) \(\delta\) 7.2-7.3 (m, 4H, Ph): 7.5-7.9 (m, 4H, Ph).

4.  **b2:** \(^1\)HNMR (CDCl\(_3\), 600MHz) \(\delta\) 7.3-7.43 (m, 4H, Ph): 7.53-7.82 (m, 4H, Ph).

5.  **c1:** IR (KBr) 3061, 1604, 1446, 1394, 927, 740 cm\(^{-1}\). \(^1\)HNMR (CDCl\(_3\), 300MHz) \(\delta\) 7.15 (m, 2H, Ph): 7.20-7.41 (m, 5H, Ph): 7.9 (m, 2H, Ph).

### 4.4.5 Synthesis of 1, 2-disubstitued benzimidazoles using SiO\(_2\)/CaCl\(_2\).2H\(_2\)O as a catalyst

The 1, 2-disubstitued benzimidazoles were prepared in good yield by microwave assisted method by the reaction of two mole equivalents of aromatic aldehyde and one mole equivalent 1, 2 phenylenediamine using silica supported calcium chloride dihydrate as catalyst without solvent. The silica supported calcium chloride dihydrate is a new catalyst reported first time for this reaction. The compounds synthesized and their yields are presented in **Table 4.24**.

**Table 4.24:** Synthesis of 1, 2 disubstitued benzimidazole derivatives catalysed by SiO\(_2\)/CaCl\(_2\).2H\(_2\)O

<table>
<thead>
<tr>
<th>Entry</th>
<th>Aldehyde</th>
<th>Product</th>
<th>MW yield</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>CHO</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>d1</td>
<td>78%</td>
</tr>
</tbody>
</table>
2. \( \text{CHO} \) \( \text{NO}_2 \) \( \text{NO}_2 \) \( 1-(3\text{-nitrobenzyl})-2-(3\text{-nitrophenyl})-1\text{H}-\text{benzo}[d]\text{imidazole} \) \( d2 \) 83% 82%

3. \( \text{CHO} \) \( \text{NO}_2 \) \( \text{NO}_2 \) \( 1-(4\text{-nitrobenzyl})-2-(4\text{-nitrophenyl})-1\text{H}-\text{benzo}[d]\text{imidazole} \) \( d3 \) 84% 82%

4. \( \text{CHO} \) \( \text{CH}_3 \) \( \text{CH}_3 \) \( 1-(4\text{-methylbenzyl})-2-(4\text{-methylphenyl})-1\text{H}-\text{benzo}[d]\text{imidazole} \) \( d4 \) 82% 80%

5. \( \text{CHO} \) \( \text{F} \) \( \text{F} \) \( 1-(4\text{-fluorobenzyl})-2-(4\text{-fluorophenyl})-1\text{H}-\text{benzo}[d]\text{imidazole} \) \( d5 \) 83% 82%
6. 1-(4-chlorobenzyl)-2-(4-chlorophenyl)-1H-benzo[d]imidazole  
   d6  84%  81%

7. 1-(2-chlorobenzyl)-2-(2-chlorophenyl)-1H-benzo[d]imidazole  
   d7  80%  77%

8. 1-(4-methoxybenzyl)-2-(4-methoxyphenyl)-1H-benzo[d]imidazole  
   d8  83%  80%

9. 1-(4-methoxybenzyl)-2-(4-methoxyphenyl)-1H-benzo[d]imidazole  
   d9  83%  79%
1, 2 phenylenediamine reacts with aldehyde to form 1, 2 disubstituted benzimidazoles. A possible mechanism is depicted in the Scheme (4) based on **Jacob et al., 2009**. The reaction takes place with the formation of dibenzilidene diamine from phenylenediamide and arylaldehyde followed by 1, 3-hydride transfer as suggested by previous workers. Herein it is proposed that SiO$_2$/CaCl$_2$.2H$_2$O acts as catalyst in the same way as ZnCl$_2$ the electrophilicity of CaCl$_2$.2H$_2$O is comparable with ZnCl$_2$. 
**Scheme 4.4:** Probable mechanism of formation of 1, 2 disubstituted benzimidazoles catalysed by SiO$_2$/CaCl$_2$.2H$_2$O (*Jacob et al., 2009*)

In conclusion we have reported here a facile method for selective synthesis of 1, 2 disubstituted benzimidazoles by the condensation of O-phenylenediamine and aldehyde using solid supported catalyst (SiO$_2$/CaCl$_2$.2H$_2$O). Moreover catalyst works well under microwave irradiation as well as under conventional methodology involving simply stirring of reaction mixture. The reaction time for conventional method is in hours (3 hours) which is reduced to minutes (1-3) under microwave irradiation.

**4.4.6 Spectral Characterization**

**4.4.6.1 FTIR spectral analysis**

The FTIR spectra of all the synthetic compounds were recorded in KBr pellets.
The FTIR spectra of benzimidazole derivatives shows bands at 3000-3100 cm$^{-1}$ for aromatic –CH str and aromatic alkenic str -C=C- at 1600-1650 cm$^{-1}$. The absorption at 1350-1500 cm$^{-1}$ show –NH str.

**4.4.6.2 1H NMR spectral analysis**

The NMR spectra of all compounds were taken in the CDCl$_3$ with Varian 600 MHz.

In the $^1$HNMR spectra of 1, 2 disubstitued benzimidazole derivatives the protons of benzene ring of imidazole moiety shows downfield δ value at 7.5-7.9 while protons of benzene ring attached with basic imidazole ring comparatively upfield δ values at 7.0-7.55. Due to the direct attachment with nitrogen and benzene ring methylene (-CH$_2$) groups gives down field δ value at 5-5.5.

**4.4.6.3 Spectral Characterisation Data**

1. **d1**: IR (KBr) 3030, 1697, 1602, 1446, 1361 cm$^{-1}$. HNMR (CDCl$_3$ 600MHz) δ 5.5 (s, 2H, CH$_2$): 7.15 (d, 2H, J=7.9 Hz, Ph): 7.2-7.3 (m, 6H, Ph): 7.35-7.40 (m, 3H, Ph): 7.9 (d, 1H, J=8Hz, Ph): 7.8 (dd, 2H, J=8.4, 2.5 Hz, Ph).

2. **d5**: IR (KBr) 3065, 2900, 1604, 1510, 1460, 1329 cm$^{-1}$. (CDCl$_3$ 600MHz) δ 5.4 (2H, s, CH$_2$): 7.0-7.2 (m, 4H, Ph): 7.3-7.8 (m, 8H, Ph).

**4.5 Synthesis of β-acetamido esters**

**4.5.1 Solventless three Component Synthesis of β-acetamido keto esters via direct Mannich-type reaction using CaCl$_2$.2H$_2$O as a Catalyst**

The β-acetamido keto esters via direct Mannich-type reaction were prepared in good yield by conventional method and microwave
method by aldehyde, acetamide, and acetylacetone/ethylacetocetate/acetophenone using CaCl$_2$.2H$_2$O as catalyst.

CaCl$_2$.2H$_2$O is a mild lewis acid promoter in this multicomponent reaction. CaCl$_2$.2H$_2$O is inexpensive and commercially available catalyst. The CaCl$_2$.2H$_2$O is a new catalyst reported first time for this reaction. The compound synthesized and their yields are presented in Table (4.24-4.29)

4.5.1.1 Mannich-type multicomponent reaction of acetamide, acetylacetone, and different aldehydes

\[
\begin{aligned}
\text{CHO} & \quad \text{CH$_3$CONH$_2$} + \text{CaCl$_2$.2H$_2$O} \\
\text{reflux} & \quad \rightarrow \text{C$_6$H$_5$CONH$_2$} + \text{HAc}
\end{aligned}
\]

R1= C$_6$H$_5$CHO, 4Cl, 4MeO, 4F, 4CH$_3$, 2Cl

Table 4.25: Mannich-type multicomponent reaction of acetamide, acetylacetone, and different aldehydes catalysed by CaCl$_2$.2H$_2$O

<table>
<thead>
<tr>
<th>Entry</th>
<th>Aldehyde</th>
<th>Product</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>CHO</td>
<td>N-(2-Acetyl-3-oxo-1-phenyl-butyl)-acetamide</td>
<td>e1 885</td>
</tr>
<tr>
<td>2.</td>
<td>Cl</td>
<td>N-[2-Acetyl-1-(4-chloro-phenyl)-3-oxo-butyl]-acetamide</td>
<td>e2 79%</td>
</tr>
</tbody>
</table>
3. \( \text{C}_8\text{H}_8\text{O}_3\text{NH}_3\text{O}_3\text{H}_3\text{C}_3\text{O}_3\text{N-}[2-\text{Acetyl}-1-(4-\text{methoxy-phenyl})-3-\text{oxo-butyl}]-\text{acetamide} \)
\[ \text{e3 } 76\% \]

4. \( \text{C}_8\text{H}_8\text{O}_3\text{F}_3\text{NH}_3\text{O}_3\text{H}_3\text{C}_3\text{O}_3\text{N-}[2-\text{Acetyl}-1-(4-\text{fluoro-phenyl})-3-\text{oxo-butyl}]-\text{acetamide} \)
\[ \text{e4 } 88\% \]

5. \( \text{C}_8\text{H}_8\text{O}_3\text{CH}_3\text{NH}_3\text{O}_3\text{H}_3\text{C}_3\text{O}_3\text{N-(2-Acetyl-3-oxo-1-p-tolyl-butyl)-acetamide} \)
\[ \text{e5 } 78\% \]

4.5.1.2 Mannich-type multicomponent reaction of acetamide, ethylacetoacetate, and different aldehydes

\[ \text{R1= C}_6\text{H}_5\text{CHO, 4Cl, 4MeO, 4F, 4CH}_3, \]
Table 4.26: Mannich-type multicomponent reaction of acetamide, ethylacetoacetate, and different aldehydes catalysed by CaCl$_2$.2H$_2$O

<table>
<thead>
<tr>
<th>Entry</th>
<th>Aldehyde</th>
<th>Product</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.</td>
<td>CHO</td>
<td>2-(Acetylamino-phenyl-methyl)-3-oxo-butyric acid ethylester</td>
<td>e6 85%</td>
</tr>
<tr>
<td>7.</td>
<td>Cl</td>
<td>2-[Acetylamino-(4-chloro-phenyl)-methyl]-3-oxo-butyricacid ethyl ester</td>
<td>e7 78%</td>
</tr>
<tr>
<td>8.</td>
<td>OCH$_3$</td>
<td>2-[Acetylamino-(4-methoxy-phenyl)-methyl]-3-oxo-butyric acid ethyl ester</td>
<td>e8 78%</td>
</tr>
<tr>
<td>9.</td>
<td>F</td>
<td>2-[Acetylamino-(4-fluoro-phenyl)-methyl]-3-oxo-butyric acid ethyl ester</td>
<td>e9 80%</td>
</tr>
</tbody>
</table>
4.5.1.3 Mannich-type multicomponent reaction of acetamide, acetophenone, and different aldehydes

\[
\begin{align*}
\text{CHO} & \quad + \quad \text{CH}_3\text{CONH}_2^+ & \text{COMe} & \quad \text{CaCl}_2.2\text{H}_2\text{O} & \quad \text{reflux} & \quad \text{NHAc}\text{O} \\
\text{R} & \quad \text{R} & \quad \text{R} & \quad \text{R} & \quad \text{R} & \quad \text{R}
\end{align*}
\]

R1= C\text{6H}_5\text{CHO}, 4\text{Cl}, 4\text{MeO}, 4\text{F}, 4\text{CH}_3, 2\text{Cl}.

**Table 4.27:** Mannich-type multicomponent reaction of acetamide, acetophenone, and different aldehydes catalysed by \(\text{CaCl}_2.2\text{H}_2\text{O}\)

<table>
<thead>
<tr>
<th>Entry</th>
<th>Aldehyde</th>
<th>Product</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>10.</td>
<td>CHO</td>
<td>N-(3-Oxo-1,3-diphenyl-propyl)-acetamide</td>
<td>e10 82%</td>
</tr>
<tr>
<td>11.</td>
<td>CHO \text{Cl}</td>
<td>N-[1-(2-Chloro-phenyl)-3-oxo-3-phenyl-propyl]-acetamide</td>
<td>e11 79%</td>
</tr>
<tr>
<td>12.</td>
<td>CHO \text{F}</td>
<td>N-[1-(4-Fluoro-phenyl)-3-oxo-3-phenyl-propyl]-acetamide</td>
<td>e12 75%</td>
</tr>
</tbody>
</table>
4.5.2 Solventless three Component Synthesis of β-acetamido keto esters via direct Mannich-type reaction using Oxalic acid as a Catalyst

The β-acetamido keto esters via direct Mannich-type reaction were prepared in good yield by conventional method as well as under microwave irradiation method by aldehyde, acetamide, and acetylacetone/ethylacetoacetate/acetophenone using Oxalic acid. It is inexpensive and commercially available catalyst. The oxalic acid is a new catalyst reported first time for this reaction. The compounds synthesized and their yields are presented in Table 4.4-4.6.

4.5.2.1 Mannich-type multicomponent reaction of acetamide, acetylacetone, and different aldehydes

\[
\text{CHO} + \text{CH}_3\text{CONH}_2 + \text{O} \xrightarrow{\text{Oxalic acid reflux}} \text{CH}_3\text{CONH}_2\text{R}_1
\]

R1 = C6H5CHO, 4Cl, 4MeO, 4F, 4CH3, 2Cl
Table 4.28: Mannich-type multicomponent reaction of acetamide, acetylacetone, and different aldehydes catalysed by Oxalic acid

<table>
<thead>
<tr>
<th>Entry</th>
<th>Aldehyde</th>
<th>Product</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>CHO</td>
<td>N-(2-Acetyl-3-oxo-1-phenyl-butyl)-acetamide</td>
<td>f1 86%</td>
</tr>
<tr>
<td>2.</td>
<td>CHO</td>
<td>N-[2-Acetyl-1-(4-chloro-phenyl)-3-oxo-butyl]-acetamide</td>
<td>f2 78%</td>
</tr>
<tr>
<td>3.</td>
<td>CHO</td>
<td>N-[2-Acetyl-1-(4-methoxy-phenyl)-3-oxo-butyl]-acetamide</td>
<td>f3 77%</td>
</tr>
<tr>
<td>4.</td>
<td>CHO</td>
<td>N-[2-Acetyl-1-(4-fluoro-phenyl)-3-oxo-butyl]-acetamide</td>
<td>f4 85%</td>
</tr>
</tbody>
</table>
4.5.2.2 Mannich-type multicomponent reaction of acetamide, ethylacetoacetate, and different aldehydes

\[
\text{CHO} + \text{CH}_3\text{CONH}_2 + \text{OEt} \xrightarrow{\text{Oxalic acid, reflux}} \text{N}-\text{(2-Acetyl-3-oxo-1-p-tolyl-butyl)-acetamide} \quad f_5 \quad 78\%
\]

R1 = 4Cl, 4MeO, 4F, 4CH3,

**Table 4.29: Mannich-type multicomponent reaction of acetamide, ethylacetoacetate, and different aldehydes catalysed by Oxalic acid**

<table>
<thead>
<tr>
<th>Entry</th>
<th>Aldehyde</th>
<th>Product</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.</td>
<td>[Chemical structure]</td>
<td>ethyl 2-(acetamido(m-tolyl)methyl)-3-oxobutanoate</td>
<td>(f_6) 86%</td>
</tr>
</tbody>
</table>

Results and Discussion
4.5.2.3 Mannich-type multicomponent reaction of acetamide, acetophenone, and different aldehydes

R1= C₆H₅CHO, 4Cl, 4MeO, 4F, 4CH₃,
Table 4.30: Mannich-type multicomponent reaction of acetamide, acetophenone, and different aldehydes catalysed by Oxalic acid

<table>
<thead>
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<th>Entry</th>
<th>Aldehyde</th>
<th>Product</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>CHO</td>
<td>N-(3-Oxo-1,3-diphenyl-propyl)-acetamide</td>
<td>f10 83%</td>
</tr>
<tr>
<td>11</td>
<td>Cl</td>
<td>N-(1-(4-chlorophenyl)-3-oxo-3-phenylpropyl)acetamide</td>
<td>f11 77%</td>
</tr>
<tr>
<td>12</td>
<td>F</td>
<td>N-[1-(4-Fluoro-phenyl)-3-oxo-3-phenyl-propyl]-acetamide</td>
<td>f12 72%</td>
</tr>
<tr>
<td>13</td>
<td>OCH₃</td>
<td>N-[1-(4-Methoxy-phenyl)-3-oxo-3-phenyl-propyl]-acetamide</td>
<td>f13 74%</td>
</tr>
</tbody>
</table>
4.5.3 Spectral Characterization

4.5.3.1 FTIR spectral analysis

The FTIR spectra of all the synthetic compounds were recorded in KBr pellets.

The FTIR spectra of β-acetamido keto esters shows bands at 3000-3100 cm\(^{-1}\) for aromatic –CH \(\text{str.}\) Aromatic alkenic \(\text{str}\) shows at 1600-1650 cm\(^{-1}\). The absorption bands at 1350-1500 cm\(^{-1}\) shows –NH \(\text{str}\) and the absorption bands at 1700-1750 cm\(^{-1}\) shows –C=O \(\text{str}\).

4.5.3.2 \(^1\)H NMR spectral analysis

The \(^1\)HNMR spectra of all compounds were taken in the CDCl\(_3\) with Varian 600 MHz and JEOL AL 300 MHz.

The \(^1\)HNMR spectrum of β-acetamido keto esters the protons of benzene ring shows \(\delta\) value at 7.0-7.5 while proton attached with –N shows \(\delta\) at 5.0-5.5 and protons of aliphatic carbon shows upfield \(\delta\) value.

4.5.4 Spectral Characterisation Data

1. **e1:** IR (KBr) 3357, 2900, 1701, 1650, 1527, 1365 cm\(^{-1}\). \(^1\)HNMR (CDCl\(_3\), 300MHz) \(\delta\) 2.27 (3H, s, COMe): 2.28 (3H, s, COMe): 5.8
Results and Discussion

2. **e3**: IR (KBr) 3200, 2961, 1711, 1657, 1601, 1425, 1369 cm⁻¹. \(^1\)HNMR CDCl₃, 300MHz) δ 2.0 (3H, s, COMe): 2.15 (3H, s, COMe): 2.85 (3H, s, COMe): 3.8 (3H, s, OMe): 4.25 (1H, d, J=5.5 Hz, CH): 5.8 (1H, dd, J=6.3, 2.9 Hz, CH): 7.03 (2H, d, J=8.3 Hz, NH): 7.22 (2H, d, J=8.2 Hz, Ph): 7.4 (2H, d, J=8.3 Hz, Ph).

3. **e7**: \(^1\)HNMR CDCl₃, 300MHz) δ 1.2 (3H, t, CH₃): 2.35 (3H, s, COMe): 2.41 (3H, s, OMe): 4.20 (1H, d, CH): 4.29 (2H, q, CH₂): 4.36 (1H, dd, CH): 7.2-7.5 (4H, m, Ph).

4. **f3**: IR (KBr) 3294, 2919, 1722, 1649, 1516, 1421, 1361 cm⁻¹. \(^1\)HNMR (CDCl₃, 300MHz) δ 2.10 (3H, s, COMe): 2.19 (3H, s, COMe): 2.79 (3H, s, COMe): 3.11 (3H, s, OMe): 4.37 (1H, d, J=5.7 Hz, CH): 5.9 (1H, dd, J=6.1, 2.4 Hz, CH): 7.16 (2H, d, J=8.2 Hz, NH): 7.29 (2H, d, J=8.4 Hz, Ph): 7.4 (2H, d, J=8.0 Hz, Ph).

The perusal of table 4.25-4.30 indicate that this reaction can give the corresponding Knoevenagel condensation product if aldehyde contains electron releasing group but if aldehyde contains any electron withdrawing group there are two possibilities i.e. formation of Knoevenagel condensation product or Mannich type product. Same result were obtained for TMSCl catalysed reaction studied by **Mao et al. (2009)** aldehyde containing NO₂ group gives
Mannich product while aldehyde containing halogen product gives Knoevenagel product.

\[
\begin{align*}
\text{CHO} & \quad + \quad \text{CH}_2\text{CONH}_2 + \quad \text{O} \\
\text{O}_2\text{N} & \quad \text{TMSCl} \\
\text{CH}_3\text{CN, reflux} & \quad \text{O} \\
\text{H}_3\text{CO} & \quad \text{NH} \\
\text{O} & \quad \text{O} \\
\text{O}_2\text{N} & \quad \text{O} \\
\text{O}_2\text{N} & \quad \text{O} \\
\end{align*}
\]
Summary and Conclusion
SUMMARY AND CONCLUSION

Five series of compounds namely cinnamic acid amides, β-benzoylacrylic acid esters, benzodiazepines, benzimidazoles and β-acetamido keto esters were synthesized and screened for their biological activities.

✓ Amongst the synthesized cinnamic acid amides, the cinnamoyl piperidine, N-cyclohexyl-1-(4′-nitro) cinnamamide, cinnamanilide, 4-nitro cinnamanilide, N-methyl cinnamanilide, 1-(4′-methoxycinnamoyl) morpholine and 2-chloro-(4′-nitro)cinnamanilide were found to exhibit significant herbicidal activity against *Echinochola colona* (weed of rice) at par with the standard butachlor and pendimethalin.

✓ Substituted 4-oxo-4-phenylbut-2-enoates have been synthesized by using substituted 4-oxo-4-phenylbut-2-enoic acids and substituted phenacetyl bromides in the presence of DMF. The compounds 2-(4-methoxyphenyl)-2-oxoethyl (2E)-4-oxo-4-phenylbut-2-enoate, 2-(4-nitrophenyl)-2-oxoethyl (2E)-4-oxo-4-phenylbut-2-enoate, 2-(4-methylphenyl)-2-oxoethyl (2E)-4-(4-methylphenyl)-4-oxobut-2-enoate and 2-oxo-2-phenylethyl (2E)-4-(4-bromophenyl)-4-oxobut-2-enoate were found to exhibit very good herbicidal activity against *Phalaris minor* (weed of wheat) at par with the standard pendimethalin.

✓ Benzodiazepines were synthesized through multicomponent reaction using trichloro acetic acid as a novel catalyst. The
synthesized benzodiazepine derivatives were also screened for antioxidant, antibacterial and duodenal smooth muscle relaxation activity of rats. The compounds were not found to exhibit significant antioxidant activity. The compounds (Z)-methyl 2-[2,3-dihydro-2-[4-methoxyphenyl]-1H-benzo[b][1,4] diazepin-4(5H)-ylidene]acetate, (Z)-benzyl 2-[2,3-dihydro-2-phenyl-1H-benzo[b][1,4] diazepin-4(5H)-ylidene]acetate, and (Z)-benzyl 2-[2,3-dihydro-2-[4-methoxyphenyl]-1H-benzo[b][1,4] diazepin-4(5H)-ylidene]acetate were found to exhibit very good antibacterial activity against *Pseudomonas aeruginosa*, *Gordonia terrae* and *Bacillus Cereus*. All the benzodiazepine derivatives exhibited duodenal smooth muscle relaxation activity of the rat.

✓ For the synthesis of benzimidazoles two new catalysts ferric chloride and silica supported calcium chloride dihydrate were developed. The reaction using these catalysts gave the product in excellent yields for conventional as well as for microwave assisted method of the synthesis. Titanium tetrachloride and oxalic acid, the catalysts used in conventional synthesis were optimized to microwave assisted method of synthesis.

✓ For the synthesis of β-acetamido keto esters, two new catalyst calcium chloride dihydrate and oxalic acid have been developed. The reaction using these catalysts gave the products in the excellent yields for conventional as well as for microwave assisted method of synthesis.
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Appendix
4-methoxy cinnamic acid

3-nitrocinnamic acid
4-hydroxycinnamic acid

2-chlorocinnamic acid
Cinnamoyl piperidine
4-hydroxy cinnamic acid

2-chloro cinnamic acid
cinnamoyl piperidine

2-(4-chlorophenyl)-2-oxoethyl (2S)-4-oxo-4-phenylbut-2-enoate \( [B2] \)
2-(4-nitrophenyl)-2-oxoethyl (2E)-4-oxo-4-phenylbut-2-enoate [B5]

2-(4-methylphenyl)-2-oxoethyl (2E)-4-(6-chlorophenyl)-4-oxobut-2-enoate [C1]
2-(4-methyl phenyl)-2-oxo ethyl (2E) -4-(4-methyl phenyl)-4-oxo but-2-enolate (E1)
The authoress was born on 16th June 1985 in Pithoragarh. She passed her High School Examination in 1999 and Intermediate Examination in 2001 from G.G.I.C., Pithoragarh. She earned her B.Sc. degree in 2004 & M.Sc. degree in Organic Chemistry in 2006 from Kumaun University (Govt. P.G. College, Pithoragarh), Nainital. In 2007 she joined G.B.P.U.A.& T., Pantnagar for her Ph.D. in Agricultural Chemicals. During her academics she is throughout first class.

She was the recipient of University fellowship during her Ph.D. programme.

Permanent Address:

Jyoti Pandey
G.I.C. Road,
P. O.-Degree College,
Pithoragarh—262502
Ph. No. 9997456306 (Mob.), 05964-264045 (H)
E-mail: jyoti.pandey16@gmail.com
jyotipandey.chem@gmail.com
ABSTRACT

Name : Jyoti Pandey  
Id. No. : 35443
Sem. & year of admission: Ist & 2007-08  
Degree : Ph.D.
Major : Agricultural Chemicals  
Deptt. : Chemistry
Minor : Biochemistry
Thesis Titled : “Synthesis of Bioactive Molecules Using Novel Methods and Evaluation of Their Biological Activities”
Advisor : Dr. Virendra Kumar

Five series of compounds namely cinnamic acid amides, β-benzoylacrylic acid esters, 1, 5 benzodiazepines, benzimidazoles and β-acetamido keto esters were synthesized. Analogues of naturally occurring cinnamic acid amides and β-benzoylacrylic acid esters were synthesized in order to evaluate their herbicidal potential against Echinochola colona (weed of rice) and Phalaris minor (weed of wheat) respectively. Some of the analogues of cinnamic acid amides and β-benzoylacrylic acid esters exhibited activity at par with the standards. Benzodiazepines, benzimidazoles and β-acetamido keto esters were undertaken in order to develop new techniques and catalysts. A new catalyst trichloro acetic acid was developed for the synthesis of benzodiazepines by the reaction of o-phenylenediamine and substituted aldehydes. Some of the synthesized benzodiazepines were found to antibacterial and duodenal smooth muscle relaxation activity. For the synthesis of benzimidazoles two new catalyst ferric chloride and silica supported calcium chloride dihydrate have been developed as novel and efficient catalysts. For the synthesis of β-acetamido keto esters two new catalyst oxalic acid and calcium chloride dihydrate have been developed.
## सारांश

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यौगिकों की पांच श्रृंखलाए सिनामिक अम्ल एमाइड, β-बेन्जोइल एक्रालिक अम्ल एस्टर, बेन्जोडाइएजिप्सिन, बेन्जीमिडाइजोल तथा β-एसीटामिडो कीटो एस्टर संश्लेषित की गयी। प्रकृति में पाये जाने वाले सिनामिक अम्ल एमाइड तथा β-बेन्जोइल एक्रालिक एस्टर को उनकी खरपतवार नाशक क्षमता का अवलोकन करने हेतु संश्लेषित किया गया। सिनामिक अम्ल एमाइड तथा β-बेन्जोइल एक्रालिक एस्टर के कुछ अनुरूप ने मानक के समान प्रतिक्रिया प्रदर्शित की।

बेन्जोडाइएजिप्सिन, बेन्जीमिडाइजोल तथा β-एसीटामिडो कीटो एस्टर को उनकी नई विधि तथा उत्तरेक्ष विकसित करने को लिया गया। नवीन उत्तरेक्ष द्राइकलोरो एसीटिक अम्ल को बेन्जोडाइएजिप्सिन के संश्लेषण के लिए विकसित किया गया। बेन्जोडाइएजिप्सिन के कुछ अनुरूप में जीवाणु रोगी तथा मृदा पेशी छोटा गतिविधि देखी गयी। बेन्जीमिडाइजोल के संश्लेषण के लिए दो नए उत्तरेक्ष फेरिक क्लोराइड तथा कैलियम क्लोराइड डाइहाइड्रेट नवीन तथा सक्षम उत्तरेक्ष विकसित किये गये। β-एसीटामिडो कीटो एस्टर के संश्लेषण के लिए आक्जेलिक अम्ल तथा कैलियम क्लोराइड डाइहाइड्रेट नवीन उत्तरेक्ष विकसित किये गये।

![Signature](image1)

(वीरेन्द्र कुमार)
(सलाहकार)

![Signature](image2)

(ज्योति पाण्डेय)
(लेखिका)