"Synthesis and Pharmacological Evaluation of 1-{[5′-substituted phenyl-1,3,4-oxadiazol-2-yl]methyl}-1*H*-benzotriazole analogues"

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BY

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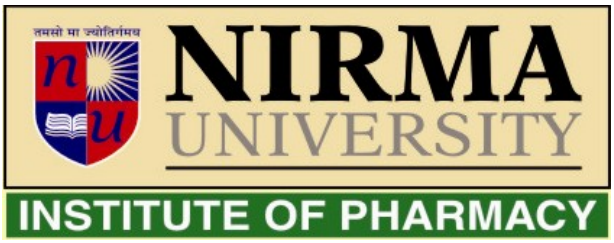

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CERTIFICATE

This is to certify that Mr. Devang Vadher (08MPH402) has prepared his thesis entitled ***"Synthesis and Pharmacological Evaluation of 1-{[5'-substituted phenyl-1,3,4-oxadiazol-2-yl]methyl}-1H-benzotriazole analogues"****, in partial fulfillment for the award of M. Pharm. degree of the Nirma University, under our guidance. He has carried out the work at the Department of Pharmaceutical Chemistry, Institute of Pharmacy, Nirma University.*

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DECLARATION

I declare that the thesis entitled ***"Synthesis and Pharmacological Evaluation of 1-{[5'-substituted phenyl-1,3,4-oxadiazol-2-yl]methyl}-1H-benzotriazole"*** *under the guidance of* Prof. Manjunath Ghate *(Guide), Professor* and *Mr. Kuntal Manna (Co-guide), Assistant Professor, Department of Pharmaceutical Chemistry, Institute of Pharmacy, Nirma University. No part of this thesis has formed the basis for the award of any degree or fellowship previously in our institute and elsewhere.*

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Date: **DEVANG S. VADHER**

A series of new 1-{[5'-substituted phenyl-1,3,4-oxadiazol-2-yl]methyl}-1*H*-benzotriazole derivatives (DS-1 to DS-9)were synthesized from 2-(1*H*-benzotriazol-1-yl) acetohydrazide (30) with different aromatic aldehyde. All the synthesized compounds were evaluated for antifungal activity against *C. albican, A. niger* and *F. oxyporum* using agar diffusion method. All the compounds exhibited moderate to good antifungal activity, compounds DS-4 and DS-5 (*para* substituted with –Cl & -F) were found most potent among all. The structures of the compounds were elucidated by FTIR, ^{1}H NMR and Mass data.

Azoles have played a crucial part in the history of heterocyclic chemistry and also extensively important synthons in organic synthesis. Owing to the versatile chemotherapeutic activities of azoles, a significant amount of research activity has been directed towards this class[1]. The azole moiety has an important biological activity like insecticidal, agrochemical, cytochrome P450 enzyme inhibitors, peptide inhibitors[2], antiprotozoal agents[3] (against *acanathamoeba castelanii*), antimicrobial[4], anticonvulsant[4], anti-inflamatory[4] and anti-cancer[4], etc. Several derivatives of benzotriazoles are reported as peroxisome proliferator activated receptors[5] NTpase/helicase inhibitors[6], UV absorbers[7], corrosion Inhibitors[8], antifading agent for metals[9], antifreeze Agent[10] and photoconductor[11].

Benzotriazole ring containing compounds *via* the fusion of 1,2,3-triazole and benzene rings, have also been extensively for biological and pharmaceutical purposes since few decades. 1,3,4-oxadiazole is well established heterocyclic ring system exhibiting broad spectrum of pharmacological activities such as antimicrobial activity[12], anti-fungal activity[13], anticancer[14], antidiarrheal agents[15], antimycobacterial activity[16] and antiinflammatory[17]. May be due to biomimetic and reactive pharmacophores of 1,3,4-Oxadiazoles ring. It also reported that 1,3,4-oxadiazole derivatives produce good monoamine oxidase inhibitors (antidepressants)[18], anti-convulsant agent[19] and potential inhibitors targeting chitin biosynthesis[20], antiviral[21], antidiabetic[22] and antimalerial[23] agents have been reported.

1.1 Aim and scope of study

Multiple numbers of semi-synthetic antifungal agents are available in the market against many fungi diseases. Out of those azoles containing agents like fluconazole miconazole are using frequently as potent anti-fungal agents against most of the fungi. But most of the azole containing compound have serious side effects like hepatotoxic, metabolic disorder through inhibition of human CYPP450 enzymes, menstrual irregularities, loss of libido, impotence, gynaecomastia in males, etc. Currently available anti-fungal drugs have another leading problems fungal resistance[24]. An increasing number of clinical resistances to antifungal agents highlight the need for understanding the molecular mechanism responsible for the development of drug

resistances. Fungi *C. albicans* is the most frequently isolated pathogen in humans has caused morbidity in seriously debilitated and immunocompromised hosts[25].

The combination of benzotriazole and 1,3,4-oxadizole moieties might be better solutions for serious clinical resistances. The present study is associated with the synthesis of 1-{[5'-substituted phenyl-1,3,4-oxadiazol-2-yl]methyl}-1*H*-benzotriazole derivatives (DS-1 to DS-9) are the good combinatorial of potent heterocyclic ring systems.

1.2 Rational Behind the Project

The treatment of uncured fungal infections, in particular systemic mycoses, is based upon the discovery and development of newly synthesized compounds that inhibiting a critical mechanism in the pathogen, but not in the host. This representation is a major challenges to the medicinal chemists and drug discovery scientists. The rational of the project to find an essential new target, that is specific for the pathogens not host. The fungi pose additional challenges to the scientists and biologist that, there are like human, they consist of eukaryotic cell(s), and hence share high homology in their genome, and thereby proteome and cellular machinery like host.

2.1 Introduction:

Fungi play a vital role in Earth's cycle of life. They decompose or break down dead bugs and plant material, such as leaves, converting their components into elements that living organisms can reuse. They are an essential source of food for plants and animals. Many plants depend on fungi for their nutrients. Fungi also have had a profound effect on human life. Take a look at a moldy fruit and you are observing a type of fungi that has transformed modern medicine. People eat fungi and use them to manufacture bread, wine, and flavorings. Fungi can also cause plant and animal diseases[26, 27].

Fungi cause a range of illnesses (mycoses) ranging from the chronic to the serious. These mycoses can manifest themselves in a variety of ways. Infections can be *superficial*, that is situated at or close to the surface of the skin, or *systemic* which means they can affect the body as a whole, rather than individual parts or organs. A list of systemic infections is given in below

Actinomycosis: Caused by *Actinomyces bovis*.
Blastomycosis: Caused by *Blastomyces dermatidis*.
Nocardiosis: Caused by *Nocardia asteroides* or *N. brasiliensis*.
Cryptococcosis: Caused by *Cryptococcus neoformans*.
Sporotrichosis: Caused by *Sporotrichum schenckii*.

Diseases such as athlete's foot (tinea pedis), 'jock' itch (tinea cruris), tinea manus (infection of the hand), thrush (oral and vaginal), and onchomycosis (affecting the nails) are examples of **superficial infections** caused by the dermatophytes from the *Trichyphyton*, *Microsporum*, *Candida* (some can also cause systemic infections) and *Epidermophyton* species. 'Ringworm' (tinea corporis) is used as a general term for a fungal infection of the skin, in particular those of the scalp and feet. These infections are contagious, and cause intense itching. They are caused by one or more of these organisms together - classification is difficult as the diseases assume such a wide variety of forms - and similar symptoms can be caused by different organisms.

An important aspect to consider when developing treatments for mycoses is that fungi are eukaryotic. That is to say they have a nucleus within the cell containing the all

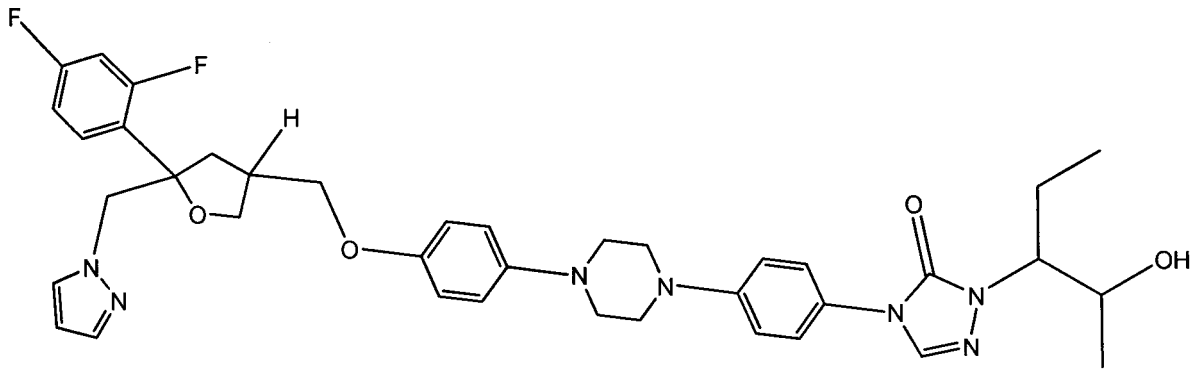
important nucleic acids. In very simplistic terms this means that some of the biochemistry regulating fungi turns out to be very similar to animal cells. They are therefore unlike the prokaryotic bacteria which do not have a cell nucleus. This can in turn pose potential problems with toxicity. For many enzymes in a fungus there are related enzymes performing the same transformations in the human cell. If you want to target one of these enzymes with your drug then absolute potency may not be as important as the difference in potency of your drug towards the different forms of the enzyme [28].

An **antifungal drug** is a medication used to treat fungal infections such as athlete's foot, ringworm, candidiasis (thrush), serious systemic infections such as cryptococcal meningitis, and others. Such drugs are usually obtained by a doctor's prescription or purchased over-the-counter. Antifungals work by exploiting differences between mammalian and fungal cells to kill the fungal organism without dangerous effects on the host. Unlike bacteria, both fungi and humans are eukaryotes. Thus fungal and human cells are similar at the molecular level. This makes it more difficult to find or design drugs that target fungi without affecting human cells. Consequently, many antifungal drugs cause side-effects. Some of these side-effects can be life-threatening if the drugs are not used properly.

Apart from side-effects like liver-damage or affecting estrogen levels, many medicines can cause allergic reactions in people. For example, the azole group of drugs is known to have caused anaphylaxis. There are also many drug interactions. For example, the azole antifungal agents such as ketoconazole or itraconazole can be both substrates and inhibitors of the P-glycoprotein, which (among other functions) excretes toxins and drugs into the intestines. Azole antifungals also are both substrates and inhibitors the cytochrome P450 family CYP3A4, causing increased concentration when administering, for example, calcium channel blockers, immunosuppressants, chemotherapeutic drugs, benzodiazepines, tricyclic antidepressants, macrolides and SSRIs.

The targets of all antifungal agents used in the clinic (and of some agents that entered or approached clinical development but have not been marketed) are summarised in Figure 1. Nevertheless, in terms of numbers of classes of agents that can be used to

treat life threatening mycoses, the targets are heavily focused, directly or indirectly, on the cell envelope (wall and plasma membrane), and particularly on the fungal membrane sterol, ergosterol, and its biosynthesis. Targets elsewhere in the cell would therefore be a welcome innovation for systemically bioavailable antifungal agents. There are six new antifungal agents that are currently generating excitement as they pass through the final developmental stages of clinical trials. Three of them (posaconazole **(1)**, ravuconazole **(2)** and voriconazole **(3)** are triazole compounds, a subset of the azoles, which are the most successful antifungal class in the clinic since the late 1960s [29].



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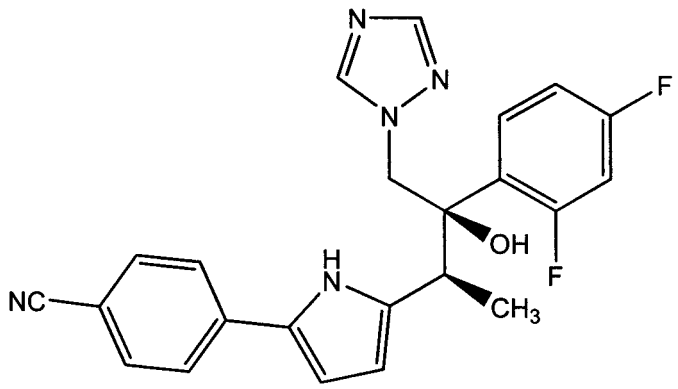

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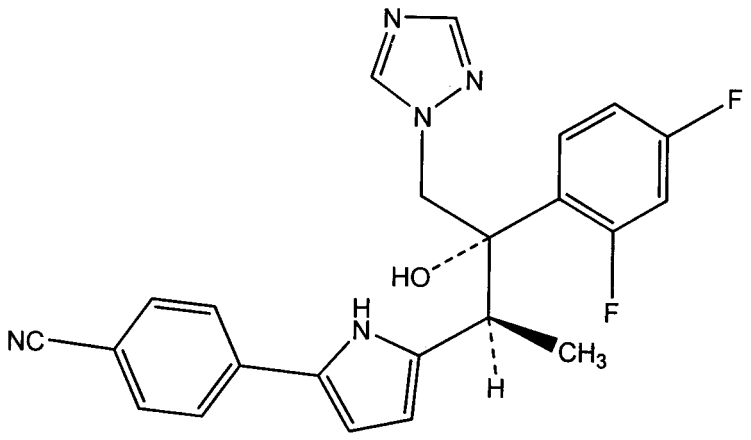

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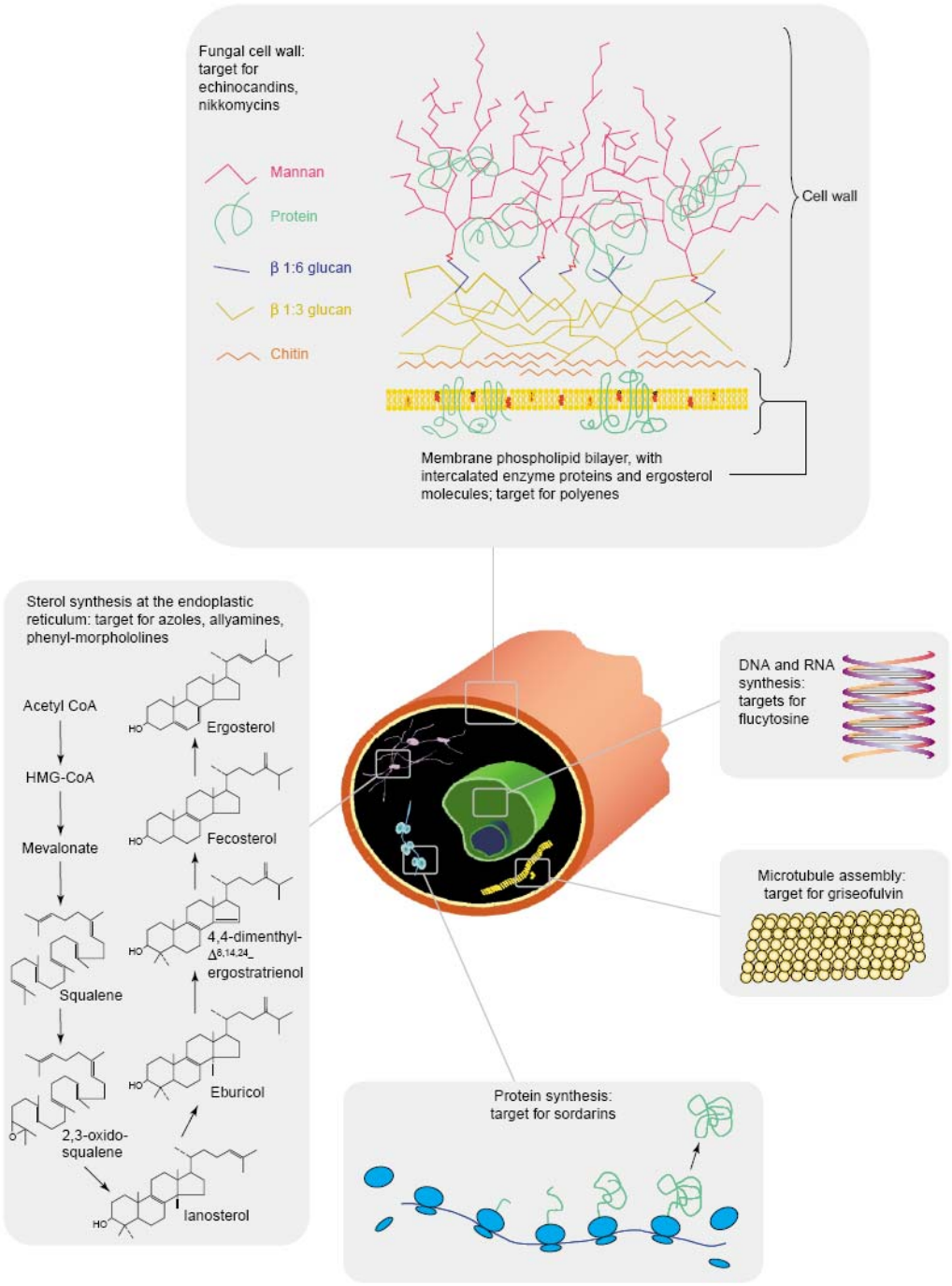
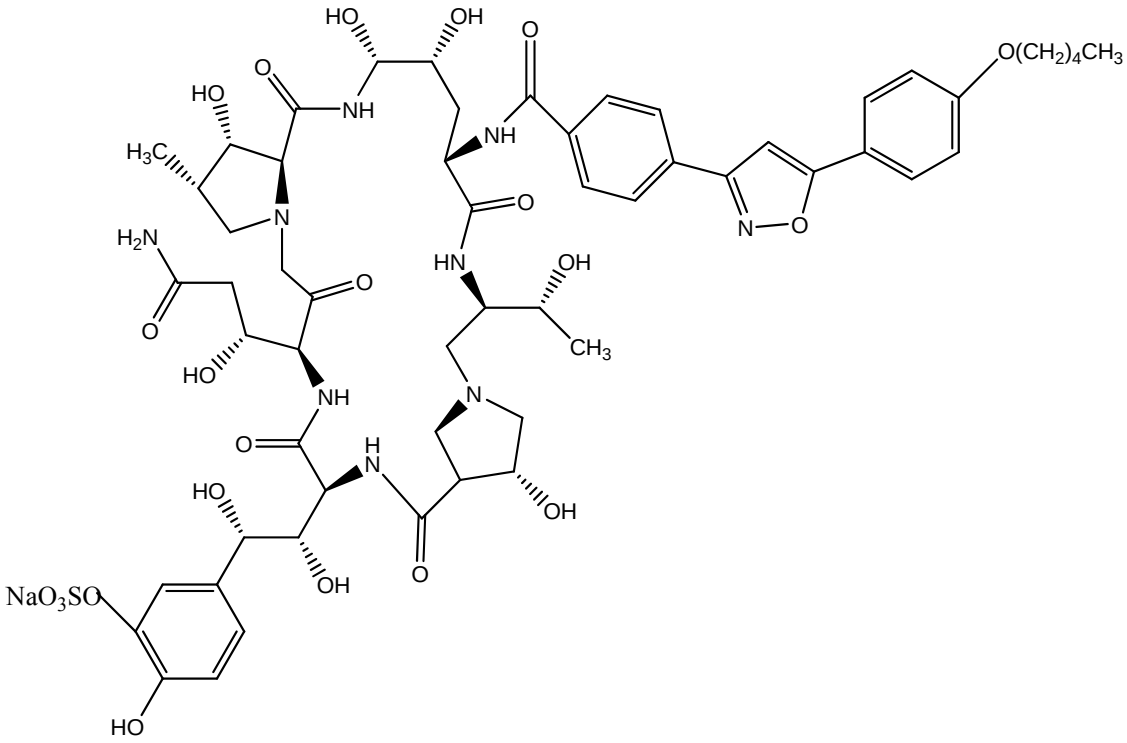


Fig. 1. Generalised cartoon showing target areas for antifungal agents. The cross-section through a fungal hypha shows the intracellular sites of action of antifungal agents. The callouts show details for each site. The cell envelope structure illustrated is based on data for Candida albicans. Other fungal species differ in the details of their cell wall composition. The steps illustrated for ergosterol synthesis are the major steps found in all fungi; species can differ in having additional steps that bypass or parallel those shown.

2. 2 Newer Antifungal Agents Under Development (2005-2009)

2. 2. 1 New polyene and other agents

The lipopeptide micafungin[38] **(4)**, like the other echinocandins,has fewer side effects than amphotericin B and other agents, but the echinocandins have not been approved as the first line therapy for invasive aspergillosis. The novel polyene SPK-843 showed less renal toxicity than both amphotericin B or liposomal amphotericin B and also better activity than micafungin and both established polyenes in a murine model of pulmonary aspergillosis. Clinical trials are presently being conducted.



(4)

2. 2. 2 New triazole

Voriconazole has no activity against the mucoraceous. The new triazole isavuconazole, (BAL4815) **(5)** in late state clinical development for the treatment of aspergillosis, appears to have *in vitro* activity against the zygomycetes (MIC_{50} and MIC_{100} of 1 and 2 μg/ml, respectively) versus voriconazole MICs of ≥ 16 μg/ml32; also, its activity was superior to that of both itraconazole and voriconazole against *Candida* spp.[39]

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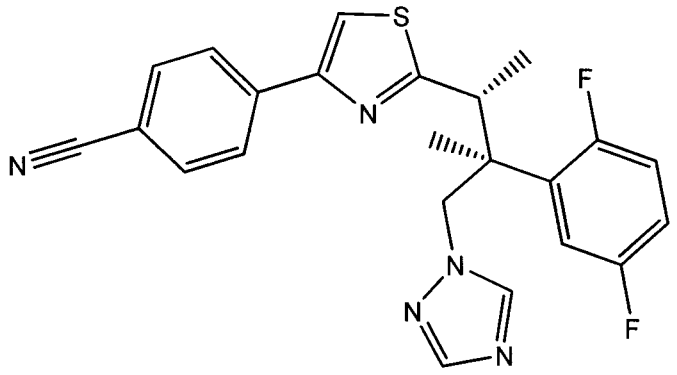


(5)

2. 2. 3 Inhibitor of ß-1,6-glucan synthesis

A pyridobenzimidazole, is a specific inhibitor of ß-1,6- glucan synthase; it has shown activity against *Candida* spp. and appears to inhibit hyphal elongation of *C. albicans*50. Genetic analysis of a resistant mutant of *Saccharomyces cerevisiae* indicated that its primary target was Kre6p (a ß-1,6-glucan synthase)[40]. Its growth inhibition is dose-dependent; since Kre6p homologous have been found in *Aspergillus fumigatus*, partial silencing of *KRE6P* expression makes *A. fumigatus* more susceptible to Congo red which appears to indicate the role of Kre6p in cell wall construction.

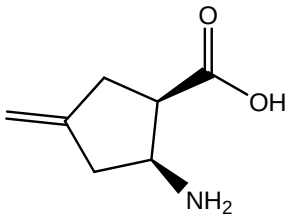
2. 2. 4 Monoclonal antibody therapy

Patient therapy

Casadeval considers serum therapy the third age of antimicrobial therapy. The results of the combination of amphotericin B and Mycograb (*Neu*tec Pharma), a human recombinant monoclonal antibody as an inhibitor of heat shock protein 90, in patients with invasive candidiasis. An 84% overall response was observed by day 10 in the combined therapy versus 48% in patients treated with amphotericin B alone; clinical and mycological response, *Candida*-attributable mortality and rate of culture-confirmed sterilization were also superior with the combined therapy. The first application of

2. 2. 5 Icofungipen

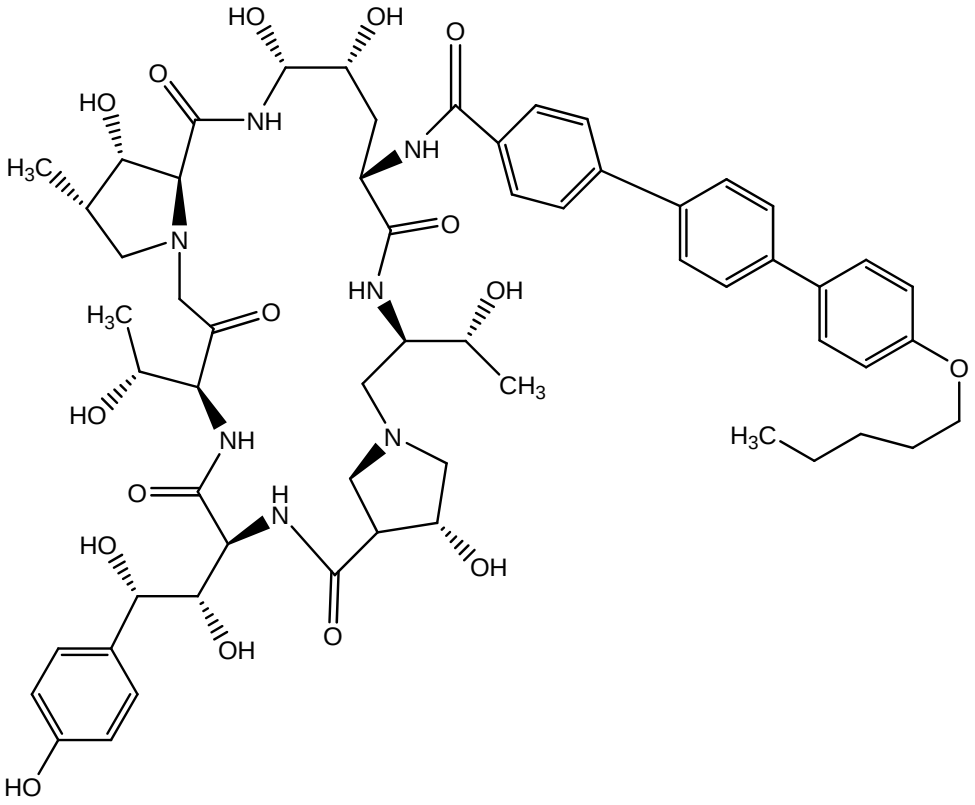
Icofungipen **(6)** (PLD-118, BAY 10-8888) is a derivative of cispentacin. It is a beta amino acid that targets isoleucyl-t-RNA synthetase; intracellular inhibitory concentrations at the target site are achieved by its active accumulation in susceptible fungal cells. Although its *in vitro* activity against *Candida albicans* is poor, it has shown strong *in vivo* activity in a neutropenic rabbit model for disseminated candidiasis, including the treatment of central nervous system infection[42, 43]. It has dose-dependent pharmacokinetics and it shows potential for the treatment of invasive candidiasis.



(6)

In addition, the mortality and morbidity of these infections is quite substantial. The most common fungal pathogens continue to be the species of *Candida* and *Aspergillus*[32]. Parallel to the increase in fungal infections, two triazoles (voriconazole and posaconazole) and three echinocandins (anidulafungin **(7)**, caspofungin **(8)** and micafungin **(9)** have been licensed for the treatment and prevention of these infections. The echinocandins have a unique mechanism of action (inhibition of β-1,3-D-glucan synthase) and a broad and similar spectrum of *in vitro* activity against *Candida* spp. and *Aspergillus* spp.[30,32,35]. During the last few years, mechanisms of resistance to most licensed agents in *Candida* spp., and to a certain point in *Aspergillus* spp., have been elucidated[30, 32, 34]. Although resistance of common *Candida* spp. And *Aspergillus* spp. to echinocandins and azoles is rare; it has been documented and continues to be reported [30,31,32,34]. The mortality rates associated with invasive candidiasis are approximately 0.4 deaths per 100,000 population/year while there was a decrease with aspergillosis from 0.42 per 100,000 in 1997 to 0.25 per 100,000 in 2003 in the United States. Although it is hoped that the introduction of these new agents will improve these

rates, the mortality rate in most aspergillosis studies is about 50%. Therefore, there is a need for new targets or strategies in antifungal therapy.



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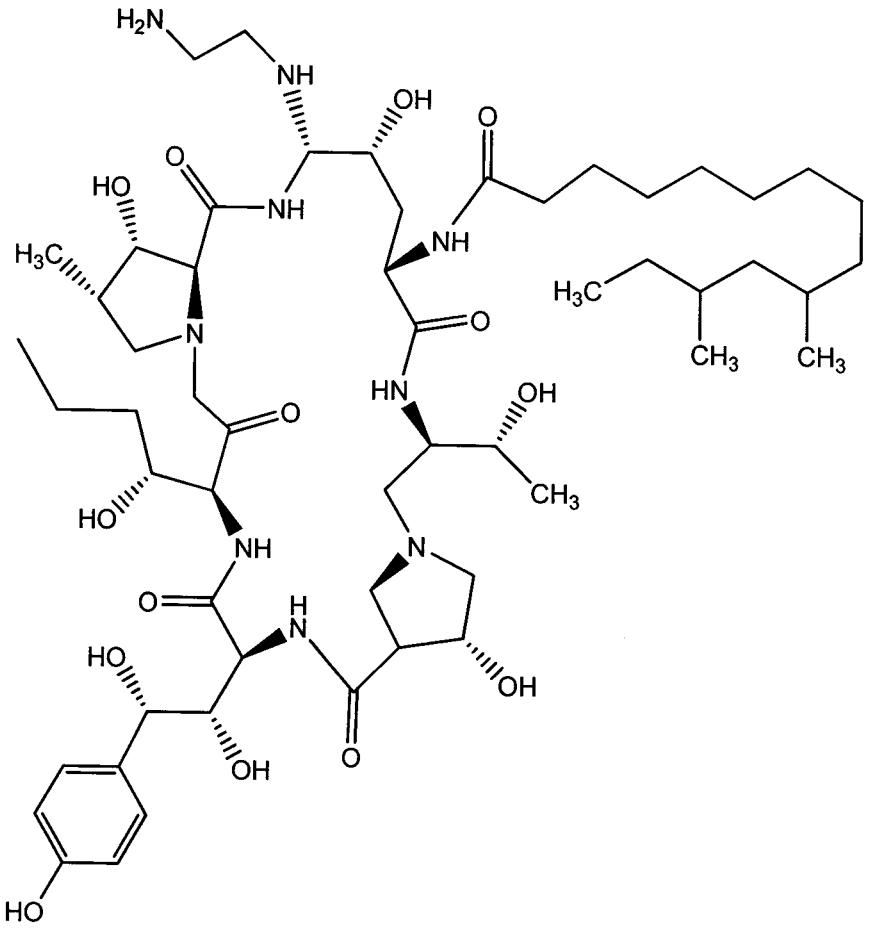

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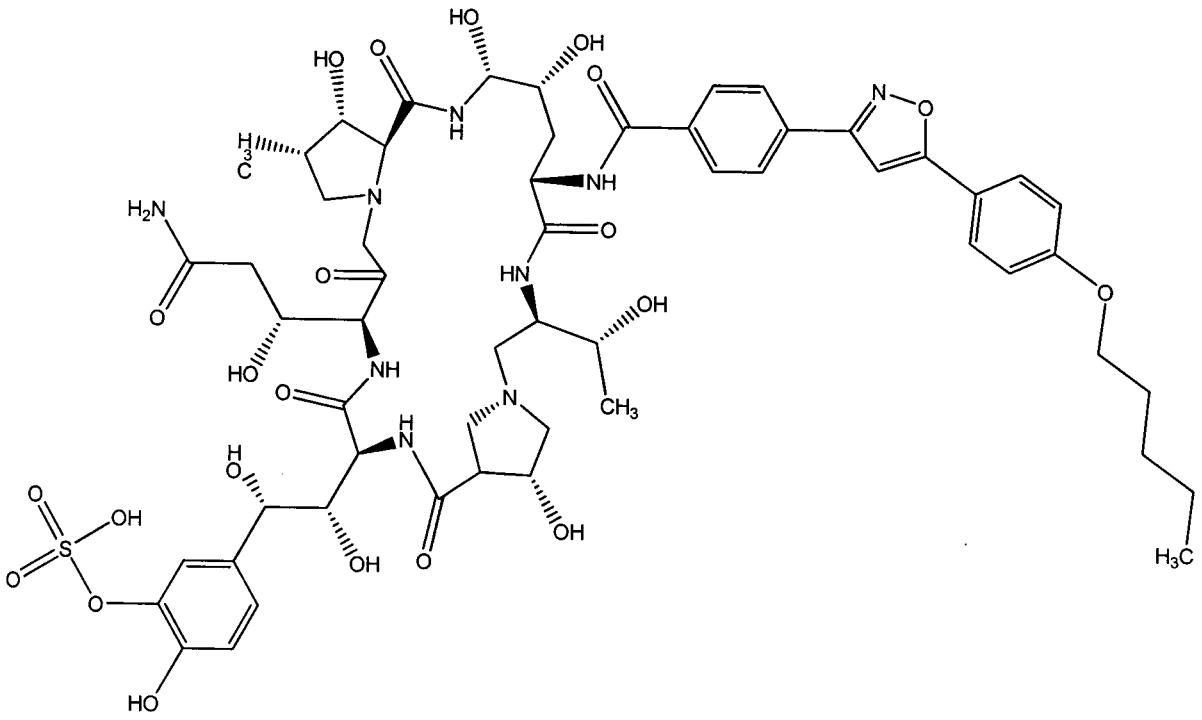

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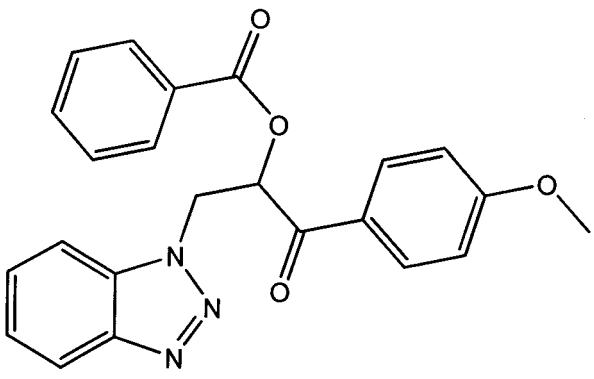
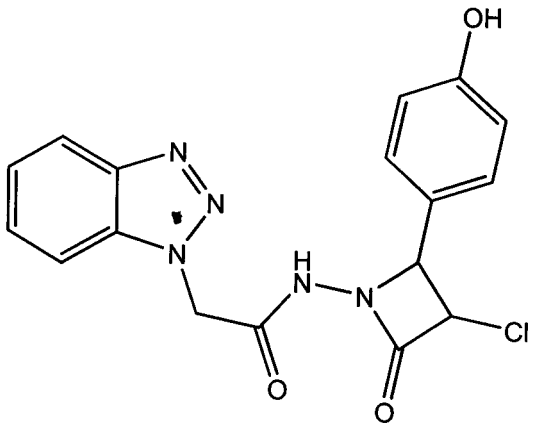
2. 3 Review Of Benzotriazole

Bin Qu et al. (2008) were synthesized bioactive compound, 3-(1*H*-benzo [d] [1,2,3] triazol-1-yl)-1-(4-methoxyphenyl)-1-oxopropan-2-yl benzoate (BmOB) **(10)**, which is a novel benzotriazole derivative. BmOB displayed anti-proliferative effects on several human tumor cell lines. Human hepatocarcinoma BEL-7402 cell line was selected as a model to illustrate BmOB's inhibition effect and its potential mechanism, since it was the highest susceptible cell line to BmOB [45].



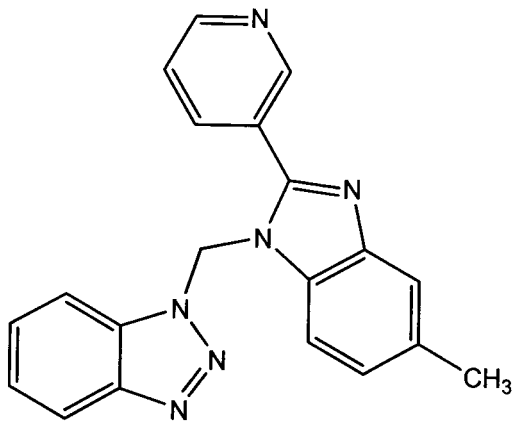
(10)

Mrunmayee P. Toraskar, (2005) was reported a compound 2-(1*H* benzotriazol-1-yl)-n'-(4-hydroxy phenylmethylidene) acetohydrazide **(11)** which exhibited moderate to good antifungal activity when tested in vitro against *C. albicans* [46].



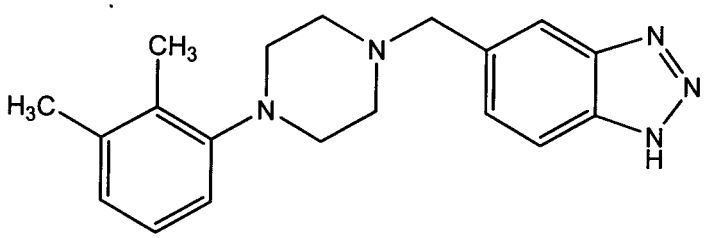
(11)

Ram Janam Singh (2009) was synthesized 1-{[(5-Methyl-2-(3-pyridyl)-1*H*-1, 3-benzimidazole-1-yl] methyl}-1*H*-1, 2, 3-benzotriazole **(12)** has been assayed for their antifungal activity against *P. oryzae, B. cinerea, A. nigar, C. albicans and T. Rubrum* and given good anti-fungal activity[47].



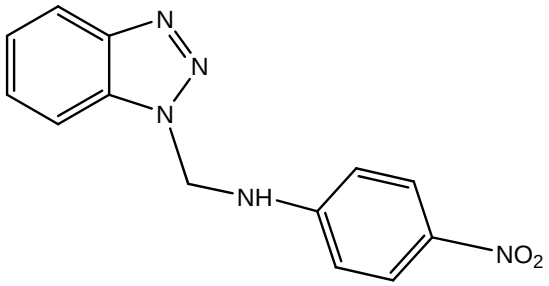
(12)

6-((4-(2,3-dimethylphenyl)piperazin-1-yl)methyl)-1*H*-benzo[d][1,2,3]triazole **(13)** was reported by Osama and Sameh (2009), to be potent atypical antipsychotic drug due to the specific structure of its heteroaryl group, that mimics catechol moiety of the dopamine[48].



(13)

S. S. Pawar (2010) has been discovered N-((1*H*-benzo[d][1,2,3]triazol-1-yl)methyl)-4-nitroaniline **(14)** which shows anthelmintic activity against Indian adult earthworms [49].



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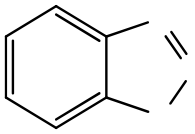
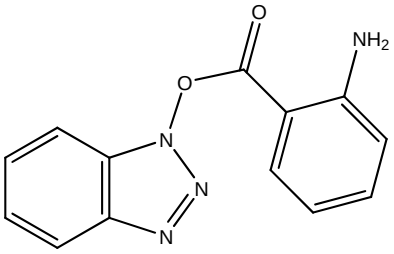
S M Tripathi and K P Singh (2008) have been reported that 1.5% benzotriazole **(15)** induced 100% pollen sterility in *H. annuus* [50].

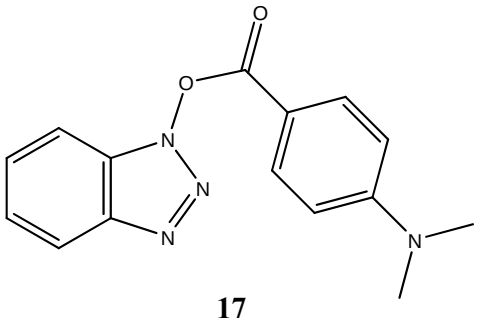


(15)

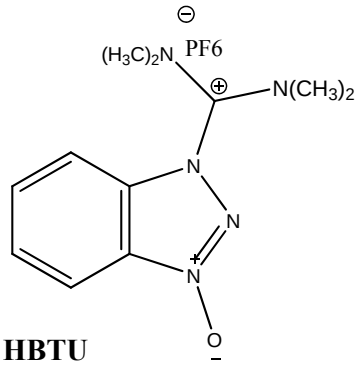
Severe acute respiratory syndrome (SARS) is caused by a newly emerged coronavirus that infected more than 8000 individuals and resulted in more than 800 fatalities in 2003. Currently, there is no effective treatment for this epidemic. SARS-3CLpro has been shown to be essential for replication and is thus a target for drug discovery. class of stable benzotriazole esters was reported as mechanism-based inactivators of 3CLpro, and the most potent inactivator exhibited a kinact of 0.0011 s21 and a Ki of 7.5 nM. It was done by Chung-Yi Wu *et al.* (1974) they were prepared a series of benzotriazole esters by condensation of HBTU with various carboxylic acids, and we found that the benzotriazole esters derived from benzoic acid containing electron-withdrawing substituents, e.g., NO_2, CN, and CF_3, were susceptible to hydrolysis, were susceptible to hydrolysis, whereas benzotriazole esters **16–18** and those with electron-donating groups were relatively stable in pH 5.0–8.0 over 24 hr at room temperature [51].



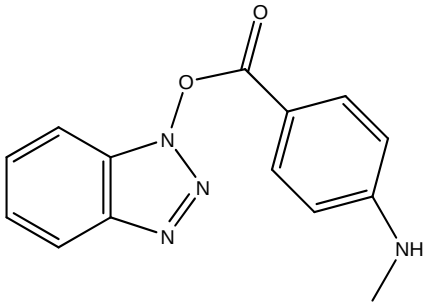
16



17



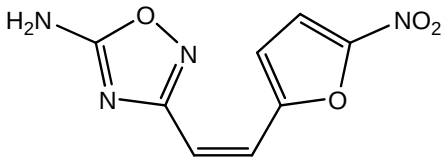
HBTU



18

2. 4 Review Of Oxadiazole

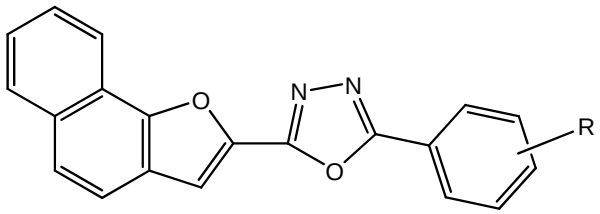
H. H. Gadebusch and H. I. Basch (1974) were reported a (Z)-3-(2-(5-nitrofuran-2-yl)vinyl)-1,2,4-oxadiazol-5-amine **(19)**, has shown microbial activity in vitro against a wide range of bacteria and fungi, and against several protozoa[52].



(19)

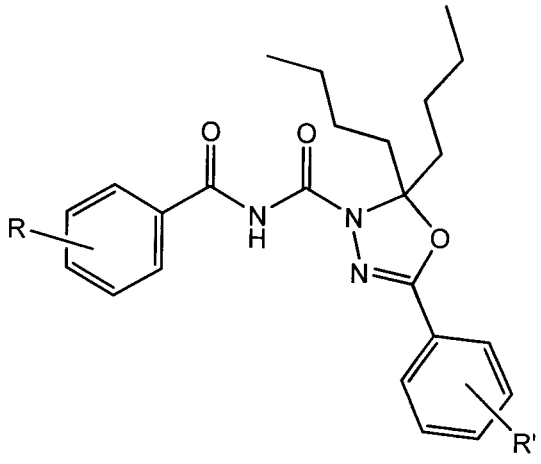
K C Ravindra et al (2006) have been synthesized a series of 1,3,4-oxadiazoles linked to naphtha [2,1-b] furan **(20)** which shown antimicrobial activity and anti-inflammatory activity[53].

Where R=4-Cl, 3-Cl, 2-OH



(20)

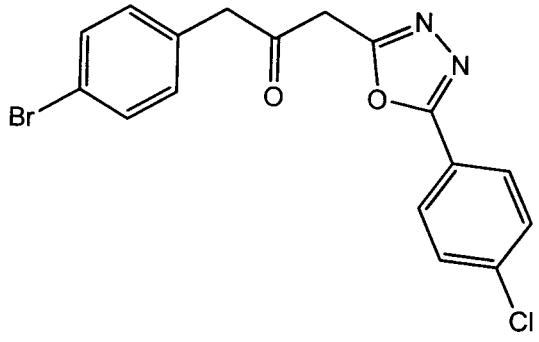
A new series of 1,3,4-oxadiazole-3(2*H*)-carboxamide **(21)** derivatives has reported by Shaoyong Ke, Zhong Li and Xuhong Qian (2008) direct heterocyclization reaction of substituted benzoylisocyanate with various aroylhydrazones as novel monoamine oxidase inhibitors (MAOIs). The preliminary results showed that most of the compounds have moderate inhibitory activities toward MAO at the concentration of 10^{-5} to10^{-3} M. This work may provide a novel class of lead compounds with potential MAO inhibitions for further optimization[54].



(21)

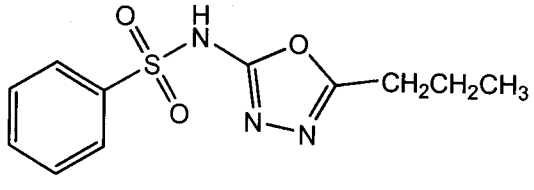
Where R, R'= Cl, F, CH_3, OCH_3

Asif Husain (2009) was reported 1-(4-bromophenyl)-3-(5-(4-chlorophenyl)-1,3,4-oxadiazol-2-yl)propan-2-one **(22)** which shows better analagesic activity , anti-inflammatory and anti bacterial activity among other derivatives synthesized[55].



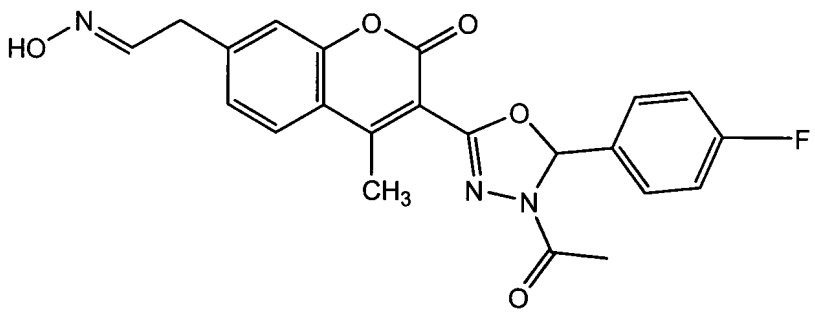
(22)

N-(5-propyl-1,3,4-oxadiazol-2-yl)benzenesulfonamide **(23)** was synthesized by Bernt Hokfelt (1962) which exhibited a powerful hypoglycemic activity in rats and rabbits following oral administration[56].



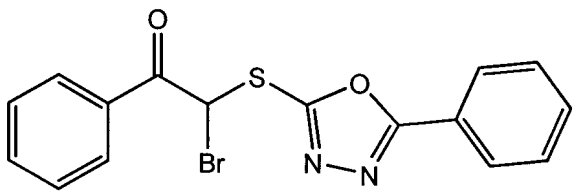
(23)

3-acetyl-2-(4-fluoro phenyl)-5-(4-methylcoumarinyl-7-oxymethyl)-2,3-dihydro-1,3,4-oxadiazole **(24)**, this compound showed promising antioxidant activity in vitro and cytotoxic activity against DLA cells and EAC cells[57]. It was synthesized by Parameswaran Manojkumar et al. (2009)



(24)

Macaev F et al. (2005) were synthesized novel 2-bromo-1-phenyl-2-(5-phenyl-1,3,4-oxadiazol-2-ylsulfanyl)-1-ethanone **(25)** and the computer-aided study of their in vitro anti-tubercular activity against *Mycobacterium tuberculosis* $H_{37}Rv$ (ATCC 27294) are reported[58]. The average accuracy of the electronic-topological method and neural network methods applied to the activity prediction in leave-one-out cross validation is 80%.



(25)

3.1 The Material Which Are Used For The Project Work Was Highlighting As Follows

Sr. No.	Reagents	Supplier company
1	o-Phenylenediamine	CDH, Mumbai
2	Acetic acid	CDH, Mumbai
3	Ammonia	SD Fine Chem Limited
4	Methanol	Merck Pvt. Ltd., Mumbai
5	Ethanol	Merck Pvt. Ltd., Mumbai
6	Benzaldehyde	Spectrochem Pvt. Ltd., Mumbai
7	2- Chloro benzaldehyde	Spectrochem Pvt. Ltd., Mumbai
8	3- Chloro benzaldehyde	Spectrochem Pvt. Ltd., Mumbai
9	4- Chloro benzaldehyde	Spectrochem Pvt. Ltd., Mumbai
10	4-Fluoro benzaldehyde	Spectrochem Pvt. Ltd., Mumbai
11	2,4-Dichloro benzaldehyde	Spectrochem Pvt. Ltd., Mumbai
12	4-Methoxy benzaldehyde	Spectrochem Pvt. Ltd., Mumbai
13	3,4-Dimethoxy benzaldehyde	Spectrochem Pvt. Ltd., Mumbai
14	N,N-Dimethyl amine benzaldehyde	Spectrochem Pvt. Ltd., Mumbai
15	4-Hydroxy benzaldehyde	Spectrochem Pvt. Ltd., Mumbai
16	Sodium nitrite	CDH, Mumbai
17	Amonium acetate	CDH, Mumbai
18	Hydrazine hydrate	CDH, Mumbai
19	Anhydrous potassium carbonate	CDH, Mumbai
20	Ethyl chloro acetate	Merck Pvt. Ltd., Mumbai
21	Acetone	Merck Pvt. Ltd., Mumbai
22	Hexen	Merck Pvt. Ltd., Mumbai
21	Chloroform	Merck Pvt. Ltd., Mumbai
22	Ethyl acetae	Merck Pvt. Ltd., Mumbai

3.2 List of instruments used in practical work.

1. Electronic balance (Dhona Bal.)

   Sensitivity upto 10mg.

2. Hot air oven (EIE Ltd, 230V)

   Temperature range from 0^{o}C-200^{o}C.

3. U. V. Chamber (EIE Ltd)

   U.V. chamber was used for detection of organic compound spot in pre-coated TLC under range of 200nm-400nm.

4. IR spectrometer

   IR spectra were taken as KBr discs on a SHIMADZU spectrometer

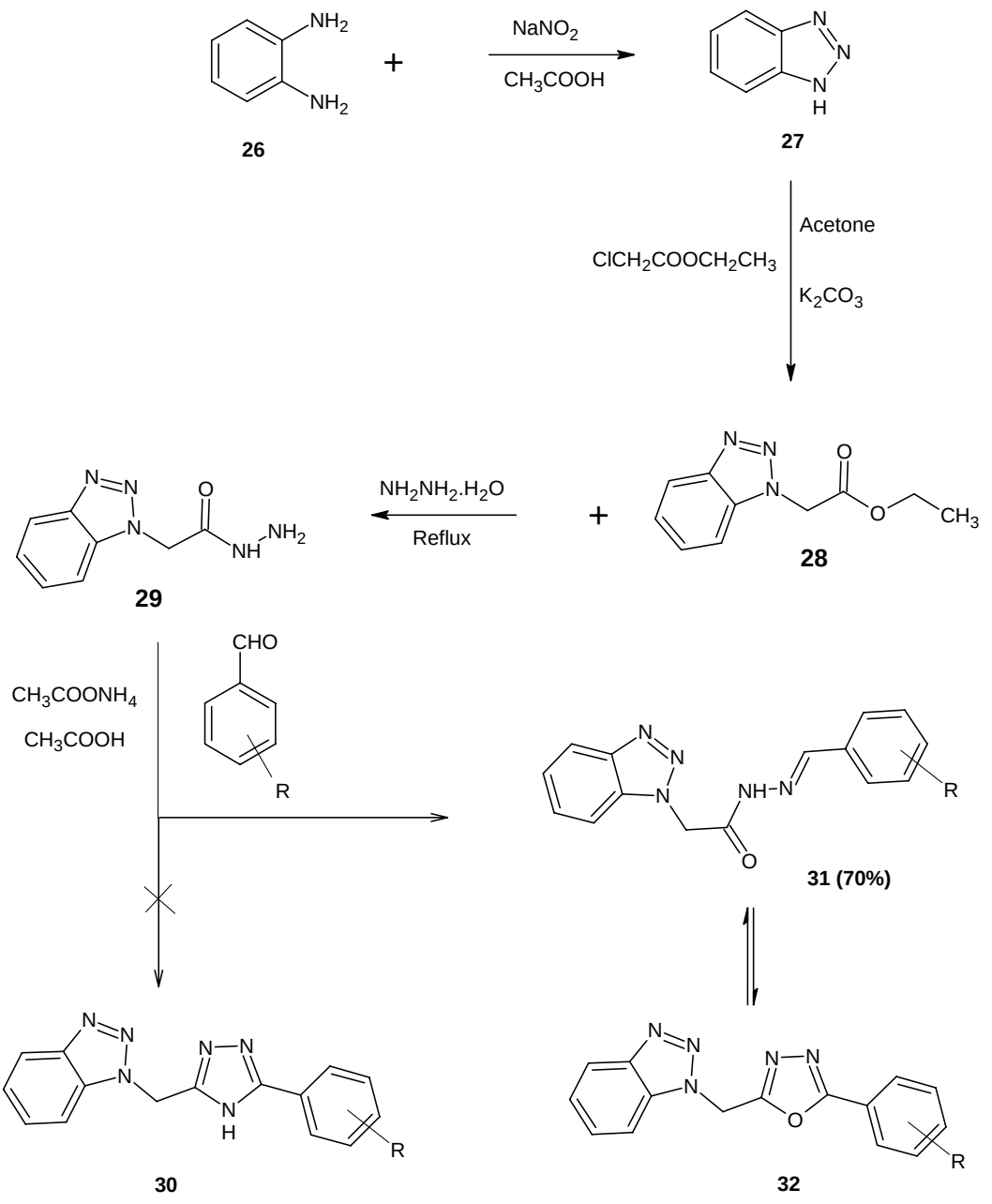
5. ^{1}H NMR spectra were obtained using a Brucker 300 MHz. Chemical shifts are recorded in ppm downfield from tetramethylsilane.

6. Melting point apparatus (Scientific, range- 50°C -300°C)

   Melting points were measured with a hot-stage apparatus Scientific and are uncorrected.

4. 1 Scheme- I: Synthesis of 1-{[5'-substituted phenyl-1,3,4-oxadiazol-2-yl]methyl}-1H-benzotriazole

Scheme - I



Where R=H, 2-Cl, 3-Cl, 4-CI, 4-F, 4-OCH_3, 3, 4-OCH_3, 4-OH, 4-N $(CH_3)_2$

4. 2 Experimental procedure for the synthesis of Benzotriazole (27) [59].

Dissolve 10.8g of *o*-phenylenediamine **(26)** in mixture of 12g (11.5ml) of glacial acetic acid and 30ml of water contained in a 250ml beaker; slight warming may be necessary. Cool the clear solution to 15^{o}C, stir magnetically and then add a solution of 7.5g of sodium nitrite in 15ml of water in one portion. The temperature of about 85^{o}C and then begins to cool while the colour changes from deep red to pale brown. Continue stirring for 15 minutes, by which time the temperature will have dropped to 35-40^{o}C, and then thoroughly chill in an ice-water bath for 30 mins. Collect by vaccum filtration the pale brown solid which separates and wash with three 30ml portion of ice-cold water. Dissolved the solid in 130ml of boiling water, add decolourising charcoal, filter and allow the filtrate to cool to about 50^{o}C before adding a few crystals of the crude benzotriazole **(27)** which have been retained for seeding. Allow the mixture to retain room temperature slowly (to avoid separation of the material as an oil) and then thoroughly chill in ice and collect the benzotriazole **(27)** which separates as pale straw coloured needles.

Yield is 69%. Melting point is 100 ^{o}C

Solvent system used for TLC **:** Chloroform **:** Methanol (4.5:0.5)

4.3 Experimental procedure for the synthesis of Ethyl-1*H*-benzotriazol-1-acetate[60] (28)

A mixture of Benzotriazole **(27)** (0.01 mole), ethyl chloro acetate (0.01 mole) and potassium carbonate 3gm in acetone 60ml was stirred for 6 hours. The solvent was removal under reduced pressure and the solid mass so obtained was extracted with ether (diethyl ether). The ether was removed under reduced pressure to get needle shaped Brown crystals.

Yield is 69%. Melting Point is 60 ^{o}C

Solvent system used for TLC : Hexen : Ethyl Acetate (3:2)

4.4 Experimental procedure for the synthesis of 2-(1*H*-benzotriazol-1-yl) acetohydrazide[61] (29)

To a solution of ethyl 1*H*-benzotriazole-1-acetate **(28)** was made in ethanol (15 ml) hydrazine hydrate (99%) was added and the reaction mixture was heated under reflux for 18-24 hours. The solution was concentrated and residue obtained thereof was added to ice-cold water and titled product 2-(1*H*-benzotriazol-1-yl) acetohydrazide **(30)** was isolated. Recrystallized from ethanol.

Yield is 50%. Melting point is 182^{o}C

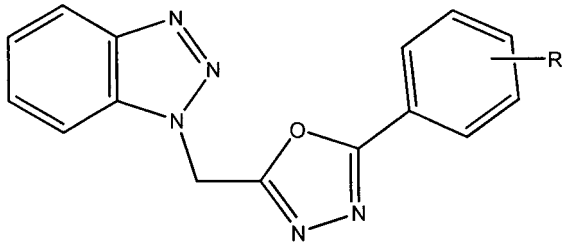
Solvent system used for TLC: Chloroform:Methanol (4.5:0.5)

4.5 Experimental procedure for the synthesis of 1-{[5'-substituted phenyl-1,3,4-oxadiazol-2-yl]methyl}-1*H*-benzotriazole[62] (31)

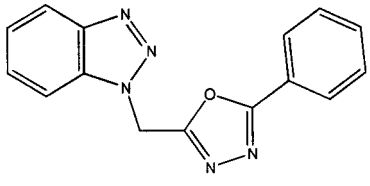
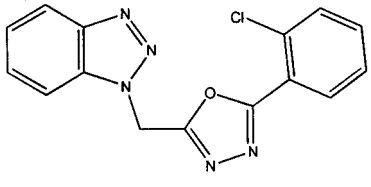
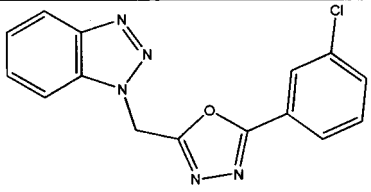
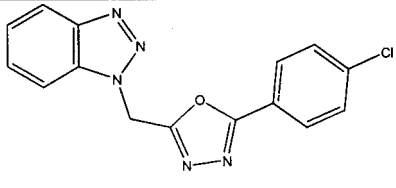
To solution of 2-(1*H*-benzotriazol-1-yl) acetohydrazide **(29)** (1.0g) in acetic acid (10ml) was added a pinch of ammonium acetate followed by the addition of different derivatives of benzaldehyde (0.52 ml) and the mixture was stirred for 24 hr at room temperature. The solution was them neutralized with liquid ammonia solution and the product **(30)** obtained was filtered washed with water and recrystallized from methanol. Reaction was monitored using pre-coated TLC. Also column was performed. But actual product obtained compound **(31)**. Because of product **(30)** is not stable and it converted into **(31).**

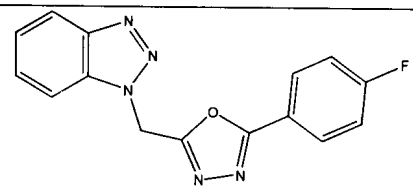
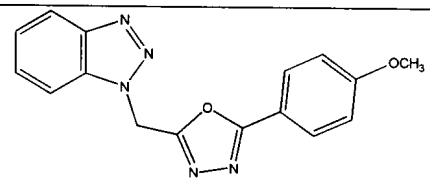
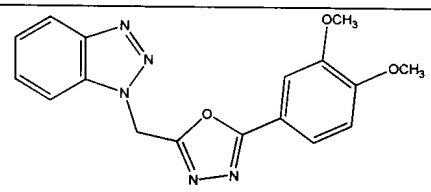
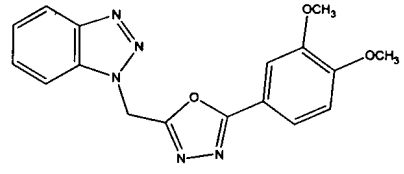
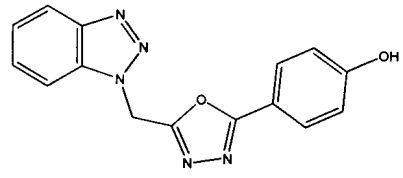
Solvent system used for TLC: Hexen : Ethyl Acetate (3:2)

4.6 Table 2. Physical data of synthesized compounds



General Structure Of Final Compound

Compound Code No.	R	Structure	Solvent for recrystallization	% Yield	R_f-Value	Melting Point (°C)
DS-1	1-H		Methanol	39.79	0.40	180
DS-2	2-Cl		Acetone	40.62	0.66	200
DS-3	3-Cl		Acetone	30.62	0.53	222
DS-4	4-Cl		Acetone	51.09	0.50	226

DS-5	4-F		Methanol	50.09	0.66	215
DS-6	4-OCH_3		Methanol	61.09	0.36	200
DS-7	4-$N(CH_3)_2$		Methanol	55.27	0.32	195
DS-8	3,4-di-OCH_3		Methanol	61.09	0.61	215
DS-9	4-OH		Methanol	40.09	0.60	200

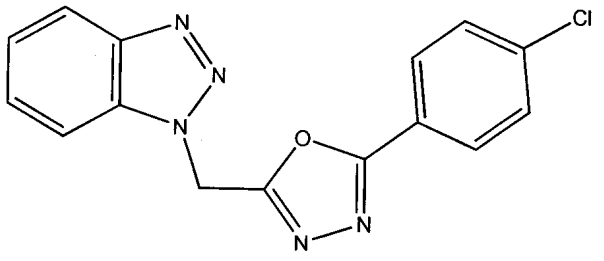
4.6.1 Solvent system used for TLC:

1. Chloroform : Methanol (4.5:0.5)
2. Hexen : Ethyl Acetate (3:2)
3. Toluene: Acetonitrile (3:2)

4.7 Spectral Data Graph Presentation

We have synthesized a series of nine compounds as illustrated in Scheme mentioned above chapter 5. Structures of all the compounds were established on the basis of analysis IR, ^{1}H NMR and Mass spectral data.

4.8.1 IR Spectra interpretation of DS-4

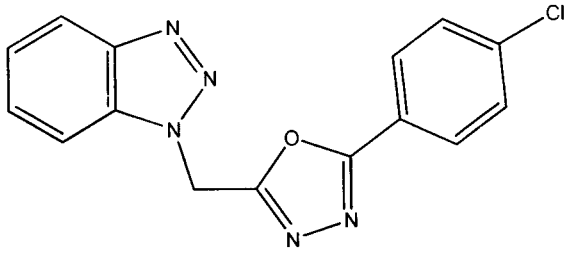


1-{[5-(4-chlorophenyl)-1,3,4-oxadiazol-2-yl]methyl}-1*H*-benzotriazole

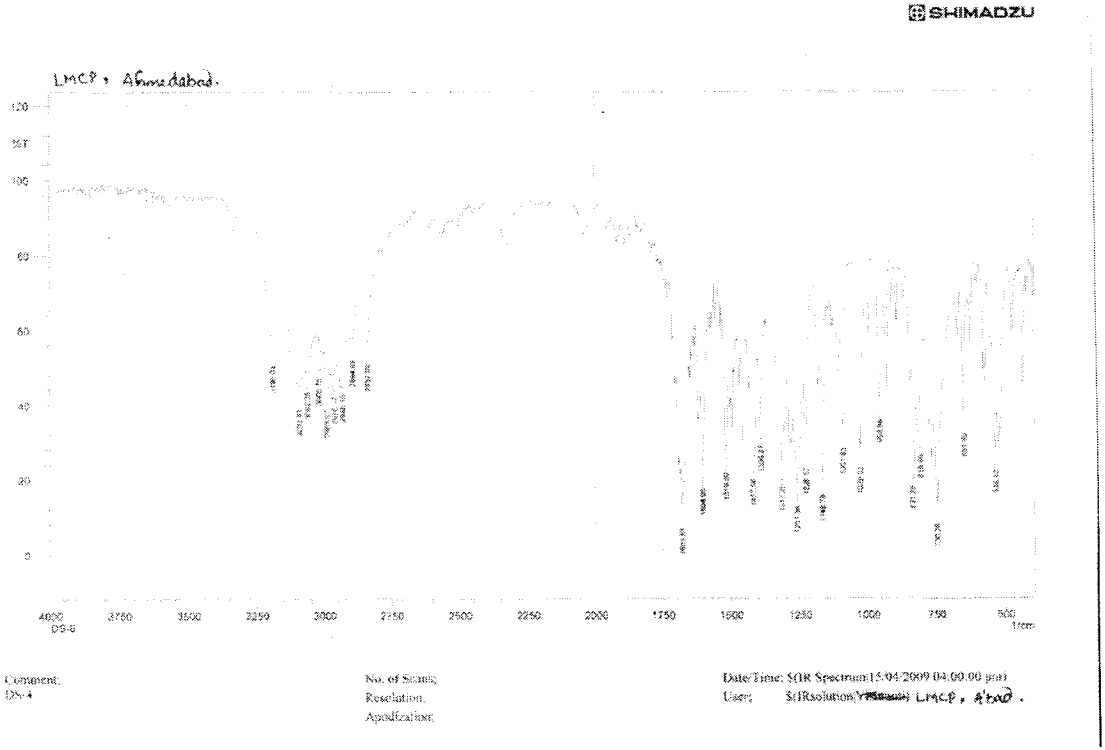
4.8.1 Table - 3 IR Spectra interpretation of DS-4

Functional Group	Expected Frequency	Acquired Frequency
-C-H (sp^2 stretch)	>3000 cm^{-1}	3097.47 cm^{-1}
-C-H (sp^3 stretch)	<3000 cm^{-1}	2985.60 cm^{-1}
-C-N (stretch)	1350-1280 cm^{-1}	1226.64 cm^{-1}
-C=N (stretch)	1690-1640 cm^{-1}	1681.61 cm^{-1}
-N-CH_2	1200-1275 cm^{-1}	1226.64 cm^{-1}
-C-O-C	1120-1180 cm^{-1}	1170.71 cm^{-1}
C-H out of plane bending	1300-1000 cm^{-1}	1386.72 cm^{-1}
-C-Cl	850-550 cm^{-1}	750.0 cm^{-1}

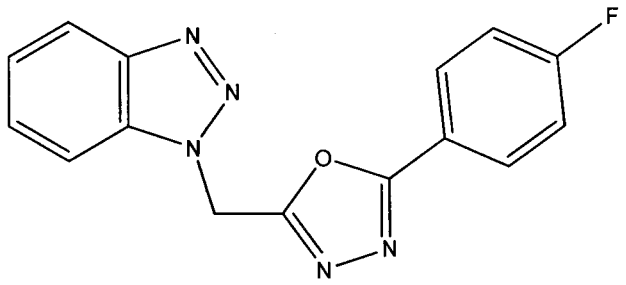
4.8.2 Figure-2 IR Spectra of DS-4



1-{[5-(4-chlorophenyl)-1,3,4-oxadiazol-2-yl]methyl}-1*H*-benzotriazole



4.8.3 IR. Spectra interpretation of DS-5

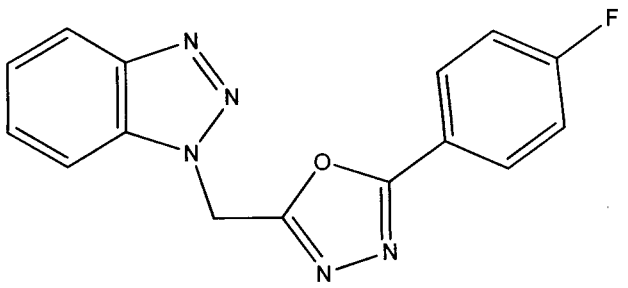


1-{[5-(4-fluorophenyl)-4*H*-1,2,4-triazol-3-yl]methyl}-1*H*-benzotriazole

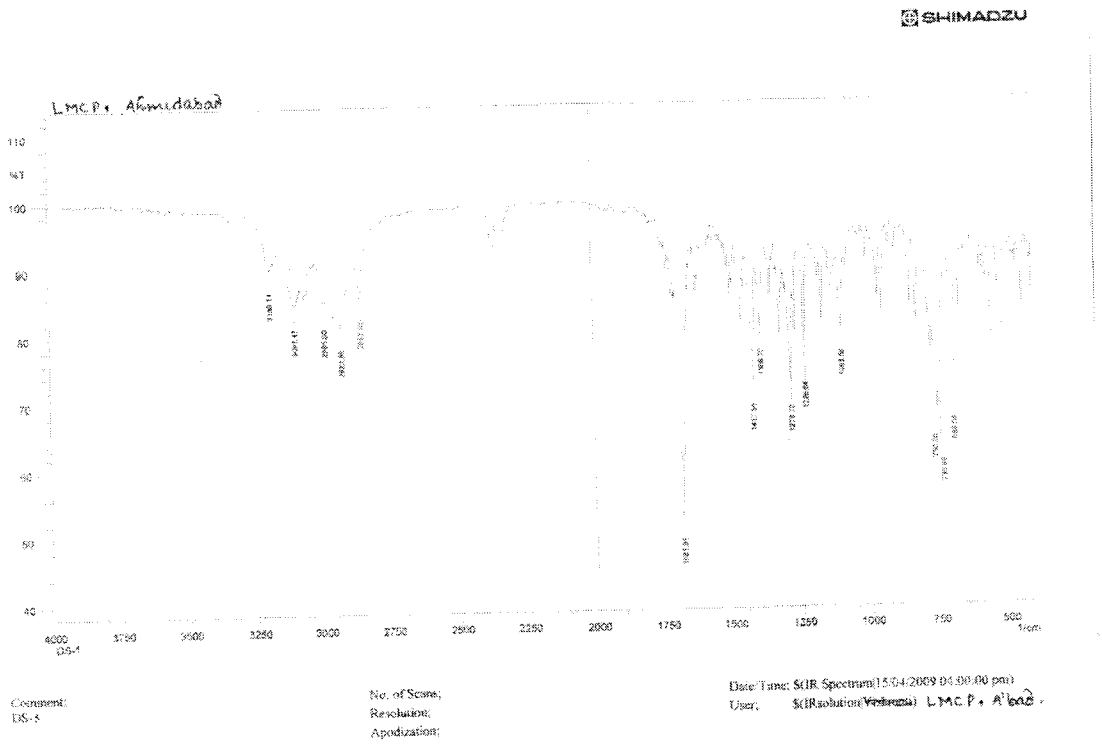
4.8.4 Table 4 IR. Spectra data of DS-5

Functional Group	Expected Frequency	Acquired Frequency
-C-H (sp^2 stretch)	>3000 cm^{-1}	3064.68 cm^{-1}
-C-H (sp^3 stretch)	<3000 cm^{-1}	2956.67 cm^{-1}
-C-N (stretch)	1350-1280 cm^{-1}	1313.43 cm^{-1}
-C=N (stretch)	1690-1640 cm^{-1}	1683.74 cm^{-1}
-N-CH_2	1200-1275 cm^{-1}	1232.43 cm^{-1}
-C-O-C	1120-1180 cm^{-1}	1168.78 cm^{-1}
C-H out of plane bending	1300-1000 cm^{-1}	1313.43 cm^{-1}
-C-F	1350-1100 cm^{-1}	1150 cm^{-1}

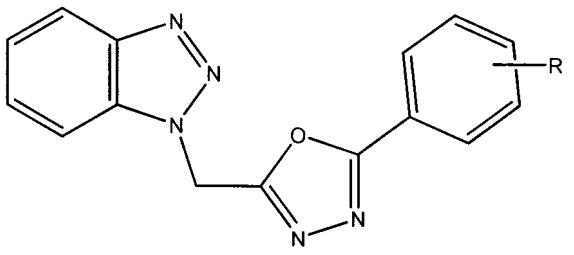
4.8.5 Figure-3 IR Spectra of DS-5



1-{[5-(4-fluorophenyl)-4*H*-1,2,4-triazol-3-yl]methyl}-1*H*-benzotriazole



4.8.6 IR Spectra data of compounds (DS-1 to DS-9)



General Structure Of Final Compound

4.8.7 Table 5 IR. Spectra data of all synthesized compounds

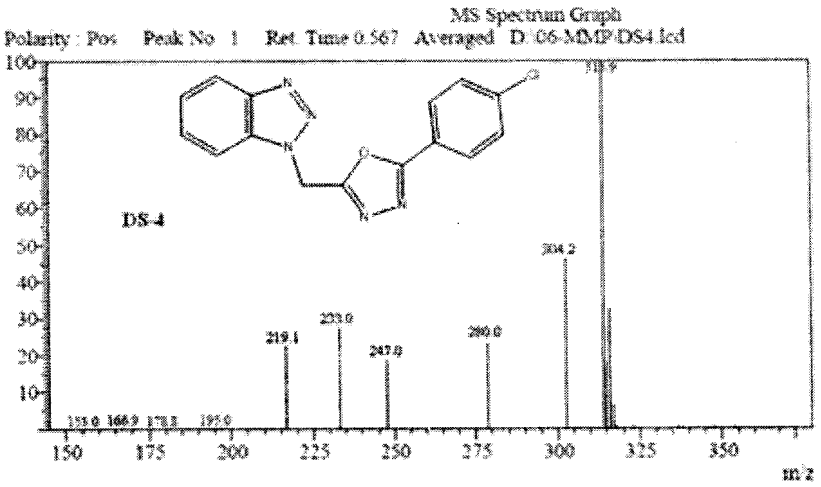
Compound code No.	**Functional Group**	**Expected Frequency**	**Acquired Frequency**
DS-1	-C-H (sp^2 stretch)	>3000 cm^{-1}	3064.68 cm^{-1}
	-C-H (sp^3 stretch)	<3000 cm^{-1}	2956.67 cm^{-1}
	-C-N (stretch)	1350-1280 cm^{-1}	1313.43 cm^{-1}
	-C=N (stretch)	1690-1640 cm^{-1}	1683.74 cm^{-1}
	$-N-CH_2$	1200-1275 cm^{-1}	1232.43 cm^{-1}
	-C-O-C-	1120-1180 cm^{-1}	1158.78 cm^{-1}
	C-H out of plane bending	1300-1000 cm^{-1}	1313.43 cm^{-1}
DS-2	-C-H (sp^2 stretch)	>3000 cm^{-1}	3064.68 cm^{-1}
	-C-H (sp^3 stretch)	<3000 cm^{-1}	2956.67 cm^{-1}
	-C-N (stretch)	1350-1280 cm^{-1}	1313.43 cm^{-1}
	-C=N (stretch)	1690-1640 cm^{-1}	1683.74 cm^{-1}
	$-N-CH_2$	1200-1275 cm^{-1}	1232.43 cm^{-1}
	-C-O-C-	1120-1180 cm^{-1}	1110.98 cm^{-1}

DS-3	-C-H (sp^2 stretch)	>3000 cm^{-1}	3064.68 cm^{-1}
	-C-H (sp^3 stretch)	<3000 cm^{-1}	2954.74 cm^{-1}
	-C-N (stretch)	1350-1280 cm^{-1}	1340.13 cm^{-1}
	-C=N (stretch)	1690-1640 cm^{-1}	1683 cm^{-1}
	-N-CH_2	1200-1275 cm^{-1}	1294.15 cm^{-1}
	-C-O-C-	1120-1180 cm^{-1}	1160.78 cm^{-1}
DS-4	-C-H (sp^2 stretch)	>3000 cm^{-1}	3068.53 cm^{-1}
	-C-H (sp^3 stretch)	<3000 cm^{-1}	2964.39 cm^{-1}
	-C-N (stretch)	1350-1280 cm^{-1}	1338.51 cm^{-1}
	-C=N (stretch)	1690-1640 cm^{-1}	1679.88 cm^{-1}
	-N-CH_2	1200-1275 cm^{-1}	1288.36 cm^{-1}
	-C-O-C-	1120-1180 cm^{-1}	1152.78 cm^{-1}
DS-5	-C-H (sp^2 stretch)	>3000 cm^{-1}	3087.76 cm^{-1}
	-C-H (sp^3 stretch)	<3000 cm^{-1}	2978.98 cm^{-1}
	-C-N (stretch)	1350-1280 cm^{-1}	1376.98 cm^{-1}
	-C=N (stretch)	1690-1640 cm^{-1}	1679.88 cm^{-1}
	-N-CH_2	1200-1275 cm^{-1}	1230.50 cm^{-1}
	-C-O-C-	1120-1180 cm^{-1}	1130.78 cm^{-1}
DS-6	C-H out of plane bending	1300-1000 cm^{-1}	1313.43 cm^{-1}
	-C-H (sp^2 stretch)	>3000 cm^{-1}	3093.61 cm^{-1}
	-C-H (sp^3 stretch)	<3000 cm^{-1}	2983.67 cm^{-1}
	-C-N (stretch)	1350-1280 cm^{-1}	1317.29 cm^{-1}
	-C=N (stretch)	1690-1640 cm^{-1}	1681.81 cm^{-1}
	-N-CH_2	1200-1275 cm^{-1}	1228.57 cm^{-1}
	-C-O-C-	1120-1180 cm^{-1}	1158.8 cm^{-1}
	-C-H (sp^2 stretch)	>3000 cm^{-1}	3043.65 cm^{-1}

DS-7	-C-H (sp^3 stretch)	<3000 cm^{-1}	2900.00 cm^{-1}
	-C-N (stretch)	1350-1280 cm^{-1}	1261.36 cm^{-1}
	-C=N (stretch)	1690-1640 cm^{-1}	1668.31 cm^{-1}
	-N-CH_2	1200-1275 cm^{-1}	1261.36 cm^{-1}
	-C-O-C-	1120-1180 cm^{-1}	1155.78 cm^{-1}
DS-8	-C-H (sp^2 stretch)	>3000 cm^{-1}	3020.21 cm^{-1}
	-C-H (sp^3 stretch)	<3000 cm^{-1}	2833.24 cm^{-1}
	-N-N (stretch)	1350-1280 cm^{-1}	1338.51 cm^{-1}
	-C=N (stretch)	1690-1640 cm^{-1}	1690.24 cm^{-1}
	-N-CH_2	1200-1275 cm^{-1}	1267.14 cm^{-1}
	-C-O-C-	1120-1180 cm^{-1}	1161.78 cm^{-1}
DS-9	-C-H (sp^2 stretch)	>3000 cm^{-1}	3097.47 cm^{-1}
	-C-H (sp^3 stretch)	<3000 cm^{-1}	2956.12 cm^{-1}
	-C-N (stretch)	1350-1280 cm^{-1}	1278.72 cm^{-1}
	-C=N (stretch)	1690-1640 cm^{-1}	1681.81 cm^{-1}
	-N-CH_2	1200-1275 cm^{-1}	1245.93 cm^{-1}
	-C-O-C-	1120-1180 cm^{-1}	1139.48 cm^{-1}

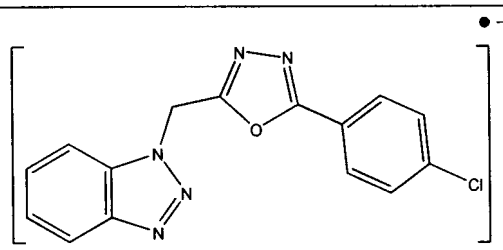
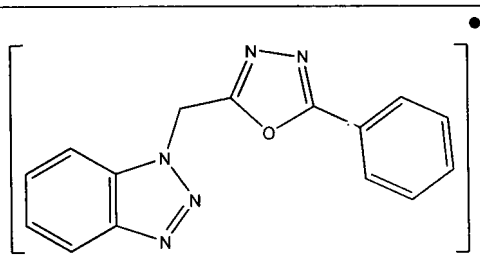
4.9 Mass spectra analysis

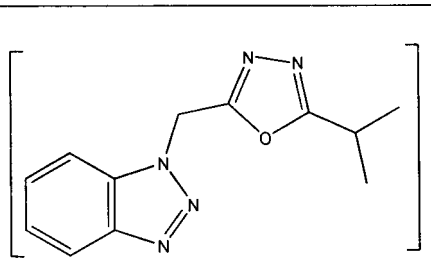
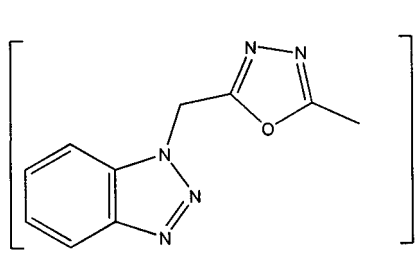
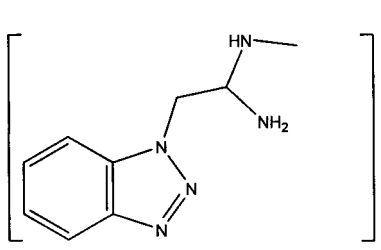
4.9.1 Figure-4: Mass spectral of compound DS-4



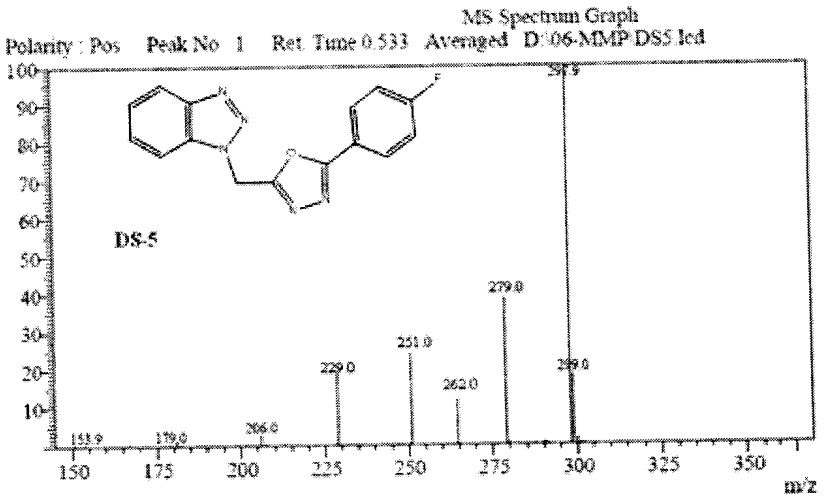
Mass (ESI-MS): m/z = 313.9 Da. (M^+, 100 %), 315.9 Da. (M+2, 35%)

4.9.2 Table-6 Mass Spectra Interpretation of DS-4

Sl. No	Fragmentation	Mol. Wt	Mol. Formula	m/z	%
1.		311.7	$C_{15}H_{11}ClN_5O$	313.9	100%
2.		277	$C_{15}H_{12}N_5O$	280.0	29%

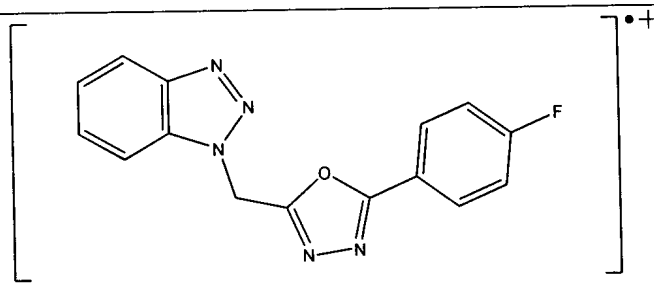
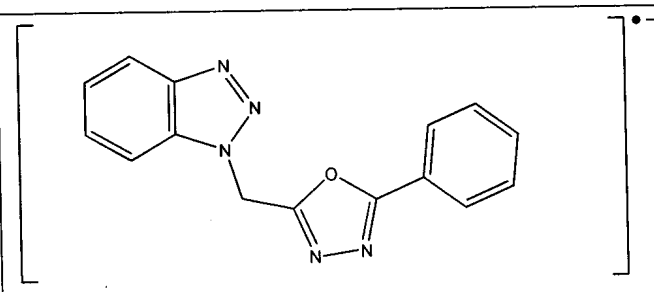
3.		243	$C_{12}H_{14}N_5O$	247.0	21%
4.		215	$C_{14}H_{21}N_5O$	219	25%
5.		191	$C_{13}H_{19}N_5$	195	9%

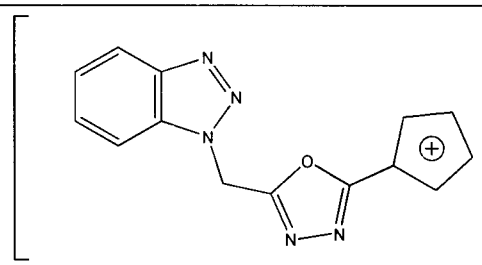
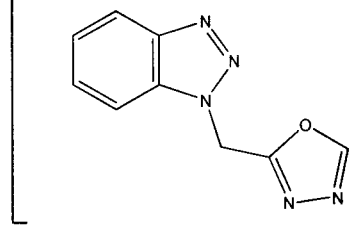
4.9.3 Figure-5 MASS spectra of DS-5



Mass (ESI-MS): m/z = 297.9 Da (M+, 100 %).

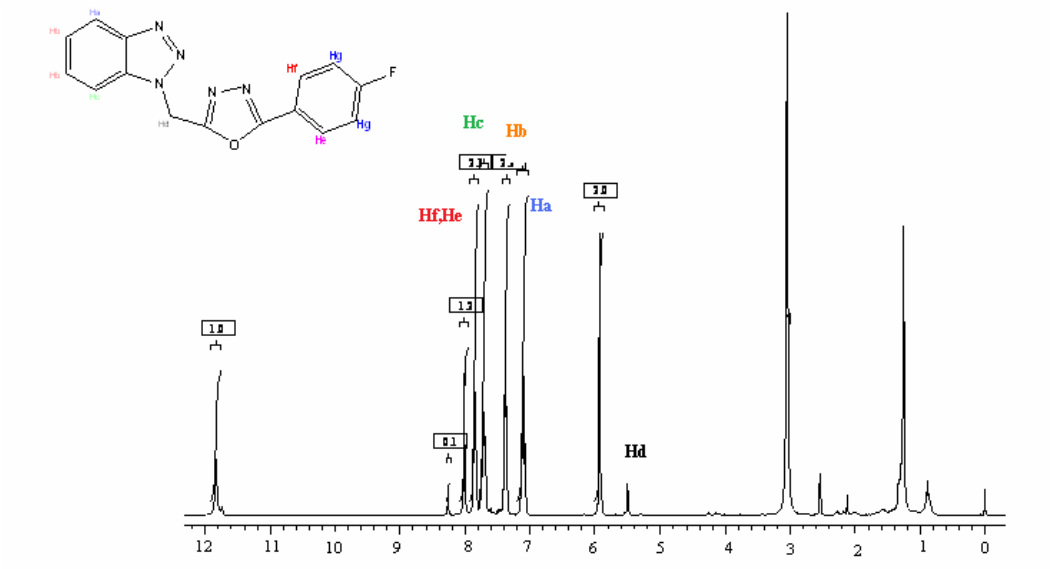
4.9.3 Table-6 Mass fragmentation interpretation of DS-5

Sl. No.	Fragmentation	Mol. Wt.	Mol. Formula	m/z	%
1.		295.2	$C_{15}H_{10}FN_5O$	297	100%
2.		277	$C_{15}H_{11}N_5O$	279	45%

3.		269	$C_{14}H_{15}N_5O$	262	18%
4.		201	$C_9H_7N_5O$	209	9%

4.10 ^{1}H NMR Spectra Data Of DS-5

4.10.1 Figure -6 ^{1}H NMR spectra of DS-5



4.10.2 Table-7 ^{1}H NMR Data Of DS-5 Compound

Sl. No.	δ value	Types of proton	Position of proton
1	7.2-7.9	(s, 4H, Ar-H)	Benzotriazole contain phenyl
2	4.9-5.9	(s, 2H)	N-CH_2
3	7.3-8.3	(s, 4H, Ar-H)	Phenyl Proton

5.1 Introduction

Numerous factors have contributed to the increase in fungal infections - most notably, increasing numbers of immunosuppressed cases e.g. AIDS, cancer or diabetes, the use of broad spectrum antibiotics, cytotoxic chemotherapy, and organ transplantation. The increasing incidence of opportunistic severe fungal infections has greatly enhanced the interest in novel antifungal agents[63]. According to literature review benzotriazole and oxadiazole moieties have antifungal activity. In present work, 1-{[5-substituted phenyl-1,3,4-oxadiazol-2-yl]methyl}-1*H*-benzotriazole derivatives were synthesized. And *in vitro* antifungal susceptibility testing performed for these synthesized derivatives.

5.2 Anti-Fungal Susceptibility testing by diffusion Method

5.2.1 Kirby-Bauer Disk Diffusion Method

In this method the cultures are streaked onto the appropriate agar media using a cotton swab and incubated for 24 hours. The plates are dried for 3 to 5 minutes followed by the application of antimicrobial impregnated disks. The plates were incubated immediately for 24 hours at 30ºC. After incubation, the diameter of zone of inhibition is measured in millimeters across the disk [64].

5.2.2 Agar Well Diffusion Method

This is very simple method. In this method, microbial strain inoculated in agar plate. Well are bored in agar plate with sterile borer. Then antimicrobial agent is loaded in well. Plates are incubated for 24 hour at 30ºC. Antimicrobial activity is determined by calculating zone of inhibition around well[65].

5.3 Experimental protocol

In present work, the synthesized compounds were evaluated for *in vitro* antifungal activity using agar well diffusion methodology against several fungal strains using Rose Bengal agar (Himedia).

We preferred the in-vitro rather than in-vivo antifungal activity because of shortage of time. We also performed only the well diffusion method rather than agar and broth dilution method and other methods due to expenses.

5.3.1 Preparation of media

Agar medium was prepared according to direction provided by manufacturer. 31.55 g of rose bengal agar was suspended in 1000 ml distilled water. It was heated to boiling to dissolve the medium completely. It was sterilized by autoclaving at 15lbs pressure (121^0C) for 15 minutes. It was cooled to 45 ^{0}C. Mixed thoroughly and poured into sterile petriplates.

Composition of rose bengal agar medium is as follow:

Ingredients	**Quantity (g/litre)**
Peptic digest of soyabean meal	5.00
Dextrose	10.00
Monopotassium phosphate	1.00
Magnesium sulphate	0.50
Rose bengal	0.05

Final pH (at 25^oC) 7.2 ± 0.2

The fungal strains used were procured from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh. Fungal strains were as follow:

1. *Aspergillus niger* (MTCC3017)
2. *Candida albicans* (MTCC1344)
3. *Fusarium oxysporum* (MTCC1755)

5.3.2 Procedure

(A) Preparation of media

Agar medium was prepared according to direction provided by manufacturer. 31.55 g of rose bengal agar was suspended in 1000 ml distilled water. It was heated to boiling to dissolve the medium completely. It was sterilized by autoclaving at 15lbs pressure (121^0C) for 15 minutes. It was cooled to 45 ^{0}C. Mixed thoroughly and poured into sterile petriplates.

(B) Preparation of test and standard solutions

All the test compounds were freely soluble in dimethyl sulfoxide. The solutions of test compounds, of required concentrations 100µg/ml, 500 µg/ml and 1000µg/ml were prepared by dissolving compounds in dimethyl sulfoxide. Fluconazole was used as the standard drug in the form of injection (Forcan,Cipla). It was diluted with water for injection to obtain required concentration of standard (25µg/1ml).

(C) Determination of zone of inhibition

The rose bengal agar, 20ml was poured into sterile petridish, under aseptic condition. The wells were prepared on agar surface by sterile cork borer of 6mm diameter. The test compounds of different concentrations (100µg/1ml, 500 µg/1ml and 1000µg/1ml) were poured into the well with the help of micropipette and kept aside to allow the

solution to diffuse totally in the medium. The plates were incubated at 25^0C for 48 hours in Biological Oxygen Demand (BOD) incubator (EIW Instruments Pvt. Ltd.). The zone of inhibition was measured in millimeters on antibiotic zone reader (Hally Instrument). Fluconazole (25µg/1ml) was used as standard drug. Also, dimethyl sulfoxide was used as a control. All the experiments were performed in triplicates.

Antifungal activity of test compounds was recorded in terms of zone of inhibition (in millimeters) shown by each compound against various fungi species.

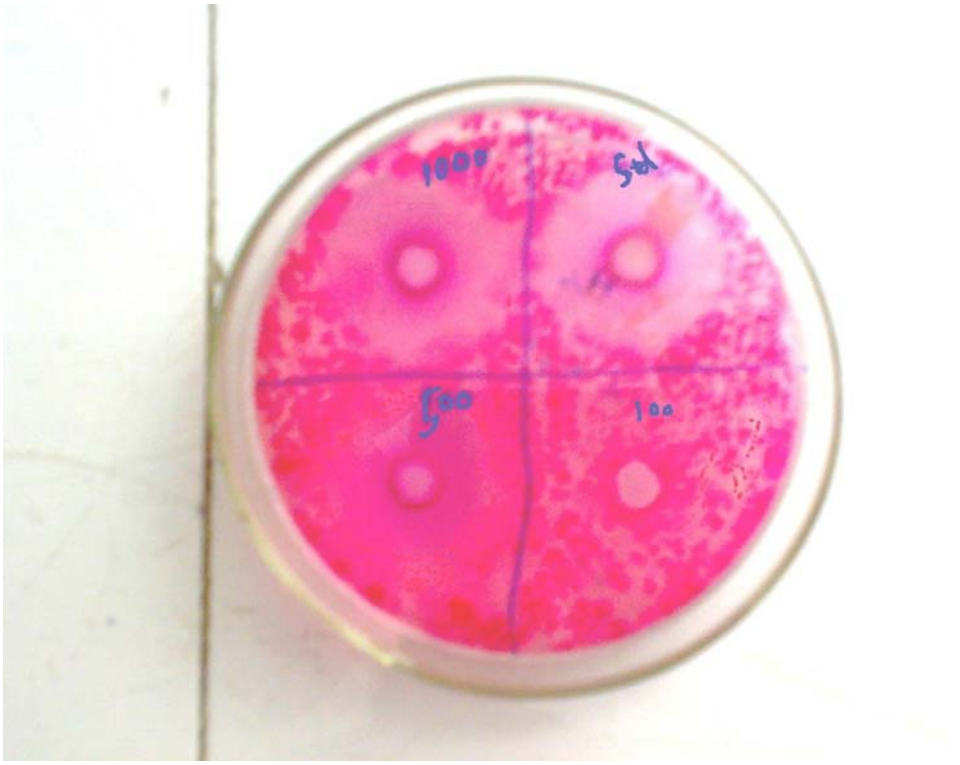


Figure 7.0 Zone of Inhibition of DS-5 against *F. oxyporum*

5.4 Observation Tables.

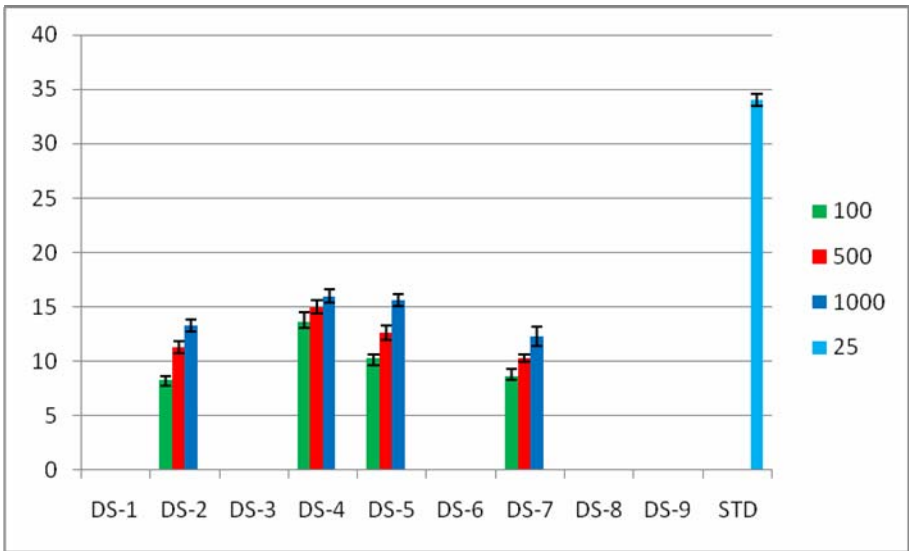
5.4.1 Table-8 Antifungal activity of synthesized compounds against *C. albican*

Compound code	Concentration (µg/1 ml)	Zone of Inhibition (mm)			
		E1*	E2*	E3*	Mean ± SEM
DS-1	100	0	0	0	0
	500	0	0	0	0
	1000	0	0	0	0
DS-2	100	8	8	9	8.33±0.333
	500	12	11	11	11.3± 0.57
	1000	14	13	13	13.3± 0.57
DS-3	100	0	0	0	0
	500	0	0	0	0
	1000	0	0	0	0
DS-4	100	12	14	15	13.66± 0.88
	500	14	16	15	15.0±0.578
	1000	15	16	17	16.0±0.57
DS-5	100	10	11	10	10.33±0.33
	500	12	12	14	12.66±0.66
	1000	16	16	15	15.6± 0.57
DS-6	100	0	0	0	0
	500	0	0	0	0
	1000	0	0	0	0
DS-7	100	8	8	10	8.6±066
	500	10	11	10	10.33±0.33
	1000	11	12	14	12.33±0.88
DS-8	100	0	0	0	0
	500	0	0	0	0
	1000	0	0	0	0
	100	0	0	0	0

DS-9	500	0	0	0	0
	1000	0	0	0	0
STD	25	33	34	35	34.0±0.578

* (E1, E2, E3 represents data of 1st, 2nd and 3rd experiment respectively, SD: Standard Deviation)

5.4.2 Figure-8 Zone of Inhibition (mm) in *C. albican*



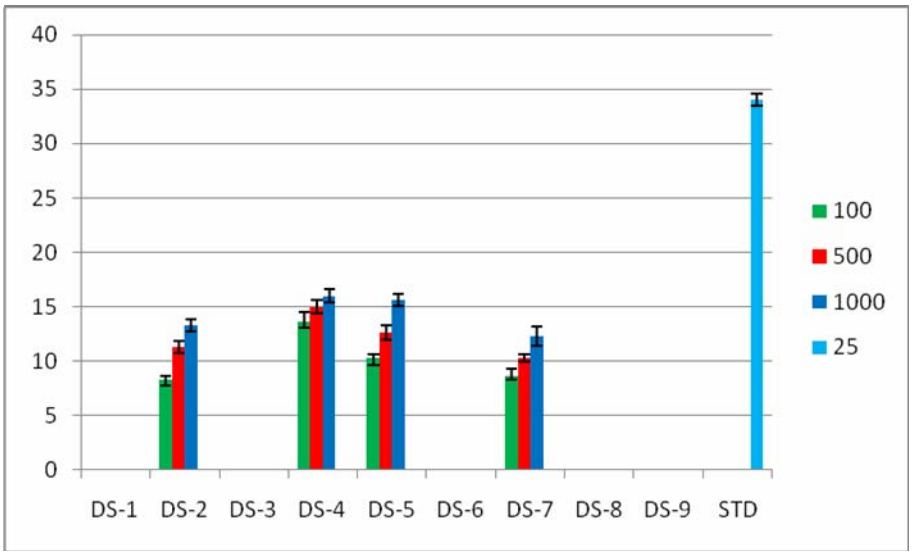
5.4.3 Table-9 Anti-fungal activity of synthesized compounds against *A. niger*

Compound code	Concentration (µg/1 ml)	Zone of Inhibition (mm)			
		E1*	E2*	E3*	Mean ± SEM
DS-1	100	0	0	0	0
	500	0	0	0	0
	1000	0	0	0	0
DS-2	100	8	10	11	9.66±0.878
	500	10	12	12.5	11.5±0.76

	1000	12	13	15	13.33±0.882
DS-3	100	0	0	0	0
	500	0	0	0	0
	1000	0	0	0	0
DS-4	100	12	12.5	13	12.5±0.289
	500	14	13.2	13.5	13.56±0.233
	1000	15	16.5	15.5	15.66±0.44
DS-5	100	16	16.5	16.8	16.43±0.23
	500	18.2	19.5	18.6	0.66± 0.38
	1000	20.1	20.9	21.5	20.83±0.405
DS-6	100	0	0	0	0
	500	0	0	0	0
	1000	0	0	0	0
DS-7	100	0	0	0	0
	500	0	0	0	0
	1000	0	0	0	0
DS-8	100	0	0	0	0
	500	8	8.5	9.4	8.6± 0.410
	1000	10.9	10.5	9.8	10.4± 0.32
DS-9	100	0	0	0	0
	500	0	0	0	0
	1000	0	0	0	0
STD	25	33	34	35	34.0±0.578

* (E1, E2, E3 represents data of 1st, 2nd and 3rd experiment respectively, SD: Standard Deviation)

5.4.4 Figure-9 Zone of Inhibition (mm) in *A. niger*



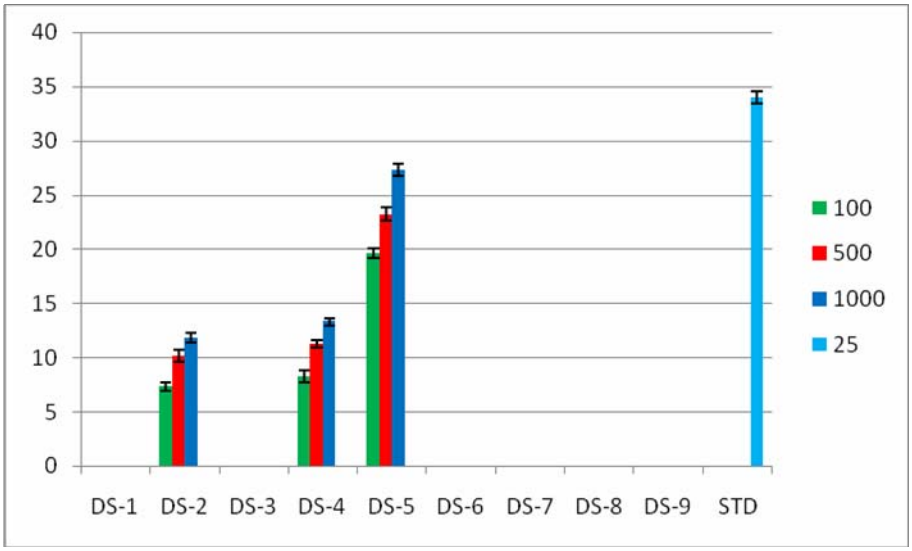
5.4.5 Table-10 Anti-fungal activity of synthesized compounds against *F. oxysporum*

Compound code	Concentration (µg/1 ml)	Zone of Inhibition (mm)			
		E1*	E2*	E3*	Mean ± SEM
DS-1	100	0	0	0	0
	500	0	0	0	0
	1000	0	0	0	0
DS-2	100	8	7	8.5	7.833±0.441
	500	11	10.5	9	10.16± 0.601
	1000	12	12.5	11	11.83±0.441
DS-3	100	0	0	0	0
	500	0	0	0	0
	1000	0	0	0	0
DS-4	100	08	09	08	08.3 ±0.57
	500	12	11	11	11.3± 0.33

	1000	14	13	13	13.33± 0.333
DS-5	100	20	19	19.5	19.666±0.441
	500	22	23.5	24.2	23.233±0.649
	1000	27	28	27	27.3 ±0.57
DS-6	100	0	0	0	0
	500	0	0	0	0
	1000	0	0	0	0
DS-7	100	0	0	0	0
	500	0	0	0	0
	1000	0	0	0	0
DS-8	100	0	0	0	0
	500	0	0	0	0
	1000	0	0	0	0
DS-9	100	0	0	0	0
	500	0	0	0	0
	1000	0	0	0	0
STD	25	33	34	35	34.0±0.578

* (E1, E2, E3 represents data of 1st, 2nd and 3rd experiment respectively, SD: Standard Deviation)

5.4.6 Figure-10 Zone of Inhibition (mm) in *F. oxyporum*



6.1 Results And Discussion

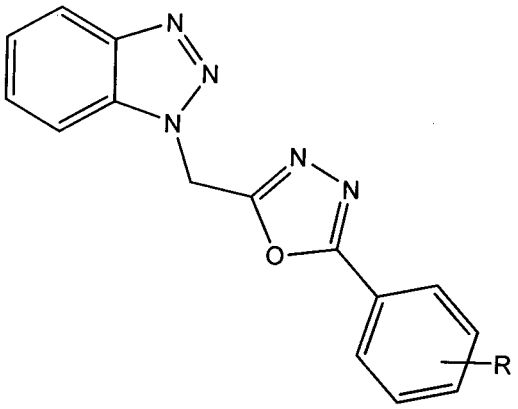
The synthetic scheme involved the synthesis of 1-{[5'-substituted phenyl-1,3,4-oxadiazol-2-yl]methyl}-1*H*-benzotriazole which consist of oxadiazole ring at 5-position. Nine compounds (**DS-1** to **DS-9**) were synthesized and confirmed the purity by TLC. All the synthesized compounds and their structures were identified on the basis of Fourier-transform infrared (FTIR) spectroscopy, ^{1}H nuclear magnetic resonance (NMR) and mass spectroscopy data. Compound, 1-{[5-(4-fluorophenyl)-1,3,4-oxadiazol-2-yl]methyl}-1*H*-benzotriazole (**DS-5**) was found light white colour solid, melting point was recorded at 215^{o}C. The purity of the compound (DS-5) was established by TLC (Rf =0.66) using mobile phase: ethyl acetate:hexen (4:6) The compound was distinguished from starting material and visualised by iodine vapour. Compound structure was established by strong IR peaks at 3041.74 (C-H stretch), 2980 [-C-H (sp^3 stretch)], 1589.90, 1232.51(N-CH_2 stretch), 1168.78 (-C-O-C), 1000.58(C-F stretch) in FTIR using KBr. Distinguished ^{1}H NMR peaks (ppm) were recorded at 7.2-7.9 (s, 4H, Ar-H) benzotriazole contain phenyl, 7.3-8.3 (s, 4H, Ar-H), 4.9-5.9 (s, 2H) N-CH_2.). In mass spectra sharper M+2 peak was found at 297 (m/z). Different fragments and important fragments peaks were recorded at 277, 269, 201 (m/z). All the spectral data and TLC assure that, compound (DS-5) was pure and formed. Compound, 1-{[5-(4-chlorophenyl)-1,3,4-oxadiazol-2-yl]methyl}-1*H*-benzotriazole (**DS-4**) was found light white colour solid, melting point was recorded at 226^{o}C. The purity of the compound (DS-4) was established by TLC (R_f =0.5) using mobile phase: Ethyl acetate:Hexen (3:7). The compound was distinguished from starting material and visualized by iodine vapor. Compound structure was established by strong IR peaks at 3097.47 (C-H, sp^2stretch), 2985.65 (-C-H, sp^3 stretch), 1589.90, 1226.64(N-CH_2 stretch), 1170.71 (-C-O-C), 750(-C-Cl stretch) in FTIR using KBr. In mass spectra sharper M+2 peak was found at 313 (m/z). Different fragments and important fragments peaks were recorded at 277, 243, 215, 191 (m/z). All the spectral data and TLC assure that, compound (DS-4) was pure and formed. Similar way we established other structures by IR, ^{1}H NMR and Mass data. The purity of the other compounds was checked on a silica gel-G plates and visualization under iodine/UV lamp. Physical and spectral data of the compounds are reported in chapter-4.

The synthesized compounds **DS-1** to **DS-9** were tested *in vitro* against three fungi species *Aspergillus niger* (MTCC3017), *Candida albicans* (MTCC1344) and *Fusarium*

oxysporum (MTCC1755) by using the cup-plate agar diffusion method against, and compared to their anti-fungal activity with fluconazole was used as the standard drug in the form of injection (Forcan, Cipla) under identical experimental conditions. All the biological results of the compounds are given in chapter 5. Rose Bengal media was prepared for fungal growth. Stock solutions of tested compounds were prepared in DMSO. Antifungal activity of test compounds was recorded in terms of zone of inhibition (in millimeters) shown by each compound against various fungi species. 5-substituted phenyl of 1,3,4-oxadiazole was produced distinguish anti-fungal activity.

5-substituted oxadiazole ring with phenyl containing *para* chlorine/fluorine (**DS-4** and **DS-5**) produced maximum antifungal activity against *Fusarium oxysporum* in table- . Substituted phenyl with oxadiazole ring, by 2-Cl, 3-Cl, 3, 4- di (OCH_3), were found less activity against all fungi. Overall pattern of activity behavior elicited by these compounds can be represented as follows: 4-F> 4-Cl> 2-Cl >3-Cl. Antifungal results indicated that some of the derivatives possessed a broad spectrum of activity against tested fungi. However, none of the derivatives showed a better spectrum of activity than the reference drug.

STRUCTURE ACTIVITY RELATIONSHIP

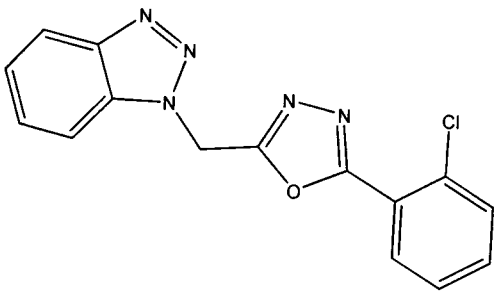


General structure of compound

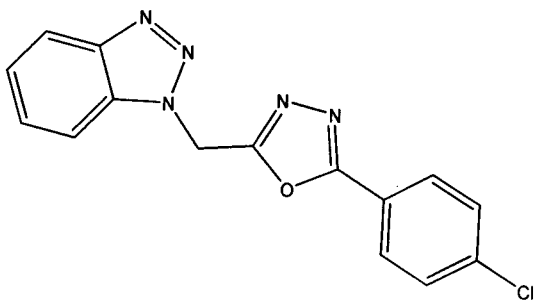
Where R=H, 2-Cl, 3-Cl, 4-CI, 4-F, 4-OCH_3, 3, 4-OCH_3, 4-OH, 4-N $(CH_3)_2$

Benzotriazole ring and 1,3,5-oxadiazole are mainly responsible for the antifungal activity because of presence of azoles and oxazole rings.

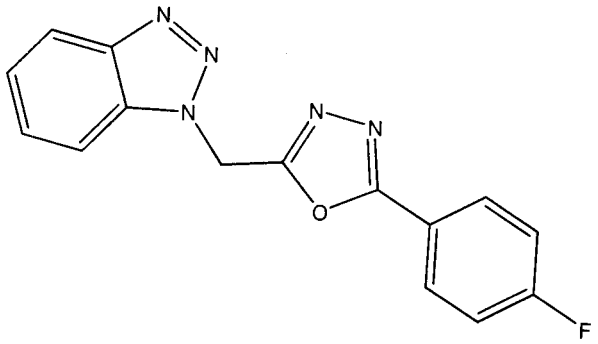
Hydrophobic substituted phenyl ring is attached at 2nd position of oxadiazole ring. The phenyl contains electron withdrawing group which gives better anti-fungal activity. Compounds are DS-2 (1-{[5-(2-chlorophenyl)-1,3,4-oxadiazol-2-yl]methyl}-1*H*-benzotriazole), DS-4 (1-{[5-(4-chlorophenyl)-1,3,4-oxadiazol-2-yl]methyl}-1*H*-benzotriazole), DS-5 (1-{[5-(4-chlorophenyl)-1,3,4-oxadiazol-2-yl]methyl}-1*H*-benzotriazole)show anti-fungal activity against *F. oxysporum* *C. albican* and *A. niger* (shown in biological activity table.). Substitution at *para* position of phenyl of oxadiazole is responsible good antifungal activity against *F. oxysporum* . Substitution at *meta* position of phenyl of oxadiazole (shows compound DS-3) not gives antifungal activity.



DS-2

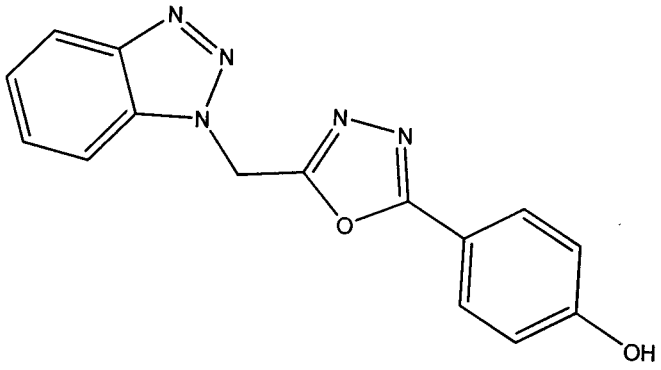


DS-4

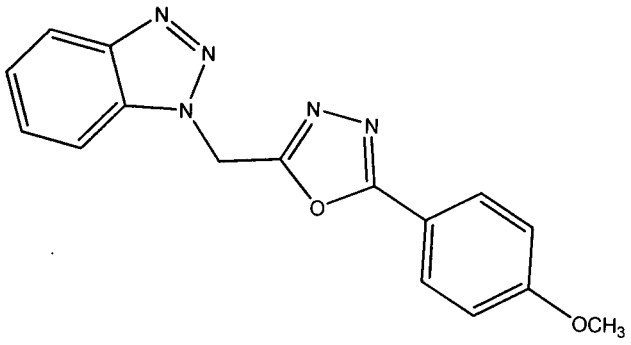


DS-5

But R position contains electron donating group like –OH, -OCH_3, -N, N-$(CH_3)_2$ which not shows anti-fungal activity. Except Compounds are **DS-6** (1-{[5-(4-methoxyphenyl)-1,3,4-oxadiazol-2-yl]methyl}-1*H*-benzotriazole), **DS-9** (4-(5-((1H-benzo[d][1,2,3]triazol-1-yl)methyl)-1,3,4-oxadiazol-2-yl)phenol) which shows antifungal activity lesser extent as compared to other derivatives due to substitution at *para* position of phenyl of oxadiazole.



DS-9



DS-6

Where electron withdrawing groups produced low yield where electron donating group produced high yield. (Show physical data chapter-4).

So, we concluded that 1,3,4-oxadiazole substituted by halogen group showing moderate good anti-fungal activity while compound substitute by electron donating groups not showing anti-fungal activity. It is better to choose halogen substitution rather than electron donating groups.

7.1 Summary And Conclusion

Azoles containing heterocyclic moiety benzotriazole connected with 5-substituted oxadiazole ring through methylene bridge in a single framework, produced good antifungal activity against different fungi. The structure activity relationship studies suggested that benzotriazole containing oxadiazole ring having promising antifungal activity, may be produce through fungi cell well penetration, which is proved by activity produced by hydrophobic analogues only. It can conclude that, benzotriazole containing oxadiazole ring and additional electron withdrawing substituted phenyl ring at 5 positions reflected better antifungal activity. In future more analogues will be synthesized and screen for *in vitro* and *in vivo* antifungal activity.

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