SYNTHESIS OF SOME SUBSTITUTED INDOLE ANALOGUES LINKED TO VARIOUS HETEROCYCLES AND THEIR BIOLOGICAL ACTIVITIES

PRABHAKER WALMIK

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DEDICATED TO MY BELOVED PARENTS
AND TEACHERS

Acknowledgement

Things that I have heard, things that I have seen, things that I have thought are my valuable experience. Things that I have suffered are my treasures. They will guide me to a certain conclusion. Here, I sincerely give my acknowledgements to those who helped me pursue my M. Phil.

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(***Prabhakar Walmik)***

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CHAPTER-1

INTRODUCTION

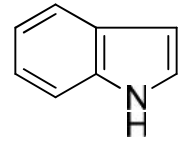
Indole and its derivatives have occupied a unique place in Chemistry of nitrogen heterocyclic compounds, because of their varied biodynamic properties[1]. Many years ago derivatives of indoles have been known for their dyeing properties, many compounds having a structural resemblance to the ancient dye indigo were known until after beginning of twentieth century. It was found that a large number of naturally occurring compounds like alkaloids possesses the indole nucleus. During this period the recognition of the plant growth hormone, heteroauxin[2], essential amino acid tryptophan[3] as the derivatives indole have added stimulus to this research.

The significant contribution of many derivatives of indole in the development of medicinal chemistry should be recognized. Serotonin, known for vasoconstrictor principle[4], plays a vital role as neurotransmitter and in psychosis. The discovery of psillicon and psilocybin[5] are the important psychotomimetics. Indole have led to extensive research on derivatives of indole-3-ethylamine or tryptamine, are reported to be potent central nervous system depressants. The anti-inflammatory[6] activity was found to be associated with many derivatives of indole, e.g. indomethacin.

The spectroscopic data collected[7] on the newer derivatives of indole, isolated from various natural sources, have immensely helped in their structure elucidation, because of this good number of alkaloids containing indole nucleus are reported in the literature. A great deal of chemistry indole and its derivatives have thus been accumulated and many monograph[8-10] on indole have already been published. Today the scope of indole research is multifarious extending from rather simple parent molecule to highly complex molecules.

Indole

Indole or benzo[b]pyrrole **1** is a planar heteroaromatic molecule in which the benzene ring is fused at position 2 and 3 of the pyrrole ring.



1

Indole is highly reactive towards electrophilic substitution reactions. Position-3 being the most preferred site for such substitution. The high reactivity at positin-3 is due to π-

electron density[11] and localization energy[12]. In presence of acids, indole is protonated at position-3 which seldom results in dimerization or polymerization. However, indole has appreciable stability in concentrated acids, when it is completely protonated [13]. The NH group of indole is relatively acidic and forms anion in presence of strong base[14]. The aromatic character of indole is explained on the basis of its ring current effect in PMR Spectrum and its appreciable resonance energy (47 kcal/mole).

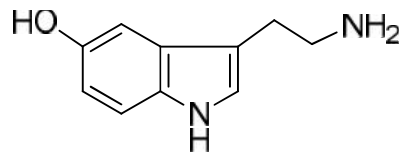
Indole is widely distributed in nature is in essential oils, coal tars, molasses tar and also found along with pus, pancreas, brain and bile. Human and animal faeces are found to contain indole, skatole. This nucleus is present in number of physiologically significant molecules like serotonin, tryptophan, indole-3-acetic acid, gramine, arbine, reserpine, yohimbine, physostigmine, lysergic acid etc, and also in important antibiotics like mitomycin and gliotoxin.

Serotonin

Serotonin or 5-hydroxytryptamine **2** was isolated for the first time from blood serum by Rapport and coworkers[15]. Serotonin was found to be present in mammalian brain[16], its highest concentration being found in basal gangalia[17-18] and pineal glands[19]. Given that basal gangalia are thought to be the area of brain concerned with emotions. These observation suggested that, any change in concentration of serotonin in the brain either by drug leads to mental disorder which would result in psychosis[15,20,21]. It antagonize by lysergic acid diethylamide at low concentrations. Due to its psychopharmacological properties[22], several compounds containing serotonin moiety have been synthesized with the hope of obtaining compounds with either serotonin agonistic or antagonistic properties.

Reserpine

Reserpine **3** has been shown to be active in reducing the concentration of serotonin in central tissues[23]. Accordingly, the synthesis of compounds which are similar to the structure of serotonin or contain a tryptamine residue or an aminoindole moiety, has gained much attention in recent years.



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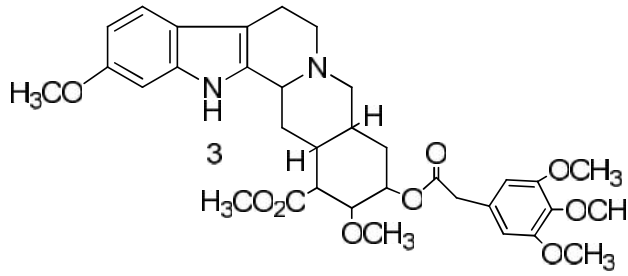
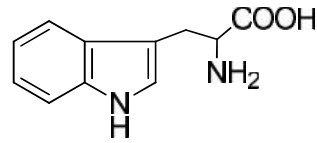


3

Tryptophan

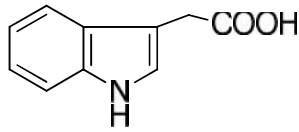
Tryptophan **4** is one of the naturally occurring essential amino acid is a protein structural unit. It is not synthesized in the animal body and hence must be supplied through diet. Deficiency of tryptophan causes a characteristics syndrome in animals. This amino acid plays a vital role in the biosynthesis of cellular proteins and porphyrins in animals.



4

Heteroauxin

Indole-3-acetic acid, is also known as heteroauxin **5** is naturally occurring plant growth hormone and is an important derivatives of indole, 1-(p-chlorobenzoyl)-5-methoxy-2-methyl indole-3-acetic acid (Indomethacin), **6** is used in the treatment of rheumatoid and related conditions[24].



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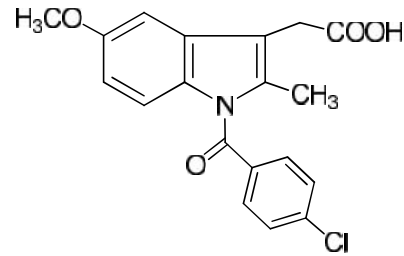
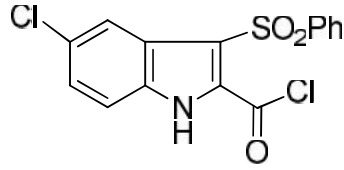


6

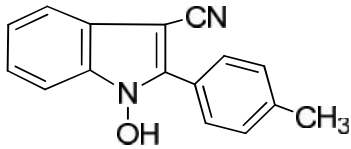
William et.al.[25] showed that the indole derivative 5-chloro-3-(phenylsulfonyl) -1H-indole-2-carboxmide **7** HIV reverse transcriptase inhibitor and claimed for the treatment of AIDS

and ARC.



7

Schleigh et.al[26]., reported the synthesis of compound **8** which has a good agrochemical fungicidal activity. Butler and coworkers[26] showed that the indole derivative **9** has activity as leakotriene receptor blockers i.e., it is potential agent for the treatment of allergic or inflammatory diseases.



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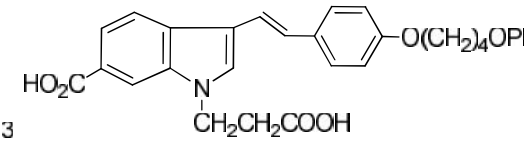
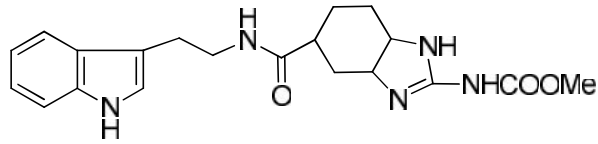


9

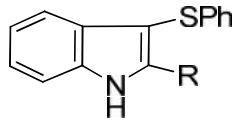
Agarwal and coworkers[27] prepared several indole derivatives and screened them for their anthelmintic activity, but the only compound **10** exhibited reduction of adult worms by 79.4% at a concentration 50 mg/kg (i.p) and 44% at 100 mg/kg (oral route) against *Brugai malayi* infection in mistomys atalensis.

Greenlee and srinivasan[28] prepared several indole derivatives **11** from ethyl indole-2-carboxylates by the series of reaction sequence, which inhibited HIV reverse transcriptase activity. Forbes and coworkers[29] synthesized several pyridyl indolyl ureas



10

12 and showed that N-(1-methyl-1-5-indolyl)-N-(3-pyridyl) urea hydrochloride (12) is selective 5-HT_{1c} receptor antagonists. This compound (12b) showed >348 fold selectivity in ligand binding studies for



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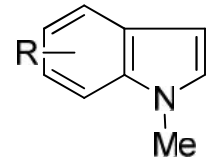


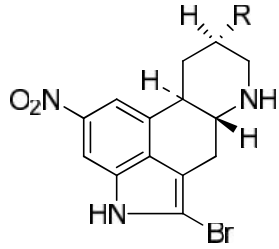
12

R= CH_2SO_2Ph
= Morpholinomethyl
= Pyrolidinomethyl or CHO

a b c d
R= 4- 5- 6- 7- NHCONHR'
R'= 3-Pyridyl

5-HT_{1c} over the 5-HT_2, 5-HT_{1D}, 5-HT_3, adrenergic α_1, α_{2A}, β_1, β_2 dopaminergic D_1 and D_2 receptors. Compound **12b** is silent competitive antagonist for 5-HT stimulated phosphonositide hydrolysis in the pig choroide liexus, a model of 5-HT receptor functional activity and was also a surmountable antagonist, of 5-HT_{1C} like receptor in the rat stomach fundus.

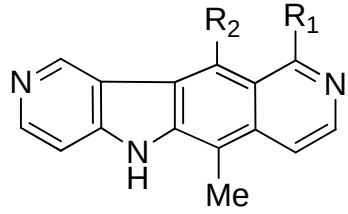
Traimer and krepelka[30] prepared 2-bromo-13-nitro derivatives of ergoline **13** by nitration of the corresponding 2-bromo compounds with nitric acid. These compounds are potential drug with action on prolactin secretion and dopaminergic receptor as well as being intermediates for drugs with these activities.



13

a b
R = α–NHCONEt_2, β–CH_2Me

Chermann et.al[31]., prepared several derivatives of dipyridoindoles **14** and tested them for the treatment of AIDS. Out of the several compounds tested 1-[(γ-diethylaminopropyl)- amino]-5-methyldipyrido[4,3-b][3,4-1]indole. 3HCl at 6.25-25 mg/ml inhibited production of human immunodeficiency virus-1 (HIV-1) by PHA stimulated peripheral lymphocytes treated with HIV-1. This compound also inhibited HIV-1 replication in macrophages.

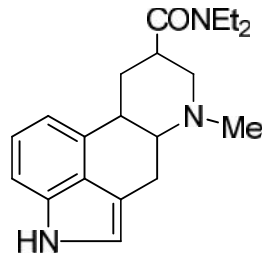


14

R_1 = H, OH,C_{1-6}-alkyl, alkylthio, alkoxy, haloges, substituted/unsubstituted amino.
R_2 = H or C_{1-4}-alkyl.

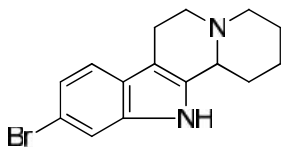
Lysergic acid diethylamide (LSD)

Ergot alkaloid and related compounds were known for their antiserotonin activity, particularly on smooth muscle. In this group lysergic acid derivatives such as diethyl amide[32], **15**, 2-bromodiethyamide and 1-methyl—lysergic acid diethyl amide buatnol amides are specially quoted. The action of LSD is selective[33,34].It prevents the antidiuretic action[35] of serotonin and is also a very potent hallucinogenic drug.



15

Chabni et.al[35]., isolated the four indole alkaloids **16**, **17**, **18a** and **18b** from the cytotoxic methylene chloride solution extracted of the marine tunicate pseudodistoma aborescens, which were characterized by their 2-D NMR. Only arborescidine **18b** showed moderate activity *in vivo* against the growth of human buccal carbinoma cells.



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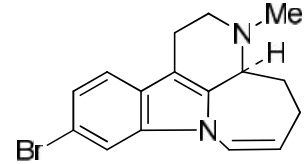

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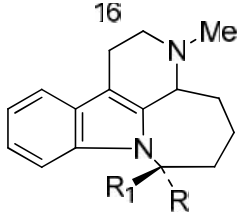
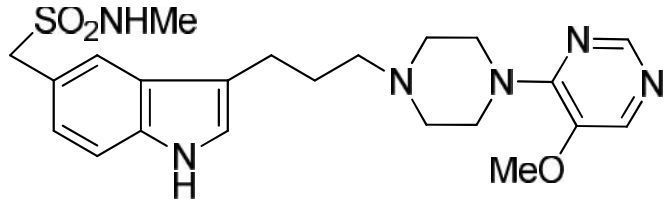


18

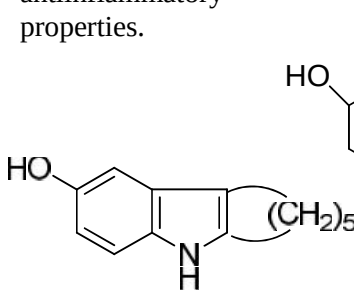
	a	**b**
R =	OH,	H
R_1 =	H,	OH

Paul R. Brodfuehrer et.al[36]., efficiently synthesized antimigrain drug candidate avitriptan (BMS 180048) **19** is reported.



19

Catherine Kuehm-Caubere and coworker[37] showed that the indole derivative **20** was proved to protect endothelial cells from the direct cytotoxicity of oxidized LDL with some additional calcium challen blocking properties and compound **21** was to found to have some antiinflammatory
properties.



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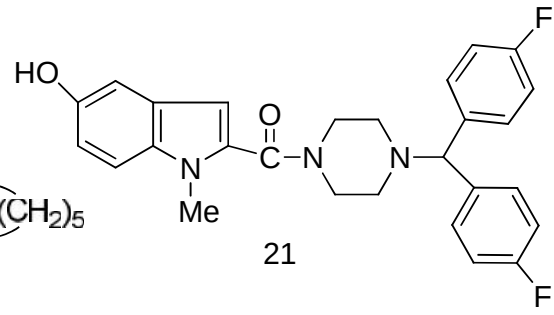
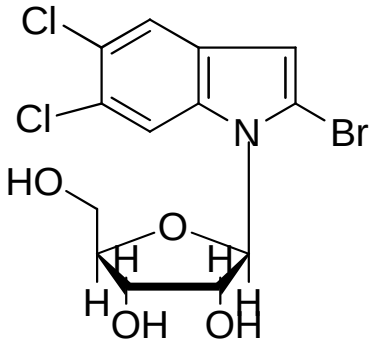


21

Jiong J. chen and coworker[38] prepared 2-bromo-5,6-dichloro-1-(β-D-ribofuranosyl) indole **22** and **23** tested for activity against HCMV, *Harpies simplese* virus type-1 (HSV-1) and human harpies six (HHV-6) and for cytotoxicity. The compound was less active against HCMV than TCRB and weakly active against HSV-1 and HHV-6.



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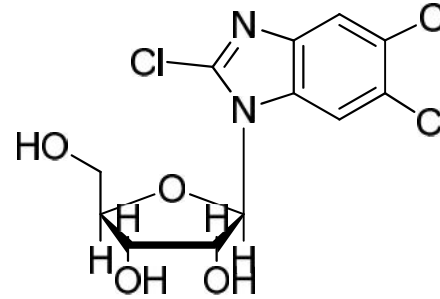
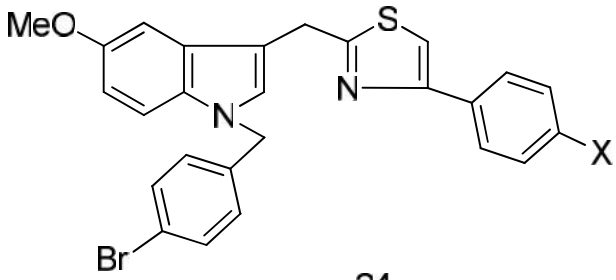


23

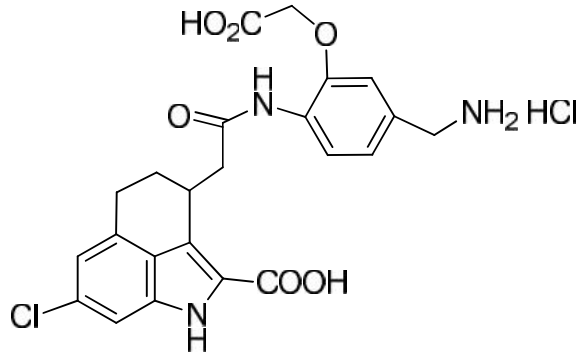
Keith w. woods et. Al[39]., reported several derivatives of indomethacin **24** these compounds are selective COX-2 inhibitors.



24

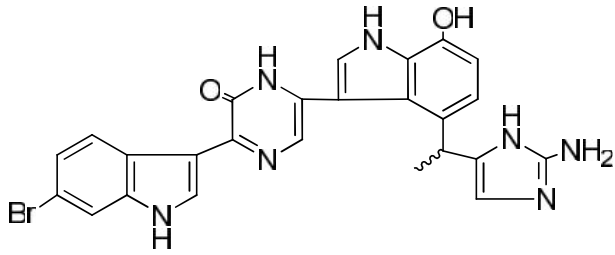
X=Br,Cl,COOH,NO_2

Seiji katayama et.al[40] reported a class of compound **25** with the highest affinity for the NMDA-Glycine binding sites.



25

Cai-Guang Yuang et. al[41]., synthesized the compound dragmacidine **26** which exhibited a broad spectrum of biological activity. It inhibited the growth of the **feline leukemia** virus, the opportunistic fungal pathogen **Cryptococcus neoforms** and **Candida albicans** and the P 388 and A 549 tumor cell line. It has been identified that compound **26** was found to be selectively inhibit neutral nitric oxide synthase (bNOS) and very useful in the treatment of Alzheimer's, Parkinson's and Huntington's diseases.



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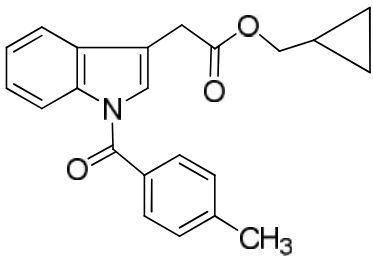
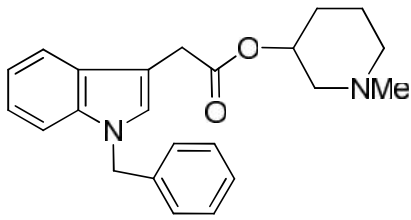


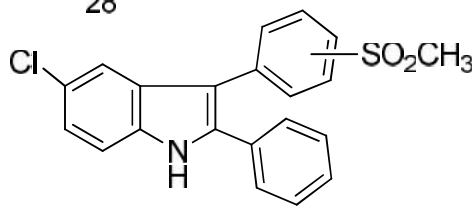
27

Suryya Olgen and dogu Nebioglu[42]., prepared several novel N-substituted carboxylic, acetic and propionic acid esters as possible selective cyclo-oxigenase-2 (COX-2) enzyme inhibitors **27**, **28.**



28

Wenhui Hu and workers[43], synthesized highly potent selective COX-2 inhibitors, compounds **29**, **30**



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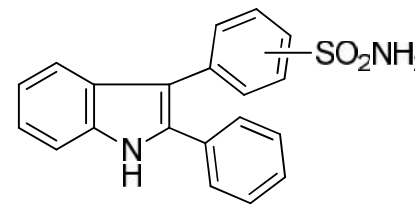
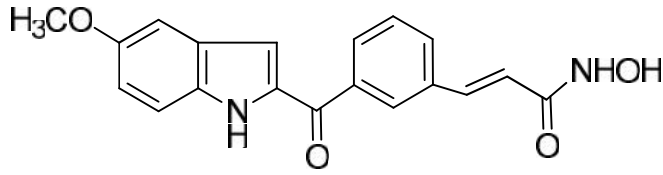


30

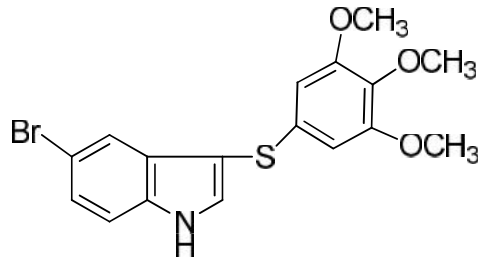
possesses higher activity than celecoxib.

Siavosh Mahboobi et.al[44], showed that the indole derivatives **31** is new class of Histone deacetylase (HDAC) inhibitor which is consider as a drug for targeted cancer therapy.



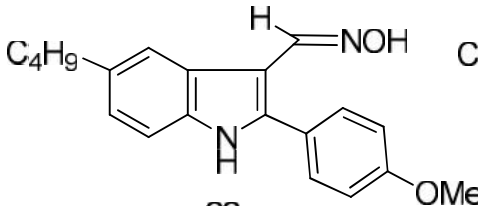
31

Giuseppe La Regina et.al[45], showed the aryl thioindole derivative **32** as a inhibitor of MCF-7 cell growth in tubulin polymerization i.e. breast cancer cells.



32

Doris Kaufmann et.al[46], synthesized several 2-phenyl indole-3-carboxaldehyde derivatives **33**, **34**, which strongly inhibits the meiotic activities in MDA-MB231 and MCF-7 breast cancer cells with IC_{50} values of 5-20 nm, due to inhibition of tubulin polymerization.



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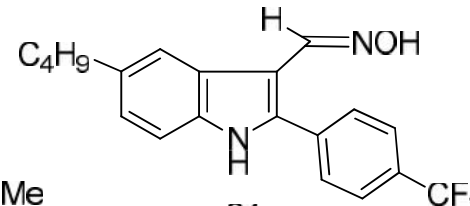


34

In view of these discoveries, research in indole field has retained its significance and search for the new compounds anticipating better pharmacological activity is still continued.

Research on Indole in this laboratory:

Indole has been the major field of research in laboratory for several years. Efforts in our laboratory centers mainly around the following derivatives of indole namely serotonin, tryptophan, heteroauxin, carbazole, carboline, pyrimidoindoles, heterocyclic system

containing the indole nucleus and also biheterocycles containing the indole nucleus.

Synthesis and biological activity of good number of tryptamines, which are structurally alike to serotonin, have reported from this laboratory[47-50].

The literature report identifying the presence of DOPA in the concentrates of *M. leprae* have prompted the above workers to undertake the synthesis of mimics of intermediates in the conversion of DOPA to melanin. As a part of this programme several derivatives of hydroxyl indoles have been prepared and screened for their *in vivo* and *in vitro* antileprotic properties. Out of the several compounds screened, indole -2-carboxylic acid and its ethyl ester, ethyl-2-methyl-5-hydroxyindole-3-carboxylate and ethyl 5,6-dimethoxy-3-methylindole-2-carboxylate have exhibited promising antileprotic activity. In particular, ethyl 5,6-dimethoxy-3-methylindole-2-carboxylate is to be found to be at the dose level of 0.08 μg/ml when tested on Fc receptors.

Hiremath and coworkers[51] have developed novel synthetic route for the synthesis Carbolines while attempting the synthesis of 2,3-benzo-γ-carbolines from 2-phenylindole-3-aldoxime under acidic condition. This compound might have been the formed by the dehydration of the Beckmann transformation product of aldoxime.

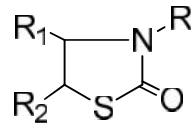
The synthesis of several derivatives has reported from this laboratory. Hiremath and Purohit[52] synthesized several alkyl substituted 3,4-dihydropyrido[4,3-b]indoles from their corresponding indole-2-(2'-yl ethylamines), which have found to possess antiserotonin activity[53]. Hiremath and co-workers[54] have developed a convenient method to synthesis 6H, 11H-indolo [3,2-c]-isoquinolin-5-ones from their corresponding ethyl 5-substituted 2-phenyl indole-3-carbomates. They[55-58] have extended this work to synthesis many substituted pyrazoles, pyrazolones and oxadiazole-2-thiones linked to indoloisoquinoline system through acetyl or thioacetyl bridge and screened them for their pharmacological and microbiological properties.

Hiremath and coworkers synthesized biheterocycles containing pyrazole[59], thiazolidino[60], imidazole[61] nucleus using indole-3-carboxaldehyde as starting compound and reported their biological activities.

Indole-3-carboxaldehyde, also reacted with various arylthioureas to get yield respective Schiff bases and there compounds were screened for various pharmacological properties[62].

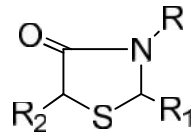
Thiazolidinone:

Thiazolidinone[63-66] are derivatives of thiazolidine, which belongs to an important group of heterocyclic compounds. Thiazolidinone **35**, **36**, and **37** with a carbonyl group 2, 4 or 5 have been subject of extensive study in recent years [67-69]



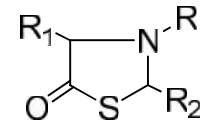
2-thiazolidinone

35



4-thiazolidinone

36



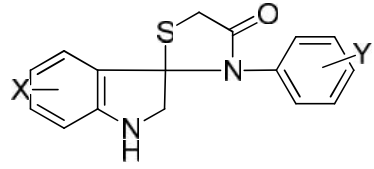
5-thiazolidinone

37

4-

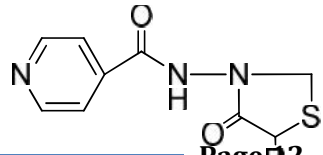
4-Thiazolidinones have been found to be associated with diverse biological activities and numerous reports have appeared in literature, which highlighted their chemistry and use [70-73].

Joshi et.al[74] prepared a number of fluorine containing Spiro[3H-indole-3-2-thiazolidine]-2,4 (1H)-dione **38** compound were screened for ant fertility activity.



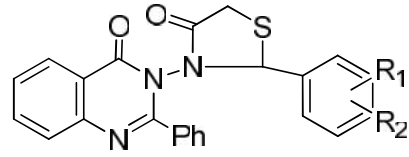
38

Desai and co-workers[75] synthesized some new 2-aryl-3-isonicotamido-4-thiazolidinones **39** compounds shows remarkable antibacterial and antituburcular activity.



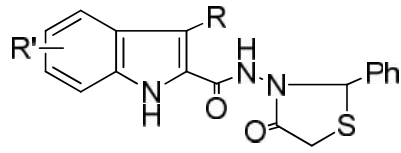
39

Husain and shukla[76] reported the synthesis, antibacterial and CNS activities of 3-(2-aryl-4-oxothiazolidin-3-yl)-2-phenyl-quinazolin-4(3H)-ones **40**.



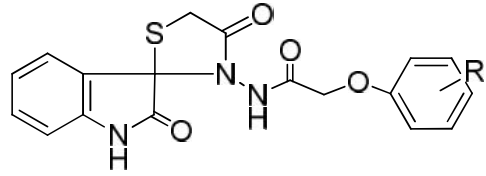
40

S. P. Hiremath and co-workers[77] synthesized derivatives of 3-(substituted indole-2-carboamido)-2-phenyl-4-oxo-3-thiazolidinones **41** these compounds were screened for their antibacterial activity.



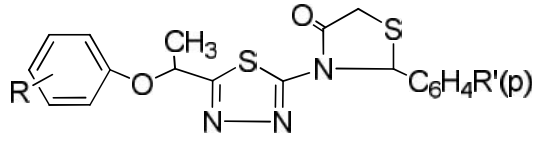
41

R. Ali and B Sharma at.al[78] synthesized derivatives of Spiro [3H-indole-3, 2-thiazolidine]-2-2-(1H)- diones **42**.



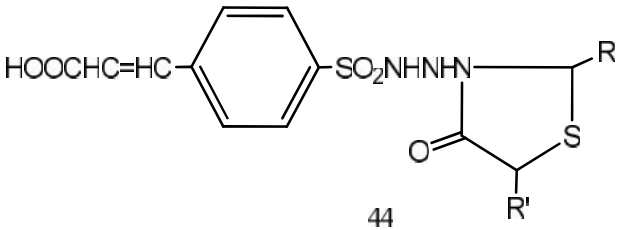
42

Srivastava et.al[79] have reported the synthesis and antifungal activity of a series of 2-aryl-3-(5-aryloxymethyl-1,3,4-thiazolidinines **43**.



43

K. C. Shah and co-workers[80] were synthesized some new 4-thiazolidinones these derivatives have been screened for their antimicrobial activity **44**.

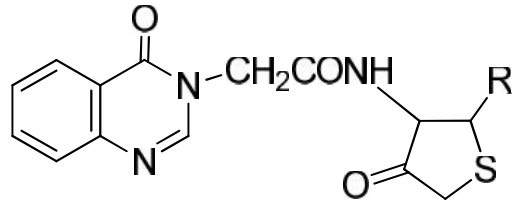


R=aryl
R'=H, Me, CH_2COOH

44

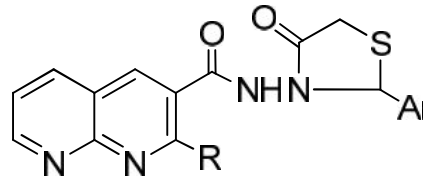
M. B. Deshmukh and co-workers[81] were synthesized derivatives of 3-(quinazolin-4-one-3-yl acetamido)-2-aryl-1, 3-thiazolidin-4-ones **45**, these compounds exhibited promising antimicrobial activity.

Sreenivasulu et. al[82] synthesized a number of new 2-aryl-3-(2-



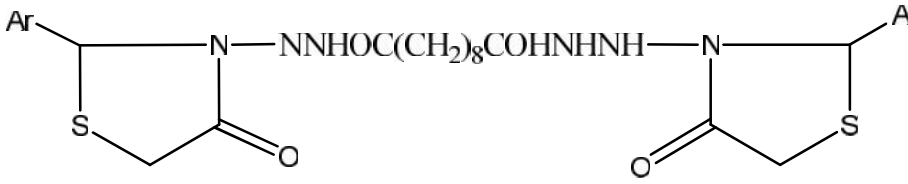
45

methyl/phenyl-1,8-naphthyridine-3-carbonyl amino)-thiazolidinone **46**.



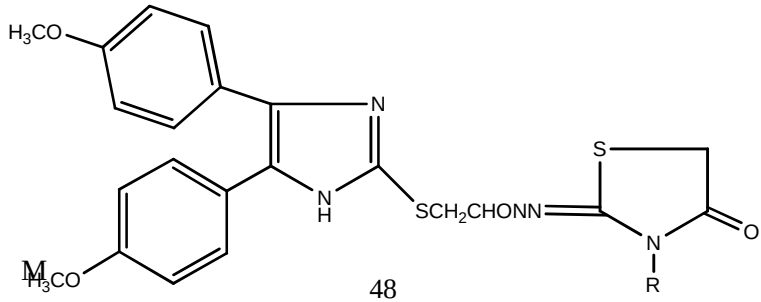
46

Kudari et. al[83] has reported the synthesis and antimicrobial activity of 1, 8-bis (thiazolidinone) octane's **47**.



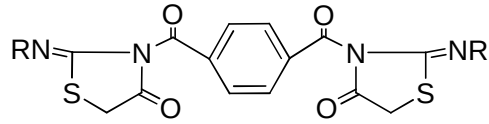
47

Gursoy et. al[84] have synthesized derivatives of 2-[4, 5-bis (methoxy phenyl) imidazol-2-yl mercapto acetyl]-hydrazone-3-alkyl/aryl-4-thiazolidinones **48**.



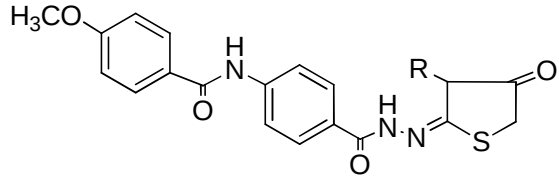
48

ruth unjayaswamy et.al[85] reported the synthesis and pharmacological evaluation of 3-terephthaloyl bis-2-(5-sybstitued-2-phenyl-3-yl) imino-thiazolidin-4-ones **49**.



49

S. G. Kucukguzel et.al[86] have been synthesized derivatives of 2-[4-(4-methoxybenzolamino) benzoylhydrazono]-3alkyl-4-thiazolidinones **50**.

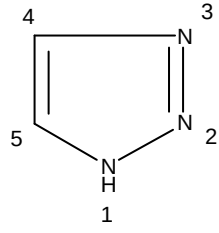


50

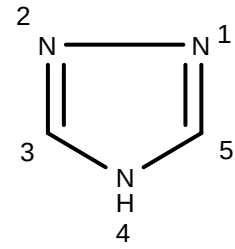
1, 2, 4-Triazoles:

The history of 1, 2, 4-triazoles is almost a century begins with pioneering work of bladin[87] who synthesized the first representative and coined the name "triazoles" for this class of compounds.

The heteroaromatic triazoles ring system is composed of five atoms, two carbon and three nitrogen, which can be arranged in two combination to give either V triazoles (V-vicinal)-1,2,3-triazole **51** or S-triazoles (S-symmetrical) or 1,2,4-triazole **52**.

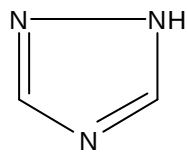


51

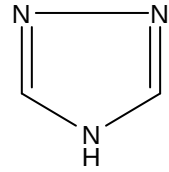


52

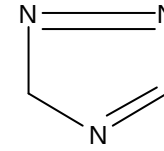
Following are the tautomeric forms of 1, 2, 4-triazoles, as a positively hydrogen associated with the resonance stabilized triazoles anion.



53



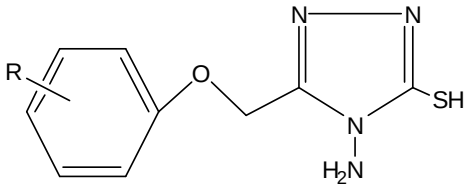
54



55

Certain types of triazoles were capable of inhibiting fog formation in photographic emulsion and that others were useful herbicide and the anticonvulsants. This led lot of interest in both simple and fused triazoles system[88]. The research on 1, 2, 4-triazoles has been extensively reviewed by pots[89] and temple[90], a large variation in the methods of synthesis of substituted triazoles has been reported. In recent years 1, 2S, 4-triazole nucleus has gained much importance in economically significant field such as pharmaceutica[91], pesticides[92], fungicides[93], paint[94], plastic[95] etc.

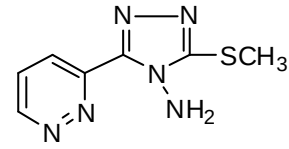
Kulkarni and co-workers[96] have reported that a number of 3-substituted phenoxymethyl 1-4-amino-5-mercapto-1, 4-amino-1,2,4-triazoles **56** these compound were screened antibacterial, antispasmodic, diuretic and herbicidal activities[103].



R= H, 2,4-Cl, 2,4,5-Cl_3, 2-Cl, 4-Cl, 4-NO_2

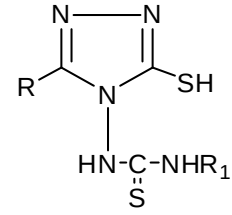
56

Sokolov and Hiller[97] have reported 3-(5'-methy-isoxazol-3-yl)-4-amino-5-mercapto-1,2,4-triazole **57** were screened for antimicrobial and fungicidal activity, the above compound shows very good activity at lower concentration.



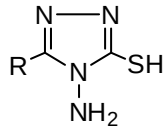
57

Chaturvedi and Tiwari[98] have reported the 5-aryl-4-substituted thiocarbamyl amino-3-mercapto-1,2,4-triazoles **58** as potential fungicide against Asporagillus niger and Helminthos Oryzae fungal species.



58

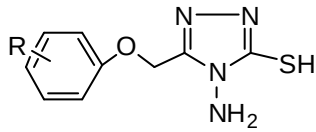
Chande and Karnik[99] reported 3-substituted-4-amino-5-mercapto-1,2,4-triazoles **59** which shows very good antimicrobial activity.



R=CH_3, C_2H_5, C_6H_5, p-$CH_3C_6H_4$, 4'-pyridyl

59

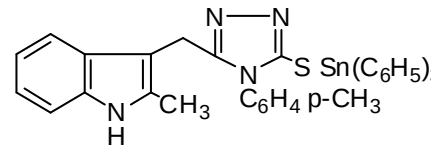
Chande and Karnik[100] have reported the synthesis of 3-substituted-aryloxy-4-amino-5-mercapto-1,2,4-triazoles **60** these compounds have shown good antibacterial properties.



R= H, CH_3.Cl

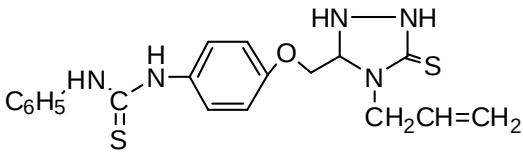
60

Anil K Sengupta and co-workers[101] were synthesized the 4-aryl-3-(2'methylindole-3-yl)-5-thiostannoxy-1,2,4-triazole **61** which exhibited antibacterial activity against B.substilus.



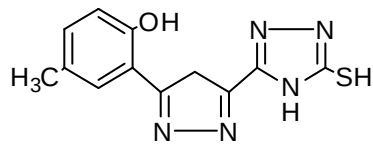
61

Iikey Kucukgugel et.al[102] synthesized the compound **62** which exhibited antituberculosis activity.



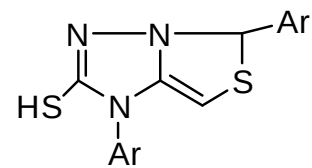
62

Dhiman et.al[103] were synthesized the 3-(3-aryl-1H-pyrazoles-5-yl)-4-substituted-5-mercapto-1,2,4-triazole **63** exhibited antibacterial activity against S.aureus.



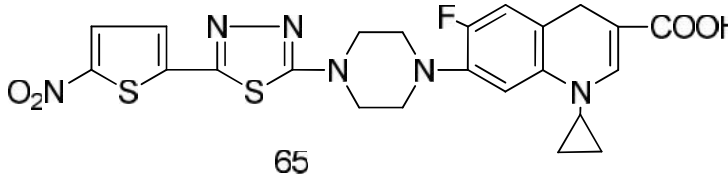
63

H.Sing and Co-workers[104] were synthesized the thiazolo [3,4-b]-1,2,4-triazole **64,** exhibited good fungicidal activity.



THIADIAZOLES:

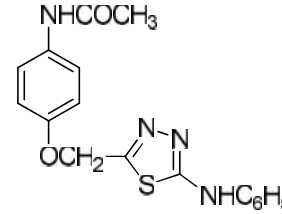
Alireza Forounadi et.al[105] were reported a class of N-[5-(5-Nitro-2-Thienyl)-1,3,4-Thiadiazole-2-yl]piperazinyl **65**. This derivatives exhibited antibacterial activity against *S.aureus and*



65

B.substilus.

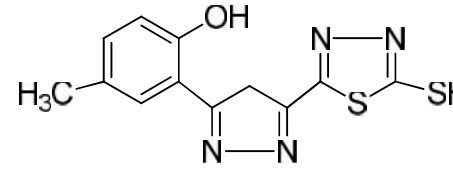
L. V. G. Nargunda et.al[106] were synthesized 1-aryl-2-amino-5[(4-acetamidophenoxy)-methyl]-1,3,4-thiadiazole **66,** which exhibited good bacterial activity against *B.substilus*



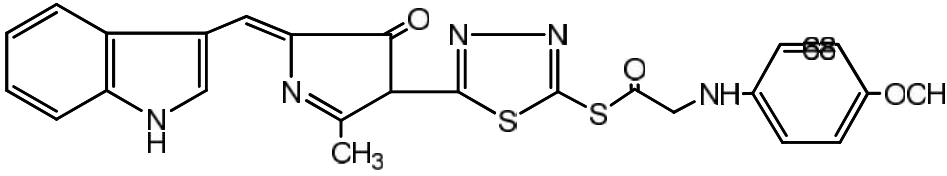
66

Meenakshi Shrimali and Co-workers[107] reported indolyl-1,3,4-thiadiazole **67**, which exhibited good anticonvulsant and analgesic activity.

A. M. Dhiman et.a[108] were reported 5-(3-aryl-1h-pyrazol-5-yl)-2-mercapto-1,3,4-thiadizole **68**, was exhibited antibacterial activity against *P.mirabilis.*



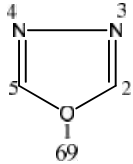
1, 3, 4-OXADIAZOLES:



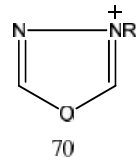
67

Literature survey reveals that 1,3,4-oxadiazole system is known since many years. But synthesis and reactivity studies of this system however received much attention only during the last three decades. A large number of patents, which have appeared in the literature recently, substantiate the importance of the 1,3,4-oxadiazoles and its derivatives. Such are used primarily in the synthesis of drugs, in the production of polymers, preparation of dyes and in photography.

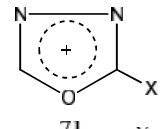
1,3,4-oxadiazole **69** is a thermally stable neutral aromatic molecule[109] . Other aromatic systems are 1,3,4-oxadiazolium cations **70,** exocyclic conjugated mesoinic 1,3,4-oxadiazoles **71** and 1,3,4-oxadizolines **72**. The derivatives of non-aromatic reduced systems are 2,3-dihydro-1,3,4-oxadiazole (Δ^2 1,3,4-oxadiazoline) **73**, 2,5-dihydro-1,3,4-oxadiazole (Δ^3 1,3,4-oxadiazoline) **74** and 2,3,4 tetrahydro 1,3,4-oxadiazole (1,3,4-oxadizolidine) **75**.



69

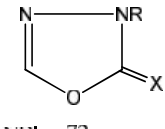


70

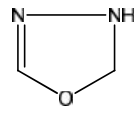


71

X=C, S, NR'



72



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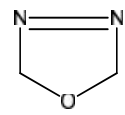

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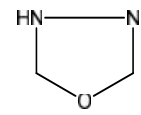
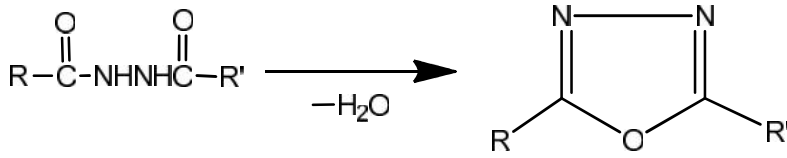


75

Preparation of 1, 3, 4-oxadiazoles:

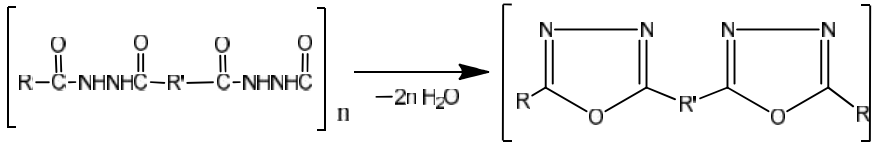
A. Condensation with elimination of water.

The starting materials are mono and di acid hydrazides, acylsemicabazides, and related compounds. The ring closure producing the oxadiazole **76** proceeds as follows.



76

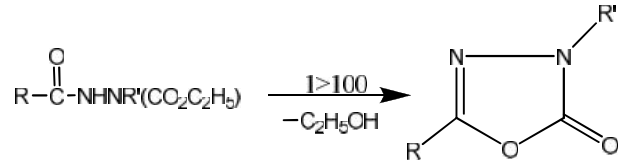
The well known conversion of N, N'-diacid hydrazides to 2,5-diaryl(alkyl)-1,3,4-oxadiazoles have been described by Stolle[110], who used dehydrating agent like fuming sulphuric acid[111-114], chlorosulphonic acid[115], diacid hydrazides may also be dehydrated by phosphoric acid and esters[116-119], phosphorus pentoxide is used especially when higher temperatures are required for ring closure. For example in the preparation of bis (perfluoroalkyl) oxadizoles[120,121]. Pellizzari[122] who employed prolonged heating above the melting point with relatively stable substituents R & R'. The preparation of bis-oxadiazoles are carried out by the above procedures[123,124]. On the other hand poly oxadiazoles **77** are formed after several hours heating at 120-300^{0}C of corresponding polyhydrazide.



77

B. Condensation with elimination of alcohol:

1-Acylhydrazne-2-carboxylic acid esters are converted on heating to 1, 3, 4-oxadiazolin-5-one **78** with elimination of alcohol[125, 126].



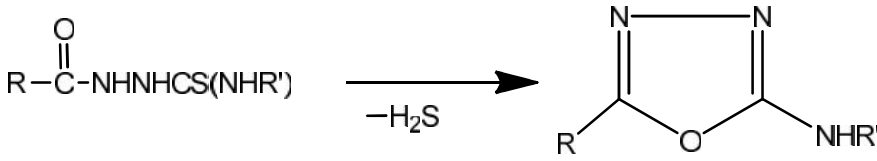
78

C. Condensation with elimination of carboxylic acids and carboxylic anhydride:

Tetracyclated hydrazines, which may be obtained a intermediates by heating diacyl diimides in an inert solvent or directly from anhydrous hydrazine and excess of carboxylic acid ester produces on heating 2,5-disubstituted-1,3,4-oxadiazoles[127, 128] with elimination of two moles of acid anhydride.

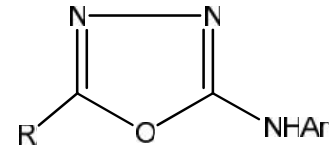
D. Condensation with elimination of H_2S or mercaptans:

The preparation of 5-substituted-2-amino-1,3,4-oxadiazoles **79** by the elimination of hydrogen sulphide from 1-acyl thiosemicarbazide using PbO[129] has been modified[130], in the modified method HgO, Pb_3O_4, $CuSO_4$ & I_2 are used as oxidative cyclizing agents.



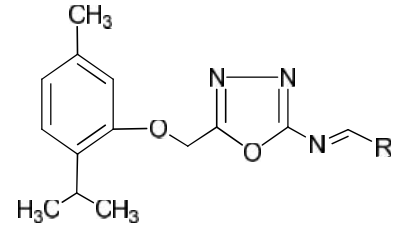
79

Daulatabad et.a[131]., reported a number of 2-alkyl-5-arylamino-1, 3, 4-oxadiazole **80** was found to have remarkable bactericidal and fungicidal activities.



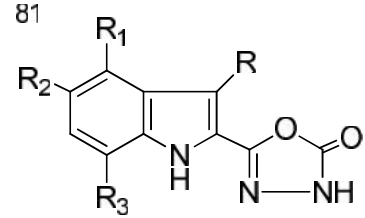
80

Roda et.al[132]., reported a number of 2-benzalamino-5-(2'-isopropyl-5'-meyhyl phenoxy methyl)-1, 3, 4-oxadiazoles **81**, were tested against show maximum activity was observed in compounds having 3-nitrophenyl and 4-chloro phenyl substitution.



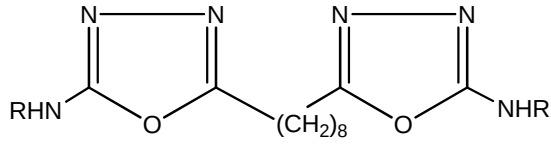
81

Hiremath and co-workers[133] have synthesized the various 2-substituted derivatives of 1, 3, 4-oxadiazoles **82** and these compounds have been screened for their antibacterial activity.



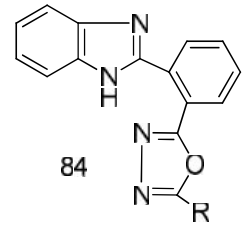
82

Kudari and co-workers[134] have reported the synthesis of 1, 8-bis (5-alkyl/aryl amino-1, 3 ,4-oxadiazole-2-yl) octanes **83** and these compounds have been screened for their antimicrobial activities.



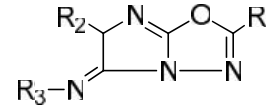
83

Parekh et.al[135]., have synthesized the 2-5-disustituted-1,3,4-oxadiazoles **84** and screened for their antimicrobial and antitubercular activity



84

Yadav and Co-workers[136] synthesized derivatives of 2,6-diaryl-5-arylamino 5,6-dihydro(2,1-b)-1,3,4-oxadiazole **85,** which exhibited good fungicidal activity.

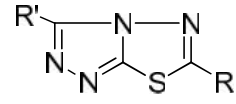


85

FUSED SYSTEM:

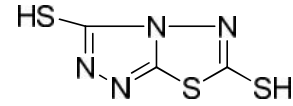
1,2,4-triazolo[3,4-b]-1,3,4-thiadiazoles:

The 1,2,4-triazoles[3,4-b]-1,3,4-thiadiazole ring system was first reported by Kanaoka[137], who has synthesized various 3-alkyl and 3-aryl derivatives by the dehydrative cyclization of 4-acyl amino 1,2,4-triazole-5-thiols **86**. Several similar synthesis were reported by starting with 4-amino-1,2,4-triazol-5-thiols and building the thiadiazole ring using carbaoxylic acid[138-141], acetic anhydride[142], acid chloride[143], aldehydes[144,145], arylisothiocyanates[146], cyanogen bromide[147] and carbon disulphide[148].



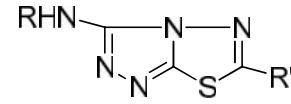
86

Cyclocondensation of thiocarbohydrazides with two equivalent of carboxylic acid[150] gave 3-6-disustituted-1, 2, 4-triazolo[3, 4-b]-1, 3, 4-thiadiazoles **87**, when condensed with carbon disulphide and pyridine it gave[151], in addition of 4-amino-2,5-dimercapto-1,2,4-triazoles and 3-6-dimercapto-1,2,4-triazolo[3,4-b]-1,3,4-thiadiazole.



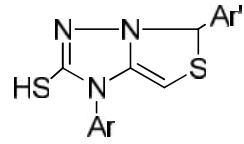
87

3,6-disustituted-amino-1,2,4-triazolo[3,4,-b]-1,3,4-tiadiazoles **88** were prepared[152] by cyclocondensation of 2-mercapto-5-sustituted-1,3,4-thiadiazole with 4-substituted thiosemicarbazide .



88

Sing and Co-workers[152], were synthesized the thiazolo[3,4-b]-1,2,4-triazole, **89** exhibited good fungicidal activity.



89

These 1, 2, 4-triazolo[3, 4-b]1,3,4-thiadiazoles possess various biological activities such as analgesic142, antiinflamatory142, fugicidal151, antimicrobial151, CNS depressant141, hypotensive activities141 and mild hypocholesterolenic141.

CHAPTER-2 EXPERIMENTAL

2-phynyl indole (4):

Acetophenone phenyl hydrazone **3** was prepared by warming a mixture of acetophenone (20 g, 0.167 mol) and phenyl hydrazine (18 g, 0.167 mol) on water bath for 1hr. The reaction mixture was cooled and separated solid filtered. It was crystallized from rectified spirit, filtered and dried under vacuum ($CaCl_2$).

An intimate mixture of powdered anhydrous zinc chloride (62 g) and acetophenone phenyl hydrazone **3** (16.6 g, 0.0120 mol) was taken in a beaker, then it was heated to 170^0 C in an oil bath. The mixture was stirred vigorously, after 5 min the mass becomes liquid with the evolution of white fumes. It was stirred with clean sand (50 g) in order to prevent solidification to hard mass. The mixture was digested for 12-16 hrs on water bath with water (200 ml) and concentrated hydrochloric acid (12 ml), to dissolve the excess of zinc chloride. It was then filtered and crude 2-phenyl indole was boiled with rectified spirit (150 ml) and filtered through preheated Buchner funnel to remove sand and finally washed with hot rectified spirit. The filtrate was cooled to room temperature to get 2-phenyl indole m. p 186-187^0C, yield 18 g (73%) (Table-1).

Phenacyl bromide:

To a solution of acetophenone (100 ml) in acetic acid (150 ml) was added bromine (30 ml) in acetic acid (150 ml) after the complete addition of bromine, the contents were shaken for 10-15 min and allowed to stand for 20-30 min. Then the mixture was decomposed in crushed ice. The solid separated out was filtered and washed with little absolute alcohol. m.p 60^0C, yield 100 g, (80 %).

Phenacyl aniline (5a-b):

A solution of phenacyl bromide (20 g, 0.01005 mol) in ethanol (100 ml) was added slowly to the corresponding substituted aniline (0.02010 mol) dissolved in ethanol (50 ml). The reaction mixture was warmed on water-bath for 15-20 min. till the color of the mixture turned to dark brown. The contents were cooled to room temperature and the solid obtained was collected, washed with rectified spirit. It was crystallized form ethanol. m. p., 158-159^0C, yield 77%.

Aniline hydrobromide (7a-b):

Substituted aniline were converted into the respective hydrobromide by adding hydrobromic acid (5 g) to a suspension of substituted anilines (5 g) in water (5 ml) and warming the mixture on a water bath. On cooling the aniline hydrobromide **7** separated out was filtered, washed with ether, dried and used as such in the next stage.

Substituted 2-phenyl indoles (8a-b):

An intimate mixture of the appropriate aniline (0.04 mol), the corresponding phenacyl aniline **5** (0.02 mol) and the respective aniline hydrobromide (0.05g) was heated in oil bath at 180-90^{0}C for 5-10 min. Then the reaction mixture was poured in to dilute hydrochloric acid (50 ml, 20%) and extracted with ether. The ether layer was washed with dilute hydrochloric acid to remove the excess of aniline and dried ($NaSO_4$). The solvent was evaporated and desired indole obtained was crystallized from suitable solvent (Table-1).

2-Phenyl indole 3-carboxaldehydes (9a-c):

Phosphors oxychloride (1.62g, 0.01 mole) was added with stirring to dimethyl formamide (3.2g, 0.0438 mole) at 10-20^{0}C, substituted indoles (0.01 mole), dissolved in N,N-dimethyl formamide was then added slowly with stirring. The temperature of the mixture was maintained between 20-30^{0}C. After the addition was over the reaction mixture was kept at 35-40^{0}C for 45 min, and poured into crushed ice. The mixture was then treated with solution of sodium hydroxide (1.9g, 0.0475 mole) in water (10%, 10 ml), it was boiled immediately for a minute and allowed to cool to room temperature. The solid that separate was filtered, washed with cold water to get 2-phenyl indole 3-carboxaldehydes**.** Product was purified by crystallization using ethanol and physical data were tabulated in table-2.

Thiosemicarbazones (10a-c):

To a solution of **2** (0.01 mole) and thiosemicarbazide (0.01 mole) in methanol (20 ml), few drops of acetic acid were added and the mixture refluxed for 5 hrs. At the end of this period, the solution was cooled to room temperature and poured into ice cold water, then neutralized with solid $NaHCO_3$, the separated solid was filtered and dried to obtain thiosemicarbazone.

The crude products were recrystallized from ethanol. Physical data were tabulated in table-3.

1-[2-(2,5-disubstituted-1H-indol-3-yl)-4-oxothiazolidin-3-yl]thiourea (11a-c):

Thiosemicarbazone (0.01 mole) was refluxed in DMF (30 ml) containing a pinch of anhydrous zinc chloride and thioglycolic acid (0.01 mol) for 8 hrs. The mixture was cooled and poured into ice cold water, the separated solid was filtered, washed with water and recrystallized from ethanol. Physical data were tabulated in table-4.

5-(2'-5'-substituted-1H-indol-3'-yl)-2-mercapto-1,5-dihydrothiazolo[3,4-b]-1,2,4-triazole (12a-c):

To 1-[2-(2,5-substituted-1H-indol-3-yl)-4-oxothiazoldin-3-yl]-4-thiourea (0.01 mole) was dissolved in 2N NaOH (10 ml) and ethanol (5 ml). The reaction mixture was refluxed for 8 hrs. The resulting mixture was cooled and poured on crushed ice, on acidification with dil.HCl gave the crude product which was filtered, washed with water, dried and recrystallized ethanol. Physical data were tabulated in table-5.

2-amino5'-(2'-5'-substituted-1H-indol-3'-yl)-5H-thiazolo[4,3-b]-1,3,4-thiadiazole
(13a-c):

To 1-[2-(2,5-substituted-1H-indol-3-yl)-4-oxothiazoldin-3-yl]-4-thiourea (0.01 mole), concentrated sulphuric acid (8 ml) was added drop wise with stirring in an ice bath at $0\text{-}2^0$c, after 45 min, the reaction mixture was poured into ice cold water and then neutralized with ammonia solution. The product thus separated was filtered, washed with water, dried and recrystallized from ethanol. Physical data were tabulated in table-6.

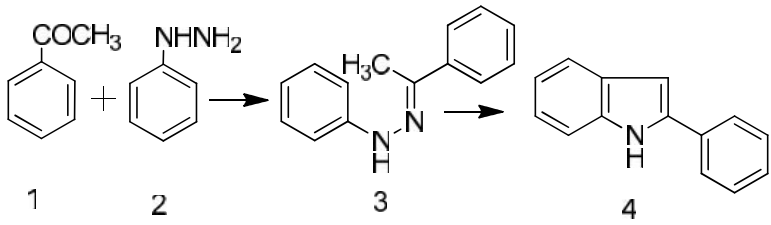
2-amino-(2'-5'-substituted-1H-indol-3'-yl)-5H-thiazolo[4,3-b] 1,3,4-oxadiazole (14a-c):

To 1-[2-(2,5-substituted-1H-indol-3-yl)-4-oxothiazoldin-3-yl]-4-thiourea (0.01 mole) in ethanol (10 ml), NaOH (8 ml, 2N) was added followed by the addition of I_2 in KI solution (5%) till permanent tinge of iodine persisted. The reaction mixture was refluxed for 2 hrs. Cooled to room temperature and poured into ice cold water. The desired product thus obtained was filtered, washed with water and recrystallized from ethanol. Physical data were tabulated in table-7.

The 2-phenyl indole **4a** was prepared by following the Fischer indolization mathod[153]. The substituted 2-phenyl indole were prepared by using Bischler method[154] **8a-b**, These 2-phenyl indoles **4** and **8a-b** on Vilsmeier Haack formylation gave 5-substituted 2-phenyl indole-3-carboxaldehydes **9a-c**. These carboxaldehydes on condensation with thiosemicarbazide afforded the 5-substituted-2-phenyl indol-3-thiosemicarbazones **10a-c.** The compounds **10a-c** on cyclization with thioglycolic acid yielded 1-[2-(2',5'-disubstituted-1H-indol-3-yl)-4-oxathiazolidin-3-yl]thioureas **11a-c**. The intermediates **11a-c** on cyclodehydration with NaOH (2N), conc. H_2SO_4 and iodine-potassium iodide afforded the respective, 5-(2',5'-disubstituted-1H-indol-3'-yl)-2-mercapto-1,5-dihydrothiozolo[4,3-b]-1,2,4-triazoles **12a-c**, 2-amino-[5-(2',5'-disubstituted-1H-indol-3'-yl)]-5H-thiazolo[4,3-b]-1,3-4-thiadiazoles **13a-c** and (2'-5'-disubstituted-1H-indol-3'yl)-5H-thiazolo[4,3-b]-1,3-4-oxadiazoles **14a-c**.

A mixture of acetophenone **1** and phenylhydrazine **2** refluxed on water bath for 1 hr gave acetophenone phenylhydrazone **3**. An intimate mixture of **3** and anhydrous zinc chloride on heating at 170^{0}C in an oil bath and stirring vigorously was followed by stirring with clean sand. After digesting the reaction mixture for 12-16 hrs on a water bath with water and conc. hydrochloric acid to dissolve the excess of zinc chloride, yielded 2-phenyl indole **4** which was purified by crystallization in benzene (Sheme-1) (Table-1).

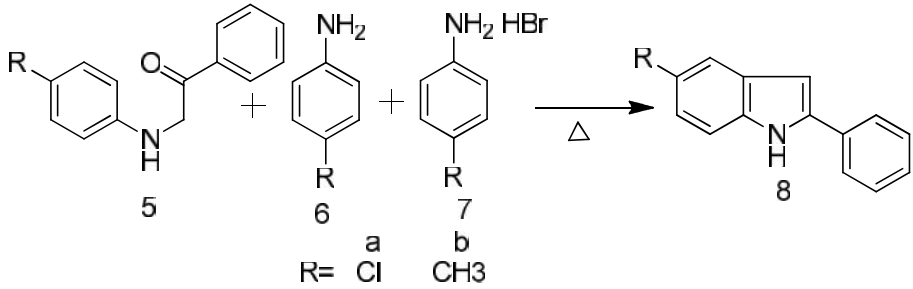
Table No.1 5-Substituted-2-phenylindoles



SCHEME-1

Comp.	R	M.P (^{0}C)	Yield (%)	Nature (Solvent)	Ref.
4	H	188-89	78	Light green needles (Benzene)	153
8a	Me	161-62	70	Colourless crystals (Benzene)	154
8b	Cl	181-82	70	Colourless crystals (Benzene)	154

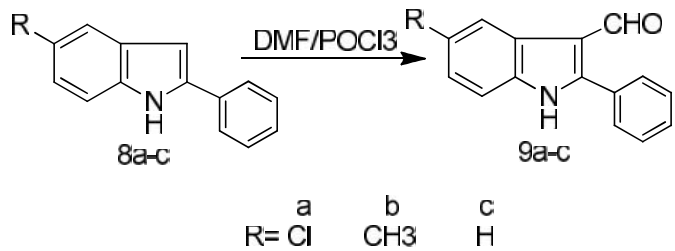
Similarly, various 5-substituted 2-phenyl indoles **8b-c** were prepared by thermal cyclization of respective phenacyl



SCHEME-2

aniline (Sheme-2).

5-chloro-2-phenyl indole **8a** on formylation by Vilsmeier Haack formylation method gave 5-chloro-2-phenyl indole-3-carboxaldehyde[155] **9a** (Sheme-3) (Table-2).



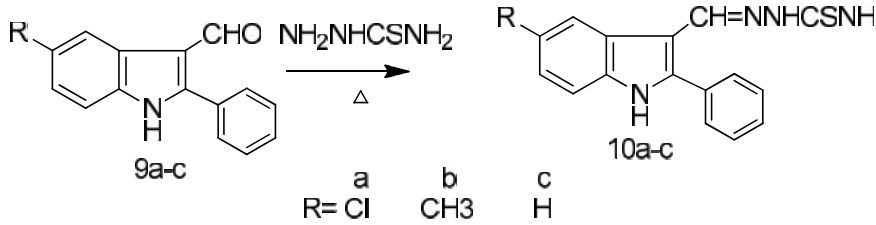
SCHEME-3

Table No.2 5-Substituted-2-phenyl Indole-3-carboxaldehydes (9a-c)

Comp.	R	M.P(^{0}C)	Yield (%)	Nature (Solvent)	Ref.
9a	Cl	284-85	71	Colorless Crystals (ethanol)	155
9b	CH_3	286-87	56	Colorless Crystals (ethanol)	155
9c	H	243-45	79	Colorless Crystals (ethanol)	155

5-Chloro-2-phenylindole-3carboxaldehyde **9a** on reaction with thiosemicarbazide in ethanol using catalytic amount of acetic acid under reflux condition for 5-6 hrs afforded 5-chloro-2-phenyl indole 3- thiosemicarbazone **10a**. The formation of **10a** was confirmed by its IR and ^{1}H NMR spectral studies. In its IR spectrum compound **10a** displayed absorption

bands at 3429/3381 cm^{-1} (double of unequal and medium intensities) due to NH_2 group of thiosemicarbazone moiety. The absorption band at 3271 cm^{-1} is attributed to indole NH, absorption peak at 3148 cm^{-1} is appeared as broad band but medium due to NH group of thiosemicarbazone residue. The absorption peak at 1591 cm^{-1} is appeared for C=N function, the sharp peak appeared at 1091 cm^{-1} is due to C=S of thiosemicarbazone and no absorption in carbonyl region indicating the absence of aldehyde group. In ^1H NMR spectrum the multiplet extending from δ 6.87-7.34 integrating for eight aromatic protons, the signals due to two protons of $CSNH_2$ resonated as singlet at δ 7.82, the sharp singlet at δ 8.12 integrating for one proton is attributed to azomethine proton, the down field broad singlet at δ 10.59 is due to CH=CS proton, the indole proton is resonated as δ 11.23 as sharp singlet. These spectral data are in agreement with the structure assigned for **10a**. Similarly, the compounds **10b-c** were prepared (Scheme-4) (Table-3).

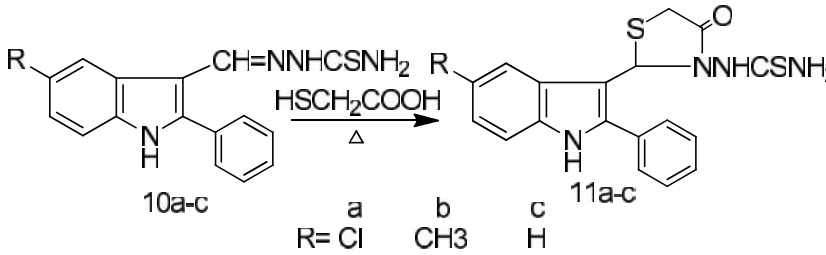


SCHEME-4

Table No.3 5-substituted-2-phenylindole-thiosemicarbazones (10a-c).

Comp	R	M.P (^0C)	Yield (%)	Nature (Solvent)	Mol. formula	Elemental Analysis. Calculated (%) (found)		
						C	H	N
10a	Cl	249-50	56	Pale yellow crystals (ethanol)	$C_{16}H_{13}N_4ClS$	58.44 (58.50)	3.98 (3.87)	17.04 (16.98)
10b	CH_3	255-56	79	Pale yellow crystals (ethanol)	$C_{17}H_{16}N_4S$	66.24 (66.28)	5.23 (5.18)	18.17 (18.18)
10c	H	220-22	71	Pale yellow crystals (ethanol)	$C_{16}H_{14}N_4S$	65.28 (65.25)	4.79 (4.83)	19.03 (19.15)

5-Chloro-2-phenylindole-3-thiosemicarbazone **10a** on cyclodehydration with thioglycolic acid in dry N,N-Dimethyl formamide with catalytic amount of anhydrous zinc chloride under reflux condition for 8 hrs, afforded 1-[2-(5'-Chloro-2'-phenyl-1H-indol-3-yl)-4-oxathiazolidin-3-yl]thiourea **11a**. The formation of compound **11a** was confirmed by its IR and ^{1}H NMR spectral studies. In its IR spectrum, the absorption peak 3414 cm^{-1} is attributed to NH_2 group, the absorption peaks at 3223/3110 cm^{-1} were assigned for NH of thiazole and the absorption peak at 1660/1608 cm^{-1} were appeared due to cyclic amide, the disappearance of bands at 1591cm^{-1} due to C=N group of **10a** clearly conforms the formation of **11a**. This is also supported by the ^{1}H NMR spectrum of **11a**. In the ^{1}H NMR spectrum sharp singlet at δ 2.8 integrating for two protons is attributed CO-CH_2-S, the multiplet appeared in the aromatic region at δ 7.03-7.59 integrating for eight aromatic protons, the singlet at δ 8.4 integrating for one proton of S-CH-N, the CS-NH_2 protons were resonated as a singlet at δ 8.9, the signal at δ 9.89 integrating for one proton of N-NH and singlet at δ 10.98 appeared due to indole NH. These spectral data are in agreement with structure assigned for **11a.** Similarly, the compounds **11b-c** were prepared (Sheme-5) (Table-4).

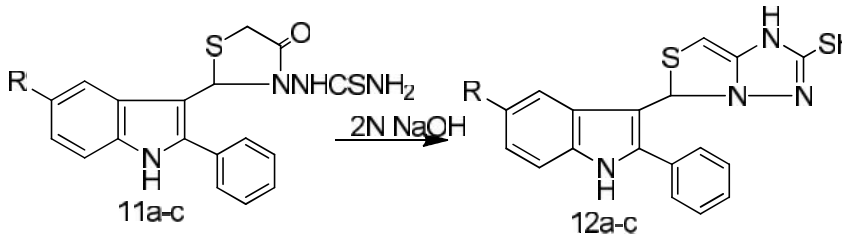


SCHEME-5

Table No.4 1-[2-(2-5-substituted-1H-indol-3-yl)-4-oxathiazolidin-3-yl]thiourea (11a-c).

Comp	R	M.P (^{0}C)	Yield (%)	Nature (Solvent)	Mol. formula	Elemental Analysis. Calculated (%) (found)		
						C	H	N
11a	Cl	220-22	63.0	Light yellow (ethanol)	$C_{18}H_{15}N_4OClS_2$	53.66 (53.62)	3.75 (3.90)	13.91 (14.03)
11b	CH_3	93-96	71.0	Light yellow (ethanol)	$C_{19}H_{18}N_4OS_2$	59.66 (59.78)	4.74 (4.92)	14.65 (14.86)
11c	H	235-39	69.0	Light yellow (ethanol)	$C_{18}H_{16}N_4OS_2$	58.67 (58.72)	4.38 (4.29)	15.21 (15.29)

1-[2-(5'-Chloro-2'-phenyl-1H-indol-3'-yl)-4-oxathiazolidin-3-yl]thiourea **11a** on cyclodehydration with NaOH (2N) in ethanol under refluxed condition for 8 hrs, afforded 5-(5'-chloro-2'-phenyl-1H-indol-3'-yl)-2-mercapto-1,5-dihydrothiozolo[4,3-b]-1,2,4-triazole **12a**. The formation of **12a** was confirmed by its IR and ^{1}H NMR spectral studies. IR Spectrum of compound **12a** showed absorption peak at 3383 cm^{-1} for indole NH, 3142 cm^{-1} peak absorption for triazole NH and C=S absorption peak at 1049 cm^{-1}. In the ^{1}H NMR spectrum, the multiplet extending from δ 6.96-7.53 accounting for eight aromatic protons and two protons of CH-S-CH= of thiazole ring. The singlet at δ 8.82 integrating for one proton may be assigned to NHCSNH protons and the indole NH was resonated at δ 11.15 as singlet. These spectral evidences substantiate the formation of compound **12a**. Similarly, the compounds **12b-c** were prepared (Sheme-6) (Table-5).

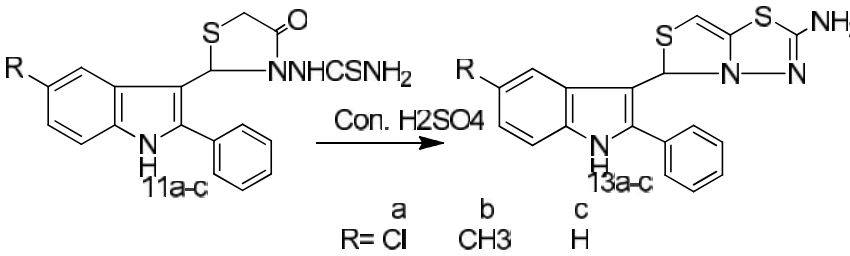


SCHEME-6

Table No.5 5-(2'-5'-substituted-1H-indol-3'-yl)-2-mercapto-1,5-dihydrothiozolo[4,3-b]-1,2,4-triazoles (12a-c).

Comp.	R	M.P (^{0}C)	Yield (%)	Nature (Solvent)	Mol. formula	Elemental Analysis. Calculated (%)(found)		
						C	H	N
12a	Cl	250-52	60.0	Pale yellow (ethanol)	$C_{18}H_{13}N_4ClS_2$	56.17 (56.29)	3.40 (3.45)	14.56 (14.49)
12b	CH_3	120-23	54.0	Pale yellow (ethanol)	$C_{19}H_{16}N_4S_2$	62.61 (62.53)	4.42 (4.38)	15.37 (15.26)
12c	H	193-95	65.0	Yellow (ethanol)	$C_{18}H_{14}N_4S_2$	61.69 (61.82)	4.03 (4.29)	15.99 (16.12)

Intramolecular chemoselective heterocyclizition of 1-[2-(5'-Chloro-2'-phenyl-1H-indol-3'-yl)-4-oxathiazolidin-3-yl]thiourea **11a** in conc. H_2SO_4 with stirring at 0-2^{0}C for 15 min, afforded 2-amino-5-(5'-Cloro-2'-Phenyl-1H-indol-3'-yl)-5H-thiazolo[4,3-b]-1,3-4-thiadiazole **13a**. The formation of **13a** was confirmed by its IR and ^{1}H NMR spectral studies. The IR spectrum of **13a** exhibited 3383 cm^{-1} absorption due to NH_2 group, 2916 cm^{-1}absoption peak due to NH and 1616 cm^{-1} absorption due to C=N function, respectively. In the ^{1}H NMR spectrum, the multiplet appeared in aromatic region at δ 6.93-7.51 integrating for eight protons of aromatic system and two protons of thiazole ring system, the sharp singlet at δ 8.8 integrating for two protons appeared due to the NH_2 functional group of thiadiazole, the singlet resonated at δ 11.17 can be assigned for the absorption of indole NH. These spectral data are in agreement with structure assigned for **13a.** Similarly, the compounds **13b-c** were prepared (Sheme-7) (Table-6).



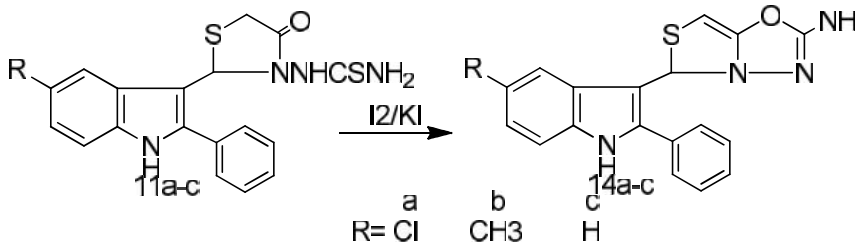
SCHEME-7

Table No.6 2-amino-(5-(2'-5'-substituted-1H-indol-3'-yl)-5H-thiazolo[4,3-b]-1,3-4-thiadiazoles (13a-c).

Comp	R	M.P (^{0}C)	Yield (%)	Nature (Solvent)	Mol. formula	Elemental Analysis. Calculated (%) (found)		
						C	H	N
13a	Cl	116-20	64.0	yellow powder (ethanol)	$C_{18}H_{13}N_4ClS_2$	56.17 (56.08)	3.40 (3.29)	14.56 (14.48)
13b	CH_3	183-85	68.0	yellow powder (ethanol)	$C_{19}H_{16}N_4S_2$	62.61 (62.32)	4.42 (4.52)	15.37 (15.45)
13c	H	160-63	61.0	yellow powder (ethanol)	$C_{18}H_{14}N_4S_2$	61.69 (61.72)	4.03 (4.23)	15.99 (16.09)

1-[2-(5'-Chloro-2'-phenyl-1H-indol-3'-yl)-4-oxathiazolidin-3-yl]thiourea **11a** on intramolecular chemoselective heterocyclizition with iodine in potassium iodide solution(5%) in ethanol refluxed for 2hrs yielded 2-amino-[5-

(5'-Chloro-2'-phenyl-1H-indol-3'yl)-5H-thiazolo[4,3-b]-1,3-4-oxadiazole **14a** the formation of **14a** was confirmed by its IR and ^{1}H NMR spectral studies. In IR spectrum of **14a** absorption at 3383 cm^{-1} due to NH_2 group, the absorption peak at 2916 cm^{-1} due to NH, the C=N functional group absorption at 1616 cm^{-1}were appeared. In the ^{1}H NMR spectrum appeared a sharp singlet at δ 8.80 integrating for two protons corresponds the NH_2 group of oxadiazole system. The multiplet at δ 6.93-7.51 in



SCHEME-8

aromatic region, integrating for eight aromatic protons and two protons of thiazole ring system, a singlet at δ 11.16 integrating of one proton of indole NH. These spectral evidences substantiates the formation of compound **14a**. Similarly, the compounds **14b-c** were prepared (Sheme-8) (Table-7).

Table No.7 2-amino-5-(2'-5'-substituted-1H-indol-3'yl)-5H-thiazolo[4,3-b]-1,3-4-oxadiazoles (14a-c).

Comp	R	M.P (^{0}C)	Yield (%)	Nature (Solvent)	Mol. formula	Elemental Analysis. Calculated (%) (found)		
						C	H	N
14a	Cl	128-30	61.0	Pale yellow powder (ethanol)	$C_{18}H_{13}N_4OClS_2$	58.61 (58.39)	3.55 (3.72)	15.19 (15.23)
14b	CH_3	120-24	65.0	Pale yellow powder (ethanol)	$C_{19}H_{16}N_4OS_2$	65.50 (65.86)	4.63 (4.72)	16.08 (15.95)
14c	H	238-39	69.0	Pale yellow powder (ethanol)	$C_{18}H_{14}N_4OS_2$	64.65 (64.36)	4.22 (4.39)	16.75 (19.98)

CHAPTER-4 ANTIMICOBIAL ACTIVITY

The development of resistance[156-160] among the various pathogenic organisms towards the antibiotics has increase the importance for investigating the new antimicrobial agents. The first, Antimicrobial drugs revolutionized in the treatment of certain protozoal infections particularly syphilis. The second major revolution in medicine is in which the antimicrobial drug has major role awaited the appearance of sulfanilamide and penicillin. Some bacteria like *Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumonia, etc*. Do not cause disease in normal human but they are opportunistic pathogens. They generally cause nosocomial disease [acquired during hospitalization]. Often these opportunistic bacteria are the cause of urinary tract infections. *P aeruginosa* is rod like and cams problems particularly in burn victums. *Klebsella pneumonia* is an encapsulated rod, often occurs, naturally in the respiratory tracts of humans. *Klebsella pneumonia* may be a primary disease or a secondary disease is characterized by gelatinous reddish brown spectrum causes pneumonia. The organisms grow on lung surface damaging the tissue, often causing death. *Staphylococcus aureus* also a rod like structure causes diarrhea by producing enterotoxin [toxin affect to gastrointestinal tract]. In order to reduce the severity of bacterial disease, it is essential to investigate new and novel antibacterial chemotherapeutic agents particularly for immuno-compromised patients.

Invasive mycoses are life-threatening infections for the immuno–compromised patients. *K. pneumonia* and *S. aureus* are the major pathogens[161]. Although triazole agents such as fluconazole and intrconazole are used to treat mycoses, their clinical efficacies are still limited. Candida strain resistant to amphotericin B[162] Ketoconazole[163] or flucytosine[164] have been reported.

Material and Methods:

Reliable test to pathogenic fungi for susceptibility to various antifungal drugs would be useful for several reasons. First, fungal infection represents significant clinical problems, particularly with the advent of aggressive immunosuppressive therapies. Second, the number of agents now available to treat fungal infections has increased and created a demand of *in vitro* methods to asses their potential *in vivo* efficacy. Third, resistance to antifungal agents has become evident. Due to these factors,

antifungal susceptibility test, which reliably predict *in vivo* response to therapy, would be valuable to investigators and practitioners.

Anti bacterial activity using cup – plate method:

The antibacterial activity of our synthesized compounds was studied comparatively with that of standard antibiotic, gentamycin[165] by cup plate method[174] using one gram positive and two gram negative organisms namely *S.aureus, K.Pneumonia and S.aureus.*

Material required

1. Medium A described in India Pharmacopoeia
2. Sterilized Petri dishes, pipettes, test tubes and beakers
3. 18 to 24hrs old growth culture in nutrient broth.
4. Sterilized test tubes.
5. Sterile 6mm cork borer
6. Sterile inoculation loops
7. Sterilized fine pointed forceps.
8. Nutrient agar.

Preparation of sub-culture:

Twenty four hours before the day of testing, the above mentioned bacteria were sub cultured separately into 25ml of sterilized nutrient broth, which was prepared in similar manner as that of nutrient agar media but without agar, inoculated sub cultured broth was kept at room temperature. The growth thus obtained was used as inoculums for the test.

Preparation of media

Bacteriological peptone [5 gm], Dextrose [1 gm], Sodium chloride [5 gm], Yeast extract [3 gm] and Agar [20 gm] were dissolved in distilled Water [1000 ml]. The pH was adjusts to 7 to 7.4 using 40% sodium hydroxide and sterilized at 15lbs pressure.

Sterilization of media [Glass wares]

The media used in the present study, nutrient agar and nutrient broth were sterilized in a conical flask of suitable capacity by autoclaving the same at 15 lbs pressure for 20min. The cork borer, glass wares, Petri dishes, test tubes and pipettes were sterilized by employing hot air oven at 160^{o} C for 1 hour.

Preparation of test solution

1. Gentamycin: 5mg of gentamycin was dissolved in 5ml of DMF [dimethyl formamide] to get a concentration of 1000 µg/ml.

2. Compound: 5mg of each the compound was dissolved in 5ml if DMF in serially and suitably sterile test tubes, thus giving a concentration of 1000 µg/ml.

Method of testing:

This method depend on the diffusion on antibiotic from a cavity through the solidified agar layer in a Petri dish to an extent such that growth of the added microorganisms is prevented entirely in a circular zone around the cavity containing a solution of antibiotic.

About 15 to 20 ml of molten nutrient agar was poured into each of the sterile plates. With the help of sterile cork borer, the cups were punched and scooped of the set agar. The agar plates so prepared are divided into different sets and each of the plates were inoculated with suspension of particular by spread plate technique particular organism by spread plate technique. The cups of inoculated plates were then filtered with 0.1ml of the test solution, gentamycin and solvent control DMF. The plates were allowed to stay for 24 hours at 38^0C to know the bacterial growth inhibition. The zone of inhibition developed, if any, was then measured for the particular organisms.

Table.8: Antibacterial activity of synthesized compounds (10-14)

Compound	Concentration (µg/ml).	Zone of Inhibition (in mm)		
		S.Aureus	*P. Aeruginosa*,	*K.Pneumonia*
10a	1000	09	14	10
10b	1000	10	11	14
10c	1000	11	12	15
11a	1000	13	12	16
11b	1000	14	12	15
11c	1000	16	14	15
12a	1000	12	14	10
12b	1000	12	13	11
12c	1000	10	13	11
13a	1000	11	14	15
13b	1000	12	15	16
13c	1000	15	15	14
14a	1000	09	09	09
14b	1000	11	10	11
14c	1000	11	11	10
Standard (Gentamycin)	1000	15	15	15

Key for interpretation	Zone of inhibition (in mm)
Inactive	less than 09
Weak active	09-11
Moderately active	12-14
Highly active	15 and above.

ANTIFUNGAL SCREENING USING CUP-PLATE METHOD.

The antifungal activity of indole analogues in comparison with that a standard antifungal drug fluconazole by cup plate method[174]. The fungal strains selected for this were *Aspergillus terrus, Aspergillus oryzae* and *Aspergillus nizer.*

Materials required

Cup plate diffusion method: Antifungal activity of the test compound was assessed against *A. terrus, A. oryzae* and *A. nizer* by cup plate diffusion method.

The following materials are used.

1. Sabouraud agar media.
2. Sterilized Petri dishes and pipettes of 0.1ml and 0.2ml
3. Cultures and nutrient broth
4. Sterilized test tubes containing solution of the test compound at known concentrations.

Preparation of Media

Twenty – four hours before the day of testing the above mentioned fungal strain were sub – cultured separately into 25ml of sterilized nutrient broth, which was prepared in similar manner as that of the nutrient agar media but without agar. Inoculated sub cultured broths were kept at room temperature. The growth thus obtained was used as inoculums for the test.

Sterilization of media and Glass wares

The media used in the present study, sabouraud agar media and nutrient broth, were sterilized in a conical flask of suitable capacity by autoclaving the same at 15 lbs pressure for 20 min. The cork borer, glassware's, Petri dishes, test tubes and pipettes, were sterilized by employing hot air oven at 160^0C for 1hr.

Preparation of solutions:

1. Fluconazole [standard drug] : 5 mg of fluconazole was dissolved in 5ml of DMF to get a concentration of 1000 µg/ml

2. Compound: 5mg of each test compounds was dissolved in 5ml of DMF in serially and suitably labeled sterile test tubes, thus giving final concentration of 1000 µg/ml.

Method of testing:

Streptomycin was added to the sterilized SDA media in order to prevent the bacterial contamination. Then 15-20 ml of molten SDA media was poured into the Petri plates and allowed to solidify. Fungal subculture was inoculated on the solidified on the media. With the help of 6mm cork borer, the cups were punched and scooped out the set agar. The cups of inoculated plates were then filled with 0.1ml of the test solution, fluconazole and solvent control DMF. The plates were allowed to stay for 24 hrs at room temperature and zone of inhibition was measured in m

Table.9: Antifungal activity of synthesized compounds (10-14)

Compound	Concentration (µg /ml).	Zone of Inhibition (in mm)		
		A. terrus,	*A. oryzae*	*A. nizer*
10a	1000	14	10	15
10b	1000	16	10	15
10c	1000	09	09	16
11a	1000	13	11	08
11b	1000	14	13	15
11c	1000	12	15	14
12a	1000	08	11	17
12b	1000	09	13	16
12c	1000	08	09	10
13a	1000	17	10	10
13b	1000	16	12	09
13c	1000	17	13	09
14a	1000	12	15	11
14b	1000	11	16	14
14c	1000	07	16	15
Standard (Fluconazole)	1000	15	16	15

Key for interpretation	Zone of inhibition (in mm)
Inactive	less than 09
Weak active	09-11
Moderately active	12-14
Highly active	15 and above.

ANTIHELMENTHIC

Anthelmentics are therapeutic agents used to destroy parasitic worms or remove them from the infected host. The majority of helminthes injections are acquired by contact with:

a. Injected animals.
b. Ground contaminated by human or animal excrement.
c. Water injected with cercanae.
d. Ingestion of injected meat.

The filarial worm requires arthropod vectors such as blood sucking mosquitoes, which transmit the parasite from one host to another.

Parasitic worms are dependent on human hosts for permanent existence. They must therefore, have some gaining asses to the body of the host and their offspring either eggs or larvae must have a means of escaping from the host's body to perpetuate the species Helminthes eggs are larvae generally are not able to produce an immediate injection in a new host. A period of time ranging from a few hours in case of oxyurids to several months with other parasites is necessary before the injective stage is reached. Before preventive care can be taken an extract knowledge of this critical period in the life cycle of Helminthes is essential.

It is estimated that over 800 million people in the world are suffer from the helminthiasis. Different types of helminthiasis were thought to be present in tropical and subtropical countries only but new helminthiasis is believed to be endemic in many parts of the world, where there is poor sanitation, poor living conditions, poor family hygiene and crowded living conditions. The treatment of helminthiasis is of great practical importance.

In addition, these diseases present a serious economic problem to the animal which is vulnerable to a large number of

parasitic worm injections. Next to Schistosomiasis, hook-worm diseases and ascaniasis are not prevalent serious human injections in animals. The serious helminthiasis are caused by flukes and round worms.

In the therapy of worm injections the following will be considered

a. The nature of the parasite.
b. The life cycle of the helminthes.
c. The site of injection.
d. The parasitic host.
e. The chemical agent to be used in treatment.

Zoological Classification:

Human and animal parasite worms are classified zoologically as follows:

a. Phylum platyhelminthes with the classes cestoda (tape worms) and trematoda (flukes).
b. Phylum nemathelminthes with the important class Nematoda (round worms), cestodes (tape worm) are flat worms.

The most serious tape-worm injections in man are caused by Diphylobothrium lactum (fish tape worm) which develops in dogs, cats and other fish eating mammals. Taenia solium (pork tape worm) and Taenia sagnato (beef tape worm), in these larval stages develop in pigs and cattles respectively. Hymenolepis none (dwary tapeworm) is the smallest adult tapeworm found in humans and Echinococcus granulosus is responsible for the larva that produces a hydatid cyst in man.

The most important flukes parasitic in man are 3 species of Schistosoma live in the mesenteric blood vessels are:

a. S. Mansoni
b. S. Haemotobium.
c. S. Japanicum.

The nematodes (round worms) are of higher organization than the flat worms. This includes such parasites of man as the hook worms (ancyclostoma deodenale nector amenicanus), the round worm (ascoris lubricoides), the whip worm (trichuris trichiura) and the pin worm (enterobius vermicularis). The filarial worms are also nematodes.

Anthelmintic activity is tested in vitro on the helminth like round worms mounted on organ both. The experimental study is unfortunately of limited significance as it is virtually impossible to stimulate the environment in which the parasite exists in the host. In vivo studies are carried out in experimental injections in animals like rat, mouse, cat, dog and finally man.

Anthelmintic activity was tested on earth worms (pheretima posthuma). The technique adopted was in vitro that of Grained et al, with modification.

Although chemotherapy of intestinal helminthiasis has made a remarkable progress during the last three decades[14-23], still lack of an ideal drug which can be used to eradicate, most of the at helminth worms inhabitation the digestive track of man.

Anthelmentic Testing Procedure:

The ultimate test of anthelmentic activity is the ability of a chemical agent to eliminate from a specifically parasitized animal with a minimum toxic effect is the host. Although at one time, a suitable in vitro test was considered a useful screening method, current thinking is directed towards in vivo screening. In vivo screening methods enable the investigator to observe the potency of various drugs on the parasite in its natural environment, thereby presenting a true picture of anthelmenting effect. With this procedure the number and condition of ova, eliminated before and after during administration can be recorded and compared with controls. After completion of dosage, laboratory examination of test animal may reveal migration of immature and mature worms of each sex, the number of parasites remaining in the host dead or alive, and any physiological changes in the host or helminth. This is also possible means of ascertaining in a general way, the mode of drug action. In many instances, laboratory animals with natural injections harbour more than one type of helminth. This is highly useful in determining the spectrum of an experimental drug. The possibility that the drugs found active against animal helminthesis may retain their activity. When used in human therapy should also be included in establishing a rational for in vivo screening. Even though, exceptions to this role are to be expected, it is as serviceable in the field of anthelmentics as it is in the field of pharmacodynamics.

Disadvantages that arise from the use of this method are the expense of maintaining large colonies of test animals, the increased quantities of synthetic chemicals required for testing and the time consumed in treating the animals at evaluation the results. Some of these difficulties can be offset by selecting relatively inexpensive experimental animals having multiple injections. The more promising drugs uncovered in initial screening can then be retested in larger animals.

The technique used for parasitic laboratory animals have been well developed, as have the methods employed in determining anthelmintic activity. Most of the parasitic injections can be produced in small test animals by oral or intraperitoneal administration of the injective stage of the parasite. In many cases parasitization can be avoided by using naturally injected animals. The degree of antiparasitic activity elicited by a chemical agent is usually determined as follows:

1. Ova count reduction or disappearance of ova from the animal excrement.
2. Passage of worms.
3. Examination of blood samples.
4. Migration of parasite within the host to an organ in which it is destroyed.

An extensive review of in vivo screening procedures against all types of helminthes has been given by standen. In vivo screening procedures offer a rapid means of deforming parasiticidal activity with minimum expenditure of drug, but extrapolation to the human or animal injection is often subject to give doubt. When suitable culture techniques are available, in vitro test has a positive part to play in biochemical and pharmacological investigations.

Materials and Methods:

1**. Normal Saline:**

Sodium chloride (9g) was exactly weighed and dissolved in sufficient distilled water to make one liter (0.9%).

2. **Albendazole solution:**

Appropriate quantity of accurately weighed Albendazole powder was dissolved in 20ml of Tween 80 suspension.

3. **Tween – 80 solution:**

Tween – 80 (0.2ml) was dissolved in small quantity of normal saline and volume was adjusted to 100ml with normal saline to get Tween-80 solution, which was used as blank.

4. **Suspension of test compound:**

Suspension of test compounds, 10mg / ml, was prepared in Tween-80 solution.

Experimental Procedures:

Earthworms (Pheretima postnuma) were collected from the fields. These worms were thoroughly washed with normal saline to remove the adhering materials and then kept in a ash containing normal saline. Petri dishes of equal size were arranged, 20 ml of normal saline was poured in first Petri dish and 20ml of tween-80 solution was poured in second Petri dish which was used as blank.20 ml of albendazole were poured in Petri dishes having concentration 20mg/ml. The suspension of the compounds under test in tween-80 were made up to 20ml to get the concentration of 20mg/ml concentration of each compounds poured in Petri dishes. Six earth worms of nearly equal size were placed in each Petri dish and time taken for complete paralysis and the death of earthworms in each Petri dish was recorded and tabulated. All the Petri dishes were observed for 2 hours. Time required by the test compounds to produce paralysis and death was compared with that of albendazole

Anthelmintheic Activity:

Compd	Concentration mg/ml	Time taken for paralysis and death of earth worms in min.		Observation
		Paralysis	Death	
10a	1	NP	ND	3 hrs
10b	1	95	ND	3 hrs
10c	1	90	ND	3 hrs
11a	1	NP	ND	3 hrs
11b	1	85	160	3 hrs
11c	1	NP	155	3 hrs
12a	1	120	ND	3 hrs
12b	1	80	160	3 hrs
12c	1	75	115	3 hrs
13a	1	NP	ND	3 hrs
13b	1	90	165	3 hrs
13c	1	95	170	3 hrs
14a	1	NP	ND	3 hrs
14b	1	60	120	3 hrs
14c	1	65	120	3 hrs
Standard	1	55	115	3 hrs

Standard: Albendazole, Control: 1% tween-80, * NP- No paralysis, ND- No death

CHAPTER-5 Conclusion

The following generalizations on the basis of the results obtained could made:

Antibacterial results, the compounds **10c, 11a, 11b, 11c, 13a** and **13b** shows highly active, where as compounds tested **10b** and **13c** shows moderately active, while other compounds are weakly active against *K. pneumonia* The compounds **13b** and **13c** shows maximum zone of inhibition, while other compounds **1a**, **10c**, **11a**, **11b**, **11c**, **12a**, **12b**, **12c** and **13a** shows moderatively active and other compounds weakly active against *P. aeruginosa*. The compounds **11c** and **14c** exhibited high activity, where as **11a**, **11b**, **14b** and **13b** showed moderatively active and other compounds are weakly active against *K. Pneumonia.* Antifungal results compounds **11b**,**13a**, **13b**, **13c** showed high activity, where as compounds **10a**, **11b**, **11c** and **14a** showed moderate activity, while other compounds were weakly active against *A.terrus.* The compounds **11c**, **14a**, **14b** and **14c** exhibited maximum zone of inhibition , where as compounds **11b**, **12b**, **13b** and **13c** were moderatively active, while other compounds shown weak activity against *A.oryzae*. Amongst the compounds tested for their antifungal activity **10a**, **10b**, **10c**, **11b**, **14c, 13a** and **13b** were highly active, where as compounds **11c** and **14b** were moderatively active, while other compounds showed weak activity against *A.niger*. Amongst the compounds tested for anthelmintic activity **14b** & **14c** shows good anthelmintic activity when compared to standard where as the activity of compounds **11b, 12b** & **13b** shows moderate anthelmintic activity remaining of the compounds shows weakly active and inactive. From the above experimental observations it is broadly concluded that, the indole moiety is very promising molecule for the biologically active nucleus.

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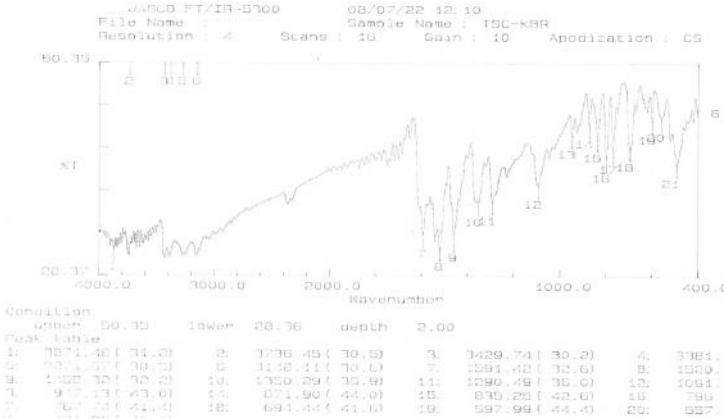
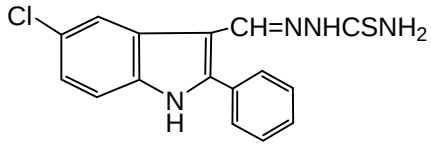
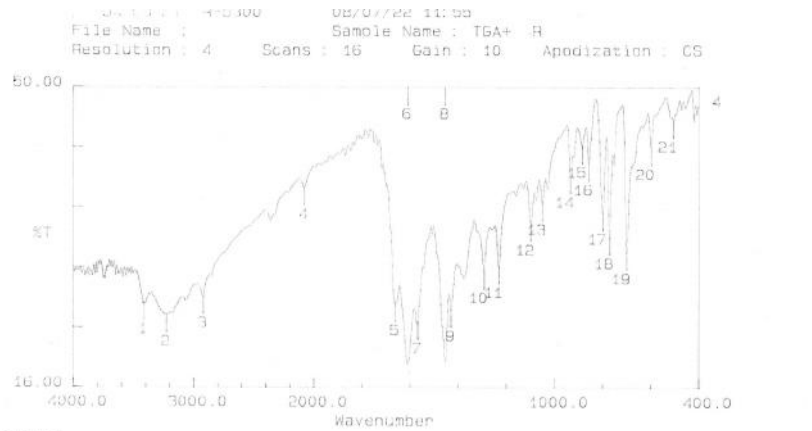
Additional Data: Spectra



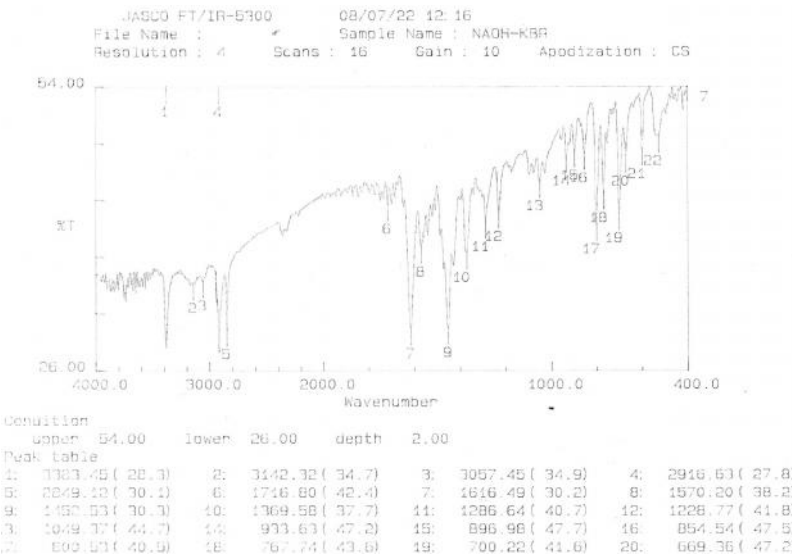


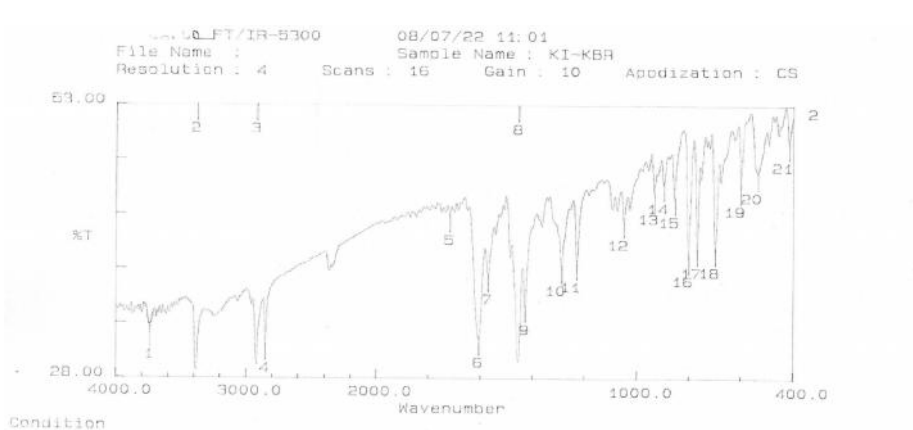
10a



ndition
Upper 50.00 lower 16.00 depth 2.00
ak table

3414.31(25.7)	2:	3223.34(24.3)	3:	2916.63(26.4)	4:	2077.52(38.7)	
1660.86(25.5)	6:	1608.78(18.7)	7:	1568.27(23.5)	8:	1452.53(19.0)	
1429.78(24.9)	10:	1290.49(29.2)	11:	1228.77(29.6)	12:	1097.60(34.5)	
1049.37(37.0)	14:	933.63(40.1)	15:	883.48(43.4)	16:	856.47(41.4)	
798.60(35.9)	18:	769.67(33.1)	19:	698.29(31.4)	20:	596.06(43.2)	


JASCO FT/IR-5300 08/07/22 12:16
File Name :
Sample Name : NAOH-KBR
Resolution : 4 Scans : 16 Gain : 10 Apodization : CS
54.00
%T
26.00
4000.0
3000.0
2000.0
1000.0
400.0
Wavenumber
Condition
upper 54.00 lower 26.00 depth 2.00
Peak table
1: 3383.45(28.3) 2: 3142.32(34.7) 3: 3057.45(34.9) 4: 2916.61(27.8
5: 2849.12(30.1) 6: 1716.80(42.4) 7: 1616.49(30.2) 8: 1570.20(38.2
9: 1450.53(30.3) 10: 1369.58(37.7) 11: 1286.64(40.7) 12: 1228.77(41.8
13: 1049.37(44.7) 14: 933.63(47.2) 15: 896.98(47.7) 16: 854.54(47.5
17: 800.53(40.9) 18: 767.74(43.6) 19: 700.22(41.6) 20: 669.36(47.2

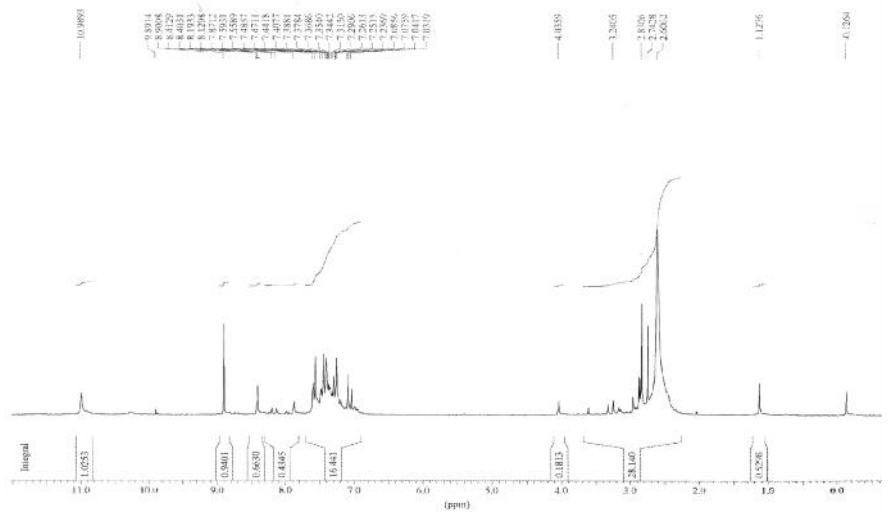


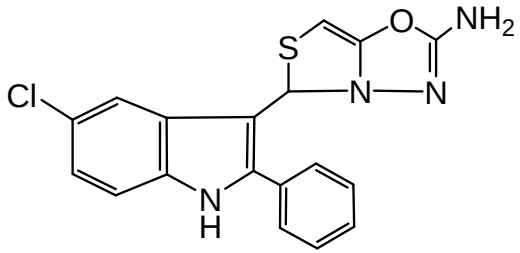
Condition

upper 53.00 lower 28.00 depth 2.00

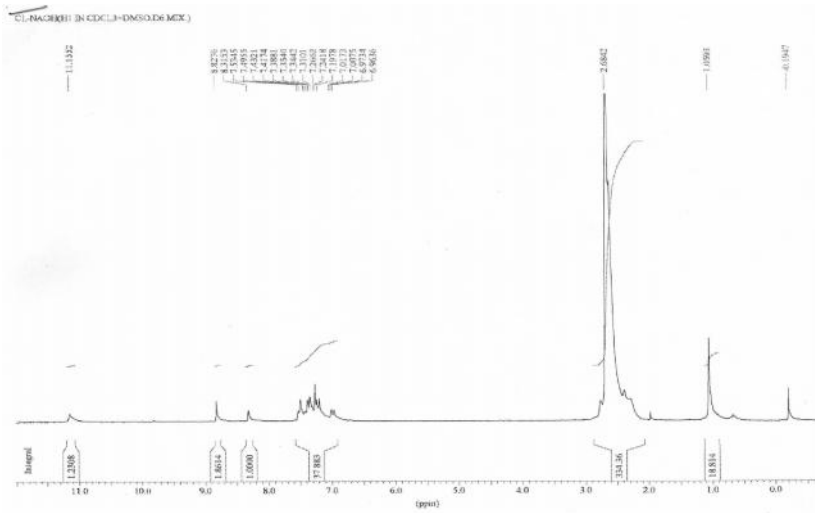
Peak table

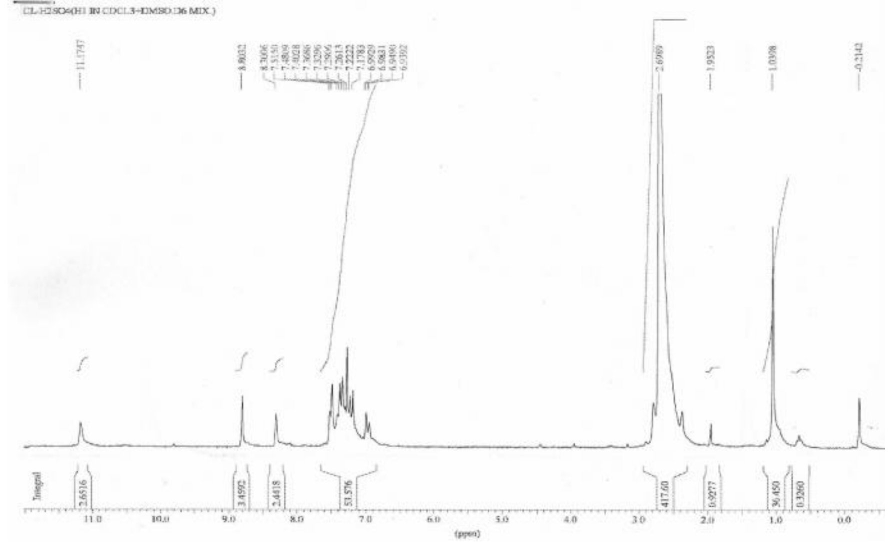
1:	3736.45(32.3)	2:	3383.45(28.8)	3:	2916.63(29.2)	4:	2849.12(31.1)
5:	1716.80(42.9)	6:	1604.92(31.7)	7:	1570.20(37.5)	8:	1454.46(29.6)
9:	1427.45(34.7)	10:	1286.64(38.3)	11:	1228.77(38.6)	12:	1049.37(42.5)
13:	933.63(44.9)	14:	896.98(45.9)	15:	854.54(44.6)	16:	800.53(39.2)
17:	767.74(40.0)	18:	698.29(40.0)	19:	599.92(45.6)	20:	534.33(46.8)

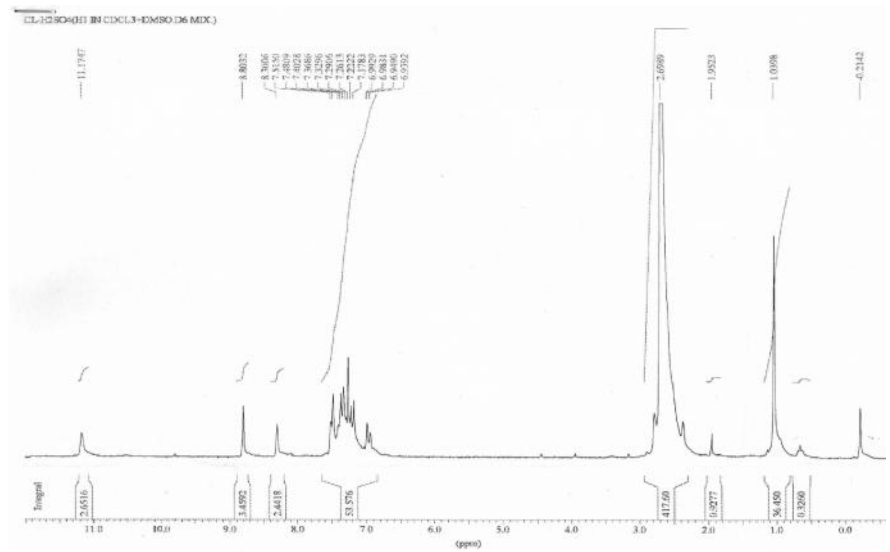




14a




CL-H2SO4(H1 IN CDCL3+DMSO D6 MIX.)
11.1747
8.8032
2.6989
1.9523
1.0398
-0.2142
Integral
2.6516
3.4592
2.4418
53.576
417.60
0.9277
36.450
0.3260
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
(ppm)

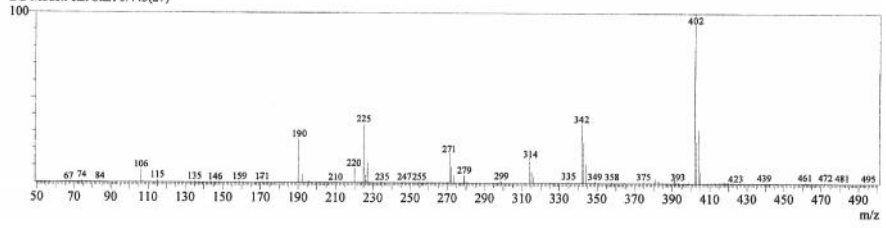


11.1747
8.8032
8.3606
7.5150
7.4809
7.4028
7.3686
7.3296
7.2906
7.2613
7.2222
7.1783
6.9929
6.9831
6.9490
6.9392
2.6989
1.9523
1.0398
-0.2142
Integral
2.6516
3.4592
2.4418
53.576
417.60
0.9277
36.450
0.3260
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
(ppm)

LCMS-2010A DATA REPORT
SHIMADZU

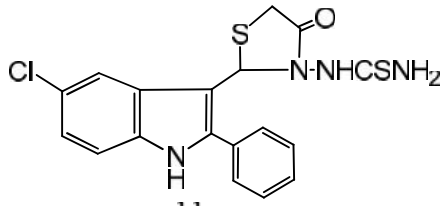
MS Spectrum

Line#:1 R.Time:0.631(Scan#:38) Positive
MassPeaks:302 BasePeak: 402.90(359086)
RawMode:Single 0.631(38)
BG Mode:Peak Start 0.443(27)



MS Peak Table

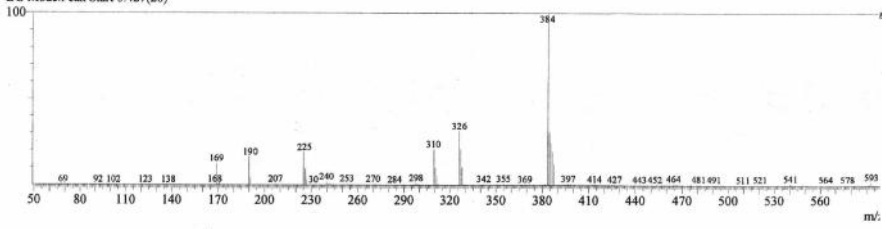
Peak#	R.Time	I.Time	F.Time	Area	Height	A/H	Mark	%Total	Name	Base m/z	Base Int.
1	0.631	0.443	0.843	9735356	931487	10.45		100.00		402.90	359086
				9735356	931487			100.00			



11a

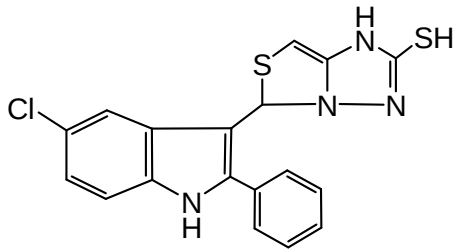
LCMS-2010A DATA REPORT
SHIMADZU

Line#:1 R.Time:0.656(Scan#:40) Positive
MassPeaks:364 BasePeak:384.70(945955)
RawMode:Single 0.656(40)
BG Mode:Peak Start 0.427(26)



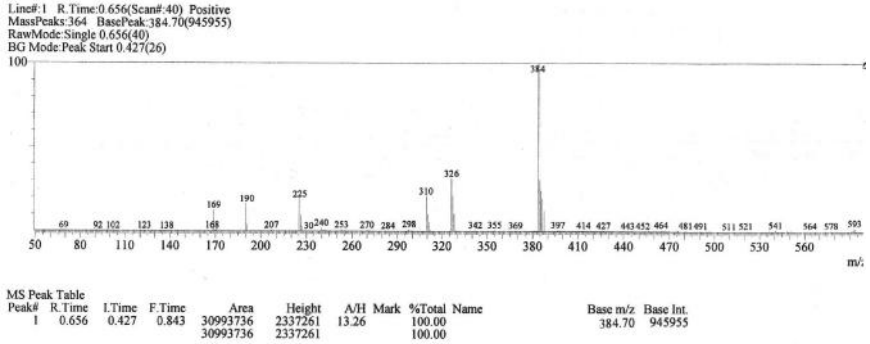
MS Peak Table

Peak#	R.Time	I.Time	F.Time	Area	Height	A/H	Mark	%Total	Name	Base m/z	Base Int.
1	0.656	0.427	0.843	30993736	2337261	13.26		100.00		384.70	945955
				30993736	2337261			100.00			



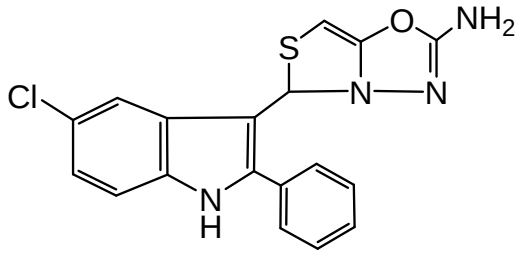
12a

LCMS-2010A DATA REPORT
SHIMADZU



MS Peak Table

Peak#	R.Time	I.Time	F.Time	Area	Height	A/H	Mark	%Total	Name	Base m/z	Base Int.
1	0.656	0.427	0.843	30993736	2337261	13.26		100.00		384.70	945955
				30993736	2337261			100.00			

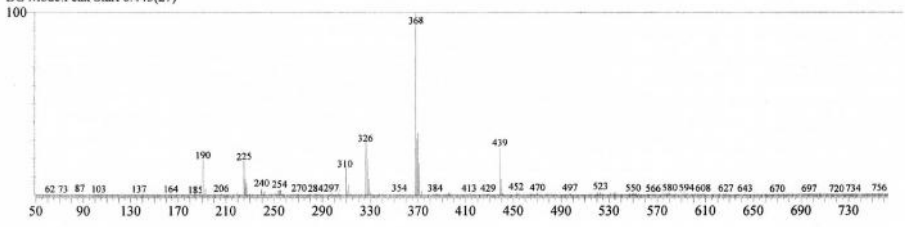


14a

LCMS-2010A DATA REPORT
SHIMADZU

MS Spectrum

Line#:1 R.Time:0.627(Scan#:38) Positive
MassPeaks:531 BasePeak:368.55(469529)
RawMode:Single 0.627(38)
BG Mode:Peak Start 0.443(27)



MS Peak Table

Peak#	R.Time	I.Time	F.Time	Area	Height	A/H	Mark	%Total	Name	Base m/z	Base Int.
1	0.627	0.443	0.843	15657983	1579388	9.91		100.00		368.55	469529
				15657983	1579388			100.00			