CHAPTER 2

LITERATURE REVIEW
Literature Review

Chapter 2

Literature Review

2.1. History of azo compounds

In early nineteenth, the dyes were obtained from natural sources for coloring the fabrics. Mauveine was the first synthetic dye synthesized in 1856. By 1970 nearly 60% of the dyes available were synthetic and prepared by diazotization mechanism. Azo compounds influenced a largest portion of synthesized organic compounds as they are very successful in drugs, dye and cosmetics. These molecules are better stable in a wide range of pH than natural dyes and even thermo stable (Awale et al., 2013). Azo compounds are synthesized by diazotization reaction of a primary aromatic amine and coupled with one or more nucleophiles, mostly an amino, active methylene and hydroxyl group (Olayinka et al., 2013). They bear functional group R-N=N-R', where R and R' can be either an aryl or heteroaryl group. The -N=N- represents as azo group. The coloring properties of those azo molecules even lasts long often exposed to light and oxygen.

Heterocyclic amines bearing dyes have pronounced bathochromic shift (Liang and Wang, 2008). Not only for coloring properties but also azo molecules are popular for their therapeutic uses and such as antiseptics (Browning et al., 1926), antimicrobial (Tiwari et al., 2012; Khalid et al., 2008; Keshavayya et al., 2013, Piste et al. 2012; Sener I & Aydi G, 2014; Malar and Abbs, 2012; Phatangare et al., 2013; Das et al., 2015; Chandrasekharan and Nagarajan, 2005; Mohammadi, 2014; Ingaral et al., 2007; Avci et al., 2012; Deshmukh et al., 2009), antidiabetics (Garg and Praksh, 1972), antitumor (Sava et al., 1982), antineoplastics (Child et al., 1977; Foye and Jeferey, 2006), transmissible spongiform encephalopathy.
Literature Review

(Rudyk et al., 2003), antiulcerative (Carceller et al., 2001) antioxidant (Manojkumar et al. 2009), analgesic (Oruc et al., 2006), anti inflammatory (Gaikwad et al. 2010), anti viral (Tonelli et al., 2009), antitubercular (Sah and Oneto, 1950) and antitumor (Thoraya and Abdallah, 2008; Wafaa et al. 2014) activities. Azo compounds are also involved in a number of biological reactions such as inhibition of DNA, RNA and protein synthesis, carcinogenesis and nitrogen fixation (Mariam et al., 2014).

Incorporation of suitable heterocyclic moieties enhances the biological potency of azo linked compounds (Shridhar et al., 2012). Azo dyes are more accepted in food processing units due to their low toxicity, no hyperactivity effect and less allergic reactions (ex- tartrazine, azorubin etc.) (Eigenmann and Haengelli, 2004). Azo molecules have their importance in various fields including textile, reprography, dye sensitizing solar cells, lasers, liquid crystalline displays, electro-optical devices and metalochromic indicators (Marchevsky et al., 1985; Peters and Freeman, 1991; Wang et al., 2000; Catino & Farris, 1985; Katz et al. 1987; Taura et al., 2014). Literatures revealed that azo bearing molecules have pesticidal activities (Samadhiya & Halve, 2001). They also used for manufacturing of cosmetics (Marmion, 1999). It was found to be observed from a lot of literatures that azo bearing ligands have modified therapeutic effect when they were combined with transition metallic ions (Abou-DobaraI et al., 2014; Esin, 2009).

Cobalt, copper, nickel and zinc are the potentially used metal ions that form low molecular weight complexes which found to be effective against various diseases. Literature support even suggests that metal complexes are more active than their ligands because the metal complexes serve as vehicle for activation of the ligands as principal cytotoxic species (Petering, 1973; Hankare et al., 2001)
Biological aspects of metallic ligands depend upon the ease of cleaving the bond between metal ion and ligand. Hence it is necessary to study the relationship between ligand and metal ion in biological system (Zahid et al., 2010). The complexes of transition metal having significant biological actions including antibacterial, antifungal and anticancer activities (Refat et al., 2013). It is well known that metal present in complexes generally accelerate the drug action and efficacy of therapeutic agents and the pharmacological efficiencies of drug based metal complexes depend upon nature of the metal ion and ligands (Siddiqi et al., 2010).

2.2. Biological activity of some azo compounds

2.2.1. Antioxidant activity of some azo compounds

At present researchers take more interest in searching of new antioxidant molecules both from synthetic and natural source. Synthetic antioxidants were often observed to be more effective than that of natural antioxidants (Ningappa et al., 2008). The innate defense may not sufficient for severe or continued oxidative stress so exogenous antioxidants are required to quench the requirements (Kareti et al., 2011). ROS (reactive oxygen species) can react with lipids, proteins, nucleic acids and various metabolic enzymes and can cause various diseases like atherosclerosis, respiratory disease, cancer etc. Antioxidants can terminate these chain reactions by removing free radical intermediates and can able to save the life of the cell by preventing its further oxidation and also supplements to prevent mortality (Goran et al., 2013). Antioxidants are the reducing agents such as thiols, ascorbic acid or polyphenols (Sies H, 1997) prevent transferring of electrons or hydrogen and thus, prevent oxidation and have been reported for exhibiting a wide range of biological activities i.e. prevention of radical-mediated cyto-toxicity, lipid peroxidation, and oxidation of low density lipoproteins (Mandal et al., 2009).
Fourteen new compounds of 1-(4-methylcoumarinyl-7-oxyacetyl) - arylazo pyrazoles were synthesized by the mixture of 3-arylazo- 2,4 pentadione and 4-methylcoumarinyl-7- oxyacetic acid hydrazide in glacial acetic acid and the synthesized compounds were evaluated their antioxidant activity by 2,2-diphenyl-1- picryl hydrazyl model. The compounds 7-((3,5-dimethyl-4-(p-tolyldiazenyl)-1H-pyrazol-1-y1)methoxy)-4-methyl-2H-chromen-2-one (3b) and 7-((3,5-dimethyl-4-(phenylldiazenyl)-1H-pyrazol-1-y1)methoxy)-4-methyl-2H-chromen-2-one (3c) were showed 50% antioxidant activity (Manoj kumar et al., 2009).

A series of heterocyclic azo dyes were synthesized by coupling of various nucleophiles viz. 8-hydroxyquinoline, 2, 6-diaminopyridine, 2-naphthol, N, N-dimethyl aniline, resorcinol, and 4, 6-dihydroxypyrimidine with 5-phenyl-1, 3, 4-thiadiazole-2-amine. The synthesized compounds were subjected to evaluation of their in-vitro antioxidant activity followed by DPPH and metal chelating model. The diazotized 5-phenyl-1, 3, 4-thiadiazole-2-amine was coupled with 8-hydroxyquinoline showed maximum antioxidant capacity than 1, 3, 4-thiadiazole azo dye coupled 2-naphthol. (Chinnagiri et al., 2013).
A series of novel pyrazole derivatives were synthesized by diazotization of 3-aminopyrazole. Antioxidant activity of the compounds was investigated from the bleaching action of ABTS derived radical cations. The compounds -2-iminopropane- hydrazonoyl cyanide (4), diazenyl)-2,3-dimethyl-1-phenyl-1,2-dihydropyrazol-5-one (20) and 2,3-dimethyl-1-phenyl-1,2-dihydropyrazol-5-one (21) substituted pyrazole showed good to moderate antioxidant activity in comparison to standard ascorbic acid (Metwally et al, 2012).

3, 5-Bis (alkyl-1, 3, 4-oxadiazole-2-yl) azo dyes were synthesized by a multi-step reactions and compounds were screened for their in- vitro antioxidant properties by DPPH
model. The compound 4b: (CH₂)₂CH₃ and 4c: (CH₂)₁₀CH₃ substituted 3, 5-Bis (alkyl-1,3,4-oxadiazole-2-yl azo dyes showed significant antioxidant activity (Shridhar et al., 2012).

![Chemical Structure](image.png)

**Scheme**

### 2.2.2. Cytotoxic activity of some azo compounds

On the basis of reports of WHO survey, cancer will be the first cause of death in the globe in future (Hoffman, et al., 2014). Nowadays research is going on to develop novel compounds that can able to stop the growth of cancer cell.

Nine tautomeric azo hydrazone compounds of N-(3-ethoxycarbonyl-4,5,6,7-tetrahydrobenzo[b]thien-2-yl)-2-aryl hydrazono-3- oxobutanamide derivatives were synthesized by coupling with diazotized aniline derivatives. The *in vitro* cytotoxic study was conducted using *Ehrlich Ascites Carcinoma* tumor cells (EAC). The compounds -(3-Ethoxycarbonyl-4,5,6,7-tetrahydrobenzo[b]thien-2-yl)-2-(4-methylphenyl hydrazono)
Literature Review

Study on medicinal interest of synthesized azo based heterocyclic compounds

oxobutanamide (4b), (3-Ethoxycarbonyl-4,5,6,7-tetrahydrobenzo[b]thien-2-yl)-2-(4-chlorophenyl hydrazono)-3-oxobutanamide (4e) and (3-Ethoxycarbonyl-4,5,6,7-tetrahydrobenzo[b]thien-2-yl)-2-(3-chlorophenyl hydrazono)-3-oxobutanamide (4f) and -(3-Ethoxycarbonyl-4,5,6,7-tetrahydrobenzo[b]thien-2- (3) showed high degree of antitumor activity against Ascites Carcinoma tumor cells (Thoraya and Abdallah, 2008).

![Scheme](image_url)

A series of new arylazothiophene and arylazopyrazole derivatives were synthesized using starting material 1-phenylbutane-1,3-dione was as key intermediate. The newly synthesized compounds were evaluated for in vitro cytotoxicity against an Ascites Carcinoma tumor cells and in vivo cytotoxicity for compound 10d using Ehrlich Ascites Cells (EAC) assay and 5-fluorouracil is used as reference drug. Compound 10d is more effective and showed the highest activity (Ali Fadda et al., Pharmacology and Pharmacy, 2012).

![Scheme](image_url)
By coupling compound 5c and 5d with benzenediazoniumchloride the compound 5,5'-Bis(phenylazo)-2-(2-hydroxybenzamido)ethyl-2-hydroxybenzoate (10c) and 5,5'-Bis(phenylazo)ethane-1,2-diyl-bis(2-hydroxybenzamide) (10d) was obtained subjected to evaluate their in vitro cytotoxicity activity against MCF7, human breast adenocarcinoma ER+, MDA-MB-231, human breast adenocarcinoma ER-, PC3, prostate cancer, HeLa S3, cervix epithelioid carcinoma, Hs 294T, human melanoma, K562, chronic myelogenous leukemia, as well as MRC-5, normal fetal lung fibroblasts by standard SRB assay method. The bis-derivatives with phenylazo groups, 10c and 10d, showed strong cytotoxicity, especially against three cell lines: MCF7, MDA-MB-231 and PC3. However compound 10c and 10d showed high cytotoxicity against MCF7 and K562 cells respectively (Djurendic et al., 2011).

<table>
<thead>
<tr>
<th>Comp</th>
<th>R</th>
<th>X</th>
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<tbody>
<tr>
<td>5c</td>
<td>H</td>
<td>O</td>
</tr>
<tr>
<td>5d</td>
<td>H</td>
<td>N</td>
</tr>
<tr>
<td>10c</td>
<td>-</td>
<td>O</td>
</tr>
<tr>
<td>10d</td>
<td>-</td>
<td>NH</td>
</tr>
</tbody>
</table>

A series of azopyrimidine derivatives were synthesized from 4-Oxo-2-thioxo-1,2,3,4-tetrahydropyrimidine (2-thiouracil). The compounds were tested for in vitro anticancer...
activity. The compounds substituted at 5 position of 2-thiouracil and 2-uracil showed good anticancer activity (Fathalla et al., 2006).

A series of 2-piperazinoethylamine azo analogues (3a-3f) were synthesized with different coupling components. The anti proliferative activity of the synthesized analogues was investigated over human breast cancer (MCF7), human glioma (U373) and astrocytoma brain tumor (C6 rat glioma) cell lines. The potential effects on cell viability were investigated by using the MTT assay method. By measuring the levels of surviving cells after incubation for 24 h with the test samples the activity was investigated by MTT colorimetric assay based on the ability of metabolically active cells to convert the pale yellow MTT to a blue formazan product which is further analyzed spectrophotometrically. The compound 3c and 3e showed more potent antiproliferative activity due to substitution of indole and pyridine respectively (Karthik et al., 2015).
A pyridine analogue of Dacarbazine was synthesized by adding the solution of sodium nitrite to the solution of 3-aminopyridine in concentrated HCl acid. The solution of sodium carbonate and dimethyl amine was added to the above prepared mixture. EC$_{50}$ values were determined by using accelerated cytotoxicity mechanism screening (ACMS) technique. It was observed that dacarbazine congener (compound III) showed two times more potent activity (Amirmostofian et al., 2013).

2.2.3. Anthelmintic activity of some azo compounds

The anthelmintics are the agents which expel the helminths or intestinal parasites by killing or stunning them without causing any harm to the host. The infection due to helminths is called helminthiasis. The report of WHO expert committee suggested that the annual death due to soil transmitted helminthiasis is more than 135,000 (Yap, P et al., 2012). Helminths harm the host by causing blood loss, injury to organs, intestinal or lymphatic obstruction and
by secreting toxins. Helminthiasis is rarely fatal but a major reason of ill health. New synthetic compounds are needed to be developed to overcome the problems related to drug resistance.

A series of 5-nitro salicylic acid based azo dyes were prepared and their anthelmintic activity was studied on Indian earth worm Pheretima posthuma. The azo derived compounds showed significant anthelmintic activity in comparison to standard drug piperazine citrate (Raghavendra and Ajay Kumar, 2013).

A series of seven novel azo derivatives of dihydropyrimidinones were synthesized by coupling various aryl diazonium salts. The anthelmintic activity of the synthesized compounds was carried out on Indian earth worm Pheretima posthuma. The compounds 4b: (2-napthol substituted dihydropyrimidinone), and 4c: (5- hydroxy Coumarin substituted dihydropyrimidinone) showed good anthelmintic activity. Whereas, 8-hydroxy quinoline and 2,6-dichloro phenol substituted dihydropyrimidinones showed moderate anthelmintic activity (Shaikh and Meshram, 2015).
2.2.4. Cholinesterase inhibitory effect of some azo compounds

Acetylcholine is an important neurotransmitter for memory. The cholinesterase enzyme helps to break down acetylcholine in the brain. Anti cholinesterases prevent the hydrolysis of acetylcholine in brain. Cholinesterase inhibitors result in higher concentrations of acetylcholine, leading to increased communication between nerve cells. Anti-cholinesterases are now days popular for treatment of Alzheimer’s disease, glaucoma, postural tachycardia syndrome and cognitive impairments in patients with schizophrenia (Choi et al., 2013).

Two series of azo coumarin analogues were synthesized. In series I, coupling of coumarin and 4-methyl coumarin was done with diazotized metoclopramide to obtain the azo compounds and in series II coupling of 7-aminocoumarin and 7-amino 4-methyl coumarin was done with diazotized diphenhydramine to obtain the azo analogues. The newly synthesized azo analogues were subjected to investigation of their in vitro cholinesterase inhibitory effect and protection ability against chlorpyrifos by modified Elman electrometric method. Diphenhydramine derivatives with coumarin show more protective ability for both plasma ChE (BchE) and erythrocyte ChE (AchE) as the compound 3 (7-aminocoumarin conjugated diphenhydramine azo compound) showed the maximum protection for all
Literature Review

Concentration while Metoclopramide derivatives (compound 1) with coumarin show selectivity protection for ChE against chlorpyriphos inhibitory effect as one derivative protected BchE and increased the inhibition of the AChE (Mahmood et al., 2014).

![Compound 3](image)

| X=H: (Z)-7-((4-((2-(dimethylamino) ethoxy)(phenyl)methyl)phenyl)diazenyl)chroman-2-one |
| X=CH3: (Z)-7-((4-((2-(dimethylamino) ethoxy)(phenyl)methyl)phenyl)diazenyl)-4-ethylchroman-2-one |

2.2.5. Antimicrobial activity

In recent years, fungal and bacterial infections have become an important complication and a major cause of morbidity and mortality. The growing incidence of microbial resistance to existing antibiotics poses a serious medical problem in treating pathogenic infections. Antimicrobials are the agents that kill or inhibit the growth of microorganisms. The clinical application of antimicrobial agents is traditionally known as chemotherapy. Azo compounds are well known for their medicinal importance and are recognized for their use as antimicrobial (Shridhar et al., 2012).

A series of m-cresol derived azo compounds were synthesized and investigated their antibacterial activities against (1) Escherichia coli (2) Staphylococcus aureus (3) Salmonella typhi (4) Pseudomonas aeruginosa by disc diffusion method. The compound 3f substituted
Literature Review

with biphenyl ring showed excellent antibacterial activities against the strains included in the study in comparison to other compounds (Rathod and Thakre, 2013).

![Scheme](image)

Scheme

3,5-Bis(alkyl-1,3,4-oxadiazole-2-yl)azo dyes were synthesized by a multi-step reaction and screened against different bacterial and fungal strains (Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Candida albicans and Candida parapsilosis) by well plate method. The compound 4c substituted with (CH₂)₁₀CH₃ showed highest significant antimicrobial activity. However the compounds 4b, 4d, and 4a substituted with ((CH₂)₂CH₃, (CH₂)₁₂CH₃ and (CH₂)₆CH₃) respectively showed significant antimicrobial activities in comparison to other compounds (Shridhar et al., 2012).

![Scheme](image)

4a= (CH₂)₆CH₃, 4b= (CH₂)₈CH₃, 4d= (CH₂)₁₂CH₃
Some new azo dyes derivatives having pyrazole and trichlorotriazine moieties were synthesized and subjected to evaluate their antimicrobial activities against different microbial strains (Staphylococcus aureus, (Gram positive bacterium), Serratia marcescens, Shigella dysenteriae, Enterobacter cloacae, Escherichia coli (Gram negative bacteria) and Candida albicans (fungus)) by cut plug method. The compound 4-((5-(4,6-dichloro-1,3,5-triazin-2-ylamino)-1phenyl-3-substituted-1H-pyrazol-4-yl)diazenyl)benzensulfonic acids substituted 7a, 7b and 7c and 2-((5-(4,6-dichloro-1,3,5- triazin-2-ylamino)-1-phenyl-3-substituted-1H-pyrazol-4-yl)diazenyl)benzoic acid substituted 7d and 7f exhibited strong antimicrobial activity. However, the compound 7a-7c only showed antifungal activities against C. albicans (Rizk et al., 2015).

Various ethyl-2-substituted phenyl hydrazono-3-oxobutyrates and 1-(4-((1H-benzo[d][1,2,3]triazol-1-yl) methyl amino) benzoyl)-3-methyl-4-(2-(4-(4- alkylpiperazin-1-ylsulfonyl) phenyl) hydrazono)-1H-pyrazol-5- (benzotriazolyl methyl amino benzoyl hydrazide)ones substituted compounds were synthesized and the antimicrobial activities of the test compounds were screened against various bacterial and fungal strains (Bacillus subtilis, Staphylococcus aureus, Kllebsiella promioe, Salmonella typhi and E. coil and Penicillium expansum, Botrydepladia thiobromine, Nigrosspora Sp., Trichothesium Sp. and Rhizopus nigricuns) by cup and plate method. The compound 1-(4-((1H-
Literature Review

benzo[d][1,2,3]triazol-1-yl)eth ylamino)benzoyl)-3-methyl-4-(2-(4-(4-benzylpiperazin-1-ylsulfonyl)phenyl) hydrazono)-1H-pyrazol-5(-benzotriazolyl methyl amino benzoyl hydrazide)-one (7c) was found more active against the above microbes. Whereas, all the compounds were observed with good fungicidal activities and can inhibit their growth up to 60% (Purvesh et al., 2013).

![Chemical Structure](image)

<table>
<thead>
<tr>
<th>Comp</th>
<th>7a</th>
<th>7b</th>
<th>7c</th>
<th>7d</th>
<th>7e</th>
<th>7f</th>
<th>7g</th>
<th>7h</th>
</tr>
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<tbody>
<tr>
<td>R</td>
<td>methyl</td>
<td>ethyl</td>
<td>benzyl</td>
<td>phenyl</td>
<td>2,3-dichlorophenyl</td>
<td>3-chlorophenyl</td>
<td>2-tetrachlorofuroyl</td>
<td>hydroxyl ethanol</td>
</tr>
</tbody>
</table>

Novel series of dialkylaminoalkyl-o cresols incorporated with purine nucleus 2a-2b, benzothiazole nucleus 5a-5b, 8a-5b and thiazole nucleus 11a-11d, 13a-13d were synthesized through Mannich reaction. The antimicrobial activity evaluation was carried out for all synthesized compounds by agar dilution technique. Most of them exerted comparable activity to Ciprofloxacin and Fluconazole. The thiazole derivatives, 2-Diethylaminomethyl-4-(4-phenylthiazol-2-ylazo)-Phenol (11a), 4-(2-Amino-4-p-tolyithiazol-5-ylazo)-2-dimethylaminomethylphenol (13c) and 4-(2-Amino-4-p-tolyl-thiazol-5-ylazo)-2-morpholin-4-ylmethylphenol (13d) are the most potent compounds (Khaled et al., 2015).
The 4-phenylazophenoxy acetic acid was synthesized by condensation of monochloroacetic acid with different sodium salts of 4-phenylazo-phenols. The antimicrobial investigation of 4-phenylazophenoxy acetic acid against *S. aureus*, *S. pyogenes*, *P. aeruginosa*, *P. vulgaris* and *C. albicans* revealed that the 2-bromo-4-phenylazophenoxyacetic and 2-allyl-4-(4-chloro-4-phenylazo)-phenoxyacetic acid showed the best antimicrobial activity against *Staphylococcus aureus* (Moanta & Radu 2009; Moanta & Radu, 2008).
Azo Schiff bases synthesized by condensation of different aromatic amines and a new azoaldehyde, 2-hydroxy-3-methoxy-5-(4-methoxyphenylazo)benzaldehyde. All the compounds exhibited antibacterial activity against *B. subtilis* and antifungal activities against several fungi including *C. albicans* and *C. neoformans* and *T. mentagrophytes*. However, the Dapsone conjugated analogue found highly effective against *Bacillus subtilis* and moderately active against *Staphylococcus aureus* (Jarrahpour et al., 2004).
Study on medicinal interest of synthesized azo based heterocyclic compounds
Literature Review

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Ar-</th>
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<tr>
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<td>phenyl</td>
</tr>
<tr>
<td>5b</td>
<td>benzyl</td>
</tr>
<tr>
<td>5c</td>
<td>3-hydroxyphenyl</td>
</tr>
<tr>
<td>5d</td>
<td>3-tolyl</td>
</tr>
<tr>
<td>5e</td>
<td>2-tolyl</td>
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<td>3-methoxyphenyl</td>
</tr>
<tr>
<td>5h</td>
<td>2-methoxyphenyl</td>
</tr>
</tbody>
</table>

Scheme

A series of (7a-j) quinazolinone based MCT reactive dyes were synthesized by coupling of diazotized 3-{4-[4-amino-2, 6-difluorobenzyl]-3, 5-difluorophenyl}-6-nitro-2-phenylquinazolin-4(3H)-one with a diverse range of 2-chloro-4-nitro anilino cyanurated coupling components (6a-j). The novel synthesized dyes were investigated for their antimicrobial activities. The Chicago acid conjugated 3-{4-[4-amino-2, 6-difluorobenzyl]-3, 5-difluorophenyl}-6-nitro-2-phenylquinazolin-4(3H)-one (7i) showed excellent antibacterial activity against *E. coli* and *P. aeruginosa* whereas, very good activity against *S. aureus* and good activity against *S. pyogenus* in comparison to standard drug Ampicillin. The 4-(4-chloro-6-(2-chloro-4-nitrophenylamino)-1,3,5-triazin-2-ylamino)-5-hydroxynaphthalene-2,7-disulfonic acid conjugated with MCT dye producing sodium 4-(4-chloro-6-(2-chloro-4-nitrophenylamino)-1,3,5-triazin-2-ylamino)-3-((4-(2,6-difluoro-4-(6-nitro-4-oxoquinazolin-3(4H)-yl)benzyl)-3,5-difluorophenyl)diazenyl)-5-hydroxynaphthalene-2,7-disulfonate (7a) showed potential antifungal activity with respect to standard drug Nystatin (Patel and Patel, 2015).
R= different o-chloro-p-nitro anilino cyanurated coupling components

Sodium 4-(4-chloro-6-(2-chloro-4-nitrophenylamino)-1, 3, 5-triazin-2-ylamino)-3-((4-(2,6-difluoro-4-(6-nitro-4-oxoquinazolin-3(4H)-yl)benzyl)-3,5-difluorophenyl)diazetyl)-5-hydroxynaphthalene- 2,7-disulfonate (7a)

Metal complexes of Cu(II), Co(II), Ni(II), Zn(II), Cd(II) and Hg (II) were synthesized using ligands 5-(p-tolulyl)-azosalicylidin-4-phenyl-2-aminothiazole (PTASPAT), 5-(p-chlorophenyl)-4-phenyl-2-aminothiazole (PCPASPAT) and 5-phenylazosalicylidin-4-phenyl-2-aminothiazole (PASPAT). The antimicrobial activity of the ligands and their complexes revealed that the metal complexes showed more potent activity than their ligands (Hankare et al., 2001).

Cu(II), Co(II), Ni(II), Zn(II), Cd(II) and Hg (II) complexes of PSPAT (R=H), PCPASPAT (R= Cl), and PTASPAT (R= CH₃)
An aqueous HCL acid solution of sulfamethoxazole was added with aqueous solution of NaNO₂ followed by coupling with acetylacetone in presence of sodium acetate to obtain 4-(((Z)-2-hydroxy-4-oxopent-2-en-3-yl)diazenyl)-N-(5-methylisoxazol-3-yl) benzenesulfonamide (HL). To the methanolic solution of Ni(OAc)₂.4H₂O, the prepared azo ligand was added and stirred continuously for 4h and then the mixture was refluxed for 6h. The obtained (complex) crystals were re-crystallized from DMF and methanol. The results of antibacterial activity of the synthesized ligand (HL) and its metal complex Ni(OAc)₂.4H₂O revealed that there is a concentration dependant decrease in the minimum inhibitory concentration (IC₅₀) against E. coli at 63.72 & 81.49 µg/ml and 77.25 & 78.28 µg/ml respectively (Das et al., 2015).

HL: 4-(((Z)-2-hydroxy-4-oxopent-2-en-3-yl) diazenyl) -N- (5-methylisoxazol-3-yl) benzenesulfonamide

2.2.6. Wound healing activity of some azo compounds

Specifically wound is a sharp injury which damages the epidermis and dermis of the skin by cutting or puncturing or by burning. Wound healing is a complex process. In normal skin the epidermis and dermis exist in steady-state equilibrium, forming a protective barrier against the external environment. Once the protective barrier is broken, the normal physiologic process of wound healing is in motion. Wound healing included haemostasis, inflammation, proliferation and remodeling like process to repair the damaged cell (Stadelmann et al., 1998; Nguyen et al., 2009). In order to promote accelerating skin repair,
many clinicians have been focused on searching of novel pharmacological agents having maximum efficiency of wound contraction and minimum toxicity.

A series containing three azo compounds substituted with hydroxytriazenes were synthesized and subjected for investigation of wound healing activity by wound excision model, incision model and dead space wound model. The compound 3-Hydroxy-3-n-propyl-1- (4-sulfonamide) phenyltriazene (HD-2) and 3-Hydroxy-3-isopropyl-1- (4-sulfonamide) phenyltriazene (HD-3) showed significant wound healing activity. There was an increased number of fibroblasts and collagen content in treated groups in contrast to control group. (Chauhan et al., 2010).

\[
\text{HD-2: } 4-(3\text{hydroxy-3-propyltriaz-1-enyl) benzenesulfonamide}\n\]
\[
\text{HD-3: } 4-(3\text{hydroxy-3-isopropyltriaz-1-enyl) benzenesulfonamide}\n\]

**Acute toxicity study of some azo compounds**

3, 5-bis (5-(furan-2-yl)-1,3,4-oxadiazol-2-yl) azo dyes were synthesized by multistep reaction sequences, which is diazotized and coupled with different coupling agents viz. 2-naphthols, 7-hydroxy quinoline, 3-hydroxy-N-phenyl-2-naphthamide, 3-hydroxy-N-(2-methoxyphenyl)-2-naphthamide, N-(4-chloro-2-methylphenyl)-3-hydroxy-2-naphthamide, 3-hydroxy-N-(4-nitrophenyl)-2-naphthamide and 3-hydroxy-N-(naphthalen-2-yl)-2-naphthamide. The acute oral toxicity study for the test compounds 5a-g were evaluated according to the OECD guidelines No.420 (2008) using Swiss albino male mice through oral route of administration. The synthesized compounds administered orally at a fixed dose of 250, 500, 1000 and 1500 mg/kg body weight. However the acute toxic symptoms and the
Literature Review

behavioral changes produced by the test compounds were observed continuously at an interval of 4 h up to 24 h. All the compounds found safe up to 1500 mg/kg except 3, 5-bis(5-(furan-2-yl)-1,3,4-oxadiazol-2-yl) azo dye (Shridhar et al., 2011).

![Structural formula of synthesized compounds](attachment:image.png)

<table>
<thead>
<tr>
<th>Comp</th>
<th>5a</th>
<th>5b</th>
<th>5c</th>
<th>5d</th>
<th>5e</th>
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</table>

2.2.7. Analgesic activity of some azo compounds

A series of 1-(2-hydroxyethyl)-3,5-dimethylpyrazolylazo dyes were synthesized by incorporation of thiosemicarbazide, 1,3,4-thiadiazole and 1,2,4-triazole-3-thione moieties. The analgesic activity of synthesized compounds was determined by hot plate and tail immersion method. Morphine was used as standard, both in spinal and supraspinal pathways. The synthesized compounds were administered at a dose of 100 mg/kg intra-peritoneal. The compound 4-[[1-(2-hydroxyethyl)-3, 5-dimethylpyrazole-4-yl)azo]phenyl]-4-(2-phenethyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione (4c) showed analgesic effect in both models (Oruc et al., 2006).
2.2.8. Anti-inflammatory activity of some azo compounds

NSAIDs are used for the treatment of acute and chronic inflammation and to relieve pain and fever. Most of the NSAID those available in the market are a constitutive form COX-1 and an inducible form, COX-2 to offer therapeutic effect that is to inhibit COX-1 and COX-2, there by the synthesis of prostaglandin and thromboxane can be blocked.

A series of seven novel azo derivatives of dihydropyrimidinones were synthesized by coupling various aryl diazonium salts. The in vivo anti inflammatory activity of the synthesized compounds was screened by carrageenan-induced hind paw oedema method. The compounds 2-naphthol, 5- hydroxy coumarin and 2, 6- diclorophenol substituted dihydropyrimidinones 4b, 4c and 4g were found to have potent anti-inflammatory effect with an appreciable percent inhibition of 53.85, 47.00 and 48.71%, respectively (Shaikh and Meshram, 2015).

A series of twelve numbers of 1-aryl-5-arylazobarbituric acid analogues were synthesized by coupling of aryl diazonium chloride to 1-aryl barbituric acid. The compounds

<table>
<thead>
<tr>
<th>Comp</th>
<th>a</th>
<th>b</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>R</td>
<td>C₄H₉</td>
<td>C₆H₁₁</td>
<td>CH₂CH₂C₆H₅</td>
</tr>
</tbody>
</table>
were screened by carrageenan-induced rat paw oedema method. The 1-aryl-5-arylazobarbituric acid substituted with 3-methyl (2a) showed 19% inhibition at a dose of 100 mg/kg body weight (Misra et al. 1986).

Novel phenyl azochalcone derivatives were prepared from 4-aminoacetophenone. Diazotization of the amino ketone followed by Claisen Schimdt condensation and finally coupled with various electrophiles gave phenylazochalcone derivatives. The in vitro anti inflammatory activity of the synthesized compounds were conducted by protein denaturation method using UV-spectro photometer. The nitrochalcone coupled with aniline 4a (I), anisidine 4a (II) and methoxy chalcone coupled with anisidine 4b(II); hydroxyl chalcone coupled with aniline 4d (I) and anisidine 4d (II); and methyl chalcone coupled with toluidine 4c (III) have shown moderate to good anti inflammatory activity (Rohini et al., 2015).

\[
\begin{align*}
4a \text{ (I)}: X=\text{NO}_2, & \quad Y=\text{NH}_2, \quad 4a \text{ (II)}: X=\text{NO}_2, & \quad Y=\text{OCH}_3, \quad 4a \text{ (III)}: X=\text{NO}_2, & \quad Y=\text{CH}_3, \quad 4a \text{ (IV)}: X=\text{NO}_2, \\
Y=\text{C}_6\text{H}_5\text{OH}; & \quad 4b \text{ (I)}: X=\text{OCH}_3, & \quad Y=\text{NH}_2, \quad 4b \text{ (II)}: X=\text{OCH}_3, & \quad Y=\text{OCH}_3, \quad 4b \text{ (III)}: X=\text{OCH}_3, & \quad Y=\text{CH}_3, \quad 4b \text{ (IV)}: X=\text{OCH}_3, \\
Y=\text{C}_6\text{H}_5\text{OH}; & \quad 4c \text{ (I)}: X=\text{CH}_3, & \quad Y=\text{NH}_2, \quad 4c \text{ (II)}: X=\text{CH}_3, & \quad Y=\text{OCH}_3, \quad 4c \text{ (III)}: X=\text{CH}_3, \\
Y=\text{CH}_3, & \quad 4c \text{ (IV)}: X=\text{CH}_3, & \quad Y=\text{C}_6\text{H}_5\text{OH}; & \quad 4d \text{ (I)}: X=\text{OH}, & \quad Y=\text{NH}_2, \quad 4d \text{ (II)}: X=\text{OH}, & \quad Y=\text{OCH}_3, \quad 4d \text{ (III)}: X=\text{OH}, & \quad Y=\text{CH}_3, \quad 4d \text{ (IV)}: X=\text{OH}, \\
Y=\text{C}_6\text{H}_5\text{OH}.
\end{align*}
\]

The azo derived 5- aminosalicylate was synthesized by diazonium salt of 5-ASA and coupled with phenol and finally immobilized on a polyethylene glycol matrix. The prodrug of azo derived 5-aminosalicylic acid congeners exhibit anti inflammatory and cytoprotective activity (Garjani et al., 2004).
2.2.9. Antitubercular activity of some azo compounds

Tuberculosis is one of the most common infectious diseases caused by *Mycobacterium tuberculosis*. About 32% of the world’s population is infected by tubercle bacillus. Tuberculosis typically attacks the lungs, but can also affect other parts of the body. It is an airborne disease, spread from infectious cough, sneezes or otherwise transmits respiratory fluids through air. Every year tentatively 8 million of infected people develop active TB and 2 millions of them face death (Rohini et al., 2015).

Novel phenyl azochalcone derivatives were prepared from 4-aminoacetophenone. Diazotization of the amino ketone followed by Claisen Schimdt condensation and finally coupled with various electrophiles gave phenylazochalcone derivatives. The antitubercular activity of compounds was assessed against *M. tuberculosis* using Microplate Alamar Blue Assay (MABA).

It was found to be observed that the chalcone derivatives showed the inhibition of growth of *M. Tuberculosis* H37 RV at 50 and 100 μg/ml concentrations due to the presence of electron releasing substituent on different aryl rings 4(a-e)(I-IV) (Rohini et al., 2015).
2.2.10. Antiviral activity of some azo compounds

Antiviral drugs are a class of drugs used specifically for the treatment of viral infections. Unlike most antibiotics, antiviral drugs do not destroy their target pathogens, instead they inhibit their development.

Heterocyclic compounds based on 3-(4-bromophenyl) azo-5-phenyl-2(3H)-furanone were synthesized by using 3-[2-(4-Bromphenyl)hydrazono]-5-phenyl-furan-2(3H)-one. The synthesized compounds were investigated for anti-avian influenza virus activity and the compounds 3-[2-(4-bromophenyl)hydrazono]-5-phenylfuran-2(3H)-one (1), 1-(4-bromophenyl)-N-hydroxy-5-phenyl-1H-pyrazole-3-carboxamide (5) and 1-(4-bromophenyl)-N-{2,3-dihydro-4-hydroxy-3-phenyl-6-oxo-2-thioxopyrimidin-1(6H)-yl}-5-phenyl-1H-pyrazole-3-carboxamide (12a) showed highest effect, revealed the promising antiviral activity against H5N1 virus [A/Chicken/Egypt/1/20 % (H5N1)] both by EC₅₀ & LD₅₀ and confirmed by plaque reduction assay on Madin-Darby canine kidney cells (Flefel et al., 2012).

A series of twelve numbers of 1-aryl-5-aryloazobarbituric acid analogues were synthesized by coupling of aryl diazonium chloride to 1-aryl barbituric acid. The compounds were screened for their in vivo and in vitro antiviral property against Tobacco Mosaic Virus (TMC) and Cucumber Green Mottle Mosaic Virus (CGMV). The 1-aryl-3-(2’-methylbenzimidazole benzoazolin-2’-thione-3’-methyl)-5-aryloazo-3-CH₃ substituted
compound (3a) showed highest 62% *in vivo* and 80% *in vitro* inhibition against TMV and 62% *in vivo* and 76% *in vitro* inhibition against CGMV (Misra *et al*., 1986).

Anti HIV potential has been reported for bis-azo compound. It exhibits anti human immuno deficiency virus (HIV) potency by inhibition of HIV virus envelop glycol protein mediated membrane. FP-21399 exhibited to inhibit the fusion of CD 4+cells (Poli and Vicenzi, 2001; Ono *et al*., 1997).

![Bisazo compound FP-21399](image)

**2.2.11. Antirheumatoidal activity of some azo compound**

The 5-aminosalicylic acid and sulphapyridine coupled to form azo compound Sulphasalazine. Normally it is given in enteric coated form as an antiulcerative drug. Sulphasalazine is an effective drug in the treatment of Rheumatoidal Arthritis with an efficacy very similar to that of injectable gold, D-penicillamine and Methotrexat (Box and Pullar, 1997).

![Sulfasalazine](image)