MICROWAVE ASSISTED SYNTHESIS OF NOVEL SCHIFF BASES AND THEIR BIOLOGICAL EVALUATION

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ABSTRACT
The aim of present study is to synthesise novel thiazolidinones from Schiff bases of salicylaldehyde by microwave assisted method. The literature review showed that Schiff bases possess a wide spectrum of biological activities such as antibacterial, anti tubercular, anti inflammatory, antiviral and antioxidant activity. Schiff bases of salicylaldehydes have also been reported as plant growth regulator and antimicrobial or antymycotic activity. Thiazolidinones found to have a broad spectrum of pharmacological properties like anti HIV, antipsychotic, anticonvulsant and antitubercular activity. In the present study, Microwave was used to make chemical synthesis more environment friendly, safe, to improve the yield and also to reduce the time consumption. The newly synthesized Schiff bases (2a and 2b) were evaluated for anticoagulant activity. Schiff bases and thiazolidinones were purified, characterized and evaluated for antibacterial and anthelmintic activities. The yield of the synthesized compounds was found to be in the range of 70-86 %. All these molecules were characterized by physical data and FT-IR, 1H NMR and mass spectral analysis. The synthesized Schiff bases (2a and 2b) at concentrations 10µg/ml, 20µg/ml, 40µg/ml and 80µg/ml were tested for anticoagulant activity using prothrombin time test. Time of coagulation for both the compounds was recorded and it was found out that Schiff base 2a showed better anticoagulant activity when compared to Schiff base 2b. All the synthesized compounds were tested for antibacterial activity on E.coli and S.aureus, using chloramphenicol as standard drug at concentrations of 0.1%, 0.2% and 0.3%. Amongst all the synthesized compounds, compounds 2a, and 3a showed highest activity. All the synthesized compounds were also screened for anthelmintic activity, using albendazole as standard. The compound 2a showed highest activity, 2b and 3a showed moderate activity.

KEYWORDS: Microwave assisted synthesis, Schiff bases, thiazolidinones, anthelmintic and antibacterial.
Where R, may be an alkyl or an aryl group. Schiff bases that contain aryl substituents are substantially more stable and more readily synthesized, while those which contain alkyl substituents are relatively unstable. Schiff bases of aliphatic aldehydes are relatively unstable and readily polymerizable\(^{911}\) while those of aromatic aldehydes having effective conjugation are more stable.\(^{12-15}\) The formation of a Schiff base from an aldehyde or ketone is a reversible reaction and generally takes place under acid or base catalysis, or upon heating.

The formation is generally driven to the completion by separation of the product or removal of water, or both. Many Schiff bases can be hydrolyzed back to their aldehydes or ketones and amines by aqueous acid or base.

1.1. b. Mechanism

The mechanism of Schiff base formation is another variation on the theme of nucleophilic addition to the carbonyl group. In this case, the nucleophile is the amine. In the first part of the mechanism, the amine reacts with the aldehyde or ketone to give an unstable addition compound called carbinolamine. The carbinolamine loses water by either acid or base catalyzed pathways. Since the carbinolamine is an alcohol, it undergoes acid catalyzed dehydration.

Typically the dehydration of the carbinolamine is the rate-determining step of Schiff base formation and that is why the reaction is catalyzed by acids. Yet the acid concentration cannot be too high because amines are basic compounds. If the amine is protonated and becomes non- nucleophilic, equilibrium is pulled to the left and carbinolamine formation cannot occur. Therefore, many Schiff bases synthesis are best carried out at mildly acidic pH. The dehydration of carbinolamines is also catalyzed by base. This reaction is somewhat analogous to the E2 elimination of alkyl halides except that it is not a concerted reaction. It proceeds in two steps through an anionic intermediate. The Schiff base formation is really a sequence of two types of reactions, i.e. addition followed by elimination.
1.1. Biological importance of Schiff bases

Schiff bases of salicylaldehydes have also been reported as plant growth regulators\textsuperscript{16} and antimicrobial\textsuperscript{17} or antifungal\textsuperscript{18} activity. Schiff bases also show some analytical applications. Schiff bases are characterized by the -N=CH- (imine) group which imports in elucidating the mechanism of transamination and racemization reaction in biological system.\textsuperscript{19,20} Schiff bases are active against a wide range of organisms for example; Candida Albicans, Escherichia coli Staphylococcus aureus, Bacillus polymxa, Trychophyton gypseum, Mycobacteria, Erysiphe graminis and Plasmopora viticola.

Schiff bases have been studied for their important properties in catalysis.\textsuperscript{21} They show catalytic activity in hydrogenation of olefins.\textsuperscript{22} They find applications in biomimetic catalytic reactions. An interesting application of Schiff bases is their use as an effective corrosion inhibitor, which is based on their ability to spontaneously form a monolayer on the surface to be protected. Many commercial inhibitors include aldehydes or amines, but presumably due to the C=N bond the Schiff bases function more efficiently in many cases.\textsuperscript{23}

Imines also have biological importance. An imine linkage between the aldehyde derived from vitamin A and the protein opsin in the retina of the eye plays an important role in the chemistry of vision. Schiff bases have been reported in their biological properties, such as, antibacterial, antifungal activities. Their metal\textsuperscript{24-27} complexes have been widely studied because they have anticancer and herbicidal applications.\textsuperscript{28-29} They serve as models for biologically important species. The Schiff bases constitute one of the most active classes of the compounds possessing diversified biological applications. The Schiff bases have been reported to possess higher degree of antitubercular, anticancer, antibacterial, anti-inflammatory, antifungal activities. Schiff bases belong to a widely used group of organic intermediates used for synthesis of pharmaceutical or rubber additives and amino protective group in organic synthesis.\textsuperscript{30}

1.2. Thiazolidinone

Thiazolidinone, a saturated form of thiazoles with carbonyl group on fourth carbon, has been considered as a moiety of choice as it possesses a broad spectrum of pharmacological activities against several targets. This array of biological response profile has attracted the attention of scientists the world over to further investigate the potential of this organic motif. 4-Thiazolidinones are derivatives of thiazolidine with a carbonyl group at the 4-position (1). Substituents in the 2-, 3-, and 5-positions may be varied, but the greatest difference in structure and properties is exerted by the group attached to the carbon atom in the 2-position (R and R’ in 2 or X in 3). Variations in the substituents attached to the nitrogen atom and the methylene carbon atom are possible for the structures represented by 2 and 3.
There are numerous biologically active molecules which contain various heteroatoms such as nitrogen, sulphur and oxygen, always drawn the attention of chemist over the years mainly because of their biological importance. Thiazolidinones are thiazolidine derivatives and have an atom of sulfur at position 1, an atom of nitrogen at position 3 and a carbonyl group at position 2, 4, or 5. However, its derivatives belong to the most frequently studied moieties and its presence in penicillin was the first recognition of its occurrence in nature. Similarly 1,3-thiazolidin-4-ones are heterocyclic nucleus that have an atom of sulfur and nitrogen at position 1 and 3, respectively and a carbonyl group at position 4 have been subjected to extensive study in the recent years.\cite{31}

### 1.2. a. Synthesis

Several methods for the synthesis of 4-thiazolidinones are widely reported in the literature. The main synthetic routes to 1,3-thiazolidin-4-ones involve three components that is an amine, a carbonyl compound, and a mercapto-acid. The classical synthesis reported can be either a one- pot three-component condensation or a two-step process. The reactions begin by formation of an imine (the nitrogen of amine attacks the carbonyl of aldehyde or ketone), which undergoes attack by generated sulphur nucleophile, followed by intramolecular cyclization on elimination of water.\cite{32-34}

![Synthesis Reaction](image)

Thiazolidinones can also be synthesized by treating a Schiff base with thioglycolic acid in an organic medium.

![Reaction Scheme](image)

### 1.2. b. Mechanism

It is based on cyclo-condensation. The reaction proceeds by the attack of mercapto acetic acid upon the C=N group, with the HS-CH2-COOH adding to the carbon atom followed by capture of the proton by nitrogen and subsequent cyclization. During the reaction an uncyclized intermediate is formed in few cases. In many instances 4-Thiazolidinones can conveniently be prepared by refluxing the mixture of thioglycolic acid and the Schiff base in benzene, dry ether or ethanol. The nucleophilic attack of the mercaptoaetic acid anion will take place on the carbon of azomethine which has got a positive character; while it is evident that the nitrogen has the negative character. Simultaneous removal of water that forms in the reaction helps in condensation and determination of the reaction time.\cite{35}
1.2. **c. Biological importance of Thiazolidinones**

4-thiazolidinone is one of the most intensively investigated classes of aromatic five member heterocyclic’s. These derivatives find a variety of applications ranging from antimicrobial, antitubercular, anti-inflammatory, anthelmintic, anticonvulsant, anti-HIV activity, anticancer, and hypoglycemic activity. Due to this, the investigation of chemistry and biology of these compounds continue to appeal the synthetic and medicinal organic researches.

The 4-thiazolidinone scaffold is very versatile and has featured in a number of clinically used drugs. A lot of research work on thiazolidinones has been done in the past. The nucleus is also known as wonder nucleus because it gives out different derivatives with all different types of biological activities.

It has been extensively reported that presence of arylazo, sulfamoylphenylazo-4 or phenylhydrazono moieties at different positions of the thiazolidone ring enhanced anti-microbial activity and its antibacterial activity may be due to its inhibitory activity of enzyme Mur B which is precursor acting during the biosynthesis of peptidoglycan. Numerous reports have appeared in the literature which highlights their chemistry and pharmacological uses. The thiazolidinones ring has been incorporated into a broad range of known biologically active compounds, either as a substituent group or as a replacement of another ring inspired researchers to synthesize several compounds containing this moiety.\(^{36}\)

1.3. **Microwave Synthesis**

In inorganic chemistry, microwave technology has been used since the late 1970s, while it has only been implemented in organic chemistry since the mid-1980s. The main reasons for this increase include the availability of commercial microwave equipment intended for organic chemistry and the development of the solvent-free technique, which has improved the safety aspects, but are mostly due to an increased interest in shorter reaction times.\(^{37}\)

The short reaction times and expanded reaction range that is offered by microwave assisted organic synthesis are suited to the increased demands in industry. In particular, there is a requirement in the pharmaceutical industry for a higher number of novel chemical entities to be produced, which requires chemists to employ a number of resources to reduce the time for the production of compounds. Chemistry databases, software for diversity selection, on-line chemical ordering systems, open-access and high throughput systems for analysis and high-speed, parallel and combinatorial synthesis equipment have all contributed in increasing the throughput. The common factors for these technical resources are automation and computer-aided control. They do not, however, speed up the chemistry itself. Developments in the chemistry have generally been concerned with novel highly reactive reagents in solution or on solid supports.

In general, most organic reactions have been heated using traditional heat transfer equipment such as oil baths, sand baths and heating jackets. These heating techniques are, however, rather slow and a temperature gradient can develop within the sample. In addition, local overheating can lead to product, substrate and reagent decomposition.

In contrast, in microwave dielectric heating, the microwave energy is introduced into the chemical reactor remotely and direct access by the energy source to the reaction vessel is obtained. The microwave radiation passes through the walls of the vessel and heats only the reactants and solvent, not the reaction vessel itself. If the apparatus is properly designed, the temperature increase will be uniform throughout the sample, which can lead to less by-products and/or decomposition products.

The use of microwaves as an energy source to heat reaction solutions has been shown to provide the following advantages.

- Broad applicability-few limitations as to types of synthesis chemistry.
- Increased reaction rates-1000 fold in best cases.
- Used to accelerate chemistries in both solution and solid phase reactions.
- Improved product yields.
- Moderately scalable (sub-milligram to milligram quantities).
- Can be conducted in either open or closed vessels.
- Broad dynamic temperature range.
- Green chemistry-reactions in supercritical water or solvent-less reactions.
- Can be used to accelerate the synthesis of peptides.
- Controlled method of heating.
- Rapid reaction optimization.

2. **REVIEW OF LITERATURE**

Kumar *et al.* reported a new series of 2-(2-aminophenyl) benzimidazole and 2-(4-amino phenyl) benzimidazole derivatives, and their corresponding Schiff bases. Compounds were synthesized by cyclo-condensation of phenylenediamines and carboxylic acids or their derivatives (nitriles, imidates, or ortho esters) under strong acidic conditions (PPA) and at high temperatures. The synthesized compounds were screened for their *invitro* antimicrobial activities against the standard strains: *Staphylococcus aureus* (ATCC – 25923) and *Bacillus subtilis* (ATCC-6633) as Gram positive, *Escherichia coli* (ATCC-11775) and *Pseudomonas aeruginosa* (ATCC-10145) as gram negative bacteria. Some of the compounds were found to be effective against gram positive bacteria at MIC values between 25 and 200 mg/mL and some were effective against ram negative
bacteria at MIC values between 25 and 200 mg/mL.\textsuperscript{[38]}

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{figure.png}
\caption{Chemical structures of benzimidazole derivatives.}
\end{figure}

**Benzimidazole derivatives**

Alang \textit{et al.} described the synthesis and antibacterial activity screening of newer Schiff bases of 2-amino-6-methylbenzothiazole derivatives. \textit{p}-toluidine on reacting with ammonium thiocyanate yielded 2-benzothiazolamines, which on subsequent reaction with hydrazine hydrate formed hydrazino derivative. Schiff bases were obtained by reacting hydrazino derivatives with different acetophenones. All the synthesized compounds showed activity against gram positive bacteria- \textit{Staphylococcus aureus} (MTCC 737), \textit{Staphylococcus epidermidis} (MTCC 3615) and gram negative bacteria- \textit{Pseudomonas aeruginosa} (MTCC 424) and \textit{Escherichia coli} (MTCC 1687) at 1 mg/mL disc concentration. The activities of all the compounds were comparable with that of standard drug, ampicillin.\textsuperscript{[39]}

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{figure.png}
\caption{Chemical structures of benzothiazole derivatives.}
\end{figure}

**Benzothiazole derivatives**

Vicini \textit{et al.} studied a comparative evaluation of three new series of benzo[d]isothiazole, benzothiazole and thiazole Schiff bases. Schiff bases were synthesized by reacting the appropriate heteroarylamine with the selected aldehyde. These compounds were evaluated \textit{in vitro} against a HIV-1 (Retrovirus), a HBV (Hepadnavirus), Yellow fever virus (YFV) and Bovine viral diarrhoea virus (BVDV). The compounds were also tested against gram positive and gram negative bacteria (\textit{Staphylococcus aureus}, \textit{Salmonella} spp.), various atypic mycobacterial strains (\textit{Mycobacterium fortuitum} and \textit{Mycobacterium smegmatis}), yeast (\textit{Candida albicans}) and mould (\textit{Aspergillus fumigatus}). None of the compounds showed antiviral or antimicrobial activity. The benzo[d]isothiazole compounds showed a marked cytotoxicity (CC50=4–9 mM) against the human CD4+ lymphocytes (MT-4) that were used to support HIV-1 growth.\textsuperscript{[40]}
Benzothiazole & thiazole derivatives
- Ali Syed et al. synthesized a novel series of Schiff bases of 2-methyl/phenyl-4-benzylidene-5-hydrazino-1,3-oxazole by condensing 2-methyl/phenyl-4-benzylidene-5-oxo-1,3-oxazole with hydrazine hydrate followed by different aromatic aldehydes. The synthesized compounds were screened for antimicrobial activities against gram positive S. aureus, C. diptheria and Gram negative E. coli, P. aeruginosa bacterial strains. Compounds showed considerable activity when compared to the standard drug, ampicillin trihydrate.[41]

Oxazole derivatives
- Somani et al. reported the synthesis of newer Schiff bases of 1, 2, 4-triazoledervatives, N’-(substitutedbenzylidene)-2-(4H-1,2,4-triazol-4-yl) acetoxyhydrazide. 1, 2, 4-triazole was converted to ethyl-5-N’-(1, 2, 4-triazolyl)-acetate using ethyl bromoacetate. It was then converted to corresponding hydrazone by hydrazine hydrate, which upon further treatment with various aromatic aldehydes under acidic condition afforded Schiff bases. All the synthesized compounds were screened for in vitro study of antibacterial efficacy against S. aureus, P. aeruginosa and E. coli at two different concentrations viz. 50 and 100 μg/mL using streptomycin as the standard. Compound showed good activity against all the organisms at both concentrations.[42]

Triazole derivatives
- Pawar and co-workers reported the synthesis and antibacterial activity of some new Schiff bases of 4-thiazolidinones. Schiff bases were synthesized by the condensation of 2-hydroxy-3-iodo-5-bromobenzaldehyde with aromatic amine in ethanol. Compounds obtained undergo cyclization with mercaptoacetic acid to afford corresponding 4-thiazolidinones. Compounds showed antibacterial activity against E. coli, B. subtilis, S. typhi, S. dysentereas as test organisms, but none of them showed better activity than standard drug tetracycline.[43]

Thiazolidinones derivatives
- Singh and co-authors synthesized some new Schiff bases of isatin and substituted isatin by condensation with 4-amino-N-carbamimidoylbenzene sulfonamide. The synthesized compounds were screened for antibacterial activity against three gram positive (S. aureus, B. subtilis, B. pumulis) and three gram negative bacterial (E. coli, S. abony, K.pneumoniae) strains. All the compounds exhibited significant antibacterial activity in comparison to the standard drug, sulfaguanidine against both gram positive and gram negative bacterial strains. None of the compounds exhibited significant antifungal activity comparable to standard antifungal drug, clotrimazol against S. cerevisiae and C. albicans.[44]

Isatin sulfonamides
- Bawa et al. synthesized a series of 4-substituted-iminomethyltetrazolo quinoline derivatives by condensation of 4-formyl-8-methyltetrazolo quinoline with appropriate aromatic amine by refluxing in dioxane. The synthesized compounds were evaluated for anti-inflammatory and antimicrobial activities. Compounds showed moderate to good activities.[45]
Tetrazolo quinoline derivatives

- Panneerselvam and coworkers synthesized a series of novel Schiff bases by condensation of 5-substituted isatin with different substituted aromatic aldehydes. The synthesized compounds were investigated for analgesic, anti-inflammatory and antibacterial activities. The minimum inhibitory concentrations (MIC) of the compounds were also determined. Most of the synthesized compounds exhibited significant antibacterial and antifungal activities. Among the synthesized compounds, few exhibited remarkable analgesic and anti-inflammatory activities. \[46\]

\[\text{Where } Ar= C_{6}H_{5}OH-o, C_{6}H_{5}OCH_{3}-o, C_{6}H_{5}Cl-o \]

\[Q= \text{quinolaxinyl-2} \]

Quinoxaline derivatives

- Sunita Bhagat et al. reported the synthesis of some salicylaldehyde Schiff bases in aqueous media. The final products generate excellent yields in a one-step procedure under microwave irradiation using a suitable solvent. The structures of the synthesized compounds were confirmed by IR, \(^1\)HNMR and Mass Spectra data. \[48\]

- Hai Jian Yang et al. reported the rapid synthesis of Schiff-base without solvent under microwave irradiation. Microwave-assisted preparations of a series of Schiff-base via efficient condensation of salicylaldehyde and aryl amines without solvent were prepared with high yield as well as environmental friendliness reaction in organic synthesis. \[49\]

- Hossein Naeimi et al. reported a convenient, mild and one-pot synthesis of double Schiff bases from three component reaction of salicylaldehyde, ammonium acetate and aliphatic aldehydes accelerated by NEt\(_3\) as a base. By this reaction, N,N\(_2\)-bis(salicylidene)-1,1-diaminoalkanes was easily obtained in excellent yields and short reaction times under mild reaction condition. \[50\]
Patel et al. reported the synthesis and antimicrobial activity of a new series of Schiff bases derived from quinazolin-4(3H)-one. The benzoazinone, 6-bromo-2-[2-(2,6-dichlorophenyl)amino]benzyl-4H-3,1-benzoxazin-4-one was prepared by the cyclization reaction of acid chloride with 5-bromoanthranilic acid. Further reaction of benzoazinone with hydrazine hydrate yielded 3-amino-6-bromo-2-[2-(2,6-dichlorophenyl)amino]benzylquinazolin-4(3H)-one, which upon subsequent condensation with different substituted aromatic aldehydes formed Schiff bases, 6-bromo-2-(2,6-dichlorophenyl)amino]-benzyl-3-substituted benzimideneamino-quinazolin-4(3H)-ones. Compounds were screened for antibacterial activity against two gram positive bacteria (Staphylococcus aureus, ATCC9144 and Bacillus subtilis, ATCC 6633) and two gram negative bacteria (Pseudomonas aeruginosa, ATCC 9027 and E. coli, ATCC 25922); while antifungal activity was screened against Candida albicans, ATCC 10231 at two different concentrations 100 and 50 μg/mL. Penicillin-G was used as a standard antibacterial agent whereas amphotericine-B was used as a standard antifungal agent. Most of the synthesized Schiff bases exhibited good antibacterial activities against gram positive and gram negative bacteria, whereas antifungal activity were found to be moderate to poor.\(^{[51]}\)

**Triazole-3-thiol derivatives**

Hunas Hal et al. reported the synthesis and antimicrobial activity of a novel series of substituted 1,2,4-triazole Schiff bases. \(N'\)[4-(substitutedbenzylidene)-3(2′4′dichlorophenoxy)methyl]1,2,4-triazol-5-ylisonicotinyl hydrazide derivatives were synthesized by the reaction between \(N'\)[4-amino-3-(2′4′dichlorophenoxy)methyl]1,2,4-triazol-5-ylisonicotinyl hydrazide and various aromatic aldehydes in presence of hydrochloric acid in alcohol. All the compounds were screened for antibacterial and antifungal activities using standard bacterial and fungal strains Staphylococcus aureus (ATCC 9144), Bacillus subtilis(ATCC 6633), Pseudomonas aeruginosa (ATCC 25668), Escherichia coli (ATCC 25922), Candida albicans (ATCC 2091), Aspergillus niger (ATCC 6275) Aspergillus fumigates(ATCC 13073) respectively. All the compounds showed moderate to good antifungal and weak antibacterial activities. Compounds were also tested for antitubercular activities by broth dilution assay method against M. tuberculosis H37Rv strain and showed very weak activities.\(^{[52]}\)
Triazol-5-ylisonicotyl hydrazide derivatives

Sridhar et al. prepared Schiff bases and hydrazones of substituted isatin by reacting isatin and aromatic primary amines/hydrazines and determined their MIC values for antimicrobial activity against seven gram positive and seven gram negative standard pathological strains. The results of MIC values indicated that 3-(4-Bromo-phenylimino)-1-[(diphenylamino)-methyl]5-nitro-1,3-dihydro-indol-2-one and 3-(4-Bromo-phenylimino)-5-nitro-1,3-dihydro-indol-2-one were found to be the most active compounds of the series.\[54\]

Substituted isatin derivatives

Prabhu et al. synthesized a new series of Schiff bases of several benzothiazole derivatives. Paranitrobenzothiazole carboxylic acid synthesized by Jacobson synthesis which was reduced to para-amino benzothiazole carboxylic acid with ammonium chloride and iron metal. The resulting product was then condensed with various aromatic or heterocyclic aldehydes in the presence of concentrated sulphuric acid as a catalyst using ethanol as solvent to yield different Schiff bases. Synthesized compounds were screened for their in-vitro antibacterial activity against *P. aeruginosa*, *E. coli*, *S. aureus*, *B. subtilis* at 100μg/ml and in-vitro antifungal activity against *Candida albicans* and *Aspergillus niger* activities at 100 μg/ml concentration. All the compounds have shown significant antibacterial activity with the reference standards, ampicillin and ketoconazole.\[55\]

Substituted benzothiazole derivatives

Vora et al. prepared some new Schiff base derivatives, of N-[(1E)-[3-(mono or di- substituted aryl)-1-phenyl-1H-pyrazol-4-yl]methylene]-4 methylpyridin-2-amine by the acid catalyzed condensation of 3-(mono- or di-substituted aryl)-1-phenyl-1H-pyrazole-4-carbaldehyde derivatives with 4-methylpyridin-2-amine. All the synthesized compounds were screened for their antimicrobial activities by using broth dilution method. Although compounds were found to be active against the tested antibacterial (*E. coli*, *P. aeruginosa*, *S. aureus*, *S. pyogenes*) and antifungal strains (*C. albicans*, *A. niger*, and *A. clavatus*), but were less active than the standard drugs.\[56\]
Pyrazol derivatives
- Atia et al. reported some new imidazole Schiff bases and screened them for antibacterial activity. 3-Aminobimidazol-4-one compounds were synthesized by the reaction of compounds, 2-[1-(2-chloroethyl)-2-nitro-1H-imidazole-5-yl]-4-arylidine 1,3-oxazol-5(4H)-ones (prepared from oxidative cyclization of compound, ([1-(2-chloroethyl)-2-nitro-1H-imidazole-5-yl]carbonyl)amino) acetic acid with aromatic aldehydes) with hydrazine hydrate. The reaction of key intermediate 3-amino-3'-(2-chloroethyl)-5-arylidine-2'-nitro-3,5-dihydro-3'H,4'H-2,4'-imidazol-4-ones with aromatic aldehydes produced newer Schiff bases in high yield. The antibacterial activity of the imidazole derivatives was tested by the agar disc diffusion method against Staph. aureus, E. coli and Proteus mirabilis bacteria. Compounds showed good activity except few ones.[57]

Imidazole derivatives
- Asiri et al. synthesized a series of 1, 5-dimethyl-2-phenyl-1, 2-dihydro-3H-pyrazol-3-one containing Schiff bases and screened for their antibacterial activities. 1, 5-dimethyl-2-phenyl-1, 2-dihydro-3H-pyrazol-3-one Schiff base derivatives were prepared by the reaction of 4-aminophenazone with different substituted aromatic aldehydes. Four compounds showed moderate to good antibacterial activity against four bacterial strains, viz. Escherichia coli, Staphylococcus aureus, Salmonella typhimurium and Streptococcus pyogenes with MIC values of 6.25 μg/ml.[58]

Pyrazol derivatives
- Pandeya et al. prepared a series of Schiff and Mannich bases derived from isatin derivatives and N-[4-(4'chlorophenyl) thiazol-2-yl]thiosemicarbazide. All the synthesized compounds were screened for antimicrobial activities against 28 pathogenic bacteria, 8 pathogenic fungi and anti-HIV-1 in MT-4 cells culture. Among the synthesized compounds, one compound showed the most pronounced activity.[59]

Thiazole thiosemicarbazone derivatives
- Hutchinson et al. synthesized fluorinated analogues of 2-(4-aminophenyl) benzothiazoles, among which 2-(4-amino-3-methylphenyl)-5-fluorobenzothiazole exhibited selective and potent anticancer activity.[60]
Benzothiazole derivatives

- Singh et al. studied substituted thiazolyl-thiazolidinylbenzothiazoles and showed that none of the compounds having 2-substituted 4-thiazolidinone ring showed any antibacterial activity but compounds were potent for insecticidal activity. Electron withdrawing group at phenyl ring such as p-OCH₃(a) enhanced its insecticidal activity. Compound containing the azetidinone moiety instead of thiazolidinone displayed antibacterial activity against gram-positive bacteria S. aureus and E. coli. Thiazolidinone derivatives synthesized from chalcones of 4-hydroxycoumarin (b) showed that compounds having the methoxy group have increased antibacterial activity while azetidinones were found to be more active than thiazolidinones.⁶¹

- Kocabalkanli et al. synthesized Mannich bases of some 2,5-disubstituted 4-thiazolidinones and evaluated their antimicrobial activity. They reported that the most active compound had a p-chlorophenyl group on the oxadiazole, a methyl and a pyrrolidinomethyl at the 5-position of the thiazolidinone (a), while the least active one has a hydrogen atom in place of a chlorine and a morpholine in place of a pyrrolidine. Further analogous of 2-phenyl-3-(4,6-diarylimidin-2-yl)thiazolidin-4-ones (b,c) have been synthesized by Gopalakrishnan et al. and tested for their antibacterial activity against Staphylococcus aureus, b-hemolytic Streptococcus, Vibrio cholera, Salmonella typhi, Shigella flexneri, Escherichia coli, Klebsiella pneumonia, and Pseudomonas aeruginosa. Ciprofloxacin was used as standard drug. Results revealed that p-(OCH₃) and p-(CH₃) groups at phenyl ring attached to the pyrimidine ring exerted strong antibacterial activity against all the tested bacterial strains on the other hand compounds with electron withdrawing p-Cl and p-F functional group at phenyl ring attached to pyrimidine ring did not improved antibacterial activity.⁶²

- El-Gaby et al. recently synthesized a series of 2-thioxo-4-thiazolidinones and 4,4'-bis(2-thioxo-4-thiazolidinone-3-yl)diphenylsulfone derivatives. Most of the compounds were found moderate in activity against...
tested strain of bacteria. Thiazolidinones (a) with sulfamoyl and thioxo moieties were found to possess highest antibacterial activity towards *Bacillus cereus* whereas thiazolidinone derivative (b) bearing pyrimidine nucleus, sulfamoyl phenyl and thioxo moieties revealed high activity against *S. aureus*.\textsuperscript{[63]}

\[ \text{a=R} = -\text{C3H5, b=R} = -\text{C6H6} \]

- Gilsoy et al. synthesised and evaluated antimicrobial activity of novel derivatives of hydrazide-hydrazones, thiosemicarbazides and thiazolidinones. All the synthesized compounds were tested for antibacterial, antifungal and antymycobacterial activity against different bacteria (*S. aureus* ATCC 6538, *S. epidermidis* ATCC 12228, *K. pneumonia* ATCC 4352, *P. aeruginosa* ATCC 1539, *E. coli* ATCC 8739, *Shigella flexneri*, *S. typhi*, *Proteus mirabilis*, *Mycobacterium tuberculosis* H37Rv) and *C.albicans* ATCC 10231. They noted that none of the compounds (a, b) showed significant activity against the selected microorganisms.\textsuperscript{[64]}

- Bondock et al. synthesized thirteen compounds and screened for antimicrobial activities against *B. subtilis*, *B. megaterium*, *E. coli*. Most of the prepared thiazolidinone derivatives (a,b) revealed comparable activity against tested strains by taking ampicillin and chloramphenicol in a concentration of 25 mg/ml as a reference drug.\textsuperscript{[65]}

- Hafez et al. synthesized a series of substituted triazolo[4,3-a]pyrimidin-6-sulfonamide with an incorporated thiazolidinone moiety and reported for their antitumor activity. Most of the synthesized compounds were found moderate in activity and compound (a) displayed a good growth inhibitory activity on all tested 60 cell lines showing GI50 values between 5.89 and 37.1*10^{-6} \text{µM. In fact, the presence of 4- methylpiprazin/ morpholine on C-5 and thienyl group at C-2 of thiazolidinone seems to be very important for anticancer activity.}\textsuperscript{[66]}

- Ottana et al. investigated 3,3’-(1,2-ethanediyl)-bis[2-aryl-4-thiazolidinone] derivatives (a), which
showed interesting stereoselective anti-inflammatory/analgesic activities and suggested that these derivatives might preferentially interact with inducible COX-2 isoform. Absence of 5-arylmethyldiene moiety in 3-[2-(4-methylphenyl)-2-oxo-1-phenylethyl]-

Amin et al. prepared several spiro [(2H,3H) quinazoline-2,10-cyclohexan]-4(1H)-one derivatives. These compounds were evaluated for their anti-inflammatory, ulcerogenic and analgesic activities. Compound (a) with 2-thiophene substitution at C-2 of thiazolidinone has shown most active anti-inflammatory activity and considerable analgesic activity.\(^{[68]}\)

Akula et al. synthesized 3-[1H-benzimidazole-2-yl-amino]-2-phenyl-1,3-thiazolidin-4- one derivatives and compound with 4-chloro (a) on phenyl ring showed promising depressant activity among all the tested compounds.\(^{[69]}\)

Chen et al. prepared various 2-(2,6-dihalophenyl)-3-(4,6-dimethyl-5-(un)substituted-pyrimidin-2-y1)-thiazolidin-4-ones. The structures of these newly synthesized compounds were confirmed by their analytical and spectral data. These compounds were also evaluated for their HIV-RT inhibitory activity. It was stated that HIV-RT inhibitory activity would be majorly affected by high value of hydrophobicity. It was reported that compound (a) and (b) having ethyl group at 5-position on N-3 position of pyrimidine ring were the most potent ones with the IC\(_50\) value of 0.26 and 0.23 lM, respectively. Their findings suggested that overall hydrophobicity of the analogues, and steric and electronic features of meta/para substituents of 3-heteroaryl moiety on thiazolidin-4-one led to a substantial increase in antiviral activity.\(^{[70]}\)

(b) Liu et al. studied a series of novel PTP1B inhibitors containing a thiazolididine-substituted biphenyl scaffold and reported that introduction of the 4- oxothiazolidine-2-thione moiety showed better inhibitory activity against PTP1B. Substitution with polar group at N of thiazolidinone ring and alkyl group at the 40-position of the biphenyl scaffold led to unfavorable for bioactivity.

Compound (a) have benzyl group lowered the fasting glucose levels and showed IC\(_50\) value of 0.48±0.07µmol/L. Replacement of carbonyl group in position 2 of the 2,4-thiazolidinedione scaffold with a phenylimino moiety enhanced its PTP1B as well as LMW-PTP inhibitory activity. Ottana et al. reported that substitution with lipophilic arylidene moiety in position 5...
particularly favored the activity; phenoxy and benzyloxy groups in the para and meta positions of the 5-benzylidene group found to be better substituents for enzyme inhibition. Compound 4-[(4-oxo-5-(4-phenoxy-}

\[
\text{benzylidene)-2-phenylimino-thiazolidin-3-yl][methyl]benzoic acid showed PTP1B and LMW-PTP inhibition at IC50 = 1.1, 3.1 \mu M, respectively.}\]

\[71\]

- Jackson et al. reported synthesis and biological evaluation of thiazolidinone-based blockers of Kv1.5. The 3,4-dimethyl derivatives (a) (IC50 = 0.069 \mu M) and 145 (IC50 = 0.270 \mu M) were found to be the most potent compounds of this series.132 In 2009, Bhandari and co-workers discovered 2-(2-(4-(4-(3-(5-substituted methylene)-4-oxo-2-(phenylimino)thiazolidin-3-yl)-2-hydroxypropylamino)benzoyl) hydrazinyl)-2-oxoethyl nitrate derivatives and evaluated them for electrocardiographic, antiarrhythmic, vasorelaxing and antihypertensive activity as well as for in-vitro nitric oxide (NO) releasing ability and found that some of these heterocycles are very potent. Compound 2-(2-(4-(3-(5-benzylidene-4-oxo-2-(phenylimino)-thiazolidin-3-yl)-2-hydroxypropylamino)benzoyl) hydrazinyl)-2-oxoethyl nitrate (c), was found to be the most potent in this series.172

- Gududuru et al. tested a series of 2-arylthiazolidine-4-carboxylic acid amides for possible cytotoxic activity in prostate cancer. Compound (a) was found to be most potent and selective cytotoxic agent with IC50 of 0.55 \mu M and 38-fold selectivity in PPC-1 cells. The SAR study showed that as the chain length increased from C7 to C18, the potency also increased but further increase in the alkyl chain by one carbon unit caused a significant loss of activity, so alkyl chain with C18 unit was optimal for effectiveness of thiazolidine analogues. Replacement of the phenyl ring with an alkyl or cyclohexyl group reduced the potency while replacement with furanyl ring derivative showed equivalent cytotoxicity. The same research group designed new series of 2-aryl-4-oxothiazolidin-3-yl amides (b) and all synthesized compounds were evaluated against five human prostate cancer cell lines. They reported that increase in the alkyl chain enhanced the antiproliferative activity while replacement of the alkyl chain with aryl group reduced the biological activity.173

\[73\]
Zitouni et al. reported the synthesis of N-pyridyl-N0-thiazolylhydrazinederivatives. Compound (a) showed high antituberculosis activity (IC50: 6.22 lg/mL and IC90: 6.78 lg/mL), its structural details revealed that 2-pyridyl and 2-hydroxy-5-methoxyphenyl group are essential for antimycobacterial activity while 3-pyridyl, 4-pyridyl group were unfavorable for activity.[74]

Kucukguzel et al. reported antimycobacterial activity of substituted 4-thiazolidinones and found those only compounds (a) and (b) showed 90 and 98% inhibitions at 6.25 µg/ml, respectively.[75]

Jaju and co-workers had synthesized isonicotinylhydrazidederivatives and screened their in vitro antimycobacterial activity against M. tuberculosis H37Rv using alamar-blue susceptibility test. They found that the antitubercular activity was considerably affected by various substituents on the aromatic ring of 4-thiazolidinone and it was proved by the fact that compounds with no substitution at the aromatic ring did not show any considerable activity. The hydroxyl and methoxyl group on aromatic ring substituted compound (a) was found to be more active (MIC = 0.31 lg/mL). Karali et al. tested 4-(3-coumarinyl)-3-cyclohexyl-4-thiazolin-2-one benzylidene hydrazone derivatives for antitubercular activity. Most of the compounds showed less than 90% inhibition and considered to be inactive and compound (b) showed maximum inhibition of 42%.[76]

Kline et al. have carried out the synthesis of some novel trisaryl substituted 2-imino-5-arylidenethiazolidin-4-one derivatives and evaluated for their inhibitory activity of bacterial type III secretion in Salmonella enterica. The results indicated that compound (b) acted directly to disrupt a protein-protein interaction. Analogous having N-3 carbalkoxy substituent (a) was shown to have significant degree of SipA inhibition at concentrations of 1M or less and the activity of compound (b) was due to the presence of combination of a cationic group and a functional group having the potential for hydrophobic interactions. [77]
Siddiqui et al. studied fungicidal activity of 4, 4ʹ-bis(200-aryl-500-methyl/unsubstituted- 400-oxothiazolidin-300-y1) bibenzyl against Fusarium oxysporium and Penicillium citrinum. The results demonstrated that the presence of 5-methyl oxothiazolidine nucleus with the bibenzyl nucleus (a) caused complete inhibition of mycelial growth of the test fungi and enhanced the fungicidal activity of these compounds. Further substitution on phenyl ring on thiazolidinone did not improve its fungicidal activity. Presence of two fluorine atoms at 2nd and 6th positions of 2-phenylthiazolidin- 4-one (b) bearing a venlafaxine moiety represented more potent antibacterial and antifungal agents.\[78\]

Khan and Yusuf synthesized steroidal (cholesterol) derivatives of thiazolidinone and evaluated against bacteria such as S.aureus, S. pyogenes, S. typhimurium and E. coli. Compounds having acetoxy (a) and chloro (b) substituents on the 3b-position of the steroidal thiazolidinone ring showed maximum potency.\[79\]

Kumar et al. synthesized a variety of new indolylthiadiazino-azetidinones and indolylthiadiazino-thiazolidinone. Out of eight indolylthiadiazino-thiazolidinone derivatives only 5-methoxy-2,3-[2”-(2”-methoxy-phenyl-4”-oxy-1”-3”-thiazolidin-3”-yl)-1”,3”,4”-thiadiazinol]indoles (a) showed moderate antibacterial activity, but none of the compounds was found to be more active than 5-methoxy-2,3-[20-(300-chloro-200-oxo- 400-methoxy-phenyl-100-azetidinyl)-10,30,40-thiadiazinol]indoles. A number of chalcone derivatives bearing the 2,4-thiazolidinedione and benzoic acid moieties has been evaluated for antimicrobial activity against six gram-positive bacteria and tested compounds did not exhibited any activity against gram-negative strains. From this series compound (b) was the best against multi drug resistant gram-positive bacterial strains (MRSA CCARM 3167, 3506; QRSACCARM 3505, 3519) with MIC values in the range of 0.5–2 l gm/ml, which showed eight-fold more potency than norfloxacin and 64-fold more activity than oxacillin. SAR study explained that free carboxyl group at para position and Cl, Br and –OCH3 groups at ortho position seem to be enhanced antibacterial activity.\[80\]
Mulwad and Mir reported antibacterial activity of N-[coumarin-6-yl] spiro- indolooazetin-2-ones/thiazolidin-4-ones derivatives. Thiazolidinone ring instead of azetidinone did not show significant activity, coumarin ring substituted with methyl group at 4th and 7th position attached with 2-isatin-4-thiazolidinone ring (a) showed moderate antibacterial activity against \( S. aureus \) and \( B. subtilis \). Aquino et al. synthesized a series of 2-[(phenylmethylene) hydrazono]-4-oxo-3-phenyl-5-thiazolidinoneacetic acids for their anti-\( T. gondii \) and antimicrobial activities.\(^4\) Thiazolidinone derivatives were initially tested for antimicrobial activity by the disc diffusion method and it was found that compound (b) revealed the best activities against \( S. aureus \), \( S. faecalis \), \( B. subtilis \) reduced and the percentage of infected cells and mean number of tachyzoites per cell in 2 IM concentration.\(^8\)

Cecilia Saiz et al. developed a tandem method for the synthesis of 2-hydrazolyl-4-thiazolidinones from commercially available materials in a 3 component reaction. The reaction connects aldehydes, thiosemicarbazides and maleic anhydride, effectively assisted by microwave irradiation. The synthesis of a new type of compound, 2-hydrazolyl-5, 5-diphenyl-4-thiazolidinone, obtained by treatment of thiosemicarbazone with benzil in basic media is also reported. HOMO/LUMO energies, orbital coefficients and charge distribution were used to explain the proposed reaction mechanism. Microwave heating for the synthesis of thiazolidinone resulted in a significantly better yield compared to thermal conditions. Microwave irradiation also allowed for a faster conversion.\(^8\)

S.J.Wadher et al. synthesized Schiff base and 4-thiazolidinones of amino salicylic acid and their derivatives and tested their antimicrobial activity. The novel molecules were synthesized and evaluated for antimicrobial studies. The synthesized compounds were screened for their in vitro antimicrobial activity. The structure of synthesized compounds was established on the basis of their spectral (IR, \(^1\)H NMR and mass) data. The purity of the synthesized compounds was confirmed by TLC.\(^8\)
Mulay Abhinit et al. reviewed the chemistry, synthesis, spectral studies and applications of 4-thiazolidinones. Work done by many researchers was explored. The literature revealed that 4-thiazolidinone has diverse biological potential, and the easy synthetic routes for synthesis have taken attention of the chemists, pharmacologists and researchers. The anticancer and anti-HIV activities are the most encouraging activities for the pharmacists. Also the research in anticonvulsant, FSH and CFTR inhibitory activity gave positive results.\[84\]

4-Thiazolidinone synthesis

3. AIM AND OBJECTIVE

Schiff bases of heterocyclic compounds are interesting class of organic compounds, being studied over the years and reported to possess a wide spectrum of biological activities such as antibacterial, anti tubercular, anti inflammatory, antiviral and antioxidant activity. Schiff bases of salicylaldehydes have also been reported as plant growth regulator and antimicrobial or antifungal activity. Schiff bases also show some analytical applications. Although a number of anticoagulant, antiplatelet, antibacterial and antifungal drugs are available in the market, the search for new molecules still continues because of the need for less costly drugs and drugs with minimal side effects. Thiazolidinones have been synthesized and screened for possible antimicrobial activity. Thiazolidinones have a broad spectrum of pharmacological properties like anti HIV, antipsychotic, anticonvulsant and antitubercular activity. In view of the above mentioned facts and need to develop some novel biologically important compounds and utilizing environmental friendly methods, an attempt has been made.

- To synthesize Schiff bases from salicylaldehyde by using a microwave as depicted in Scheme I.
- To purify the new compounds by recrystallization.
- To synthesize Thiazolidinones using the above
Schiff bases.

- To purify the new compounds by recrystallization.
- To characterize the new compounds by physical and spectral data (TLC, IR, $^1$H NMR and Mass spectral analysis).
- To screen the Schiff bases for in-vitro anticoagulant activity.
- To screen Schiff bases and thiazolidinones for in-vitro antibacterial and anthelmintic activities by standard protocol available in literature.

4. EXPERIMENTAL SECTION

4.1. Methodology

The present work is based on Schiff base reaction which involves reaction between different amines with salicylaldehyde without using a solvent to get imines. Schiff bases were screened for anticoagulant activity and using these Schiff bases, thiazolidinones were synthesized and were screened for in vitro antibacterial activity and anthelmintic activity.

Chemicals and Reagents

The chemicals and reagents (Table 1) used in the present project were of AR and LR grade, procured from S.D-Fine Chem.Ltd.

<table>
<thead>
<tr>
<th>Chemicals and Reagents</th>
<th>Chemicals and Reagents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salicylaldehyde</td>
<td>DL Isoleucine</td>
</tr>
<tr>
<td>3 aminobenzoic acid</td>
<td>acetone</td>
</tr>
<tr>
<td>Ethanol</td>
<td>methanol</td>
</tr>
<tr>
<td>Tetrahydrofuran</td>
<td>Silica gel G</td>
</tr>
</tbody>
</table>

Table no.1 List of chemicals and reagents.

SCHEME I

Aldehyde: R= C₆ H₅ OH (phenol)/ C₆ H₆ (benzene)
Amine: R= C₆ H₅ OH (phenol)/ C₅ H₅ N (pyridine)

Analytical Techniques

1. Physical data: Melting points of the synthesized compounds were recorded using melting point apparatus with open capillary tubes.

2. Thin Layer Chromatography: Purity of the compounds was determined by thin layer chromatography using silica gel G as stationary phase and various combinations of methanol: water: tetrahydrofuran in the ratio 2:2:1.

3. Instrumentation: The techniques employed for the characterization of the synthesized compounds were IR, $^1$H NMR and Mass spectral analysis.

a. IR spectra: The IR spectra of the synthesized compounds were recorded on a Fourier Transform IR spectrometer (model Shimadzu 8700) in the range of 400-4000 cm$^{-1}$ using KBr pellets and values are reported in cm$^{-1}$ and the spectra were interpreted.

b. $^1$H- NMR: $^1$H-NMR spectra were recorded on DPX-200 MHz NMR spectrometer using DMSO-d$_6$ and chemical shifts (δ) are reported in parts per million down field from internal reference tetramethylsilane (TMS) and the spectra were interpreted.

c. Mass spectra: Mass spectra were recorded on Mass spectrophotometer (model Shimadzu) by LC-MS and the spectra were interpreted.
Schiff base: R = C₆ H₅ OH (phenol)/ C₆ H₆ (benzene), R¹ = C₆ H₅ OH (phenol)/ C₅ H₅ N (pyridine)
Thiazolidinones: R¹ = C₆ H₅ OH (phenol)/ C₆ H₆ (benzene), R = C₆ H₅ OH (phenol)/ C₅ H₅ N (pyridine)

SCHEME FOR INDIVIDUAL COMPOUNDS SCHEME FOR SYNTHESIS OF SCHIFF BASE 2a

SCHEME FOR SYNTHESIS OF SCHIFF BASE 2b
SCHEME FOR SYNTHESIS OF THIAZOLIDINONE 3a

Schaiff base 2a + Thioglycolic acid

DMF (Dimethyl formamide) Microwave irradiation for 15s, dissolved in water, filtered.

2-(2-(2-hydroxyphenyl)-4-oxo-thiazolidin-3-yl)benzoic acid

SCHEME FOR SYNTHESIS OF THIAZOLIDINONE 3b

Schaiff base 2b + Thioglycolic acid

DMF (Dimethyl formamide) Microwave irradiation for 30s, dissolved in water, filtered.

2-(2-(2-hydroxyphenyl)-4-oxo-thiazolidin-3-yl)-3-methylpentanoic acid
4.2. General procedures: Synthesis of Schiff base 2a

1.06 ml of salicylaldehyde and 1.37 g of 3-aminobenzoic acid were taken in a clean and dry conical flask and subjected to microwave irradiation for 2 min 30 s giving 10 s pulses to synthesize 0.01 mole of the compound. The product was then cooled and recrystallized using ethanol and to get pure crystals of the product. Melting point was determined using melting point apparatus with open capillary tubes. TLC was performed to determine the Rf value.

Synthesis of Schiff base 2b

1.06 ml of salicylaldehyde and 1.31 g of DL Isoleucine were taken in a clean and dry conical flask and subjected to microwave irradiation for 3 min 20 s giving 10 s pulses to synthesize 0.01 mole of the compound. The product was then cooled and recrystallized using tetrahydrofuran to get pure crystals of the product. Melting point was determined using melting point apparatus with open capillary tubes. TLC was performed to determine the Rf value.

Table no. 2: Physical data of Schiff bases.

<table>
<thead>
<tr>
<th>Code</th>
<th>Mol.F</th>
<th>Mol.wt (g.mol-1)</th>
<th>M.P (ºC)</th>
<th>% Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>2a</td>
<td>C14H11NO3</td>
<td>241</td>
<td>163</td>
<td>86</td>
</tr>
<tr>
<td>2b</td>
<td>C13H17NO</td>
<td>219</td>
<td>185-190</td>
<td>70</td>
</tr>
</tbody>
</table>

Synthesis of Thiazolidinone 3a.

The above synthesized Schiff base 2a (0.01 mole) was taken in a clean and dry conical flask. To this 0.7 ml of dimethylformamide (0.01 mole) was added. 0.7 ml of thioglycolic acid (0.01 mole) was added slowly to the above contents and subjected to microwave irradiation for 15 s. To this solution ice cold water was added immediately when the solution was still hot. The product was filtered, dried and recrystallized with ethanol to get pure crystals of the product. Melting point was determined using melting point apparatus with open capillary tubes. TLC was performed to determine the Rf value.

Synthesis of Thiazolidinone 3b.

The above synthesized Schiff base 2b (0.01 mole) was taken in a clean and dry conical flask. To this 0.7 ml of dimethylformamide (0.01 mole) was added. 0.7 ml of thioglycolic acid (0.01 mole) was added slowly to the above contents and subjected to microwave irradiation for 30 s. To this solution ice cold water was added immediately when the solution was still hot. The product was filtered, dried and recrystallized with ethanol to get pure crystals of the product. Melting point was determined using melting point apparatus with open capillary tubes. TLC was performed to determine the Rf value.

4.3. Biological Activity

1. Antibacterial activity

Even though pharmacological industries have produced a number of new antibiotics in the last three decades, resistance to these drugs by microorganisms has increased. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents. Such a fact is cause for concern, because of the number of patients in hospitals who have suppressed immunity, and due to new bacterial strains, which are multi-resistant. Consequently, new infections can occur in hospitals resulting in high mortality.[65]

The problem of microbial resistance is growing and the outlook for the use of antimicrobial drugs in the future is still uncertain. Therefore, actions must be taken to reduce this problem, for example, to control the use of antibiotic, develop research to better understand the genetic mechanisms of resistance, and to continue studies to develop new drugs, either synthetic or natural. The ultimate goal is to offer appropriate and efficient antimicrobial drugs to the patient.

Antibacterials are drugs used to treat infections caused due to bacteria, by either inhibiting their growth or by killing them. In view of the above mentioned problems, an attempt was made to synthesize new Schiff bases and thiazolidinones and tested their antibacterial activity.

Procedure

The synthesized Schiff bases and thiazolidinones were screened for antibacterial activity using two cultures, *E.coli* for gram negative species and *Staphylococcus aureus* for gram positive. The compounds were tested by agar well diffusion assay. The cultures were prepared 24 hrs prior to testing the antibacterial activity. Nutrient agar medium was prepared and sterilized in an autoclave for 45 minutes. This medium was inoculated with the above cultures and then poured into different petriplates under a laminar airflow chamber in an aseptic room. The medium was allowed to solidify. Using aseptic borers, small holes were made at equal distances in the petriplates. The synthesized compounds were dissolved in ethanol to make 10, 20 and 30 μg/ml concentration solutions. The standard drug solution was also prepared in concentrations of 10, 20 and 30 μg/ml. Using aseptic borers, holes were made in the media at equal distances. These holes were filled with the above solutions using an automatic pipette. Ethanol was used as control.

The petriplates were placed in a refrigerator for a short period of time and then transferred to an incubator where they were incubated in an inverted position at 37º C for
24hrs. These petriplates were then observed for zone of inhibition and the distance was measured in mm. The zone of inhibition for different test compound and standard drug are tabulated in the table no 8.

2. Anthelmintic Activity
Helminthes infections are among the most widespread infections in humans, distressing a huge population of the world. Although the majority of infections due to helminthes are generally restricted to tropical regions and cause enormous hazard to health and contribute to the prevalence of undernourishment, anaemia, eosinophilia and pneumonia. Parasitic diseases cause ruthless morbidity affecting principally population in endemic areas. The gastro-intestinal helminthes becomes resistant to currently available anthelmintic drugs therefore there is a foremost problem in treatment of helminthes diseases. [86-87]

Anthelmintics are drugs that are used to treat infections due to parasitic worms. This includes both flat worms, e.g. flukes, tapeworms and roundworms, i.e. nematodes. They are of huge importance for human tropical medicine and for veterinary medicine. The World Health Organization estimates that a staggering 2 million people harbor parasitic worm infections. [38-69]

Despite the prevalence of parasitic worms, anthelmintic drug discovery is the poor relation of the pharmaceutical industry. The simple reason is that the nations which suffer most from these tropical diseases have little money to invest in drug discovery and therapy. It comes as no surprise therefore that the drugs available for human treatment were first developed as veterinary medicines.

Procedure
The synthesized Schiff bases and thiazolidinones were screened for anthelmintic activity using Pheretima Posthuma (earthworm). 11 earthworms of nearly equal size were placed in control, standard and test compound’s solutions at room temperature. Normal saline was used as control. The standard drug used was albendazole. The standard drug and test compound were dissolved in minimum quantity of ethanol and adjusted the volume up to 10ml with normal saline solution to get the concentrations 0.1%, 0.2% and 0.5%/w/v.

The compounds were evaluated by the time taken for complete paralysis and death of earthworms. The mean lethal time for each test compound was recorded and compared with standard drug.

The time taken by earthworms to become motionless was noted as paralysis time. To ascertain the death of motionless earthworms, they were frequently applied with external stimuli, which stimulates and induces movement in earthworms, if alive. The mean lethal time and paralysis time of the earthworms for different test compounds and standard drug are tabulated in the table no 9.

3. Anticoagulant activity
Anticoagulants are medicines that prevent the blood from clotting as quickly or as effectively as normal. Anticoagulants are used to treat and prevent blood clots that may occur in blood vessels. Blood clots can block an artery or a vein (blood vessels). A blocked artery stops blood and oxygen from getting to a part of our body. The tissue supplied by a blocked artery becomes damaged, or dies, and this results in serious problems such as a stroke or heart attack. A blood clot in a large vein, such as a deep vein thrombosis (a clot in the leg vein), can lead to serious problems such as a pulmonary embolism (a clot that travels from the leg vein to the lungs). [90]

A number of anticoagulants are available including: warfarin, acenocoumarol, phenindione, dabigatran, aspirin and rivaroxaban. However, due to its side effects like bleeding and other disadvantages like its chemical inhomogeneity and the variability of its physiological activities, an important field of research are alternatives to genuine anticoagulants.

Despite the prevalence of these anticoagulants, they suffer from common side effects like haemorrhages, headaches, backpain, nose bleeds etc.

Therefore the aim of the present study was to develop newer Schiff bases with anticoagulant activity and to minimize the side effects and the cost associated with the presently available anticoagulants.

Procedure
The synthesized Schiff bases (2a and 2b) were screened for anticoagulant activity by prothrombin time test. The prothrombin time test (also known as the pro test or PT test) is a useful screening procedure for the extrinsic coagulation mechanism including the common pathway. It detects deficiencies in factor II, V, VII, and X. The prothrombin time test is frequently used to follow oral anticoagulant therapy that inhibit factors II, VII, IX and X. The normal prothrombin time ranges between 11-15 seconds.

Collection of blood sample
The blood sample was obtained from an individual having normal prothrombin time, not suffering from any cardiovascular diseases (hypertension, congestive heart failure, coagulation disorders such as, Hemophilia A or B) or diabetes, not recently using nonsteroidal anti inflammatory drugs, not obese or smokers and free from dyslipidemic disorders and placed separately in container containing tri-sodium citrate to prevent the clotting process. Centrifugation (15 minutes at rate 3000 rpm) was carried out to separate the blood cells from plasma in order to obtain pure platelet plasma (PPP) for prothrombin time test. The obtained plasma sample was poured separately in plane containers using automatic pipette and stored at room temperature. [91-92]
Collection of blood and Plasma re-calcification
0.2 ml plasma, 0.1 ml of Schiff base of different concentrations and CaCl2 [(25 mili M) thereby reversing the effect of citrate which enables the blood to clot again] were added together in a clean fusion tube and incubated at 37°C in water bath. For control experiment Schiff base solution was replaced by same volume of 0.9% saline water. The clotting time of Schiff bases (2a and 2b) were recorded with stopwatch by tilting the test tubes every 5 seconds. This time is called the prothrombin time.  

The Schiff bases (2a and 2b) were dissolved in methanol to make 10, 20, 40, and 80 μg/ml concentration solutions.

Time of coagulation of Schiff bases 2a and 2b at different concentrations are tabulated in table no 10 and table no 11 respectively.

5. RESULTS AND DISCUSSION
Chemistry
As a result of microwave assisted synthesis, it was observed that the reaction was completed in a short time with higher yields compared to the conventional method.

Schiff bases and thiazolidinones show a wide spectrum of pharmacological applications. The synthesis of all compounds was carried out as depicted in Scheme – I. Novel Schiff bases were obtained by microwave irradiation of different aryl amines with different aldehydes. These Schiff bases were then treated with thioglycolic acid in the presence of DMF (dimethyl formamide) in a microwave to give thiazolidinones. The resulting compounds were purified by recrystallization using ethanol and methanol.

The synthesized compounds were characterized by both physical and spectral data like 1H-NMR, Mass spectra and FT-IR. Schiff bases were evaluated for anticoagulant activity. Newly synthesized compounds were evaluated for antibacterial and anthelmintic activities.

The synthesized novel Schiff bases were characterized from the spectral analysis, it is revealed that, IR shows presence of C=N peak at around 1600 cm⁻¹ for all the Schiff bases. IR also shows presence of –OH peak at around 3300 cm⁻¹ for Schiff bases containing –OH group. 1H-NMR shows chemical shift (δ) of protons in –COOH at around 13 ppm, Ar-OH at around 9 ppm and Ar-H at around 6-8 ppm.

The synthesized novel thiazolidinones bases were characterized from the spectral analysis, it is revealed that, IR shows presence of C=O peak at around 1700 cm⁻¹ and presence of N-CH-S peak at around 730 cm⁻¹ for all the thiazolidinones. 1H-NMR shows chemical shift (δ) of protons in –COOH at around 13 ppm, Ar-OH at around 9 ppm and 6.7-7.9 ppm.

All the compounds showed characteristic base peak for M+1 peak and the mass spectra of all the synthesized compounds obeying the nitrogen rule.

| Table no: 4 Physical characterization data of compound 2a. |
|----------------|----------------------------------------------------------------------------------------------------|
| IUPAC Name    | (E)-3-(2-hydroxybenzylideneamino) benzoic acid                                                    |
| Mol.F         | C14H11NO3                                                                                         |
| Mol.wt        | 241 g mol⁻¹                                                                                        |
| M.P           | 163 oC                                                                                             |
| Solubility    | Methanol and ethanol                                                                              |
| TLC solvent   | Methanol : Water : Tetrahydrofuran-2:2:1                                                          |
| Rf value      | 0.65                                                                                              |
| % Yield       | 86%                                                                                               |

<table>
<thead>
<tr>
<th>Compound 2b.</th>
</tr>
</thead>
<tbody>
<tr>
<td>(E)-2-(benzylideneamino)-3-methylpentanoic acid</td>
</tr>
</tbody>
</table>

| Table no: 5 Physical characterization of compound 2b. |
|----------------|--------------------------------------------------------------------------------------------------------|
| IUPAC Name    | (E)-2-(benzylideneamino)-3-methylpentanoic acid                                                      |
| Mol.F         | C13H17NO2                                                                                           |
| Mol.wt        | 219 g mol⁻¹                                                                                        |
| M.P           | 185-190 oC                                                                                          |
| Solubility    | Tetrahydrofuran                                                                                     |
| TLC solvent   | Methanol : Water : Tetrahydrofuran-2:2:1                                                             |
| Rf value      | 0.56                                                                                               |
| % Yield       | 70%                                                                                                 |
Compound 3a

![Chemical structure of compound 3a](image)

2-(2-(2-hydroxyphenyl)-4-oxothiazolidin-3-yl)benzoic acid

Table no: 6 Physical characterization data of compound 3a.

<table>
<thead>
<tr>
<th>IUPAC Name</th>
<th>2-(2-(2-hydroxyphenyl)-4-oxothiazolidin-3-yl) benzoic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mol.F</td>
<td>C16H13NSO4</td>
</tr>
<tr>
<td>Mol.wt</td>
<td>315 g mol-1</td>
</tr>
<tr>
<td>M.P.</td>
<td>215 oC</td>
</tr>
<tr>
<td>Solubility</td>
<td>Ethanol</td>
</tr>
<tr>
<td>TLC solvent</td>
<td>Methanol : Water : Tetrahydrofuran-2:2:1</td>
</tr>
<tr>
<td>Rf value</td>
<td>0.43</td>
</tr>
<tr>
<td>% Yield</td>
<td>84%</td>
</tr>
</tbody>
</table>

Compound 3b

![Chemical structure of compound 3b](image)

2-(2-(2-hydroxyphenyl)-4-oxothiazolidin-3-yl)-3-methylpentanoic acid

Table no: 7 Physical characterization data of compound 3b.

<table>
<thead>
<tr>
<th>IUPAC Name</th>
<th>2-(2-(2-hydroxyphenyl)-4-oxothiazolidin-3-yl)-3-methylpentanoic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mol.F</td>
<td>C15H19NSO4</td>
</tr>
<tr>
<td>Mol.wt</td>
<td>309 g mol-1</td>
</tr>
<tr>
<td>M.P.</td>
<td>247 oC</td>
</tr>
<tr>
<td>Solubility</td>
<td>Ethanol</td>
</tr>
<tr>
<td>TLC solvent</td>
<td>Methanol : Water : Tetrahydrofuran-2:2:1</td>
</tr>
<tr>
<td>Rf value</td>
<td>0.34</td>
</tr>
<tr>
<td>% Yield</td>
<td>75%</td>
</tr>
</tbody>
</table>

Chemical characterization data of synthesized compounds: Compound 2a.

![Chemical structure of compound 2a](image)

(E)-3-(2-hydroxybenzylideneamino)benzoic acid

IR data

<table>
<thead>
<tr>
<th>Functional group</th>
<th>Frequency in cm⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>C=O stretching</td>
<td>1603.88</td>
</tr>
<tr>
<td>CH=O stretching</td>
<td>1501.65</td>
</tr>
<tr>
<td>OH stretching</td>
<td>3089.13</td>
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<tr>
<td>COOH stretching</td>
<td>2995.58</td>
</tr>
<tr>
<td>Aromatic C-H stretching</td>
<td>2570.26</td>
</tr>
</tbody>
</table>

H¹NMR data

<table>
<thead>
<tr>
<th>Protons</th>
<th>δ in ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>COOH</td>
<td>s(12.8)</td>
</tr>
<tr>
<td>Ar-H</td>
<td>m(7.4-7.9)</td>
</tr>
<tr>
<td>Ar-OH</td>
<td>s(9.0)</td>
</tr>
<tr>
<td>CH</td>
<td>m(6.9-7.0)</td>
</tr>
</tbody>
</table>

Compound 2b

![Chemical structure of compound 2b](image)

(E)-2-(benzylideneamino)-3-methylpentanoic acid

IR data

<table>
<thead>
<tr>
<th>Functional group</th>
<th>Frequency in cm⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>C=O stretching</td>
<td>1594.23</td>
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<tr>
<td>CH=O stretching</td>
<td>1499.72</td>
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<tr>
<td>CH-H stretching</td>
<td>3119.03</td>
</tr>
<tr>
<td>COOH stretching</td>
<td>2581.83</td>
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<td>Aromatic C-H stretching</td>
<td>2850.91</td>
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</table>

H¹NMR data.

<table>
<thead>
<tr>
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<th>δ in ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>COOH</td>
<td>s(13.7)</td>
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<tr>
<td>Ar-H</td>
<td>m(6.6-8.6)</td>
</tr>
<tr>
<td>CH3</td>
<td>s(0.9)</td>
</tr>
<tr>
<td>CH2</td>
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<td>CH</td>
<td>s(2.5)</td>
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<tr>
<td>CH</td>
<td>s(3.9)</td>
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</table>
MASS data

<table>
<thead>
<tr>
<th>Molecular weight</th>
<th>Fragmentation</th>
</tr>
</thead>
<tbody>
<tr>
<td>219</td>
<td>248(35)</td>
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<tr>
<td></td>
<td>219(50)</td>
</tr>
<tr>
<td></td>
<td>203(30)</td>
</tr>
<tr>
<td></td>
<td>175(40)</td>
</tr>
</tbody>
</table>

Compound 3a.

2-(2-(2-hydroxyphenyl)-4-oxothiazolidin-3-yl)benzoic acid

IR data

<table>
<thead>
<tr>
<th>Functional group</th>
<th>Frequency in cm⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>C=O stretching</td>
<td>1725.46</td>
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<tr>
<td>N-CH-S stretching</td>
<td>741.00</td>
</tr>
<tr>
<td>OH stretching</td>
<td>3311.92</td>
</tr>
<tr>
<td>Aromatic C-H stretch</td>
<td>2982.08</td>
</tr>
<tr>
<td>COOH stretching</td>
<td>2493.10</td>
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</tbody>
</table>

H¹NMR data

<table>
<thead>
<tr>
<th>Protons</th>
<th>δ in ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>COOH</td>
<td>s(12.8)</td>
</tr>
<tr>
<td>Ar-OH</td>
<td>s(9.0)</td>
</tr>
<tr>
<td>Ar-H</td>
<td>m(7.4-7.9)</td>
</tr>
<tr>
<td>CH</td>
<td>m(6.97-7.01)</td>
</tr>
<tr>
<td>CH₂</td>
<td>s(3.6)</td>
</tr>
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</table>

Figure 1: FT-IR spectra of (E)-3-(2-hydroxybenzyldeneamino)benzoic acid (Schiff base 2a)
Figure 2: FT-IR spectra of 2-(2-(2-hydroxyphenyl)-4-oxothiazolidin-3-yl)benzoic acid (Schiff base 2b)

Figure 3: FT-IR spectra of 2-(2-(2-hydroxyphenyl)-4-oxothiazolidin-3-yl)benzoic acid. (Thiazolidinone 3a)

Figure 4: H¹NMR spectra of (E)-3-(2-hydroxybenzyldieneamino) benzoic acid (Schiff base 2a)
Figure 5: $^1$H NMR spectra of (E)-2-(benzylideneamino)-3-methylpentanoic acid (Schiff base 2b).

Figure 6: $^1$H NMR spectra of 2-(2-(2-hydroxyphenyl)-4-oxothiazolidin-3-yl) benzoic acid (Thiazolidinone 3a)

Figure 7: $^1$H NMR spectra of 2-(2-(2-hydroxyphenyl)-4-oxothiazolidin-3-yl)-3- methylpentanoic acid (Thiazolidinone 3b)
Figure 8: Mass spectra of (E)-2-(benzylideneamino)-3-methylpentanoic acid (Schiff base 2b)

Figure 9: Mass spectra of 2-(2-(2-hydroxyphenyl)-4-oxothiazolidin-3-yl) benzoic acid: (Thiazolidinone 3a)

Figure 10: Mass spectra of 2-(2-(2-hydroxyphenyl)-4-oxothiazolidin-3-yl)-3-methylpentanoic acid (Thiazolidinone 3b)
Antibacterial activity
The synthesized compounds (2a-3b) were evaluated for antibacterial activity on *E. coli* and *S. aureus* for gram negative and gram positive species respectively. All compounds showed antibacterial activity. Among the compounds tested, all the compounds showed significant zone of inhibition compared to standard drug chloramphenicol at (10, 20 and 30 µg/ml) concentration of compounds.

Table no: 8 Antibacterial activities of novel Schiff bases and Thiazolidinones.

<table>
<thead>
<tr>
<th>S.No</th>
<th>COMPOUND</th>
<th>ZONE OF INHIBITION Distance in mm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><strong>E.coli</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td>10µg/ml</td>
</tr>
<tr>
<td>1</td>
<td>Ethanol (control)</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>Chloramphenicol (std)</td>
<td>20</td>
</tr>
<tr>
<td>3</td>
<td>2a</td>
<td>17</td>
</tr>
<tr>
<td>4</td>
<td>2b</td>
<td>16</td>
</tr>
<tr>
<td>5</td>
<td>3a</td>
<td>17</td>
</tr>
<tr>
<td>6</td>
<td>3b</td>
<td>12</td>
</tr>
</tbody>
</table>

Figure 11: Antibacterial activity *E.coli* (gram negative)

Graphical representation of antibacterial activity against gram negative bacteria *E.coli*. of compounds (2a, 2b, 3a, 3d).

Where 2a:- (E)-3-(2-hydroxybenzylimideneamino) benzoic acid.
2b:- (E)-2-(benzylimideneamino)-3-methylpentanoic acid.
3a:- 2-(2-(2-hydroxyphenyl)-4-oxothiazolidin-3-yl) benzoic acid.
3b:- 2-(2-(2-hydroxyphenyl)-4-oxothiazolidin-3-yl)-3-methylpentanoic acid.
Figure 12: Antibacterial activity *S.aureus* (gram positive).
Graphical representation of antibacterial activity against gram positive bacteria *S.aureus* of compounds (2a, 2b, 3a, 3b).
Where 2a:- (E)-3-(2-hydroxybenzylideneamino) benzoic acid
2b:- (E)-2-(benzylideneamino)-3-methylpentanoic acid
3a:- 2-(2-(2-hydroxyphenyl)-4-oxothiazolidin-3-yl) benzoic acid
3b:- 2-(2-(2-hydroxyphenyl)-4-oxothiazolidin-3-yl)-3-methylpentanoic acid.

Figure 13: Photograph of various novel Schiff bases and thiazolidinones-Antibacterial activity against gram negative bacteria (*E.coli*).
Figure 14: Photograph of various novel Schiff bases and thiazolidinones-Antibacterial activity against gram positive bacteria (S.aureus).

**Anthelmintic activity**

The synthesized compounds (2a-3b) were evaluated for anthelmintic activity on Indian earthworms (*Pheretima posthuma*). All compounds showed anthelmintic activity.

Among the compounds tested, all the compounds showed significant paralytic time for earthworms compared to standard drug albendazole at (0.1%, 0.2% and 0.5%) concentration of compounds.

**Table no 9: Anthelmintic activities of novel Schiff bases and thiazolidinones.**

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Name</th>
<th>Time in minutes</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>For paralysis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>% Drug concentration</td>
<td>0.1</td>
<td>0.2</td>
<td>0.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Control</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Albendazole</td>
<td>14</td>
<td>12</td>
<td>9</td>
<td>42</td>
<td>32</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>2a</td>
<td>29</td>
<td>23</td>
<td>10</td>
<td>50</td>
<td>43</td>
<td>37</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>2b</td>
<td>24</td>
<td>17</td>
<td>11</td>
<td>45</td>
<td>37</td>
<td>28</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>3a</td>
<td>27</td>
<td>17</td>
<td>13</td>
<td>53</td>
<td>47</td>
<td>31</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>3b</td>
<td>28</td>
<td>24</td>
<td>14</td>
<td>51</td>
<td>44</td>
<td>36</td>
<td></td>
</tr>
</tbody>
</table>

|       |         | For death       |   |   |   |   |   |   |
|       |         | % Drug concentration | 0.1 | 0.2 | 0.5 |   |   |   |
| 1     | Control | -               | -  | -  | -  | -  | -  | -  |
| 2     | Albendazole | 42 | 32 | 25 |   |   |   |
| 3     | 2a      | 50              | 43 | 37 |   |   |   |
| 4     | 2b      | 45              | 37 | 28 |   |   |   |
| 5     | 3a      | 53              | 47 | 31 |   |   |   |
| 6     | 3b      | 51              | 44 | 36 |   |   |   |

Figure 15: Anthelmintic activity.
Graphical representation of anthelmintic activity of compounds (2a, 2b, 3a, 3b). Where 2a: (E)-3-(2-hydroxybenzylideneamino) benzoic acid.
2b: (E)-2-(benzylideneamino)-3-methylpentanoic acid.
3a: 2-(2-(2-hydroxyphenyl)-4-oxothiazolidin-3-yl) benzoic acid.
3b: 2-(2-(2-hydroxy phenyl)-4-oxothiazolidin-3-yl)-3-methylpentanoic acid.

**Figure 16: Anthelmintic activity.**

Graphical representation of Anthelmintic activity of compounds (2a, 2b, 3a, 3b). Where 2a: (E)-3-(2-hydroxybenzylideneamino) benzoic acid.
2b: (E)-2-(benzylideneamino)-3-methylpentanoic acid.
3a: 2-(2-(2-hydroxyphenyl)-4-oxothiazolidin-3-yl) benzoic acid.
3b: 2-(2-(2-hydroxyphenyl)-4-oxothiazolidin-3-yl)-3-methylpentanoic acid.

**Figure 17: Photograph of various novel Schiff bases and thiazolidinones- Anthelmintic activity.**
Anticoagulant activity

The synthesized Schiff bases (2a and 2b) were screened for in vitro anticoagulant activity using prothrombin time test. 10 µg/ml, 20 µg/ml, 40 µg/ml and 80 µg/ml concentrations of compounds (2a and 2b) were prepared. Time of coagulation at different concentrations was recorded for both the compounds. From the time of coagulation of the compounds it was found out that Schiff base 2a possess better anticoagulant activity when compared to Schiff base 2b.

Table no: 10 Determination of coagulation time of Schiff base 2a.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Name of compound</th>
<th>Amount of plasma</th>
<th>Amount of extract</th>
<th>Calcium chloride solution</th>
<th>Time of coagulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Control</td>
<td>0.2ml</td>
<td>0.1ml</td>
<td>0.3ml</td>
<td>1min</td>
</tr>
<tr>
<td>2.</td>
<td>Schiff base 2a(10µg/ml)</td>
<td>0.2ml</td>
<td>0.1ml</td>
<td>0.3ml</td>
<td>6min 23s</td>
</tr>
<tr>
<td>3.</td>
<td>Schiff base 2a(20µg/ml)</td>
<td>0.2ml</td>
<td>0.1ml</td>
<td>0.3ml</td>
<td>7min 12s</td>
</tr>
<tr>
<td>4.</td>
<td>Schiff base 2a(40µg/ml)</td>
<td>0.2ml</td>
<td>0.1ml</td>
<td>0.3ml</td>
<td>8min 10s</td>
</tr>
<tr>
<td>5.</td>
<td>Schiff base 2a(80µg/ml)</td>
<td>0.2ml</td>
<td>0.1ml</td>
<td>0.3ml</td>
<td>9min 19s</td>
</tr>
</tbody>
</table>

Table no: 11 Determination of coagulation time of Schiff base 2b.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Name of compound</th>
<th>Amount of plasma</th>
<th>Amount of extract</th>
<th>Calcium chloride solution</th>
<th>Time of coagulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Control</td>
<td>0.2ml</td>
<td>0.1ml</td>
<td>0.3ml</td>
<td>1min</td>
</tr>
<tr>
<td>2.</td>
<td>Schiff base 2b(10µg/ml)</td>
<td>0.2ml</td>
<td>0.1ml</td>
<td>0.3ml</td>
<td>5min 53s</td>
</tr>
<tr>
<td>3.</td>
<td>Schiff base 2b(20µg/ml)</td>
<td>0.2ml</td>
<td>0.1ml</td>
<td>0.3ml</td>
<td>6min 25s</td>
</tr>
<tr>
<td>4.</td>
<td>Schiff base 2b(40µg/ml)</td>
<td>0.2ml</td>
<td>0.1ml</td>
<td>0.3ml</td>
<td>6min 44s</td>
</tr>
<tr>
<td>5.</td>
<td>Schiff base 2b(80µg/ml)</td>
<td>0.2ml</td>
<td>0.1ml</td>
<td>0.3ml</td>
<td>7min 39s</td>
</tr>
</tbody>
</table>

The IR data showed the presence of major functional groups present in the synthesized compounds, $^1$H-NMR data showed the chemical shift of protons and Mass spectral analysis confirmed the molecular weight of the synthesized compounds.

The synthesized Schiff bases were screened for anticoagulant, antibacterial and anthelmintic activities and thiazolidinones were screened for antibacterial and anthelmintic activities. Schiff base 2a ((E)-3-(2-hydroxybenzylideneamino) benzoic acid) showed better anticoagulant, antibacterial and anthelmintic activities compared to Schiff base 2b and thiazolidinone 3a (2-(2-(2-hydroxyphenyl)-4-oxothiazolidin-3-yl) benzoic acid) showed better antibacterial and anthelmintic activities compared to thiazolidinone 3b.

Antibacterial, anthelmintic and anticoagulant activities seen in Schiff bases (2a and 2b) is due to the presence of highly reactive azomethine group (RHC=N-R1). Presence of phenolic hydroxyl group in Schiff base 2a is also important for biological activity particularly...
antibacterial activity.

Antibacterial and anthelmintic activities seen in thiazolidinones (3a and 3b) are mainly due to the presence of thiazolidine ring. Presence of N-CH-S grouping and phenolic hydroxyl groups in thiazolidinones (3a and 3b) confers the presence of anthelmintic and antibacterial activities.

6. CONCLUSION

The objective of the present work was to employ microwave for the synthesis of some novel Schiff bases and novel thiazolidinones Schiff bases have been reported to possess higher degree of antitubercular, anticancer, antibacterial, anti-inflammatory, antifungal activities and even thiazolidinones find a variety of applications ranging from antimicrobial, antitubercular, anti-inflammatory, anthelmintic, anticonvulsant, Anti-HIV activity, Anticancer, and hypoglycemic activity. Microwave was used microwave to make chemical synthesis more environment friendly, safe, to improve the yield and also to reduce the time consumption. This method is safe, more environment friendly and by using this method compounds can be synthesized in absence of solvents. Microwave synthesis gives improved product yields, provides controlled method of heating and also increases reaction rates.

The newly synthesized Schiff bases (2a and 2b) were evaluated for anticoagulant activity. Schiff bases and thiazolidinones were purified, characterized and evaluated for antibacterial and anthelmintic activities. The yield of the synthesized compounds was found to be in the range of 70-86%. All these molecules were characterized by physical data and FT-IR, 1H NMR and mass spectral analysis. The functional groups in the title compounds were indicated by their IR spectra. The no. of protons in the compound was confirmed by their 1H NMR spectra. The structure of title compounds were confirmed by their Mass spectra.

The synthesized Schiff bases (2a and 2b) at concentrations 10µg/ml, 20µg/ml, 40µg/ml and 80µg/ml were tested for anticoagulant activity using prothrombin time test. Time of coagulation for both the compounds was recorded and it was found out that Schiff base 2a showed better anticoagulant activity when compared to Schiff base 2b.

All the synthesized compounds were tested for antibacterial activity on E.coli and S.aureus, using chloramphenicol as standard drug at concentrations of 0.1%, 0.2% and 0.3%. Amongst all the synthesized compounds, compounds 2a, and 3a showed highest activity.

All the synthesized compounds were also screened for anthelmintic activity, using albendazole as standard. The compound 2a showed highest activity, 2b and 3a showed moderate activity.

From the data of antibacterial and anthelmintic activity of Schiff bases and thiazolidinones it is clearly concluded that the synthesized compounds are having good antibacterial and anthelmintic activities. The data of anticoagulant activity of Schiff bases also showed that the synthesized Schiff bases possess good anticoagulant activity.

7. ACKNOWLEDGEMENTS

On this occasion of successful completion of my work, I offer my salutations to the Almighty, with whose showering of blessings, this task was ventured without any hindrance. It affords me an immense pleasure to acknowledge with gratitude the help and guidance rendered to me by a host of people, whom, I owe a substantial measure for the completion of this project.

It is with pleasure of immense gratitude that I express my most cordial and humble thanks to my esteemed research Guide, Mr. SURESH, Scientist Glochem industries pvt. ltd, for his valuable guidance, keen interest, perennial inspiration and everlasting encouragement. I shall forever remain indebted to him for having inculcated in me a quest for excellence, a spirit of diligence and perseverance, a sense of humility, honesty and respect for the moral and ethics which govern our sciences and without whom this work would not have seen the light of the day.

It gives me an immense pleasure and pride to express my deep sense of gratitude and respect for my teacher and Guider (Mrs.) P. Madhuri, Asst Professor at Sultan-U-Uloom College of Pharmacy, Hyderabad and for his evergreen expertise and inspiring guidance throughout the period of my project work. I am indebted to Dr. J. Venketashwara Rao for enlightening me on the finer skills of dealing with synthetic problems. I consider myself fortunate to be associated with him.

I take this opportunity to reveal my gratitude, regards and sincere thanks to Miss.Sharmila Sutradhar and faculty of Sultan-U-Uloom college of pharmacy for their guidance, constant encouragement and valuable suggestions throughout this course. I am very much thankful for their unflinching support, affection, which helped me a lot in exploring my abilities and made it possible for me to carry out the present work which otherwise, would be very difficult.

I am indebted to my seniors, research scholars Mr. Adil Shareef, Ms. Shabnam Dobani for their restless and patient support throughout my work period. I have the pleasure of collaborating with them for my thesis work.

It gives me a great pleasure to express my thanks to my Sultan-U-Uloom friends Mohsin abbas Khabmaty, Shaik Md Muzakir, Faisal Ahmed, syed wajahat ullah, mirza fathe ullah baig and many more for their constant support, encouragement during the entire period of my research.
I would like to present my sincere thanks towards Analysis Departments for their support and helping me in analysis of my work and making it possible for me to carry out the present work which otherwise, would be very difficult. I am at loss of words while thanking my Family for their support, sacrifice and pain they have taken in bringing me up to this position. Finally, I take this opportunity to thank one and all who have directly or indirectly helped me in completing this task.

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