1.1 Biological Importance of Tetrahydro Furano and Pyrano Quinolines:

Tetrahydroquinoline derivatives are an important class of natural products and exhibits\(^1\) biological activities in various fields. Pyrano-quinoline-alkaloids-simulenoline\(^2,3\) and huajiaosimuline\(^4\) were isolated from root barks of zanthoxylum simulans, a shrub found in Taiwan and China.

While simulenoline\(^1\) was the most recently isolated of the two alkaloids, a third pyranoquinoline alkaloid zanthodioline\(^5\) from the same species was just recently disclosed in the literature.\(^2\) These novel monoterpenoid pyranoquinolines are potent inhibitors of platelets aggregation or example, at a concentration of 100µg/ml. Simulenoline\(^1\) demonstrates a nearly complete suppression of platelet aggregation induced in vitro by collagen, arachidonic acid and PAF in general.\(^2\) While simulenoline\(^1\) and Zanthodiolin\(^5\) are not cytotoxic, huajiaosimuline\(^6\) is toxic to ward several human cultured cell lines especially the estrogen receptor positive breast cancer cells.

Therefore pyrano and furano quinoline derivatives are synthesized due to their broad spectrum of activity against microbacterial and various parasites more over hetero fused quinoline are known to bind DNA with high affinity inhibit DNA topo isomers and they also display cytotoxic and antitumor activities.
2.1 Biological importance of Cis-iso-Quinolonic acid:

Tetrahydroquinoline derivatives are an important class of compound in the field of pharmaceuticals and exhibits a wide spectrum of biological activities. Including psychotropic, antiallergic, antiinflammatory, estrogenic behavior in particular, iso quinolonic acids are found to possess a vast range of pharmacological activities particularly iso quinolonic acids are useful precursors for a total synthesis of naturally occurring phenanthridine alkaloids, such as corynolive, oxocorynolive and epicorynolive as well as Indino Isoquinolines possessing significant antitumor activities.
3.1 Biological importance of β-Amino carbonyl Compounds:

The aza-Michel reaction is an important reaction in organic chemistry especially for the synthesis of hetero cycles containing a β- amino carbonyl unit. β-amino carbonyl compounds are useful building blocks for the molecules with applications in pharmaceuticals and fine chemicals. They are versatile intermediates for the synthesis of biologically important natural products and antibiotics and chiral auxiliaries and other nitrogen containing molecules. β-amino carbonyl ingredients, which have attracted great attention for their use as key intermediates of anticancer agents, antibiotics and other drugs.

They are also useful precursors for the generation of β- amino alcohols, which are common reagents in the preparation of fine chemicals pharmaceuticals.

β-amino carbonyl compounds used as essential intermediates in the synthesis of β-amino acid and β-lactam antibiotic. Consequently, the development of novel synthetic methods for their synthesis has attracted sustained interest in organic synthesis.
4.1 Biological importance of Schiff’s bases:

The literature survey of the antimicrobial activity of Schiff bases have shown that many of them are useful as the best bactericides and fungicides against the various gram +ve and gram – ve bacterial and fungi. Schiff bases constitute one or the most active class of compounds possessing diversified biological applications. The Schiff bases have been represented to possess higher degree of antitubercular and anticancer activity.

Schiff bases are reagents, which are becoming increasing important in the pharmaceutical, dye and plastic industries as well as for liquid-crystal technology and mechanistic investigations of the drugs used in pharmacology, biochemistry, and physiology.

Procedure:

Petriplates containing 20 ml. Mllre Hinton medium were seeded with 24 hr.culture of bacterial strains. Wells were cut and 20µl of the plant extract (namely aqueous, methanol and chloroform extracts) were added. The plates were then incubated at 37 °C for 24 hours. The antibacterial activity was assayed by the inhibition zone formed around the well. Chloramphenicol dilution was used as appositive.
REAGENTS:

1. Muller Hinton Agar Medium (1L):

   The medium was prepared by dissolving 33.9 gm. of the commercially available Muller Hinton Agar Medium (Hi Media) in 1000 ml of distilled water. The dissolved medium was autoclaved at 15 lbs. pressure at 121°C for 15 minutes. The autoclaved medium was mixed well and poured onto 100 mm petriplates (25-30ml/plate) while still molten.

2. Nutrient broth (1L):

   One litre of nutrient broth was prepared by dissolving 13 gm of commercially available nutrient medium (Hi Media) in 1000 ml distilled water and boiled to dissolve the medium completely. The medium was 44 dispensed as desired and sterilized by autoclaving at 15 lbs. pressure (121°C) for 15 minutes.

3. Chloramphenicol/Streptomycin solution/disc: (standard antibacterial agent)

   Prepare solution of standard antibiotic as 10, 20……100 microgram/ml. This will help in discrimination between test sample and standard antibiotic solution. One can able to compare effectiveness of sample.

   Synthesized compounds in our present study were screened for their antibacterial and antifungal activities. Compounds are tested for antimicrobial and antifungal nature.

   As per result shown in Fig. A & B It is concluded that, in Figure A show that there is an moderate concentration after immediate addition of mixture while, in
Figure B, it clearly noticed that there is an high concentration of compound after 4-hr in incubation period. This is indicate that less period of incubation there is less concentration where as more incubation then there is more or high concentration.

**Test Methodology:**

- Well diffusion test
- Broth micro dilution test

a) **Well diffusion test:**

Petri plates containing 20 ml. muller Hinton medium were seeded with 24 hr culture of bacterial strains. Wells were cut and 20 ml of the preparation (namely aqueous DMSO and Methanol preparation) were added. The plates were then incubated at 37 ºC for 24 hrs. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the well. Chloramphenicol dilution was used as a positive control.

After 16 to 18 hours of incubation, each plate was examined. The resulting zones of inhibition were uniformly circular with a confluent lawn of growth. The diameters of the zones of complete inhibition were measured, including the diameter of the disc where the chloramphenicol was used as control (NCCLS, 1997, National committee for clinical laboratory standard.)

b) **Micro dilution method:**

Dilution susceptibility testing methods are used to determine the minimum concentration of anti-microbial activity needed to inhibit or kill the micro organism. This can be achieved by dilution of antimicrobial in either agar or broth media.
The minimum inhibitory concentration (MIC) was determined by micro
dilution method using serially diluted extracts following the procedures
recommended by the NCCLS.\textsuperscript{27-28} The aqueous DMSO and suspected ‘powder’
preparations were diluted to get series of concentration from 10 mg/ml to 100
mg/ml in sterile nutrient broth. The micro organism suspension at 50 ml was
added to the broth dilution. These were incubated for 18 hrs at 37\degree C MIC at each
product was taken as the lowest concentration that did not give any visible
bacterial growth.\textsuperscript{29}

1.1 Biological Screening of Tetrahydro Furano and Pyrano Quinolines:

In our present investigation all the synthesized compounds were evaluated
in vitro for their antibacterial activity against,

1) Gram positive bacterial strain \textit{S.aureus}.\textsuperscript{*}

2) Gram negative bacterial strain \textit{p.aeruginosa}.\textsuperscript{*}

In the anti-microbial test, investigated compounds were found effective as
antibacterial agents. Antimicrobial testing was performed by well-diffusion
methods. Purified products were found to be inhibitory for both ‘Gram positive
and Gram negative’ organisms \textit{P.seudomonas aeruginosa} and \textit{S.aureus}
respectively as shown in (\textbf{Fig.5.01}) and (\textbf{Table No.5.1})
Table No. 5.1

Antimicrobial Effect of on ‘Gram positive and Gram negative’ Organism.

<table>
<thead>
<tr>
<th>Product code</th>
<th>Gram Negative</th>
<th>Gram Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>P. aeruginosa</em></td>
<td><em>S.aureus</em></td>
</tr>
<tr>
<td>1A</td>
<td>Negative*</td>
<td>Positive**</td>
</tr>
<tr>
<td>1B</td>
<td>Positive</td>
<td>Positive**</td>
</tr>
<tr>
<td>1C</td>
<td>Negative</td>
<td>Positive**</td>
</tr>
<tr>
<td>1D</td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>1E</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>1F</td>
<td>Negative</td>
<td>Positive**</td>
</tr>
<tr>
<td>1G</td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>1H</td>
<td>Positive**</td>
<td>Positive</td>
</tr>
<tr>
<td>1I</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>1J</td>
<td>Positive**</td>
<td>Positive</td>
</tr>
<tr>
<td>1K</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>1L</td>
<td>Positive**</td>
<td>Positive**</td>
</tr>
<tr>
<td>1M</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>1N</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>1O</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>1P</td>
<td>Positive</td>
<td>Positive**</td>
</tr>
</tbody>
</table>

*Negative- Medium effective
** Positive- More effective

(N -Although the activity found against *P.seudomonas* was not to be persistent, as it has a resistance plasmid in it, Strain had reflected resistant activity against respective drugs after 2 successive data.)
2.1 Biological Screening of Cis-Iso-Quinolonic acid:

In microbial test we found that investigated compounds to be more inhibitory for *P. aeruginosa* but it show minimum inhibitory for *S.aureus* as in (Fig.5.02) and in (Table No.5.2).

**Table No.5.2**

‘Antimicrobial’ Effect on ‘Gram positive and Gram negative’ Organism.

<table>
<thead>
<tr>
<th>Product code</th>
<th>Gram Negative</th>
<th>Gram Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>P. aeruginosa</em></td>
<td><em>S. aureus</em></td>
</tr>
<tr>
<td>2A</td>
<td>Negative*</td>
<td>Positive**</td>
</tr>
<tr>
<td>2B</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>2C</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>2D</td>
<td>Negative</td>
<td>Positive**</td>
</tr>
<tr>
<td>2E</td>
<td>Positive**</td>
<td>Negative</td>
</tr>
<tr>
<td>2F</td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>2G</td>
<td>Positive**</td>
<td>positive**</td>
</tr>
<tr>
<td>2H</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>2I</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>2J</td>
<td>Positive</td>
<td>Positive**</td>
</tr>
<tr>
<td>2K</td>
<td>Positive**</td>
<td>Positive</td>
</tr>
<tr>
<td>2L</td>
<td>Positive</td>
<td>Positive</td>
</tr>
</tbody>
</table>

*Negative-Medium effective  
**Positive-More Effective

(N.B-Although the activity found against *Pseudomonas* was not to be persistent, as it has a resistance plasmid in it, Strain had reflected resistant activity against respective molecules after 1 day).
3.1 Biological Screening of β- Amino carbonyl Compounds:

In microbial test we found that investigated compounds to be more inhibitory for \( P. \) aeruginosa and for \( S. \) aureus as in (Fig.5.03) and in (Table No.5.2).

Table No. 5.3
‘Antimicrobial’ Effect on ‘Gram positive and Gram negative’ Organism.

<table>
<thead>
<tr>
<th>Product code</th>
<th>Gram -ve</th>
<th>Gram +ve</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( P. ) aeruginosa</td>
<td>( S. ) aureus</td>
</tr>
<tr>
<td>3A</td>
<td>Negative*</td>
<td>Positive**</td>
</tr>
<tr>
<td>3B</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>3C</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>3D</td>
<td>Positive**</td>
<td>Positive**</td>
</tr>
<tr>
<td>3E</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>3F</td>
<td>Positive**</td>
<td>Positive</td>
</tr>
<tr>
<td>3G</td>
<td>Positive</td>
<td>Positive**</td>
</tr>
<tr>
<td>3H</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>3I</td>
<td>Positive**</td>
<td>Positive</td>
</tr>
<tr>
<td>3J</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>3K</td>
<td>Positive**</td>
<td>Positive</td>
</tr>
<tr>
<td>3L</td>
<td>Positive</td>
<td>Positive</td>
</tr>
</tbody>
</table>

*Negative-Not effective

**Positive-Effective
4.1 Biological Screening of Schiff’s bases:

Biological activity can take many different form and may be measured in different ways depending on the level at which the investigation is conducted.

**Microbial Testing:**

Antimicrobial compounds mainly reveal their activity either by inhibiting microbial cell division or by interrupting their metabolism transcription or translation. Many of antibiotic known to date may also interrupt lipid metabolism, which has been proven to be very effective against gram – ve organisms.

Synthesized compounds in our present study were screened for their antibacterial and antifungal activities. Testing of compounds for antimicrobial and antifungal nature.

As per result shown in A and B, it is concluded that, in figure A show that there is an moderate concentration after immediate addition of mixture while, in figure B, it clearly noticed that there is an high concentration of compound in after 4 - hours incubation period. This is indicate that, less period of incubation there is less concentration where as more incubation then there is more or high concentration.

**Test Methodology:**

a) Well diffusion test

b) Broth micro dilution test
a) **Well diffusion test:**

Petri plates containing 20 ml. muller Linton medium were seeded with 24 hr. Culture of bacterial strains. Wells were cut and 20 ml. of the preparation (namely aqueous, DMSO and Methanol preparation) were added. The plates were then incubated at 37 °C for 24 hrs. The antibacterial activity was assayed by measuring the diameter of the zone of inhibition formed around the well. Chloramphenicol dilution was used as a positive control.

After 16 to 18 hours of incubation, each plate was examined. The resulting zones of inhibition were uniformly circular with a confluent lawn of growth. The diameters of the zones of complete inhibition were measured, including the diameter of the disc where the chloramphenicol was used as control (NCCLS, 1997, National committee for clinical laboratory standard.) (Fig.5.04)

b) **Micro dilution method:**

Dilution susceptibility testing methods are used to determine the minimum concentration of antimicrobial activity needed to inhibit or kill the micro organism. This can be achieved by dilution of antimicrobial in either agar or broth media.

The minimum inhibitory concentration (MIC) was determined by micro dilution method using serially diluted extracts following the procedures recommended by the NCCLS. The aqueous DMSO and suspected ‘powder’ preparations were diluted to get series of concentration from 10 mg/ml to 100 mg/ml in sterile nutrient broth. The micro organism suspension at 50 mL was added to the broth dilution. These were incubated for 18 hours at 37 °C MIC at each product was taken as the lowest concentration that did not give any visible bacterial growth. (Fig.5.05)
Table No. 5.4
Antimicrobial activity of Schiffs base derived from Pyrimethamine

<table>
<thead>
<tr>
<th>Product code</th>
<th>Test Organism Gram Positive</th>
<th>Test Organism Gram Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S. aureus</td>
<td>E. coil</td>
</tr>
<tr>
<td></td>
<td>ZI</td>
<td>MIC</td>
</tr>
<tr>
<td>4A</td>
<td>15 mm</td>
<td>275</td>
</tr>
<tr>
<td>4B</td>
<td>20 mm</td>
<td>300</td>
</tr>
<tr>
<td>4C</td>
<td>25 mm</td>
<td>100</td>
</tr>
<tr>
<td>4D</td>
<td>30 mm</td>
<td>95</td>
</tr>
<tr>
<td>4E</td>
<td>35 mm</td>
<td>90</td>
</tr>
<tr>
<td>4F</td>
<td>40 mm</td>
<td>90</td>
</tr>
<tr>
<td>4G</td>
<td>45 mm</td>
<td>85</td>
</tr>
</tbody>
</table>

ZI = Zone of Inhibition.

MIC = Minimum Inhibition of concentration

From Table No. 5.4 observed that the 4B (compound) Product show highest MIC against S.aureus and p.aeruginosa followed by E.coil fungi. The S.aureus show Gram positive activity where as E.coil and p. aeruginosa show gram negative activity. The minimum inhibition of concentration was found against 4C- product followed by 4A and 4B, where as in case of E.coil. 4C, 4 B and 4 A and in case of p.aeruginosa. 4 A and 4 C is common and fallowed by 4 B product. The zone of inhibition was also more or less similar of all products against all tested fungal species.
Minimum in 2 i.e 15 mm of 4 A product against *S. aureus*, where as the maximum is 48 mm zone of inhibition of 4 G product against *E. coli*. The 4 D product show very similar MIC against *p. aures* and *E. coli* except *p. aureginosa* which is zero. The 4 A, 4F and 4 G product have similar action in all tested fungal species regarding minimum inhibition of concentration.

**Antifungal Activity:**

In present investigation some newly synthesized Schiff bases were assessed for their antifungal activity against fungi. For the assessment activity, spore germination method in Petridish was followed. The stain was prepared by dissolving the chemicals with gentle heating for complete dissolution.

**Reagent- Lactophenol cotton blue stain:**

- Phenol crystal (20gm.)
- Cotton blue (0.05)
- Lactic acid (20 ml.)
- Glycerol (20 ml.)
- Distilled water (20 ml.)

The stain was prepared by dissolving the chemicals with gentle heating for complete dissolution.

**Procedure:**

Aliquots of spore were prepared by mixing loop full of fungal spores in sterile distilled water 25 ml of spore suspension was added to 10 mL of the plant extracts and placed in separate glass slides. Slides with 25 ml of spore suspension
alone served as the controls. Slides were then incubated in moist chamber at 25 ± 20 °C for 24 hours. Each slide was fixed in lacto phenol cotton blue stain. The mold was mixed gently with the stain using two teasing needles. A cover slip was placed on the preparation and examined under the phase contrast microscope for spore germination. Percentage germination with effect of these compounds after a period of 24 hours was recorded by observing petridishes directly under microscope. The results of antifungal activity are give in Table no. 5.6

Table No. 5.5

Antifungal activity of Schiffs base derived from Pyrimethiamine

<table>
<thead>
<tr>
<th>Product code</th>
<th>Concent. of product (Micro/ml)</th>
<th>% of metabolic activity FLC values</th>
<th>C. albicans</th>
<th>A. niger</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (100)</td>
<td>100</td>
<td></td>
<td>Inhibitory lowest conc.</td>
<td>Inhibitory lowest conc.</td>
</tr>
<tr>
<td>4A</td>
<td>55</td>
<td>95</td>
<td>60</td>
<td>50</td>
</tr>
<tr>
<td>4B</td>
<td>48.2</td>
<td>85</td>
<td>80</td>
<td>125</td>
</tr>
<tr>
<td>4C</td>
<td>40.0</td>
<td>70</td>
<td>75</td>
<td>120</td>
</tr>
<tr>
<td>4D</td>
<td>135.7</td>
<td>75</td>
<td>40</td>
<td>100</td>
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<tr>
<td>4E</td>
<td>22.2</td>
<td>57.84</td>
<td>25</td>
<td>50</td>
</tr>
<tr>
<td>4F</td>
<td>20.9</td>
<td>71.91</td>
<td>50</td>
<td>78</td>
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<tr>
<td>4G</td>
<td>41.22</td>
<td>87.31</td>
<td>40</td>
<td>85</td>
</tr>
</tbody>
</table>

FLC– Fluconazole concentration
The Table (5.5) shows that seven products were tested against *C.albicans* and *A.niger* for inhibitory lowest concentration as well as concentration of product in control and % of metabolic activity in FLC values. The inhibitory lowest concentration is 25 from 4 E product against *C.albicans*. The effect on *C. albicans* and *A.niger* of product were highest from 4 D and lowest from 4E. The highest concentration of product in control 135.7 from 4 D product and the lowest control is 20.9 from 4 F- product. The more germination of spore from 4A product followed by 4 A and 4 B, while the minimum spore germination was found from 57.84 in 4 E product.
Fig. 5.03

(A)  (B)

Fig. 5.04

(A)  (B)
A. Immediate after addition of mixture

B. Incubation after 24 hr

Fig. 5.05

A. Negative Control

B. Effect of antifungal activity

Fig. 5.06
Result and Discussion:

All the synthesized compounds exhibited significant antibacterial and antifungal activity. All the compounds were active against all tested microorganisms with a range of MIC values for *P. aeruginosa* and *S. aureus*. Compounds 8H, 9 I and 12 L are shows active against both Gram positive and Gram negative bacteria. Compound 3 G, 3 F, 3 J are highly effective against *P. aeruginosa* and *S. aureus*.

From the antimicrobial screening results it revealed that the degree of inhibition varied in compound compound 4A to 4G. Among the tested compounds, it is observed that the introduction of electron donating group in phenyl ring enhances the antibacterial activity against *P. aeruginosa* and *S. aureus*.

MIC values of Pyrimethiamine in range from 0.002 to 0.6 mg/l. In our study, the synthesized product shows that more MIC value than trimethoprim i.e product have highly anti bacterial.

Pyrimethiamine product is highly antibacterial agent compound containing pyrimidine rings play significant role in many biological systems. MIC of trimethoprim is compared with prepared compounds are more bioactive than reference. The MIC of prepared derivatives are higher than alone. Common food spoilage causing organism i.e. *Staphylococcus aureus* was interestingly responding to many products. It’s notably interesting that all purified molecules are mostly active against ‘Gram positive’ microorganisms.

In antifungal screening results it revealed that the degree of inhibition is varied in all compounds 4 A to 4 G.
References:


Chapter-V


25) Comparision of disc diffusion agar dilution and broth micro dilution for anti microbial susceptibility testing of five chitosan.


