University of Basrah

Collage of pharmacy

Synthesis, Characterization and evaluation of antibacterial activity of Sulfadiazine- pyrrole-2-carboxylaldehyde as a new Schiff base

supervised by

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Done by

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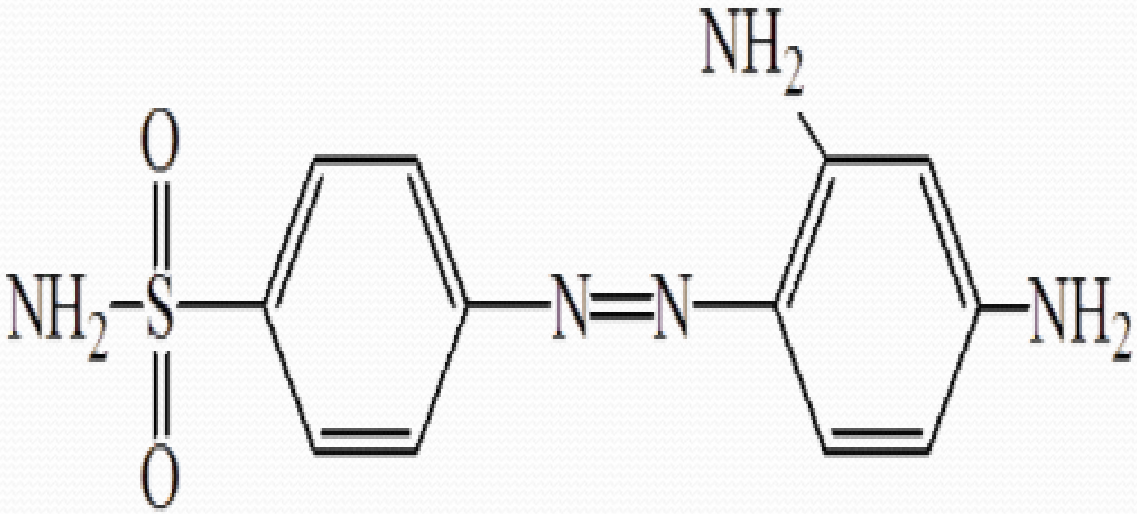
- **Introduction**
  - **Sulfonamides**
  - **Schiff base**
- **Material and method**
- **Chemical synthesis**
- **Antibacterial evaluation**
- **Conclusions**
- **Recommendations**

History of sulfonamides

In 1932, Domagk started to study a brilliant red dye, soon after named Prontosil 4-[(2,4-diaminophenyl) azo]benzenesulfonamide shown in figure (1-1).

Which protect against, and treat, streptococcal infections in mice [1], but Prontosil showed that it was inert on bacterial cultures.

Figure (1-1): Structure of prontosil.



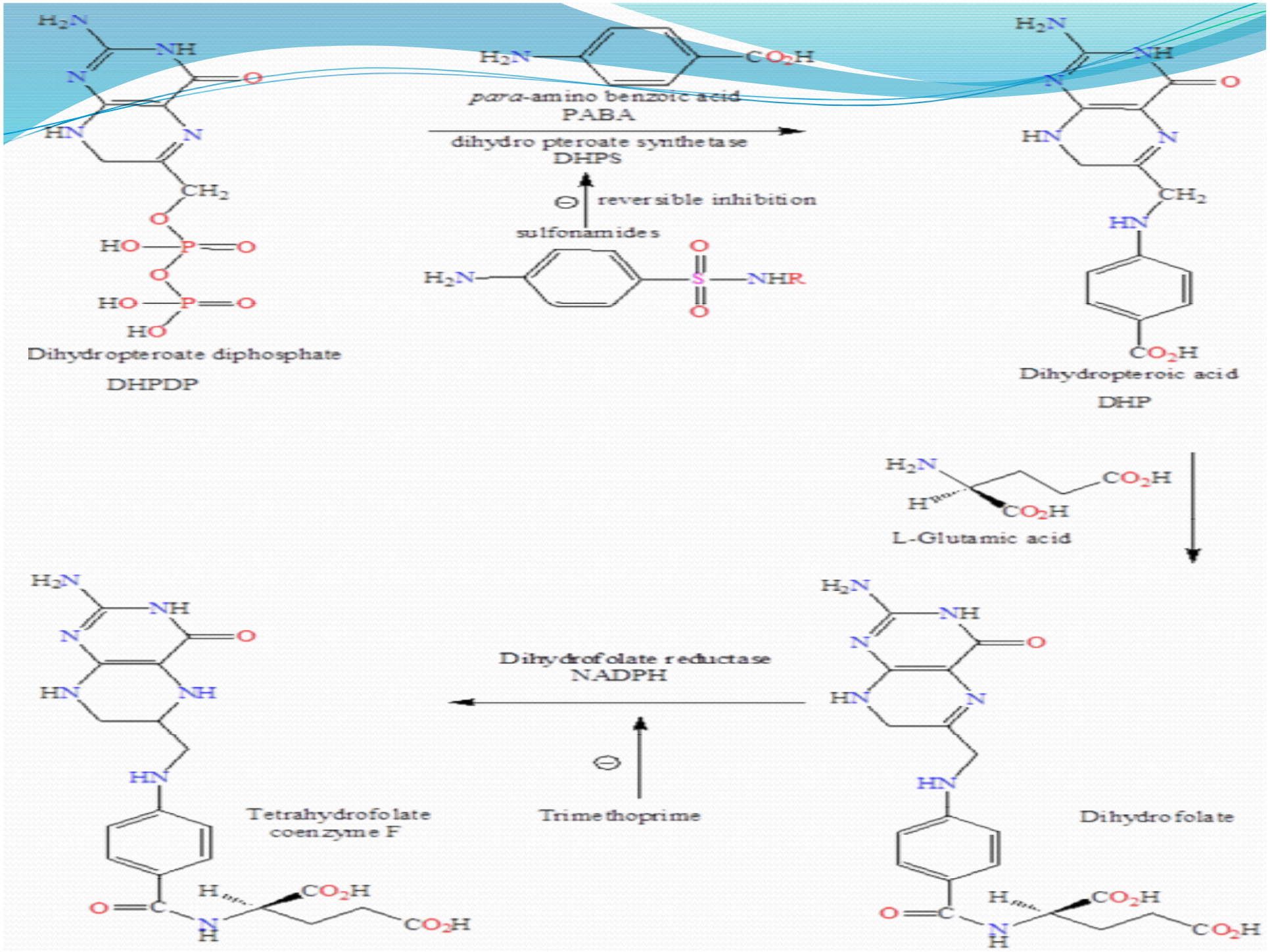
Mechanism of action of sulfonamides as antibacterial

Sulfonamides act on DHPS enzyme whereas trimethoprim act on DHFR enzyme [7]. Sulfonamides produce their antibacterial effect *in-vivo* by targeting bacterial metabolic pathway.

As mentioned before due to the lack of ability of bacteria to obtain dihydrofolic acid from their environment, to be used as a part of the bacteria's DNA biosynthesis, inhibition of dihydrofolic acid synthase posses a attractive target for bacteriostatic agents [8].

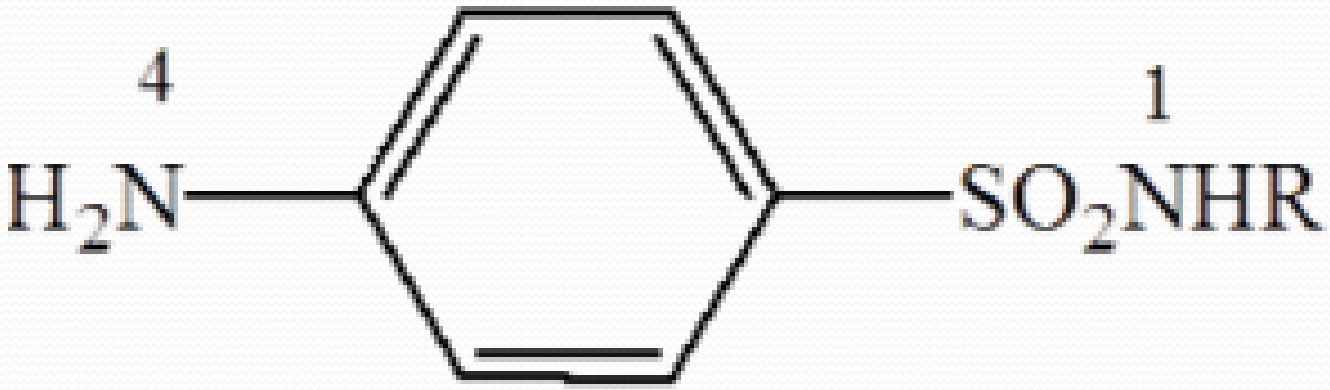
The formation of dihydrofolic acid is launched via coupling para-aminobenzoic acid with pteridine diphosphate, which then undergo formation of dihydrofolic acid through an amide coupling with glutamic acid (scheme (1-1).

Sulfonilamide shows structural similarity to that of *p*-aminobenzoic acid and act as a competitive inhibitor.


Dihydropteroate diphosphate
DHPDP
para-amino benzoic acid
PABA
dihydro pteroate synthetase
DHPS
reversible inhibition
sulfonamides
Dihydropteroic acid
DHP
L-Glutamic acid
Dihydrofolate reductase
NADPH
Trimethoprime
Dihydrofolate
Tetrahydrofolate
coenzyme F

Biological activities of sulfonamides

Sulfonamides can be divided into two categories; where the first group are the antibiotics (eg, sulfamethoxazole, sulfisoxazole, sulfacetamide) and the second are the non-antibiotic sulfonamides (e.g., thiazides, glyburide, furosemide, sumatriptan, celecoxib).


4
1
H2N
SO2NHR

The antimicrobial action of the sulfa drugs is not only because of the occurrence of pharmacologically active sulfonamide group (-SO2NH2), but in addition is related to presence of amino group (-NH2) at the para position of the benzene ring.

Sulfanilamide is a simple molecule. In order to arise with effective derivatives of this molecule, three successful strategies have generally been employed: (1) Sulfonamide group modification (2) The amino group modification and finally (3)The instantaneous modifications of both groups.

Sulfonamides have a broad range of activity both gram positive and gram negative bacteria, *nocardia*, *Chlamydia trachomatis*, and a number of protozoa .

In addition a number of enteric bacteria, for example *E. coli* and *Klebsiella sp*, *Salmonella*, *Shigella*, and *Enterobacter* sp. are inhibited.

Microbial resistance to sulfonamides

Sulfonamides are drugs of choice for a few types of infections, but their use is relatively limited in current antimicrobial chemotherapy due to development of resistance [31]. Additionally indiscriminate use of sulfonamides has lead to the appearance of many drug-resistant strains of bacteria.

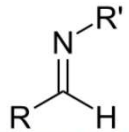
since bacterial mutations lead to over production of PABA although other mechanisms for example alterations in the binding strength of sulfonamides to the pathway enzymes i.e folic acid synthesized enzyme protein has low affinity for sulfonamides (reduced affinity of dihydropteroate synthase for sulfonamides with keep of affinity for PABA) , reduced permeability of the cell membrane(altered permeability), and active efflux of the sulfonamide may play a role [2, 33]

History of Schiff base

Schiff bases, named after Hugo Schiff [35] , are formed when any primary amine reacts with an aldehyde or a ketone under specific conditions.

Structurally, a Schiff base (also known as imine or azomethine) (Fig. 1-4) is a nitrogen analogue of an aldehyde or ketone in which the carbonyl group (CO) has been replaced by an imine or azomethine group

General structure of a imine.



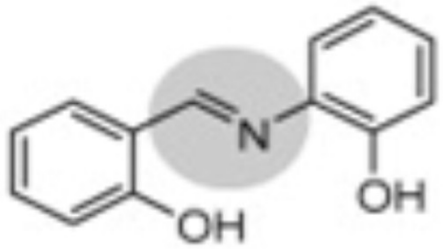
General structure of an azomethine compound

Schiff bases are imines in which R^2 is an alkyl or aryl group (not a hydrogen). R^1 and R^2 may be hydrogens

Fig. 1-4

The Antibacterial activity of Schiff base

Schiff bases have been pointed to as promising antibacterial agents. For example, *N*-(salicylidene)-2-hydroxyaniline (compound 4; Fig. 1-5) is effective against *Mycobacterium tuberculosis* H37Rv, exhibiting an MIC value of 8 μg/mL [39]. The selectivity of compound 4 was checked by performing experiments with J774 macrophages. No cytotoxic effect on J774 macrophages was observed for compound 4, even when it was tested at concentrations as high as 1000 μg/mL. More than 80% of macrophage cells were viable at such experimental conditions, demonstrating the high selectivity of compound 4.



N-(Salicylidene)-2-hydroxyaniline (**4**)

(*Antibacterial activity*)

Non-natural Compound

Materials and Equipments

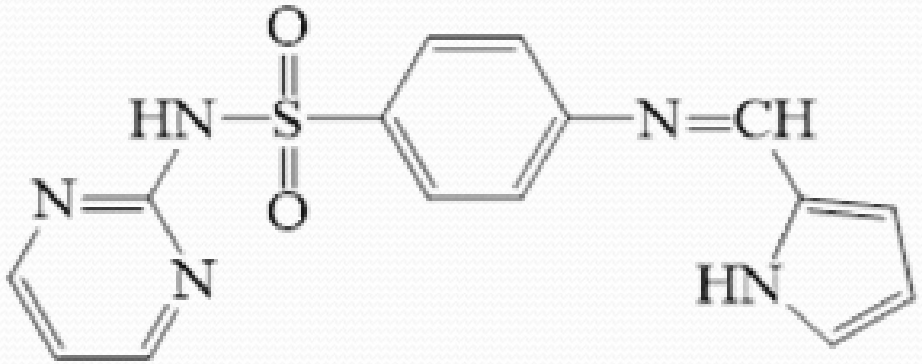
Chemical substance and slovent	Molecular formula	Supplied company	country
Acetic acid (glacial)	C_2H_4O	Fisher	England
chloroform	$CHCl_3$	MERCK	Germany
Ethanol absolute	C_2H_6O	Scharlau	Spain
Methanole	CH_4O	Scharlau	Spain
Pyrrole-2-carboxaldehyde	C_5H_5NO	Sigma-Aldrich	USA
sulfadiazine	$C_{10}H_{10}N_4O_2S$	Gerhard buchmann tuttingen	Germany
Dimethyl sulfoxide	C_2H_6OS	Sigma-Aldrich	USA

Equipments and instruments:

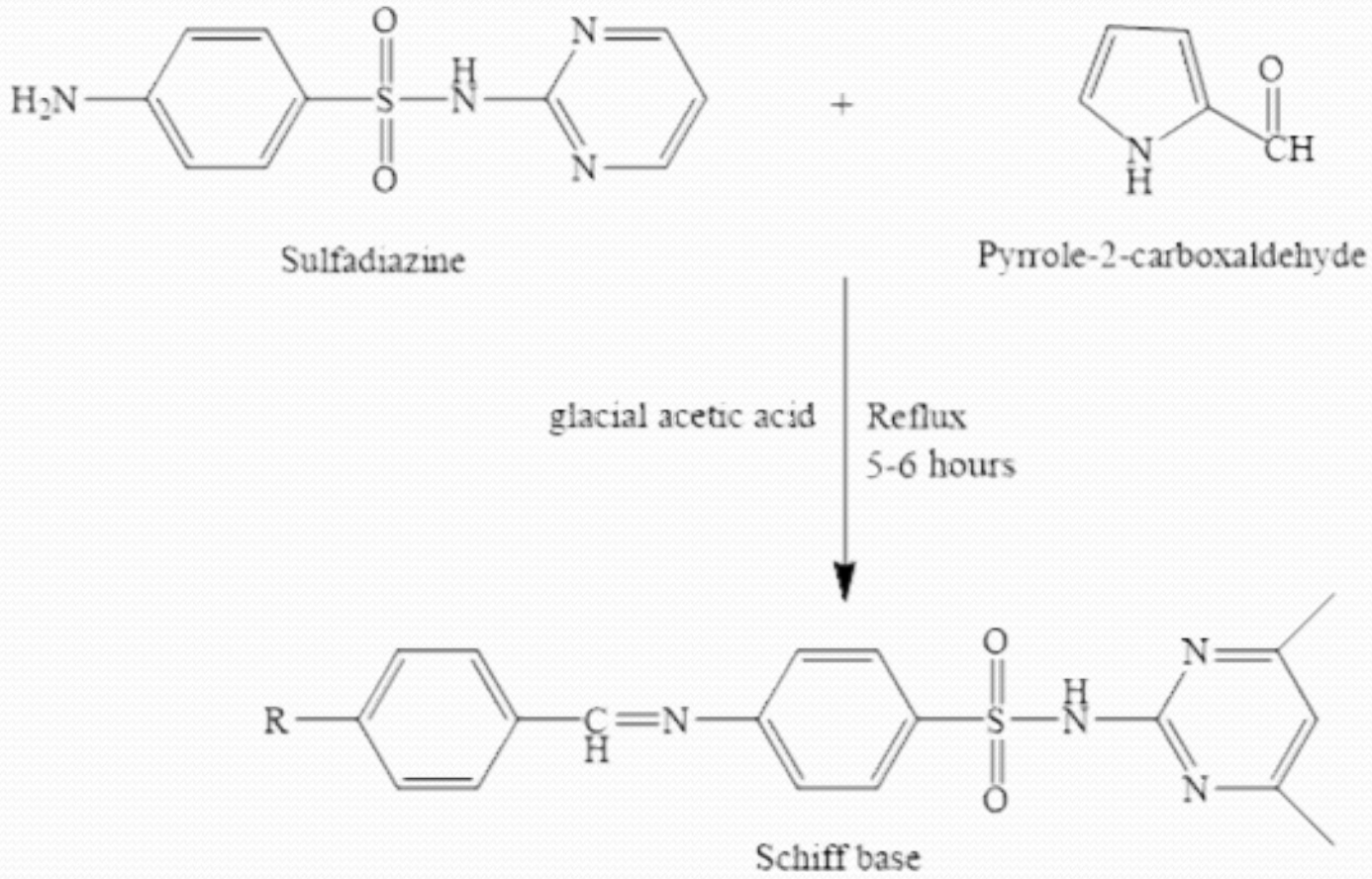
Equipments	company	country
Melting point apparatus	Stuart melting point aaparatus	Germany
FT-IR IRAffinity-1	Shimadzu	Japan
Thin layer chromatography plates: aluminum sheets, coated with silica gelG60 F_{254}thickness 0.25 mm	Merck	Germany

Chemical synthesis:

The Synthesis of [4-(((1*H*-pyrrol-2-yl) methylene) amino)-*N*-*(*pyrimidin-2-yl)benzenesulfonamide].(Schiff base compound) .



The synthetic pathways:



Bacterial isolates (tested bacteria):

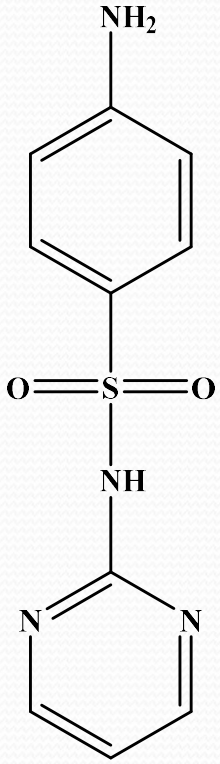
The synthesized chemical compound have been studied for its antibacterial activity *in vitro* against two types of clinical isolates (tested bacteria) which were taken from the laboratory of researches at Biochemistry and Clinical sciences Department, College of pharmacy /University of Basrah. These clinical isolates (tested bacteria) include:

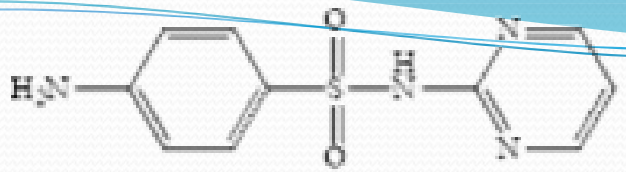
Staphylcoccusaureus (as gram positive bacteria).

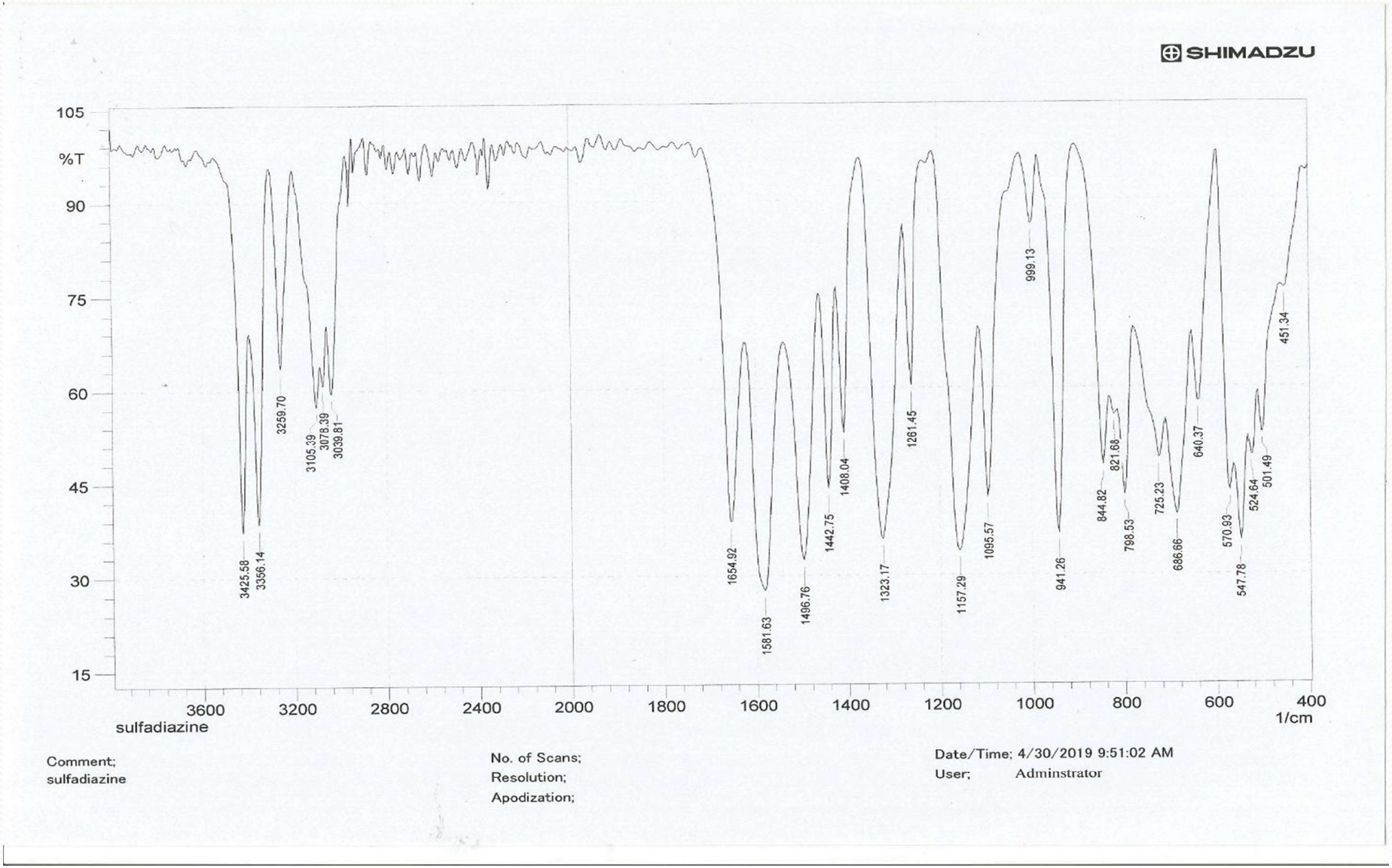
Escherichia coli (as gram negative bacteria).

Identification and characterization of synthesized compounds:

Compound's symbol	Empirical formula	Molecular weight (g/mol)	Physical appearance	% yield	Observed melting point (C^0)	R_f value
sulfadiazine	$C_{10}H_{10}N_4O_2S$	250.27	White powder	100%	253-256	R_f=0.31
Schiff base compound	$C_{15}H_{13}N_5O_2S$	327.36	Bright orange powder	83%	256-260	R_f=0.55

sulfdiazine	Band (cm^{-1})	Interpretation
	3425	NH asymmetric stretching of aromaticprimary amine (Ar-NH_2).
	3356	NH symmetric stretching of aromatic primary amine (Ar-NH_2).
	3259	NH asymmetric stretching of secondary amine (-SO_2NH-).
	3105	Aromatic CH stretching.
	3078	Aromatic CH stretching.
	3039	Aromatic CH stretching.
	1654	Aromatic C=N stretching of pyrimidine ring.
	1581	Aromatic C=C stretching.
	1496	Aromatic C=C stretching of pyrimidine ring.
	1323	S=O asymmetric stretching
	1261	C-N stretching.
	1157	S=O symmetric stretching
	1095	Aromatic CH bending (in plane).
	941	S-N stretching.
	844	Aromatic C-H bending (out of plane).




SHIMADZU
105
%T
90
75
60
45
30
15
3425.58
3356.14
3259.70
3105.39
3078.39
3039.81
1654.92
1581.63
1496.76
1442.75
1408.04
1323.17
1261.45
1157.29
1095.57
999.13
941.26
844.82
821.68
798.53
725.23
686.66
640.37
570.93
547.78
524.64
501.49
451.34
3600
3200
2800
2400
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1600
1400
1200
1000
800
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1/cm
sulfadiazine
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sulfadiazine
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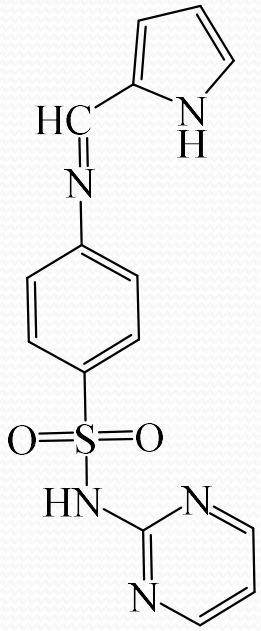
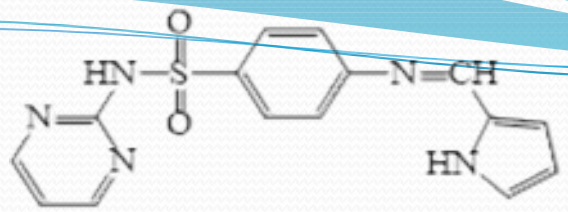
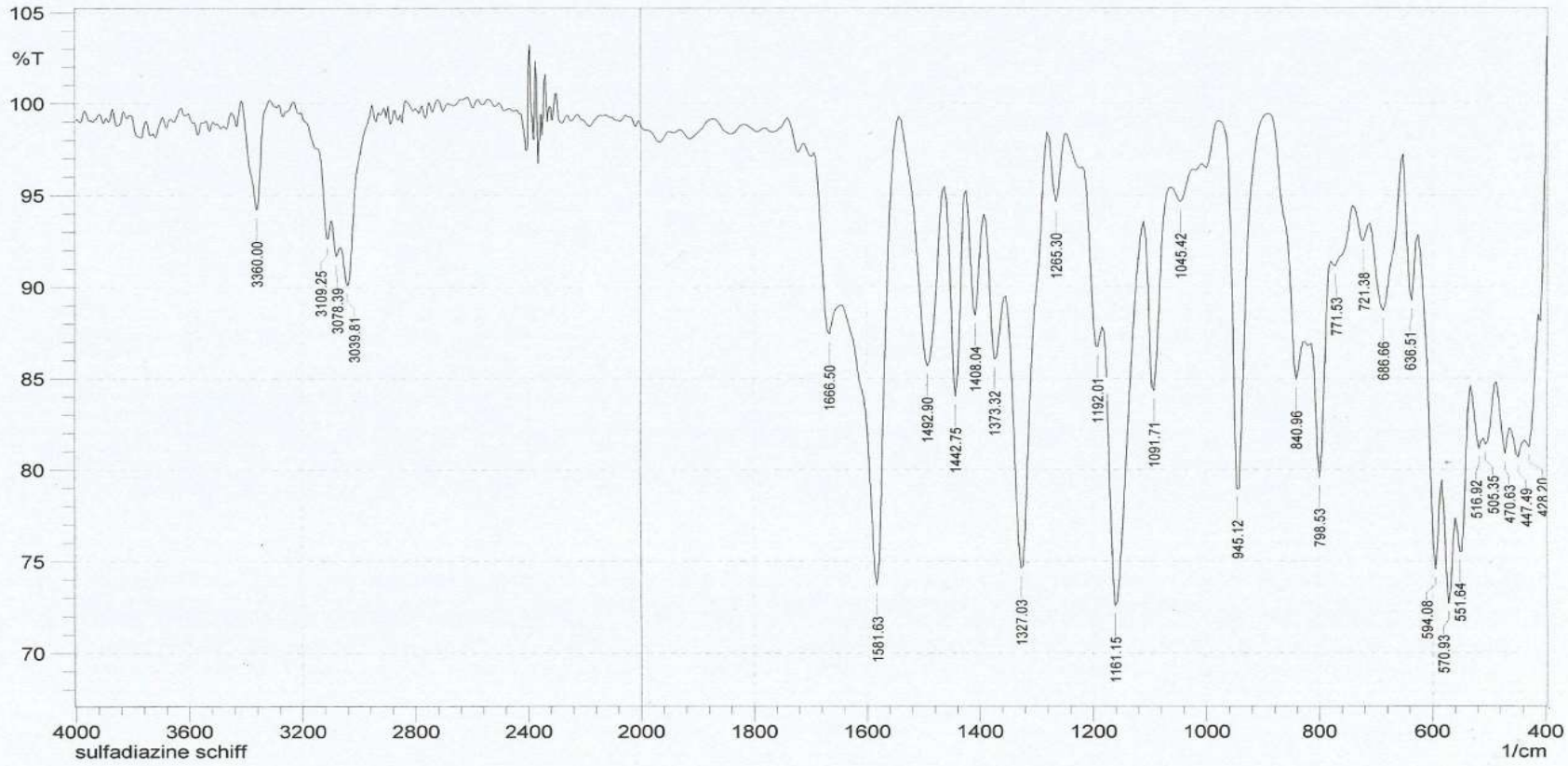
Fourier transforms infrared spectroscopy :

compound	Band (cm^{-1})	Interpretation
	3360	NH stretching of secondary amine (SO_2NHR).
	3109	Aromatic CH stretching.
	3078	Aromatic CH stretching.
	3039	Aromatic CH stretching.
	1666	C=N stretching of imine.
	1581	Aromatic C=N stretching of pyrimidine ring.
	1492	Aromatic C=C stretching.
	1327	S=O asymmetric stretching
	1161	S=O symmetric stretching
	1091	Aromatic CH bending (in plane).
	945	S-N stretching.
	840	Aromatic CH bending (out of the plane).



SHIMADZU



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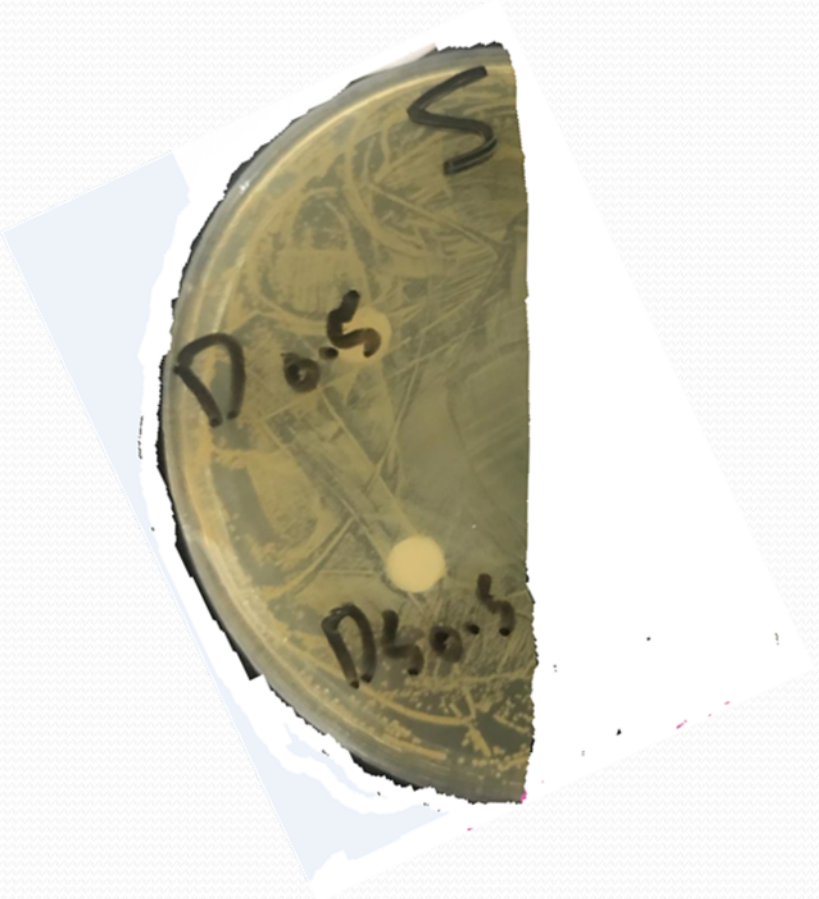
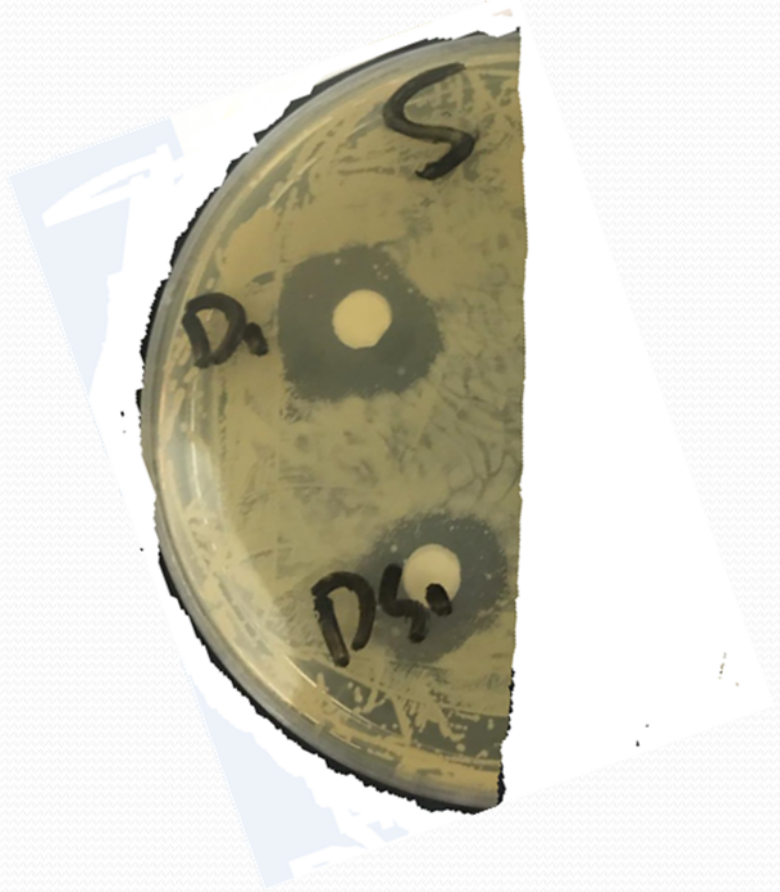
The in vitro antibacterial evaluation of sulfadiazine and the synthesized chemical compound against *Staphyllococusaureus.*

Chemical compounds	**Diameter of inhibition zone (mm) of *Staphyllococus aureus*(per 1ml of DMSO).**	
	1000 mcg	**500 mcg**
sulfadiazine	18	R
Schiff base compound	13	R


S
D 0.5
D50.5

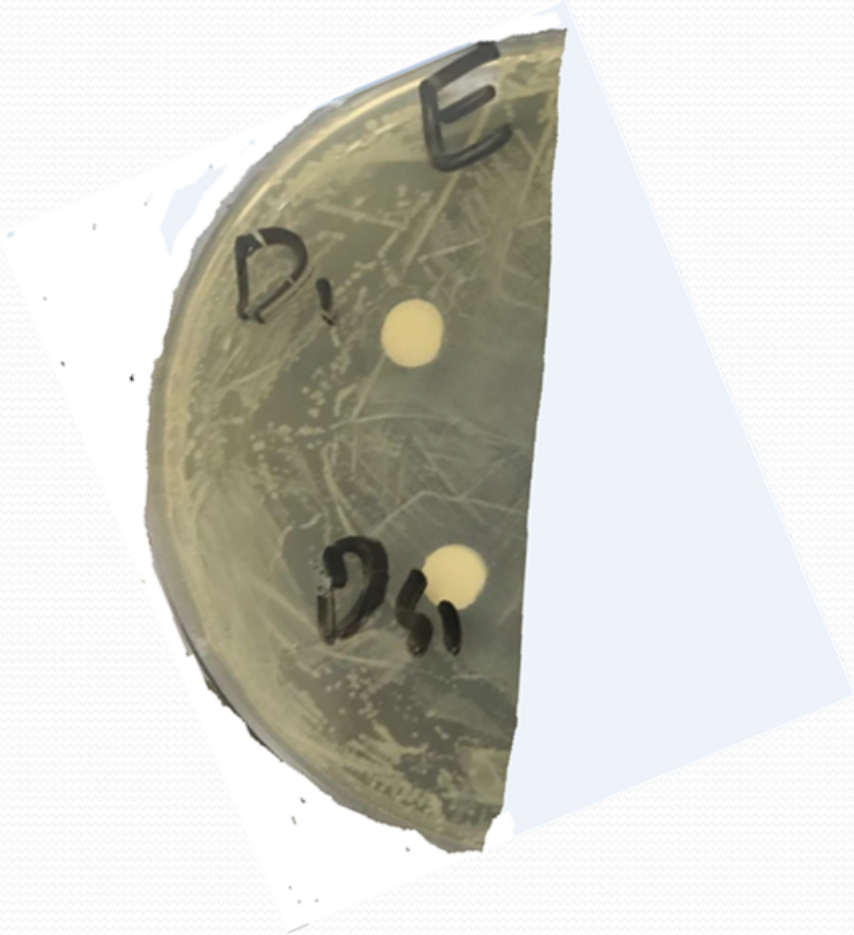

S
D
D5

Staphylococcus aureus is a G +ve bacteria .Sulfadiazine is a (bacteriostatic broad spectrum) shows antibacterial activity against gram +ve bacteria. After formation of Schiff base compound the p-amino group converted to imine group, so the antibacterial activity of the synthesized compound may be due to -SO2NH- group , imine group itself and pyrrole ring .

The in vitro antibacterial evaluation of sulfadiazine and the synthesized chemical compound against *Escherichia coli.*

Chemical compounds	**Diameter of inhibition zone (mm) of *Escherichia coli*(per 1ml of DMSO).**	
	1000mcg	**500 mcg**
sulfadiazine	R	R
Schiff base compound	R	R


E
D0.5
D0.5


E
D1
D1

Escherichia coli is gram -ve bacteria , the absence of antibacterial activity of sulfadiazine and Schiff base compound may be due to development of bacterial resistant for both compounds and this may result from reduced permeability of the cell membrane(altered permeability), and active efflux of the sulfonamide may play a role [2,33]. In addition to that E-Coli resistant strains to sulfonamide has been revealed because of their containing sulfonamide- resistance dihydropteroate Synthase [34] .

Conclusions

- The synthesis of designed compound has been successfully achieved.
- The purity and structural formula of synthesized compound were confirmed by melting points determination, R_f values & FT-IR spectroscopy
- In vitro antibacterial activity of synthesized compound has successfully achieved against ***Staphyllococus aureus*** gram +ve . However , it was less than the activity of the sulfadiazine .

Recommendations

1. Utilizing another types of sulfonamides in Schiff base approach with other types of heterocyclic aldehydes and compare their antimicrobial activities.
2. Studying the antimicrobial activities of synthesized compound on large types of bacteria.
3. Studying the anti tuberculoses activity of synthesized compound.
4. Studying the anticancer activity of synthesized compound.
5. Determination of partition coefficient (P) of synthesized compound.

Thank you