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Transition Metal Catalyzed Synthesis of Biologically Active Heterocycles

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Abstract:

A variety of highly efficient methodologies for the synthesis of aromatic heterocycles and their derivatives have been reported in the past, the development of novel methodologies is in continuous demand. Particularly, development of new synthetic approaches toward heterocycles, aiming at achieving greater levels of molecular complexity and better functional group compatibilities in a convergent and atom economical fashions from readily accessible starting materials and under mild reaction conditions, is one of the major research endeavors in modern synthetic organic chemistry. Transition metal-catalyzed transformations, which often help to meet the above criteria, are among the most attractive synthetic tools. In the present research a series of 7 Spiro-heterocyclic derivatives against antibacterial proteins and anti-fungal. The antibacterial results showed that highest value for the compounds 1, 2 in antibacterial activity. The compounds were isolated with TLC, purified with column chromatography and evaluated by spectroscopic method.

Key words: Spiro-heterocyclic compounds, anti-bacterial and anti-fungal activity copper transition metal

Introduction

Heterocyclic chemistry is a very important branch of organic chemistry accounting for nearly one-third of modern

publications (**Katritzky *et al*., 1984).** In fact two thirds of organic compounds are heterocyclic compounds. A cyclic organic compound containing all carbon atoms in ring formation is referred to as a carbocyclic compound. If at least one atom other than carbon forms a part of the ring system then it is designated as a heterocyclic compound. Nitrogen, oxygen and sulfur are the most common heteroatoms but heterocyclic rings containing other hetero atoms are also widely known. An enormous number of heterocyclic compounds are known and this number is increasing rapidly. Heterocyclic compounds may be classified into aliphatic and aromatic **(Mauras *et al*., 2009)**. Although, a variety of highly efficient methodologies for synthesis of aromatic heterocycles and their derivatives have been reported in the past, the development of novel methodologies is in continuous demand. Particularly, development of new synthetic approaches toward heterocycles, aiming at achieving greater levels of molecular complexity and better functional group compatibilities in a convergent and atom economical fashions from readily accessible.

Starting materials and under mild reaction conditions, is one of a major research endeavor in modern synthetic organic chemistry.

Transition metal-catalyzed transformations, which often help to meet the above criteria, are among the most attractive synthetic tools.

Biological importance of Heterocyclic compounds

Besides the vast distribution of heterocycles in natural products, they are also the major components of biological molecules such as DNA and RNA. DNA is without doubt the most important macromolecule of life. Nucleotides, the building blocks of our genes are derivatives of pyrimidine and purine ring structures. Chlorophyll and heme, the oxygen carriers in plants and animals respectively are derivatives of large porphyrin rings. Heterocycles are present in a wide variety of

drugs **(Brown *et al.*, 2003).** Most vitamins, many natural products, biomolecules, and biologically active compounds, including antitumor **(Maponya *et al.*, 1998)** antibiotic, anti-inflammatory, antidepressant, antimalarial, anti-HIV, antimicrobial, antibacterial, antifungal, antiviral, antidiabetic, herbicidal, fungicidal, and insecticidal agents. Also, they have been frequently found as a key structural unit in synthetic pharmaceuticals and agrochemicals. Some of these compounds exhibit a significant solvatochromic, photochromic.

Spiro-hetero cyclic compounds:

The chemistry of heterocyclic compounds has been an interesting field of study for a long time. The name `spirocyclane' was first introduced by Baeyer in 1900 — Spirocyclane structures are found in wide range of natural compounds isolated from various sources **(Grover &Kini, 2003).** The complexities of Spiro moleculas are represented by the quaternary carbon center and two fused rings. The construction of the spirocycles can be roughly categorized into alkylation, rearrangement, cycloaddition and cleavage of bridged system. For example a vinylcyclopropanolvinylcyclobutanol rearrangement **fig** had been reported for the synthesis of Spiro cycles by Trost's lab.

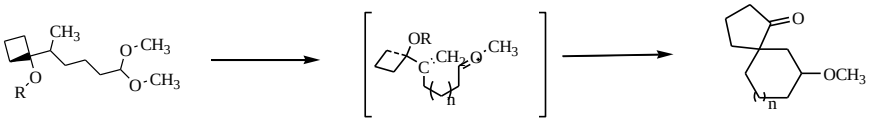


Fig :- synthesis of spirocycles by Trost's lab

Bacteria.

Microbes belonging to the bacteria group are made up of only one cell under a microscope, bacteria look like balls, rods, or spirals. Life in any form on Earth could not exist without these tiny cells.

Bacterial diseases

Bacterial diseases include any type of illness caused by bacteria. Millions of bacteria normally live on the skin, in the intestines, and on the genitalia. (**Abraham *et al.*, 1996).** The vast majority of bacteria do not cause disease, and many bacteria are actually helpful and even necessary for good health. These bacteria are sometimes referred to as "good bacteria" or "healthy bacteria." Harmful bacteria that cause bacterial infections and disease are called pathogenic bacteria. Bacterial diseases occur when pathogenic bacteria get into the body and begin to reproduce and crowd out healthy bacteria, or to grow in tissues that are normally sterile.

Treatment

Small Heterocyclic molecules are very important building blocks in the synthesis of biologically active compounds. These building blocks include nitrogen and oxygen-containing heterocycles which are active against different bacteria as 2-oxazolidinones, 1,3-oxazinan-2-ones, 2-oxazolines, oxazines, morpholine, benzofurans, benzooxazines, Pyrrole derivatives, and many more.

Material and Methods

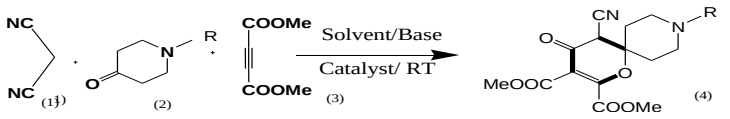


Fig : Synthesis of spiropyranopiperidone in CuO using Water at 60°C.

Where (R) is:-

(a) -CH_3
(b) -C_2H_5
(c) -OCH_3
(d) -Cl
(e) -Br
(f) -F
(g) –C_6H_5

Table:- Influence of catalyst on product yield under different solvents

Entry	solvent	base	catalyst	Product Yield%
1	Ethanol	KOH	CuI_2	47
2	DCM	K_2CO_3	$InCl_3$	43
3	DMF	Cs_2CO_3	SiO_2	27
4	1,4dioxane	KOH	$ZnCl_2$	46
5	water	K_2CO_3	$MgCl_2$	21
6	Methanol	K_2CO_3	CuI_2	33
7	DCM	K_2CO_3	$ZnCl_2$	40
8	Water	-	CuO	87

Anti- bacterial and anti-fungal activity.

Anti-bacterial Activity of synthesized derivatives has been tested on P.aeruginonsa and *S.aureus* Anti-fungal activity has been screened on A.*niger*.

Preparation of Discs:

Whatman No: 1 filter paper discs of 6mm diameter are prepared and autoclaved by keeping in a clean and dry Petri plate. The filter paper discs were soaked in plant extracts for 6 hours are taken as test material. After 6 hours the discs were shade dried. The concentrations of plant extracts per disc are accounted for 0.1 grams/1ml. Subsequently they are carefully transferred to spread on cultured Petri plates. Filter paper discs immersed in ethanol, benzene, distilled water are prepared and used as control

Preparation of medium for bacterial and fungal cultures:

For testing of the anti-bacterial and anti-fungal cultures which were mentioned above. The following medium is used

Nutrient Agar medium (NAM)

Beef extract	--	500mg
Sodium chloride	--	500mg
Peptone	--	1gm

Ph	--	7.0 – 7.2
Distilled water	--	100ml
Agar	--	2gm

The medium was steamed for 30 min neutralized at 37°c and steamed for half an hour and filtered. The medium was sterilized at 15 lbs for 20 min at 121°c.

Testing of anti-bacterial and anti-fungal activity:

To test the anti-bacterial and anti-fungal activity on agar plates, LB agar medium was prepared using the ingredients mentioned above. The medium was sterilized at 121°c for 30 min's.

The agar test plates were prepared by pouring about 15ml of the medium into 10cm Petri dishes under aseptic condition and left undisturbed for 2hrs to solidify the medium.1ml of inoculum (containing suspension) ofP.aeruginosa and *S.aureus*was poured to the respective plates separately containing solidified agar media. Six replicates were maintained. The prepared sterile whatman no :1 filter paper discs of 6mm diameter were impregnated with the extracts and shaken thoroughly and this test plates incubated for a period of 48 hrs. In BOD at 37°c for the development of inhibitory zones and the average of 2 independent readings for each organism in different extracts were recorded. The control Petri plates and also maintained above respective cultures.

Results and Discussion

Synthesis of characterization of spiropyranopiperidone derivatives:-

The synthesized products and their properties have been given below:-

Table. Properties of synthesized derivatives.

S. NO.	Derivatives	Name	Molecular Formula	Yield %	M.P (^{0}C)	Elemental Analysis (in %)
1`	4a	Methyl-substituted spiropyrano-piperidone	$C_{15}H_{18}N_2O_6$	87	69-73	C, 56.30; N, 3.62; H, 6.53
2	4b	Ethyl-substituted spiropyrano-piperidone	$C_{16}H_{20}N_2O_6$	84	102-104	C, 55.28; N, 3.79; H, 6.28.
3	4c	Methoxy-substituted spiropyranopiperidone	$C_{15}H_{17}N_2O_7$	81	95-98	C, 58.25; H, 6.30; N, 7.87
4	4d	Chloro-substituted spiropyrano-piperidone	$C_{14}H_{15}N_2O_6Cl$	73	60-63	C, 55.05; H, 5.61; N, 8.63
5	4e	Bromo-substituted spiropyrano-piperidone	$C_{14}H_{15}N_2O_6Br$	75	77-79	C, 54.05; H, 5.95; N, 3.93
6	4f	Floro-substituted spiropyrano-piperidone	$C_{14}H_{15}N_2O_6F$	77	73-75	C, 54.03; H, 5.93; N, 3.91;
7	4g	Phenyl-substituted spiropyrano-piperidone	$C_{20}H_{20}N_2O_6$	79	61-63	C, 55.30; N, 3.80; H, 6.25

The characterizations of synthesized derivatives have been done by ^{1}HNMR and Mass spectral analysis.

(4a) - ^{1}HNMR

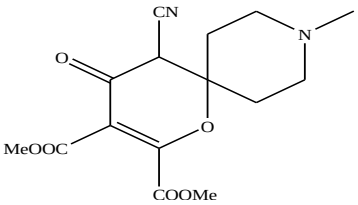


Fig :-structure of derivative (4a)

Data of ^{1}HNMR: (400MHz, $CDCl_3$/TMS) δ: 1.29 (t, 3H), 1.31(t, 3H), 1.77(t, 2H), 1.80 (t, 2H), 2.23 (t, 2H), 2.25(t, 2H), 2.29(s, 3H), 3.49 (s, 1H), 3.65 (s, 3H of CH_3), 4.22(q, 3H), 4.23 (q, 3H),
EIMS: (m/z): 383 [M+],

(4b)-^{1}HNMR

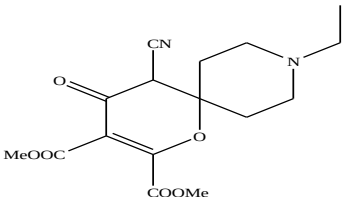


Fig :-structure of derivative (4b)

Data of ^{1}HNMR:(400MHz, $CDCl_3$/TMS) δ: 1.31 (t, 3H), 1.32 (t,3H),1.78 (t,2H), 1.77(t, 2H),2.22 (t,2H), 2.23 (t,2H), 2.26 (s, 3H), 3.63 (s,1H), 4.18(q, 2H), 4.17(q,2H)**,** ;
EIMS: (m/z): 350 [M+],

(4c)-^{1}HNMR

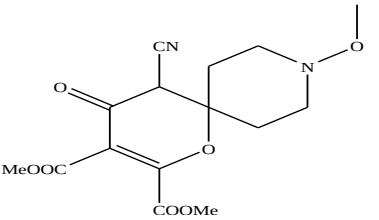


Fig :-structure of derivative (4c)

Data of ^{1}HNMR: (400MHz, $CDCl_3$/TMS) δ: 1.31 (t, 3H), 1.32 (t, 3H), 1.29 (s, 3H), 1.77(t, 2H),1.78 (t, 2H), 2.22 (t, 2H), 2.23 (t, 2H), 2.26 (s, 3H), 3.45 (s, 1H), 4.11(q, 2H), 4.18(q, 2H), 4.17(q, 2H)**,**;
EIMS: (m/z): 352 [M+].

(4d)-^{1}HNMR

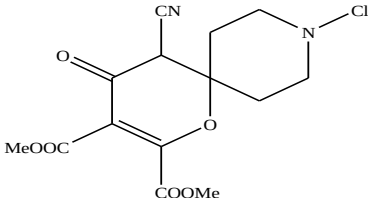


Fig :-structure of derivative (4d)

Data of ^{1}HNMR:(400MHz, $CDCl_3$/TMS) δ: 1.31 (t, 3H), 1.32 (t, 3H), 1.29 (s, 3H), 1.77(t, 2H),1.78 (t, 2H), 2.22 (t, 2H), 2.23 (t, 2H), 2.26 (s, 3H), 3.45 (s, 1H), 4.11(q, 2H), 4.18(q, 2H), 4.17(q, 2H)
EIMS: (m/z): 355 [M+].

(4e)-^{1}HNMR

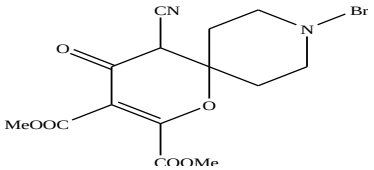


Fig :-structure of derivative (4e)

Data of ^{1}HNMR:(400MHz, CDCl3/TMS) δ: 1.77 (t, 2H), 1.78(t, 2H), 2.22 (t, 2H), 2.23 (t, 2H), 2.26 (s, 3H), 3.45(s, 1H), 3.65 (s, 3H), 3.75(s, 3H), 3.74(s, 3H)
EIMS: (m/z): 322 [M+].

(4f)-^{1}HNMR

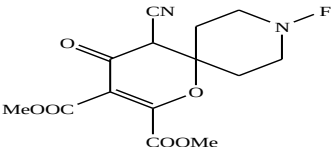


Fig :-structure of derivative (4f)

Data of ^{1}HNMR:(400MHz, $CDCl_3$/TMS) δ: 1.27(t, 3H) 1.75 (t, 2H), 1.77 (t, 2H), 2.21 (t, 2H), 2.22 (t, 2H), 2.25 (s, 3H), 3.45 (s, 1H), 3.73 (s, 3H of OCH_3), 3.72 (s, 3H, CH_3), 4.10 (q, 3H).

EIMS: (m/z):354 (M^+).

(4g)- ^{1}HNMR

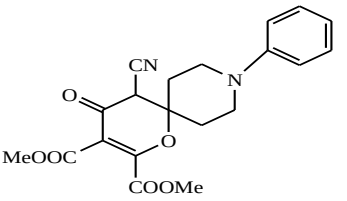


Fig :-structure of derivative (4g)

Data of ^{1}HNMR:(400MHz, $CDCl_3$/TMS) δ: 1.30 (t, 3H), 1.79 (t, 2H), 1.79 (t, 2H), 2.23 (s, 2H), 2.24 (t, 2H), 2.28(s, 3H), 3.47(s, 1H), 3.67(s, CH_3), 3.76(s, 3H, CH_3), 4.19 (q, 2H).
EIMS: (m/z):369 (M^+).

Anti-Bacterial and Anti-Fungal Activity.

The anti-bacterial activity for synthesized derivatives have been scanned by cell death%

Table:- Anti-bacterial effect of compounds on bacterial cell line

Derivatives	Conc (µg/ml)	O.D (optical density)	Cell Viability (%)	Cell death (%)
4a	1000	0.35	37.5	91.1
4b	1000	0.37	36.63	86.37
4c	1000	0.36	53.4	75.15
4d	1000	0.39	45.1	73.5
4e	1000	0.41	43.7	53
4f	1000	0.40	41.2	61
4g	1000	0.37	42.9	65
Cell control benzene, distilled water, and ethanol	-	0.48	100	0.0

Table: - Anti-fungal effect of compounds on fungal cell line.

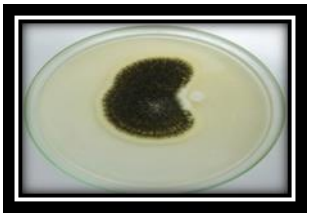
Derivatives	Conc (µg/ml)	O.D (optical density)	Cell Viability (%)	Cell death (%)
4a	1000	0.37	36.5	90.14
4b	1000	0.35	38.63	85.37
4c	1000	0.36	50.4	77.15
4d	1000	0.38	44.1	73.00
4e	1000	0.40	42.7	52.00
4f	1000	0.39	40.2	59.00
4g	1000	0.37	41.9	66.00
Cellcontrol benzene, distilled water,and ethanol	-	0.47	100	0.0

The plates of compounds showing best inhibition on bacterial and fungal growth has been shown below:-

Compound (4a)

Pseudomonas aeruginosa ***Staphylococcus aureus***

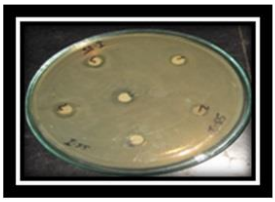
Fig:- Plates of compound (4a) showing best inhibition on bacterial growth

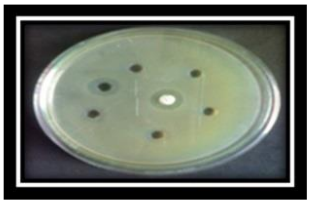


Aspergillusniger

Fig : Plate of compound (4a) showing best inhibition on fungal growth

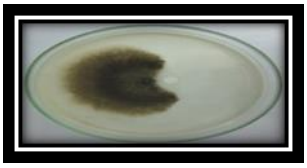
Compound (4b)





Pseudomonasaeruginosa ***Staphylococcusaureus***

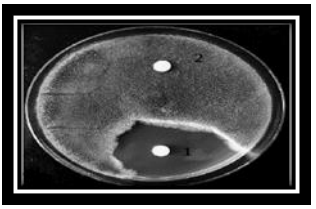
Fig: Plates of compound (4b) showing best inhibition on bacterial growth



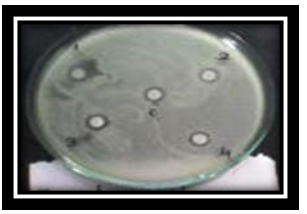
Aspergillusniger

Fig: Plate of compound (4b) showing best inhibition on fungal growth

Compound (4c)

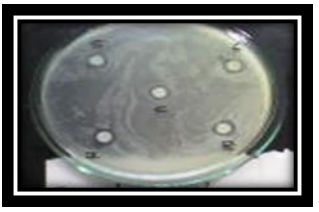


Pseudomonas aeruginos



Staphylococcus aureus

Fig : Plates of compound (4c) showing best inhibition on bacterial growth



Pseudomonas aeruginosa

Fig : Plate of compound (4c) showing best inhibition on fungal growth

Synthesized spiropyranopiperidone derivatives showed excellent anti-bacterial andanti-fungal activities. The inhibitory properties of these compounds were compared with standard controls for standards cell lines respectively. The cell inhibition profiles were found to be highly dependent on structure of the applied spiropyranopiperidone. The maximum concentration (µg/ml) used in the study was 1000 µg/ml of the seven derivatives. The bacterial and fungal cell lines grown in DMEM, when subjected to different synthesized compounds resulted in 91.1 %, 83.21%, 75.15%, 53%, 61% and65% for bacterial inhibition and 90.14%,85.37%, 77.15%,73.00%,52.00%,59.00% and 66.00% for fungal inhibition. It was observed from the result that a gradual decrease in percentage inhibition was observed in all the cases.

The less activities of the compounds against bacterial and fungal cells can be attributed to expression of the gene expressed targets having minimal affinity to the chemical constituents present in these fungal cells. It might also be due to the low penetration power of the active substituents which might have resulted in low growth inhibitions. It has been seen that for bacterial growth inhibition , the rate of cell control for the compound 4a is more than (91%) when compared to plate 4b, (86%), 4c , (75.15%), 4d, (73.5%), 4e, (53%), 4f,(61%), and 4g, (65%).Fungal death control and the rate of cell control in the compound 4a is more than(90.14%) compared to plates 4b(85.37%). 4c,(77.15%), 4d,(73.00%), 4f (52.00%) and 4g, (66.00%).The compound (4a)emerged out as an outstanding anti-bacterial and anti-fungal inhibitor, it is observed 91% control which is best as only 9% bacteriasurvive and around 10%fungus survive.

Compound having electron donating group i.e. alkyl group in (4a) derivative showed the best result against bacteria (91.1% of cell death) and also (4a) derivative showed the best result against fungus (90.14%of cell death) whereas group having electron withdrawing capacity i.e. Cl, F, Br and C_6H_5 showed less anti- bacterial and anti-fungal activity.

Since the derivatives 4b, 4c, 4d, 4e, 4f and 4g increases the polarity of the compounds due to which they are not able to penetrate bacterial and fungal cell wall and thus showed less inhibition.

Summary and Conclusion

Synthetic chemistry can rightfully be considered a prerequisite of our modern society. This discipline supplies many valuable resources to our world enabling us to produce the quantities of fertilizer needed to feed a growing world's population and produce the numerous customized materials without which society could not progress. Importantly, synthetic chemistry has

a huge impact on public health where treatments for almost any disease can be developed resulting in a steady increase in life expectancy. All these advances have been enabled by the curiosity of generations of scientists constantly searching for new solutions to the assembly of functional molecules. Importantly this has required the development of several new methods for selectively forming new chemical bonds allowing the generation of more complex drug candidates. The most common synthetic routes to the top-marketed drugs containing heterocyclic compounds, which allowed us to analyse how medicinal chemists have addressed challenges over the past 30 years. An enormous number of heterocyclic compounds are known and this number is increasing rapidly.Heterocyclic chemistry is a very important branch of organic chemistry accounting for nearly one-third of modern publications.

A cyclic organic compound containing heteroatom is reffered to as heterocycle. It is mainly of two types' simple heterocyclics and spiroheterocycles. This work represents chemical transformations employed for spiropyranopiperidone derivatives. We wish to present a complementary compilation of the synthesis route using transition metal catalyst which worked efficiently with solvent and generate spiropyranopiperidone heterocycle. We synthesized seven derivatives of targeted molecule and then passed them through anti-bacterial and anti-fungalactivity. It was found that almost all derivatives have been found active against bacteria and fungi, but the methyl derivative having least polarity shows the best inhibition.

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