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Research Article

EVALUATION OF ANTIMICROBIAL ACTIVITY OF NOVEL OXAZOLO-PYRIDINE DERIVATIVE

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Abstract

Oxazolopyridines derivatives (All five)were screened for *in vitro* antimicrobial activity against gram-positive bacteria *B. Subtilis* andgram-negative bacteria and *E. Coli*, cutaneous fungi *C. Albicans* and *A. Niger*. oxazolopyrineOP-5 exhibited excellent antibacterial activity against E. coli in comparison to standard drug – Ciprofloxacin. Moreover, OP-3 showed excellent antifungal activity against A. Niger in comparison to standard drug – griseofulvin.Gram-negative bacteria were found to be more sensitive towards the OP-5 in comparison to gram-positive bacteria.

Keyword : Oxazolopyridines derivatives, antibacterial activity, antifungal activity

Introduction

Synthetic organic chemistry is the art of building-up organic compounds from smaller entities. This science has found application in the production of organic compounds of commercial interest, in the construction of new, potentially bioactive molecules derived from rational design, in the challenge to synthesize very complex natural products, in finding new methods and strategies to render this science more efficient.

The synthesis of a complex organic compound requires a synthetic analysis and planning;[2] the most efficient method consists in the retrosynthetic analysis which is based on proper disconnections that virtually generate smaller fragments that are in turn disconnected till commercially available compounds are reached. Each reaction in the synthesis scheme must affect only the required functional group leaving intact the others, which therefore must be protected. The protection-deprotection strategy is of fundamental importance in a synthetic plan.[3] Stereoselectivity is also fundamental in the synthetic strategy, as most target molecules are chiral. Different approaches have been developed to perform stereoselective syntheses: chiral substrates of natural origin (the chiral pool) have been used as starting materials; chiral auxiliaries or chiral catalysts have been exploited to induce stereoselectivity; the chiral resolution of a stereoisomeric mixture has been performed.

In order to simplify and fasten the synthetic procedures a solid phase approach been developed.[4] This method allows automation of some repetitive procedures, such as peptide or oligonucleotide synthesis.

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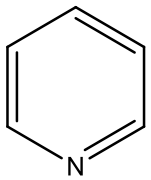
The solid phase technique allowed the development of combinatorial synthesis, an approach that generates a high number of organic compounds, based on the combinatorial disposition of different building blocks in the construction of the products. Finally, nowadays there is an effort to render synthetic chemistry more environment friendly.

Pyridine is a fundamentally important chemical compound with the formula C_5H_5N. It is a liquid with a distinctively putrid, fishy odor. Its molecules have a six membered ring structure that can be found in many compounds, including the nicotinamides. This compound has numerous applications. It is both a versatile solvent and it is a building block for a variety of other organic compounds.



Pyridine

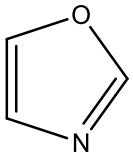
Pyridine involved in Medicine

- Pyridines find omnipresent applications in medicaments and in agrochemicals.[6]
- The leading groups are **antimicrobials (isoniazid)** and histamine h1 antagonists such as pheniramine, but also anticancer, analgesic, and antidepressant agents.
- Pyridine derivatives continue to attract great interest due to the wide variety of interesting biological activities observed for these compounds, such as anticancer, analgesic, **antimicrobial,** and antidepressant, activities.
- Pyridine is used in the pharmaceutical industry as a raw material for various drugs, vitamins, and **fungicides** and as a solvent.
- In many enzymes, the prosthetic pyridine nucleotide (NADP) is involved in various reduction-oxidation reactions, pyridine in biological systems is its presence in the vitamins niacin and pyridoxine (vitamin B6) but also in highly toxic alkaloids such as nicotine.[7]

. **Oxazole**

. The chemistry of oxazole was come in concern during the world war when penicillin was considered to contain the oxazole ring system, but the invention of oxazole's

as dienes in Diels-Alder reaction and in 1, 3-dipolar cycloaddition reaction of mesonic heterocycles give idea for advance of oxazole chemistry. Oxazole contains an oxygen atom and a pyridine type nitrogen atom at the1 and 3 positions of the ring and like pyridine, oxazole are weekly basic substances. Oxazole be considered as derived from furan by thereplacement of –CH= (methane group) from the position-3 by the azomethine nitrogen (-N=) group.[8] Oxazole ring is numbered as follows:



Antimicrobials are chemicals that kill or inhibit the growth of microorganisms and are used to treat microbial infections. Some are produced naturally by microbes but many are synthetic. Antimicrobials include antibiotics, antivirals, antifungals and other drugs such as antimalarials.

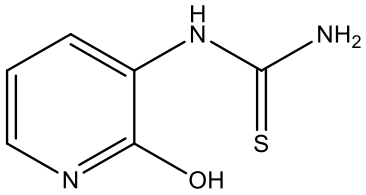
With increase in the incidence of multidrug-resistant gram-positive and gram-negative bacteria it becomes imperative to continuously search for small molecules as anti-infective agents.[43]

Multiply resistant organisms render therapy more precarious and costly and sometimes unsuccessful. Individuals may succumb to MDR infections because all available drugs have failed, especially in the developing world.[44] Notable global examples include hospital and community MDR strains of Mycobacterium tuberculosis, Enterococcus faecium, Enterobacter cloacae, Klebsiella pneumoniae, S. aureus, Acinetobacterbaumanii and Pseudomonas aerugi. In developing countries, MDR enteric disease agents such as Salmonella enteritidis, Shigellaflexneri and Vibrio cholerae threaten and circumvent public health measures.

OXAZOLO-PYRIDINE DERIVATIVE : Five compound of oxazol derivative are listed below which are selected for evaluation of Anti-Microbial activity

Compounds

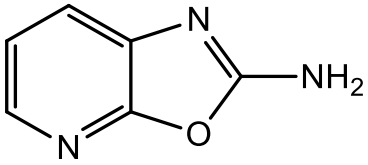
Intermediate Compound-1



IUPAC name: 1-(2-hydroxy-3-pyridyl)thiourea

Molecular formula : $C_6H_7N_3OS$
Molecular weight : 169.20
Physical state : Crystalline solid
Color : Reddish yellow
Melting point : 171°C
Yield : 82.10%,
Solubility : DMSO and DMF
R_f-value : 0.61

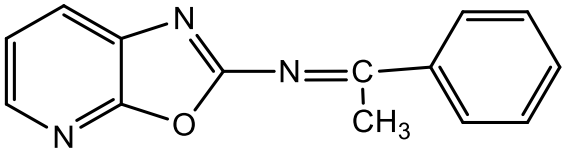
Intermediate Compound-2 [OP-0]



IUPAC Name: 2-aminoxazolo [5,4-b] pyridine [OP-0]

Molecular formula : $C_6H_5N_3O$
Molecular weight : 135.12
Physical state : Crystalline solid
Color : Pale red
Melting point : 155°C
Yield : 74.4%,
Solubility : DMSO and DMF
R_f value : 0.79

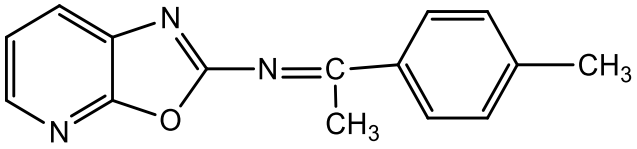
Compound [OP-1]



IIUPAC name: N-benzylideneoxazolo[5,4-b]pyridin-2-amine

Molecular formula : $C_{14}H_{11}N_3O$
Molecular weight : 237.26
Physical state : Crystalline
Color : Brown
Melting point : 143°C
Yield : 63.8%
Solubility : DMSO and DMF
R_f value : 0.63

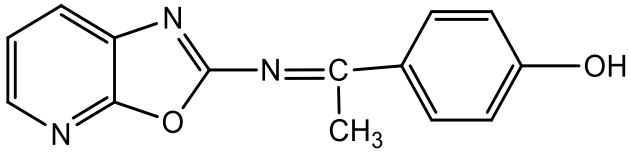
Compound [OP-2]



IUPAC Name: N-(4-methylbenzylidene)oxazolo[5,4-b]pyridin-2-amine

Molecular formula : $C_{15}H_{13}N_3O$
Molecular weight : 251.28
Physical state : amorphous
Color : Brownish Yellow
Melting point : 112 °C
Yield : 68.6%,
Solubility : DMSO and DMF
R_f value : 0.57

Compound [OP-3]



IUPAC Name: 4-((oxazolo[5,4-b]pyridin-2-ylimino)methyl)phenol

Molecular formula : $C_{14}H_{11}N_3O_2$
Molecular weight: 253.26
Physical state : Amorphous
Color : Yellow
Melting point : 128 ^{O}C
Yield : 62.5%
Solubility : DMSO and DMF
R_f value : 0.6

Materials of Characterization[7-9]

Method of Screening of Antimicrobial Activity

Evaluation of Antimicrobial Activity

A drug is considered as bacteriostatic or fungistatic when it inhibits the growth or multiplication of bacteria or fungi respectively and considered as bactericidal or fungicidal when it actually results in the death of bacteria or fungi. Drugs that are bactericidal under certain circumstances may have an apparent bacteriostatic effect at the other times. Important factors for the antimicrobial activity are size of the inoculums, metabolic state of organisms, pH, temperature and duration of interaction, concentration of the inhibitor and presence of interfering substance. In Vitro tests are used as screening procedure for new agents and for testing susceptibility of individual isolates from infections to determine which of the available drugs might be useful therapeutically. In general, minimum inhibitory concentration (MIC) and sensitivity tests are used to express the effectiveness of a compound as an antimicrobial agent. MIC is the smallest concentration of the substance required to inhibit the growth of a test organism under specified conditions. MIC can be determined by the tube dilution method. Sensitivity testing is done to determine the range of microorganisms that are susceptible to the compound under specified conditions. This method is suitable for the organisms that grow well overnight such as most of the common aerobes and facultative anaerobes and rapidly growing fungi such as *Candida Albicans*. Several forms of discdiffusion methods have been advocated. Among this Kirby Bauer method is the official method of the USA Food & Drug Administration.

Preparation of the Nutrient Media

The following broths were used in the present work. Compositions of broths are as follows:

Table no. 1: Composition of Nutrient broth

S.no.	Component	Amount
1	Peptone (bacteriological)	10 gm
2	Beef extract	10 gm
3	Sodium chloride	5 gm
4	Purified water	1000 ml
5	pH	7.2 ± 0.2

Table no. 2: Composition of Sabouraud's broth

S. no.	Component	Amount
1	Dextrose	20 gm
2	Peptone (mycological)	10 gm
3	Agar	15 gm
4	Purified water	1000 ml
5	pH	7.2 ± 0.2

The broths were prepared by dissolving the specified quantities of the dehydrated broth (Hi media) in purified water and were distributed 4 ml quantities in to each test tube. The tubes were closed with cotton plugs and sterilized by autoclaving at 121ºC for 15 minutes.

Cultivation of Microorganisms

The bacterial cultures were aseptically inoculated into nutrient broth and incubated under aerobic conditions at 37ºC for 24 h. Fungal cultures were inoculated into Sabouraud's broth and incubated under aerobic conditions at 25 ºC for 48 h.The following bacterial and fungal cultures were used for the study:

Table no. 3: Different microbial cultures used

S. no.	Name of Microorganisms	Status
1	*BacilliusSubtilis*	Gram positive bacteria
2	*Escherichia Coli*	Gram negative bacteria
3	*Candida Albicans*	Fungi (yeast)
4	*Aspergillus Niger*	Fungi (mold)

The bacterial cultures were aseptically inoculated into nutrient broth and incubated under aerobic conditions at

37ºC for 24 h. fungal cultures were inoculated in to Sabouraud's broth and incubated under aerobic conditions at 25ºC for 48 h.

Determination of Antimicrobial Activity

Modified Kirby-Bauer method, one of the official methods among disc diffusion methods, was used for the evaluation of antimicrobial activity of the synthesized compounds. Circular paper disks of 6 mm diameter was impregnated with the specific amount of the test sample and were placed on a suitable nutrient/sabraud's agar medium in a petri plate which was inoculated on its surface with one of the test organisms. After incubation, the plates were observed for the growth inhibition zones around the disks. The diameter of the zone of inhibition is proportional to the antimicrobial activity of the substance. The diameters of the zone of inhibition were compared with that produced by the standard antibiotics.

Preparation of the Disks and Samples

Paper disks of 6 mm diameter and 2 mm thickness were used for the test. These disks were sterilized by autoclaving at 121 ºC (15 lb PSIG) for 15 minutes. Almost all samples were tested at 50 µg level. To obtain this, sample solutions containing 10 mg/ml were prepared in sterile dimethylformamide (DMF) and 5µl each of the solutions was added on each disk using a micropipette. All the solutions were added on each disk using a micropipette. All the solutions were prepared using aseptic precautions. Ciprofloxacin (10 µg/disk) was taken as standard antibiotics for the comparison of the antibacterial activity of the synthesized compounds and Clotrimazole (10 µg/disk) were used as standard drugs for antifungal activity studies.

General Procedure

Each Petri plate containing nutrient/ sabouraud's agar medium was inoculated with one bacterial/ fungal culture by spreading the suspension of the organism with a sterile cotton swap. Each plate was divided into six equal portions along the diameter. Each portion was used to place one disk. Four disks of each sample were placed on four portions, one disk with standard drug and a disk impregnated with the solvent (DMF). All the plates were kept in the refrigerator for 30 minutes to allow the diffusion of the sample in to the refrigerator for 30 minutes to allow the diffusion of the sample into the surrounding agar medium. Then the plates inoculated with bacterial cultures were incubated at 37 ºC for 18 h and those with incubated at 25 ºC for 48 h. Diameter of the zones of inhibition wherever produced were measured and the average diameter for each sample was calculated. The diameters obtained for the test samples were compared with that produced by the standard antibiotics, ciprofloxacin for antibacterial activity and clotrimazole for antifungal activity. The results of antibacterial and antifungal activity are given in Tables and figures.

Result and Discussion

Oxazolopyridines derivatives (All five)were screened for *in vitro* antimicrobial activity against gram-positive bacteria *B. Subtilis* andgram-negative bacteria and *E. Coli*, cutaneous fungi *C. Albicans* and *A. Niger*.

oxazolopyrine**OP-5** exhibited excellent antibacterial activity against *E. coli* in comparison to standard drug – Ciprofloxacin. Moreover, **OP-3** showed excellent antifungal activity against *A. Niger* in comparison to standard drug – griseofulvin.Gram-negative bacteria were found to be more sensitive towards the **OP-5** in comparison to gram-positive bacteria.

Analysis of the antifungal activity data concluded that**OP-2** possessed significant bioactivity against both fungus *Candida Albicans* and *Aspergillus Niger*when compared to the reference drug griseofulvin. Also **OP-4** and **OP-5** possessed significant bioactivity against fungus *Aspergillus Niger*. **OP-4** possessed significant antibacterial activity against *E. Coli.*

The analysis of bioactivity data, it is observed that **OP-2** and **OP-4** has moderate antifungal activity against *Candida Albicans.* Moreover, a moderate level of activity was observed against bacteria *B. subtilis* for the newly synthesized **OP-5**, in comparison to the standard drug ciprofloxacin.

However, **OP-1, OP-2** and **OP-3** hadshownno significant or poor activity against neither Gram-positive nor Gram-negative bacteria. And **OP-4** hadshown poor activity against Gram positive bacteria only.

In addition, the analysis of the pharmacological activity data revealed that displayed a poor bioactivity against pathogenic fungus *Candida Albicans* and *Aspergillus Niger* for **OP-1**. And it is observed that **OP-5** possessed poor bioactivity against *Candida Albicans* only.

Table No. 4: Anti-Bacterial Activity of all synthesized compound

Compounds	*B. subtilis*	*E. coli*
OP-1	12(6.25)[a]	10(12.5)
OP-2	8(12.5)	12(12.5)
OP-3	10(12.5)[a]	15(12.5)
OP-4	15(12.5)	18(12.5)
OP-5	17(25)	23(25)
Control	-	-
Ciproflaxacin	20(6.25)	20(12.5)

[a] Values in brackets are MIC values (g mL^{-1}).

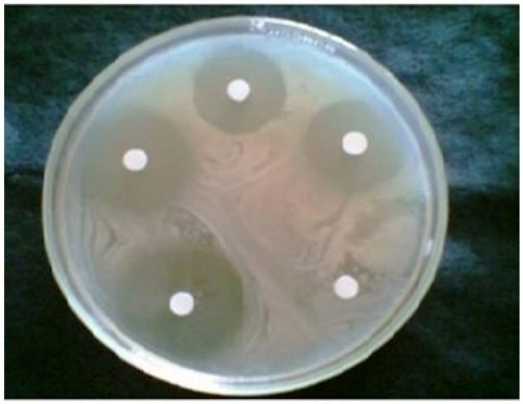


Figure no. 1: Antibacterial Activity of synthesized compounds

Table No. 5: Anti-Fungal Activity ofall synthesized compound

Compounds	*C. albicans*	*A. niger*
OP-1	11(6.25)[a]	16(6.25)
OP-2	13(6.25)	18(6.25)
OP-3	20(6.25)[a]	21(6.25)
OP-4	15(6.25)	18(6.25)
OP-5	10(25)	17(6.25)
Control	-	-
Griseofulvin	20(6.26)	19(6.25)

[a] Values in brackets are MIC values (g mL^{-1})

Figure no. 2: Antifungal Activity of synthesized compounds

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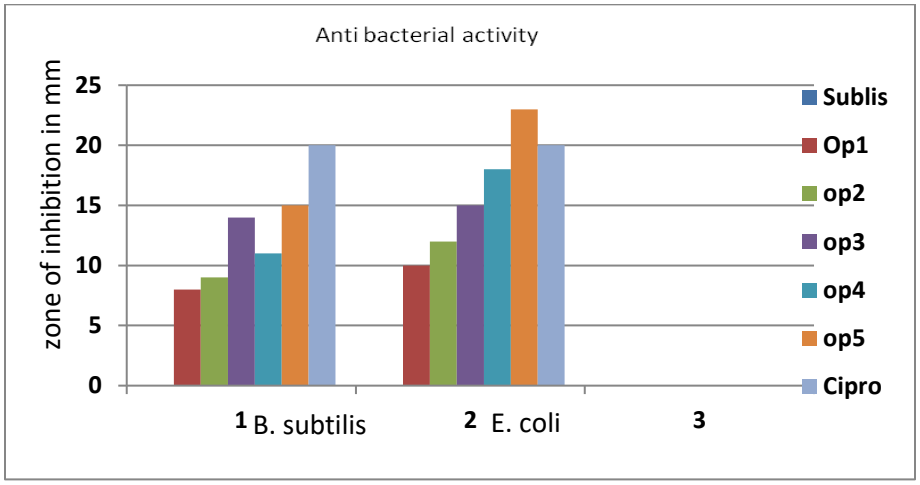

Figure no. 3: Anti-bacterial activity of synthesize compound

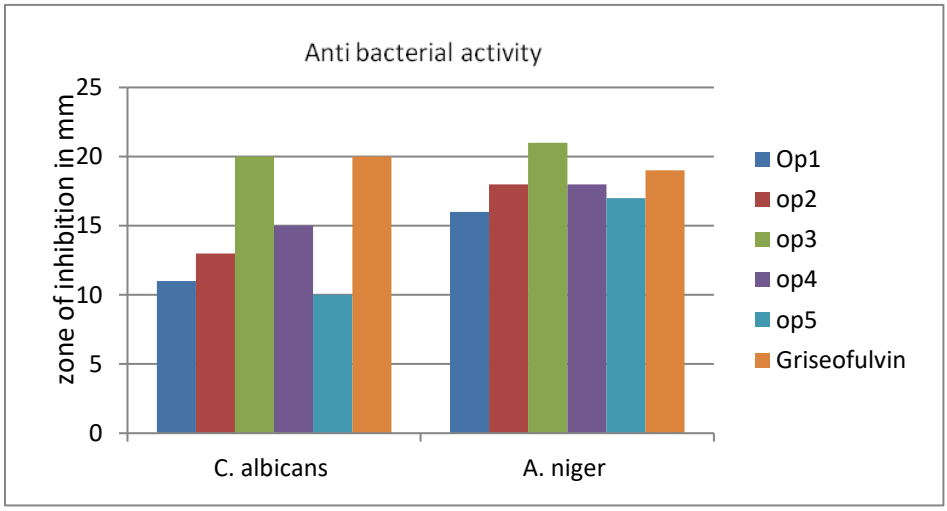

Figure no. 5: Anti-fungal activity of synthesize compound

Table no. 6: Comparative antimicrobial study of Oxazolopyridines derivatives

	B. Subtilis	***E. coli***	***C. Albicans***	***A. Niger***
Excellent	-	Op-5	-	Op-3
Significant	-	Op-4	Op-2	Op-2, op-4, op-5
Moderate	Op-5	-	Op-2, op-4,	-
Poor	Op-1,op-2, op-3, op-4,	Op-1, op-2, op-3	Op-1, op-5	Op-1

-