Research Article

Antimicrobial Activity of Some Diaminobenzophenone Derivatives

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ABSTRACT

Heterocyclic chemistry offers an example for the lack of distinct demarcations; in fact, it pervades the plurality of the other chemical disciplines. Heterocycles are inextricably woven into the life processes. More than 90% of new drugs contain heterocycles and the interface between chemistry and biology, at which so much new scientific insight, discovery and application is taking place is crossed by heterocyclic compounds. Benzimidazole derivatives of 3, 4-diaminobenzophenone derivatives (IV) & (V), have been synthesized after the synthesis of 4-isothiocyanato-3-methylbutanal (CIT), a reagent. All the synthesized derivatives have been screened with various bacterial and fungal strains viz. *Escherichia coli, Salmonella typhi, Pseudomonas neumonaie, Bacillus cereus, Candida albicans, Penicillium chrysogenum*, *Saccharomyces cerevisiae*, *Aspergillus niger*. After the antimicrobial studies, it were found that compound (IV) & (V) can be act as a standard drug against fungal strain *Aspergillus niger, Candida albicans, Saccharomyces cerevisiae*, as these showed more inhibition zone than the standard drug Ketoconazole respectively. Compound (IV) & (V) showed very good activity against fungal strains *Penicillium chrysogenum* respectively.

Keywords: 3, 4-diaminobenzophenone acid, antibacterial activity, antifungal activity.

INTRODUCTION

The research have shown that most of the heterocycles contain considerable biological actions such as antibiotic, antifungal, anti-inflammatory, antiviral, anticancer, anticonvulsant, anthelmintic, antihistamine, antidepressant activities. Lactum antibiotics are useful and frequently prescribed an antimicrobial agent that share a common structure and this class includes Penicillin G which is active against susceptible gram-positive cocci[1]. An Antifungal drug is a medication used to treat fungal infection such as athletes' foot, ringworm, candidiasis serious systemic infection such as *Cryptococcal meningitis* & others. The azoles antifungal include two broad classes i.e. imidazole and triazoles which inhibit the cytochrome. The anticonvulsants are a diverse group of pharmaceuticals used in the treatment of epileptic seizures. The heterocyclic compounds mostly used in the anticonvulsant are barbexaclone, phenobarbital, nimatazepam, lorazepam. Taking into account that compound bearing a thiazole, pyridyl and indol moieties possess a wide spectrum of biological activities which is related their capacity to transfer electrons to scavenge reactive oxygen species presence of NCS linkage, this properties are responsible for antibacterial, anticonvulsant, fungicidal, and antiviral activities.

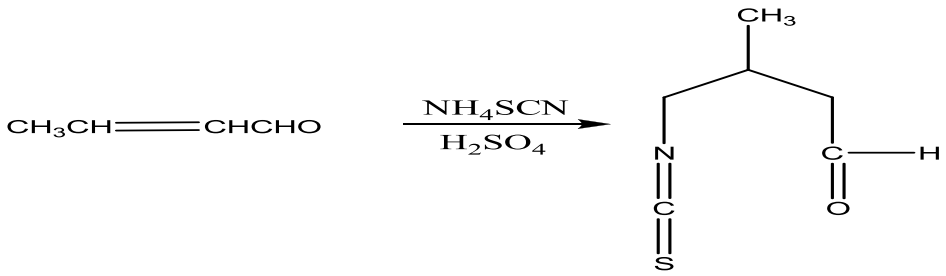
The heterocycles nucleus is one of the most important and well known heterocycles which is a common and integral feature of a variety of natural products and Medicinal agents. Heterocycles nucleus is present as a core structural component in an array of drug categories such as antimicrobial[2-10], anti-inflammatory[11], analgesic[12], anticancer[13], antiviral[14], anti-neoplastic[15], antihypertensive[16], ant malarial[17], local anesthetic[18], ant anxiety[19], antidepressant[20], antihistaminic[21], antioxidant[22], ant-tubercular[23], anti-Parkinson's[24], antidiabetic[25], antiobesity[26] and immunomodulatory agents[27] etc. The thiadiazole are the heterocyclic compounds which have the five member ring containing nitrogen & sulphur. The biological activities of 1,3,4-thiadiazole derivatives have been investigated by Sondhi et.al.[28].

Heterocycles play an important role in biochemical processes because the side groups of the most typical and essential constituents of living cells, DNA and RNA, are based on aromatic heterocycles. The presence of heterocycles in all kinds of organic compounds of interest in biology, pharmacology, optics electronics and material sciences and so on is very well known. Between them, sulfur and nitrogen-containing 3 heterocyclic compounds have maintained the interest of researchers through decades of historical development of organic synthesis. The grounds of this interest were their biological activities and unique structures that led to several applications in different areas of pharmaceutical and agrochemical research or, more recently, in material sciences. The present studies are an attempt to review the pharmacological activities of reported for heterocycles in the current literature with an update of recent research findings on this nuclei.

METHODOLOGY

Synthesis of 4-isothiocyanato-3-methylbutanal (CIT)

4-isothiocyanato-3-methylbutanal was prepared by adding H_2SO_4 (49gm; 0.5mol), diluted with distilled water (50ml) to croton aldehyde (70gm; 1mol) over a period of 25 minutes at 15ºC. Ammonium thiocyanate (76gm; 1mol) dissolved in H_2O (100ml), was added to the mixture at 21ºC. After stirring for 15 minutes the upper oily layer was separated with the help of separating funnel and washed with aqueous sodium carbonate (saturated solution) and finally with water to free it from acid. The content were left over fused calcium chloride for 24 hours and subjected to fractionalation. The yield was 25.68gm.



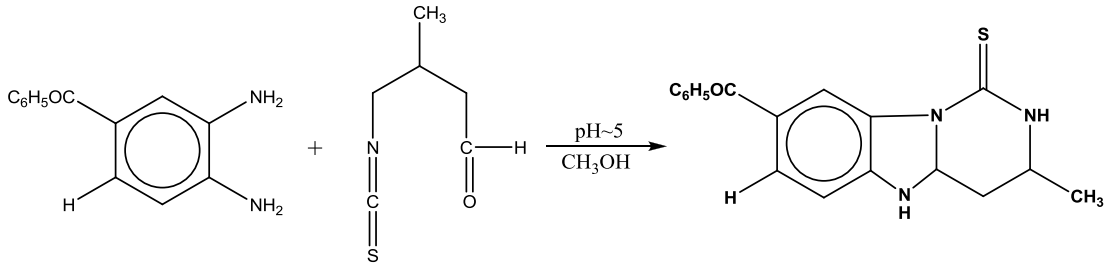
but-2-enal
Chemical Formula: C_4H_6O
Molecular Weight: 70.09
I

4-isothiocyanato-3-methylbutanal
Chemical Formula: C_6H_9NOS
Molecular Weight: 143.21
II

Figure 1: Schematic representation of the synthesis of 4-isothiocyanato-3-methylbutanal.

General procedure for the condensation of 3, 4-diaminobenzophenone with CIT

4-isothiocyanato-3-methylbutanal (0.7ml) was added to a solution of 3, 4-diaminobenzophenone (1.0gm) in methanol (10ml). The pH of the reaction medium was adjusted to about ~5 by adding few drops of sulphuric acid (10% sulphuric acid in methanol). The reaction mixture was heated under reflux for 8 hours. After 15 minutes ppt. appears and after 1 hour 40 minutes ppt. separated out (yield = 0.320 gm). After cooling, the solid was collected and washed with methanol to give compound. The remaining compound in reaction solution is separated by the column chromatography. The total yield is 0.876gm.



3,4-diaminobenzophenone derivative
Chemical Formula: $C_{13}H_{12}N_2O$
Molecular Weight: 212.25
III

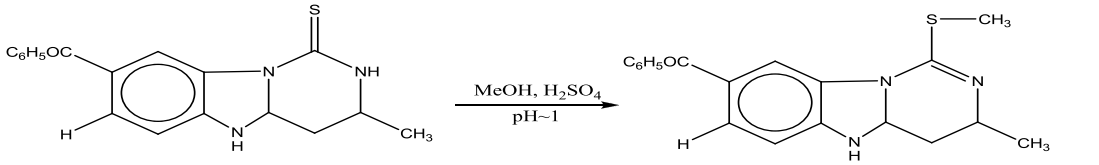
4-isothiocyanato-3-methylbutanal
Chemical Formula: C_6H_9NOS
Molecular Weight: 143.21
II

3,4-diaminobenzophenone derivative
Chemical Formula: $C_{18}H_{17}N_3OS$
Molecular Weight: 323.41
IV

Figure 2: Schematic representation of condensation of 3, 4-diaminobenzophenone.

General procedure for S-Methylation of 3, 4-diaminobenzophenone derivative

3, 4-diaminobenzophenone (0.2 g) was dissolved in CH_3OH (20 ml) and in this solution 2 drops of concentrated sulphuric acid was added. Reaction contents having pH~1 was heated under reflux for 8 hrs under reduced pressure and gets no compound. The rest mixture was used in packing the column and performed the column chromatography. At last we get the final compound of yield 0.142gm.



3,4-diaminobenzophenone derivative
Chemical Formula: $C_{18}H_{17}N_3OS$
Molecular Weight: 323.41
IV

S-Methylated derivative of 3,4-diaminobenzophenone
Chemical Formula: $C_{19}H_{19}N_3OS$
Molecular Weight: 337.44
V

Figure 3: Schematic representation of S-Methylation of 3, 4-diaminobenzophenone derivative.

Detection of elements

a) **Test for Sulphur-** Sodium extract take in small test tube; add in excess of acetic acid and few drops of lead acetate, black precipitate obtained. Sulphur is confirmed.

b) **Test for Nitrogen-** Treat 2ml sodium extract with 2-3 drops of dil H_2SO_4. Now added a small amount of solid $FeSO_4$, blue colour appears. Nitrogen is present.

c) **Test for Halogen-** Taking about 2-3ml of sodium extract and acidity with some drops of dil HNO_3. Now evaporates the solution to almost 1ml. This will expel hydrogen cyanide and hydrogen sulphide. Dilute the solution with equal volume of water and add 2-3ml of silver nitrate solution. If no precipitate is found, it indicate, no halogen atom is present.

General experimental procedure

Melting points (m.p.) were determined by a (JSGW) apparatus and are uncorrected. Only principle sharply defined I.R. peaks are reported. Thin layer chromatography (TLC) was performed on silica gel-G for TLC and spots were visualized by iodine vapour. Column chromatography was performed by using Molychem silica gel for column chromatography (100-200mesh). For all compounds yield, m.p. and spectral data are reported.

Antimicrobial Activity

In this study we reported the antimicrobial activity of 3, 4 diaminobenzophenone against various pathogenic microorganisms.

a) **Microorganisms**

The bacterial and fungal cultures *Escherichia coli, Bacillus cereus, Salmonella typhi, Pseudomonas pneumonia, Aspergillus niger, Candida albicans, Penicillium chrysogenum, Saccharomyces cerevisiae* were provided by department of Microbiology, Dolphin (PG) Institute of Bio-Medical and Natural Sciences, Manduwala, Dehradun. The bacterial and fungal cultures were stored on Nutrient Broth and Sabouraud Dextrose Broth respectively at 4°C. The bacterial and fungal cultures were grown on the Muller Hinton and Sabouraud Dextrose Agar respectively.

b) **Culture Media**

For bacterial culture purpose Nutrient Broth and Mueller Hinton Agar media (HIMEDIA) were used. For fungal culture purpose, Sabouraud Dextrose Broth and Sabouraud Dextrose Agar (HIMEDIA) were used.

c) **Antimicrobial assay**

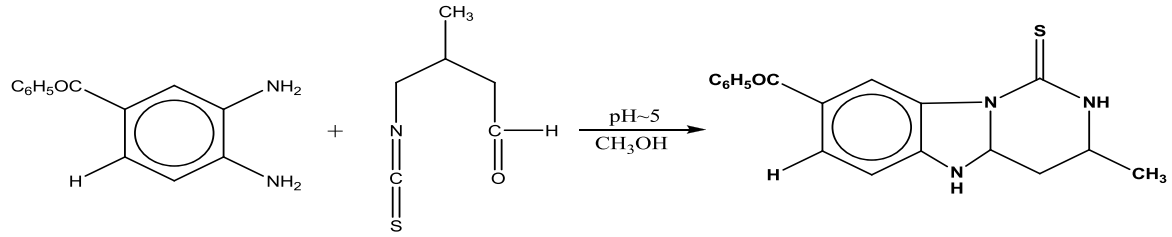
The *invitro* antibacterial and antifungal effect of 3, 4-diaminobenzophenone derivatives were determined by Disc and Hole method. The bacterial strains were sub-cultured in Muller-Hinton Agar and incubated at 37ºC for 24 hours. Turbidity of the suspension was adjusted to the Mac Farland Standard (0.5) and 100µl of suspension plated on Muller Hinton Agar, wells were made with the help of (6mm) borer. Prepare the solution of each derivative (1st and 2nd) and standard drug in 200mg/ml concentration and 100µl of each solution (Dimethyl sulfoxide) loaded in each well against the control (solvent) and standard drug Amoxicillin. Plates were incubated at 37ºC for 24 hours and recorded the zone of inhibition or sensitivity against *Escherichia coli, Bacillus cereus, Salmonella typhi, Pseudomonas pneumonia,.*

For antifungal test, the fungal cultures were grown in Sabouraud Dextrose Agar for 96 hours adopting the above procedure, made suspension of subcultured organisms. Plates were incubated at 27ºC for 72 hours and recorded the zone of inhibition or sensitivity against *Aspergillus niger, Candida albicans, Penicillium chrysogenum, Saccharomyces cerevisiae* against the standard drug Ketoconazole.

RESULTS AND DISCUSSION

4-isothiocyanato-3-methylbutanal (II) on condensation with 3,4-diaminobenzophenone (III) by refluxing for eight hours in methanol at pH~5. During the reaction no compound separated out. After refluxing of eight hours, reaction mixture was used in packing column and performed column chromatography. In column chromatography, we used different polarity combination of several solvents.

Step 2: Synthesis of 3, 4-diaminobenzophenone with CIT



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Chemical Formula: $C_{18}H_{17}N_3OS$
Molecular Weight: 323.41
IV

Table 1: Polarity during column chromatography

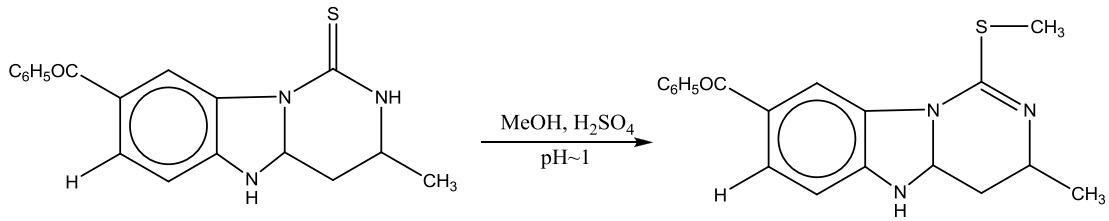
S.No.	Combination of different solvents	Observations
1.	Petroleum ether	Absence of any spot
2.	Petroleum ether : CCl_4 (5:5)	Absence of any spot
4.	Petroleum ether : CCl_4 (5:5)	Absence of any spot
5.	Pure CCl_4	Absence of any spot
6.	Petroleum ether : Ethyl acetate (5:2)	Single spot
7.	Petroleum ether : Ethyl acetate (8:2)	Single spot
8.	Petroleum ether : Ethyl acetate (5:5)	Single spot
9.	Petroleum ether : Ethyl acetate (2:8)	Single spot
10.	Ethyl acetate Pure	Mixture
11.	Petroleum ether : Methanol (8:2)	Mixture

During reaction and from the column chromatography, we get 0.876gm compound (IV).

The compound (IV) having following properties

1. Yield - 0.876gm
2. % Yield - 57.63%
3. Melting point - 240ºC
4. Solubility - DMSO, Partially soluble in methanol
5. Element detection - Nitrogen and sulphur are present and halogen are absent
6. Elution - Petrolium ether : Ethyl acetate, Ethyl acetate : Petrolium ether
7. Solvent of crystallization - Methanol
8. I.R. Spectra - Wave number(cm^{-1}) 3237.15(N-H Stretching), 3056.53 (C-H Stretching in aromatic), 2969.44(C-H Stretching in methyl), 1570(C=C Stretching in aromatic, 1387.69(C-H Stretching in methyl), 1097.65(C-H Def in methyl)

Step 3: S-Methylation of 3, 4-diaminobenzophenone derivative



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S-Methylated derivative of 3,4-diaminobenzophenone
Chemical Formula: $C_{19}H_{19}N_3OS$
Molecular Weight: 337.44
V

Table 2: Polarity during column chromatography

Combination of different solvents	Observations
Petroleum ether	Absence of any spot
Petroleum ether : $CHCl_3$ (5:5)	Absence of any spot
$CHCl_3$ (pure)	Absence of any spot
Petroleum ether : Ethyl acetate (8:2)	Single spot
Petroleum ether : Ethyl acetate (5:5)	Single spot
Petroleum ether : Ethyl acetate (5:5)	Single spot
Petroleum ether : Ethyl acetate (2:8)	Single spot
Ethyl acetate pure	Single spot
Methanol : Petroleum ether (2:8)	Mixture

3, 4-diaminobenzophenone derivative (IV) was dissolved in methanol and adds conc. H_2SO_4 till the pH becomes 1. Reaction contents having pH~1 was heated under reflux for 8 hrs under reduced pressure and gets no compound. The rest mixture was used in packing the column and performed the column chromatography. At last we get the final compound S-Methylated derivative of 3, 4-diaminobenzophenone. In column chromatography, we used different polarity combination of several solvents.

During reaction and from the column chromatography, we get 0.015gm compound (V).

The compound **(V)** having following properties:

9. Yield - 0.142gm
10. % Yield - 13.42%
11. Melting point - 240ºC
12. Solubility - DMSO, Partially soluble in methanol
13. Element detection - Nitrogen and sulphur are present and halogen are absent
14. Elution - Petrolium ether : Ethyl acetate, Ethyl acetate : Petrolium ether
15. Solvent of crystallization - Methanol

16. I.R. Spectra - Wave number(cm^{-1}) 2969.44 (C-H Stretching in methyl), 1631.10(C=N Stretching), 1387.69(C=H Def in methyl), 782(C-S Stretching)

Antimicrobial activity of prepared compounds

The prepared 3, 4-diaminobenzophenone derivatives were screened for antibacterial and antifungal action. The compounds under investigation were found active against both bacterial and as well as fungal strains tested.

Microorganism used

Bacteria

1. *Escherichia coli*
2. *Bacillus cereus*
3. *Salmonella typhi*
4. *Pseudomonas pneumonia*

Fungi

1. *Aspergillus niger*
2. *Candida albicans*
3. *Penicillium chrysogenum*
4. *Saccharomyces cerevisiae*

Antibacterial activity

The agar well diffusion method was used. The test organisms (bacterial cultures) were spread on the prepared Muller Hinton Agar plates. Well of 6 mm diameter were punched into the agar medium. The each well 100 µl of each compound (IV) and (V) and standard drug Ciprofloxacin were added and allowed to diffuse. The plates were then incubated at 37ºC for 24 hours.

The antibacterial activities for different strains of bacteria were tested for each compound against the standard drug Ciprofloxacin and recorded the zone of inhibition in millimeter.

Table 3: Antibacterial activity of 3, 4-diaminobenzophenone derivatives and standard drug Ciprofloxacin

S. No	Name of the test organisms	Antibacterial activity of compound (IV)	Antibacterial activity of compound (V)	Antibacterial activity of standard drug Ciprofloxacin
1.	*Escherichia coli*	16mm	14mm	43mm
2.	*Bacillus cereus*	23mm	17mm	50mm
3.	*Salmonella typhi*	14mm	13mm	42mm
4.	*Pseudomonas pneumonia*	15mm	17mm	40mm

Antibacterial activities indicated that the both compounds possess a less spectrum of activity against the tested bacterial strains. Compound (IV) showed mild activity against *Escherichia coli* (16mm inhibition zone), *Bacillus cereus* (23mm inhibition zone), *Salmonella typhi* (14mm inhibition zone) and *Pseudomonas pneumonia* (15mm inhibition zone).

Compound (V) showed mild activity against *Escherichia coli* (14mm inhibition zone), *Bacillus cereus* (17mm inhibition zone), *Salmonella typhi* (13mm inhibition zone) and *Pseudomonas pneumonia* (17mm inhibition zone).

Antifungal activity

The agar well diffusion method was used. The test organisms (fungal cultures) were spread on the prepared Sabouraud Dextrose Agar plates. Well of 6 mm diameter were punched into the agar medium. The each well 100 µl of each compound (IV) and (V) and standard drug Ketoconazole were added and allowed to diffuse. The plates were then incubated at 37ºC for 72 hours.

The antifungal activities for different strains of fungal were tested for each compound against the standard drug Ketoconazole and recorded the zone of inhibition in millimeter.

Table 4: Antifungal activity of 3, 4-diaminobenzophenone derivatives and standard drug Ketoconazole

S. No.	Name of the test organisms	Antifungal activity of compound (IV)	Antifungal activity of compound (V)	Antifungal activity of standard drug Ketoconazole
1.	*Aspergillus niger*	**23mm**	**21mm**	23 mm
2.	*Candida albicans*	**18mm**	**19mm**	12 mm
3.	*Penicillium chrysogenum*	17mm	19mm	21 mm
4.	*Saccharomyces cerevisiae*	**15mm**	**17mm**	13mm

Antifungal activities indicated that the both compounds possess an excellent spectrum of activity against the tested fungal strains. Compound (IV) was active against all the fungal strains and showed very good activity against

Aspergillus niger (23mm inhibition zone), *Candida albicans* (18mm inhibition zone), *Penicillium chrysogenum* (17mm inhibition zone) and *Saccharomyces cerevisiae* (15mm inhibition zone).

Compound (V) showed excellent activity against against *Aspergillus niger* (21mm inhibition zone), *Candida albicans* (19mm inhibition zone), *Penicillium chrysogenum* (19mm inhibition zone) and *Saccharomyces cerevisiae* (17mm inhibition zone).

CONCLUSION

In bacterial strains the compound (IV) showed mild activity: *Bacillus cereus, Escherichia coli, Salmonella typhi, and Pseudomonas pneumonia.* In bacterial strains the compound (V) showed mild activity with *Escherichia coli, Pseudomonas pneumonia, Salmonella typhi, Bacillus cereus.* In fungal strain compound (IV) showed excellent activity: *Aspergillus niger, Candida albicans, Saccharomyces cerevisiae, Penicillium chrysogenum.* In fungal strain compound (V) showed Excellent activity with *Aspergillus niger*, *Penicillium chrysogenum, Candida albicans*, *Saccharomyces cerevisiae*So we concluded that compound (IV) & (V) can be act as a standard drug against fungal strain *Aspergillus niger, Candida albicans*, *Saccharomyces cerevisiae,* as these showed more inhibition zone than the standard drug Ketoconazole respectively. Compound (IV) & (V) showed very good activity against fungal strains *Penicillium chrysogenum* respectively.

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