

Synthesis and Antimicrobial Effects of Some Phenylazopropanedinitriles and Their Derivatives

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In this study, phenylazopropanedinitriles and their derivatives were synthesized and their antimicrobial effects were investigated against five different strains of bacteria and yeast. The antimicrobial effects of these compounds were examined at six different doses. It was shown that phenylazopropanedinitriles have an antimicrobial effect on the bacterial and yeast strains within the range 25–1000 µg. Generally, when the dose was increased, the inhibition zone expanded on the growth media. When the phenylazopropanedinitriles are compared with some antibiotics, it is seen that generally these compounds have more antimicrobial effects than some antibiotics.

Key Words: Phenylazopropanedinitriles, Antimicrobial effect, Bacterial and yeast cells, Antibiotics.

INTRODUCTION

Phenylazopropanedinitriles are compounds that contain alternating nitrile and azo groups in their skeleton. They are used as starting materials for synthesis of adenine and aminopyrimidine derivatives^{1–9}. Phenylazopropanedinitriles are a new type of sulfhydryl enzyme inhibitors of high biological activity and showing a high degree of structural specificity¹⁰. Phenylazopropanedinitrile and derivatives possess marked antimicrobial activity¹⁰ which seems to be related to the structure $R-N=N-CH(CN)_2$ or to its tautomeric form $R-NH-N=C(CN)_2$.

Aryl azo compounds have been shown to possess significant biological activities¹¹ and an aryl azo group appears to promote antineoplastic activity¹². Similar compounds are used as starting materials in the synthesis of some potential antitumour drugs¹³.

In this study, the synthesis of phenylazopropanedinitriles and their antimicrobial effects on different strains of bacteria and yeast are reported.

EXPERIMENTAL

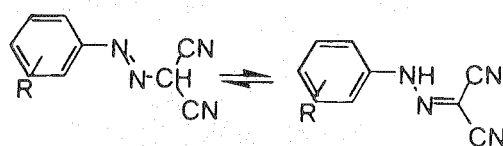
Aniline and its derivatives, propane dinitrile, sodium nitrite and sodium, were supplied by Merck. Solvents and other liquids used in experimental works were dried by conventional methods. All reactions were monitored using Kieselgel 60PF254 (silica gel) precoated TLC plates. The purity of each phenylazopropanedinitrile and its derivatives was determined by thin layer chromatog-

raphy. FTIR spectra were recorded with a Matson 1000 spectrometer as KBr pellets.

Synthesis of phenylazopropanedinitrile and its derivatives

2-Chlorophenylazopropanedinitrile: The phenylazopropanedinitrile and its derivatives were prepared by using diazonium salts. 2-Chloroaniline (5.4 mL, 0.05 mol) and 13.5 mL (0.2 mol) of conc. HCl was mixed slowly. The anilinium chloride formed was filtered and air-dried and this salt was dissolved in 30 mL of absolute alcohol. The stirred mixture was cooled (-5 to 0°C) and diazotized by the careful dropwise addition (over a period of 30 min) of ethyl nitrite (5 mL, 0.03 mol). The mixture and ethyl nitrite (b.p. 17°C) must be kept in cold (-5 to 0°C) during the addition.

A stirred solution of sodium ethoxide prepared by the careful addition of pieces of sodium (0.7 g, 0.03 mol) to absolute alcohol (25 mL) was added in propanedinitrile (1.98 g, 0.3 mol). Then, the diazotized amine solution in alcohol was added cautiously and slowly to a stirred solution of the carbanion and was mixed at 30 min. The reaction mixture was filtered. The product was precipitated by addition of 20 mL of water. The precipitate was recrystallized from EtOH-water. Similarly, other derivatives were prepared on the same outline as mentioned above. The physical characteristics of the synthesized compounds are given in Table-1.



R(*p*-Cl, -H, *o*-NO₂, *p*-Br)

Fig. 1. Phenylazopropanedinitrile and derivatives

TABLE-1
PHYSICAL AND FTIR SPECTRAL DATA OF PHENYLAZOPROPANEDINITRILE
AND ITS DERIVATIVES

Substituents	m.p. (°C)	Lit. m.p. (°C)	Yield (%)	FTIR (KBr, cm ⁻¹)
<i>p</i> -Cl	178	185	76	3276, 3016
<i>o</i> -NO ₂	140-142	143	96	3442, 3095
-H	140-142	145	92	3231, 3078
<i>p</i> -Br	178	—	65	3235, 3057

Bacterial and Yeast Strains

Bacterial cells: *Escherichia coli* ATCC 35218; *Bacillus subtilis* ATCC 6633; *Bacillus cereus* RSKK 863; *Micrococcus luteus* NRLL B-4375.

Yeast Cell: *Candida albicans* (ATCC 10239)

Preparation of Microbial Culture Media

Bacterial strains were inoculated into nutrient broth (Difco) and incubated at

30 ± 0.1°C for 24 h. Yeast cells were inoculated into YPD Broth (Difco) and incubated at 30 ± 0.1°C for 48 h. In order to test the antimicrobial effects, phenylazopropanedinitriles and 15 mL of YPD Agar (Merck) were placed in petri dishes which were then inoculated with strains of yeast by taking 100 µL from cell culture media. It was left to solidify at room temperature for a while and then holes were made on top with a sterile stick. Solutions in quantities stated above were then added to these holes. Petri dishes were left at 4°C for 2 h. Then, bacterial cultures were incubated at 34 ± 0.1°C for 24 h and yeast cultures were incubated at 30 ± 0.1°C for 72 h. At the end of the incubation time, the inhibition zones on the bacterial and yeast nutrient media were measured.

RESULTS AND DISCUSSION

The results indicate that while phenylazopropanedinitrile has antimicrobial effects on all the microorganisms at high doses, it has no antimicrobial effect on some microorganisms (*Bacillus subtilis*, *Bacillus cereus* and *Micrococcus luteus*) at low doses (Table-2). In addition, its derivatives showed antimicrobial effect on the microorganisms in all the doses (Tables 3–5). The results are collected in Tables 3–5. But 3-(*o*-bromo-phenylazo)-3-pentene-4-ol-2-one has no antimicrobial effect on all the studied microorganisms between 25 µg and 1000 µg. When the compounds having antimicrobial effect are compared, it is observed that these compounds are different from each other in terms of cyano, halogen and nitro groups in their structure skeletons. It is known that some compounds containing these groups have antimicrobial effect on the microbial growth media¹⁵.

TABLE-2
ANTIMICROBIAL EFFECT OF PHENYLAZOPROPANEDINITRILE COMPOUND ON
BACTERIAL AND YEAST CELLS (inhibition zone = mm)

	10 µg	25 µg	50 µg	300 µg	600 µg	1000 µg
Bacterial strains						
<i>Escherichia coli</i> (ATCC 35218)	8.75±0.49	16.60±0.58	17.00±1.15	17.00±1.00	22.00±1.00	32.25±0.64
<i>Bacillus subtilis</i> (ATCC 6633)	0.00±0.00	0.00±0.00	17.00±1.15	17.00±1.00	22.00±1.00	32.25±0.64
<i>Bacillus cereus</i> (RSKK 863)	0.00±0.00	0.00±0.00	3.25±0.49	23.66±0.57	26.00±1.00	29.00±1.00
<i>Micrococcus luteus</i> (NRLL, B-4375)	0.00±0.00	0.00±0.00	0.00±0.00	21.00±1.00	22.66±0.57	30.33±0.57
Yeast strain						
<i>Candida albicans</i> (ATCC 10239)	5.50±0.57	6.00±0.81	7.50±0.57	10.25±0.76	16.50±1.91	17.50±0.57

The antimicrobial effects of compounds used in this study are derived from

cyano groups rather than the groups containing halogen and nitro. Because when the phenylazopropanedinitrile compounds were compared with derivatives, it was that they had nearly the same inhibition zone radius. On the other hand, 3-(*o*-bromo-phenylazo)-3-pentene-4-ol-2-one¹⁴ compound (it has no cyano group) did not show any antimicrobial effect on the microorganism in the 25–1000 µg concentration ranges. This result has supported the above mentioned case.

TABLE-3
ANTIMICROBIAL EFFECT 2-(2-NITROPHENYLAZO) PROPANEDINITRILE COM-
POUND ON THE BACTERIAL AND YEAST CELLS (inhibition zone = mm)

	10 µg	25 µg	50 µg	300 µg	600 µg	1000 µg
Bacterial strains						
<i>Escherichia coli</i> (ATCC 35218)	5.60±0.54	9.83±0.39	13.75±0.95	32.66±1.15	34.75±0.95	41.25±0.95
<i>Bacillus subtilis</i> (ATCC 6633)	11.75±0.95	15.50±0.57	16.80±0.83	22.00±1.41	25.40±0.89	30.00±0.81
<i>Bacillus cereus</i> (RSKK 863)	8.40±0.54	14.25±0.49	17.20±0.83	23.50±2.08	25.20±1.64	33.00±2.00
<i>Micrococcus luteus</i> (NRLL, B-4375)	6.25±0.49	8.60±0.54	11.40±0.89	22.25±0.89	25.40±0.89	28.25±1.20
Yeast strain						
<i>Candida albicans</i> (ATCC 10239)	4.25±0.49	4.25±0.95	5.20±0.44	7.50±0.57	9.00±1.00	12.00±1.00

TABLE-4
ANTIMICROBIAL EFFECT 2-(4-CHLOROPHENYLAZO) PROPANEDINITRILE COM-
POUND ON THE BACTERIAL AND YEAST CELLS (inhibition zone = mm)

	10 µg	25 µg	50 µg	300 µg	600 µg	1000 µg
Bacterial strains						
<i>Escherichia coli</i> (ATCC 35218)	9.50±0.57	12.60±0.89	18.00±1.09	26.33±1.52	28.50±1.00	37.00±1.15
<i>Bacillus subtilis</i> (ATCC 6633)	20.25±0.95	32.00±0.70	32.20±0.44	32.50±0.57	34.66±0.57	44.00±1.41
<i>Bacillus cereus</i> (RSKK 863)	14.60±0.54	23.50±1.73	24.50±0.54	21.25±0.49	26.25±1.89	28.50±2.12
<i>Micrococcus luteus</i> (NRLL, B-4375)	18.60±0.54	22.50±1.29	28.20±1.48	31.33±0.57	31.50±0.70	32.00±2.64
Yeast strain						
<i>Candida albicans</i> (ATCC 10239)	14.50±0.70	16.33±2.07	11.50±0.70	17.66±0.57	18.50±0.70	18.33±0.57

TABLE-5
ANTIMICROBIAL EFFECT 2-(4-BROMOPHENYLAZO)PROPANEDINITRILE
COMPOUND ON THE BACTERIAL AND YEAST CELLS (inhibition zone = mm)

	10 µg	25 µg	50 µg	300 µg	600 µg	1000 µg
Bacterial strains						
<i>Escherichia Coli</i> (ATCC 35218)	6.75±0.49	10.50±1.00	10.50±0.57	13.25±0.95	16.50±0.57	22.00±1.41
<i>Bacillus subtilis</i> (ATCC 6633)	26.25±0.95	28.80±1.09	26.40±1.51	25.33±1.15	28.00±0.81	34.25±1.25
<i>Bacillus cereus</i> (RSKK 863)	13.80±1.03	16.60±1.14	16.80±1.83	20.40±1.14	22.00±0.70	25.33±1.52
<i>Micrococcus luteus</i> (NRLL, B-4375)	19.66±1.15	19.75±1.49	20.00±1.41	17.75±0.95	18.66±1.15	26.50±1.00
Yeast strain						
<i>Candida albicans</i> (ATCC 10239)	10.00±0.00	10.50±0.70	11.00±0.00	11.25±0.49	12.66±0.57	13.00±0.00

The development of microorganisms may be inhibited by these compounds by affecting the enzymes which contain sulfhydryl group which has a very important role in the metabolism of microorganisms. The study of Zsolnai¹⁰ supports this point of view. Besides, the antimicrobial effects of some antibiotics were investigated on the studied microorganisms. The results are collected in Table-6. The present findings clearly demonstrate that the synthesized compounds have greater antimicrobial effect than antibiotics on the microorganisms.

TABLE-6
ANTIMICROBIAL EFFECT OF SOME ANTIBIOTICS ON THE
BACTERIAL AND YEAST CELLS (inhibition zone = mm)

<i>Antibiotics</i>	<i>E. coli</i> (ATCC 35218)	<i>B. subtilis</i> (ATCC 6633)	<i>B. cereus</i> (RSKK 863)	<i>M. luteus</i> (NRLL, B-4375)
Cefadroxil (30 µg)	25.00	27.10	23.00	40.00
Amino acids (30 µg)	0.00	15.00	17.50	19.00
Ampicillin (10 µg)	8.70	8.70	19.70	33.60
Tetracycline (30 µg)	16.80	30.00	22.00	30.40
Linkomicin (2 µg)	0.00	0.00	8.70	27.60
Erythromycin (15 µg)	11.00	24.50	19.00	32.00
Vankomycin (30 µg)	0.00	22.00	17.00	28.30
Azithromycin (15 µg)	0.00	33.40	19.20	38.00
Amoxycillin/Clavulanic acid (20 µg)	24.00	19.40	0.00	48.00
Penicillin G (10 units)	16.60	0.00	0.00	5.00
Chloramphenicol (30 µg)	22.70	22.70	22.70	38.50
Cephalothin (30 µg)	15.60	18.20	19.20	23.40
Polymyxine B (300 units)	0.00	0.00	1.60	0.00
Cefoxitin (30 µg)	10.00	16.00	7.40	24.20

Antifungal ($\mu\text{g/mL}$)	Yeast strain (mm)	Antifungal ($\mu\text{g/mL}$)	Yeast strain (mm)
Oxiconazole	<i>Candida albicans</i> (ATCC 10239)	Isoconazole	<i>Candida albicans</i> (ATCC 10239)
0.2	17	0.2	17
0.1	17	0.1	14
0.05	14	0.05	14

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