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Short communication

SYNTHESIS, CHARACTERIZATION AND ANTI-BACTERIAL ACTIVITY OF CERTAIN 2,3,4,5-TETRAHYDROPYRIDAZINONE ANALOGUES

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Abstract

*In the present study, six new derivatives of Pyridazinone were synthesized and evaluate their anti-bacterial activity. The experimental work involves the synthesis of benzoyl propionic acid **(a)**, then 6-phenyl-2,3,4,5-tetrahydro pyridazin-3-one **(b)** which was then condensed with various aldehydes to form respective derivatives. All the synthesized compounds were identified by IR, ^{1}HNMR and antimicrobial activity was performed on the compounds synthesized against Staphylococcus aureus (MTCC 737), Staphylococcus epidermidis (MTCC 3615), Pseudomonas aeruginosa (MTCC 424) and Escherichia coli (MTCC 1687).*

Keywords: *Pyridazinone analogues, substituted benzaldehydes, anti-bacterial activity.*

Bazı 2,3,4,5-Tetrahidropiridazinon Analoglarının Sentezi, Karakterizasyonu ve Antibakteriyel Aktiviteleri

*Bu çalışmada; altı yeni Piridazinon türevleri sentezlenmiş ve antibakteriyel aktiviteleri değerlendirilmiştir. Deneysel çalışmamız sırasıyla, benzoil propiyonik asit **(a)**, 6-fenil-2,3,4,5,-tetrahidro piridazin-3-on **(b)** ve daha sonra çeşitli aldehitlerle ilgili türevlerinin sentezlenmesini içermektedir. Tüm sentezlenmiş olan bileşiklerin yapıları IR ve HNMR teknikleri ile tayin edilmiştir. İlaveten Staphylococcus aureus (MTCC 737), Staphylococcus epidermidis (MTCC 3615), Pseudomonas aeruginosa (MTCC 424) ve Escherichia coli (MTCC 1687) kullanılarak sentezlenen bileşiklerin antimikrobiyal aktiviteleri tespit edilmiştir.*

Anahtar kelimeler: *Piridazinon analogları, Sübstitüe benzaldehidler, Anti-bakteriyel aktivite*

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INTRODUCTION

From the literature survey, it has been found that the available antibacterial compounds still have some of the disadvantages. These are microbial resistance, associated disorders like GIT disturbances and dose required may be high. Tremendous work has been reported on Pyridazinone derivatives in past and evaluated for different activities like antiviral (1), antibacterial (2-4), antifungal (5,6), anticancer (7,8), anti-inflammatory (9-12), analgesic (11,12), cardiotonic(13,14), antihypertensive (15), antiepileptic (16), molluscicidal (17) and herbicidal (18) activities. The present study was undertaken in order to synthesize some new compounds build upon this nucleus with the hope to enhance the biological properties of newly designed compounds.

EXPERIMENTAL

All the solvents and reagents used were of laboratory grade (LR). All the reactions were monitored by TLC using Toluene: Ethyl Acetate: Formic Acid (5:4:1) as solvent system. Anhydrous sodium sulphate or potassium carbonate was used for drying various solvents. Melting points of all synthesized compounds were determined using open capillary tube and were uncorrected. The Proton Resonance Spectra (^{1}H-NMR) were recorded on Bruker 30 of NMR Spectrometer 400 MHz Spectrometer (Chemical Shift in ppm) in $CDCl_3$/DMSO using tetra methyl Silane (Me_4Si) as internal reference. IR spectra were recorded by making KBr pellets on Fourier Transform IR (Jasco-4100 typeA) Spectrometer.

Chemistry

6-phenyl-4(benzylidene)-2, 3, 4, 5-tetrahydro pyridazin-3-one (D_1)

The compound 6-phenyl-2, 3, 4, 5-tetrahydro pyridazin-3-one (1g) was refluxed for 10 hours with benzaldehyde (1ml) in methanol containing sodium hydroxide (0.5g). Contents were concentrated, then poured into ice-cold water, filtered and recrystallized with ethanol to get the crystals.

6-phenyl-4(4'-methoxybenzylidene)-2, 3, 4, 5-tetrahydro pyridazin-3-one (D_2)

The compound 6-phenyl-2, 3, 4, 5-tetrahydro pyridazin-3-one (1g) was refluxed for 10 hours with anisaldehyde (1ml) in methanol containing sodium hydroxide (0.5g). Contents were concentrated, then poured into ice-cold water, filtered and recrystallized with ethanol to get the crystals.

6-phenyl-4(4'-cholorobenzylidene)-2, 3, 4, 5-tetrahydro pyridazin-3-one (D_3)

The compound 6-phenyl-2, 3, 4, 5-tetrahydro pyridazin-3-one (1g) was refluxed for 10 hours with benzaldehyde (1ml) in methanol containing sodium hydroxide (0.5g). Contents were concentrated, then poured into ice-cold water, filtered and recrystallized with ethanol to get the crystals.

6-(p-chlorophenyl)-4-(benzylidene)-2, 3, 4, 5-tetrahydro pyridazin-3-one (D_4)

The compound 6-(p-Chlorophenyl)-2, 3, 4, 5-tetrahydro pyridazin-3-one (1g) was refluxed for 10 hours with benzaldehyde (1ml) in methanol containing sodium hydroxide (0.5g). Contents were concentrated, then poured into ice-cold water, filtered and recrystallized with ethanol to get the crystals.

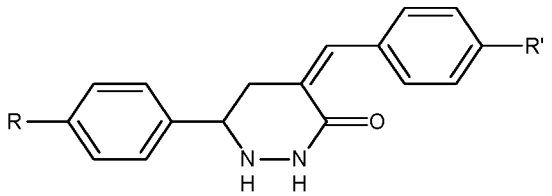
6-(p-chlorophenyl)-4(4-methoxybenzylidene)-2, 3, 4, 5-tetrahydro pyridazin-3-one (D_5)

The compound 6-(p-Chlorophenyl)-2, 3, 4, 5-tetrahydro pyridazin-3-one (1g) was refluxed for 10 hours with anisaldehyde (1ml) in methanol containing sodium hydroxide (0.5g). Contents were concentrated, then poured into ice-cold water, filtered and recrystallized with ethanol to get the crystals.

6-(p-chlorophenyl)-4(4'-cholorobenzylidene)-2, 3, 4, 5-tetrahydro pyridazin-3-one (D_6)

The compound 6-(p-Chlorophenyl)-2, 3, 4, 5-tetrahydro pyridazin-3-one (1g) was refluxed for 10 hours with benzaldehyde (1ml) in methanol containing sodium hydroxide (0.5g). Contents were concentrated, then poured into ice-cold water, filtered and recrystallized with ethanol to get the crystals.

Table 1. Some physical characteristics of the synthesized compounds.



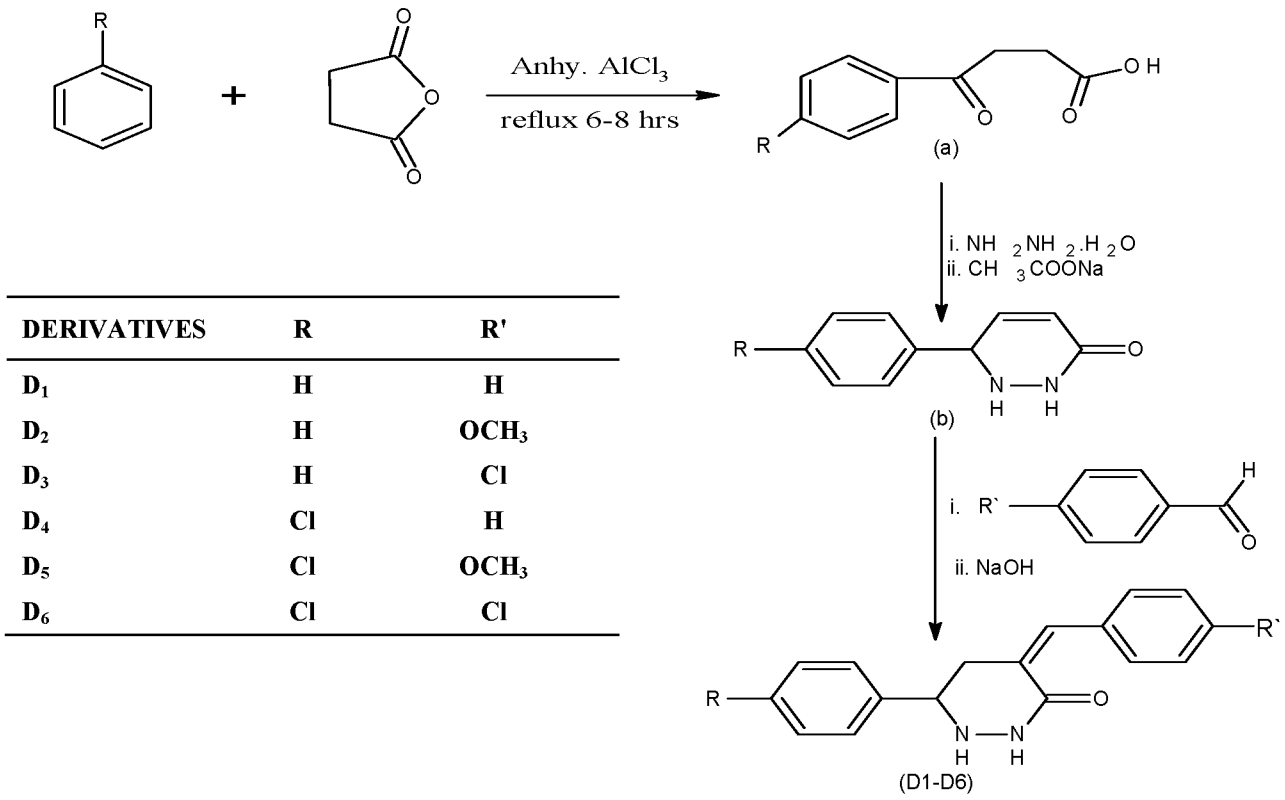
Compound	R	R'	m.p.(°C)	Yield (%)	Formula	Mol. Wt.
D_1	H	H	181-183	63	$C_{17}H_{20}N_2O$	260
D_2	H	OCH_3	167-169	57	$C_{18}H_{22}N_2O_2$	298
D_3	H	Cl	194-196	51	$C_{17}H_{19}ClN_2O$	302.5
D_4	Cl	H	205-207	58	$C_{17}H_{19}ClN_2O$	302.5
D_5	Cl	OCH_3	186-189	47	$C_{18}H_{21}ClNO_2$	318.5
D_6	Cl	Cl	219-221	53	$C_{17}H_{18}Cl_2NO$	323

Table 2. Spectral data of the compounds

Compound	FTIR(KBr) cm^{-1}	^{1}HNMR(DMSO)ppm(δ)
D_1	3400(Ar-H), 3350(NH), 2862(C-H), 1642.09 (lactam ring), 1603.52 (-C=C), 1258.32(C-N)	12.09(1H,s,N-H), 10.04(1H,s,C-H), 7.917-7.898(2H,d,C-H), 7.576-7.557(2H,t,C-H), 7.539-7.372(4H,t,C-H), 7.331(2H,d,C-H), 7.27(1H,t,C-H), 4.032(2H,d,CH_2), 1.749(1H,s, N_1)
D_2	3400(Ar-H), 3435(NH), 2831(C-H), 1682.59(lactam ring), 1602.56(-C=C), 1509.99(-C-O-CH_3), 1255.32(C-N), 1165.76(OCH_3)	9.782(1H,s,N_2), 8.496(1H,s,C-H), 7.757-7.737(2H,d,C-H), 7.694-7.675(2H,t,C-H), 7.192(1H,t,C-H), 6.95-6.93(2H,d,C-H), 6.770(1H,t,C-H), 4.448(2H,d,CH_2), 4.435(3H,s,OCH_3), 2.498(1H,s,N_1)
D_3	3400(Ar-H), 3444(NH), 2857(C-H), 1698.98(lactam ring), 1602.5(-C=C), 1484.92(C-Cl), 1292.07(C-N)	10.209(1H,s,N_2), 8.621(1H,s,C-H), 7.782(2H,d,C-H), 7.642(2H,t,C-H), 7.431(2H,t,C-H), 7.096(1H,t,C-H), 7.007(2H,d,C-H), 7.27(1H,t,C-H), 4.383(2H,d,CH_2), 1.768(1H,s,N_1)
D_4	3402(Ar-H), 34359(NH), 2831(C-H), 1682.59(lactam ring), 1602.56(-C=C), 1255.3(C-N)	8.688(1H,s,N_2),5.25(1H,s,C-H), 7.476(2H,d,C-H), 7.375(2H,t,C-H), 7.425(2H,d,C-H), 7.268(1H,t,C-H), 7.455(2H,d,C-H), 3.924(1H,t,C-H), 1.26(2H,d,CH_2), 0.886(1H,s,N_1)
D_5	3402(Ar-H), 3435(NH), 2831(C-H), 1682.59(lactam ring), 1602.56(-C=C), 1255.32(C-N), 1166.72(OCH_3)	9.779(1H,s,N_2), 8.491(1H,s,C-H), 7.753-7.733(2H,d,C-H), 6.947-6.927(2H,d,C-H), 7.690-7.670 (2H,d,C-H), 6.886-6.887 (2H,d,C-H, 3.809(1H,t,C-H), 2.495(2H,d,CH_2), 3.051(3H,s,$OCH)_3$, 2.064(1H,s,N_1)
D_6	3400(Ar-H), 3444(NH), 2857(C-H), 1698.98(lactam ring), 1602.56(-C=C), 1489.79(C-Cl), 1292.07(C-N)	8.625(1H,s,N_2), 7.803(1H,s,C-H), 7.783(2H,d,C-H), 7.455-7.333(4H,d,C-H), 7.575-7.524(2H,d,C-H), 4.689(1H,t,C-H), 2.048-2.002(2H,d,CH_2), 1.441(1H,s,N_1)

RESULTS AND DISCUSSIONS

The efficient synthetic route for the synthesis of pyridazinone compounds was underlined in Fig 1: Synthesis of benzoyl propionic acid **(a)**, then 6-phenyl-2, 3, 4, 5-tetrahydro pyridazin-3-one **(b)** which was then condensed with various aldehydes to form respective derivatives **(D1-D6)**.



DERIVATIVES	R	R'
D_1	H	H
D_2	H	OCH_3
D_3	H	Cl
D_4	Cl	H
D_5	Cl	OCH_3
D_6	Cl	Cl

Figure 1. Synthetic design for the synthesis of 2,3,4,5-tetrahydropyridazinone derivatives

Antimicrobial activity

For present work efficacy of six compounds were detected against *Staphylococcus aureus* (MTCC 737), *Staphylococcus epidermidis* (MTCC 3615), *Pseudomonas aeruginosa* (MTCC 424) and *Escherichia coli* (MTCC 1687). The concentration of the test compound used was 50mg/ml and ampicillin was taken as the standard drug (Table 1 and 2). The zone of inhibition obtained in different strains of bacteria are shown graphically of *S. aureus* (Figure 2), *S. epidermidis* (Figure 4), *P. aeruginosa* (Figure 3), *E. coli* (Figure 5).The reference strains *Staphylococcus aureus* (MTCC 737), *Staphylococcus epidermidis* (MTCC 3615), *Pseudomonas aeruginosa* (MTCC424) and *Escherichia coli* (MTCC 1687) were taken from Microbial type Culture collection (MTCC) and gene bank, Chandigarh, India.

Table 3. Comparison of zone of inhibition of various derivatives synthesized

S. No.	Derivatives	Zone of inhibition (mm)			
		Staphylococcus aureus (MTCC 737)	*Staphylococcus epidermidis* (MTCC 3615)	*Pseudomonas aeruginosa* (MTCC 424)	*Escherichia coli* (MTCC 1687)
1	D_1	11 (64.7%)	12 (75%)	7 (46.67%)	7 (43.75%)
2	D_2	9 (52.94%)	11 (68.75%)	-	6 (37.5%)
3	D_3	7 (41.17%)	8 (50%)	12 (80%)	13 (81.25%)
4	D_4	10 (58.82%)	12 (75%)	7 (46.67%)	6 (37.5%)
5	D_5	7 (41.17%)	8 (50%)	13 (86.67%)	12 (75%)
6	D_6	-	-	8 (53.33%)	10 (62.5%)
7	Ampicillin	17	16	15	16

Table 4. Comparison of antimicrobial activity with different derivatives synthesized

S.No.	Derivative	Anti-bacterial activity			
		Staphylococcus aureus (MTCC 737)	*Staphylococcus epidermidis* (MTCC 3615)	*Pseudomonas aeruginosa* (MTCC 424)	*Escherichia coli* (MTCC 1687)
1	D_1	++	++	+	+
2	D_2	++	++	-	+
3	D_3	+	+	++	+++
4	D_4	++	++	+	+
5	D_5	+	+	+++	++
6	D_6	-	-	+	++
7	Ampicillin	+++	+++	+++	+++

+++ Diameter of zone of inhibition between 13-17 mm, ++ Diameter of zone of inhibition between 9-12 mm, + Diameter of zone of inhibition between 4-8 mm, - No zone of inhibition observed.

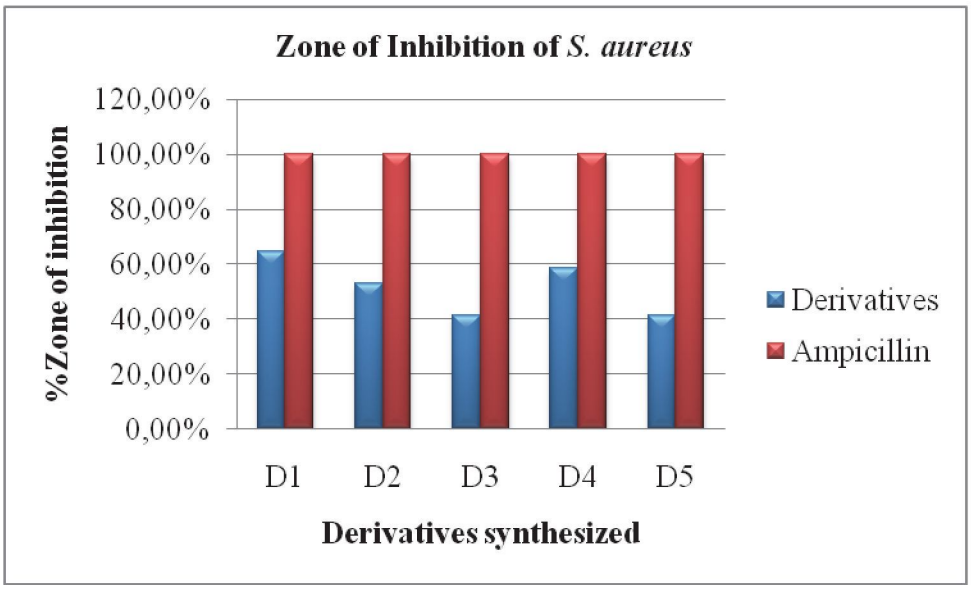


Figure 2. Comparison of % zone of inhibition and derivatives synthesized in case of *S. aureus*

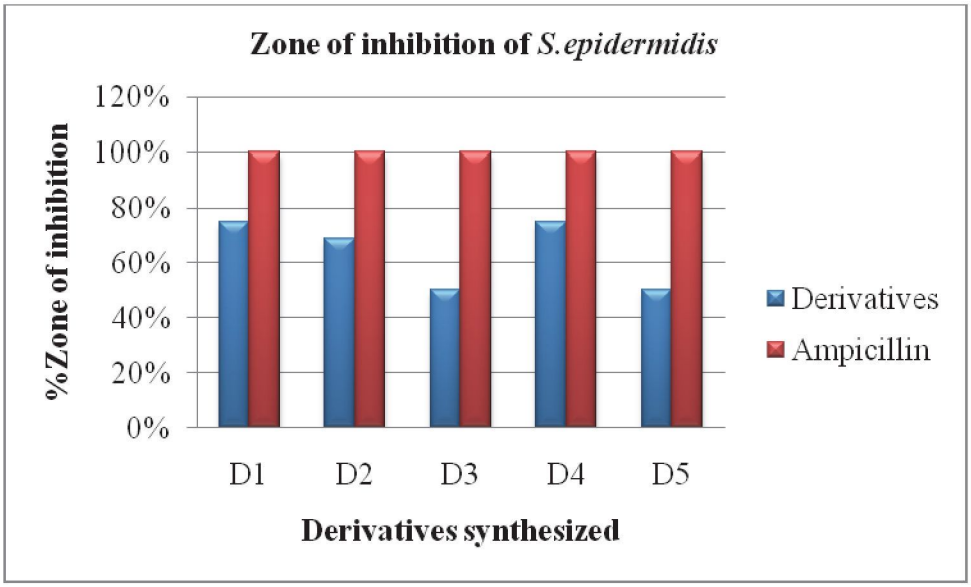


Figure 3. Comparison of % zone of inhibition and derivatives synthesized in case of *S. epidermidis*

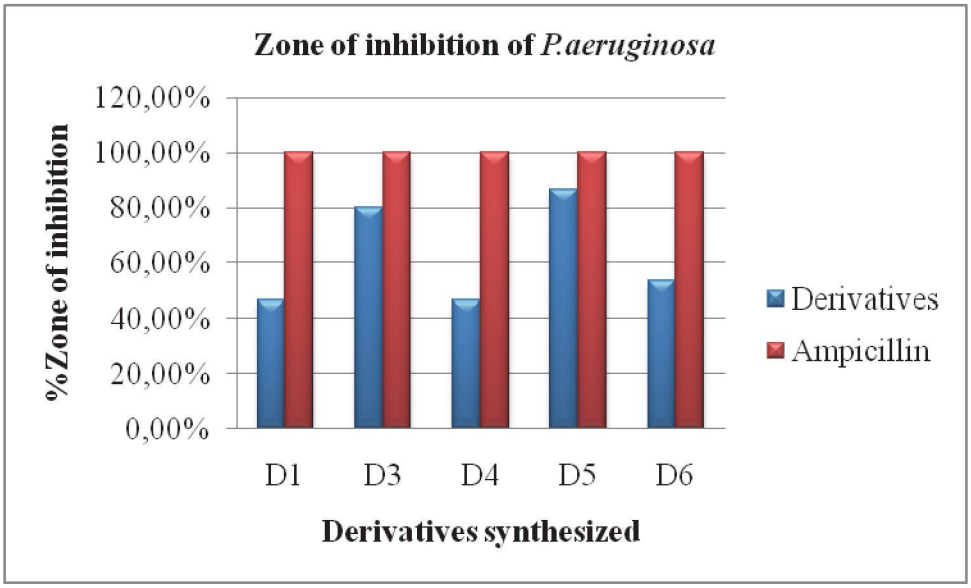


Figure 4. Comparison of % zone of inhibition and derivatives synthesized in case of *P. aeruginosa*

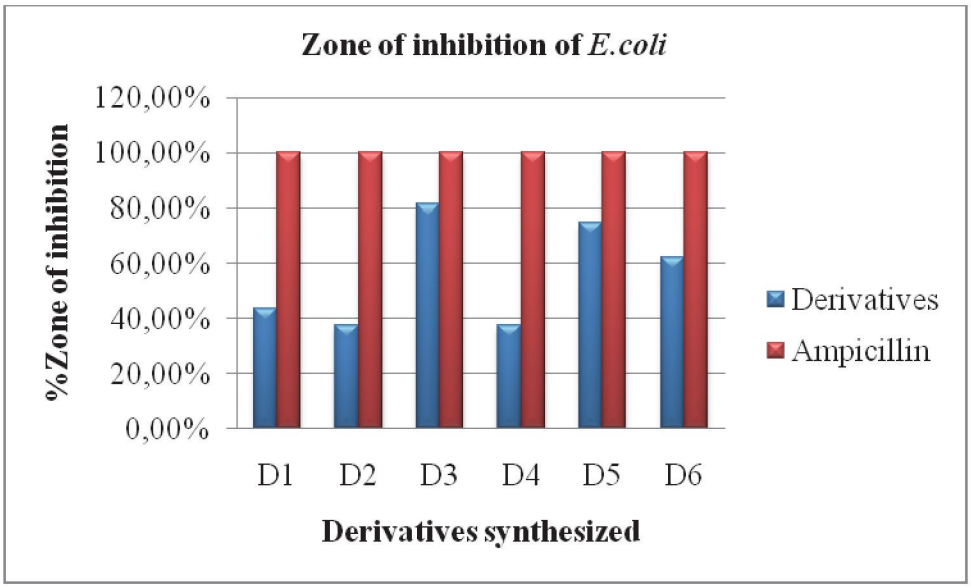


Figure 5. Comparison of % zone of inhibition and derivatives synthesized in case of *E. coli*

CONCLUSION

Compounds D3 and D5 showed excellent activity against *E. coli* and *P. aeruginosa* when tested at 50 mg/ml concentration taking ampicillin as the standard. From the above results, it may be concluded that the derivatives of pyridazinone posses moderate to potent antimicrobial activity when compared to standard, ampicillin. Therefore, the experimental study justifies the therapeutic application of the pyridazinone moiety in the present era.

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