MEDWIN PUBLISHERS
Committed to create value for Researchers

Open Access Journal of Microbiology & Biotechnology
ISSN: 2576-7771

Synthesis, Antibacterial and Antifungal Properties of Cyclohexane Tosyloxyimine Derivative

Shoaib M[1,2]*

[1]Department of Microbiology, Faculty of Biology, Baku State University, Azerbaijan

[2]Department of Parasitology and Microbiology, PMAS Arid Agriculture University Rawalpindi, Pakistan

***Corresponding author:** Muhammad Shoaib, Department of Microbiology, Faculty of Biology, Baku State University, Azerbaijan, Tel: (+99450) 4474229; Email: shoaib1676@gmail.com

Research Article

Volume 4 Issue 3

Received Date: July 01, 2019

Published Date: August 13, 2019

DOI: 10.23880/oajmb-16000150

Abstract

Due to increasing antimicrobial resistance, functionally substituted cyclohexane derivatives are being explored as potential antimicrobial agents. Reaction of diethyl 4-hydroxy-6-(hyd-roxyimino)-4-methyl-2-phenylcyclohexane-1,3-dicarboxylate with 4-toluenesulfonyl chloride in boiling acetone in the presence of equimolar triethylamine resulted in formation of diethyl - 4-hydroxy-4-methyl-2-phenyl-6-((tosyloxy)imino) cyclohexane-1,3-dicarboxylate. The structure of novel compound was characterized by ^{1}H and ^{13}C NMR spectra and elemental analysis was performed. Agar well diffusion assay was used to screen novel compound against Gram-positive bacteria, Gram-negative bacteria and fungi. Test compound showed better antimicrobial properties against Gram-negative bacteria as compared to Gram-positive bacteria and fungi. *Acinetobacter baumannii BDU-32* was found to be most sensitive bacteria while *Candida pseudotropicalis BDU MA88* was found to be most sensitive yeast.

Keywords: *Acinetobacter baumannii;* Agar well diffusion; Antimicrobial resistance; Cyclohexane; Tosyloxyimine derivatives

Introduction

Antimicrobial resistance is a global health problem and major obstacle in the eradication of infectious diseases. This leads to exploration of synthetic organic compounds as new antimicrobial agents with unique mode of action [1]. Functionally substituted derivatives of cyclohexane possess diverse biological properties. Anticancer activity, antioxidant activity, cytotoxic activity, anti-inflammatory activity and antimicrobial activity of different derivatives of cyclohexane has been reported in literature [2-4]. Keeping this in mind, wide range of functionally substituted cyclohexane derivatives are being explored as potential antimicrobial agents.

Diacetyl (diethoxycarbonyl) hydroxycyclohexanones act as valuable construction blocks of organic synthesis. This is due to existence of an extensive source of raw materials in the form of available 1,3- dioxocompounds (acetylacetone, ethyl acetoacetate), aliphatic and aromatic aldehydes. Furthermore, these substances have high chemical potential due to presence of oxogroups of various types [5-15]. The interaction of diethoxycarbonyl hydroxycyclohexanones with hydroxylamine corresponding oximes is studied in literature [16].

However, transformations of these oximes with use of hydroxyl group as reactionary center, has yet not been investigated. Here we report the synthesis and antimicrobial characterization of diethyl -4- hydroxy -4- methyl -2- phenyl -6- ((tosyloxy)imino) cyclohexane -1, 3- dicarboxylate which is formed by reaction of diethyl 4- hydroxy-6- (hyd-roxyimino)- 4-methyl- 2- phenylcyclohexane-1,3 -dicarboxylate with 4- toluenesulfonyl chloride in boiling acetone in the presence of equimolar triethylamine.

Material and Methods

Synthesis of Diethyl -4-hydroxy-4-methyl-2-phenyl-6-((tosyloxy) imino) cyclohexane-1,3-dicarboxylate

A solution of 5 mmol of diethyl 4-hydroxy-6-(hydroxyimino)-4-methyl-2-phenylcyclohexane-1,3-dicarboxylate, 5 mmol of 4-toluene-sulfonyl chloride and 5 mmol of triethylamine in 20 ml of acetone is boiled for 6 hours. To the resultant solution, 50 ml of cold water is added. After 24 hours, the precipitated powder is filtered, recrystallized from ethanol.

^{1}H and ^{13}C NMR spectra recorded on a Bruker AC-300 instrument (300 MHz on 1H and 75 MHz nuclei at 13C cores) in a ($CDCl_3$ solution, residual signals of the solvent used as the standard. The melting points were determined on a Kofler's table. TLC monitored the purity of the resulting compounds on *Silufol UV-254* plates, eluent acetone-hexane 1:2, developer-iodine vapor, UV detector.

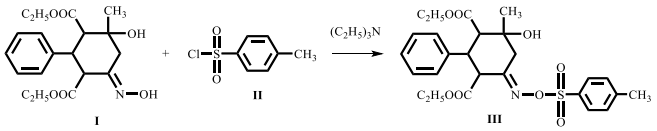
Agar Well Diffusion Assay

Standard agar well diffusion method [17] was used to evaluate antimicrobial properties of newly synthesized derivative. Mueller-Hinton agar was used to determine *in vitro* antibacterial properties against four Gram-positive bacteria (*Staphylococcus aureus BDU-23, Bacillus Subtilis BDU-50, Bacillus mesentericus BDU-51* and *Bacillus megaterium BDU-N20*) and four Gram-negative bacteria (*Escherichia coli BDU-12, Klebsiella pneumoniae BDU-44, Acinetobacter baumannii BDU-32* and *Pseudomonas aeruginosa BDU-49*). Test compound was screened for antifungal properties against *Candida tropicalis BDU LK30, Candida pelliculosa BDU KT55* and *Candida pseudotropicalis BDU MA88* using sabouraud dextrose agar (SDA). All the test cultures were obtained from collection of Department of Microbiology, Baku State University. Test compound was dissolved in dimethyl sulphoxide (DMSO) and three different concentrations of test compound (0.3%, 0.1% and 0.05%) were evaluated for antimicrobial activity. All the experiments were repeated three times and DMSO was used as control due to its inert nature.

Results and Discussion

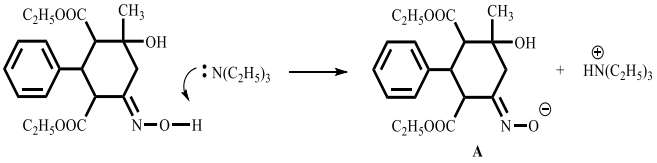
Synthesis of Diethyl - 4-hydroxy-4-methyl-2-phenyl-6-((tosyloxy) imino) cyclohexane-1,3-dicarboxylate

As shown in scheme 1, the reaction of diethyl 4-hydroxy-6-(hyd-roxyimino)-4-methyl-2-phenylcyclohexane-1,3-dicarboxylate (I) with 4-toluenesulfonyl chloride (II) in boiling acetone in the presence of equimolecular triethylamine resulted in formation of diethyl - 4-hydroxy-4-methyl-2-phenyl-6-((tosyloxy)imino) cyclohexane-1,3-dicarboxylate (III):



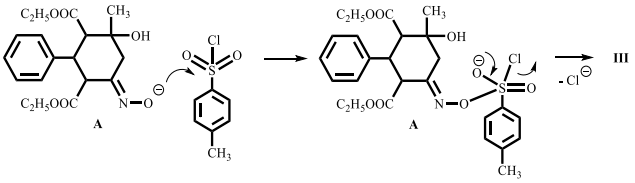
Scheme 1: Synthesis of test compound.

The catalytic role of Triethylamine (Scheme 2) act as catalyst and as a base, it chips off a proton of hydroxyl group of an oxime fragment forming an anion intermediate (A):



Scheme 2: Catalytic role of triethylamine.

Further this anion reacts with 4-toluenesulfonyl chloride leading to formation of a product (III) (Scheme 3):



Scheme 3

Diethyl - 4-hydroxy-4-methyl-2-phenyl-6-((tosyloxy) imino) cyclohexane-1,3-dicarboxylate: The compound was colorless crystals and yield was 63%. Melting point was found to be 190˚C. **^{1}H NMR spectrum (300 MHz, $CDCl_3$, δ, ppm**: 0.77 (t, 3H, OCH_2CH_3); 0.99 (t, 3H, OCH_2CH_3); 1.31 (3H, s, CH_3); 2,0 (1H, d, CH_2); 2,43 (3H, s, CH_3); 2.78 (1H, d, CH_2); 3.51 (1H, s, OH); 3.56 (1H, d, CH); 3.58 (1H, d, CH); 3.73-4.00 (5H, m, OCH_2 , CH); 7.15-7.32 (7H, m, CH_{ar}), 7.80 (2H, d, CH_{ar}). **^{13}C NMR spectrum (75 MHz, $CDCl_3$, δC, ppm:** 13.56 (OCH_2CH_3); 13.83 (OCH_2CH_3); 28.48 (CH_2); 37.95(CH); 44.93 (CH); 53.69 (CH); 57.08 (OCH_2); 60.99(OCH_2); 70.92 (C); 46.27 (CH); 50.32 (CH); 63.86 (OCH_2); 127.84 (CH_{ar}); 128.11 (CH_{ar}); 128.61 (CH_{ar}); 129.08 (CH_{ar}); 129.46 (CH_{ar}); 132.46 (CH_{ar}); 137.98 (CH_{ar}); 145.00(CH_{ar}); 163.01 (C=N); 167.50 ($CO_2C_2H_5$) ; 173.64 ($CO_2C_2H_5$). **Found, %:** C-60.51; H-6.14; N-2.88; **$C_{26}H_{31}NO_8S$, Calculated, %:** C-60.33; H-6.04; N-2.71 (Figure 1).

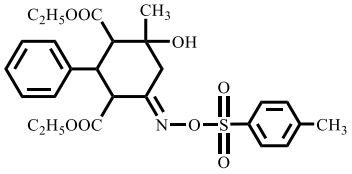


Figure 1: Structure of synthesized compound.

Antimicrobial activity

Table 1 show that test compound showed variable antimicrobial properties against different test cultures. Test compound was found to be more active against gram-negative bacteria as compared to gram-positive bacteria. At 0.3% concentration, cyclohexane derivative showed remarkable antimicrobial activity against *Escherichia coli, Acinetobacter baumannii, Bacillus subtilis* and *Candida pseudotropicalis.* Weak to moderate antibacterial activity was observed against *Escherichia coli, Acinetobacter baumannii* and *Candida pseudotropicalis* at 0.1% concentration. At 0.05% concentration, test compound was inactive against all the test cultures.

Acinetobacter baumannii was found to be most sensitive Gram-negative bacteria (20 mm zone of inhibition), while *Bacillus subtilis* was most sensitive Gram-positive bacteria (16.7 mm zone of inhibition). *Klebsiella pneumoniae, Pseudomonas aeruginosa, Bacillus megaterium* and *Bacillus mesentericus* were found to be resistant against test compounds. Among the fungal cultures, *Candida tropicalis* and *Candida pelliculosa* were found to be resistant while *Candida pseudotropicalis* was highly sensitive. Thus, cyclohexane tosyloxyimine derivatives can act as potential antimicrobial agents in future.

Test Culture	Concentration of test compound		DMSO
	0.3%	0.1%	
Escherichia coli	18.3±0.3	14±0.3	-
Klebsiella pneumoniae	-	-	-
Acinetobacter baumannii	20±0.5	17.3±0.6	-
Pseudomonas aeruginosa	-	-	-
Staphylococcus aureus	14.7±0.4	-	-
Bacillus subtilis	16.7±0.9	-	-
Bacillus megaterium	-	-	-
Bacillus mesentericus	-	-	-
Candida tropicalis	-	-	-
Candida pelliculosa	-	-	-
Candida pseudotropicalis	20.7±0.3	14.7±0.	-

(-): Inactivity

Table 1: Average diameter of inhibition zone in mm.

References

1. Shoaib M, Ganbarov G Kh (2019) Functionally Substituted Chemical Organic Compounds: Potential Antimicrobial Substances. J Microbiol Biotechnol 4(1): 000136.

2. Shoaib M, Israyilova AA, Ganbarov K (2019) Cyclohexane and its functionally substituted derivatives: important class of organic compounds with potential antimicrobial activities. JMBFS 9(1).

3. Song L, Kang H, Liu D, Dai Z, He J Wang, et al. (2015) Dimedone Derivative {2-[(4-Hydroxy-phenylamino)-methylene]-5,5-dimethyl-cyclohexane-1,3-dione} Plays an Important Role in Breast Cancer Treatment. Tropical Journal of Pharmaceutical Research 14(9): 1719-1722.

4. Flefel EM, Sayed HH, Hashem AI, Shalaby EA, El-Sofany W, et al. (2014) Pharmacological evaluation of some novel synthesized compounds derived from spiro(cyclohexane-1,2′-thiazolidines). Medicinal Chemistry Research 23(5): 2515-2527.

5. Ismiev AI, Magerramov AM, Sukach VA, Vovk MV (2016) Zh Org Farm Khim 14(4): 16.

6. Krivenko AP, Kozlova EA, Grigor`ev (2003) Regioselective Ethanolamination and Ketalization of

3-Ph-2,4-diacetyl(diethoxycarbonyl)-5-hydroxy-5-methylcyclohexanones. Molecules 8(2): 251-255.

7. Poplevina NV, Kuznetsova AA, Krivenko AP (2010) Synthesis and structure of substituted triazoloquinazolines. Chem Heterocycl Compd 46(9): 1148-1150.

8. Dyachenko VD, Sukach SM (2012) Synthesis of 1H-pyrazolo[3,4-c]isoquinolin-1-ones by the condensation of cyclohexanone derivatives with 3-amino-1-phenyl-1H-pyrazol-5(4H)-one. Russ J Gen Chem 82(2): 305-309.

9. Ismiyev AI, Maharramov AM, Aliyeva RA, Askerov RK, Mahmudov KT, et al. (2013) Syntheses and some features of five new cyclohexane-1,3-dicarboxylates with multiple stereogenic centers. Journal of molecular structure 1032: 83-87.

10. Magerramov AM, Ismiev AI, Kadyrova NA, Gadzhieva KE, Askerov RK, et al. (2013) Crystal structure of diethyl- 1- isobutyl- 9- hydroxy- 9- methyl- 7- phenyl- 1, 4- diazaspiro [4,5] decane - 6, 8 - dicarboxylate $C_{25}H_{38}N_2O_5$. Journal of Structural Chemistry 54(6): 1137-1139.

11. Hote BS, Lokhande PD (2014) Novel and Efficient Synthesis of 4-Indazolyl-1,3,4-trisubstituted Pyrazole Derivatives. Synth Commun 44(10): 1492-1500.

12. Dyachenko VD, Karpov EN (2014) Synthesis of functionalized alkyl-substituted cyclohexanones, pyridines, and 2,3,5,6,7,8-hexahydroisoquinolines by condensation of aliphatic aldehydes with CH acids. Russ J Org Chem 50(12): 1787-1796.

13. Dyachenko VD, Sukach SM, Dyachenko AD (2015) Synthesis of partially hydrogenated isoquinoline derivatives by condensation of 2,4-diacetyl-3-aryl(hetaryl)-5-hydroxy-5-methylcyclohexanones with malononitrile and its dimer and a study of their alkylation. Chem Heterocyc Comp 51(1): 51-55.

14. Sukach SM, Dyachenko VD (2015) Multicomponent synthesis of 3-(alkylsulfanyl)-8-aryl(hetaryl)-7-acetyl-6-hydroxy-1,6-dimethyl-5,6,7,8-tetrahydroisoquinoline-4-carbonitriles. Russ J Org Chem 51(7): 1020-1025.

15. Maharramov AM, Ismiyev AI, Allahverdiyev MA, Maleev AV, Potekhin KA (2016) Crystal structure of phenylhydrazine with diacetyl substituted ketol of the cyclohexane series. Journal of Structural Chemistry 57(4): 764-770.

16. Gein VL, Nosova NV, Potemkin KD, Aliev ZG, Krivenko AP (2005) Synthesis and Structure of Diisopropyl 6-Hydroxy-6-methyl-4-oxocyclohexane-1,3-dicarboxylates and Their Reactions with Nucleophilic Reagents. Russ J Org Chem 41(7): 1016-1022.

17. Balouiri M, Sadiki M, Ibnsouda SK (2016) Methods for in vitro evaluating antimicrobial activity: A Review. Journal of Pharmaceutical Analysis 6(2): 71-79.