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SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF NEW FUNCTIONALLY SUBSTITUTED DIALKYL CARBOXYLATE CYCLOHEXANE DERIVATIVES

Abstract. *We report synthesis and antimicrobial activity of new functionally substituted dialkyl carboxylate cyclohexane derivatives. The new cascade carboxylation reaction involving multicomponent interaction of alkyl 2-cyanoacetate (ethyl, isopropyl and butyl) with benzaldehyde and acetylacetone resulted in formation of three new dialkyl 1,3-dician-2,6-diphenyl-5-acetyl-4-hydroxy-4-methylcyclo-hexane-1,3-dicarboxylate derivatives (1a-1c). Correct elemental analysis and spectral data (IR, 1H-NMR, 13C-NMRspectra and MS) were used to characterize and confirm the structure of newly synthesized compounds. Agar well diffusion assay was used to determine antimicrobial activity of derivatives against Gram-positive and Gram-negative bacteria, and fungi. The synthesized compounds showed variable antimicrobial activity and were found to be more active against Gram-negative bacteria as compared to gram-positive bacteria and fungi.*

Keywords: *antimicrobial activity, cyclohexane derivatives, alkyl 2-cyanoacetate, benzaldehyde, acetylacetone, agar well diffusion*

Introduction. Due to ever increasing antimicrobial resistance and shortage of new antimicrobial drugs, synthetic organic compounds are extensively synthesized and explored as probable antimicrobial agents. Functionally substituted alicyclic compounds are best available targets [1]. One of the developed trends of modern organic chemistry in recent years is related with the development of productive methods for the synthesis of complex carboxy-heterocyclic compounds. The effectiveness of these methods is provided through creation of synthesis schemes with minimal stages, total convergence and minimal consumption of reagents, solvents and other material resources. The trend of multicomponent reactions has rapidly increased in the synthetic arsenal of organic chemistry. Multicomponent reactions are carried out in a single reactor on the basis of interaction of at least three substances. All the added reagents simultaneously constitute the final product through consistently happening elementary transformations i.e. cascade or tandem reactions [2-5]. Methylene active nitriles (malononitrile, malonodinitrile, alkyl 2-cyanoace-tates etc.) are one of the bifunctional reagents that perform as basis of various multicomponent reactions, generating multi vectoral transformations [6-8]. The chemistry of these compounds has not yet fully expanded its synthetic potential. Therefore in present study, we report the new cascade carboxylation reaction involving multicomponent interactions for synthesis of new dialkyl 1,3-dician-2,6-diphenyl-5-acetyl-4-hydroxy-4-methylcyclo-hexane-1,3-dicarboxylate derivatives and evaluation of their antimicrobial potential.

Purpose of study. The aim of the current research focuses to determine the new directions of the interaction of benzaldehyde, alkyl 2-cyanoacetate and acetylacetone in multicomponent cascade environment. The reaction of these reagents usually results in formation of 2-aminopyrene compounds [9-14]. But here, with help of new cascade caboxylation system, we focus on synthesis and antimicrobial activity of novel dialkyl dicarboxylate cyclohexane derivatives.

Material and methods. Synthesis of functionally

substituted dialkyl dicarboxylate cyclohexane derivatives. Cascade reaction involving multicomponent interactions of alkyl 2-cyanoacetate (ethyl, isopropyl and butyl) with benzaldehyde and acetylacetone led to development of three new dialkyl 1,3-dician-2,6-diphenyl-5-acetyl-4-hydroxy-4-methylcyclo-hexane-1,3-dicarboxylate derivatives: Diethyl 1,3-dician-2,6-diphenyl-5-acetyl-4-hydroxy-4-methylcyclohexane-1,3 dicarboxylate(1a), Diisopropyl 1,3-dician-2,6-diphenyl-5-acetyl-4-hydroxy-4-methylcyclo-hexane-1,3-dicarboxylate(1b) and Dibutyl 1,3-dician-2,6-diphenyl-5-acetyl-4-hydroxy-4-methylcyclohexane-1,3-dicarboxylate(1c).

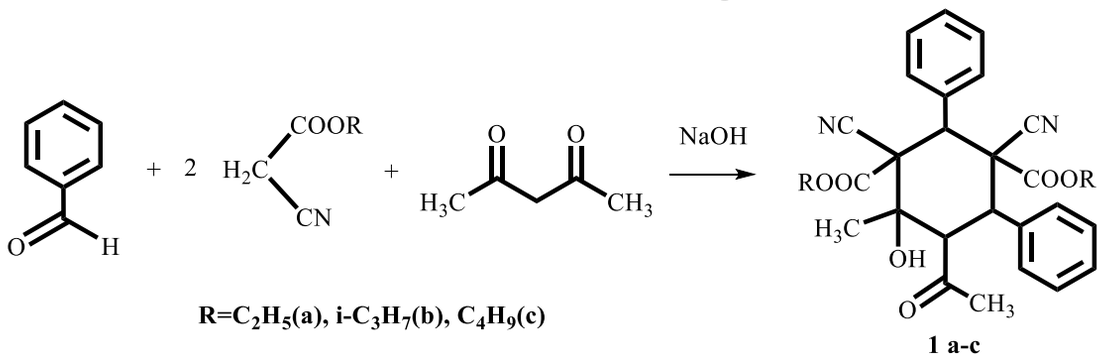
10 ml of ethanol, 0.53g of benzaldehyde (5 mmol) and 10 mmol of alkyl 2-cyanoacetates (ethyl for 1a, isopropyl for 1b and butyl for 1c) were placed in 50ml flat flask supplied with magnetic mixer. Afterwards, 3 ml of water solution of 0.1g of NaOH was added to the reaction medium in mixing condition. After half an hour, 0.5g of acetylacetone (5 mmol) and 0.53g of benzaldehyde (5 mmol) were added to the medium. The reaction medium was kept at room conditions for 48 hours after mixing for one more hour. The precipitated crystals were filtered and recrystallized in ethanol.

At the first stage, the intermediates of benzaldehyde and alkyl 2-cyanoacetates (A), and benzaldehyde and acetylacetone (B) are taken in the presence of NaOH as shown in scheme 2.

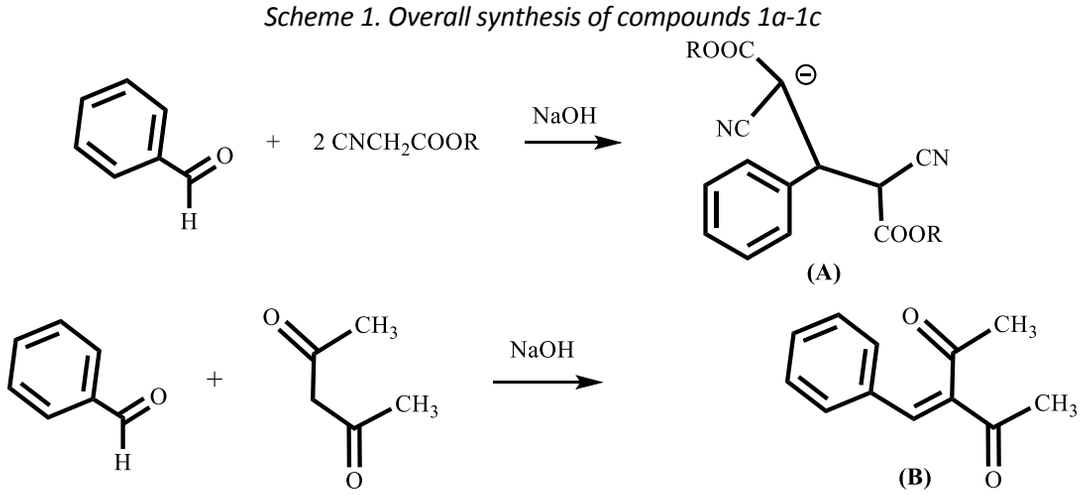
Subsequently, the phases of these intermediates are formed by the cascade carboxylation reaction as shown in the following scheme 3.

^{1}H and ^{13}C NMR spectra were recorded on a Bruker AC-300 instrument (300 MHz on ^{1}H and 75 MHz nuclei at ^{13}C cores) in a $(CD_3)_2SO$ solution, as residual signals of the solvent were 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 1:1, developer-iodine vapor, UV detector. Carlo Erba 1106 analyzer was used to perform elemental analysis for C, H, and N.

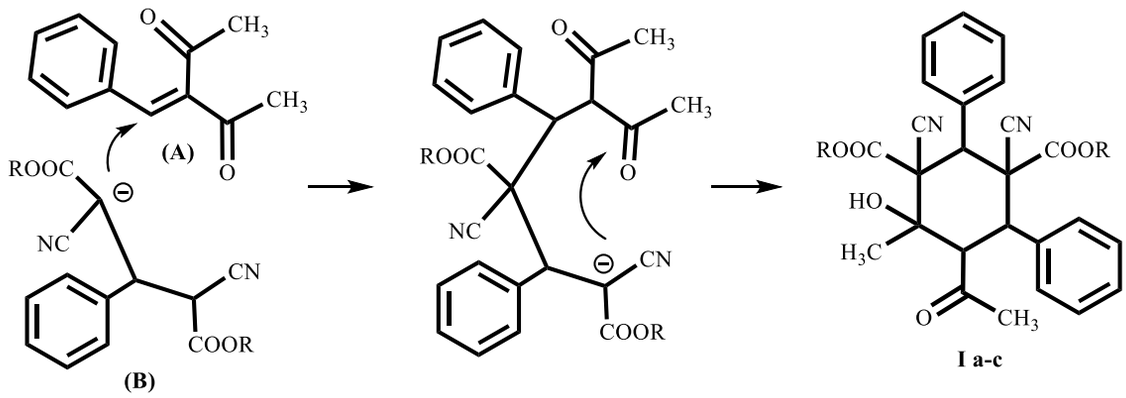
Determination of antimicrobial activity. Standard agar well diffusion assay [15] was used to determine antimicrobial properties of novel compounds. *In vitro* antibacterial properties were evaluated against *Escherichia coli BDU-12,*



Scheme 1. Overall synthesis of compounds 1a-1c



Scheme 2. Formation of intermediates A and B



Scheme 3. Interaction of intermediates A and B

Klebsiella pneumoniae BDU-44, Acinetobacter baumannii BDU-32, Pseudomonas aeruginosa BDU-49, Staphylococcus aureus BDU-23, Bacillus Subtilis BDU-50, Bacillus mesentericus BDU-51 and *Bacillus megaterium BDU-N20* using Mueller-Hinton agar and antifungal screening was performed against *Candida tropicalis BDU LK30, Candida pelliculosa BDU KT55* and *Candida pseudotropicalis BDU MA88* using sabouraud dextrose agar. All the test cultures were obtained from our own collection at Department of Microbiology, Baku State University. Three different concentrations of test compounds; 0.3% (3 mg of test compound per mL of DMSO), 0.1% (1 mg of test compound per mL of DMSO) and 0.05% (0.5 mg of test compound per mL of DMSO) were selected. DMSO was used as control and all the experiments were performed in triplicates.

Results. Diethyl 1,3-dician-2,6-diphenyl-5-acetyl-4-hydroxy-4-methylcyclohexane-1,3 dicarboxylate(1a). The synthesized compound is colorless solid in the yield of 67%. Melting point was found to be 163^{0}C. ^{1}H-NMRspectrum (300 MHz, $(CD_3)_2SO$), δ, ppm:1.00 (t, 3 H, $OCH_2C\underline{H}_3$); 1.07 (t, 3 H, $OCH_2C\underline{H}_3$); 1.10 (3H, s, $C\underline{H}_3$);2.1 (3H, s, $C\underline{H}_3$);2.65 (1H, d, C<u>H</u>); 3.55 (1H, d, C<u>H</u>); 3.90 (1H, s, C<u>H</u>); 4.04-4.10 (m, 4 H, 2 $OC\underline{H}_2CH_3$,); 4.90 (1H, s, O<u>H</u>); 7.39-7.41 (m, 6 H, Ph); 7.64-7.65 (m, 4 H, Ph). ^{13}C-NMRspectrum (75 MHz, $(CD_3)_2SO$), δC, ppm:13.46 ($OCH_2\underline{C}H_3$); 13.49 ($OCH_2\underline{C}H_3$); 19.32 ($\underline{C}H_3$); 31.17 ($\underline{C}H_3$); 40.52 (<u>C</u>H); 46.26 (<u>C</u>H); 50.33 (<u>C</u>H);63.86 ($O\underline{C}H_2CH_3$); 63.89 ($O\underline{C}H_2CH_3$); 69.59(<u>C</u>-OH);116.22 (C≡N); 117.37 (C≡N); 128.61(Ph); 129.63 (Ph); 130.66 (Ph); 131.79 (Ph); 163.41 ($\underline{C}O_2Et$); 165.13 ($\underline{C}O_2Et$); 207.20 (C=O). Found, %: C-69.40, H-6.10, N-5.60; $C_{29}H_{30}N_2O_6$, Calculated, %: C-69.31, H-6.02, N-5.57.

Diisopropyl 1,3-dician-2,6-diphenyl-5-acetyl-4-hydroxy-4-methylcyclo-hexane-1,3-

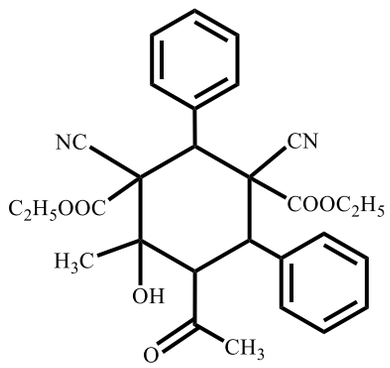


Fig.1. Structure of compound 1a

dicarboxylate(1b). The synthesized compound is colorless solid in the yield of 63%. Melting point was found to be 178^{0}C. ^{1}H-NMRspectrum (300 MHz, $(CD_3)_2SO$), δ, ppm: 1.17–1.33 m [12H, $CH(C\underline{H}_3)_2$], 1.10 (3H, s, $C\underline{H}_3$); 2,12 (3H, s, $C\underline{H}_3$); 2.74 (1H, d, C<u>H</u>); 3.55 (1H, d, C<u>H</u>); 3.85 (1H, s, C<u>H</u>);5.00–5.12 m [2H, $CH(CH_3)_2$];7.30- 7.57,(m, 10H, Ph). ^{13}C-NMRspectrum (75 MHz, $(CD_3)_2SO$), δC, ppm: 20.10 ($\underline{C}H_3$); 21.13 [$CH(\underline{C}H_3)_2$], 21.15 [$CH(\underline{C}H_3)_2$], 21.22 [$CH(\underline{C}H_3)_2$], 21.26 [$CH(\underline{C}H_3)_2$], 30.17 ($\underline{C}H_3$);40.56 (<u>C</u>H); 46.28 (<u>C</u>H); 50.39 (<u>C</u>H); 72.74 [<u>C</u>H(CH$_3$)$_2$]; 73.13[<u>C</u>H(CH$_3$)$_2$]; 115.22 (C≡N), 116.19 (C≡N), 128.60 (Ph); 129.63 (Ph); 130.61 (Ph); 131.87 (Ph); 163.15 (CO_2Pr-i); 164.92 (CO_2Pr-i), 206.13 (C=O). Found, %: C-70.25; H-6.52; N-5.32; $C_{31}H_{34}N_2O_6$, Calculated, %: C-70.17; H-6.46; N-5.28.

Dibutyl 1,3-dician-2,6-diphenyl-5-acetyl-4-hydroxy-4-methylcyclohexane-1,3-dicarboxylate(1c). The synthesized compound is colorless solid in the yield of 55%. Melting point was found to be 205^{0}C. ^{1}H-NMRspectrum (300 MHz, $(CD_3)_2SO$), δ, ppm: 0,98 (t, 4H, $OCH_2CH_2CH_2C\underline{H}_3$);1.00(t,3H, $OCH_2CH_2CH_2C\underline{H}_3$);1.10 (3H, s, $C\underline{H}_3$); 1,20-1,30 (8H, m,$C\underline{H}_2$); 2,17 (3H, s, $C\underline{H}_3$); 2.75 (1H, d, C<u>H</u>); 3.55 (1H, d, C<u>H</u>); 3.85(1H, s, C<u>H</u>); 3.95-4.10 (m, 4 H, 2$OC\underline{H}_2CH_2CH_2CH_3$); 5.06(1H, s, O<u>H</u>); 7.30-7.55 (m,10H, Ph). ^{13}C-NMRspectrum (75 MHz, $(CD_3)_2SO$), δC, ppm: 13.26 ($OCH_2CH_2CH_2\underline{C}H_3$); 13.50 ($OCH_2CH_2CH_2\underline{C}H_3$);

17.25($\underline{C}H_2$); 18.20($\underline{C}H_2$); 19.33($\underline{C}H_3$); 31.20 ($\underline{C}H_3$); 32.18($\underline{C}H_2$);33.37($\underline{C}H_2$);40.55 ($\underline{C}H$); 46.27 ($\underline{C}H$); 50.32 ($\underline{C}H$); 63.86 ($O\underline{C}H_2$); 63.89 ($O\underline{C}H_2$); 69.59 ($\underline{C}$-OH);116.17 (C≡N); 117.63 (C≡N); 128.21 (Ph); 129.43 (Ph); 131.23 (Ph); 132.37 (Ph); 164.44 ($\underline{C}O_2C_4H_9$); 165.71 ($\underline{C}O_2C_4H_9$); 208.80 (C=O). Found, %: C-71.05; H-7.04; N-5.11; $C_{33}H_{38}N_2O_6$, Calculated, %: C-70.95; H-6.96; N-5.01.

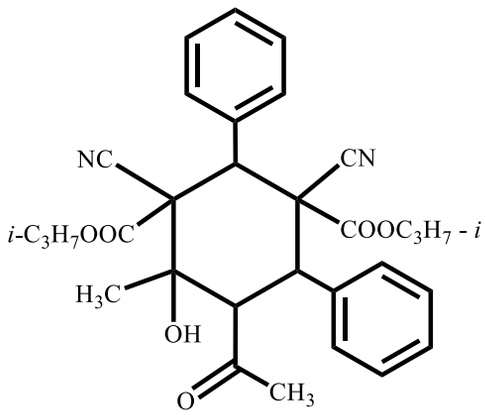


Fig.2. Structure of compound 1b

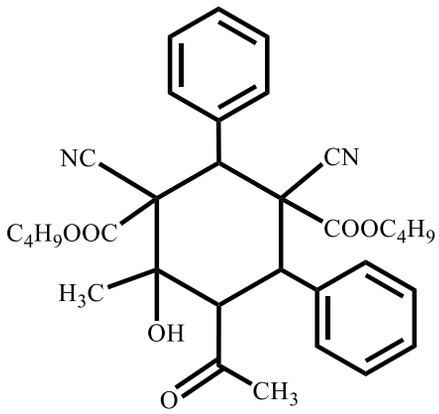


Fig.3. Structure of compound 1c

Antimicrobial activity. The antimicrobial activity of newly synthesized compounds (1a-1c) was tested against four Gram-negative bacteria, four Gram-positive bacteria and three yeast species. Tested compounds exhibited variable antimicrobial activity at concentration of 0.3% and 0.1%. DMSO was used as control and showed no activity against any of tested cultures. Overall results of antimicrobial activity are listed in table 1.

Table 1.

Average diameter of inhibition zone in mm

Test Culture	1a		1b		1c		DMSO
	0.3%	0.1%	0.3%	0.1%	0.3%	0.1%	
Escherichia coli	14.7±0.3	-	18±0.5	-	25±0.6	13±0.3	-
Klebsiella pneumoniae	-	-	-	-	-	-	-
Acinetobacter baumannii	17.3±0.9	14.7±0.7	19.3±1	16.3±0.3	23±0.1	-	-
Pseudomonas aeruginosa	12.3±1	-	15.3±0.9	-	-	-	-
Staphylococcus aureus	-	-	12.7±0.2	-	19.7±0.7	12±0.3	-
Bacillus Subtilis	14.7±0.3	-	-	-	-	-	-
Bacillus megaterium	-	-	11.7±0.6	-	11.7±0.7	-	-
Bacillus mesentericus	13.3±0.7	-	-	-	-	-	-
Candida tropicalis	-	-	-	-	-	-	-
Candida pelliculosa	-	-	-	-	-	-	-
Candida pseudotropicalis	-	-	-	-	-	-	-

(-)*: Inactivity*

Discussions. In the present study, it has been found that the use of benzaldehyde, alkyl 2-cyanoacetate and acetylacetone in a sequence of reactions and the use of sodium hydroxide as a catalyst by the acquisition of functional cyclohexane derivatives results in an original carboxylation reaction. Previously such multicomponent cascade reactions have been used for synthesis of 2-aminopyrene compounds [12-14]. The interaction of intermediates of benzaldehyde and alkyl 2-cyanoacetates (A) and benzaldehyde and acetylacetone (B) in the presence of sodium hydroxide is manifestation of new cascade carboxylation reaction.

All the synthesized compounds (1a-1c) are colorless solids with the yield between 67% and 55%. It is observed that with the increase in Carbon chain length of alkyl group, yield of the compound decreases and melting point increases. That's why minimum yield and maximum melting point was found for butyl derivative (1c) as compared to ethyl and isopropyl (1a and 1b, respectively) derivatives.

Compounds exhibited weak to moderate activity against different test cultures at 0.3% and 0.1% concentration. All the compounds were inactive at concentration of 0.05%. None of the compound was found to be active against fungal strains. Generally, compounds showed better

antimicrobial activity against Gram-positive bacteria as compared to Gram-negative bacteria. Benzofuran cyclohexane-5-carboxylate derivatives show more potent activity against Gram-positive bacteria as compared to Gram-negative bacteria [16]. These results contradict with our findings, which is due to presence of dialkyl group in our compounds. *Acinetobacter baumannii* and *Escherichia coli* were most sensitive test cultures. Compound 1c was found to be most active antibacterial compound, as zone of inhibition (25mm, 23 mm for *Escherichia coli* and *Acinetobacter baumannii,* respectively) was observed. Thus, increase in carbon chain length of alkyl group resulted in better antimicrobial activity of the synthesized compounds.

Conclusion. The novel cascade carboxylation system involving interactions of alkyl 2-cyanoacetate (ethyl, isopropyl and butyl) with benzaldehyde and acetylacetone led to formation of three new dialkyl 1,3-dician-2,6-diphenyl-5-acetyl-4-hydroxy-4-methylcyclo-hexane-1,3-dicarboxylate derivatives. New transformations i.e. cascade carbo-cyclation were determined during interaction of benzaldehyde, alkyl 2-cyanoacetates and acetylacetone. The structure of compounds was confirmed by elemental analysis and spectral data. These compounds exhibited moderate antibacterial activity against Gram-negative bacteria and weak activity against Gram-positive bacteria, and showed no activity against fungi.

Prospects for further research. Due to perpetual increasing antimicrobial resistance and dearth of new classes of antimicrobial drugs, cascade carboxylation system should be envisioned as productive method for synthesis of new compounds having better antimicrobial potential due to their unique mode of action.

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