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Synthesis and Antimicrobial Activities of a Series of 2,5-Substituent 1,4-Cyclohexanedione Derivatives

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A series of 2,5-substituent 1,4-cyclohexanedione derivatives compounds were designed and synthesized. Their structures were identified by elemental analysis, ^{1}H NMR, IR spectra. Their assayed antibacterial (*Escherichia coli*, *Bacillus subtilis*) and antifungal (*Candida albicans*) activities were also evaluated by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) method. The results of biological test showed 2,5-dibenzylformate 1,4-cyclohexanedione (**11**) and 2,5-diformanilide 1,4-cyclohexanedione (**12**) have favorable antimicrobial activity with MICs of 26.7, 33.6, 24.2 and 32.3 μg/mL against *Escherichia coli* and *Bacillus subtilis*, respectively.

Key Words: Cyclohexanedione derivatives, Antimicrobial activities, Structure-activity relationships.

INTRODUCTION

The rapid development of pathogen resistance to most of the known antibiotics is becoming a serious health problem[1,2]. Therefore, the development of new and different antimicrobial drugs is a very important objective and much of the research program efforts are directed towards the design of new agents[3-5].

2,5-Substituted 1,4-cyclohexanedione are important organic intermediate. The Claisen-Dieckmann condensation of the respective succinates is very straight forward approaches for the synthesis of the 1,4-cyclohexanedione-2,5-dicarboxylate[6-8]. However, yield and purification of this procedure were not satisfying (the maximum yield 65 %). Recently, our group made a remarkable progress in improving the synthesis of 1,4-cyclohexanedione 2,5-dicarboxylate. Using this new method, 1,4-cyclohexanedione 2,5-dicarboxylate can be prepared under mild conditions in a high yield (up to 93 %).

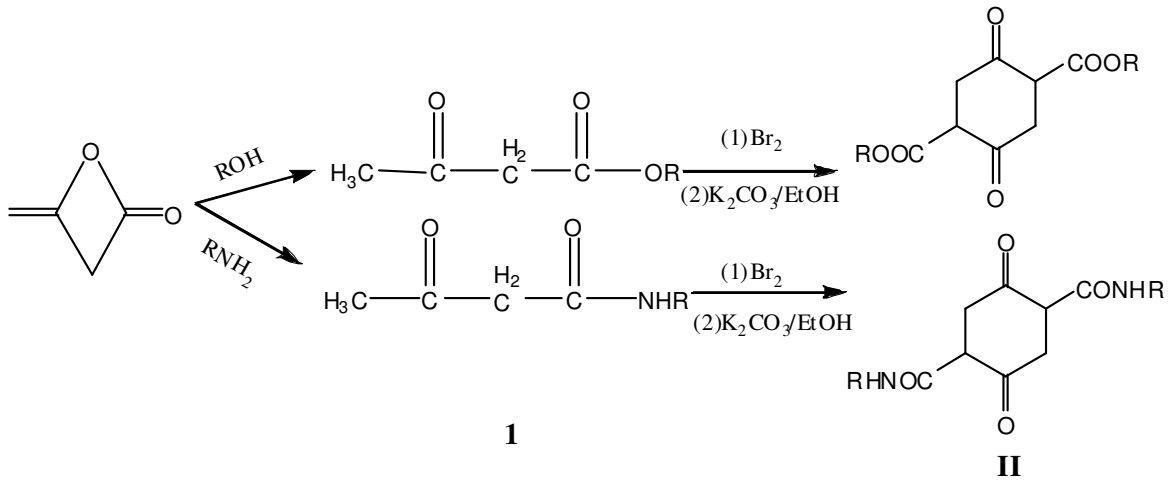
2,5-Substituted 1,4-cyclohexanedione are vital for all life, occurring in key biological processes as diverse as the oxidative maintenance of biological amine levels. Their derivatives are the basic structure of the quinones, which are not only found in bacteria, plants and arthropods but are also the base for a large number of chemical derivatives with pharmacological applications[9-11]. However, the antibacterial activity of 2,5-substituted 1,4-cyclohexanedione was less reported. In this paper, we first introduced the direct synthesis of 2,5-substituted 1,4-cyclohexanedione derivatives from acetoacetyl derivatives and using the diketene as starting materials. The antibacterial activity of the compounds were tested. The synthesis route is described in **Scheme-I**.

EXPERIMENTAL

All chemicals and reagents were obtained from commercial sources and used without further purification. Melting points (m.p.) were measured on a Boetius micro melting point apparatus. The IR spectra were recorded in the 4000-400 cm^{-1} region using KBr pellets on a Nicolet 170SX spectrophotometer. All the NMR spectra were recorded on a Bruker DRX500 model spectrometer in $CDCl_3$. Chemical shifts for ^{1}H NMR spectra were reported in parts per million to residual solvent protons.

General synthetic procedure: The mixture of diketene with alcohol and amine in mole ratio 1:1 afforded the intermediate **I**. To a 100 mL flask 15 mmol of intermediate **I**, 10 mmol of bromine was dropwise added with stirring. The reaction was maintained until the mixture was turned into colourless or light yellow for 0.5 h. The organic layer was isolated, dried over sodium sulfate. Then dissolved in 30 mL EtOH, K_2CO_3 0.15 mol, reaction 3-4 h, washed with water, filtered. The light yellow or white solid produces have been obtained. The yields of fifteen title compounds are list in Table-1.

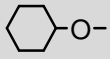
Synthesis of 2,5-dimethylformate 1,4-cyclohexanedione (1): Prepared according to general procedure above using methyl acetoacetate. Recrystallization with EtOH afforded the desired compound as a light yellow crystal. Yield: 93.1 %.



1 **II**

Scheme-I

TABLE-1
PRODUCTS AND YIELDS FROM THE DIFFERENT STARTING MATERIALS IN K_2CO_3/EtOH SYSTEM

Entry	X Groups	m.p. (ºC)	Yield (%)
1	CH_3-	155.5-157.2	93.1
2	CH_3CH_2-	127.5	91.5
3	$CH_3CH_2CH_2$-	93.5-95.4	88.7
4	$(CH_3)_2CH$-	124.7	73.8
5	$CH_3(CH_2)_2CH_2$-	95.5-97.5	85.2
6	$CH_3CH_2(CH_3)CH$-	104-106	82.0
7	$(CH_3)_2CHCH_2$-	103.5-105	83.6
8	$CH_3(CH_2)_3CH_2$-	99-101	84.6
9	$(CH_3)_2CHCH_2CH_2$-	58-60	82.3
10		130.9-132	78.8
11	$PhCH_2$-	180.2	80.9
12	Ph-NH	145.1-146.9	70.5
13	*o*-MePh-	177.4-178.8	75.2
14	*o*-MeOPh-	172.4-173.4	76.1
15	*p*-MePh-	152.5-154.6	73.2

Yellow solid. m.p. 155.5-157.2 ºC. IR (KBr, ν_{max}, cm^{-1}): 3112, 2994, 2915, 1635, 1445, 1229, 1063, 807, 796. ^1H NMR (400Hz, $CDCl_3$): δ 12.02 (s, 2H); 3.94 (s, 4H); 2.88 (s, 6H). Elemental analysis (anal. calcd. (%) for $C_{10}H_{12}O_6$): C 52.63, H 5.30; found (%): C 52.16, H 4.87.

Synthesis of 2,5-diethylformate 1,4-cyclohexanedione (2): Prepared as described for **1** except using ethyl acetoacetate. Yield: 91.5 %. Yellow solid. m.p. 127.5 ºC. IR (KBr, ν_{max}, cm^{-1}): 3126, 2985, 2914, 1673, 1399, 1217, 1066, 801, 782. ^1H NMR (400 Hz, $CDCl_3$): δ 12.23 (s, 2H); 4.24-4.45 (m, 4H); 3.20 (s, 2H); 1.32-1.46 (m, 6H). Elemental analysis (anal. calcd. (%) for $C_{12}H_{16}O_6$): C 56.25, H 6.29; found (%): C 56.16, H 5.92.

Synthesis of 2,5-dipropylformate 1,4-cyclohexanedione (3): Prepared as described for **1** except using propyl acetoacetate. Yield: 88.7 %. White solid. m.p. 93.5-95.4 ºC. IR (KBr, ν_{max}, cm^{-1}): 3207, 2978, 2899, 1674, 1407, 1231, 1069, 816, 796. ^1H NMR (400Hz, $CDCl_3$): δ 12.08(s, 2H); 4.38-4.03 (t, 4H); 3.47-3.41(m, 4H); 3.21(s, 4H); 0.86-1.07 (m, 6H). Elemental analysis (anal. calcd. (%) for $C_{14}H_{20}O_6$): C 59.14, H 7.09; found (%): C 58.86, H 6.92.

Synthesis of 2,5-diisopropylformate 1,4-cyclohexanedione (4): Prepared as described for **1** except using isopropyl acetoacetate. Yield: 73.8 %. White solid. m.p. 124.7 ºC. IR (KBr, ν_{max}, cm^{-1}): 3129, 2973, 2929, 1662, 1379, 1208, 1060, 815, 780. ^1H NMR (400Hz, $CDCl_3$): δ 12.14 (s, 2H); 3.47-3.35 (m, 2H); 3.16(s, 4H); 1.27-1.23 (d, 12H). Elemental analysis (anal. calcd. (%) for $C_{14}H_{20}O_6$): C 59.14, H 7.09; Found (%): C 58.43, H 6.8.

Synthesis of 2,5-dibutylformate 1,4-cyclohexanedione (5): Prepared as described for **1** except using butyl acetoacetate. Yield: 85.2 %. White solid. m.p. 95.5-97.5 ºC. IR (KBr, ν_{max}, cm^{-1}): 3187, 2978, 2899, 1672, 1344, 1234, 1069, 817, 796. ^1H NMR (400 Hz, $CDCl_3$): δ 12.07(s, 2H); 4.37-4.10 (t, 4H); 3.13 (s, 4H); 1.75-1.69 (m, 4H); 1.67-1.60(m, 4H); 0.99-0.90 (t, 6H). Elemental analysis (anal. calcd. (%) for $C_{16}H_{24}O_6$): C 61.52, H 7.74; found(%): C 61.32, H 7.92.

Synthesis of 2,5-di-α-methylpropylformate 1,4-cyclohexanedione (6): Prepared as described for **1** except using α-methylpropyl acetoacetate. Yield: 82.0 %. White solid. m.p. 104-106 ºC. IR (KBr, ν_{max}, cm^{-1}): 3181, 2977, 2934, 1660, 1389, 1224, 1096, 827, 795. ^1H NMR (400 MHz, $CDCl_3$): δ 12.15 (s, 2H); 5.06-4.84 (m, 2H); 3.11 (s, 4H); 1.75-1.56 (m, 4H); 1.34-1.19 (d, 6H); 1.05-0.76 (t, 6H). Elemental analysis (anal. calcd. (%) for $C_{16}H_{24}O_6$): C 61.52, H 7.74; found (%): C 60.86, H 7.45.

Synthesis of 2,5-diisobutylformate 1,4-cyclohexanedione (7): Prepared as described for **1** except using isobutyl acetoacetate. Yield: 83.6 %. White solid. m.p. 103.5-105 ºC. IR (KBr, ν_{max}, cm^{-1}): 3126, 2968, 2910, 1663, 1383, 1237, 1073, 828, 775. ^1H NMR (400 MHz, $CDCl_3$): δ 12.82 (s, 2H); 4.85-4.71(d, 4H); 3.25(s, 4H); 1.73-1.62 (m, 2H); 0.89-0.76 (d, 12H). Elemental analysis (anal. calcd. (%) for $C_{16}H_{24}O_6$): C 61.52, H 7.74; found (%): C 61.41, H 7.38.

Synthesis of 2,5-diamylformate 1,4-cyclohexanedione (8): Prepared as described for **1** except using amyl acetoacetate. Yield: 84.6 %. White solid. m.p. 99-101 ºC. IR (KBr, ν_{max}, cm^{-1}): 3123, 2973, 2936, 1657, 1341, 1226, 1073, 824, 780. ^1H NMR (400 Hz, $CDCl_3$): δ 12.07(s, 2H); 4.31-4.14(t, 4H); 3.34 (s, 4H); 3.34-3.14 (m, 4H); 1.73-1.62 (m, 4H); 1.39-1.27 (m, 4H); 0.92-0.0.82 (t, 6H). Elemental analysis (anal. calcd. (%) for $C_{18}H_{28}O_6$): C 63.51, H 8.29; found (%): C 62.97, H 7.92.

Synthesis of 2,5-diisoamylformate 1,4-cyclohexanedione (9): Prepared as described for **1** except using isoamyl acetoacetate. Yield: 82.3 %. White solid. m.p. 58-60 ºC. IR (KBr, ν_{max}, cm^{-1}): 3128, 2957, 2868, 1652, 1349, 1217, 1075, 827,

777. ^{1}H NMR (400 Hz, $CDCl_3$): δ 12.24 (s, 2H); 4.25-4.22 (t, 4H); 3.20 (s, 4H); 1.79-1.71 (m, 2H); 1.62-1.57 (m, 4H); 0.81-0.79 (d, 12H). Elemental analysis (anal. calcd. (%) for $C_{18}H_{28}O_6$): C 63.51, H 8.29; found (%): C 62.64, H 7.79.

Synthesis of 2,5-dicyclohexane formate 1,4-cyclohexanedione (10): Prepared as described for **1** except using cyclohexane acetoacetate. Yield: 78.8 %. White solid. m.p. 130.9-132 ºC. IR (KBr, ν_{max}, cm^{-1}): 3125, 2931, 2857, 1656, 1389, 1218, 1063, 793, 776. ^{1}H NMR (400 Hz, $CDCl_3$): δ 10.23(s, 2H); 3.47-3.41 (m, 8H); 3.34 (s, 4H); 2.60-2.47 (t, 2H); 1.87-1.56 (m, 8H); 1.39-1.34 (t, 4H). Elemental analysis (anal. calcd. (%) for $C_{20}H_{28}O_6$): C 65.92, H 7.74; found (%): C 65.43, H 7.42.

Synthesis of 2,5-dibenzylformate 1,4-cyclohexanedione (11): Prepared as described for **1** except using benzyl acetoacetate. Yield: 80.9 %. White solid. m.p. 180.2 ºC. IR (KBr, ν_{max}, cm^{-1}): 3126, 3027, 2895, 1654, 1398, 1218, 1069, 805, 737. ^{1}H NMR (400 Hz, $CDCl_3$): δ 11.95 (s, 2H); 7.49-7.30 (m, 10H); 3.34 (s, 4H); 3.34-3.20 (t, 4H). Elemental analysis (anal. calcd. (%) for $C_{22}H_{20}O_6$): C 69.46, H 5.30; found (%): C 68.98, H 4.92.

Synthesis of 2,5-diformanilide 1,4-cyclohexanedione (12): Prepared as described for **1** except using acetoacetyl aniline. Yield: 70.5 %. White solid. m.p. 145.1-146.9 ºC. IR (KBr, ν_{max}, cm^{-1}): 3294, 3138, 2957, 1730, 1655, 1406, 1052, 753, 692. ^{1}H NMR (400 Hz, $CDCl_3$): δ^1 8.59 (s, 2H); 7.56-6.96 (m, 10H); 4.12-4.08 (t, 2H); 3.89-3.83 (d, 4H). Elemental analysis (anal. calcd. (%) for $C_{20}H_{18}N_2O_4$): C 68.57, H 5.14, N 8.00; found (%): C 68.36, H 4.92, N 7.93.

Synthesis of 2,5-di(*o*-methylformanilide) 1,4-cyclohexanedione (13): Prepared as described for **1** except using acetoacetyl *o*-methylaniline. Yield: 75.2 %. White solid. m.p. 177.4-178.8 ºC. IR (KBr, ν_{max}, cm^{-1}): 3243, 3134, 1701, 1644,1542, 1400, 750. ^{1}H NMR (400 Hz, $CDCl_3$): δ^1 8.16 (s, 2H); 7.72-7.06 (m, 8H); 3.00-2.80 (t, 2H); 2.40-2.38 (d, 4H); 2.32 (s, 6H). Elemental analysis (anal. calcd. (%) for $C_{22}H_{22}N_2O_4$): C 69.84, H 5.82, N 7.41; found (%): C 68.68, H 5.92, N 7.24.

Synthesis of 2,5-di(*p*-methoxylformanilide) 1,4-cyclohexanedione (14): Prepared as described for **1** except using acetoacetyl *p*-methoxylaniline. Yield: 76.1 %. White solid. m.p. 172.4-173.4 ºC. IR (KBr, ν_{max}, cm^{-1}): 3292, 3132, 2937, 1730, 1650,1513, 1253, 1028, 830. ^{1}H NMR (400 Hz, $CDCl_3$): δ^1 8.43(s, 2H); 7.48-6.88 (m, 8H); 4.31-4.08 (t, 2H); 3.89-3.60 (d, 4H); 1.28 (s, 6H). Elemental analysis (anal. calcd. (%) for $C_{22}H_{22}N_2O_6$): C 64.39, H 5.37, N 6.83; found (%): C 63.98, H 5.29, N 6.80.

Synthesis of 2,5-di(*p*-methylformanilide) 1,4-cyclohexanedione (15): Prepared as described for **1** except using acetoacetyl *p*-methylaniline. Yield: 73.2 %. White solid. m.p. 152.5-154.6 ºC. IR (KBr, ν_{max}, cm^{-1}): 3289, 3129, 2937, 1731, 1404, 1052, 820, 793. ^{1}H NMR (400 Hz, $CDCl_3$): δ^1 8.44 (s, 2H); 7.54-6.92 (m, 8H); 4.14-4.10 (t, 2H); 3.89-3.76 (d, 4H); 2.50 (s, 6H). Elemental analysis (anal. calcd. (%) for $C_{22}H_{22}N_2O_4$): C 69.84, H 5.82, N 7.41; found (%): C 68.71, H 5.53, N 7.39.

Antimicrobial activity: The antibacterial activity of the synthesized compounds was tested against *Escherichia coli* and *Bacillus subtilis* using Mueller-Hinton medium, the antifungal activity of the compounds was tested against *Candida albicans* using RPMI-1640 mediun. The MICs (minimum inhibitory concentrations) of the test compounds were determined by a colorimetric method using the dye MTT[12,13]. Suspension of the microbes prepared to contain *ca.* 10^5 cfu/mL and applied to 96-well microplates with serially diluted compounds in DMSO to be tested and incubated at 37 ºC for 24 and 48 h for bacteria and fungi, respectively. After the MICs were visually determined on each of microtitration plates, 50 µL MTT solution was added to each well for 4-5 h. The supernatant of each well was removed and 100 µL of isopropanol containing 5 % 1 mol/L HCl was added to extract the dye. The optical density (OD value) was measured with a microplate reader at 550 nm.

RESULTS AND DISCUSSION

The MICs of the compounds against three bacterias are presented in Table-2. The compounds **1-10** and **13-15** also showed to be inactive against *Escherichia coli* and *Bacillus subtilis*. To the contrary, compounds **11** and **12** exhibited antimicrobial activity against the two bacteria strain with an MIC value of 24.2-33.6 µg/mL, which was comparable to the positive control kanamycin and penicillin. Although all the inhibiting activities were lower than the positive control, the activities of compounds **11** and **12** exhibited similar antibacterial activities with commercial antibiotics. Compounds **1-15** were also tested against *Candida albicans* which they had no antifungal activity.

TABLE-2
ANTIMICROBIAL ACTIVITY OF THE SYNTHESIZED COMPOUNDS

Compound	Minimum inhibitory concentration (µg/mL)		
	Escherichia coli	*Bacillus subtilis*	*Candida albicans*
1	>50	>50	>50
2	>50	>50	>50
3	>50	>50	>50
4	>50	>50	>50
5	>50	>50	>50
6	>50	>50	>50
7	>50	>50	>50
8	>50	>50	>50
9	>50	>50	>50
10	>50	>50	>50
11	26.7	33.6	>50
12	24.2	32.3	>50
13	>50	>50	>50
14	>50	>50	>50
15	>50	>50	>50
[a]Ketoconazole	>50	>50	4.2
[b]Kanamycin	4.5	1	>50
[c]Penicillin	>50	2	>50

[a,b,c]:Used as positive control.

Compounds **11** and **12** showed strong antimicrobial activities. According to structure-activity relationships, it is assumed that the non-substitutional group phenyl ring may play a key role. Other compounds showed no significant inhibition. This may be due to the large substituents hindering the compounds to permeate the cell membrane. Nevertheless, the biological activities of their potential metabolites seems to be worth studying.

Conclusion

We have synthesized a series of 1,4-cyclohexanedione-2,5-dicarboxylate derivatives using a successful method under mild conditions in a high yield. All the compounds were tested for their antibacterial (*Escherichia coli* and *Bacillus subtilis*) and antifungal (*Candida albicans*) activities by MTT method. It may be concluded that compounds **11** and **12** showed strong antimicrobial activities. The results may be useful to researchers attempting to gain more understanding of the antimicrobial activity of 2,5-substituted 1,4-cyclohexanedione compounds.

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REFERENCES

1. D.T.W. Chu, J.J. Plattner and L. Katz, *J. Med. Chem*., **39**, 3853 (1996).
2. K.M. Overbye and J.F. Barrett, *Drug Discov. Today*, **10**, 45 (2005).
3. L. Otvos Jr., J.D. Wade, F. Lin, B.A. Condie, J. Hanrieder and R. Hoffmann, *J. Med. Chem*., **48**, 5349 (2005).
4. H. Xin and K.A. Reynolds, *Antimicrob. Agents Chemother*., **46**, 1310 (2002).
5. Y. Cui, Y. Dang, Y. Yang, S. Zhang and R. Ji, *Eur. J. Med. Chem*., **40**, 209 (2005).
6. A.T. Nielsen, *Org. Synth*., **45**, 25 (1965).
7. A.J. Fatiad, *Synthesis*, 65 (1976).
8. K.J. Boosen, *Helv. Chim. Acta*, **60**, 1256 (1977).
9. S.X. Wang, M. Mure, K.F. Medzihradsky, A.L. Burlingame, R.A. Brown, D.M. Dooley, A.J. Smith, H.M. Kagan and J.P. Klinman, *Science*, **273**, 1076 (1996).
10. C. Basavaraja, N.R. Kim, H.T. Park and D.S. Huh, *Bull. Korean Chem. Soc*., **30**, 4907 (2009).
11. T. Bánsági Jr. and O. Steinbock, *Chaos*, **18**, 026102 (2008).
12. P. Cao, X.F. Huang, H. Ding, H.M. Ge, H.Q. Li, B.F. Ruan and H.L. Zhu, *Chem. Biodivers*, **4**, 881 (2007).
13. J. Meletiadis, J.F. Meis, J.W. Mouton, J.P. Donnelly and P.E. Verweij, *J. Clin. Microbiol*., **38**, 949 (2000).