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Synthesis of bis-chalcones based on 5, 5´-methylene bis(2-hydroxybenzaldehyde) and screening their antibacterial activity

Sogol Motallebi Tala-Tapeh [1], Nosratollah Mahmoodi[2,*], Ali Vaziri[3]

[1]Department of chemical Engineering, Science and Research branch, Islamic Azad university, Tehran Iran[2]Department of Chemistry, Faculty of Sciences, University of Guilan, Rasht, Iran, E-mail:mahmoodi@guilan.ac.ir
[3]Department of chemical Engineering, Science and Research branch,Islamic Azad university, Tehran Iran, E-mail:a.vaziri@srbiau.ac.ir

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Abstract

A one-pot synthesis of novel derivatives of bis-chalcones by condensation of prepared bis-aldehyde and different ketones in basic media have been described. Compounds were characterized by IR, ^{1}H NMR, ^{13}C NMR and EI-Mass spectra. The antibacterial activities of bis-chalcones were evaluated against Staphylococcus Aureus, Escherichia coli, Pseudomonas aeruginosa and Bacillus subtilis. Biological data indicated that some products exhibit promising activities. A significant reasonable reaction time, a nearly quantitative yield, and high atom economy in the product were observed. The simplicity of the reaction, ease of execution, simple workup, and good yields, with the use of easily accessible starting materials is hallmarks of this process.

Keywords: Bis-chalcones, Bis-aldehyde, Trioxane, Antibacterial.

1. Introduction

Chalcones (1,3-diaryl-2-propen-1-ones) are abundant in nature and have been synthesized during years possessing a variety of biological activities such as antimalarial [14-28], antifungal [24], antibacterial [25], anti-inflammatory [13-26], antitumor [29], inhibition of key enzymes [32], cytotoxicity [19], antiplatelet [17], antileishmanial [12], antiviral [15], inhibitor of colon cancer cell growth [4], Radical-scavenging [9], antidyslipidemic [23], anti-diabetic agents [5], vasorelaxant [31], antiprotozoal [8]. Chalcones are important and useful intermediates in synthesis of many heterocyclic compounds such as pyrimidine [6], pyrazole [18], pyrazoline [10], isoxazolidine [7], flavonoids [30], flavan [21], pyridine [11], N- phenylpyrazole [1], xanthenes [3].

2. Materials and equipments

2.1. Experimental

Chemicals were purchased from Merck. IR absorption band maxima were measured with a Shimadzu UV-2100 spectrophotometer. Melting points are uncorrected and determined using a Mettler Fp5 melting point apparatus.

[*] Corresponding Author: Email: sogol.motallebi@yahoo.com

All NMR data were recorded in DMSO-d6 using a Bruker Avance 400-MHz spectrometer. Chemical shifts are reported in ppm (δ) using deuterated solvents as internal references. EI- Mass spectra were recorded on 5973 Network Mass Selective Detector.

2.2. Synthesis of 5,5′-methylenbis (2-hydroxybenzaldehyde)

To a two-necked flask was added a well solved mixture of trioxane (0.3 g), AcOH (1 mL) and H_2SO_4 (0.02 mL, 4 drops), then 2-hydroxybenzaldehyde (30 mmol, 3 mL) and AcOH (5 mL) was added to this mixture and refluxed at 85 ˚C under N2 for 22 hours. A pink solution was formed during the reaction. After completion of the reaction, the mixture was poured into an ice-water mixture and kept at refrigerator for one night. The pink solid was filtered, dried and recrystallized from EtOH. Yield 60%, pink solid, m.p = 138-140°C, FT-IR (KBr cm-1): 3492 (O-H, Stretch), 2923, 1847 (C-H aliphatic, Stretch), 1651 (C=O, Stretch), 1480, 1440 (C=C, Stretch).

2.3. General process for synthesis of bis-chalcones (5a,b)

To a flask containing bis-aldehyde (1 mmol), corresponding ketone (2.2 mmol) and EtOH (4 mL) was added KOH 60% (4 mL) dropwise, during addition flask was put in an ice bath at 4-5 ˚C. After completion of the reaction the reaction mixture was monitoring by TLC (petroleum ether:EtOAc 6:3), the mixture was acidified by HCl 5%, product precipitate during acidifying. Solid was filtered, dried and washed with acetone.

2.4. 3,3′-(methylene bis(6-hydroxy-3,1-phenylene)) bis(1-(3-nitrophenyl)prop-2-ene-1-one) (5a)

Yield 78%, brown solid m.p = 189-192 °C. IR (KBr, cm^{-1}): 3400 (O-H, Stretch), 1620 (C=O, Stretch), 1560 (NO_2, asymmetric Stretch), 1489 (C=C, Stretch), 1350 (NO_2, Symmetric Stretch), 1260 (C-O, Stretch). Mass (m/z): $C_{31}H_{22}N_2O_8$, 552 (M_{+2}) (14%), 109 (100%).

2.5. 3,3′-(methylenebis(6-hydroxy-3,1-phenylen)) bis-(1-(furan-2-yl)prop-2-ene-1-one) (5b)

Yield 88%, orange solid, m.p = 175-184 °C. IR (KBr, cm-1): 3400 (O-H, Stretch), 1640 (C=O, Stretch), 1580 (C=C, Stretch), 1260, (C-O, Stretch). 1H NMR (400 MHz, DMSO-d6): δ; 10.24 (s, OH), 8.04-6.55 (m, 16H), 3.92 (s, 2H, CH2) ppm. 13C NMR (100 MHz, DMSO-d6): δ; 177.0 (C=O), 155.8, 153.1, 148.0, 147.6, 136.7, 128.0, 120.5, 118.7, 118.7, 116.3, 112.6, 42.0 (CH2) ppm.

General process for synthesis of bis-chalcones (5i-5j) A solution of bis-aldehyde (1 mmol), corresponding ketone (2.2 mmol), EtOH (7 mL), AcOH (4 drops) and piperidine (0.1 mL) was refluxed for 12 hours. Progress of the reaction was controlled by TLC (petroleum ether:EtOAc 6:3), after completion of the reaction the solid was filtered, dried and washed with EtOH.

2.6. 5,5′-((methylenebis(6-hydroxy-3,1-phenylene)) bis(methaneylidene))bis(imidazoleine-2,4-dione) (5c)

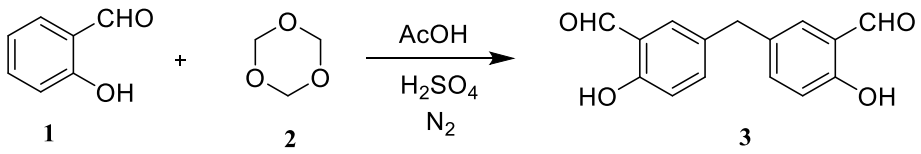
Yield 83%, brown solid, m.p= 208-210 °C. IR (KBr, cm-1): 3500, 3400, 3100 (2N-H, OH, Stretch), 2850 (C-H, Stretch), 1700, 1660 (C=O, Stretch), 1580, 1530, 1490 (C=C, Stretch), 1280 (C-O, Stretch). Mass (m/z): $C_{21}H_{16}N_4O_6$, 420 (M^+) (1.5%), 276 (100%).

2.7. 5,5′-((methylenebis(6-hydroxy-3,1-phenylene)) bis(methaneylidene))bis(pyrimidine-2,4,6(^1H,^3H,^5H)-trione) (5d)

Yield 87%, yellow solid, m.p= 101-104 °C. IR (KBr, cm-1): 3400 (O-H, stretch), 1670 (C=O, Stretch), 1600, 1490 (C=C, Stretch), 1250 (C-O, Stretch). Mass (m/z): $C_{23}H_{16}N_4O_8$, 478 (M^{+2}) (2.187), 262 (100%).

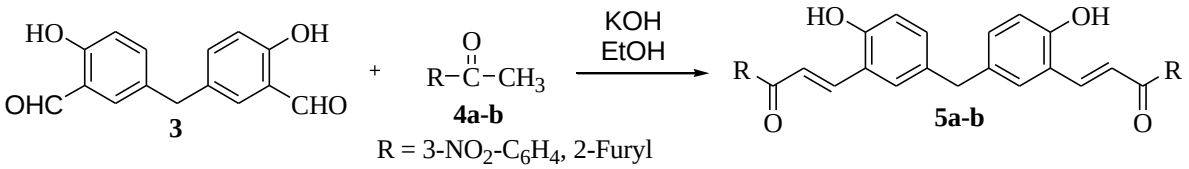
3. Results and discussion

In continuation to our previous works in synthesis of bis- and tris- compounds [2,20,33], in the present work we report synthesis of new derivatives of bis-chalcones possessing anti-bacterial activity. Bis-aldehyde 3 (5,5′-methylenbis(2-hydroxybenzaldehyde)) was prepared *via* the reaction of 2-hydroxy benzaldehyde 1 and trioxane 2 in presence of acetic acid under reflux and N_2 atmosphere [27] (scheme 1).



Scheme 1. Preparation of 5,5´-methylenbis(2-hydroxybenzaldehyde) 3.

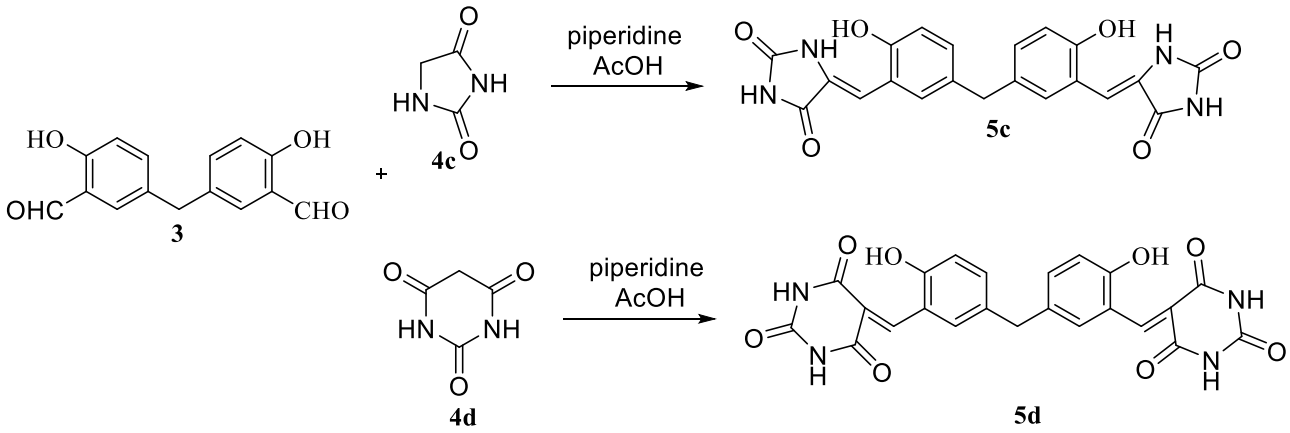
Bis-chalcones (5a,b) were synthesized by Claisen-Schmidt reaction of 1 mmol bis-aldehyde 3 and 2.2 mmol of ketone (4a,b) such as, 3-nitroacetophenone, 1-(furyl-2-yl)ethanone, , in the presence of KOH 60% at room temperature (Scheme 2).



R = 3-NO_2-C_6H_4, 2-Furyl

Scheme 2. Synthesis of bis-chalcones 5a and 5b.

In the first attempt the reaction of 1 mmol of bis-aldehyde 3 and 2 mmol of 2-furyl methyl ketone was preformed as a typical reaction, after completion of the reaction TLC showed small amount of bis- aldehyde together with final product, increasing the amount of ketone up to 2.2 mmol yielded pure final product. As far as we looked the synthesis of novel bis-chalcones such as 5,5´-((methylenebis(6-hydroxy-3,1-phenylene))bis(methaneylidene))bis(imidazoleine-2,4-dione) 5c and 5,5´-((methylenebis(6-hydroxy-3,1-phenylene))bis(methaneylidene))bis(pyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione 5d have not been reported until now. These two bis-chalcones 5c and 5d were synthesized *via* the reaction of bis-aldehyde 3 and hydantoin 4c or barbituric acid 4d respectively in EtOH solution at reflux condition (Scheme 3).



Scheme 3. Synthesis of bis-chalcones 5c and 5d.

All products were obtained as ultrapure colored solid powder with high yield. The structures of compounds were established by IR, ^{1}H NMR, ^{13}C NMR and mass spectroscopy. The NMR spectra of these products could not be recorded due to its low solubility in common deuterated solvents. These compounds are certainly polar due to their

acidic protons and zwitterionic character however for these compounds their mass spectra were recorded. The ^{1}H NMR spectra of bis-chalcones showed singlet at 10.24-9.80 ppm due to phenolic OH, protons of *α,β*–unsaturated appeared in aromatic region and CH_2 group appeared in 3.93-3.66 ppm. In the ^{13}C NMR spectra of bis-chalcones C=O appeared in 193.3-177.0 ppm and CH_2 appeared around 40 ppm, in most cases CH_2 peak joint with DMSO-d_6 signals. EI-Mass spectra of 5,5´-((methylenebis(6-hydroxy-3,1-phenylene))bis(methaneylidene))bis(imidazoleine-2,4-dione) 5c revealed exact mass while, mass spectra of 3,3´-(methylene bis(6-hydroxy-3,1-phenylene))bis(1-(3-nitrophenyl)prop-2-ene-1-one) 5a, and 5,5´-((methylenebis(6-hydroxy-3,1-phenylene))bis(methaneylidene)) bis(pyrimidine-2,4,6(1H,3H,5H)-trione) 5d revealed [M+2], [M-4] and [M+2], respectively.

The *in vitro* antibacterial activities of bis-chalcones were screened against Staphylococcus aureus (S. aureus) ATCC 29213 and Bacillus subtilis (MTCC 121) as gram-positive and Escherichia coli (E. coli) ATCC 25922 and Pseudomonas aeruginosa ATCC 27853 gram-negative bacteria. Gentamycin was used as model. According to the Table 1 all bis-chalcones were inactive against E. Coli. Compounds 5b-d showed antibacterial activity against Bacillus subtilis moreover, compound 5d had good antibacterial activity. Compounds 5a-d had good antibacterial activity against S. aureus and compound 5c had remarkable activity against S. aureus. Compounds 5b,5c showed moderate antibacterial activity against Pseudomonas aeruginosa.

Table 1. Antimicrobial activity of the compounds

Antimicrobial activity (zone of inhibition in mm)				
compound	Pseudomonas aureus aeruginosa	Escherichia coli	Staphylococcus aureus	Bacillus subtilis
5a	-	-	18	-
5b	8	-	17	8
5c	9	-	20	10
5d	-	-	17	20
DMSO	-	-	-	-
Gentamycin	21	22	23	20

3.1. Pharmacological Screening

Minimum inhibition concentration (MIC) was evaluated for compounds 5a-d against Staphylococcus aureus, Bacillus subtilis and Pseudomonas aeruginosa. Compound 5c and 5d were active against Staphylococcus aureus in minimum concentration of 32 µg/mL see Table 2.

Table 2. MIC of compounds 5a-d.

(minimum inhibition concentration(µg/ml)			
compound	Pseudomonas aureus Aeruginosa	Staphylococcus aureus	Bacillus subtilis
5a	-	32	-
5b	-	128	-
5c	-	32	-
5d	-	32	128

3.2. Antibacterial activity

A sterilized glass tube (5 mm diameter) was used aseptically to make wells on plates. The antibacterial activity of

compounds was assayed biologically using the Agar well-diffusion method. A colony of each standard test organism was sub-cultured in order to obtain fresh bacteria on the nutrient agar plates at 37 ˚C for 18 h. To preparations of suspensions of microorganisms (0.5 McFarland), one to two colonies from each plate was dissolved in isotonic saline solution. Then Mueller–Hinton agar (Merck) plates were prepared according to manufacturers' instructions in order to evaluate the antibacterial activities of compounds. The sterile Mueller–Hinton agar plates were inoculated with the bacteria. 0.001 g of test samples was dissolved in 1 mL dimethyl sulfoxide (DMSO) to obtain a stock solution. A concentration of 1 mg/mL or 100 μg/0.1 mL of each sample was prepared. 0.1 mL of prepared samples was dropped into each respective labeled well aseptically. The inoculated plates were left on the table for 1 h to allow the each sample to diffuse into the agar. Gentamycin was used as a positive control and DMSO as a negative control. Test organism growth may be affected by the inhibitory action of the test compound and so, a clear zone around the disc appeared as an indication of the inhibition of the test organism growth. The results of our tests were presented as the inhibition zones, given in millimeters (mm). Measurements were obtained after 24 h for bacteria.

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