ISSN (Online) 2249-6084 (Print) 2250-1029

International Journal of Pharmaceutical and Phytopharmacological Research (eIJPPR)
[Impact Factor – 0.7826]

Journal Homepage: www.eijppr.com

Research Article

Synthesis, *in vitro* Activity of Some New Class of Ethyl 2-oxo-4, 6-di (hetar-2-yl) cyclohex-3-encarboxylate

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Article info

Article History:
Received 07 May 2013
Accepted 10 July 2013

Keywords:
Green chemistry, Hetarylchalcones, Michael addition, Cyclocondensation, Cyclohexenone.

Abstract

In an attempt to find a new class antimicrobial and antifungal agent, series of hetaryl cyclohexenone from new series of heterocyclic chalcones via Micheal addition of ethyl acetoacetate [1] to 1, 3-Dihetarylprop-2-en-1-one [2] under solvent-free base catalyzed condensation were synthesized.

1. INTRODUCTION

The major challenges in organic and medicinal chemistry are the design, synthesis and production of compounds which are of high therapeutic nature.

Cyclohexenones are known to be efficient synthones in building intermediate in the synthesis of fused heterocyclic[1] such as benzothiadiazoles[1], benzisoxazoles and benzopyrazoles [2,3], or carbazole derivatives[4].

These units proved to have biological activity. On the other hand chalcones have drawn the attention of organic chemist due to biological activity such as antimicrobial and antifungal.

The majority of synthetic reactions are preformed in molecular solvents. In many instances however assuming that one of the reactant is solid, the same reaction can be undertaken by simple grinding. The procedure can provide for shorter reaction time, higher yields and conversions than reactions in conventional solvents [5].

In view of above considerations it was thought inters to the synthesis some new hetaryl cyclohexenones by solvent-free methodology as compared to conventional method.

2. MATERIALS AND METHODS

Melting points were taken on a Boëtius melting point microscope and are uncorrected, ^{1}HNMR analysis was conducted on a Mercury-300BB (300 MHz) instrument in DMSO-d_6 ; Infrared spectra were recorded on a Perkin Elmer FTIR-1710 spectrometer using KBr disc and Mass spectra on a Jeol JMS D-300 spectrometer operating at 75 eV.

2.1 General Procedure for the Synthesis of 1, 3-di (2-hetaryl) propenone [2] {2(a-e)}

Hetarylmethyl ketone (5 mmol), hetaryl aldehyde (5 mmol) and $NaOH_{(s)}$ (5mmol) were combined using a mortar and pestle, and the yellow medium was aggregated until a pale yellow powder was formed within10 min. The cross- aldol product was washed with water, dried and crystallized from ethanol; the product gave very pure and absolute yields.

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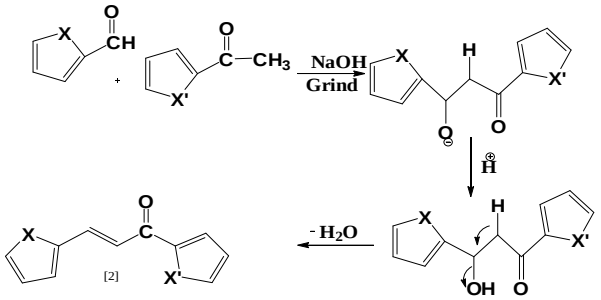
The base catalyzed cross-aldol condensation between a hetaryl aldehyde and hetaryl methyl ketone afforded the 1, 3-dihetarylprop-2-en-1-ones [2(a-e)] under solvent-less reaction medium, in excellent yields with high steroselectivity scheme(I)



(E)-1,3-di(hetar-2-yl)prop-2-en-1-one

X= S, O
X'= S, O, N

Fig.1: Scheme (I) cross-aldol condensation

2.2 General Procedure for the Synthesis of Cyclohexenone [3 (a-e)]

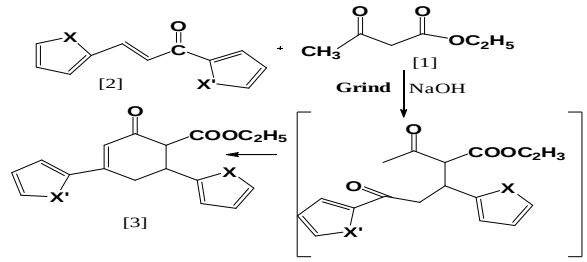
2.2.1 Conventional Procedure

Heterocyclic chalcone [2] (3 mmol) and ethyl acetoacetate [1] (3mmol) were refluxed for 2 h in 10-15 mL ethanol in the presence of 0.5 ml 10% NaOH. The reaction mixture was then poured with good stirring into 200 ml ice-cold water and kept at room temperature until the reaction product separated as a solid, filtration and recrystallization from ethanol gave compounds 3(a-d).

2.2.2 Solvent-free Methodology

Solvent-less organic reactions based on grinding together reactants involves the formation of a liquid phase prior to the reaction [6]. The liquid phase formed is an eutectic melt a mixture of a liquid and a solid phase that has a fine grain structure. Formations of the melt

explains the fast kinetics because the uniform distribution of the reacting components leads to put them in close proximity and are poised to react in a controlled way[7,8].
Compound [1] and [2] and NaOH were taken together in an equimolar ratio. The reagents were ground in a mortar using pestle. The mixture melted, turned to a pale yellow paste and then yellow powder within 5min. The reaction was monitored by TLC (Hexane: ethyl acetate:: 4:1). The workup of the product gave very pure and absolute yields.
The synthetic strategy focuses on the fact that acetoacetate[1] having and active methylene moiety undergoes Micheal cyclocondensation under base catalyze condition scheme (II) to give the cyclohexenones 3(a-d) under solvent free medium in quantitative yields as compared conventional methods.

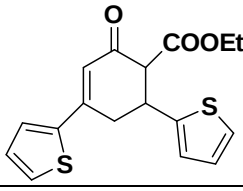
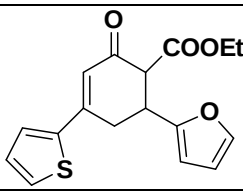
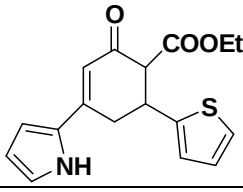
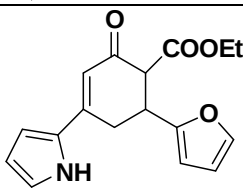
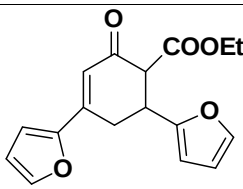


X= S, O
X'= S, O, N

Fig.2: Scheme (II) Micheal addition-cyclocondensation

3. RESULTS AND DISCUSSION

Table 1: Physical properties of Ethyl 2-oxo-4, 6-di (hetar-2-yl) cyclohex-3-encarboxalate

No.	Compound	mp°C	Yields		RF {Hexane: Ethyl acetate (4:1)}
			Conventional	Solvent-free	
3a		133	75.8	97.0	0.67
3b		125	70.4	95.0	5.1
No.	**Compound**	**mp°C**	**Yields**		**RF {Hexane : Ethyl acetate (4:1)}**
			Conventional	**Solvent-free**	
3c		195	65.1	93.1	0.39
3d		146	63.3	90.0	0.46
3e		oily	60.5	78.1	0.35

3.1 Spectral Data

Structure analysis for the elucidation of structures of the new Ethyl 2-oxo-4, 6-di (hetar-2-yl) cyclohex-3-encarboxylate 3(a-e) was comprised of IR, ^{1}HNMR and mass spectra.

3.2 Ethyl 2-oxo-4, 6-di (thiophen-2-yl) cyclohex-3-encarboxalate (A)

Yellow crystal, The reaction was monitored by TLC (hexane: ethyl acetate:: 4:1).
Rf=0.67. **^{1}H NMR** (DMSO-d_6, 300.0 MHz) δ 1.063(3H, t, J=6.8Hz, -CH_2CH_3), 2.488-2.512(1H, m, CHAr), 3.122 (1H, dd, -$CHCOOC_2H_5$), 3.274 (2H, m, CH_2CHAr), 4.039(2H, q, J=6.8Hz, CH_2CH_3), 6.427(1H, s, J=5.25Hz, =CH-CO), 6.942-6.971(2H, m,

HAr) 7.023 (1H, d, J=7.20Hz, HAr), 7.739(1H, t, J=6.96Hz,HAr), 7.755-7.852(2H, m, HAr).
FTIR (KBr, cm^{-1}) 1736.58($\nu_{C=O}$ ester), 1656.55($\nu_{C=O}$ketone), 1590($\nu_{C=C}$), 1206.26(ν_{C-S}), 825.31(ν_{C-H}). **MS: m/z** 332(M^+, 68.9%), 258(100%), 150(93.3%), 97(66.7%).

Elemental analysis Calculate $C_{17}H_{16}O_3S_2$**:** C 61.42, H 4.85, O 14.44, S 19.29

3.3 Ethyl 2-oxo-6-(furan-2-yl)-4-(thiophen-2-yl) cyclohex-3-encarboxalate (B)

Brown crystal, The reaction was monitored by TLC (hexane: ethyl acetate:: 4:1).
Rf=0.51**, ^{1}H NMR** (DMSO-d_6, 300.0 MHz) δ 1.101(3H, t, J=6.8Hz,-CH_2CH_3), 2.495-2.507(1H,m,CHAr), 3.270(1H, dd, J=1.50Hz, -$CHCOOC_2H_5$), 3.808-3.850(2H, m, J=1.37Hz, CH_2CHAr), 4.099(2H, q, J=6.8Hz, CH_2CH_3), 6.267(1H, d, J=6.30 Hz C_3H furan) 6.413(1H, s, =CH-CO), 7.201-7.593 (3H, m, HAr), 7.76(1H, t, J=7.38Hz,HAr), 7.854(1H, d, J=7.20Hz,HAr)
FTIR (KBr,cm^{-1}), 1732.0($\nu_{C=O}$ ester),1658.7($\nu_{C=O}$ketone), 1604.7($\nu_{C=C}$), 1161.1($\nu_{=C-O}$),729.0(ν_{C-H}), 686.6(ν_{C-S}) , **MS:m/z** 316(M^+,28.1%), 243(100%),121(37.1%), 65(17.7%). **Elemental analysis Calculate $C_{17}H_{16}O_4S$:** C 64.54, H 5.10, O 20.23, S 10.14

3.4 Ethyl 2-oxo-4-(1H-pyrrol-2-yl)-6-(thiophen-2-yl) cyclohex-3-encarboxalate

Yellow crystal, The reaction was monitored by TLC (hexane: ethyl acetate:: 4:1) .
Rf=0.39. **^{1}H NMR** (DMSO-d_6, 300.0 MHz) δ 1.061(3H , t, J=6.8Hz, -CH_2CH_3), 2.941-3.201(1H, m, CHAr), 3.301(dd, 1H, -$CHCOOC_2H_5$), 3.75-3.829(2H, m, -CH_2CHAr), 4.026(2H, q, J=6.8Hz, CH_2CH_3), 6.229 (1H, s, =CH-CO), 6.221(1H, t, J=6.96Hz,HAr), 6.777-7.681(4H, m, HAr), 7.81(1H, d, HAr), 11.611(1H, s, J=5.00Hz, H 2-pyrrol). **FTIR** (KBr, cm^{-1}),
3255(ν_{NH} stretching), 1730($\nu_{C=O}$ ester), 1641($\nu_{C=O}$ ketone), 1581($\nu_{C=C}$),
1110.92(ν_{C-S}) ,703(ν_{C-H}). **MS:m/z** 315(M^+,25.3%), 242(58.8%), 180(24.2%), 137(41.8%), 104(100%). **Elemental analysis Calculate $C_{17}H_{17}NO_3S$:** C 64.74, H 5.43, N 4.44, O 15.22

3.5 Ethyl 2-oxo-4-(1H-pyrrol-2-yl)-6-(furan-2-yl) cyclohex-3-encarboxalate

Yellow crystal, The reaction was monitored by TLC (hexane: ethyl acetate:: 4:1).
Rf=0.46,**^{1}HNMR** (DMSO-d_6, 300.0MHz) δ 1.29(3H , t, J=6.8Hz, -CH_2CH_3), 2.13-2.38(1H, m, CHAr), 3.56(1H,dd, -$CHCOOC_2H_5$), 3.73-3.89(2H, m,-CH_2CHAr), 4.21(2H, q, J=6.8Hz, CH_2CH_3), 6.05-6.17(3H, m, ,HAr), 6.40 (1H, s, =CH-COAr), 6.51(1H, d , J=6.30Hz, HAr), 6.95(1H, d, J=6.62Hz, HAr), 7.581(1H, t, J=7.38Hz,HAr), 11.30(1H, s, J=5.0Hz, H 2-pyrrol). **FTIR** (KBr, cm^{-1}),
3252(ν_{NH} stretching), 1728($\nu_{C=O}$ester), 1641($V_{C=O}$ ketone), 1583.45($\nu_{C=C}$), 1135.99(ν_{C-O}), 748.33(ν_{C-H}). **MS:m/z**:300(M^+,18.7%), 227(100.0%).
Elemental analysis Calculate $C_{17}H_{17}NO_4$**:** C 68.21, H 5.76, N 4.68, O 21.38.
IR spectra depicted in the region of the carbonyl stretching vibration two sharp absorption at $\nu_{max(KBr)}$ (1728-1736), (1641-1658) cm^{-1}, corresponding respectively to the ester and the vinylic carbonyls. The ^{1}H-NMR spectra substantiated the results of the IR analysis. The characteristic signals of an ethyl ester moiety δ (1.06-1.29) (3H,t)

3.6 Biological Activity for Ethyl 2-oxo-4,6-di(thiophen-2-yl) cyclohex-3encarboxalate (A) and Ethyl 2-oxo-6-(furan-2-yl)-4-(thiophen-2-yl) cyclohex-3-encarboxalate B

3.6.1 Antimicrobial and Antifungal Study Procedure
Antibacterial activities of all the compounds were studied against Gram-positive bacteria [Staphylococcus aureus (RCMB000108), Bacillis subtilis (RCMB000109)] and Gram negative bacteria [Pseudomonas aeruginosa (RCMB000103), Escherichia coli (RCMB000106)].

3.6.2 Antifungal Activity
The synthesized compounds were also screened for their antifungal activity against [Candida albicans (RCMB0005003), Aspergillus fumigates (RCMB002006), Geotrichum candidum(052008), Syncephlastrum racemosum (005004)] .
Mean zone inhibition in mm ± standard deviation beyond well diameter (6mm) produced on a range of environmental and clinically pathogenic microorganisms using (10mg\ml) concentration of tested samples.
The test was done using the diffusion agar technique, well diameter: 6mm …… (100µl was tested), (RCMB): Regional Center for Mycology and Biotechnology Culture Collection *NA: No Activity, data are expressed in the form of mean ± SD.
The table no. 2 and 3 represents the biological activity of compounds A and B.

Table 2: Antibacterial Activity for Ethyl 2-oxo-4,6-di(thiophen-2-yl) cyclohex-3encarboxalate (A) and Ethyl 2-oxo-6-(furan-2-yl)-4-(thiophen-2-yl) cyclohex-3-encarboxalate B

Test Organism	***Staphylococcus aureus***		***Bacillis subtilis***		***Pseudomonas aeruginosa***		***Escherichia coli***	
Standard used	Penicillin G	Streptomycin	Penicillin G	Streptomycin	Penicillin G	Streptomycin	Penicillin G	Streptomycin
Zone of Inhibition (mm)	30.1±0.06	28.1±0.07	31.6±0.05	29.7±0.06	28.3±0.08	25.2±0.09	33.1±0.09	29.7±0.07
Zone of Inhibition (mm) for Test Compounds								
A	17.3±0.3		19.9±0.05		NA		14.1±0.2	
B	19.4±0.2		20.2±0.08		11.3±0.06		14.9±0.2	

Table 3: Antifungal for Ethyl 2-oxo-4,6-di(thiophen-2-yl)cyclohex-3encarboxalate (A) and Ethyl 2-oxo-6-(furan-2-yl)-4-(thiophen-2-yl) cyclohex-3-encarboxalate B

Test Organism	*Aspergilluss fumigates*		*Geotrichum candidum*		*Candida albicans*		*Syncephlastrum racemosum*	
Standard used	Itraconazole	Clotrimazole	Itraconazole	Clotrimazole	Itraconazole	Clotrimazole	Itraconazole	Clotrimazole
Zone of Inhibition (mm)	27.4±0.05	26.3±0.08	24.2±0.09	23.2±0.03	25.2±0.07	20.8±0.02	23.9±0.04	21.4±0.05
Zone of Inhibition (mm) for Test Compounds								
A	13.2±0.09		10.2±0.1		9.4±0.2		NA	
B	19.4±0.03		16.3±0.2		15.2±0.09		12.8±0.05	

4. CONCLUSION

Synthesis of Ethyl 2-oxo-4,6-di(hetar-2-yl)cyclohex-3-encarboxylate by solvent-less reactions were given excellent yield. There have good antimicrobial activity and useful intermediates in the synthesis of structurally diverse heterocycles.

5. ACKNOWLEDGEMENT

The author is grateful to Professor Nadia.H.Metwally, Department of Chemistry, Faculty of Science, Cairo University-Egypt; for providing laboratory facilities and the access to carry the spectroscopic analysis.

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