SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF NOVEL CHALCONE DERIVATIVES

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Summary

Aiming at the development of antimicrobial agents, we have synthesized novel dimethylamino and ethoxy chalcones by condensing p-dimethylamino benzaldehyde and p-ethoxy benzaldehyde with acetophenone derivatives in dilute ethanolic sodium hydroxide solution at room temperature according to Claisen – Schmidt condensation. Structures of these compounds were elucidated by their IR, 'H NMR and Mass spectral analysis. The antimicrobial activity of the novel products was evaluated by Filter Paper Disc diffusion Method. The 1h and 1c showed excellent activity against *S. aureus* at both concentration i.e. 500µg/ml and1000 µg/ml.

Key Words: Chalcones, Antimicrobial activity.

Introduction

Chalcones are the aromatic ketones belonging to 1, 3-diaryl-2-propen-1-ones. Chalcones are also known as benzylideneacetophenones or phenyl styryl ketones. Naturally occurring chalcones belong to the flavanoids[1-3]. Chemically, they consist of open chain flavonoids in which the two aromatic rings are joined by a three carbon α,β unsaturated carbonyl system The presence of a reactive α,β unsaturated keto function in chalcones is found to be responsible for their antimicrobial activity[4]. Chalcones are one of the major classes of natural products with widespread distribution in fruits vegetables, spices, tea & soya based foodstuff and variety of trees and plants. Chalcones exist as either *E* or *Z* isomers. *E* isomer is the most stable form and consequently majority of chalcones are isolated as *E* isomer[5].

The compounds with the backbone of chalcones have been reported to possess various biological activities such as antimicrobial[6], anti-inflammatory, analgesic, anticancer antioxidant[7], antiulcerative[8], antimalarial, antileishmania[9], antifungal`[10], inhibition of chemical mediators release[11], inhibition of leukotriene B_4[12], inhibition of tyrosinase[13-14] and inhibition of aldose reductase[15] activities. Appreciation of these

findings motivated us to synthesize a novel series of chalcones as a potential template for antimicrobial agents. It must be noted that this scaffold provides substitution pattern on benzylidenacetophenones nucleus.

In the present communication we report the reaction of various acetophenone derivatives with different aromatic aldehyde derivatives to form chalcones (1a-j). The structures of the various synthesized compounds were assigned on the basis of IR, ^{1}HNMR and Mass spectral analysis. These compounds were also screened for their antimicrobial activity.

Experimental

The melting points were recorded in open sulphuric acid or oil bath using thermometer and were uncorrected. IR spectra (KBr disks) were recorded using Perkin-Elmer FTIR-RX_1 spectrophotometer. A ^{1}HNMR spectrum was recorded on Bruker Avance (400 MHz) spectrometer in $CDCl_3$ solutions, with tetra methyl silane (TMS) as internal standard. Mass spectra were recorded on a Waters Q-T of micro MS. Progress of the reactions was monitored using TLC, performed on aluminium plates precoated with silica gel-G, using chloroform: methanol (92:8) as the solvent systems and the spots were visualized by exposure to iodine vapors.

General Procedure

Chalcones were synthesized by base catalyzed Claisen-Schmidt condensation reaction of appropriately substituted acetophenones and aldehydes by known literature method[16].A mixture of benzaldehyde derivatives (0.01 mol) and acetophenone derivatives (0.01 mol) was dissolved in 10 ml rectified spirit in a 250 ml round-bottomed flask equipped with a magnetic stirrer. Then 10 ml NaOH solution (1g in 10ml H_2O) was added drop wise to the reaction mixture on vigorous stirring for 30 minutes when solution became turbid. The reaction temperature was maintained between 20-25˚C using a cold water bath on the magnetic stirrer. After vigorous stirring for 4-5 hours the reaction mixture was neutralized by 0.1-0.2N HCl whereby the precipitation occurred. On filtering off, the crude chalcone was dried in air and recrystallized by rectified spirit. The purity of the compounds was determined by TLC using chloroform: methanol (92:8) as the solvent systems. The chemical profile of the compounds is as shown in Table 1.

The physical and spectral data of chalcones are given below(1a-1j)

3-(4-dimethylamino phenyl)-1-(4-chloro phenyl)-2-propen-1-one (1a)

M.p.190-192°C, IR (KBr) cm^{-1}: 1649 (>C=O in conjugation with C=C), 1590,1552(>C=C< in conjugation with C=O), 744(-Cl); ^{1}HNMR ($CDCl_3$,400Hz),δ (ppm): 7.44(d, 2H, Ar 3', 5'H), 7.92 (d, 2H, Ar 2',6'H), 7.29(d,1H_a, J = 16 Hz, =CH), 7.80(d, 1H_b, J =16Hz, =CH), 7.54 (d, 2H, Ar 2",6"-H), 6.69 (d,2H, Ar 3", 5"- H), 3.04(s,6H,-$N(CH_3)_2$); Mass spectrum

(ESI),*m/z*: 286($M^{+\cdot}$ +1); Exact mass of molecular ion *m/z* = 285.0920, calculated for $C_{17}H_{16}ClNO$: 285.0921.

3-(4-dimethylamino phenyl)-1-(4-bromophenyl)-2-propen-1-one (1b)

M.p.220-222°C, IR (KBr) cm^{-1}: 1647 (>C=O in conjugation with C=C), 1563,1551(>C=C< in conjugation with C=O), 665(-Br); ^{1}HNMR ($CDCl_3$),δ (ppm): 7.62(d, 2H, Ar 3', 5'H), 7.87 (d, 2H, Ar 2',6'H), 7.28(d,1H_a, J = 16 Hz, =CH), 7.80(d, 1H_b, J =16Hz, =CH), 7.54 (d, 2H, Ar 2",6"-H), 6.69 (d,2H, Ar 3", 5"- H), 3.04(s,6H,-N(CH_3)$_2$;. Mass spectrum (ESI) *m/z*: 330($M^{+\cdot}$ +1) Exact mass of molecular ion *m/z* = 329.2188, calculated for $C_{17}H_{16}BrNO$: 329.2190.

3-(4-dimethylamino phenyl)-1-(4-iodophenyl)-2-propen-1-one (1c)

M.p.223-225°C, IR (KBr) cm^{-1}: 1644 (>C=O in conjugation with C=C), 1561,1548(>C=C< in conjugation with C=O), 560(-I); ^{1}HNMR ($CDCl_3$),δ (ppm): 7.72(d, 2H, Ar 3', 5'H), 7.85 (d, 2H, Ar 2',6'H), 7.29(d,1H_a, J = 16 Hz, =CH), 7.75 (d, 1H_b, J =16Hz, =CH), 7.54 (d, 2H, Ar 2",6"-H), 6.69 (d,2H, Ar 3", 5"- H) 3.04(s,6H,-N(CH_3)$_2$); Mass spectrum (ESI) *m/z*: 378($M^{+\cdot}$ +1) Exact mass of molecular ion *m/z* = 377.20, calculated for $C_{17}H_{16}INO$: 377.21.

3-(4-dimethylamino phenyl)-1-(4-methoxy phenyl)-2-propen-1-one (1d)

M.p.195-197°C, IR (KBr) cm^{-1}: 1632 (>C=O in conjugation with C=C), 1567,1529 (>C=C< in conjugation with C=O), 1166(-OCH_3); ^{1}HNMR ($CDCl_3$),δ (ppm): 3.78(s,3H,-OCH_3), 7.46(d, 2H, Ar 3', 5'H), 7.96 (d, 2H, Ar 2',6'H), 7.39(d,1H_a, J = 16 Hz, =CH), 7.82(d, 1H_b, J =16Hz, =CH), 7.63 (d, 2H, Ar 2",6"-H), 6.66(d,2H, Ar 3", 5"- H), 3.02(s,6H, -N(CH_3)$_2$); Mass spectrum (ESI) *m/z*: 283($M^{+\cdot}$ +2) Exact mass of molecular ion *m/z* = 281.1414, calculated for $C_{18}H_{19}NO_2$: 281.1415.

3-(4-dimethylamino phenyl)-1-(4-ethoxy phenyl)-2-propen-1-one (1e)

M.p.207-209°C, IR (KBr) cm^{-1}: 1630 (>C=O in conjugation with C=C), 1563,1520 (>C=C< in conjugation with C=O), 1048(-OC_2H_5); ^{1}HNMR ($CDCl_3$),δ (ppm): 1.30-1.32(t,3H,-CH_3), 4.03-4.08(q,2H,-OCH_2) 7.14(d, 2H, Ar 3', 5'H), 8.02 (d, 2H, Ar 2',6'H), 7.39(d,1H_a, J = 16 Hz, =CH), 7.82(d, 1H_b, J =16Hz, =CH), 7.65 (d, 2H, Ar 2",6"-H), 6.67(d,2H, Ar 3", 5"- H), 3.02(s,6H, -N(CH_3)$_2$); Mass spectrum (ESI) *m/z*: 296($M^{+\cdot}$+1) Exact mass of molecular ion *m/z* = 295.36, calculated for $C_{19}H_{21}NO_2$: 295.37.

3-(4-ethoxyphenyl)-1-(4-chlorophenyl)-2-propen-1-one (1f)

M.p.182-184°C, IR (KBr) cm^{-1}: 1658 (>C=O in conjugation with C=C), 1602,1510(>C=C< in conjugation with C=O), 1092 (-OC_2H_5), 813(-Cl); ^{1}HNMR ($CDCl_3$),δ (ppm): 7.45(d, 2H, Ar 3', 5'H), 7.95 (d, 2H, Ar 2',6'H), 7.36(d,1H_a, J = 16 Hz, =CH), 7.79(d, 1H_b, J =16Hz, =CH), 7.58 (d, 2H, Ar 2",6"-H), 6.91 (d,2H, Ar 3", 5"- H),1.40-1.44(t, 3H, -CH_3), 4.03-4.08 (q, 2H, -CH_2); Mass spectrum (ESI) *m/z*: 288($M^{+\cdot}$+2) Exact mass of molecular ion *m/z* = 286.0760, calculated for $C_{17}H_{15}ClO_2$: 286.0761.

3-(4-ethoxyphenyl)-1-(4-bromo phenyl)-2-propen-1-one (1g)

M.p. 212-215°C, IR (KBr) cm^{-1}: 1657 (>C=O in conjugation with C=C), 1584,1566(>C=C< in conjugation with C=O), 1070 (-OC_2H_5), 665(-Br); ^{1}HNMR ($CDCl_3$),δ (ppm): 7.64(d, 2H, Ar 3', 5'H), 7.88 (d, 2H, Ar 2',6'H), 7.36(d,1H_a, J = 16 Hz, =CH), 7.80(d, 1H_b, J =16Hz, =CH), 7.59 (d, 2H, Ar 2",6"-H), 6.92 (d,2H, Ar 3", 5"- H), 1.41-1.45(t, 3H, -CH_3), 4.05-4.10

(q, 2H, -CH_2); Mass spectrum (ESI) *m/z*: 331($M^{+\cdot}$+1) Exact mass of molecular ion *m/z* = 330.0254, calculated for $C_{17}H_{15}BrO_2$: 330.0255.

3-(4-ethoxyphenyl)-1-(4-iodo phenyl)-2-propen-1-one (1h)

M.p. 210-213°C, IR (KBr) cm^{-1}: 1652 (>C=O in conjugation with C=C), 1545,1520(>C=C< in conjugation with C=O), 1055 (-OC_2H_5), 560(-I); ^{1}HNMR ($CDCl_3$),δ (ppm): 7.88(d, 2H, Ar 3', 5'H), 7.66 (d, 2H, Ar 2',6'H), 7.36(d,1H_a, J = 16 Hz, =CH), 7.79(d, 1H_b, J =16Hz, =CH), 7.49 (d, 2H, Ar 2",6"-H), 6.94 (d,2H, Ar 3", 5"- H), 1.42-1.46(t, 3H, -CH_3), 4.03-4.08 (q, 2H, -CH_2); Mass spectrum (ESI) *m/z*: 379($M^{+\cdot}$+1) Exact mass of molecular ion *m/z* = 378.203, calculated for $C_{17}H_{15}IO_2$: 378.204.

3-(4-ethoxyphenyl)-1-(4-methoxyphenyl)-2-propen-1-one (1i)

M.p.186-188° C, IR (KBr) cm^{-1}: 1654(>C=O in conjugation with C=C), 1596,1571(>C=C< in conjugation with C=O),1171(-OCH_3), 1075 (-OC_2H_5); ^{1}HNMR ($CDCl_3$),δ (ppm): 3.85(s,3H,-OCH_3), 6.94 (d, 2H, Ar 3', 5'H), 8.03 (d, 2H, Ar 2',6'H), 7.43(d,1H_a, J = 16 Hz, =CH), 7.78 (d, 1H_b, J =16Hz, =CH), 7.58 (d, 2H, Ar 2",6"-H), 6.91 (d,2H, Ar 3", 5"- H), 1.40-1.43(t, 3H, -CH_3), 4.02-4.07 (q, 2H, -CH_2); Mass spectrum (ESI) *m/z*: 283 ($M^{+\cdot}$ +1) Exact mass of molecular ion *m/z* = 282.1253, calculated for $C_{18}H_{18}O_3$: 282.1255.

3-(4-ethoxyphenyl)-1-(4-ethoxy phenyl)-2-propen-1-one (1j)

M.p.195-198°C, IR (KBr) cm^{-1}: 1655 (>C=O in conjugation with C=C), 1596,1570(>C=C< in conjugation with C=O), 1045 (-OC_2H_5),1033(-OC_2H_5); ^{1}HNMR ($CDCl_3$),δ (ppm): 1.40-1.45(m, 6H, -CH_3), 4.03-4.12 (m, 4H, -CH_2), 6.96 (d, 2H, Ar 3', 5'H), 8.03 (d, 2H, Ar 2',6'H), 7.44(d,1H_a, J = 16 Hz, =CH), 7.78(d, 1H_b, J =16Hz, =CH), 7.59 (d, 2H, Ar 2",6"-H), 6.91 (d,2H, Ar 3", 5"- H); Mass spectrum (ESI) *m/z*: 297($M^{+\cdot}$+1) Exact mass of molecular ion *m/z* = 296.1410, calculated for $C_{19}H_{20}O_3$:296.1412.

Antibacterial Activity

Antimicrobial activity of all synthesized compounds were determined by disc diffusion method[17]. All human pathogenic bacteria viz *Staphylococcus aureus* (737), *Pseudomonas aeruginosa* (1688) were procured from Institute of Microbial Technology, Chandigarh. The agar medium was purchased from Hi-media Laboratories Ltd., Mumbai, India. Preparation of nutrient broth, subculture, base layer medium, agar medium and peptone water was done as per the standard procedure. Discs measuring 6.25 mm in diameter were punched from Whatman No.1 filter paper. Stock solutions of compounds diluted in dimethyl sulphoxide (1% DMSO) to give final concentration of 500µg/ml and 1000 µg/ml. A reference standard for both gram positive and gram negative bacteria was made by dissolving accurately weighed quantity of chloramphenicol (500 and 1000 µg/mL, respectively) in sterile distilled water, separately. The incubation was carried out at 37ºC for 24h. All the experiments were carried out in triplicate. Simultaneously, controls were maintained by employing 0.1 mL of dimethylsulfoxide which did not reveal any inhibition. Zones of inhibition produced by each compound was measured in mm. The results of antibacterial studies are given in Table 2.

Result and Discussion

The structures of synthesized compounds were confirmed by thin layer chromatography (TLC), mp, IR, ^{1}HNMR spectral analysis. The titled compounds were confirmed by IR spectral data showing sharp bands in the range between 1630-1660 cm^{-1} indicated the presence of C=O group. Compounds (1a-1k) were also confirmed by ^{1}HNMR spectral analysis. Inspection of the ^{1}HNMR spectra suggested that the chalcones were geometrically pure and configured trans (J_{Ha-Hb} = 16 Hz)

The results of the compounds of preliminary antibacterial testing are shown in Table 2. The results revealed that majority of the synthesized compounds showed varying degrees of inhibition against Gram positive bacteria. The compounds 1h and1c showed excellent activity against *S. aureus* at both concentration i.e. 500µg/ml and1000 µg/ml. The compounds 1g, 1f , 1j, 1b,1e & 1a have shown good to moderate activity against *S aureus* at both concentration i.e. 500µg/ml and 1000 µg/ml. Two of the chalcones with antistaphylococcal activity (1d &1i) gave no inhibitory zones probably due to their low diffusion potential into agar media.

Finally, no activity was observed for compounds against *P.aeruginosa,* a Gram negative organism. It is widely known that Gram positive and negative organisms have significantly different membrane compositions and architecture[18] which would explain the selective activity of the present compounds against Gram positive *S. aureus.*

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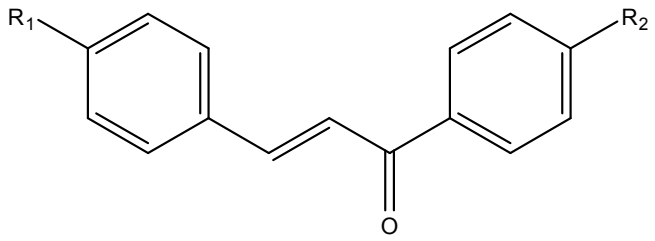
Table 1. Physical constants of the synthesized compounds.



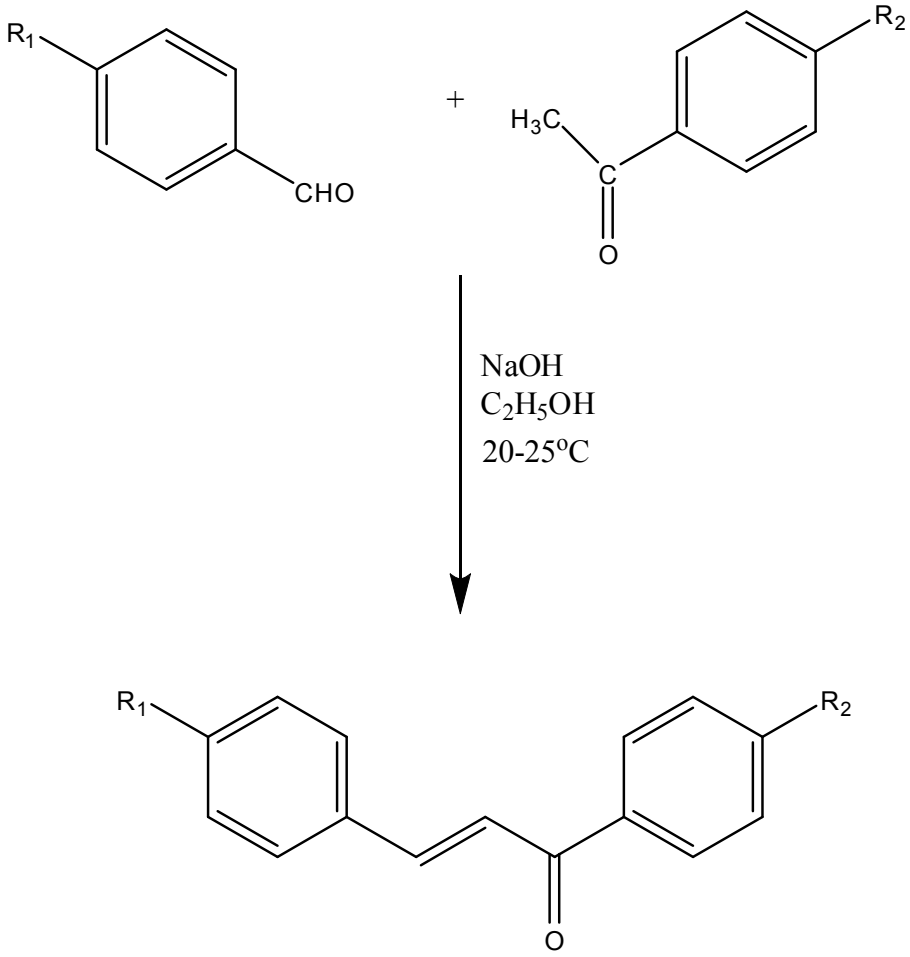
Comp Code	R_1	R_2	Molecular Formula	Molecular Wt	Reaction Time (hr)	Yield[a] %	mp(˚C)
1a	$-N(CH_3)_2$	Cl	$C_{17}H_{16}ClNO$	285.092	4	58	190-192
1b	$-N(CH_3)_2$	Br	$C_{17}H_{16}BrNO$	329.219	4	55	220-222
1c	$-N(CH_3)_2$	I	$C_{17}H_{16}INO$	377.211	5	53	221-223
1d	$-N(CH_3)_2$	$-OCH_3$	$C_{18}H_{19}NO_2$	281.141	4	18	195-197
1e	$-N(CH_3)_2$	OC_2H_5	$C_{19}H_{21}NO_2$	295.370	5	16	207-209
1f	$-OC_2H_5$	Cl	$C_{17}H_{15}ClO_2$	286.076	4	74	182-184
1g	$-OC_2H_5$	Br	$C_{17}H_{15}BrO_2$	330.025	4	72	212-215
1h	$-OC_2H_5$	I	$C_{17}H_{15}IO_2$	378.204	4	72	210-213
1i	$-OC_2H_5$	$-OCH_3$	$C_{18}H_{18}O_3$	282.125	4	68	186-188
1j	$-OC_2H_5$	OC_2H_5	$C_{19}H_{20}O_3$	296.141	4	67	195-198

[a] isolated yield

Table 2. Antimicrobial activity of the active compounds

Comp. No	Antimicrobial activity (%inhibition)			
	Staphylococcus aureus (737)		*Pseudomonas aeruginosa* (1688)	
	500 µg/mL	1000 µg/mL	500 µg/mL	1000 µg/mL
1a	27.1	38.6	-	-
1b	29.3	39.8	-	-
1c	31.8	43.9	-	-
1d	-	-	-	-
1e	25.4	35.9	-	-
1f	30.5	40.7	-	-
1g	31.4	43.1	-	-
1h	32.7	44.2		
1i	-	-	-	-
1j	26.7	37.8	-	-
Chloramphenicol	42.3	55.2	63.7	78.9
DMSO		1.4	-	1.2

Scheme 1. synthesis of Chalcones



References

1. Dhar DN, Chemistry of chalcones and related compounds, 1st ed., John Wiley, New York, 1981:5-9.
2. Roger CR. The nutritional incidence of flavonoids: some physiological and metabolic considerations. Experientia 1988;44:725-731.
3. Bent HH. The biochemistry and medical significance of the flavonoids. Pharmacology & Therapeutics 2002;96:67– 202.
4. Daniela B, Stoyan P, Bistra S, Mariana N, Hristo N. Examination of growth inhibitory properties of synthetic chalcones for which antibacterial activity was predicted.European Journal of Medicinal Chemistry 2009;44:2211-2218.
5. Nowakowska Z, K˛edzia B, Schroeder G. Synthesis, physicochemical properties and antimicrobial evaluation of new (E)-chalcones. European Journal of Medicinal Chemistry 2008;43: 707-713.
6. Anjani S, Kishor K, Ana C, Marina S, Irini D, Athina G, Synthesis of some new S-triazine based chalcones and their derivatives as potent antimicrobial agents. European Journal of Medicinal Chemistry 2010;45:510–518.
7. Babasaheb P, Shrikant SG, Ragini GB, Jalinder VT, Chandrahas NK. Synthesis and biological evaluation of simple methoxylated chalcones as anticancer, anti-inflammatory and antioxidant agents. Bioorganic & Medicinal Chemistry 2010; 18:1364–1370
8. Parmar NS, Parmar S. Anti-ulcer potential of flavonoids. Indian J Physiol Pharmacol 1998;42:343–351.
9. Mei L, Prapon WS, Croft L, Agnes LC, Mei L. Structure activity relationships of antileishmanial and antimalarial chalcones. Bioorganic And Medicinal Chemistry 2003;11:2729-2738.
10. Lahtchev KL, Batovska DI, , Parushev P, Ubiyvovk VM, Sibirny AA. Antifungal activity of chalcones: A mechanistic study using various yeast strains. European Journal of Medicinal Chemistry 2008;43:2220-2228.
11. Horng HK, LoTT, Kun L Y, Cheng TL, Jih PW, Chun NL. Structure–activity relationship studies on chalcone derivatives: the potent inhibition of chemical mediators release. Bioorganic & Medicinal Chemistry 2003;11:105-111.
12. Anastasia D, Maya M, Christos A, Dimitra HL, Panagiotis K, Natural and synthetic 20-hydroxy-chalcones and aurones: Synthesis, characterization and evaluation of the antioxidant and soybean lipoxygenase inhibitory activity. Bioorganic & Medicinal Chemistry 17 (2009) 8073–8085.
13. Ohad N, Ramadan M, Soliman K, Snait T, Jacob V. Chalcones as potent tyrosinase inhibitor: the effect of hydroxyl positions and numbers. Phytochemistry 2004;65: 1389–1395.
14. Soliman K, Ohad N, Ramadan M, Maayan S, Snait T, Jacob V. Chalcones as potent tyrosinase inhibitors: the importance of a 2,4-substituted resorcinol

moiety. Inorganic & Medicinal Chemistry 2005;13:433–441.
15. Fabio S, Stefania B, Luca C, Gabriella V, Michele M, Luciano A. Synthesis and activity of a new series of chalcones as aldose reductase inhibitors. European Journal of Medicinal Chemistry 1998;33(11):859-866.
16. Ahmed MG, Romman UKR, Ahmed SM, Akhter K, Halima ME. Synthesis and Correlation of Spectral Properties of Some Substituted 1,3-Diphenyl-2-Propen-1-Ones. Bangladesh J Sci. Ind. Res. 2007;42:45-49.
17. Berry A, Corian V. Antibiotics in Laboratory Medicine. Williams and Wilkins, Baltimore 1991:1-16.
18. Papo N, Shai Y. A novel lytic peptide composed of dl-amino acids selectively kills cancer cells. Peptides 2003;24:1693-1703.