Indian Journal of Chemistry
Vol. 47B, June 2008, pp. 927-931

Note

Microwave studies on synthesis of biologically active chalcone derivatives

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Received 16 October 2006; accepted (revised) 29 January 2008

Synthesis of pyrazole derivative using microwave energy has been carried out. The compound 1-[4-(2-hydroxy-ethoxy)-phenyl]-ethanone, is prepared from *p*-hydroxy-acetophenone and 2-chloro-ethanol. Chalcone derivative has been prepared by the condensation reaction of 1-[4-(2-hydroxy-ethoxy)-phenyl]-ethanone with 4-chlorobenzaldehyde. This chalcone derivative cyclised with hydrazine hydrate and glacial acetic acid under microwave irradiation conditions give pyrazole derivative. These products have been characterized by detailed spectral analysis and have been screened for their antimicrobial activity, against *Escherichia coli*, *Proteus vulgaris*, and *Salmonella typhimurium*.

Keywords: Antimicrobial activity, chalcones, pyrazole

Pyrazole is a class of compounds, which has many applications in different field. One of the method for the synthesis of such compound is from α,β-unsaturated carbonyls (chalcone) by the cyclization with hydrazine and substituted hydrazines.

The chemistry of chalcones has generated intensive scientific interest due to their biological and industrial applications. Chalcones are natural biocides[1-4] and are well known intermediates in the synthesis of heterocyclic compounds exhibiting various biological activities[4-8]. Chalcones and their derivatives possess some interesting biological properties such as anti-bacterial[9], antifungal[10], insecticidal[11], anaesthetic[12], antiinflammatory, analgesic, ulcerogenic[13], *etc*.

Pyrazole and their derivatives are considered to be important for drugs and agricultural chemicals. Some substituted pyrazoles and their derivatives have been reported to possess several interesting biological activities such as hypnotic properties, antimicrobial, antitumor and antifungal. Many pyrazoles are used for the treatment of thyroid and leukaemia. It has incidental antiviral activity against Herpes and Vaccinia infections.

Experimental Section

Preparation of potassium salt of *p*-hydroxy aceto-phenone

To an ethanolic solution of KOH (5.4 g in 72 mL), *p*-hydroxy acetophenone (10.0 g) was added with stirring. The solution was stirred at room temperature for 1 hr and concentrated under reduced pressure. Diethyl ether (40 mL) was added to it. Solid of potassium salt of *p*-hydroxy acetophenone (11.0 g) was separated out. It was washed with diethyl ether and kept for drying in a desiccator under vacuum.

O-Alkylation

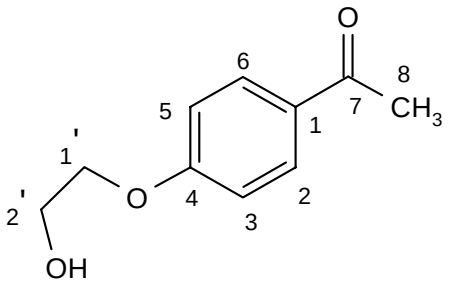
2-Chloroethanol (5.52 mL) was added in potassium salt of *p*-hydroxy acetophenone (10.0 g) suspended in dry DMF (20 mL). The contents of the flask were refluxed in the oil bath at 80-90°C for 18 hr under anhydrous conditions. The reaction was monitored by thin layer chromatography. After the removal of DMF under vacuum, viscous liquid was yielded. Purification of the product was carried out by passing through column using silica gel with hexane: ethyl acetate (4:3) solvent system. The compound on repeated crystallization with ethanol gave white crystals (**1**, 5.50 g, 53.19%).

Preparation of chalcone

To a solution of compound **1** (5.0 g, 0.01 mole) and 4-chloro benzaldehyde, in ethanol (20 mL), a catalytic quantity of sodium hydroxide was added. The reaction required 50 seconds in microwave unit. The reaction was monitored by TLC and it was kept at room temperature and then cooled in an ice bath. After filtration, the product was washed with ethanol (5 mL) followed by distilled water, dried and crystallized from ethanol to yield a pure compound **2**.

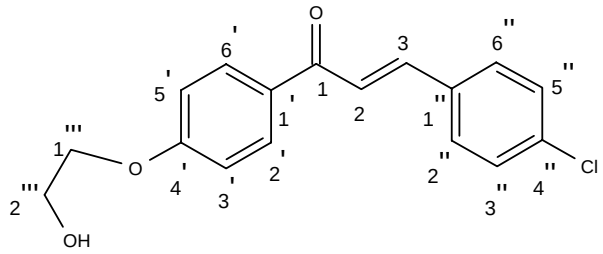
Preparation of chalcone derivative (pyzoline)

To the compound **2** (2.0 g, 0.01 mole) in glacial acetic acid (10 mL), hydrazine hydrate (99%,



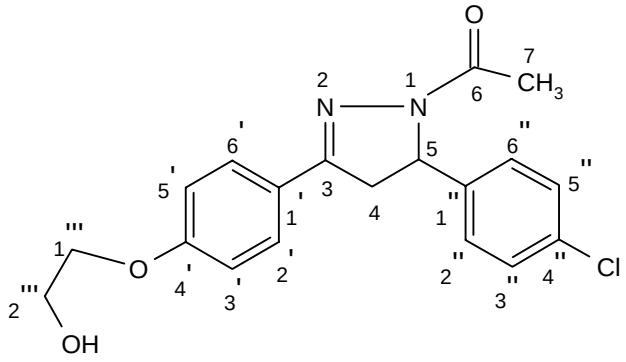
1

1-[4-(2′-hydroxy-ethoxy)-phenyl]-ethanone



2

3-[4"-Chloro-phenyl)-1-[4′-(2‴-hydroxy-ethoxy)-phenyl]-propenone



3

1-{5-(4"-Chloro-phenyl)-3-[4′-(2‴-hydroxy-ethoxy)-phenyl]-4,5dihydro- pyrazol-1yl}-ethanone

0.015 mole) was added. Kept in microwave for 120 seconds. The mixture was kept at room temperature and cooled. The resulting solid was filtered, washed with distilled water, dried and crystallized from ethanol to yield white crystals of chalcone derivative **3** (0.850 g, 42%, **Scheme I**).

Biological Assay

Bacterial strains

Strains of *Pseudomonas aeroginosa* (resistant to piperacilline, ceftazidime, cefepime and gentamicine), *Staphylococcus aureus* (resistant to oxacilline), *Aspergillus niger*, *Escherichia coil*, *Proteus vulgaris*, *Bacillus subtilis*, and *Salmonella typhimurium* were used. The bacterial strains were clinical isolates from the National Collection of Industrial Microorganisms (NCIM), Biochemical Sciences Division, National Chemical Laboratory, Pune. Bacterial strains were maintained on MH Agar. For inoculums preparations, bacteria were sub cultured in peptone water, at 37ºC for 18 hr. The total viable count of each culture was average 1×10^{12}/mL.

Assay for inhibition of bacterial growth:

The antimicrobial activity of compounds **1**, **2** and **3** were determined by the "hole-plate diffusion method"[9]. The tested bacterial suspensions were homogenously seeded onto petri dishes containing 15 mL of the MH agar medium. Holes were aseptically bored into the agar with a hallow punch and 25 µL aliquots of the extract were placed into wells with sterile pipette. The plate was kept for 2 hr at room temperature for the diffusion of the extract into the agar. Bacterial growth inhibition was determined as the diameter of the inhibition zones around the holes. Ethyl acetate was used as a solvent for dilution of the extracts.

Determination of Minimum Inhibitory Concentration (MIC)

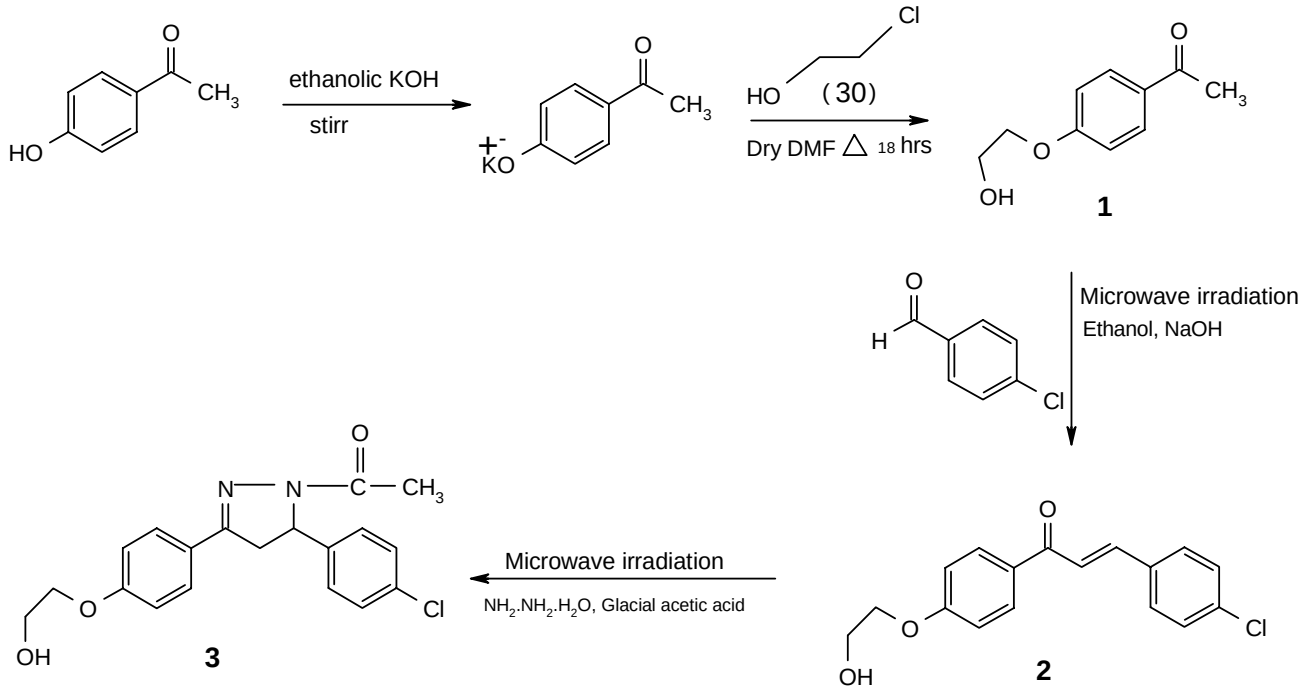
The plates were incubated at 37ºC for 18 hr. The MIC was considered the lowest concentration of the sample that prevents visible growth.

Results and Discussion

Compound 1

Compound **1**, 1-[4-(2′-hydroxy-ethoxy)-phenyl]-ethanone, a white crystalline solid, was purified by crystallization using ethanol; m.p. 75.0°C; In IR spectrum showed frequencies at 3409.9 (-OH), 1602.7 (Phenyl ketone), 1652.9, 1571.7 (aromatic region), 1253, 1178.4, 1078.1 (-C-O stretching) cm^{-1} and 846.7 (*p*-disubstituted aromatic ring); ^{1}H NMR spectrum showed characteristic *doublets* of *para* disubstituted aromatic protons at δ 7.90 (*d*, *J* = 10 Hz, 2H) for H-2 & H-6 and at δ 6.92 (*d*, *J* = 8 Hz, 2H) for H-3 & H-5 positions. The signals at δ 4.14 (*dd*, *J* = 2 Hz, 4 Hz, 2H) and δ 3.98 (*dd*, *J* = 4 Hz, 4 Hz, 2H) were assigned for H-1′ and H-2′ of etheral side chain of aromatic ring respectively. An intense peak at δ 2.54 (*s*, 3H) for acetoxy methyl group. A singlet appears at δ 2.54 for one proton of hydroxy function, confirmed the product formed.

The ^{13}C NMR spectrum of compound **1** showed 10-carbon atoms. Multiplicities of carbon signals



Scheme I

were determined by the DEPT plus sequence. Compound showed the presence of four CH, two CH_2, one CH_3 and (by difference) three quaternary carbons. The signal appeared at δ 196.77 (*s*) for C-7 carbonyl carbon in conjugation with aromatic ring 162.54 (*s*) and 130.41 (*s*) for C-1 and C-4 carbon of aromatic ring 130.56 (*d*) and 114.11 (*d*) have been assigns for aromatic carbons of C-2 and C-6 and C-3 & C-5 respectively. The signals at δ 69.36 (*t*) allocated for C-1′ methylene attached to ethereal oxygen atom and at δ 61.02 (*t*) for C-2′, attached to hydroxy group of side chain respectively. A quartet appears at δ 26.24 has been assigned for acetoxy methyl group.

Compound 2

Compound **2**, 3-[4-cloro-phenyl)-1-[4-(2-hydroxy-ethoxy)-phenyl]-prop-2-enone, a yellow crystalline solid, was purified by crystallization using ethanol; m.p. 150.0°C; elemental analysis for $C_{17}H_{15}O_3Cl$ requires: C, 67.44; H, 4.95; Cl, 11.75% and Found : C, 66.53 ; H, 4.77; Cl, 12.13%, In IR spectrum showed characteristic absorption bands at 3450.0 (hydroxy group), 3010 (olefinic –C-H stretching frequency), 1656.7 (α, β unsaturated ketone in conjugation with aromatic ring), 1602.7 (α, β unsaturated double bond in conjugation with ketone and aromatic ring), 1600.0, 1580.0, 1566.1 and 1500.0 (aromatic region), 1259.4, 1222.8, 1176.5, 1080.1, 1028.0 and 1020.0 (-C-O stretching), 819.7 (paradisubstituted aromatic rings) and 505.3 (-C-Cl) cm^{-1}. In ^1H NMR spectrum showed characteristic *doublets* of *para* disubstituted aromatic protons at δ 8.04 (*d*, *J* = 8 Hz, 2H) for H-2′ & H-6′ and at δ 7.01 (*d*, *J* = 8 Hz, 2H) for H-3′ & H-5′ and δ 7.58 (*d*, *J* = 8 Hz, 2H) for H-2″ & H-6″ and at δ 7.39 (*d*, *J* = 8 Hz, 2H) for H-3″ & H-5″. Signals shows at δ 7.76 (*d*, *J* = 16 Hz, 1H) and δ 7.51 (*d*, *J* = 16 Hz, 1H) have been assigned for H-3 olefinic proton attached to aromatic ring and for H-2 olefinic proton attached to carbonyl carbon respectively. This shows *trans* nature of olefinic bond. The signals at δ 4.19 (*dd*, *J* = 6 Hz, 4 Hz, 2H) and at δ 4.02 (*dd*, *J* = 4 Hz, 8Hz, 2H) assigned for H-1‴ and H-2‴ of etheral side chain of aromatic ring respectively.

The ^{13}C NMR spectrum of compound **2** shows 17-carbon atoms. Multiplicities of carbon signals were determined by the DEPT plus sequence. Compound shows the presence of ten CH, two CH_2, and (by difference) five quaternary carbons. The signals appeared at δ 185.0 (*s*) for C-1 carbonyl carbon in conjugation with aromatic ring, δ 130.92 (*s*) & δ 153.44 (*s*) for C-1′ & C-4′ carbon aromatic ring, δ 130.0 (*d*) and 114.73 (*d*) have been assigns for aromatic carbons of C-2′ & C-6′ and C-3′ & C-5′

respectively. The signals at δ 69.29 (*t*) are allocated for C-1′′′′ methylene attached to ethereal oxygen atom and at δ 61.32 (*t*) for C-2′′′ attached to hydroxy group respectively. Signals at δ 129.54 (*d*) and δ 129.23 (*d*) are assigned for aromatic carbons of C-2′′ & C-6′′ and C-3′′ & C-5′′ respectively. Two singlets observed at δ 134.0 (*s*) and 137.0 (*s*) represent the tetrasubstituted aromatic carbons for C-1′′ and C-4′′ positions of chlorophenyl ring.

Compound 3

Compound **3** 1-{5-(4′′-chloro-phenyl)-3-[4′-(2′′′-hydroxy-ethoxy)-phenyl]-pyrazol-1yl}-ethanone, a white crystalline solid, $C_{19}H_{19}O_3N_2Cl$ was purified by crystallization in ethanol; m.p. 176.0°C; elemental analysis $C_{19}H_{19}O_3N_2Cl$ requires: C, 63.60; H, 5.30; N, 7.81; Cl, 9.90. and Found: C, 62.89; H, 5.03; N, 7.90; Cl, 11.59%. IR spectrum showed characteristic absorption bands at 3361.7 (hydroxy group), 3010 (olefinic –C-H stretching frequency) 1656.7 (acetyl carbonyl), 1604.7 (α, β unsaturated double bond in conjugation with aromatic ring), 1633.7, 1568.0 and 1519.8 (aromatic region), 1257.5, 1224.7, 1180.4, 1020.0 (-C-O stretching) and 846.7 (*para* disubstituted aromatic rings) and 545.8 (-C-Cl) cm^{-1}. ^1H NMR spectrum showed characteristic doublets of *para* substituted aromatic protons at δ 7.78 (*d*, *J* = 8 Hz, 2H) for H-2′ & H-6′ and at δ 7.07 (*d*, *J* = 10 Hz, 2H) for H-3′ & H-5′ and at δ 7.39 (*d*, *J* = 8 Hz, 2H) for H-2′′ & H-6′′ and at δ 7.28 (*d*, *J* = 8 Hz, 2H) for H-3′′ & H-5′′reapectively. The signal appeared at δ 4.02 (*dd*, *J* = 2 Hz, 4 Hz, 2H) and at δ 4.15 (*dd*, *J* = 4 Hz, 4 Hz, 2H) assigned for H-1′′′ and H-2′′′ of etheral side chain of aromatic ring respectively. Signals observed at δ 3.20 (*dd*, *J* = 4Hz, 6 Hz, 2H) and δ 5.63 (*dd*, *J* = 4Hz, 6 Hz, 1H) have been assigns for H-4 and H-5 protons of pyrazol ring respectively. An intense peak at δ 2.51 (*s*, 3H) is acquire for acetoxy methyl group. A broad singlet appears at δ 2.27 for one proton of hydroxy function.

The ^{13}C NMR spectrum of compound **3** shows 19-carbon atoms. Multiplicities of carbon signals were determined by the DEPT plus sequence. Compound shows the presence of nine CH, three CH_2, one CH_3 and (by difference) six quaternary carbons. The signals appeared at 153.42 (*s*) for C-3 tetrasubstituted carbon of pyrazol ring which is conjugation with aromatic ring, δ 124.18 (*s*), 160.4 (*s*) for C-1′ & C-4′ carbon aromatic ring, δ 127.11 (*d*) and 114.84 (*d*) are assigns for aromatic carbons of C-2′ & C-6′ and C-3′ & C-5′ respectively. From dept spectrums, presence of three methylene carbon is confirms. Out of three, the signals observed at δ 69.46 (*t*) are allocated for C-1′′′ methylene attached to ethereal oxygen atom and at δ 61.28(*t*) for C-2′′′ attached to hydroxy group respectively. A methylene signal notice at δ 42.30 (*t*) for C-4 position of pyrazol ring. Signals at δ 129.01 (*d*) and δ 128.24 (*d*) are assigns for aromatic carbons of C-2′′ & C-6′′ and C-3′′ & C-5′′ respectively. Two singlets are observed at δ 133.40 (*s*) and 140.45 (*s*) represents the tetrasubstituted aromatic carbons for C-1′′ and C-4′′ positions of chlorophenyl ring. The signal is appears at δ168.74 (*s*) for C-6 carbonyl carbon attached to pyrazol ring, signal was appeared δ 59.33 (*d*) assigns for C-5 position of pyrazol ring. A methyl signal was notice at 21.82 (*q*) acquire for C-7 position of acetyl group.

Biological activity

Compounds **1**, **2** and **3** were tested for antimicrobial activity against *E. coli*, *Salmonella typhimurium*, *Staphylococcus aureus*, *Bacillus subtilis*, *Proteus vulgaris*, *Pseudomonas aeroginosa*, *Aspergillus niger* (**Table I**). The results revealed that the growth of bacterial strains against *Escerichia coli, Salmonella*

Table I — Antimicrobial activity of compounds **1**, **2** and **3**

Compd	Concentrations (mg/mL)	M.I.C.- Zone*(mm) details	Active Organism
1	2.00, 4.00, 6.00, 8.00 and 10.00	8 mm for 10.00 mg/mL	*Escerichia coli, Salmonella typhimurium* and *Proteus vulgaris*
2	2.00, 4.00, 6.00, 8.00 and 10.00	7 mm for 10.00 mg/mL	*Escerichia coli, Salmonella typhimurium* and *Proteus vulgaris*
3	2.00, 4.00, 6.00, 8.00 and 10.00	8 mm for 10.00 mg/mL	*Escerichia coli, Salmonella typhimurium* and *Proteus vulgaris*

*The values for zone diameter are given in brackets and are measured excluding the diameter of the disc.

typhimurium and *Proteus vulgaris* get affected by compounds **1**, **2** and **3** which is seen by inhibition zone of 8, 7 and 8 mm. respectively.

Conclusion

Synthesis of chalcone **2** and pyrazole derivative **3** from ketone **1** by microwave irradiation were carried out. These compounds shows antimicrobial activity against *Escerichia coli, Salmonella typhimurium* and *Proteus vulgaris*.

Acknowledgement

Thanks are due to the Principal and Head of the Department of Chemistry, Sir Parshurambhau College, Pune, India for co-operation

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