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Solvent-Free Synthesis of Chalcones and Antioxidant Activity

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ABSTRACT

Claisen-schmidt base catalyzed solvent-free condensation reaction of 4'-amino acetophenone with different substituted benzaldehyde afford some substituted 4'-amino chalcones using grinding method. The method is suitable in terms of short reaction time, ease of reaction procedure, no need of catalyst, giving better yield of desired product. Newly synthesized chalcones screened for testing antioxidant activity.

1. Introduction

The diverse nature of chemical / catalyst, regents and reaction solvent universe requires simple, efficient and solvent-free synthetic protocols for biologically active compounds [1-4]. For these purposes emerging area of green chemistry play an important role into development of synthetic strategic [5, 6]. The main criterion of green synthetic chemistry is to reduce minimum hazardous and make simple reaction procedure while design new synthetic molecules [7]. Many researchers have reported grinding mode solvent-free synthetic route for some well-known reaction such as Grignard reactions [8], Reformatsky reactions [9], Aldol condensations [10], Dieckmann condensations [11], Reductions [12], and others [13, 14]. Most of these reactions are carried out at room temperature in absence of solvent-free environment, using only a mortar and pestle. As part our interest towards synthesis of chalcones due to its broad spectrum biological activity including antimalarial, antibacterial, antifibrogenic, anticancer, antitrichomonal, antinflammatory, antileishmanial, cytotoxic and anti-*Trypanosoma cruzi* activities [15-22]. In present communication we reported solvent-free synthesis of some novel 1,3-diaryl-2-propen-1-one the reaction of 4-amino acetophenone and substituted benzaldehyde under grinding technique in presence of mild basic condition without using any organic solvent (Scheme 1).

2. Experimental Methods

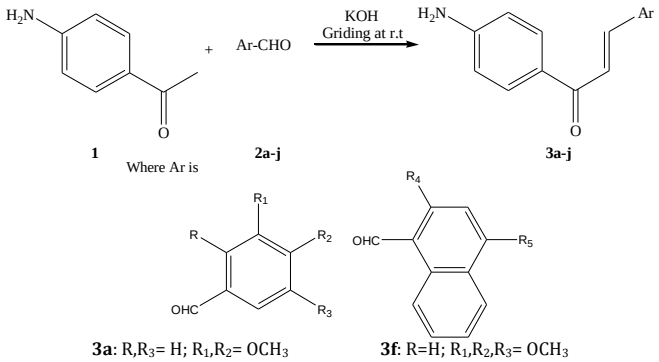
2.1 Materials

Melting points were determined in an open capillary tube and are uncorrected. IR spectra were recorded in KBr on a Perkin-Elmer spectrometer. ^{1}H NMR spectra were recorded on a Gemini 300-MHZ instrument in DMSO as solvent and TMS as an internal standard. The mass spectra were recorded on EISHIMADZU-GC-MS spectrometer. Elemental analyses were performed on a Perkin-Elmer 240 CHN elemental analyzer. The reactions were carried out in open glass mortar and pestle. Purification of the compound indicated using TLC (ethyl acetate / hexane as mobile phase).

2.2 Typical Procedure for Synthesis of 4'-Amino Chalcones

*Preparation of 1-(4-Amino-phenyl)-3-(3,5-dibromo-2-hydroxy-phenyl)-propenone: **3d***

4'-aminoacetophenone (0.01 mmol) and 2-hydroxy-3,5-dibromobenzaldehyde (0.01 mmol) and solid pallete of KOH (0.02 mmol) were taken in morter and grind for several minute (Table-1). On completion of grinding as monitored by TLC, the obtained solid mixture was diluted with cold water, acidified by dil HCl and neutralize with acetic acid (0.002 mmol). The resultant crude solid product on crystallization using ethyl alcohol to give pure 1-(4-Amino-phenyl)-3-(3,5-dibromo-2-hydroxy-phenyl)-propenone **3d**. Similarly other derivatives of substituted 4'-amino chalcones were synthesized.



3a: R,R$_3$= H; R$_1$,R$_2$= OCH$_3$
3b: R= OH; R$_1$,R$_2$,R$_3$= H
3c: R= OH; R$_1$,R$_3$= I; R$_2$= H
3d: R= OH; R$_1$,R$_3$= Br; R$_2$= H
3e: R= OH; R$_1$,R$_2$= H; R$_3$= Cl
3f: R=H; R$_1$,R$_2$,R$_3$= OCH$_3$
3g: R,R$_1$,R$_3$= H; R$_2$= (NCH$_3$)$_2$
3h: R,R$_1$,R$_3$= H; R$_2$= Cl
3i: R$_4$,R$_5$= H
3j: R$_4$= OCH$_3$; R$_5$= H

Scheme 1 Solvent-free synthesis of some novel 4'-amino chalcones

*1-(4-Amino-phenyl)-3-(3,4-dimethoxy-phenyl)-propenone (**3a**):*

Yellow crystalline solid. M.P. 122 °C. Yield 70 %. FT-IR (KBr, ν, cm^{-1}): 3446 (N-H *Stretch*), 3070 (C-H *Stretch*), 1637 (C=O *Stretch*), 1510, 1444 (*Aromatic* C=C *Stretch*), 1255 (C-O *Stretch*), 1026, 827. ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm: 3.89 (s, 3H, OCH$_3$), 3.91 (s, 3H, OCH$_3$), 6.13 (s, 2H, NH$_2$), 6.61 (d, J = 15.8 Hz, 1H, H$_\alpha$), 7.25 (d, J = 15.8 Hz, 1H, H$_\beta$), 6.82-7.23 (m, 7H, ArH). MS (EI, m/z (%): 283 (M$^+$, 62 %). Anal. calcd. For C$_{17}$H$_{17}$O$_3$N: C, 72.08; H, 6.0; N, 4.94. Found: C, 72.15; H, 5.84; N, 4.92.

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*1-(4-Amino-phenyl)-3-(2-hydroxy-phenyl)-propenone (**3b**)*

Light black crystaline solid. M.P. 208 °C. Yield 68 %. FT-IR (KBr, ν, cm^{-1}): 3452 (N-H Stretch), 3088 (C-H Stretch), 1635 (C=O Stretch), 1530, 1455 (Aromatic C=C Stretch), 1253 (C-O Stretch), 1023, 825. ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm: 6.13 (s, 2H, NH_2), 5.24 (s, 1H, OH), 6.65 (d, J = 15.6 Hz, 1H, H_α), 7.22 (d, J = 15.7 Hz, 1H, H_β), 6.90-7.38 (m, 8H, ArH). MS (EI, m/z (%): 239 (M^+, 75 %). Anal. calcd. For $C_{15}H_{13}O_2N$: C, 75.31; H, 5.43; N, 5.85. Found: C, 75.37; H, 5.41; N, 5.89.

*1-(4-Amino-phenyl)-3-(2-hydroxy-3,5-diiodo-phenyl)-propenone (**3c**)*

Brown crystalline solid. M.P. 172 °C. Yield 71 %.FT-IR (KBr, ν, cm^{-1}): 3442 (N-H Stretch), 3075 (C-H Stretch), 1636 (C=O Stretch), 1525, 1440 (Aromatic C=C Stretch), 1252 (C-O Stretch), 1030, 827. ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm: 6.13 (s, 2H, NH_2), 6.65 (d, J = 15.8 Hz, 1H, H_α), 7.28 (d, J = 15.8 Hz, 1H, H_β), 6.78-7.15 (m, 6H, ArH). MS (EI, m/z (%): 491 (M^+, 45 %). Anal. calcd. For $C_{15}H_{11}O_2I_2N$: C, 36.65; H, 2.24; N, 2.85, I, 51.73. Found: C, 36.62; H, 2.26; N, 2.84; I, 51.70.

*1-(4-Amino-phenyl)-3-(3,5-dibromo-2-hydroxy-phenyl)-propenone (**3d**)*

Brown crystalline solid**.** M.P. 148 °C. Yield 73 %. FT-IR (KBr, ν, cm^{-1}): 3440 (N-H Stretch), 3072 (C-H Stretch), 1636 (C=O Stretch), 1528, 1440 (Aromatic C=C Stretch), 1252 (C-O Stretch), 1034, 827. ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm: 6.13 (s, 2H, NH_2), 6.63 (d, J = 15.8 Hz, 1H, H_α), 7.26 (d, J = 15.8 Hz, 1H, H_β), 6.67-7.18 (s, 6H, ArH). MS (EI, m/z (%): 397 (M^+, 58 %). Anal. calcd. For $C_{15}H_{11}O_2Br_2N$: C, 45.34; H, 2.77; N, 3.52; Br, 40.30. Found: C, 45.30; H, 2.74; N, 3.55; Br, 40.35.

*1-(4-Amino-phenyl)-3-(5-chloro-2-hydroxy-phenyl)-propenone (**3e**)*

Redish crystalline solid. M.P. 178 °C. Yield 68 %. FT-IR (KBr, ν, cm^{-1}): 3442 (N-H Stretch), 3060 (C-H Stretch), 1635 (C=O Stretch), 1518, 1435 (Aromatic C=C Stretch), 1254 (C-O Stretch), 1028, 827. ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm: 6.13 (s, 2H, NH_2), 6.61 (d, J = 15.8 Hz, 1H, H_α), 7.25 (d, J = 15.8 Hz, 1H, H_β), 6.79-7.08 (m, 7H, ArH). MS (EI, m/z (%): 273.5 (M^+, 73 %). Anal. calcd. For $C_{15}H_{12}O_2ClN$: C, 65.81; H, 4.38; N, 5.11; Cl, 12.97. Found: C, 65.85; H, 4.36; N, 5.08; Cl, 12.94.

*1-(4-Amino-phenyl)-3-(3,4,5-trimethoxy-phenyl)-propenone (**3f**)*

Pale yellow crystalline solid. M.P. 168 °C. Yield 74 %. FT-IR (KBr, ν, cm^{-1}): 3445 (N-H Stretch), 3072 (C-H Stretch), 1637 (C=O Stretch), 1515, 1440 (Aromatic C=C Stretch), 1255 (C-O Stretch), 1026, 827. ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm: 3.89 (s, 6H, two OCH_3), 3.91 (s, 3H, OCH_3), 6.13 (s, 2H, NH_2), 6.63 (d, J = 15.8 Hz, 1H, H_α), 7.25 (d, J = 15.8 Hz, 1H, H_β), 6.82-7.29 (s, 6H, ArH). MS (EI, m/z (%): 313 (M^+, 80 %). Anal. calcd. For $C_{18}H_{19}O_4N$: C, 69.0; H, 6.0; N, 4.47. Found: C, 68.97; H, 6.08; N, 4.49.

*1-(4-Amino-phenyl)-3-(4-dimethylamino-phenyl)-propenone (**3g**)*

Yellow brown crystalline solid**.** M.P. 183 °C. Yield 66 %. FT-IR (KBr, ν, cm^{-1}): 3442 (N-H Stretch), 3075 (C-H Stretch), 1637 (C=O Stretch), 1518, 1446 (Aromatic C=C Stretch), 1250 (C-O Stretch), 1026, 827. ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm: 3.17 (s, 6H, $N(CH_3)_2$), 6.13 (s, 2H, NH_2), 6.65 (d, J = 15.8 Hz, 1H, H_α), 7.25 (d, J = 15.8 Hz, 1H, H_β), 6.87-7.32 (m, 8H, ArH). MS (EI, m/z (%): 266 (M^+, 85 %). Anal. calcd. For $C_{17}H_{18}N_2O$: C, 76.69; H, 6.76; N, 10.52. Found: C, 76.73; H, 6.74; N, 10.49.

*1-(4-Amino-phenyl)-3-(4-chloro-phenyl)-propenone (**3h**)*

Yellow crystalline solid**.** M.P. 132 °C. Yield 76 %. FT-IR (KBr, ν, cm^{-1}): 3446 (N-H *Stretch*), 3070 (C-H *Stretch*), 1637 (C=O *Stretch*), 1522, 1465 (*Aromatic* C=C *Stretch*), 1254 (C-O *Stretch*), 1030, 827. ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm: 6.13 (s, 2H, NH_2), 6.67 (d, *J* = 15.8 Hz, 1H, H_α), 7.25 (d, *J* = 15.8 Hz, 1H, H_β), 6.70-7.20 (m, 8H, ArH). MS (EI, *m/z* (%): 257.5 (M^+, 48 %). Anal. calcd. For $C_{15}H_{12}OClN$: C, 69.90; H, 4.66; Cl, 13.78; N, 5.43. Found: C, 69.86; H, 4.69; Cl, 13.81; N, 5.41.

*1-(4-Amino-phenyl)-3-naphthalen-1-yl-propenone (**3i**)*

Yellow crystalline solid. M.P. 143 °C. Yield 72 %. FT-IR (KBr, ν, cm^{-1}): 3442 (N-H *Stretch*), 3072 (C-H *Stretch*), 1635 (C=O *Stretch*), 1528, 1480 (*Aromatic* C=C *Stretch*), 1252 (C-O *Stretch*), 1037, 824. ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm: 6.15 (s, 2H, NH_2), 6.65 (d, *J* = 15.7 Hz, 1H, H_α), 7.20 (d, *J* = 15.6 Hz, 1H, H_β), 6.76-7.09 (m, 11H, ArH). MS (EI, *m/z* (%): 289 (M^+, 64 %). Anal. calcd. For $C_{19}H_{15}ON$: C, 78.89; H, 5.19; N, 4.84. Found: C, 78.92; H, 5.17; N, 4.85.

*1-(4-Amino-phenyl)-3-(2-methoxy-naphthalen-1-yl)-propenone (**3j**)*

Yellow crystalline solid. M.P. 154 °C. Yield 75 %. FT-IR (KBr, ν, cm^{-1}): 3445 (N-H *Stretch*), 3072 (C-H *Stretch*), 1637 (C=O *Stretch*), 1525, 1463 (*Aromatic* C=C *Stretch*), 1254 (C-O *Stretch*), 1030, 827. ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm: 6.13 (s, 2H, NH_2), 3.83 (s, 3H, OCH_3), 6.65 (d, *J* = 15.8 Hz, 1H, H_α), 7.27 (d, *J* = 15.8 Hz, 1H, H_β), 6.79-7.20 (m, 10H, ArH). MS (EI, *m/z* (%): 303 (M^+, 70 %). Anal. calcd. For $C_{20}H_{17}O_2N$: C, 79.20; H, 5.61; N, 4.62. Found: C, 79.25 H, 5.58, N, 4.60.

3. Results and Discussion

Literature survey reveals that there is less work done on synthesis of chalcones in which amino functional group is present. Therefore in continuation of earlier devotion towards solvent-free synthesis [23-26] herein, report (Scheme 1), we describe an efficient method for the synthesis of 4-amino-1,3-diaryl-2-propen-1-one under solvent-free grinding technique. The chalcones **3a-j** was prepared by conventional Claisen-schmidt reaction and studied the antimicrobial activity [27]. A variety of methods have been reported for the preparation of this class of compounds. However, in spite of their potential utility, some of the reported methods suffer from drawback such as long reaction time, cumbersome product isolation procedure, and environmental concern.

The present studies describe that reactions were carried out using grindstone technique simply by mixing corresponding 4-aminoacetophenone **1** and substituted benzaldehyde **2a-j**. The mixture was ground together in mortar with pestle at room temperature for 2-3 minutes, and then a catalytic amount of KOH was added to this grinded reaction mixture. The grinding was continued for 4-6 minutes and progress of reaction was monitored on thin layer chromatography (TLC) using ethyl acetate and hexane (1:1). The completion of reaction was indicated by wetting with the formation of light colored reaction mixture. Obtained solid was easily separated by using cold water and simple Buchner filtration; final purification was achieved by crystallization from ethanol. This method does not need expensive reagents or special care to exclude moisture from the reaction medium. The reaction proceeded efficiently and smoothly at room temperature, and the products were obtained in excellent yields. Formation of compounds established on the basis of spectral data. IR of condensed compound **3a-j** revealed the presence of band near 1637 due to carbonyl C=O stretch. The band which in region 1535, 1455 shows stretching of α,β-unsaturated C=C double bond. Proton NMR of **3a-j** show characteristic peak at δ 6.65 and 7.25 with *J* = 15.7 revealed the presence of H_α & H_β proton.

Results of antioxidant values expressed as IC_{50} with different antioxidant are as shown in Table 1. The compound no. 3a, 3c, 3d and 3f showed IC_{50} at 65.08, 70.06, 63.03, 64.02 μM when compared with that of the standard ascorbic acid at 62.07 μM. However the compound 3b and 3h did not show significant activity. The antioxidant activity by NO scavenging method showed the IC_{50} greater than 300 μM except for 3b and 3h in comparison with standard ascorbic acid at 125.34 μM.

3.1 Antioxidant Activity

The following antioxidant methods were used to evaluate the antioxidant properties of our test compounds.

3.1.1 DPPH· Scavenging Activity

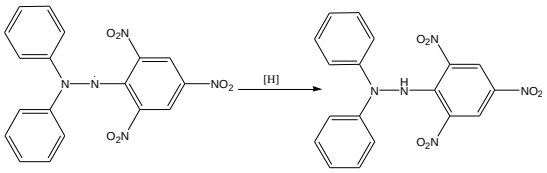
DPPH is a stable free radical that can accept an electron or hydrogen radical to become a stable diamagnetic molecule. Due to its odd electron, the methanolic solution of DPPH shows a strong absorption band at 517 nm. DPPH radical reacts with various electron donating molecules (reducing agents or antioxidants). When electrons become paired off, bleaching of the DPPH solution is the result. This results in the formation of the colourless 2,2'-diphenyl-1-picryl hydrazine (Fig. 1). Reduction of the DPPH radicals can be estimated quantitatively by measuring the decrease in absorbance at 517 nm.

Procedure

Equal volumes of 100 μM 2,2'-diphenyl-1-picrylhydrazyl (DPPH) in methanol was added to different concentrations of test compounds (0 – 200 μM/mL) in methanol, mixed well and kept in dark for 20 min. The absorbance at 517 nm was measured using the spectrophotometer UV-1650, Shimadzu [28]. Plotting the percentage DPPH· scavenging against concentration gave the standard curve and the percentage scavenging was calculated from the following equation:

$$\% \text{ scavenging} = (\text{Absorbance of blank} - \text{Absorbance of test}) \times 100 / \text{Absorbance of blank}$$

IC50 was obtained from a plot between concentration of test compounds and % scavenging. Ascorbic acid was used as standard for comparison.



Structural changes occurs of DPPH during oxidation

Fig. 1 Structural changes occurs of DPPH during oxidation

3.1.2 Nitric Oxide Scavenging Activity

Nitric oxide (NO) will be generated by sodium nitropruside in solution. In the presence of an antioxidant or nitric oxide scavenger the amount of NO generated will be less. The excess NO will be estimated by Griess reagent is the mixture of sulphanilic acid and naphthylethylenediamine dihydrochloride. The nitric oxide will give pink colour complex estimated at 540 nm.

Procedure

To a reaction mixture (6 ml) containing sodium nitroprusside (10 mM, 4 mL), phosphate buffer saline (PBS, 1.0 mL) and 1.0 mL of different concentration of test compounds/standard were incubated at 25 °C for 150 min. After incubation, 0.5 mL of the reaction mixture containing nitrate was removed and 1.0 mL of sulphanilic acid was added, mixed well and allowed to stand for 5 min for completion of diazotization. Then 1.0 mL of naphthylethylenediaminedihydrochloride was added, mixed and allowed to stand for 30 min in dark at room temperature. The absorbance of these solutions was measured at 540 nm against corresponding blank solution without sodium nitroprusside [29]. The % scavenging and IC50 values were determined as explained in DPPH assay.

Table 1 Antioxidant scavenging of synthesized some 4-amino chalcones **3a-j**

Compound	DPPH scavenging (μM)	NO scavenging (μM)
3a	65.08	325
3b	NSA	245
3c	70.06	410
3d	63.03	525
3e	85.07	510
3f	64.02	625
3g	145	575
3h	NSA	225
3i	140	335
3j	88.04	370
Standard	†62.07	† 125.34

NSA- Not significant activity † Ascorbic acid

4. Conclusion

In summary we have reported first time the synthesis of chalcones bearing amino substituent in basic nucleus under solvent-free grindstone technique. Method is efficient in terms of simple reaction procedure, short reaction time, and ease work-up of product giving high yields. Antioxidant activity of amino chalcones revealed that the compound **3a**, **3c**, **3d** and **3f** shows significant activity.

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