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Novel pyrano[3,2-c]chromene derivatives via a green one-pot three component: Synthesis, characterization, antioxidant, antibacterial and anti-inflammatory activities

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Abstract: A simple, green, efficient and economical procedure for the synthesis of , pyrano[3,2-c]chromene derivatives by using a one-pot three-component condensation of 4-Hydroxycoumarin, various arylaldehydes and malonitrile in 1-butyl-3-methylimidazolium triflate as green media is described. This reaction proceeded under mild conditions with the use of an inexpensive and readily available catalyst, high to excellent yields, and simple workup procedure. In addition; the obtained pyrano[3,2-c]chromenes were investigated for anti-oxidant activities by super oxide radical; DPPH (2,2-Diphenyl-1-picrylhydrazyl); and hydroxyl radical scavenging assays; in which most of them displayed significant antioxidant activities. Furthermore the pyrano[3,2-c]chromene were evaluated for anti-inflammatory activity by indirect haemolytic and lipoxygenase inhibition assays and revealed good activity. Most of the new pyrano[3,2-c]chromenes exhibited moderate antibacterial activity.

Keywords: Multicomponent reaction; 4-Hydroxycoumarin; pyrano[3,2-c]chromene; 1-butyl-3-methyl-imidazolium triflate; Malononitrile; Ammonium acetate; antibacterial and anti-inflammatory activities.

Introduction

Recently, environmental pollution and the economic crisis have become very important global challenges. As a result, industrial and manufacturing units including chemicals and pharmaceutical companies show propensity to environmentally friendly protocols that is so called green and sustainable protocols. One of the important strategies in green chemistry is multicomponent reactions (MCRs).

The strategies of MCRs offer significant advantages over conventional linear-type syntheses because of high degree of atom economy, high selectivity, and procedural simplicity due to formation of carbon-carbon and carbon-heteroatom bonds in one-pot procedure [1-5].MCRs, particularly those performed in green and eco-friendly media, have become increasingly useful tools for the synthesis of chemically and biologically important compounds because of their environmentally friendly atom economy and green characteristics, and the "greening" of global chemical processes has became a major issue in the chemical industry[6-10]. Organic reactions in ionic liquid (IL) media have received the considerable attention of synthetic organic chemists in recent years; IL is an environmentally friendly solvent with unique properties such as high ionic conductivity, non-volatility, high thermal stability.

In continuation of our interest in the application of new catalysts in organic synthesis via MCRs [11-13], herein, we aim to develop efficient synthetic processes using green and eco-friendly methods for the synthesis of pyrano[3,2-c]chromene derivatives,in ionic liquid through a three component condensation reaction of benzaldehydes, malononitrile and a 4-Hydroxycoumarin in the presence of catalytic amount of ammonium acetate as a catalyst (Scheme 1).

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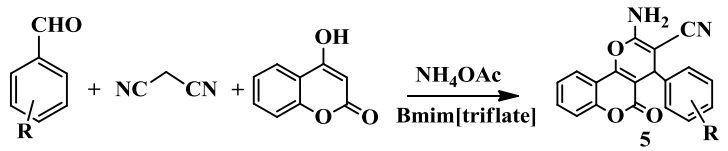


Scheme 1. Synthesis of pyrano[3,2-c]chromene derivatives **5**

Experimental Section

Materials and equipment

Melting points were recorded in open capillary and were uncorrected. Thin Layer Chromatography (TLC) was carried out using aluminum sheets pre-coated with silica gel 60F254 purchased from Merck. All reagents were obtained from Fluka and Aldrich.The purity of the products was tested in each step by TLC (SiO_2, $CHCl_3$/MeOH and THF/MeOH). Melting points were determined using an Electrothermal apparatus and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were carried on a Varian Gemini 300 (300 MHz) spectrometer using TMS as internal standard (δ = 0 ppm). IR spectra were recorded on a Perkin-Elmer 398 Spectrophotometer. MS were recorded on a LC-MS-MS 8030 Shimadzu. Elemental analyses were performed on Perkin-Elmer 2400 elemental analyzer, and the values found were within ±0.3% of the theoretical values. The UV spectra were recorded on a Perkin Elmer Lambda 11 spectrophotometer.

General procedure for the synthesis of 3,4-dihydropyrano[3,2-c]chromenes 5

A mixture of aldehyde (1 mmol), malononitrile (1 mmol), 4-hydroxycoumarin (1 mmol) and ammonium acetate (15 mol%, 0.15 mmol, 11.7 mg) in 1-butyl-3-methylimidazolium triflate (5 mL) was refluxed for an appropriate time. Upon completion of the reaction, monitored by TLC, the reaction mixture was allowed to cool to room temperature. The solid separated was filtered off, washed with ethanol and purified by recrystallization from aqueous ethanol to afford the products **5**.

2-Amino-4-(m-methoxyphenyl)-5-oxo-4,5-dihydropyrano [3,2-c] chromene-3-carbonitrile 5c
Yield: (90%), mp=325°C
FT-IR (KBr) ν, cm-1: 3441, 3368 (NH_2); 3323, 3183 (C-H) arom; 2195 (CN); 2836 (-CH3); 1709 (C=O); 1679, 1613 (C=C); 1061 (C-O).
^{1}H NMR. (DMSO-d_6, 300 MHz) (δ: ppm): 2.5 (s, 2H, NH_2); 3.8 (s, 3H, CH3); 4.45 (s, 1H, CH); 6.7-7.9 (m, arom, 8H). ^{13}C NMR. (DMSO-d6, 75 MHz) (δ : ppm): 161.3 (C2); 159.5 (C3'); 159.4 (C4); 159.2 (C11); 152.2 (C9); 142.0 (C1'); 129.6 (C5'); 128.9 (C7); 124.6 (C6); 123.5 (C5); 120.1 (C6'); 118.2 (CN); 116.5 (C8); 115.1 (C10); 113.1 (C2'); 111.9 (C4'); 103.9 (C3); 81.8 (C12); 55.3 (C7'); 40.3 (C13).
Anal. Calcd for $C_{20}H_{14}N_2O_4$: C, 69.359%, H, 4.074%N, 8.088%, Found: C, 69.4%, H, 4.1% N, 8.2%,

2-Amino-5-oxo-4-(pentafluorophenyl-4,5-dihydropyrano [3,2-c] chromene-3-carbonitrile 5e
Yield: (94%), mp=330°C
FT-IR (KBr) ν, cm^{-1}: 3427, 3323 (NH_2); 3183 (C-H) arom; 2351; 2195 (CN); 1717 (C=O); 1679, 1613, 1503 (C=C); 1377, 1061 (C-O); 994; 934; 758.
^{1}H NMR. (DMSO-d_6, 300 MHz) (δ : ppm): 2.1 (s, 2H, NH_2); 5 (s, 1H, CH); 7.2-7.9 (m, arom, 4H).^{13}C NMR. (DMSO-d_6, 75 MHz) (δ : ppm): 162.7 (C_2); 159.41 (C_4); 159.1 (C_{11}); 152.3 (C_9); 135.3 ($C_{2'}$, $C_{3'}$, $C_{5'}$, $C_{6'}$); 130.2 (C_7); 124.8 (C_6); 123.5 (C_5); 117.8 (CN); 116.5 (C_8); 115.5 (C_{10}); 112.1 ($C_{1'}$, $C_{4'}$); 101.9 (C_3); 89.4 (C_{12}); 27.1 (C_{13}).
Anal. Calc. for $C_{19}H_7F_5N_2O_3$. C, 56.171%, H, 1.737% N, 6.895%, Found: C, 56.2%, H, 1.67% N, 6.9%,

2-Amino-4-(3-fluorophenyl)-5-oxo-4,5-dihydropyrano [3,2-c] chromene-3-carbonitrile 5g
Yield: (89%), mp=320°C
FT-IR (KBr) ν, cm^{-1}: 3383, 3317 (NH_2); 3190 (C-H) arom; 2195 (CN); 1702 (C=O); 1679, 1613 (C=C); 1377, 1061 (C-O); 1244; 773.
^{1}H NMR. (DMSO-d_6, 300 MHz) (δ : ppm): 2.25 (s, 2H, NH_2); 4.45 (s, 1H, CH); 6.9-7.9 (m, arom, 8H). ^{13}C NMR. (DMSO-d_6, 75 MHz) (δ : ppm): 163.9 ($C_{3'}$); 160.8 (C_2); 158 (C_4); 153.7 (C_{11}); 152.4 (C_9); 144.4 ($C_{1'}$); 130.7 ($C_{5'}$); 129.6 (C_7); 125.4 (C_6); 123.4 (C_5, $C_{6'}$); 118.7 (CN); 116.5 (C_8); 116.1 ($C_{2'}$); 115.6 (C_{10}); 112.4 ($C_{4'}$); 103.5 (C_3); 86.5 (C_{12}); 40.3 (C_{13}).
Anal. Calcd for $C_{19}H_{11}FN_2O_3$. C, 68.263%, H, 3.317% N, 8.380%, Found: C, 68.1%, H, 3.27% N, 8.2%,

2-Amino-4-(4-carboxyphenyl)-5-oxo-4,5-dihydropyrano [3,2-c] chromene-3-carbonitrile 5h
Yield: (87%), mp=330°C
FT-IR (KBr) ν, cm^{-1}: 3434, 3323 (NH_2); 3183 (C-H) arom; 3213 (O-H); 2195 (CN); 1717, 1694 (C=O); 1672, 1606 (C=C); 1377, 1259, 1061 (C-O);
^{1}H NMR. (DMSO-d_6, 300 MHz) (δ : ppm): 2.1 (s, 2H, NH_2); 4.4 (s, 1H, CH); 7.2-8.1 (m, arom, 8H); 10.1 (s, 1H, OH).^{13}C NMR. (DMSO-d_6, 75 MHz) (δ : ppm): 167.3 ($C_{7'}$); 166.1 (C_2); 164.7 (C_4); 160.4 (C_{11}); 152.3 (C_9); 146.4 ($C_{1'}$); 129.1 (C_7); 128.3 ($C_{2'}$, $C_{6'}$); 128.1 ($C_{4'}$); 126.8 ($C_{3'}$, $C_{5'}$); 123.9 (C_6); 123.5
(C_5); 118.3 (CN); 115.8 (C_8); 113.6 (C_{10}); 103.6 (C_3); 83.92 (C_{12}); 41.7 (C_{13}).
Anal. Calc. for $C_{20}H_{12}N_2O_5$. C, 66.667%, H, 3.357% N, 7.775%, Found: C, 66.57%, H, 3.4% N, 7.6%,

Antioxidant activity

DPPH radical scavenging assay:

The free radical scavenging activity for DPPH radicals wasperformed as described previously [14]. In brief, reaction mixture containing 200 µL of 0.1 mM DPPH-ethanol solution, 90 µL of 50 mM Tris-HCl buffer (pH 7.4) and 10 µL of deionised water and various concentrations of compounds **5** (3.0 - 16.0 µM), and ascorbic acid was used as a control. Reaction mixture was incubated for 30 min at room temperature and absorbance was read at 540 nm. The percentage radical scavenging activity was calculated according to the following formula:

Inhibition (%) = [(Absorbance control-Absorbance sample)/Absorbance Control] X 100.

Hydroxyl radical scavenging assay:

The hydroxyl radical (˙OH) scavenging activity of newly synthesized compounds was determined previously [15]. We used Fe (III) - ascorbate - ethylenediaminetetraacetic acid - hydrogen peroxide system (Fenton' s reaction) to generate hydroxyl radical. In brief, reaction mixture containing 0.01 mL of $FeCl_3$ (10 mM), 0.1 mL of EDTA (1 mM),0.36 mL of deoxyribose (10 mM), 0.1 ml of H_2O_2 (10 mM), 1 mL of the compounds **3-12** (concentrations ranging from 3.0 - 16 .0 µM), 0.33 mL of phosphate buffer (50 mM, pH 7.4) and 0.1 mL ascorbic acid (1 mM) was added. The mixture was incubated at 37^0C for 1 h and 1 mL of the incubated mixture was mixed with 1 mL of 10% trichloro acetic acid (TCA) and 1mL of thiobarbituric acid (TBA) (1% in 0.025 M NaOH), the resulting mixture was incubated in water bath at 90^0C for 20 min. The absorbance was measured at 532 nm. Ascorbic acid was used as a positive control. The percentage of hydroxyl radical scavenging activity was calculated using the formula:

Inhibition (%) = [(Absorbance control-Absorbance sample)/Absorbance Control] X 100

Superoxide anion radical scavenging assay:

The superoxide anion radical scavenging activity of newly synthesized compounds were determined previously [16]. 1 mL of Nicotinamide adenine dinucleotide (NADH) (468 µM in 100 mM phosphate buffer of pH 7.4), 1 mLofNitro blue tetrazolium (NBT) (156 µM NBT in 100 mM phosphate buffer of pH 7.4), and different concentration of compounds **5** (3.0-16.0 µg/mL) were added to get the final volume of 3 mL. The reaction was started by the addition of 100 µL of PMS (60 µM in 100 mM phosphate buffer of pH 7.4). The mixture was incubated for 5 min at 25^0C and the absorbance was measured at 560 nm. Quercetin was used as a control. The percentage radical scavenging activity was calculated using the formula:

Inhibition (%) = [(Absorbance control-Absorbance sample)/Absorbance Control] X 100.

Anti-inflammatory activity

Lipoxygenase inhibition assay:

The lipoxygenase inhibition assay was performed according to the method previously described [17]. Briefly, to a solution of 0.1 mL of 0.2 M borate buffer (pH 9.0), 0.1 mL of 1000 units lipoxydase enzyme solution, test compounds **5** dissolved in DMSO (3 - 16 µM) was added, agitated and incubated at room temperature for 5 min. Later, 2.0 mL of 0.6 mM linoleic acid was added and the absorbance was measured at 234 nm. Indomethacin was used as control. The percent (%) inhibition was calculated by the following equation:

Inhibition (%) = [(Absorbance control-Absorbance sample)/Absorbance Control] X 100.

Indirect haemolytic assay:

Indirect haemolytic assay was performed according to the reported method [18-19]. One mL of fresh human red blood cells and 1 mL of fresh Hen's egg yolk in 8 mL of phosphate buffered saline was mixed to prepare the substrate for indirect hemolytic activity. One mL of this suspension was incubated with 4-28 µg of partially purified venom for 45 min at 37^0C and 9 mL ice cold sodium perborate was used to stop the reaction. The reaction mixture was centrifuged at 2000 rpm for 20 min then the released hemoglobin was read at 540 nm. For inhibition studies 10 µg of venom sample (secretory-PLA_2 purchased from sigma) was incubated with various concentrations of compounds **3-12** (20-100 µM in DMSO) for 30 min at room temperature and mixed with 1 mL of substrate solution and incubated at room temperature for 30 min. The reaction was stopped by adding 9 mL of ice cold sodium perborate and extent of hemolysis is measured at 540 nm. Aristolochic acid was used as reference drug. The percent (%) inhibition was calculated as follows:

Inhibition (%) = [(Absorbance control-Absorbance sample)/Absorbance Control] X 100.

Antibacterial activities

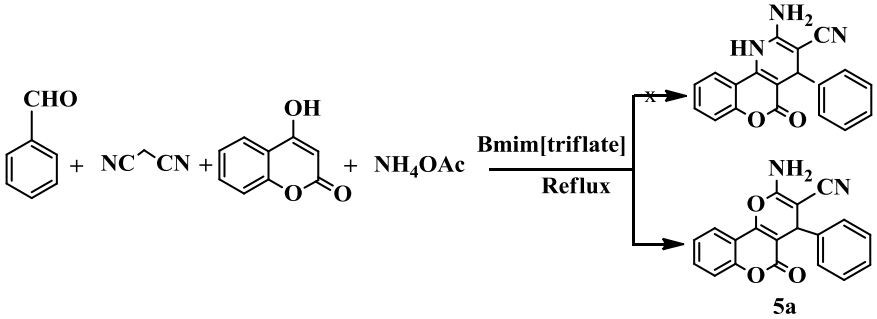
The antimicrobial activities of the synthesized compounds were determined by the minimum inhibitory concentration (MIC) in accordance with NCCLS guideline M7-A_6 and M38-P,

Precultures of the tested bacteria were made by inoculating 10 mL of Luria- Bertani (LB) and incubating for 24h at 37°C. The tested fungus, Candia albicans, was made by grown on potato dextrose agar (PDA) for more than three days at 28°C. The colonies were harvested, suspended in sterile saline, and adjusted to a concentration that yielded an absorbance similar to that of a 0.5 Mc

Farlan 625 nm or fungi at 530 nm, the equivalence of 1-2 108 cfc/ml. Then the simples were further diluted 1:1000 In LB or PDA to 104cfc/ml.

Results and Discussion

Organic reactions in ionic liquid (IL) media have received the considerable attention of synthetic organic chemists in recent years; (IL) is an environmentally friendly solvent with unique properties such as high ionic conductivity, non volatility, high thermal stability, non flammability, and miscibility with organic compounds, especially with the heterocyclic compounds [20-22]. Because of these useful properties numerous works have been published in the last decades reporting the possibility to perform several organic reactions and catalyzed processes in these green media [23-25]. To achieve suitable conditions for the synthesis of pyrano[3,2-c]chromene derivatives we run a model reaction by stirring an equimolecular amounts of 4-hydroxycoumarin (**1**) with benzalaldehyde (**2**) and malononitrile (**3**) in the presence of ammonuim acetate (2.5 mol %) in 1-butyl-3-methylimidazolium triflate (5 mL) at reflux that result in the formation of the desired compound with 85% yield . The product was identified by spectral data revealed that ammonium acetate was not involved in the reaction and the product could be **5a**. Scheme 2



Scheme 2. Model reaction for the synthesis of 2-amino-4,5-dihydro-5-oxo-4-phenylpyrano[3,2-c]chromene-3-carbonitrile **5a** catalyzed by NH_4OAc.

^{1}H NMR spectra of 5a displayed to prominent singlets at δ 4.45 and 2.50 due to CH and NH_2 proton respectively confirming the cyclization. ^{13}C NMR spectra of 5a are consistent with the proposed structure by displaying the absorption peaks at 41.8, 164.7 and 118. 2 due to CH, C_4 and CN carbons respectively. All the synthesized compounds were characterized by IR, NMR and microanalysis; in particular the spectral characteristics of products **5a**, **5b**, **5d** and **5f** correspond to those found in the literature [26-29].

From this observation it is evident that ammonium acetate was used as a catalyst. In order to seek an optimal solvent, the model reaction was explored using different solvents such as DMC, DEC, ethanol, tetrahydrofuran (THF), dichloro-methane, THF, bmim[triflate] and toluene at reflux temperature in the presence of a catalytic amount of ammonium acetate as an inexpensive and readily catalyst. The results are summarized in Table 1.

Table 1. Synthesis of pyrano[3,2-c]chromene derivatives in the presence of different solvents

Entry	Solvent	Temp (°C)	Time (min)	Yield (%)[b]
1	DMC	90 °C	30	85
2	DEC	128 °C	30	75
3	EtOH	78 °C	120	72
4	$CHCl_3$	61 °C	120	60
5	Bmim[triflate]	>100°C	30	90
6	THF	66 °C	120	65
7	Toluene	110 °C	120	60
8	CH_2Cl_2	40 °C	120	62

[a] Reaction conditions: 4-Hydroxycoumarin (1mmol), malonitrile (1mmol), solvent (5mL), room temperature.
[b] Isolated yield of product.

It was found that polarity of solvent and presence of ammonium acetate play an important role for the success of the reaction. The results indicated that solvents were also affected on the yield of (Table 1, entries 1-8). In the organic solvents such as dichloromethane, THF, ethanol, or

toluene, the yield of **5** were lower and longer reaction times were required,

It was observed that among all solvents and media, the best result was obtained when 1-butyl-3-methylimidazolium triflate was chosen in the presence of catalytic amount of *ammounim acetate* at 90°C. The desired product was obtained in excellent yield and high purity.

Based on the results, bmim[triflate] was chosen to be the best in terms of the yield of the product and reaction time in comparison to common organic solvents. (Table 1, entry 5). In order to optimize the ammonuim acetate loading, the model reaction was performed with different amounts of catalyst at reflux, Table 2.

Table 2. Effect of catalyst amount on the condensation of benzaldehyde **1**, malononitrile **2**, and 4-hydroxycoumarin **3** in Bmim[triflate] [a].

Entry	Catalyst	Mol(%)	Time (mn)	Yield (%)[b]
1	NH_4OAc	5	30	55
2	NH_4OAc	10	10	90
3	NH_4OAc	15	3	92
4	NH_4OAc	20	3	94
5	NH_4OAc	30	3	92
6	NH_4OAc	35	620	90
7	NH_4OAc	40	2	88
8	No Catalyst	-	620	10

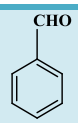
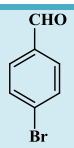
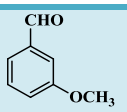
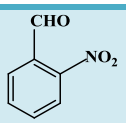
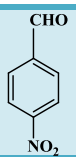
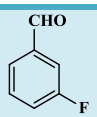
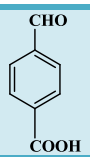
[a] Reaction conditions: Benzaldehyd 1a (1 mmol), malononitrile 2 (1 mmol), and 4-hydroxycoumarin 3 (1 mmol), Bmim[triflate] (5 mL).
[b] Isolated yields after purification.

In order to optimize the more suitable reaction conditions for the preparation of pyrano[3,2-c]chromene derivatives derivatives **5** via this novel green chemical approach, quantity of the catalyst required was determined. It was found that, when the reaction was carried out in the presence of 5mol%of catalyst, 55% of yield was obtained. As we increase the percentage of the catalyst to 10mol%, 15 mol%, and 20 mol%, the yields were also found to be increased up to 90%, 92%, and 94%, respectively, but beyond 20mol% there is no significant improvement of the rate as well as yield of the reaction, and further increase in the quantity of catalyst did not show appreciable improvement in the yield of product. Thus, 20mol% of catalyst was chosen as maximum quantity of the catalyst for the reaction.

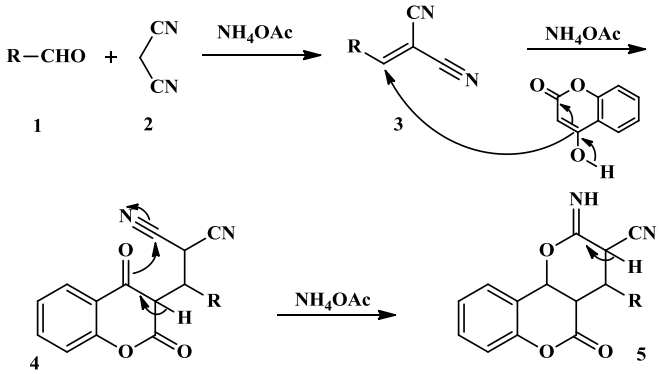
After optimization the reaction conditions, the scope of the method was investigated with a series of substituted aromatic aldehydes. The results are summarized in Table 3. As seen from Table 3, the aromatic aldehydes carrying both electron-withdrawing (Entries **4,5**) and electron-donating functional groups (Entry **8**) underwent successful condensation with malononitrile and 4-hydroxycoumarin in the presence of catalytic amount of ammonium acetate in Bmim[triflate] at reflux to afford the corresponding products in good yields. It seems that the electronic effects and the nature of the substituents on the aryl aldehyde ring have slight effect on both reaction yield and necessary time for the completion of the reaction. The electron-donating groups somewhat increased reactivity and afforded higher yields compared to electron-withdrawing groups. In addition, this reaction was affected by steric effect. For example, 2-nitrobenzaldehyd required longer reaction time compared to 4-nitrobenzaldeyde owing to sterically hindered *ortho* position, substituted by nitro group.

However, when the reaction was carried out with pentafluoro benzaldehyd the corresponding products were obtained in good yields after longer reaction times. In this case, the effects of functional groups in the aromatic aldehyde ring were opposite. Remarkably, the reactions were clean and all the products were obtained after only a filtration and simple washing with water and ethanol. Thus, a simple work-up gives the title products without of need of chromatographic purification.

Table 3. Synthesis of pyrano[3,2-c]chromene derivatives **5** catalyzed by NH_4OAc.

Entry	**1**	**2**	**3**	**4**	**5**	**5**	**7**	**8**
Aldehyde								
Product	5a	5b	5c	5d	5e	5f	5g	5h
Time(min)	50	60	45	80	60	50	75	45
Yield(%)	94	92	90	-	94	90	89	87
Reported	27	27	-	26	-	27	-	-

The plausible mechanism for the formation of pyrano[3,2-c]chromene derivatives **5** is offered in Scheme 3. Firstly, intermediate **3** was formed via the Knoevenagel condensation reaction between arylaldehyde **1** and malonitrile **2** followed by Michael addition reaction between 4- Hydroxycoumarine and **3** furnished **4**, which upon intermolecular cyclization and dehydration gave rise to **5**.



Scheme 3. The plausible mechanism for the formation of pyrano[3,2-c]chromene derivatives **5**

Pharmacology

Antibacterial activity

In vitro antibacterial activities of the synthesized pyrano[3,2-c]chromene derivatives **5** against the Gram positive bacteria Staphylococcus aureus (ATCC29213), Bacillus subtilis (ATCC 33712), Staphylococcus aureus (ATCC700699) and the Gram negative bacteria Escherichia coli (ATCC11303) were evaluated by the microtiter broth dilution method and susceptibility testing according to the National Committee for Clinical Laboratory Standards (NCCLS) [30].

The minimal inhibitory concentration (MIC_{80}) defined as the amount of compound required for the 80 inhibition of bacterial growth was recorded [31]. Ceftazidime and Cefotaxime were used as control drugs. The observed data on the antimicrobial of the complexes and control drugs were given in Table 4.

Table 4. In vitro antibacterial activity of pyrano[3,2-c]chromene derivatives **5**.

Compound	**Minimum inhibitory concenration (µg/ml)[a]**			
	Escherichia coli	*S.aureus*	*B.subtilis*	*C.albicans*
5a	0.80	<0.39	0.80	>220
5b	0.80	0.80	0.80	0.80
5c	0.80	<0.39	0.80	>220
5d	1.56	1.56	1.56	1.56
5e	0.80	≤0.39	0.80	>220
5f	0.80	<0.35	1.25	>220
5g	0.80	<0.39	0.80	>220
5h	>220	>220	>220	>220
Ceftazidime[b]	>220	0.80	12.5	0.80
Cefotaxime[b]	>220	3.12	3.12	<0.39

[a] Minimum inhibitory concentration values are means of three experiments
[b] Positive control

In general compound **5d** showed the marked antibacterial activities against all four bacteria, whereas **5h** show no inhibitory effect against bacterial lines tested.

Compound **5b** displayed the excellent antibacterial activities against Escherichia coli, B.subtilis with values of 0.80 µg/ml.

Antioxidant activity

We considered that it was worthwhile to study the potential aspects of these pyrano[3,2-c]chromene derivatives **5** for antioxidant activity according to our initial planning. The synthesized compounds **5** were tested for in vitro antioxidant activity by DPPH radical, hydroxyl radical and superoxide radical scavenging assays. The IC50 values of the standards and test samples are summarized in **Table 5**. In all three antioxidant assays, pyrano[3,2-c]chromene derivatives **5c** and **5f** showed antioxidant activities at 9.8 - 12.8 µM concentration. But, pyrano[3,2-c]chromene derivatives **5b** and **5e** showed good antioxidant activity (7.0 - 8.8 µM). Surprisingly, pyrano[3,2-c]chromene derivatives **5a** is inactive in all antioxidant assays.

Table 5. IC50 values of pyrano[3,2-c]chromene derivatives 5 in anti-oxidant assays.

Compounds	IC_{50} values in µM		
	DPPH radical scavenging assay	Hydroxy radical Scavenging assay	Superoxide radical scavenging assay
5a	NA	NA	NA
5b	15.4	14.8	11.8
5c	13	13	14
5d	14	15	16
5e	13	13	14
5f	15	13	14
5j	14	15	16
5h	14	15	16
Ascorbic acid	3.9	3.8	-
Quercetin	-	-	4.8

Anti-inflammatory activity

As per our objective, we next examined the anti-inflammatory activities of pyrano[3,2-c]chromene derivatives **5** by lipoxygenase inhibition and phospholipase A_2 (PLA_2) inhibition assays. The IC_{50} values of the standards and test samples in both assays are given **Table 4**. In both the assays, pyrano[3,2-c]chromene derivatives **5b** and **5c** showed potent anti-inflammatory activity in lipoxygenase inhibition assay (5.0 - 5.1 µM) and PLA_2 inhibition assay (26.5 - 34.9 µM). Notably, compound **5a** with is not active. It should be noted that, **5b** and **5c** nearly have anti-inflammatory activities, as that of standards Indomethacin and Aristolochic acid.

Table 6. IC_{50} values of pyrano[3,2-c]chromene derivatives **5** for anti-inflammatory activity

Compounds	IC_{50} values in µM	
	Lipoxygenase inhibition assay	PLA_2 inhibition assay
5a	NA	NA
5b	6.0	25.5
5c	5.2	33.9
5d	11.5	62.1
5e	11.4	62.4
5f	13	63
5g	13	66
5h	14	67
Indomethacin	4.9	-
Aristolochic acid	-	26.0

Conclusions

In conclusion, we demonstrated a mild and efficient *ammonuim acetate* catalyzed synthesis of pyrano[3,2-c]chromene derivatives **5** using one-pot three-component Domino reaction. The results indicate that NH_4OAc is an efficient, eco-friendly and cost-effective catalyst for this reaction. The obvious advantages of the method are 1) operational simplicity, 2) high atom economy, 3) excellent yields, and 4) products are isolated in pure form without intervention of chromatography. The newly synthesized pyrano[3,2-c]chromene derivatives **5** might exhibit interesting pharmacology activities and may act as potential drug candidates.

The preparations of the new products are supported by elemental analyses, IR, $^{1}H/^{13}C$ NMR, two-dimensional ^{1} H-^{13}C HMBC, MS and UV-Vis spectroscopy.

The compounds **5** were investigated for anti-oxidant activities by super oxide radical; DPPH (2,2-Diphenyl-1-picrylhydrazyl); and hydroxyl radical scavenging assays; in which most of them displayed significant antioxidant activities. Furthermore; compounds **5** were evaluated for anti-inflammatory activity by indirect haemolytic and lipoxygenase inhibition assays and revealed good activity.

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References

1- I.Ugi, A. Domling, W. Horl, *Endeavor.*, **1994**, 18, 115.
2- C.Hulme, V. Gore, *Med. Chem.*,**2003**, 1, 51.
3- K. S Nirav, P. P. Manish, G. P. Ranjan, *Phosphorus, Sulfur Silicon Relat. Elem.*, **2009**, 184, 2704-2719.
4- H. Naeimi, Z. Rashid, A.H. Zarnani, R. Ghahremanzadeh, *J.Chem.*, **2013**, Article ID 169695, 9.
5- E. Rajanarendar, P. Venkateshwarlu, S.R. Krishna, K. G . Reddy, K. Thirupathaiah, *Green and Sustainable Chemistry.*, **2015**, 5, 107-114.
6- K. Kumaravel and G. Vasuki, " *Green Chemistry.*, **2009**,vol. 11, no. 12, pp. 1945-1947.
7- S. L. Jain, S. Singhal and B. Sain, *Green Chemistry.*, **2007**,vol. 9, no. 7, pp. 740-741.
8- J. Wang, S. Daqing, Q. Zhuang, W. Xiangshan, Hu. Hongwen, *Journal of Chemical Research.* **2004**, 12, 818 -820.
9- S. Rupam, S. Manas, M. Lekhok, C. Kushal, D.Prajapati, *Synlett.*, **2010**, 19 , 2847 - 2852 .
10- M. P. Goncharenko, Y. Sharanin, *Russian Journal of Organic Chemistry.* **1993**, 29, 1218
11- L.E. Kaïm, L. Grimaud, J. Oble, *Angew. Chem. Int. Ed*., **2005**, 44, 48, 7961.
12- M. Sinha, K. Khoury, E. Herdtweck, A. Dömling, *Chem. Eur. J.*, **2013**, 19, 25, 8048-8052.
13- M. Sinha, K. Khoury, E. Herdtweck, A. Dömling, *Org. Biomol. Chem.*, **2013**, 11, 29, 4792-4796.
14- Y.M.D. Chuanga, Y.S.Wanga, Y.Y. Kuob, H. P. Tsaia, W.L. Shyura, **2004**. *J. Ethnopharmacol.*, 95, 409-419.
15- B. Halliwell, J.M. C.Gutteridge, O. L. Arnoma, *Anal. Biochem.*, **1987**, 165, 215-219.
16- M. N. Nishimiki, K. Yagi, *Biochem. Biophys. Commun.*, **1972**, 46, 849-854.
17- U .A.Shinde, K. R.Kulkarni, A. S. Phadke, A. M. Nair, D. V. J. Mungantiwar, M. N. Saraf, *Ind. J. Exp. Biol.*, **1999**, 371, 258-261.
18- H. G.Boman, U. Kaletta, *Biochem. Biophys. Acta.*, **1957**, 24, 619- 623.
19- K. H. Ahn, H. Kim, J. Kim, R. Jeomg, S. C. Kang, T. S. H. T. Shin, G. Lim, *J. Bull. Korean Chem. Soc.*, **2002**, 23, 626-628.
20- R. Hagiwara, Y. Ito, *Journal of Fluorine Chemistry.*, **2000**. 105, 2, 221-227.
21- T. Welton, *Chemical Reviews.*, **1999**, 99, 8, 2071-2083.
22- M. Earle, J. Seddon, *Pure and Applied Chemistry.*, **2000**, 72, 7, 1391-1398.
23- H. Olivier, *Journal of Molecular Catalysis A.*, **1999**, 146, 1-2, 285-289.
24- J .S. Yadav, B. Reddy, P. Sreedhar, *Journal of Molecular Catalysis A.*, **2007**, 270, 160-163.
25- R. Sheldon, *Chemical Communications.*, **2001**, 23, 2399-2407.
26- K. Niknam, A. Piran, *Green and Sustainable Chemistry*, **2013**, 3, 1-8.
27- S. S. Mansoor, K. Logaiya, K. Aswin,P. N. Sudhan, Journal of Taibah University for Science, **2015**, 9 ,213–226.
28- J. M. Khurana , B. Nand, P. Saluja, Tetrahedron,2010, 66 , 5637-5641.
29- M. Ziarani, G.Badiei, A.Azizi, M. Zarabadi, Iran. J. Chem. Chem. Eng. **2011**,Vol. 30, No. 2, 59-65.
30- P.A.Wayne, National Committee for clinical laboratory standard. Referece method for broth dilution antifungal susceptibility testing of conidium – forming filamentous fungi. Proposed standard M38-P. **1998**. *National Committee for clinical laboratory standard*.
31- Y.S.Shim, K.C.Kim, D.Y.Chi, K.H.Lee, H. Cho, *Bioorg. Med. Chem. Lett*, **2003**, 13, 2561-2563.