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Synthesis and in vitro antibacterial study of dihydropyrano[3,2-c]chromene derivatives by nano fluoro apatite doped with Mg and Si as a cooperative catalyst

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ABSTRACT

One of the important Multi-component reaction (MCRs) is the synthesis of dihydropyrano chromene derivatives by one-pot reaction. In this research, an inexpensive and effective one pot three component procedure with good yields for the synthesis of dihydro pyrano chromenes is reported. In this method, the synthesis of pyrano chromene derivatives was followed by using 4-hydroxycoumarin, malononitrile and aldehydes in the presence of catalytic amount of nano fluoro apatite doped with Mg and Si (Mg-Si-FA). It should be cited that all reactions were carried out in H_2O/EtOH as the solvent under reflux condition and the corresponding products were obtained in good yields and pretty short reaction time. Moreover, the anti-bacterial properties of the present products were studied by determination of their minimum inhibitory concentration (MIC).

Keywords: *Dihydropyrano[3,2-c]chromene, Multi-component reaction, Cooperative catalyst, Antibacterial activity.*

1. Introduction

Multicomponent reactions (MCRs), have played an important role in organic and medicinal chemistry [1,2]. Moreover, this approach is known as an important, economical and environmentally benign process in medicinal chemistry and modern organic synthesis because it not only decreases the number of reaction steps and times, energy consumption and waste [3,4] but also increases high purity and excellent yields[5]. Among different starting materials for the synthesis of heterocyclic compounds, especially oxygen-containing molecules which due to their physicochemical characteristics are an important and essential category of heterocycles [6]. Many compounds containing the 2H-chromene core show good biological activities. For example, Daurichromene D displays strong histamine release-inhibiting activity on rat peritoneal mast cells, also Gramniphenol C, a natural 2H-chromene is a strong antiviral agent (Fig. 1) [7]. Meanwhile, as shown in Fig. 2 some pyrano[3,2-c] chromene derivatives exhibit pharmaceutical properties [8].

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3,4-dihydropyrano[3,2-c]chromene derivatives are important groups of heterocycles with a broad range of biological and pharmacological properties, such as diuretic, spasmolytic, analgesic, anti-cancer, anticoagulant, anti-anaphylactic, anti-tumor and anti-fungal [9–13]. Also they can be used for the treatment of neurodegenerative diseases, including Alzheimer's disease, Parkinson's disease, Huntington's disease, AIDS associated dementia, Down's syndrome and schizophrenia [14,15]. Moreover, aminochromene derivatives show a wide spectrum of biological activities including antihypertensive and anti-ischemic behavior [16–18]. In general, 3,4-dihydropyrano[3,2-c]chromenes are available through the one-pot three-component reaction of aldehyde, malononitrile, and 4-hydroxycoumarin in the presence of basic catalyst in organic solvent. In recent studies, several methods for the preparation of these heterocyclic compounds have been reported with various catalysts such as tetrabutyl ammonium bromide (TBAB) [19], Sodium Dodecyl sulfate (SDS) [20], Hexa-methylenetetramine (HMT) [21], 4-(dimethyl amino) pyridine (DMAP) [22], 1,8-diazabicyclo[5.4.0] undec-7-ene (DBU) [23], poly (ethylene glycol) grafted N,N-dimethylaminopyridine

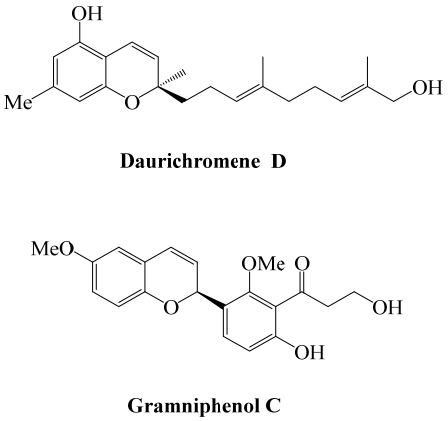


Fig. 1.

functionalized dicationic ionic liquid [24], CuO nanoparticles [25], silica-grafted ionic liquid [26], Magnetic Fe_3O_4-supported sulfonic acid-functionalized graphene oxide [27], Poly(4-Vinylpyridine) [28], Nano-SiO_2 [29], MgO [30] and o-benzenedisulfonimide [31].

Some of these procedures displayed disadvantages such as the use of toxic, highly acidic and expensive catalysts, require organic solvents, tedious work-up and use of stoichiometric amount of catalyst. Therefore, cleaning processes have been in permanent focus.

Recently, $Ca_{9.5}Mg_{0.5}(PO_4)_{5.5}(SiO_4)_{0.5}F_{1.5}$ (Si-Mg-FA) synthesized by simultaneous incorporation of F, Mg and Si into the hydroxyapatite (HA) structure and greatly enhanced biological, mechanical, physical and chemical properties and it has been observed as a new nano cooperative catalyst in organic synthesis [32–34].

In continuation of our ongoing program to develop the environmentally benign methods in organic synthesis, herein we report an efficient method for the synthesis of 3,4-dihydropyrano[c] chromene derivatives by using a three-component reaction of 4-hydroxycuomarin, aldehyde and malononitrile in the presence of $Ca_{9.5}Mg_{0.5}(PO_4)_{5.5}(SiO_4)_{0.5}F_{1.5}$ (Si-Mg-FA), as a new nano-biocatalyst under mild and heterogeneous conditions (Scheme 1).

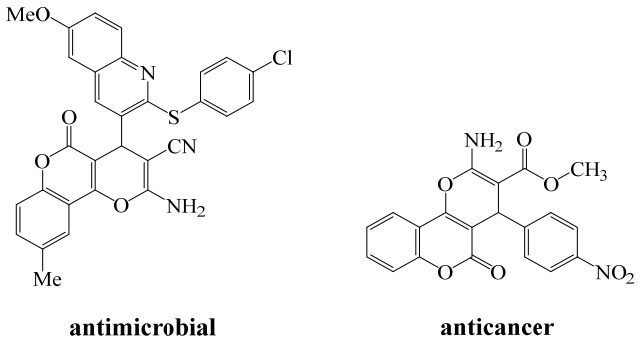


Fig. 2.

2. Experimental

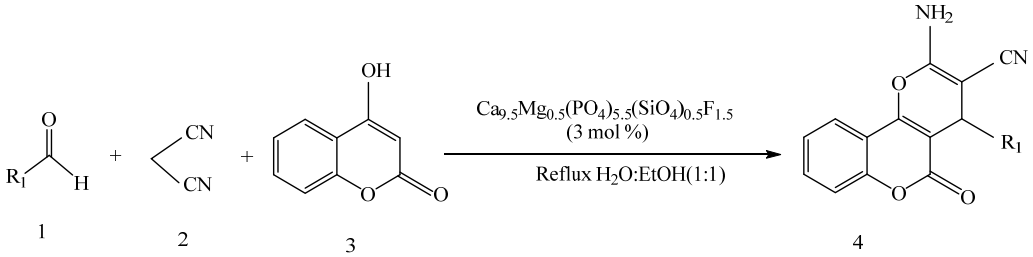
2.1. General

All reagents were purchased from Merck and Aldrich with more than 98% purity and used without further purification. Products were characterized by comparison of authentic samples (IR, ^{1}HNMR, ^{13}CNMR spectrum, melting point) with those obtained by literature. All melting points were taken on an Electro Thermal Amstead 9200 apparatus and are uncorrected.

^{1}HNMR spectra were recorded on a Bruker DRX-400 spectrometer operating at 400 MHz in DMSO-d6 as the solvent. FT-IR Spectra were obtained using Analyst Data PerkinElmer. Fluoro apatite doped with Mg and Si (Mg-Si-FA) as Nano powder was synthesized according to previous works [33,34].

2.2. General Procedure for the synthesis substituted 3,4-dihydropyrano[3,2-C]chromenes (4a-o)

A mixture of aldehyde (1mmol), malononitrile (1mmol) and 4-hydroxycoumarine (1 mmol) in the presence of $Ca_{9.5}Mg_{0.5}(PO_4)_{5.5}(SiO_4)_{0.5}F_{1.5}$ (0.03 g, 3 mol%) as a catalyst was added in 5 mL of EtOH/H_2O (1:1) and was stirred at reflux conditions. After the completion of the reaction, monitored by TLC (ethyl acetate, n-hexane 3:1), the mixture was cool and cold water was added to the mixture. The solid product was filtered. Then for the separation of the catalyst, the solid products were recrystallized in EtOH/H_2O (9:1).



Scheme 1. Synthesis of dihydropyrano[3,2-c]chromene derivatives using Si-Mg-FA.

2.3. Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentration of synthesized compounds were tested against a panel of bacteria strains by Macro-dilution method. Brain Heart Infusion (BHI) was used as a medium to grow and dilute the compound suspension for the bacterial test. Penicillin V and Tetracycline were bought from local pharmacy as tablets form (500 mg) and were used as standard antibacterial drugs. At first, colonies grown over night on an agar plate was inoculated into Brain Heart Infusion (BHI) (0.5 ml) in sterile conditions. Then the tube was incubated for 24 h at 37 ℃. Due to the growth of bacteria the solution became turbid. Turbidity was measured by the spectrophotometric device. In order to set the spectrophotometric device, after zeroing the instrument against the pure BHI solution, then the bacteria (0.5 ml) inoculated into BHI tube and was compared with 0.5 Mc Farland turbidity standard. Absorption in the range of (0.08-0.1) suggests 0.5 McFarland standard that, this range equals concentration (1.5×10^8 cuf/ml). In the following, the synthesized 3,4-dyhidropyrao[c] chromenes and also standard antibacterial drugs (0.004 gr) were dissolved in dimethyl sulfoxide (DMSO) (2 ml) and the solution was diluted with BHI (2 ml). Further progressive serial dilution was performed to obtain the required concentrations of 1000, 500, 250. 125, 62.5, 31.25, 15.6, 7.8, 3.9, 1.9 (µg/ml). Also to each of tubes were inoculated 100 µl of prepared bacterium suspension and incubated at 37 ℃ for 24 h. At the end of the incubation period, MIC was determined as the last dilution so that no increase in visual turbidity was observed.

3. Results and Discussion

Our preliminary investigations were focused on optimize the reaction condition and to evaluate the amount of silicon and magnesium co-doped fluorapatite ($Ca_{9.5}Mg_{0.5}(PO_4)_{5.5}(SiO_4)_{0.5}F_{1.5}$, Si–Mg-FA) as a catalyst. The reaction of 4-chlorobenzaldehyde, malononitrile, and **.**4-hydroxycoumarin was carried out under different conditions (Table 1). When the reaction was carried out in the absence of the catalyst, the corresponding product was obtained in low yield after lengthy reaction time (Table 1, entry 1). It was found that the best results was obtained when the reaction was carried out in H_2O/EtOH (1:1) at reflux condition by using 3 mol% of the catalyst (Table 1, entry 6). It should be mentioned that an increase of the amount of the catalyst did not improve the yield of the reaction (Table 1, entry 8).

After optimizing the reaction conditions, we studied the generality of this method. Using this procedure, different kinds of aromatic, aliphatic, α,β-unsaturated and heterocyclic aldehydes were treated with 4-hydroxycoumarin and malononitril to produce the corresponding 3,4- dihydropyrano[3,2-c] chromenes in good to high yields (Table 2).

As can be seen from Table 2, aromatic aldehydes having electron-withdrawing as well as electron-donating groups were transformed into the corresponding dihydropyrano [3,2-c] chromenes in good to high yields at short reaction times. It is obvious that the substituents of the aromatic aldehyde dramatically influence in the reaction time. Aromatic aldehydes with electron-withdrawing groups (such as halid, cyano and nitro) (Table 2, entries 2-9) required shorter reaction times but provided higher yields than those aldehydes with electron-donating groups (such as methyl and methoxy) (Table 2, entries 10,11). It is worth noting that the steric effects of ortho substitutions on the aromatic aldehydes increased the reaction times (Table 2, entries 8,10). The notable advantage of this method is that heterocyclic aldehydes such as thiophene-2-carbaldehyde and 3-pyridinecarbaldehyde were respectively converted to the corresponding products in high yields without making any side products (Table 2, entries 13,14).

Table 1. Optimization of condition of the synthesis of 3,4- dihydropyrano[c] chromenes.

Entry	Catalyst (mol%)	Solvent	Temp. (℃)	Time (min)	Yield (%)
1	-	H_2O/ EtOH	Reflux	120	12
2	2	EtOH	Reflux	120	60
3	3	H_2O	Reflux	120	50
4	4	EtOH	Ultrasonic irradiation	66	10
5	2	H_2O/ EtOH	Reflux	90	62
6	3	H_2O/ EtOH	Reflux	60	90
7	4	H_2O/ EtOH	Reflux	60	90
8	3	H_2O/ EtOH	Room temperature	240	8

Reaction conditions: 4-chlorobenzaldehyde (1mmol), malononitrile (1 mmol) and 4-hydroxycoumarin(1mmol). The yields refer to the isolated pure products.

Unlike most reported methods, by employing the present procedure, the aliphatic aldehydes were easily converted to the corresponding dihydropyrano[3,2-c]chromene products in suitable yields (76 %), (Table2,entry 15). Acetophenone didn't react and produce the corresponding 3,4- dihydropyrano[3,2-c] chromenes in this condition it may be due to the hindrance effect and lower electrophilicity of carbonyl group in the structure of the ketones in comparison with aldehydes. (Table 2, entry 16).

It should be mentioned that TON (turn over number) and TOF (turn over frequency) were investigated in the present work and the obtained results were summarized in Table 2.

The proposed mechanism for the condensation reaction of aldehyde, malononitrile and 4-hydroxycoumarin to their corresponding of 3,4- dihydropyrano[c] chromenes by using a catalytic amount of Si–Mg–FA is shown in Scheme 2. Initially Knoevenagel condensation between aldehyde and malononitrile is carried out. After that Michael addition between enolized 4-hydroxycoumarin and intermediate (I) is followed by cyclization and tautomerization [33,34].

Also, to show the quality of this method, the efficacy of the present catalyst was compared with some reported catalysts for the synthesis of 2-amino-4,5-dihydro-4-(4-chloro)-5-oxo-pyrano[2,3-c]chromen-3-carbonitrile.

As shown in Table 3, Si–Mg–FA is comparable with the most of the reported catalysts in views of yield, reaction time and reaction conditions. Moreover, the present catalyst is superior in terms of using low amount of the catalyst. The use of green and reusable catalyst, non-toxic solvent, mild reaction conditions and simple procedure are the other advantages of the present work.

The reusability of the catalyst was studied for the reaction of 4-chlorobenzldehye, malononitrile and 4-hydroxycoumarin under optimized conditions. After each run, hot ethanol was added to the reaction mixture to dissolve the product and the catalyst was separated by filtration. The catalyst dried at 120 ℃ for 2 h to use for the next reaction. The results showed that the catalyst could be employed three times, although its activity gradually decreased (Table 4).

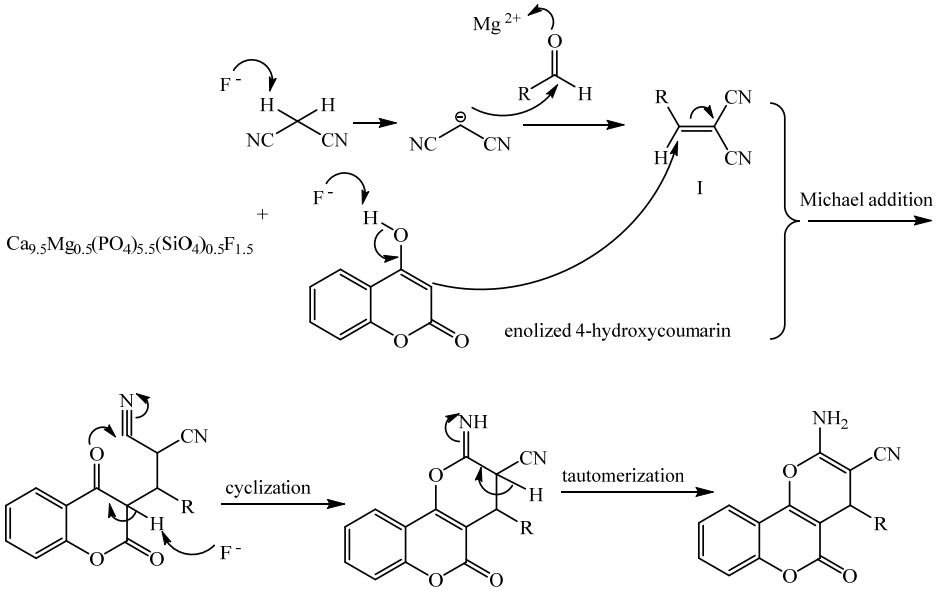
3.1. Antibacterial assay

As reported in the literature, the compounds with chromene core have notable antibacterial activity, for example indolyl-4H-chromenephenylprop-2-en-1-one derivatives show good antibacterial activity against Gram-posetive organisms *Staphylococcus aureus* and *B. subtilis* and also Gram-negative organisms *Klebsiella* and *Escherichia coli* [38], also biological activity of 2-Amino-4H-chromene Derivatives was studied and exhibit the good antibacterial activity against *Staphylococcus aureus* and *Bacillus anthracic* [39].

Table 2. Synthesis of 3,4-dihydropyrano[c]chromenes by using catalytic amount of Si-Mg-FA.

Entry	Carbonyl compounds	Products	Time (min)	Yield (%)[a]	m.p. (℃)		TON	TOF (hr^{-1})	Ref.
					Found	Reported			
1	C_6H_5CHO	4a	90	92	257-258	256-257	30.67	20.44	[24]
2	4-Cl-C_6H_4CHO	4b	60	90	261-263	263-264	30.00	30.00	[24]
3	4-F-C_6H_4CHO	4c	90	87	265-267	262-263	29.00	19.33	[24]
4	3-Br-C_6H_4CHO	4d	30	82	274-275	274-276	27.33	54.66	[35]
5	3-NO_2-C_6H_4CHO	4e	40	85	266-267	264-265	28.33	42.28	[24]
6	4-NO_2-C_6H_4CHO	4f	30	95	261-263	258-260	31.67	63.34	[36]
7	4-CN-C_6H_4CHO	4g	60	88	288-290	289-290	29.33	29.33	[21]
8	2,4-Cl_2-C_6H_3CHO	4h	60	90	256-257	257-259	30.00	30.00	[36]
9	4-CH_3OCO-C_6H_4CHO	4i	15	92	242-246	-	30.67	122.68	-
10	2-OCH_3-C_6H_4CHO	4j	90	84	244-246	247-249	28.00	18.66	[21]
11	4-CH_3-C_6H_4CHO	4k	120	95	260-261	254-256	31.67	15.83	[8]
12	Cinnamaldehyde	4l	30	86	191-193	-	28.67	57.34	-
13	2-Thienyl-CHO	4m	90	85	260	263-265	28.33	18.89	[35]
14	3-Pyridyl-CHO	4n	90	92	248-250	251-253	30.67	20.44	[37]
15	Hexanal	4o	90	76	188-190	-	25.33	16.88	
16	Acetophenone	4p	180	-	-	-	-		

[a]The yields refer to the isolated pure products. [8,21,23,34,35,36].



Scheme 2. A possible mechanism for the formation of 3,4-dihydropyrao [c] chromene derivatives.

In order to investigation on the antimicrobial properties of compounds with chromene core some of the synthesized products and standard drugs were screened for their in-vitro antibacterial activities by Minimum inhibitory concentration (MIC) method. All the bacteria were procured from Iranian Research Organization for Science and Technology. The antibacterial assay was carried out against two Gram-Negative bacteria [*Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853)] and two Gram-Positive bacteria [(*Staphylococcus aureus* (ATCC 25923), *Staphylococcus Epidermidis* (ATCC 12228)]. For the antibacterial activity the Penicillin, Tetracycline was used as a standard reference.

The results reported in the table 5 showed among all the screened derivatives compound 4a, 4j, 4l, 4n, have good antibacterial activity with MIC value of 150µg/ml against Gram-positive bacterium such as, *Staphylococcus aureus* (ATCC 25923) compared to Penicillin and Tetracycline drug. Also compound 4a showed good antibacterial activity with MIC value of 150µg/ml against another Gram-positive bacterium *Staphylococcus Epidermidis* (ATCC 12228). Moreover, review of the results reported in table 3 have showed compound 4a, 4j, good antibacterial activity against *Escherichia coli* bacterium (ATCC25922) as Gram-negative bacterium with MIC value of 135µg/ml compared to control drug (Penicillin, Tetracycline) and also, other compound has good antibacterial activity.

Table 3. Comparison of Si–Mg-FA with different catalysts for the synthesis of 2-amino-4,5-dihydro-4-(4-chloro)-5-oxo-pyrano[2,3-c]chromen-3-carbonitrile.

Entry	Catalyst	Amount of catalyst	Conditions	Time (min)	Yield (%)	Ref.
1	Tetrabutylammoniom bromide	10 mol (**%**)	H_2O (reflux)	45	93	[19]
2	Sodium Dodecyl Sulfate	20 mol (**%**)	H_2O (reflux)	180	88	[20]
3	Hexa-methylenetetramine	10 mol (**%**)	EtOH (reflux)	40	95	[21]
4	Nano-SiO_2	20 mol (**%**)	H_2O (70 ˚C)	21	92	[31]
5	MgO nanoplates	50 mol (**%**)	H_2O (reflux)	120	84	[32]
6	o-Benzenedisulfonimide	50 mol (**%**)	Solvent free/ 120 ℃	40	85	[33]
7	$(NH_4)_2HPO_4$	10 mol (**%**)	EtOH/H_2O (reflux)	120	85	[37]
8	ZnO	10 mol (**%**)	H_2O (reflux)	180	91	[35]
9	Silica-supported molybdic acid	5 mol (**%**)	EtOH/H_2O (reflux)	40	94	[38]
10	Si-Mg-FA	3 mol (**%**)	EtOH/H_2O (reflux)	60	90	This work

Table 4. Reusability of the catalyst.

Run	Time (min)	Yield (%)
1	60	90
2	80	87
3	80	82

Also, against *Pseudomonas aeruginosa* bacterium (Gram-negative bacteria) all of the compounds except 4j, 4m have showed good antibacterial activity with MIC value of 150µg/ml compared to control drug. According to this conclusion, it is worth to mention that the synthesized 3,4-dihydropyrano[c]chromenes compounds emerged as a spot of antimicrobial medicine research.

4. Conclusions

In conclusion, we have developed the one-pot synthesis of 3,4-dihydropyrano [c] chromenes derivatives in excellent yields by a simple and efficient procedure under mild conditions in the presence of $Ca_{9.5}Mg_{0.5}(PO_4)_{5.5}(SiO_4)_{0.5}F_{1.5}$ (Si-Mg-FA) as a co-operative catalyst. The advantages of this method are the short reaction time, high yields, simple workup, and non- chromatographic purification of products. The present method does not involve any hazardous organic solvent, the reaction was carried out in eques ethanol reflux. Therefore, this procedure could be classified within green chemistry. The synthesized compounds were evaluated for their antibacterial activities against Gram-Negative bacteria and Gram-Positive bacteria. Some of the compound, showed good antibacterial activity.

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Table 5. Anti-microbial activity (MIC) (µg/ml) of the synthesized 3,4-dihydropyrano chromenes derivatives.

Compound	Gram-Positive Bacteria		Gram-Negative Bacteria	
	Staphylococcus aureus ATCC 25923	*Staphylococcus Epidermidis* ATCC 12228	*Escherichia coli* ATCC 25922	*Pseudomonas aeruginosa* ATCC 27853
4a	150	150	135	150
4j	150	250	135	300
4k	270	270	150	150
4h	250	250	150	150
4l	150	250	150	150
4n	150	250	150	150
4g	500	250	150	150
4m	270	270	150	300
Penicillin	35	80	62.5	35
Tetracycline	15	3.9	3.9	70

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