Iranian Journal of Organic Chemistry Vol. 8, No. 1 (2016) 1693-1701

Synthesis and antibacterial of oxopyrano chromene derivatives through the multicomponent reaction of 4-hydroxycumarin derivatives with dialkylacetylene dicarboxylates and isocyanides catalyzed by piperdine

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Received: November 2015; Revised: January 2016; Accepted: January 2016

Abstract: In this work, we report a new family of chromene from the one-pot three-component reaction of 4-hydroxycumarin **1** with dialkylacetylene dicarboxylates **2** and isocyanides **3** catalyzed by piperidine, under condition of room temperature and ethanol as a solvent. This reaction leads to the compounds dialkyl-2-(tert-butylamino)-4,5-dihydro-5-oxopyrano[3,2-c]chromene-3,4-dicarboxylate **4a, 4b, 5** and dialkyl-2-(cyclohexylamino)-4,5-dihydro-5-oxopyrano[3,2-c]chromene-3,4-dicarboxylate **4c-4f** and are evaluated as antibacterial agents against a panel of Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Candida albicans, **B**. subtilis and Proteus bacteria. Among synthesized compounds, **4a** exhibited more potent inhibitory activity against Escherichia coli and Staphylococcus aureus (0.5-2 µg/ml.) in high yields.

Keywords: Oxopyrano-[3,2-*c*]chromene derivatives, Isocyanides, Ugi reaction, Piperidine, Antibacterial activity.

Introduction

The synthesis of coumarins and their derivatives play an important role in organic chemistry not only due to their presence as key structural units in many natural products and in important pharmaceuticals, but they can also be employed in synthetic chemistry as building blocks and has attracted considerable attention from organic and medicinal chemists for many years as a large number of natural products contain this heterocyclic nucleus. They are widely used as additives in food, perfumes, agrochemicals, cosmetics, pharmaceuticals [1] and in the preparations of insecticides, optical brightening agents and dispersed fluorescent [2]. Cumarin is an important class of benzopyrones that is found in nature and acts as a structural subunit of more complex natural products [3].These molecules generally have a broad range of biological activities and found in many plants, notably in high concentration in the Tonka bean, woodruff, and bison grass [4]. They are the structural motif of many natural and a synthetic compound that endows them with a wide range of biological activities, given the development of coumarins as photosensitizes [5], anti HIV agents [6], antibiotics [7], anticancer [8], rodenticides and oral anticoagulants [9]. It is also used as a gain medium in some dye lasers [10, 11]. And also act as intermediates for the synthesis of fluorocumarins, chromenes, cumarone, and 2-acylresorcinols [12]. Their properties turn cumarins very interesting targets to organic chemists, and several strategies for their synthesis were already developed. There is continuing interest in the synthesis of these materials. Cumarins can be synthesized by various methods such as Pitchman [13], Perkin [14], Knoevenagel [15], Reformat sky [16] and Witting reactions [17].

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Chromenes are of special interest as photo chromic compounds [18] due to their photo-induced reversible electro cyclic opening of the pyran ring. [2]H- and [3] H-Chromenes represent an important class of molecules [19, 20] being the main subunits in numerous natural and biologically active compounds. They are widely adopted for fabrication of opto-electronic devices and ophthalmic lenses [21]. Development of chromenes with programmed photo chromic properties are of foremost importance.

Multicomponent reactions (MCRs), [22, 23] involving at least three starting materials in a one-pot reaction, remain the most efficient method of rapidly introducing molecular diversity. As such they have found widespread use in organic and diversity-oriented synthesis by their ability to access highly functionalized molecules in simple and straightforward one-step transformations. [24] Compared to conventional multistep organic syntheses MCRs are advantageous owing to their greater atom efficiency, accessibility to large numbers of compounds and complex molecules, wide structural diversity and simplicity of their one-pot procedures making them amenable to combinatorial synthesis. Among the known multicomponent reactions to date, the most valuable reactions are those based on isocyanides.

In recent years, isocyanide-based multi-component reactions (MCRs) have gained much attention because of their synthetic potential to obtain a diverse array of heterocycles [22], especially in drug discovery [23]. The fact that complex products can be formed in a single operation by simultaneous reactions of several reagents has caused IMCRs to be among the most powerful methods for the synthesis of organic molecules [24].

It has been shown that alkyl or aryl isocyanides add to dialkylacetylene dicarboxylates to generate zwitterionic species, which serve as intermediates in many different reactions. [25-28], recently, these highly reactive zwitterionic intermediates have been captured by suitable CH- [26], NH- [27], and OH– acids [28] substrates. The reaction between isocyanides and dimethyl acetylene dicarboxylate (DMAD) in the presence of naphthols and phenols respectively yields benzochromene [29] and chromene derivatives [30]. Similar products were reported from the reaction between DNAD, cyclohexyl isocyanide or *tert*-butyl isocyanide and 4-hydroxycumarin in water, in the presence of a phase-transfer catalyst at 80 ˚C after four hours and in 70% yield [31]. The similar reaction also has been carried out under dichloromethane at room temperature and 24 hr. or under acetone and reflux after 24 hours [32-33].

The inhibition of DNA gyrase or DNA topoisomerase IV and cell permeability of the quinolones are greatly influenced by the nature of the C-7 substituent on the standard structure of 4-quinolone-3-carboxylic acids. In addition, the substitution of bulky groups is permitted at the C-7 position [34-37].

With these in mind, previously, we have reported the synthesis and antibacterial activity of gatifloxacin hybrids, carrying N-[nitroaryl)-1,3,4-thiadiazol-2-yl]-gatifloxacin derivatives that exhibit high activity against-positive and less activity against Gram-negative bacteria [38].

In view of our general multi-component reactions (MCRs), we have reported the reaction of isocyanides, dialkylacetylene dicarboxylates and urea derivatives in the presence of DMF at room temperature and 48 h. [39].

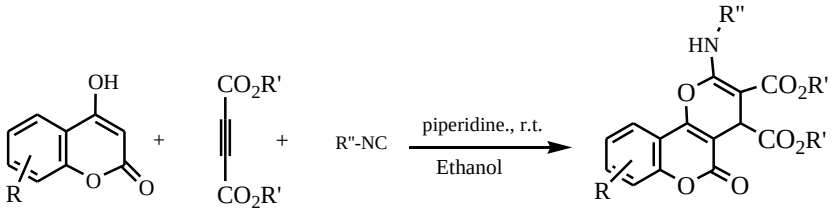
In the present work, we report a new family of chromene from the one-pot three-component reaction of 4-hydroxycumarin **1** with dialkylacetylene dicarboxylates **2** and isocyanides **3** in the presence of piperidine, under condition of room temperature and ethanol as a solvent after **1** hr. This reaction leads to the compounds dialkyl-2-(*tert*-butylamino)-4,5-dihydro-5-oxopyrano[3,2-*c*]chromene-3,4-dicarboxylate **4a, 4b, 5** and dialkyl-2-(cyclohexylamino)-4,5-dihydro-5-oxopyrano[3,2-*c*]chromene-3,4-dicarboxylate **4c**-**4f** in high yields (Scheme **1**).

When the reaction was carried out with 4-hydroxycumarin and ethyl phenyl acetylene carboxylate in the presence of *tert*-butyl isocyanide, it afforded compound **5** (Scheme **2**).

A single-crystal X-ray diffraction study confirmed the identity of compound **4c** [40]. An ORTEP diagram of **4c** is shown in Figure **1.**

Mechanistically, for the **4a**-**4f** and **5** products, it is conceivable that the reaction involves the initial formation of a 1:1 zwitterionic intermediate **10** between *tert-but*-isocyanide or cyclohexyl isocyanide and the acetylenic ester, which react with 4-hydroxycumarin. Cyclization leads to the dimethyl-2-(*tert*-butylamino)-4,5-dihydro-5-oxopyrano-[3,2-*c*] chromene-3,4-dicarboxlate **4a**, diethyl-2-(*tert*-butylamino)-4,5-dihydro-5-oxopyrano-[3,2-*c*] chromene-3,4-dicarboxlate **4b**, dimeithyl-2-(cyclohexylamino)-4,5-dihydro-5-oxopyrano-[3,2-*c*] chromene-3,4-dicarboxlate **4c**, diethyl-2-(cyclo hexylamino)-4,5-dihydro-5-oxopyrano-[3,2-

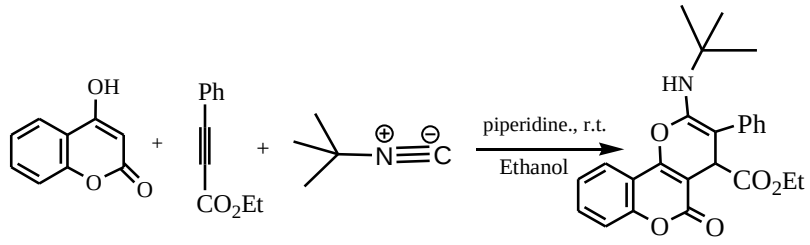
c]chromene-3,4-dicarboxlate **4d**, dimethyl-2-(cyclohexylamino)-9-fluro-5-oxo-4H,5H-pyrano-[3,2-c]chromene-3,4-dicarboxlate **4e,** dimethyl-2-(cyclohexylamino)-9-fluro-5-oxo-4*H*,5*H*-pyrano-[3,2–c]chromene-3-4-dicarboxylate **4f** andethyl-2-(*tert*-butylamino)-4,5-dihydro-5-oxo-3-phenylpyrano[3,2-*c*]chromene-4-carboxylate **5** (Scheme **3**).



1 **2** **3** **4a-f, 5**

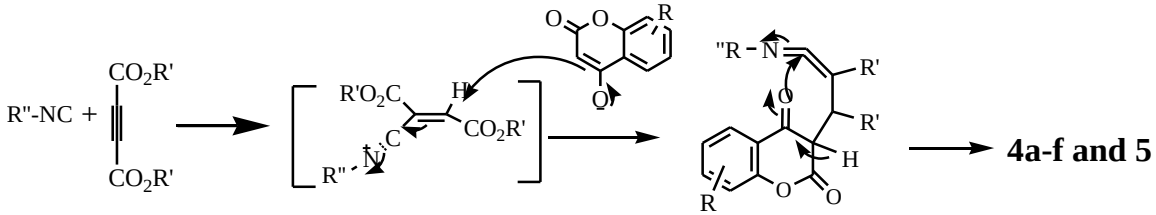
	R	R'	R"	Yield%
4a	H	Me	*tert*-buthyl	88
4b	H	Et	*	95
4c	H	Me	cyclohexyl	78
4d	H	Et	*	90
4e	F	Me	*	92
4f	F	Et	*	95
5	H	Et, Ph	*tert*-buthy	70

Scheme 1: The reaction of isocyanides, dialkylacetylene dicarboxylates and 4-hydroxycumarine derivatives.



5

Scheme 2: The reaction of *tert*-butyl isocyanide, ethyl phenyl acetylene carboxylate and 4-hydroxycumarine.



Scheme 3: The mechanism of the reaction of isocyanides, dialkylacetylene dicarboxylates and 4-hydroxycumarine Derivatives.

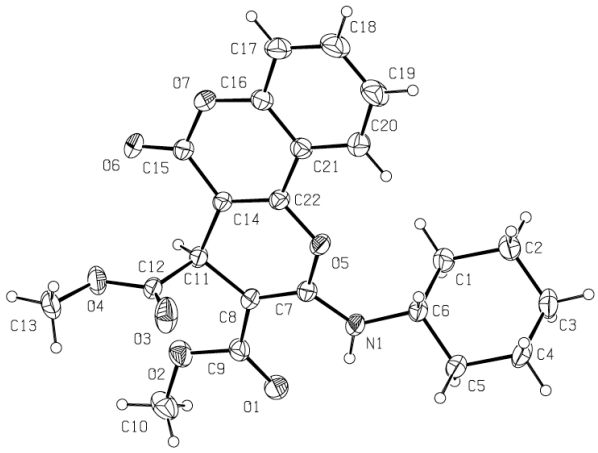


Figure 1: ORTEP diagram of the X-ray crystal structure of **4c.**

The antimicrobial activity of compounds (**A-E**) was determined by Agar disc diffusion technique from the *Punctuation Method and Well Method*. The chromenes obtained were preliminarily evaluated for their *in vitro* antibacterial activity against a narrows spectrum of bacterial species procured from the Laboratory of Microbial Biochemistry (Chem. Dept. Faculty of Science, North Tehran Branch, Azad Univ.) [41]. *Staphylococcus aureus, Escherichia coli.tui*, *pseudomonas aeruginosa* and also *Candida albicans* fungus were incubated at 37˚C during a period 24 hrs. The compound that showed best antimicrobial activity was further tested by the dilution method. After the incubation for 24 hrs, the last tube with no growth of microorganisms was taken to represent MIC (Minimum Inhibitory Concentration). The concentration of the prepared solutions was 0.5, 0.25, 0.125, 0.0625, 0.03125, 0.0156, 0.0078, 0.0039, 0.00195, 0.00097, 0.00048 and 0.00024µg/ml. The aim of this method was to determine the exact concentration of the investigated compound which will have an inhibitory effect on the growth of selected microorganisms. This concentration was considered as minimal inhibition concentration (MIC). MBC (minimum bactericidal concentration) is determined by sub culturing the solution of substances with no visible opacity onto the culture medium.

Among synthesized compounds, dimethyl-2-(cyclohexylamino)-9-fluro-5-oxo-4H,5H-pyrano-[3,2-c]chromene-3,4-dicarboxlate **(4e)**, exhibited the most potent inhibitory activity against *Escherichia Coli.uti* and *Staphylococcus aureus* bacteria.

The compounds were tested at two concentrations, 0.5 and 2 µg mL^{-1} in $CHCl_3$ Table **1,** Figures **2-3**.

As seen in the Table **2,** we obtain concentrations of synthesized compounds MIC and MBC for *Staphylococcus aureus* and *Escherichia coli* bacteria.

Table 1: microbiological activity of newly synthesized chromene derivatives, A=DMAD TERT Q, B=DEAD TERT Q, C=DMAD CYCLO Q, D=DEAD CYCLO Q, E=DMAD CYCLO QF.

Test compound	Concentration	E. Coli	Pseudomonas aeruginosa	Staphylococcus aureus	Candida albicans
A	0.5	0	6	6	0
	2	9	8	10	8
B	0.5	0	0	0	0
	2	0	6	0	5
C	0.5	7	5	5	0
	2	10	11	9	6
D	0.5	0	0	0	0

	2	0	4	0	0
E	0.5	15	11	14	0
	2	25	28	18	19

Table 2: MIC and MBC of synthesized compounds against *Staphylococcus aureus* and *Escherichia coli*.

Test compound	*Staphylococcus aureus*		*Escherichia coli*	
	MIC(mg/mL)	MBC(mg/mL)	MIC(mg/mL)	MBC(mg/mL)
A=DMAD- TERT Q	0.125	0.25	0.25	0.125
C=DMAD-CYCLO Q	0.0625	0.125	0.0156	0.0625
E=DMAD-CYCLO QF	0.156	0.0039	0.00195	0.0078

Results and discussion

In the present study we report our results on the one-pot three-component reaction of 4-hydroxycumarin **1** with dialkylacetylene dicarboxylates **2** and isocyanides **3** catalyzed by piperidine, under condition of room temperature and ethanol as a solvent.

The Mass spectra of all compounds are fairly similar and display molecular ion peaks. The IR spectra of **4a,** showed signal at about 3241 cm^{-1}, assignable to the NH and strong absorption at about 1728 and 1661 cm^{-1}, assignable to the two ester carbonyls. The strong absorptions in the 1607 cm^{-1}, is assignable to the C=C. The ^{1}H NMR spectrum of **4a,** exhibited 8 signal lines, readily recognized as arising from three methyl (δ = 1.56), methoxy (δ = 3.72 and 3.76), methin (δ = 4.76) and NH group at (δ = 9.02)protons, together with a fairly complex multiple in the aromatic region. The ^{13}C NMR spectrum of **4a**, showed 17signals in agreement with the proposed dimethyl-2-(*tert*-butylamino)-4,5-dihydro-5-oxopyrano-[3,2-*c*]chromene-3, 4-dicarboxlate structure. The ^{1}H and ^{13}C NMR spectra of **4b-4f** and **5** are similar to those of **4a** except for the ester groups, which exhibit characteristic signals with appropriate chemical shifts. A single-crystal X-Ray diffraction study confirmed the identity of compound **4c** [40]. An ORTEP diagram of **4c** is shown in Figure **1.** The antimicrobial activity of compounds (A-E) from the ***well method*** and ***Punctuation method*** was determined by disc diffusion technique. Among synthesized compounds, dimethyl-2-(cyclohexylamino)-9-fluro-5-oxo-4H,5H-pyrano-[3,2-c]chromene-3,4-dicarboxlate **(4e)**, exhibited the most potent inhibitory activity against *Escherichia Coli.uti* and *Staphylococcus aureus* bacteria. That is shown in Table **1**.

Table 1: Model reaction and optimization under various conditions for the synthesis of arylmethyledene-isoxazole-5(4H)-one[a].

Entry	Catalyst	Solvent[b]	Temp ^{0}C	Time (min)	Yield (%)[c]
1	5mol%	EtOH	R.T.	70min	65
2	10mole%	EtOH	R.T.	60min	90

3	15mol%	EtOH	R.T.	50min	90
4	10mol%	EtOH/H_2O(1:1)	R.T.	50min	90
5	10mole%	EtOH/H_2O(1:2)	R.T.	60min	88

[a]*Reaction Conditions*: ethyl acetoacetate (1mmol), 4-OMe benzaldehyde (1mmol), hydroxylamine hydrochloride (1mmol), [b] 4 mL, [c] isolated yield.

Table 2: Synthesis of Arylmethyledene-isoxazole-5(4H)-ones[a].

Entry	Ar	Product	Yield (%)[b]	Time(min)	m. p (^oC)		
					Found	Reported	Ref
1	2-OHC_6H_4	4a	92	60min	197-199	198-201	[16]
2	4-$CH_3OC_6H_4$	4b	90	50min	172-174	175-177	[16]
3	C_6H_5	4c	90	55 min	138-140	141-143	[16]
4	4-$NO_2C_6H_4$	4d	40	80min	178-180	--------	-----
5	4-OHC_6H_4	4e	90	70min	212-214	210-211	[19]
6	N,N$(CH_3)_2C_6H_4$	4f	89	80min	222-225	220-221	[19]
7	3-OCH_3,4-OHC_6H_3	4g	45	80min	210-212	212-215	[16]
8	4-CH_3 C_6H_4	4h	93	70min	132-134	135-135	[19]
9	C_6H_5CH=CH	4i	92	65min	174-176	171-173	[16]

[a]*Reaction Conditions*: ethyl acetoacetate (1mmol), aldehydes(1mmol), hydroxylamine hydrochloride(1mmol), imidazole(20mol%), EtOH:H_2O(1:1/4ml),R.T, [b]Isolated yield.

Conclusion

In conclusion, we have developed an efficient and green approach for the one-pot three component synthesis a new family of chromene derivatives from the 4-hydroxycumarin **1** with dialkylacetylene dicarboxylates **2** and isocyanides **3** catalyzed by piperidine, under condition of room temperature and ethanol as a solvent. Moreover, the advantages of this method are the experimental simplicity, inexpensive reagents, short reaction times and easy workup procedure. The reaction leads to the compounds dialkyl-2-(tert-butylamino)-4,5-dihydro-5-oxopyrano [3,2-c]chromene-3,4-dicarboxylate **4a, 4b, 5** and dialkyl-2-(cyclohexylamino)-4,5-dihydro-5-oxopyrano [3,2-c]chromene-3,4-dicarboxylate **4c-4f** and are evaluated as antibacterial agents against a panel of Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Candida albicans, **B**. subtilis and Proteus bacteria. Among synthesized compounds, dimethyl-2-(cyclohexylamino)-9-fluro-5-oxo-4H, 5H-pyrano-[3,2-c]chromene-3,4-dicarboxylat **4a** exhibited more potent inhibitory activity against Escherichia coli and Staphylococcus aureus (0.5-2 µg/ml.).

Experimental

General:

All reagents were purchased from Merck, Fluka and Aldrich with high-grade quality. Melting points were measured on an Electro thermal 9100 apparatus. IR

spectra were recorded on a Shimatzu IR-460 spectrometer. Mass spectra were recorded on a FINNIGAN-MAT 8430 Mass spectrometer operating at an ionization potential of 70 eV. The NMR spectra were recorded at 400, 300 and 250 (^{1}H), 100, 75.4 and 62.9 (^{13}C), on a Bruker Avance DPX-400 MHz NMR instrument with $CDCl_3$ as solvent. Chemical shifts (δ) are reported relative to TMS as the internal standard.

Typical experimental procedure for preparation of Dimethyl-2-(tert-butylamino)-4,5-dihydro-5-oxopyrano-[3,2-c]chromene-3,4-dicarboxlate (4a):

To a magnetically stirred solution of 4-hydroxycumarin **1** (0.162 g, 1 mmol) and dimethyl acetylene dicarboxylate **2** (0.122ml, 1 mmol) in ethanol (15 ml), was added drop wise *tert-but*-isocyanide **3** (0.113 ml, 1 mmol) at room temperature over 10 min. The reaction mixture was stirred at room temperature and 0.1 ml piperidine for 1 h. The solvent was removed under vacuum and the solid residue was washed with ethanol and afforded the correspondingdimeithyl-2-(cyclohexylamino)-4,5-dihydro-5-oxopyrano-[3,2-*c*]chromene-3,4-dicarboxlate (**4a**) as white crystals in a **88%** yield; mp= 214-216°C.

Selected spectral data:

*Dimethyl-2-(tert-butylamino)-4,5-dihydro-5-oxopyrano-[3,2-c]chromene-3,4-dicarboxlate (**4a**):*

White crystals (0.341g, 88%); mp: 214–216°C; IR (KBr)(ν_{max},cm^{-1}): 3241 (N–H), 1728, 1681 (C=O), 1607 (C=C); ^{1}H NMR (300 MHz, $CDCl_3$, Me_4Si): δ_H 1.57 (9H, s, $3CH_3$), 3.72 and 3.76 (6H, 2s, $2OCH_3$), 4.76 (1H, s, CH), 7.38 (1H, t, J=7.5 and 7.8 Hz), 7.40 (1H, d, J=7.8 Hz, CH), 7.61 (1H, t, J=7.8 and 7.8 Hz, CH), 7.85 (1H, d, J=7.5 Hz, CH), 9.02 (1H, brs., NH). ^{13}CNMR (75.4 MHz, $CDCl_3$, Me_4Si): δ_C 30.9 (3C*Me*$_3$), 51.6 (*C*Me$_3$), 53.0 and 53.2 ($2OCH_3$), 73.3(*C*H), 103.5, 113.9, 117.6, 122.7, 125.0 and 133.0 (6C) 153.1, 155.4 and 159.9 (*3*C), 161.1, 169.9 and 173.4 (3C=O); MS (*m/z*, %): 320, 240, 121, 57, $C_{20}H_{21}O_7N$,388 (M+.60).

*Diethyl-2-(tert-butylamino)-4,5-dihydro-5-oxopyrano-[3,2-c]chromene-3,4-dicarboxlate (**4b**):*

White crystals (0.39g, 95%); mp: 211–213°C; IR (KBr)(ν_{max}/cm^{-1}): 3233 (N–H), 1731, 1687, 1653 (3C=O), 1601 (C=C); ^{1}HNMR ($CDCl_3$, Me_4Si): δ_H 1.23 (9 H, s, CMe_3), 1.05- 2.07 (6 H, m, 2 CH_3), 4.14 (4H, m, $2OCH_2$), 4.70 (1H, s, CH), 7.35–7.70 (4H, m, arom), 8.73 (1H, s, NH). ^{13}CNMR ($CDCl_3$, Me_4Si): δ_C 14.1 (C*Me*$_3$), 33.4 ($2CH_3$), 50.6 (*C*Me$_3$), 59.8 and 61.4 ($2OCH_2$), 63.1 (CH), 73.2 ,103.2 and 113.5 (3C), 117.1, 121.9, 124.5, and 132.6 (4 CH), 152.7, 154.8, 1589.1 (=*C*–N and 2=C–O), 160.7, 169.1 and 172.9 (3CO); MS (*m/z*%): $C_{22}H_{25}O_7N$,415 (M+.25).

*Dimeithyl-2-(cyclohexylamino)-4,5-dihydro-5-oxopyrano-[3,2-c]chromene-3,4-dicarboxlate (**4c**):*

White crystals (0.32g, 78%); mp: 199–201°C; IR (KBr)(ν_{max},cm^{-1}): 3250 (N–H), 1732, 1688 and 1664 (C=O), 1605 (C=C); ^{1}HNMR (400 MHz, $CDCl_3$, Me_4Si): δ_H 1.25–2.10 (10H, m, $5CH_2$), 3.70 and 3.72 (6H, 2s, $2OCH_3$), 3.74 (1H,m,N–*CH*), 4.74 (1H, s, CH), 7.36 (1H, t, $^3J_{HH}$ = 8.2 and 7.6 Hz, CH), 7.40 (1H, d, $^3J_{HH}$ = 8.2 Hz, CH), 7.61 (1H, t, $^3J_{HH}$ = 7.6 and 8.0 Hz, CH),7.71 (1H, d, $^3J_{HH}$ = 8.2 Hz, CH), 8.71 (1H, d, NH); ^{13}CNMR (100.6 MHz, $CDCl_3$, Me_4Si): δ_C 24.4, 25.4, 33.4, 33.7 and 36.3 ($5CH_2$), 50.7 (*C*H methin), 51.2 and 52.7 ($2OCH_3$), 72.1 (HN–*C*H) 103.1, 113.6, 117.1, 121.9, 124.6 and 132.7 (6*C*), 152.7, 154.9 and 158.2 (=*C*-N and 2 =C-O), 160.6, 169.3 and 173.1 (3 C=O); MS (*m/z* %): $C_{22}H_{23}O_7N$, 413 (M+.15).

*Diethyl-2-(cyclohexylamino)-4,5-dihydro-5-oxopyrano-[3,2-c]chromene-3,4-dicarboxlate(**4d**):*

White crystals(0.42g, 90%); mp: 166–167°C; IR (KBr)(ν_{max}/cm^{-1}): 3230 (NH), 3120 (=C-H), 2930 and 2856 (-CH), 1736, 1727, 1689 (3C=O), 1602 (C=C);^{1}HNMR ($CDCl_3$, Me_4Si): δ_H 1.24-2.06 (16H, m, $5CH_2$ and $2CH_3$), 3.85 (1H, s, N-*CH*), 4.10- 4.19 (4H, m, $2OCH_2$), 4.69 (1H, s, CH), 7.33–7.69 (4H, m, arom), 8.70(1H, d , $^3J_{HH}$ = 7.5 Hz, NH); ^{13}CNMR ($CDCl_3$, Me_4Si): δ_C 14.1 and 14.4 ($2CH_3$), 24.4, 25.3, 33.4, 33.7 and 36.5 ($5CH_2$), 50.59 (NH-*C*H), 59.8 and 61.4 ($2OCH_2$), 72.2 (CH), 103.0 (=*C*-CH), 113.6 (=*C*-C-O), 117.0, 121.9, 124.5 and 132.6 (4C), 152.7 (=*C*-NH), 154.8 and 158.1 (2C), 160.7, 168.9 and 172.9 (3 C= O); MS (*m/z* %): $C_{24}H_{27}O_7N$, 441 ($M^{+\cdot}$15).

*Dimethyl-2-(cyclohexylamino)-9-fluro-5-oxo-4H,5H-pyrano-[3,2-c]chromene-3,4-dicarboxlate (**4e**):*

White crystals (0.36g, 92%); mp: 240-243°C; IR (KBr)(ν_{max}/cm^{-1}): 3360 (NH),1732, 1687, 1655 (3C=O), 1602 (C=C), 1085 (C-F); ^{1}HNMR (400 MHz, $CDCl_3$, Me_4Si): δ_H 1.32–2.09 (10H, m, $5CH_2$), 3.72 and 3.76 (6H, s, $2OCH_3$), 3.86 (1H, m, NH-*CH*), 4.74 (1H, s, CH), 7.33 (1H, t, $^3J_{HH}$ = 6 Hz, CH), 7.37 (1H, d , $^3J_{HH}$ = 6.4 Hz, CH), 7.40 (1H, t, $^3J_{HH}$ = 6.4 Hz, CH), 8.71 (1H, sbr., NH); ^{13}CNMR ($CDCl_3$, Me_4Si): δ_C, 24.4, 25.3,33.4, 33.7 and 36.3 (5CH2), 50.7 (NH-*C*H), 72.1 (-*C*H), 51.3 and 52.7 ($2OCH_3$), 104.0, 107.7, and 108.0 (=*C*-C-F), 118.8 and 118.9 (2O-*C*=C), 120.2 (*C*=C-C-F), 120.4 (=*C*-C-C=C-F), 154.1 (O-*C*-C=C-O), 157.6 (C-F,J_{CF} =244 Hz), 158.1 (O-CO), 160.0

(=*C*-NH), 160.3,169.2 and 172.8 (3 C=O). MS (*m/z* %): 432 ($M^{+\cdot}$60); ^{19}F NMR (90 MHz, $CDCl_3$, CF_3COOH): δ_F -38.76 (1H, d, $^3J_{HH}$ = 6.4 Hz, CH),

*Diethyl-2-(cyclohexylamino)-9-fluro-4,5-dihydro-5-oxopyrano-[3,2–c]chromene-3-4-dicarboxylate(**4f**):*

Whit crystals (0.4g, 95%); m.p.168–171°C;IR (KBr)(ν_{max}/cm-1): 3250 (NH), 3132 (=C-H), 2933 and 2854 (-CH), 1732,1688,1652 (3C=O), 1604(C=C);1HNMR (400 MHz, $CDCl_3$, Me4Si): δ_H 1.24–1.85 (16H,m,5CH2 and 2CH_3), 3.85–3.87 (1H, m, NH-*CH*),4.12–4.25 (4H,m, 2OCH_2), 4.73 (1H, s, CH), 7.32–7.40 (3H, m, arom), 7.32 (1H, t, $^3J_{HH}$ = 6.4 Hz), 7.37 (1H, d, $^3J_{HH}$ = 6.8 Hz, CH), 7.40 (1H, t, $^3J_{HH}$ = 6.8 Hz, CH), 8.74 (1H, d, $^3J_{HH}$ = 7.6 Hz, NH);^{13}CNMR (100.6 MHz, $CDCl_3$, Me_4Si): δ_C 14.1 and 14.4 (2CH_3), 24.4, 25.3, 33.4, 33.7 and 36.5 (5CH_2), 50.6 (N-CH), 72.2 (N-CH), 59.9 and 61.5 (2OCH_2), 107.7 (*C*=C–F), 108.0 (=*C*–C–F), 118.8 and 118.90 (2 O–*C*=C), 120.1 (*C*=C–C–F), 120.4 (=*C*–C–C=C–F), 154.1 (O-*C*-C=C-O), 157.6 (C–F,$^2J_{CF}$ = 2.88 Hz), 157.9 (O–CO), 160.0, (=*C*–NH), 160.4, 168.9 and 172.7 (3 CO); MS (*m/z* %): 460 (M+.74); ^{19}F NMR (90 MHz, $CDCl_3$, CF_3COOH):δ_F 3.506.

*Ethyl 2-(tert-butylamino)-4,5-dihydro-5-oxo-3-phenylpyrano[3,2-c]chromene-4-carboxylate (**5**):*

Brown crystals (0.0.29g, 70%); mp: 210–215 °C; IR (KBr)(ν_{max}/cm^{-1}): 3320 (N–H),3120 (=C-H), 2932 and 2844 (-CH), 1728, 1684, and 1640 (3C=O), 1601 (C=C), 1237 (C-O). ^{1}HNMR (250 MHz, $CDCl_3$, Me_4Si): δ_H 0.83 (3H, t, $^3J_{HH}$= 6 Hz, CH_3), 1.22 (9 H, s, 3CH_3), 4.15 (2H, t, 2OCH_2), 5.12 (1H, s, CH), 7.10-7.93 (9H, m, aromatic), 9.77 (1H, s br., NH); ^{13}C NMR (62.9 MHz, $CDCl_3$, Me_4Si): δ_C 14.1 (CH_3), 22.6 (3*C*H_3),29.6 (CH_3), 44.6 (OCH_2), 61.4 [*C*(Me3)$_3$], 88.1 (CH), 116.4-135.8 (9C aromatic), 154.2 (=*C*–N),167.5 (=C-O),168.1 and 176.7(2C=O); MS (*m/z* %): $C_{25}H_{25}NO_5$, 419 ($M^{+\cdot}$15).

Acknowledgement

The authors would like to thank Islamic Azad University North Tehran Branch for providing the fund to perform this research.

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