European Journal of Chemistry 5 (3) (2014) 457-462

European Journal of Chemistry

Journal homepage: www.eurjchem.com

Synthesis of new 3,4-dihydropyrano[*c*]chromene derivatives and their evaluation as acetyl cholinesterase inhibitors

Younes Bouazizi [a,b,*], Anis Romdhane [b], and Hichem Ben Jannet [b]

[a] *Chemistry Department, Faculty of Science, Jazan University, 2097, Saudi Arabia*
[b] *Laboratory of Heterocyclic Chemistry, Natural Products and Reactivity, Bioorganic Chemistry and Natural Products, Faculty of Sciences, University of Monastir, Monastir, 5000, Tunisia*

**Corresponding author at: Chemistry Department, Faculty of Science, Jazan University, 2097, Saudi Arabia.*
Tel.: +96.653.7135063. Fax: +96.653.7135065. E-mail address: bouazizi_younes@yahoo.fr (Y. Bouazizi).

ARTICLE INFORMATION

DOI: 10.5155/eurjchem.5.3.457-462.1040

Received: 02 March 2014
Received in revised form: 26 April 2014
Accepted: 27 April 2014
Online: 30 September 2014

KEYWORDS

Chromene
Iminoethers
Pyrrolochromene
Pyridinochromene
Dihydropyrano[*c*]chromene
Anti-acetylcholinesterase activity

ABSTRACT

2-Amino-4-phenyl-4,5-dihydro-5-oxopyrano[2,3-*c*]chromen-3-carbonitrile derivatives (8a-d) have been isolated in good yields by the reaction of corresponding 4-hydroxycoumarin (1) with substituted aldehydes (2a-d) and malononitrile (3) under reflux conditions. The reactivity of α-functionalized iminoethers (9a-d) with hydrazine, hydroxylamine and piperidine was studied. The synthesized compounds were characterized by various techniques including spectroscopy. Compounds 8-11 were also evaluated as potential acetylcholinesterase inhibitors.

1. Introduction

4-Hydroxycoumarins (2*H*-1-benzopyran-2-ones) have evoked a great deal of interest due to their biological properties and characteristic conjugated molecular architecture. Many of them display important pharmacological effects, including analgesic [1], anti-arthritis [2], anti-inflammatory [3], anti-pyretic [4], anti-bacterial [5], anti-viral [6], and anti-cancer [7] properties. 4-Hydroxycoumarin and its derivatives have been effectively used as anticoagulants for the treatment of disorders in which there is excessive or undesirable clotting, such as thrombophlebitis [8], pulmonary embolism [9], and certain cardiac conditions [10]. A number of comparative pharmacological investigations of the 4-hydroxycoumarin derivatives have shown good anticoagulant activity combined with low side effects and little toxicity [11].

Our research has been devoted to the development of several heterocyclic systems derived from 4*H*-pyrans (chromenes) as starting material a new class of heterocyclic systems with the hope that they may be biologically active. We report here, facile syntheses approaches to several heterocyclic systems derived from 4*H*-pyrans (chromenes) as starting material, for which we have evaluated their anti-acetylcholin-esterase activity.

2. Experimental

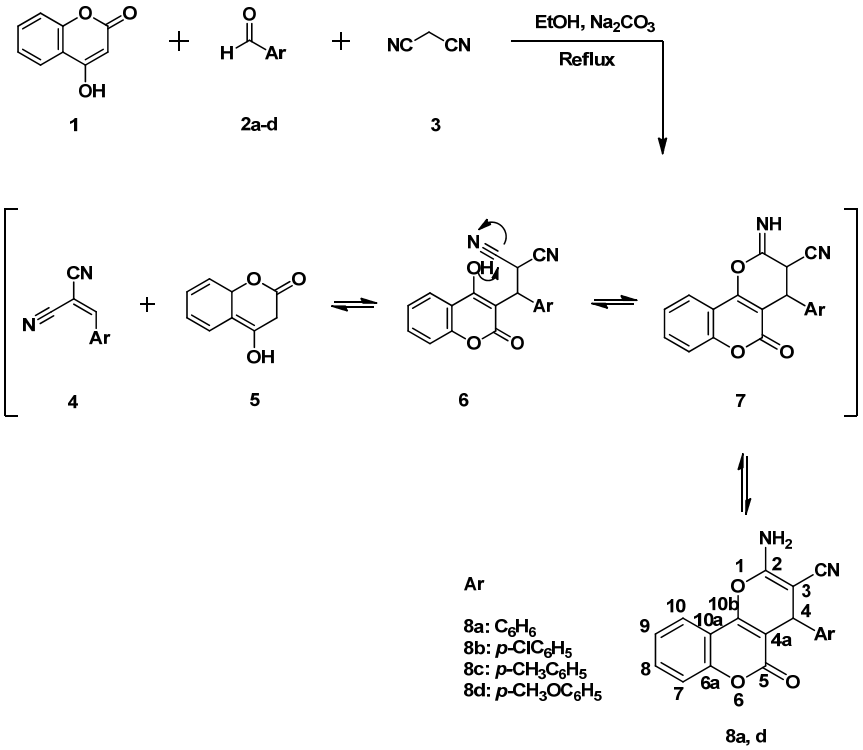
2.1. Instrumentation

^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectra were recorded in deuterated $CDCl_3$ and DMSO-d_6 on a Bruker AC-300 using non-deuterated solvents as internal reference. All chemical shifts were reported as δ values (ppm) and coupling constants (*J*) were expressed in Hz. All reactions were monitored by TLC using aluminium sheets of SDS silica gel 60 F_{254}, 0.2 mm.

2.2. Biological properties

Acetylcholinesterase enzymatic activity was measured by the Ellman test [12], 98 µL (50 mM) Tris-HCl buffer pH = 8. 30 µL sample and 7.5 µL acetylcholinesterase solutions containing 0.26 U/mL were mixed in an ELISA plate well and left to incubate for 15 min. Subsequently, 22.5 µL of AtchI (Acetyl thiocholine iodide, substrate concentration = 0.023 mg/mL) and 142 µL of DTNB (5,5-Dithio-*bis*(2-nitrobenzoic acid), chromogen concentration = 3 mM) were added.

ISSN 2153-2249 (Print) / ISSN 2153-2257 (Online)
http://dx.doi.org/10.5155/eurjchem.5.3.457-462.1040



Scheme 1

The absorbance at 405 nm was read when there action reached the equilibrium. A control action was carried out using water instead of compound.

The absorbance value obtained was considered 100% activity. Inhibition (%) was calculated with Equation (1).

$$I\ \% = 100\text{-}(A_{sample}/A_{control}) \times 100 \quad (1)$$

where, A_{sample} is the absorbance of the reaction containing the extract and $A_{control}$ the absorbance of the reaction control. Tests were carried out in triplicate and a blank with Tris-HCl buffer instead of enzyme solution was done. Sample concentration providing 50% inhibition (IC_{50}) was obtained plotting the inhibition percentage against compound solution concentrations.

2.3. Synthesis

Starting materials were prepared using standard methods [13,14].

2.3.1. Reaction of 4-hydroxycoumarin (1) with compounds 2a-d

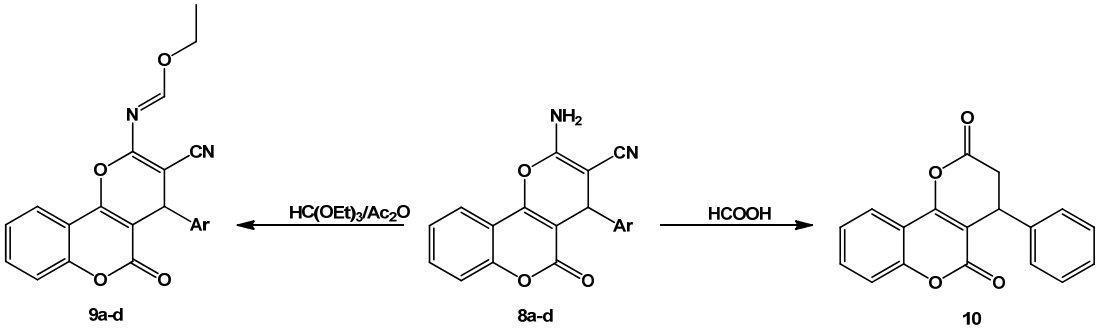
General procedure: To a stirred mixture of 4-hydroxy coumarin (**1**) (3 mmol) and benzaldyde (**2a-d**) (3 mmol) and malononitrile (**3**) (3 mmol) in absolute ethanol (30 mL) was added anhydrous sodium carbonate (32.68 mg, 0.308 mmol) and the mixture was heated under reflux. A TLC control showed that the reaction was completed after an hour. After cooling, the mixture diluted with cold ethanol when a solid formed which collected by filtration, washed several times with cold ethanol and dried and recrystallized from ethanol to afford the chromenes **8a-d** (Scheme 1).

2-Amino-5-oxo-4-phenyl-4,5-dihydropyrano[3,2-c]chromene-3-carbonitrile (**8a**): Color: White. Yield: 80%. ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 4.45 (s, 1H, CH-Ph), 7.28-7.38 (m, 5H, NH_2, Ar-H), 7.47-7.53 (m, 4H, Ar-H), 7.79 (t, 1H, *J* = 8.1 Hz, Ar-H), 7.90 (d, 1H, *J* = 7.8 Hz, Ar-H). ^{13}C NMR (75 MHz, DMSO-d_6, δ, ppm): 159.9 (1C, CO), 158.3 (1C, Ar-C), 153.7 (1C, Ar-C), 152.5 (1C, Ar-C), 143.7 (1C, Ar-C), 133.3 (1C, Ar-C), 128.8 (2C, Ar-C), 127.9 (2C, Ar-C), 127.4 (1C, Ar-C), 125.0 (1C, Ar-C), 122.8 (1C, Ar-C), 119.6 (1C, Ar-C), 116.9 (1C, Ar-C), 113.3 (1C, CN), 104.3 (1C, Ar-C), 58.2 (1C, Ar-C), 37.3 (1C, CH-Ph). Anal. calcd. for $C_{19}H_{12}N_2O_3$: C, 72.15; H, 3.82; N, 8.86. Found: C, 72.20; H, 3.85; N, 8.90%.

2-Amino-4-(4-chlorophenyl)-5-oxo-4,5-dihydropyrano[3,2-c]chromene-3-carbonitrile (**8b**): Color: White. Yield: 75%. ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 4.72 (s, 1H, CH-Ph), 7.40-8.13 (m, 10H, NH_2, Ar-H). ^{13}C NMR (75 MHz, DMSO-d_6, δ, ppm): 160.4 (1C, CO), 158.9 (1C, Ar-C), 154.4 (1C, C-NH_2), 153.2 (1C, Ar-C), 143.1 (1C, Ar-C), 133.8 (1C, C-Cl), 130.6 (2C, Ar-C), 128.8 (2C, Ar-C), 127.9 (1C, Ar-C), 127.4 (1C, Ar-C), 125.0 (1C, Ar-C), 122.8 (1C, Ar-C), 119.6 (1C, Ar-C), 113.3 (1C, CN), 104.4 (1C, Ar-C), 58.6 (1C, Ar-C), 38.3 (1C, CH-Ph).

2-Amino-5-oxo-4-(p-tolyl)-4,5-dihydropyrano[3,2-c]chromene-3-carbonitrile (**8c**): Color: White. Yield: 80%. ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 2.25 (s, 3H, CH_3), 4.40 (s 1H, CH-Ph-CH_3), 7.12-7.93 (m, 10H, NH_2, Ar-H). ^{13}C NMR (75 MHz, DMSO-d_6, δ, ppm): 159.8 (1C, CO), 158.4 (1C, Ar-C), 153.7 (1C, C-NH_2), 152.5 (1C, Ar-C), 140.8 (1C, Ar-C), 136.7 (1C, Ar-C), 133.2 (1C, Ar-C), 129.5 (2C, Ar-C), 128.9 (2C. Ar-C), 127.9 (1C, Ar-C), 122.8 (1C, Ar-C), 119.7 (1C, Ar-C), 116.9 (1C, Ar-C), 113.4 (1C, CN), 104.6 (1C, Ar-C), 58.6 (1C, Ar-C), 37.0 (1C, CH-Ph-Me), 21.0 (1C, CH_3). Anal. calcd. for $C_{20}H_{14}N_2O_3$: C, 72.12; H, 4.27; N, 8.48. Found: C, 72.16; H, 4.30; N, 8.50%.

2-Amino-5-oxo-4-(4-methoxyphenyl)-4,5-dihydropyrano[3,2-c]chromene-3-carbonitrile (**8d**): Color: White. Yield: 85%. ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 3.71 (s, 3H, CH_3O), 4.39 (s, 1H, CH), 6.80-7.85 (m, 10H, NH_2, Ar-H).



Scheme 2

^{13}C NMR (75 MHz, DMSO-d_6, δ, ppm): 161.2 (1C, CO), 158.8 (1C, Ar-C), 158.5 (1C, C-NH_2), 154.5 (1C, C-O-Me), 151.7 (1C, Ar-C), 135.9 (1C, Ar-C), 133.1 (2C, Ar-C), 129.0 (1C, Ar-C), 125.1 (1C, Ar-C), 121.4 (1C, Ar-C), 119.8 (1C, Ar-C), 117.2 (1C, Ar-C), 115.0 (2C, Ar-C), 111.7 (1C, CN), 106.4 (1C, Ar-C), 59.1 (1C, Ar-C), 56.1 (1C, CH_3-O), 36.7 (1C, CH-Ph-Me).

2.3.2. Reaction of compounds 8a-d with triethylortho formate

General procedure: A mixture of compounds **8a-d** (0.01 mmol), triethylorthoformate (0.01 mmol) and Ac_2O (30 mL) was refluxed for 6 h. The solid product that precipitated during the reflux was filtered off, dried and recrystallized from ethanol to give compounds **9a-d** (Scheme 2).

Ethyl-N-(3-cyano-5-oxo-4-phenyl-4,5-dihydropyrano[3,2-c] chromen-2-yl)formimidate (**9a**): Color: White solid. Yield: 80%. ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 1.34 (t, 3H, *J* = 9 Hz, CH_3-CH_2-O), 4.38 (q, 2H, *J* = 9 Hz, CH_3-CH_2-O), 4.76 (s, 1H, CH-Ph), 7.27-8.22 (m, 9H, Ar-H), 8.92 (s, 1H, N=CH). ^{13}C NMR (75 MHz, DMSO-d_6, δ, ppm): 162.4 (1C, C-N=CH), 159.4 (1C, CO), 158.6 (1C, Ar-C), 155.0 (1C, -N=CH-O), 153.4 (1C, Ar-C), 152.1 (1C, Ar-C), 133.5 (2C, Ar-C), 133.0 (1C, Ar-C), 132.0 (1C, Ar-C), 129.3 (2C, Ar-C), 124.6 (1C, Ar-C), 123.4 (1C, Ar-C), 116.8 (1C, Ar-C), 116.3 (1C, Ar-C), 113.9 (1C, CN), 103.1 (1C, Ar-C), 82.9 (1C, Ar-C), 64.1 (1C, -O-CH_2-CH_3), 37.5 (1C, CH-Ph), 13.8 (1C, CH_3-CH_2-O).

Ethyl-N-(4-(4-chlorophenyl)-3-cyano-5-oxo-4,5-dihydropyra no[3,2-c]chromen-2-yl)formimidate (**9b**): Color: White. Yield: 75%. ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 1.35 (t, 3H, *J* = 9 Hz, CH_3-CH_2-O), 4.39 (q, 2H, *J* = 9 Hz, CH_3-CH_2-O), 4.76 (s, 1H, CH-Ph), 7.42-8.22 (m, 8H, Ar-H), 8.93 (s, 1H, N=CH). ^{13}C NMR (75 MHz, DMSO-d_6, δ, ppm): 162.7 (1C, Ar-C), 159.4 (1C, CO), 155.0 (1C, O-CH=N), 153.9 (1C, Ar-C), 152.2 (1C, Ar-C), 140.5 (1C, Ar-C), 133.2 (1C, Ar-C), 132.3 (1C, Ar-C), 130.2 (2C, Ar-C), 128.6 (2C, Ar-C), 124.7 (1c, Ar-C), 123.6 (1C, Ar-C), 116.6 (1C, Ar-C), 116.4 (1C, Ar-C), 112.8 (1C, CN), 102.5 (1C, Ar-C), 82.2 (1C, Ar-C), 64.2 (1C, O-CH_2-CH_3), 37.7 (1C, CH-Ph), 13.8 (1C, CH_3-CH_2-O).

Ethyl-N-(3-cyano-5-oxo-4-(p-tolyl)-4, 5-dihydropyrano [3,2-c]chromen-2-yl)formimidate (**9c**): Color: White. Yield: 70%. ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 1.35 (t, 3H, *J* = 9 Hz, CH_3-CH_2-O), 3.32 (s, 3H, CH_3), 4.38 (q, 2H, *J* = 9 Hz, O-CH_2-CH_3), 4.65 (s, 1H, CH-Ph), 7.18-8.20 (m, 8H, Ar-H), 8.92 (s, 1H, N=CH-O). ^{13}C NMR (75 MHz, DMSO-d_6, δ, ppm): 162.4 (1C, Ar-C), 159.4 (1C, CO), 155.2 (1C, O-CH=N), 153.6 (1C, Ar-C), 152.1 (1C, Ar-C), 138.6 (1C, Ar-C), 136.9 (1C, Ar-C), 133.0 (1C, Ar-C), 129.2 (2C, Ar-C), 128.0 (2C, Ar-C), 124.7 (1c, Ar-C), 123.5 (1C, Ar-C), 116.7 (1C, Ar-C), 116.4 (1C, Ar-C), 112.9 (1C, CN), 103.0 (1C, Ar-C), 82.8 (1C, Ar-C), 64.2 (1C, O-CH_2-CH_3), 37.9 (1C, CH-Ph), 20.6 (1C, CH3), 13.8 (1C, CH_3-CH_2-O).

Ethyl-N-(3-cyano-4-(4-methoxyphenyl)-5-oxo-4,5-dihydro pyrano[3,2-c]chromen-2-yl)formimidate (**9d**): Color: White. Yield: 78 %. ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 1.35 (t, 3H, *J* = 9 Hz, CH_3-CH_2-O), 3.33 (s, 3H, OCH_3), 4.38 (q, 2H, *J* = 9 Hz, O-CH_2-CH_3), 4.63 (s, 1H, CH-Ph), 6.83-8.21 (m, 8H, Ar-H), 8.91 (s, 1H, N=CH-O). ^{13}C NMR (75 MHz, DMSO-d_6, δ, ppm): 162.4 (1C, Ar-C), 159.4 (1C, CO), 158.7 (1C, O-CH=N), 155.1 (1C, Ar-C), 153.4 (1C, Ar-C), 152.1 (1C, Ar-C), 133.6 (1C, Ar-C), 133.0 (1C, Ar-C), 129.4 (2C, Ar-C), 124.7 (1C, Ar-C), 123.5 (1c, Ar-C), 116.8 (1C, Ar-C), 116.4 (1C, Ar-C), 113.9 (2C, Ar-C), 112.9 (1C, CN), 103.2 (1C, Ar-C), 82.9 (1C, Ar-C), 64.2 (1C, O-CH_2-CH_3), 55.1 (1C, O-CH_3), 37.5 (1C, CH_3), 13.8 (1C, CH_3-CH_2-O).

2.3.3. Reaction of compound 8a with formic acid

A mixture of the chromene **8a** (0.01 mmol) and formic acid (20 mL) was refluxed for 5 h. The mixture cooled it as a solid started to form and the precipitate filtered off, then washed with water and diethyl ether. The solid recrystallized from ethanol and afforded the 4-phenyl-3,4-dihydropyrano[3,2-*c*] chromene-2,5-dione, **10** (Scheme 2).

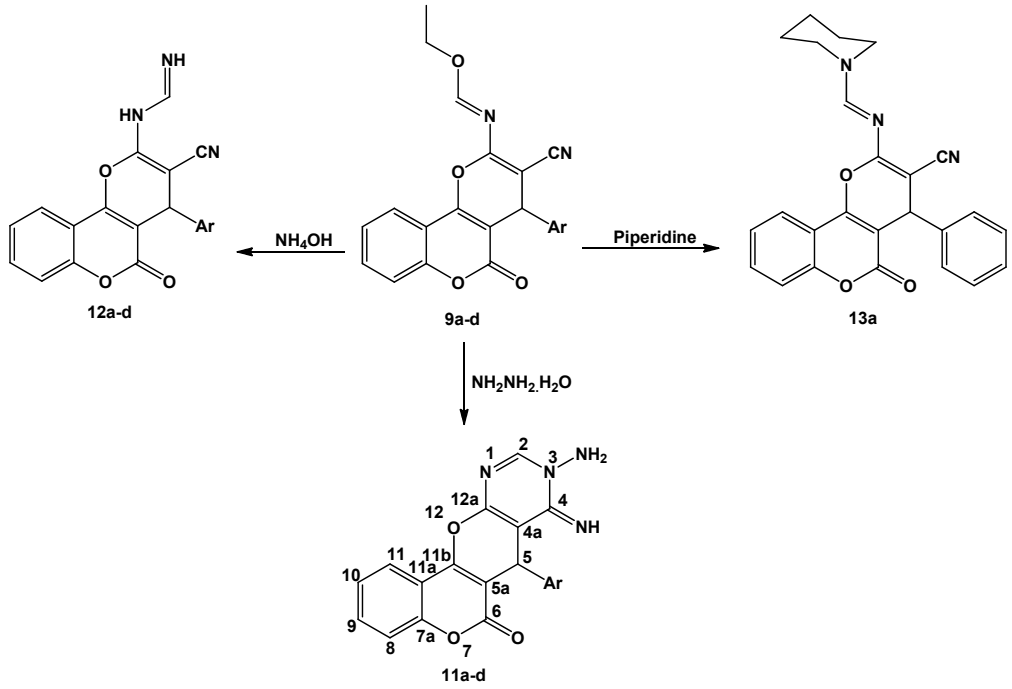
4-Phenyl-3,4-dihydropyrano[3,2-c]chromene-2,5-dione (**10**): Color: White. Yield: 80%. ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 2.99 (dd, 1H, *J* = 1.2 Hz, CH_2), 3.57 (dd, 1H, *J* = 7.8 Hz, CH_2), 4.45 (d, 1H, *J* = 7.8 Hz, CH-Ph), 7.22-7.87 (m, 9H, Ar-H). ^{13}C NMR (75 MHz, DMSO-d_6, δ, ppm): 165.6 (1C, O-CO-CH_2), 160.7 (1C, O-CO-C), 157.7 (1C, Ar-C), 153.1 (1C, Ar-C), 140.5 (1C, Ar-C), 133.6 (1C, Ar-C), 129.6 (1C, Ar-C), 128.0 (2C, Ar-C), 127.1 (2C, Ar-C), 125.4 (1C, Ar-C), 123.0 (1C, Ar-C), 117.2 (1C, Ar-C), 113.8 (1C, Ar-C), 106.6 (1C, Ar-C), 36.7 (1C, CH-Ph), 35.8 (1C, CH_2).

2.3.4. Reaction of compounds 9a-d with hydrazine hydrate

General procedure: A solution of compounds **9a-d** (0.01 mmol) and hydrazine hydrate (5 mL) in EtOH (50 mL) was sttired at room temperature for 1 h. The solid product was collected by filtration and recrystallized from ethanol to give compounds **11a-d** (Scheme 3).

9-Amino-8-imino-7-phenyl-8,9-dihydrochromeno[3',4',5,6] pyrano[2,3-d]pyrimidin-6(7H)-one (**11a**): Color: White. Yield: 72%. ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 4.99 (s, 1H, CH-Ph), 5.73 (s, 2H, NH_2), 7.14-7.94 (m, 10H, NH, Ar-H), 8.17 (s, 1H, CH=N). ^{13}C NMR (75 MHz, DMSO-d_6, δ, ppm): 163.1 (1C, CO), 161.0 (1C, Ar-C), 160.3 (1C, Ar-C), 157.2 (1C, C=NH), 155.0 (1C, Ar-C), 152.5 (1C, Ar-C), 142.1 (1C, N=C-N-NH_2), 133.3 (1C, Ar-C), 128.9 (2C, Ar-C), 128.7 (1C, Ar-C), 127.7 (2C, Ar-C), 125.3 (1C, Ar-C), 123.1 (1C,Ar-C), 117.0 (1C, Ar-C), 113.9 (1C, Ar-C), 106.2 (1C, Ar-C), 96.6 (1C, Ar-C), 34.0 (1C, CH).

9-Amino-7-(4-chlorophenyl)-8-imino-8,9-dihydrochromeno [3',4',5,6]pyrano[2,3-d]pyrimidin-6(7H)-one (**11b**): Color: White. Yield: 70%. ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 4.99 (s, 1H, CH-Ph), 5.76 (s, 2H, NH_2), 7.12-7.93 (m, 9H, NH, Ar-H), 8.17 (s, 1H, CH=N). ^{13}C NMR (75 MHz, DMSO-d_6, δ, ppm): 164.0 (1C, C=NH), 163.1 (1C, CH=N), 159.8 (1C, CO), 154.2 (1C, Ar-C),



Scheme 3

152.0 (1C, Ar-C), 151.3 (1C, Ar-C), 140.8 (1C, Ar-C), 132.8 (1C, Ar-C), 131.6 (1C, Ar-C), 130.7 (2C, Ar-C), 127.9 (2C, Ar-C), 124.8 (1C, Ar-C), 122.5 (1C, Ar-C), 116.5 (1C, Ar-C), 113.2 (1C, Ar-C), 104.3 (1C, Ar-C), 99.6 (1C, Ar-C), 34.8 (1C, CH).

9-Amino-8-imino-7(p-tolyl)-8,9-dihydrochromeno[3',4',5,6] pyrano[2,3-d]pyrimidin-6(7H)-one (**11c**): Color: White. Yield: 78%. ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 2.50 (s, 3H, CH_3), 4.97 (s, 1H, CH-Ph), 5.77 (s, 2H, NH_2), 7.15-7.91 (m, 9H, NH, Ar-H), 8.16 (s, 1H, CH=N). ^{13}C NMR (75 MHz, DMSO-d_6, δ, ppm): 159.8 (1C, CO), 154.2 (1C, Ar-C), 152.0 (1C, Ar-C), 151.3 (1C, C=NH), 148.4 (1C, Ar-C), 147.2 (1C, Ar-C), 140.9 (1C, CH=N), 132.8 (1C, Ar-C), 131.6 (1C, Ar-C), 130.7 (2C, Ar-C), 127.9 (2C, Ar-C), 124.7 (1C, Ar-C), 122.5 (1C, Ar-C), 116.5 (1C, Ar-C), 113.1 (1C, Ar-C), 104.3 (1C, Ar-C), 99.8 (1C, Ar-C), 54.9 (1C, CH), 34.8 (1C, CH_3).

9-Amino-8-imino-7(4-methoxyphenyl)-8,9-dihydrochromeno [3',4',5,6]pyrano[2,3-d]pyrimidin-6(7H)-one (**11d**): Color: White. Yield: 78%. ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 2.50 (s, 3H, OCH_3), 4.90 (s, 1H, CH-Ph), 5.76 (s, 2H, NH_2), 6.79-7.92 (m, 9H, NH, Ar-H), 8.15 (s, 1H, CH=N). ^{13}C NMR (75 MHz, DMSO-d_6, δ, ppm): 159.8 (1C, CO), 158.2 (1C, Ar-C), 154.0 (1C, Ar-C), 153.8 (1C, C=NH), 151.9 (1C, C-OCH_3), 150.8 (1C, Ar-C), 148.0 (1C, Ar-C), 146.8 (1C, Ar-C), 133.9 (1C, Ar-C), 132.6 (1C, Ar-C), 129.8 (2C, Ar-C), 124.7 (1C, Ar-C), 122.4 (1C, Ar-C), 116.5 (1C, Ar-C), 113.5 (2C, Ar-C), 113.2 (1C, Ar-C), 104.9 (1C, Ar-C), 54.9 (1C, OCH_3), 34.8 (1C, CH).

2.3.5. Reaction of compounds 9a-d with hydroxylamine

General procedure: A mixture of compounds **9a-d** (0.01 mmol) and hydroxylamine (10 mL) in methanol:THF (*v:v*, 14:6) (20 mL) was stirred at room temperature for 1 h. The solid product was collected and recrystallized from ethanol to give compounds **12a-d** (Scheme 3).

N-(3-Cyano-5-oxo-4-phenyl-4,5-dihydropyrano[3,2-c]chro men-2-yl)formimidamide (**12a**): Color: White. Yield: 85%. ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 4.55 (s, 1H, CH-Ph), 7.26-8.06 (m, 10H, NH, Ar-H), 8.44 (d, 1H, *J* = 13.8 Hz, -NH), 8.56 (dd, 1H, *J* = 9.6 Hz, C*H*=NH). ^{13}C NMR (75 MHz, DMSO-d_6, δ, ppm): 160.0 (1C, CO), 158.4 (1C, Ar-C), 156.0 (1C, Ar-C), 154.2 (1C, C=NH), 152.5 (1C, C-OCH_3), 143.0 (1C, Ar-C), 133.2 (1C, Ar-C), 128.9 (2C, Ar-C), 128.3 (1C, Ar-C), 127.6 (2C, Ar-C), 125.0(1C, Ar-C), 123.3 (1C, Ar-C), 119.3 (1C, Ar-C), 116.8 (1C, Ar-C), 113.6 (1C, CN), 103.7 (1C, Ar-C), 74.4 (1C, Ar-C), 34.8 (1C, CH).

N-(4-(4-Chlorophenyl)-3-cyano-5-oxo-4,5-dihydropyrano [3, 2-c]chromen-2-yl)formimidamide (**12b**): Color: White. Yield: 85%. ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 4.55 (s, 1H, CH-Ph), 7.25-8.04 (m, 9H, NH, Ar-H), 8.44 (d, 1H, *J* = 13.8 Hz, -NH), 8.56 (dd, 1H, *J* = 9.6 Hz, C*H*=NH). ^{13}C NMR (75 MHz, DMSO-d_6, δ, ppm): 162.6 (1C, CO), 160.5 (1C, Ar-C), 159.8 (1C, Ar-C), 156.8 (1C, C=NH), 154.6 (1C, C-OCH_3), 152.1 (1C, Ar-C), 140.5 (1C, Ar-C), 132.9 (1C, Ar-C), 131.8 (1C, C-Cl), 130.3 (2C, Ar-C), 128.1 (2C, Ar-C), 124.8 (1C, Ar-C), 122.6 (1C, Ar-C), 116.5 (1C, Ar-C), 113.3 (1C, CN), 105.2 (1C, Ar-C), 95.6 (1C, Ar-C), 33.0 (1C, CH).

N-(3-Cyano-5-oxo-4-(p-totyl)-4,5-dihydropyrano[3,2-c]chro men-2-yl)formimidamide (**12c**): Color: White. Yield: 80%. ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 2.50 (s, 3H, CH_3), 5.1 (s, 1H, CH-Ph), 7.25-8.04 (m, 9H, NH, Ar-H), 8.44 (d, 1H, *J* = 13.8 Hz, -NH), 8.56 (dd, 1H, *J* = 9.6 Hz, C*H*=NH). ^{13}C NMR (75 MHz, DMSO-d_6, δ, ppm): 162.5 (1C, CO), 160.4 (1C, Ar-C), 159.7 (1C, Ar-C), 156.6 (1C, C=NH), 154.3 (1C, C-OCH_3), 152.0 (1C, Ar-C), 138.6 (1C, Ar-C), 136.3 (1C, Ar-C), 132.7 (1C, Ar-C), 128.8 (2C, Ar-C), 128.2 (2C, Ar-C), 124.8 (1C, Ar-C), 122.5 (1C, Ar-C), 116.5 (1C, Ar-C), 113.3 (1C, CN), 105.7 (1C, Ar-C), 96.1 (1C, Ar-C), 33.1 (1C, CH), 20.5(1C, CH_3).

N-(3-Cyano-4-(4-methoxyphenyl)-5-oxo-4,5-dihydropyrano [3,2-c]chromen-2-yl)formimidamide (**12d**): Color: White. Yield: 85%. ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 3.80 (s, 3H, OCH_3), 4.80 (s, 1H, CH-Ph), 7.34-8.02 (m, 9H, NH, Ar-H), 8.40 (d, 1H, *J* = 13.8 Hz, -NH), 8.50 (dd, 1H, *J* = 9.6 Hz, C*H*=NH). ^{13}C NMR (75 MHz, DMSO-d_6, δ, ppm): 160.5 (1C, CO), 159.4 (1C, Ar-C), 158.7 (1C, Ar-C), 155.6 (1C, C=NH), 153.2 (1C, C-OCH_3), 151.5 (1C, Ar-C), 138.5 (1C, Ar-C), 136.6 (1C, Ar-C), 130.7 (2C, Ar-C), 128.3 (1C, Ar-C), 125.0 (1C, Ar-C), 123.7 (1C, Ar-C), 115.1 (1C, Ar-C), 116.0 (1C, Ar-C), 113.9 (2C, Ar-C), 113.2 (1C, CN), 104.7 (1C, Ar-C), 96.1 (1C, Ar-C), 38.7(1C, CH).

2.3.6. Reaction of compound 9a with piperidine

Compound **9a** (1 mmol), piperidine (3 mL) and toluene (20 mL) was refluxed for 3 h. The solvent was removed under reduced pressure and the resulting solid was recrystallized from ethanol:petrol ether (*v*:*v*, 14:6) (20 mL) to give compound **13a** (Scheme 3).

5-Oxo-4-phenyl-2-((piperidin-1-ylmethylene)amino)-4,5-di hydropyrano[3,2-c]chromene-3-carbonitrile (**13a**): Color: White. Yield: 80%. ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 1.56-1.64 (m, 6H, CH_2-CH_2-CH_2), 3.65-3.71 (m, 4H, CH_2-N-CH_2), 4.55 (s, 1H, CH-Ph), 7.22-8.32 (m, 9H, Ar-H), 8.57 (s, 1H, N=CH-N). ^{13}C NMR (75 MHz, DMSO-d_6, δ, ppm): 160.1 (1C, Ar-C), 157.7 (1C, CO), 154.3 (1C, Ar-C), 153.2 (1C, Ar-C), 152.5 (1C, Ar-C), 143.1 (1C, Ar-C), 133.2 (1C, Ar-C), 128.9 (2C, Ar-C), 128.2 (1C, Ar-C), 127.6 (2C, Ar-C), 124.9 (1C, Ar-C), 123.9 (1C, Ar-C), 119.5 (1C, Ar-C), 116,7 (1C, Ar-C), 113,6 (1C, Ar-C), 103,7 (1C, Ar-C), 74,1 (1C, Ar-C), 50.8 (1C, CH_2), 43.3 (1C, CH_2), 38.7 (1C, Ar-C), 26.5 (1C, CH_2), 25.1 (1C, CH_2), 24.0 (1C, CH_2).

3. Results and discussion

3.1. Synthesis

Treatment of 4-hydroxycoumarin (**1**) with aryl aldehydes (**2a**-**d**) and malononitrile (**3**) in the boiling ethanol during several hours in the presence of anhydrous sodium bicarbonate as a catalyst gave 2-amino-5-oxo-4-phenyl-4,5-dihydropyrano[3,2-*c*]chromene-3-carbonitrile derivatives (**8a**-**d**) in high yields (75-85%) (Scheme 1).

The ^{1}H NMR spectra of compound **8a** displays a signal at δ 4.45 ppm that ascribable to the proton H_4. In addition, the aromatic protons are observed between δ 7.28 and 7.90 ppm (see experimental), and the expected singlet for the proton H_8 is observed at δ 7.79 ppm (Scheme 1).

The observed high regioselectivity is most probably associated with the reaction sequence outlined in Scheme 1. Initial Knoevenagal reaction between aldehydes substitutes **2a**-**d** and malonitrile (**3**) produces the unsaturated nitrile **4**, which, undergoes a Michael reaction with the base derived coumarin anion, **5**. The resulting Michael adduct **6** then undergoes intra molecular cyclization producing the annelatediminopyran **7**. Subsequent tautomeric[1,3] sigmatropic shift gives compounds **8a**-**d**.

Under these conditions, the reaction proceeds sufficiently rapidly and smoothly to afford the target chromenes **8a**-**d** in high yields without Michael adducts **6** being detected. However, the proposed mechanism is supported to some degree by isolation of analogous Michael adducts in the previously studied reaction of 4-hydroxycoumarin with arylidenecyano acetamides [3].

Refluxing compounds **8a**-**d** with triethylorthoformate in acetic anhydride at reflux afforded the corresponding ethyl-*N*-(3-cyano-5-oxo-phenyl-4,5-dihydropyrano[3,2-*c*]chromen-2-yl)formimidate (**9a**-**d**) while with formic acid, chromene-2,5-one-2,5-dione (**10**) were formed, Scheme 2.

Hydrazinolysis of compound **9a**-**d** in ethanol at room temperature afforded the 9-amino-8-imino-7-phenyl-8,9-di hydrochromeno [3',4',5,6]pyrano[2,3-*d*]pyrimidin-6(7*H*)-one derivatives, **11a**-**d**.

Reaction of compounds **9a**-**d** with hydroxylamine in MeOH-THF at room temperature yielded the *N*-(3-cyano-5-oxo-4-phenyl-4,5-dihydropyrano[3,2-*c*]chromen-2-yl)formimidamide deriva-tives, **12a**-**d**. Interaction of compounds **9a**-**d** with piperidine in toluene afforded the chromen 5-oxo-4-phenyl-2-((piperidin-1-ylmethylene) amino)-4,5-dihydropyrano[3,2-*c*]chromene-3-carbonitrile, **13a**, Scheme 3.

The structures of products are characterized by ^{1}H NMR along with ^{13}C NMR are in agreement with the proposed structures.

3.2. Biological properties (Acetylcholinesterase inhibition)

Inhibition of acetylcholinesterase (AChE), the key enzyme in the breakdown of acetylcholine, is considered one of the treatment strategies against several neurological disorders such as Alzheimer's disease, senile dementia, ataxia, and myasthenia gravis [15,16]. The acetylcholinesterase (AChE) inhibition was determined using an adaptation of the method described in the literature [17]. All compounds were analyzed on what concerns their acetylcholinesterase inhibition activity (Table 1). Values oscillating between 0.010 and 0.130 mg/mL were obtained. Compared to those given in the literature for crude pure products [17], we can say that the synthesized compounds **8**, **9**, **10** and **11** are considered good inhibitors of acetylcholinesterase. The greatest inhibitory activity was exhibited by compound **11a** (Ar = Ph) (IC_{50} = 0.110 µg/mL). It has been shown that the activity of these derivatives depends in general on the nature of Ar. In compound **10**, the acetylcholinesterase inhibition decreases from Ar = Ph (**9a**) to Ar = *p*-MeOPh (**9d**).

The same phenomena have been observed with compounds **9a**-**d** and **8a**-**d**. It has been also shown that the activity decreases considerably when Ar varied in the order Ph, *p*-ClPh, *p*-MePh and *p*-MeOPh. The substitution of both the phenyl seems to affect the activity of the chromene skeleton.

Table 1. Acetylcholinesterase inhibition capacity represented by IC_{50} (mg/mL) of compounds **8**, **9**, **10** and **11**.

Compound	Acetylcholiinesterase inhibition capacity represented by IC_{50} (mg/mL)
8a	0.091
8b	0.065
8c	0.064
8d	0.035
9a	0.130
9b	0.048
9c	0.030
9d	0.021
10	0.077
11a	0.110
11b	0.033
11c	0.025
11d	0.010

4. Conclusion

In conclusion, this work reports the synthesis of 3,4-dihydropyrano[c]chromene derivatives and their evaluation as acetyl cholinesterase inhibitors, via the simple and useful 4-hydroxycoumarin (**1**) with substituted aldehydes **2a**-**d** and malonitrile (**3**) under reflux reaction conditions.

Reference

[1]. Adami, E.; Marazzi-Uberti, E.; Turba, C. *Arch. Ital. Sci. Farmacol.* **1959**, *9*, 61-69.
[2]. Arvind, B. T.; Venkat, S. S.; Narayan, D. S.; Devanand, B. S. *Bull. Environ. Pharmacol. Life Sci.* **2012**, *7*, 30-33.
[3]. Luchini, A. C.; Rodrigues-Orsi, P.; Cestari, S. H.; Seito, L. N.; Witaicenis, A.; Pellizzon, C. H. *Biol. Pharm. Bull.* **2008**, *31*, 1343-1350.
[4]. Jae-Chul, J; Qee-Sook, P. *Molecules* **2009**, *14*, 4790-4803.
[5]. Chohan, Z. H.; Shaikh, A. U.; Rauf, A.; Supuran, C. T. *J. Enz. Inhib. Med. Chem.* **2006**, *21*, 741-748.
[6]. Kirkiacharian, B. S.; Clercq, E.; Kurkjian, R.; Pannecouque, C. *J. Pharm. Chem.* **2008**, *42*, 265-270.
[7]. Velasco-Velazquez, M. A.; Agramonte-Hevia, J.; Barrera, D.; Jimenez-Orozco, A.; Garcia-Mondragon, M. J.; Mendoza-Patino, N.; Landa, A.; Mandoki, J. *Cancer. Lett.* **2003**, *198*, 179-186.
[8]. Shapiro, S.; Sherwin, B. *N. Y. State J. Med.* **1943**, *43*, 45-52.
[9]. Butsch, W. L.; Stewart, J. D. *Arch. Surg.* **1942**, *45*, 551-553.
[10]. Hintz, K. K.; Ren, J. *Vascul. Pharmacol.* **2003**, *40*, 213-217.
[11]. Manolov, I.; Maichle-Moessmer, C.; Danchev, N. *Eur. J. Med. Chem.* **2006**, *41*, 882-890.
[12]. Howes, M. R.; Houghton, P. *J. Pharmacol. Biochem. Behav.* **2003**, *75*, 513-527.
[13]. Saudi, M. N. S.; Gaafar, M. R.; El-Azzouni, M. Z.; Ibrahim, M. A.; Eissa, M. M. *Chem. Res.* **2008**, *17*, 541-563.

[14]. Sunduru, N.; Agawal, A.; Katiyar, S.; Nishi, B.; Goyal, N.; Gupta, S.; Chauhan, P. M. S. *Bioorg. Med. Chem.* **2006**, *14*, 7706-7715.
[15]. Huisgen, R. *J. Org. Chem* **1976**, *41*, 403-419.
[16]. Pinner, A.; Klein, F. *Ber. Dtsch. Chem. Ges* **1877**, *10*, 1889-1897
[17]. Orhan, G.; Orhan, I.; Sener, B. *Lett. Drug. Desig. Discov.* **2006**, *3*, 268-274.