European Journal of Chemistry 1 (2) (2010) 134-139

European Journal of Chemistry

Journal homepage: www.eurjchem.com

Novel isoquinoline derivatives from isochromen-1,3-dione

Mahmoud Refaee Mahmoud, Manal Mohamed El-Shahawi,
Eman Abd El-Fattah El-Bordany and Fatma Saber Mohamed Abu El-Azm*

Chemistry Department, Faculty of Science, Ain Shams University, Abbassia 11566, Cairo, Egypt

**Corresponding author at: Chemistry Department, Faculty of Science, Ain Shams University, Abbassia 11566, Cairo, Egypt. Tel.: +2.202.4821096; fax: +2.202.831836. E-mail address: ftmsaber@yahoo.com (F. Saber).*

ARTICLE INFORMATION

Received: 21 April 2010
Received in revised form: 12 May 2010
Accepted: 12 May 2010
Online: 30 June 2010

KEYWORDS

Isochromene-1,3-dione
Isoquinolines
2-Cyanoethanoic hydrazide
Nitrogen nucleophiles

ABSTRACT

A number of novel isoquinoline derivatives have been synthesized using the readily obtainable (E)-4-(3,4-dimethoxybenzylidene)-4H-isochromene-1,3-dione, **2**, via the reaction with different nitrogen nucleophiles such as cyanoethanoic hydrazide, cyclohexylamine, 2-aminothiophenol and *p*-toluenesulfonohydrazide. Furthermore, the reactivity of **2** towards thiophenol, and ethylcyanoacetate has been investigated.

1. Introduction

In recent years, there has been increasing interest in the synthesis of natural products, since; they are an excellent and reliable source for the development of new drugs. In this context, chromene derivatives are a class of natural products that often occurs as microbial metabolites and that have been found to exhibit interesting biological properties, [1-7] including antifungal, anti-inflammatory, anti-allergic, narcotic, anti-angiogenic, anti-malarial, [8] anti-bacterial, [9] anti-cancer, anti-virus [10] and anti-microbial activities [11,12]. In view of their natural occurrence, biological activities and utility as synthetic intermediates, [13-27] we report here the synthesis of novel isochromene and isoquinoline derivatives starting from the readily obtainable Z-2-[1-carboxy-2-(3,4-dimethoxy phenyl)vinyl]-benzoic acid, **1** [28].

2. Experimental

All melting points were taken on Griffin and Geory melting point apparatus and are uncorrected. IR spectra were recorded on Pye Unicam SP 1200 spectrophotometer using KBr Wafer technique. ^{1}H-NMR spectra were determined on a Varian Gemini 300 MHz using TMS as internal standard (chemical shifts in δ-scale). EI-MS were measured on a Schimadzu-GC-MS, QP 1000 EX instrument operating at 70 eV. Elemental analyses were carried out at the microanalytical unit, faculty of science, Ain Shams University by using Perkin-Elmer 2400 CHN elemental analyzer and satisfactory analytical data (± 0.4) were obtained for all compounds.

2.1. (E)-4-(3,4-dimethoxybenzylidene)-4H-isochromene-1,3-dione (2)

A mixture of the diacid **1** (3.28 g, 0.01 mole) and acetyl chloride (30 mL) was refluxed for 10 minutes on water bath. The reaction mixture was allowed to cool then the separated solid was filtered off, dried and then recrystallized from ethanol to give **2** as orange crystals. M.p.: 138-140 °C, yield (2.89 g) 90%. IR (ν): 1773, 1725 cm^{-1} (C=O$_{\text{coupling bands}}$). ^{1}H-NMR ($CDCl_3$) δ 8.1-7 (m, 7H$_{\text{arom.}}$), 6.9 (s, 1H, olefinic H), 3.93 (s, 3H, OMe) and 3.69 (s, 3H, OMe). MS: 310 (100), 295 (12.6), 222 (11.2), 165 (14.0), 151 (12.9), 89 (3.8). Anal. Calcd. for $C_{18}H_{14}O_5$ (310.3): C, 69.76; H, 4.55. Found: C, 69.63; H, 4.61.

2.2. (Z)-4-(3,4-dimethoxybenzylidene)-4H-isochromene-1,3-dione (3)

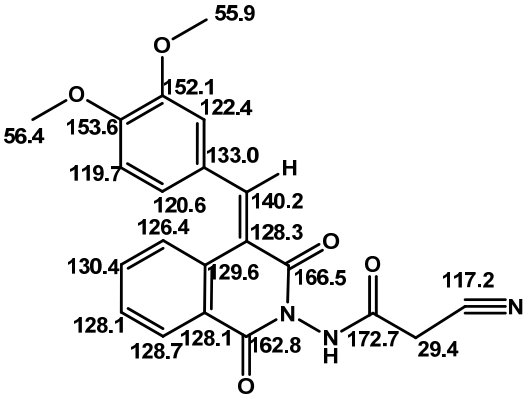
A mixture of the diacid **1** (3.28 g, 0.01 mole) and *N*,*N*-dicyclohexyl carbodiimide DCC (2 g) in dry benzene (30 mL) was stirred overnight at room temperature. The deposited dicyclohexylurea was separated. Evaporation of benzene left a yellow solid which filtered off, dried and then recrystallized from ethanol to give **3** as yellow crystals; M.p.: 163-165 °C, yield (2.64 g) 85%. IR (ν): 1768, 1720 cm^{-1} (C=O$_{\text{coupling bands}}$). ^{1}H-NMR ($CDCl_3$) δ 8.3-7 (m, 7H$_{\text{arom.}}$), 6.4 (s, 1H, CH=), 3.8 (s, 3H, OMe), 3.74 (s, 3H, OMe). MS: 310 (100). Anal. calcd. for $C_{18}H_{14}O_5$ (310.3): C, 69.76; H, 4.55. Found: C, 69.69; H, 4.63.

2.3. (E)-N-(4-(3,4-dimethoxybenzylidene)-1,3-dioxo-3,4-dihydroisoquinolin-2(1H)-yl)-2-cyano-acetamide (4) and (E)-N'-(3,4-dimethoxybenzylidene)-2-cyanoacetohydrazide (5)

A mixture of compound **2** (3.1 g, 0.01 mole) and cyanoethanoic hydrazide (0.99 g, 0.01 mole) in dioxane (30 mL) was refluxed for 6 h. After concentration of the solvent, a yellow solid was deposited which filtered off, dried and then recrystallized from ethanol to give **4**. Slow evaporation of the filtrate leave oil which solidify after trituration with small amount of ethanol. The separated solid was filtered off, dried

ISSN 2153-2249 (Print) / ISSN 2153-2257 (Online)
DOI:10.5155/eurjchem.1.2.134-139.71

and then recrystallized from ethanol to give **5** as pale yellow crystals. **4:** M.p.: 210-213 °C, yield 45%. IR (ν): 3210 cm^{-1} (NH), 2257 cm^{-1} (C≡N), 1748, 1682 cm^{-1} (C=O). ^{1}H-NMR (DMSO-d_6) δ 11.07 (s, 1H, NH, exchangeable with D_2O), 8.09-7.08 (m, 8$H_{arom.}$ + olefinic H), 4.05 (s, 2H, $COCH_2CN$), 3.85 (s, 6H, 2OMe). MS: 391 (71.3), 323 (21.4), 309 (17.1), 216 (100), 176 (43.5), 151 (50.8), 145 (32.2), 90 (49.5), 64 (13.8). Anal. calcd. for $C_{21}H_{17}N_3O_5$ (391.38): C, 64.45; H, 4.38; N, 10.74. Found: C, 64.24; H, 4.18; N, 10.44. ^{13}C-NMR (DMSO-d_6) for compound **4** is given in Scheme 1.



Scheme 1

5: M.p.: 150-152°C, yield 13%. IR (ν): 3194 cm^{-1} (NH), 2261 cm^{-1} (C≡N), 1680 cm^{-1} (C=O). ^{1}H-NMR ($CDCl_3$) δ 10.1 (br.s, 1H, NH), 8.6 (s, 1H, CH=N), 7.7-7.1 (m, 3$H_{arom.}$), 4.0 (s, 2H, $COCH_2CN$), 3.88 (s, 3H, OMe), 3.8 (s, 3H, OMe). MS: 247 (61.6), 163 (100), 148 (35.6), 120 (11.8), 92 (25.4). Anal. calcd. for $C_{12}H_{13}N_3O_3$ (247.25): C, 58.29; H, 5.30; N, 16.99. Found: C, 58.4; H, 5.12; N, 17.09.

2.4. Pyrido[1,2-b]-1,2,4-triazolo[5,1-a]isoquinolinone derivative (6)

A mixture of the isoquinolin-1,3-dione **4** (3.91 g, 0.01 mole) and p-chlorobenzylidene-malononitrile (1.88 g, 0.01 mole) in 50 mL ethanol and drops of piperidine was refluxed for 6 h. After cooling, the reaction mixture was acidified with cold dilute hydrochloric acid. The precipitated solid was collected by filtration, washed with water, dried and then recrystallized from benzene/ethanol to give **6** as buff crystals. M.p.: 310-312°C, yield 55%. IR (ν): 2220 cm^{-1} (C≡N), 1672 cm^{-1} (C=$O_{cyclic\ amide}$). ^{1}H-NMR (DMSO-d_6) δ 8.7-7.2 (m, 11 $H_{arom.}$), 6.9 (s, 1H, CH=), 4.08 (d, 1H, COCHCN), 3.9 (s, 3H, OMe), 3.82 (s, 3H, OMe), 3.66 (d, 1H, CH). MS: 298 ([M-$C_{17}H_{12}O_3$], 65.0), 265 (83.3), 185 (100), 129 (40.8), 105 (54.2), 78 (25.0), 77 (23.3). Anal. calcd. for $C_{31}H_{20}ClN_5O_4$ (561.97): C, 66.25; H, 3.59; Cl, 6.31; N, 12.46. Found: C, 66.4; H, 3.36; Cl, 6.09; N, 12.11.

2.5. (E)-1-[4-(3,4-dimethoxybenzylidene)1,3-dioxo-3,4-dihydroisoquinolin-2-(1H)-yl]-6-amino-2-oxo-1,2-dihydropyridin-3,5-dicarbonitrile (7)

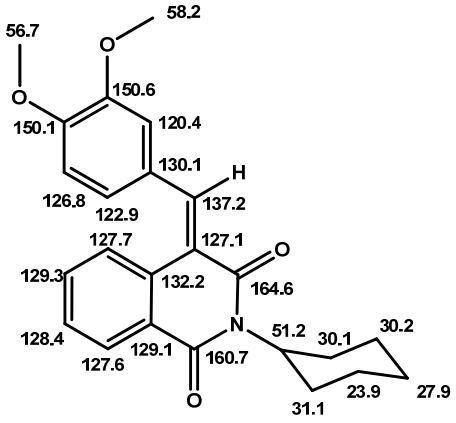
A mixture of the isoquinolin-1,3-dione **4** (3.91 g, 0.01 mole) and ethoxymethylene-malononitrile (1.22 g, 0.01 mole) in 50 mL ethanol and drops of piperidine was refluxed for 6 h. After cooling, the reaction mixture was acidified with cold dilute hydrochloric acid. The precipitate was collected by filtration, washed with water, dried and then recrystallized from ethanol to give **7** as brown crystals. M.p.: 200-203°C, yield 35%. IR (ν): br. 3332 cm^{-1} (NH), 2210 cm^{-1} (C≡N), 1682 cm^{-1} (C=O). ^{1}H-NMR (DMSO-d_6) δ 8.8-7.3 (m, 8$H_{arom.}$), 7.0 (s, 1H, olefinic H), 3.9 (s, 3H, OMe), 3.76 (s, 3H, OMe), 3.0 (br.s, 2H, NH_2, exchangeable with D_2O). MS: 329 ([M-Ar·, -H·], 67.9), 328 (100), 302 (29.9), 301 (77.4), 247 (67.9), 151 (59.9), 91 (17.7), 65 (13.4). Anal. calcd. for $C_{25}H_{17}N_5O_5$ (467.43): C, 64.24; H, 3.67; N, 14.98. Found: C, 64.2; H, 3.53; N, 14.66.

2.6. (E)-2-(2-(cyclohexylcarbamoyl)phenyl)-3-(3,4-dimethoxyphenyl)acrylic acid (8)

A mixture of compound **2** (3.1 g, 0.01 mole) and cyclohexyl amine (0.99 mL, 0.01 mole) in 30 mL dioxane was refluxed for 3 h. After concentration of the solvent, a yellowish white solid was deposited then filtered off, washed with ethanol, dried and recrystallized from ethanol to give **8** as yellowish white crystals. M.p.: 162-164°C, yield 70%. IR (ν): br. NH, OH centered at 3309 cm^{-1}, 1707 cm^{-1} (C=O_{acid}), 1640 cm^{-1} (C=O_{amide}). ^{1}H-NMR ($CDCl_3$) δ 11.6 (br.s, 1H, COOH), 9.2-9.4 (br.s, 2H, exchangeable with D_2O), 8.3-6.4 (m, 7$H_{arom.}$), 5.8 (s, 1H, olefinic H), 3.7 (s, 6H, 2OMe) and 1.8-0.96 (m, 11H, C_6H_{11}-). MS: 409 (4.5), 311 (5.6), 310 (20.5), 295 (3.4), 222 (4.7), 165 (10.8), 151 (33.0), 98 (9.2), 89 (8.5), 56 (100). Anal. Calcd. for $C_{24}H_{27}NO_5$ (409.47): C, 70.40; H, 6.65; N, 3.42. Found: C, 70.36; H, 6.41; N, 3.5.

2.7. (E)-4-(3,4-dimethoxybenzylidene)-2-cyclohexyl isoquinoline-1,3(2H,4H)-dione (9)

A mixture of **8** (4.1 g, 0.01 mole) and freshly distilled acetic anhydride (20 mL) was refluxed for 2 h. The reaction mixture was allowed to cool then poured on ice-cold water. The deposited solid was filtered off, washed several times with water, dried and then recrystallized from benzene to give **9** as orange crystals. M.p.: 162-4°C, yield 80%. IR (ν): 1759, 1723 cm^{-1} (C=$O_{coupling\ bands}$). ^{1}H-NMR ($CDCl_3$) δ 7.9-6.8 (m, 8$H_{arom.}$ + olefinic H), 3.8 (s, 3H, OMe), 3.77 (s, 3H, OMe), 2.6-1.14 (m, 11H, C_6H_{11}-). MS: 391 (3.0), 310 ([M-C_6H_{11}], 100). Anal. calcd. for $C_{24}H_{25}NO_4$ (391.46): C, 73.64; H, 6.44; N, 3.58. Found: C, 73.7; H, 6.32; N, 3.29. ^{13}C-NMR ($CDCl_3$) for compound **9** is given in Scheme 2.



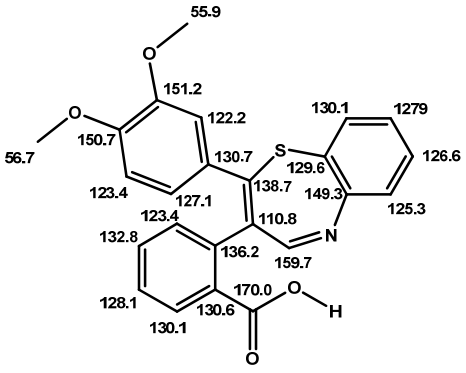
Scheme 2

2.8. (E)-N-((E)-4-(3,4-dimethoxybenzylidene)-3-oxo-3,4-dihydroisochromen-1-ylidene)-cyclo-hexanaminium perchlorate (10)

A suspension of compound **8** (4.1 g, 0.01 mole) in acetic anhydride (20 mL) and (1 mL, 0.01 mole) perchloric acid was stirred at room temperature in an ice bath for a few minutes until an orange crystals were formed. The deposited crystals were filtered off, washed with dry diethyl ether to give **10**; yield 85%. IR (ν): 3345 cm^{-1} (NH^+), 1809 cm^{-1} (C=O), 1643 cm^{-1} (C=N^+). MS: 391 ([M-$HClO_4$], 17.9), 308 (22.5), 294 (12.1), 278 (13.9), 151 (36.4), 89 (15.6), 55 (100). Anal. Calcd. for $C_{24}H_{26}ClNO_8$ (491.92): C, 58.60; H, 5.33; Cl, 7.21; N, 2.85. Found: C, 58.3; H, 5.1; Cl, 7.4; N, 3.0.

2.9. 2-((2E,4E)-2-(3,4-dimethoxyphenyl)benzo[b][1,4] thiazepin-3-yl)benzoic acid (11)

A mixture of compound **2** (3.1 g, 0.01 mole) and 2-aminothiophenol (1.25 g, 0.01 mole) in 30 mL dioxane was refluxed for 8 h. After concentration of the solvent, a yellowish white solid was deposited which filtered off, dried and recrystallized from benzene/ethanol to give **11** as yellowish white crystals. M.p.: 176-178°C, yield 73%. IR (ν): br. 3422-2836 cm^{-1} (OH_{acid}), 1684 cm^{-1} (C=O). ^{1}H-NMR ($CDCl_3$) δ 10.5 (s, 1H, COOH, exchangeable with D_2O), 7.8-6.5 (m, $12H_{arom.}$ + 1H, N=CH), 3.85 (s, 3H, OMe), 3.7 (s, 3H, OMe). MS: 417 (12.5), 310 (49.6), 240 (6.3), 151 (69.7), 134 (9.4), 108 (13.8), 91 (15.6), 76 (57.5), 65 (43.1). Anal. calcd. for $C_{24}H_{19}NO_4S$ (417.48): C, 69.05; H, 4.59; N, 3.36; S, 7.68. Found: C, 68.8; H, 4.32; N, 3.09; S, 7.41. ^{13}C-NMR ($CDCl_3$) for compound **11** is given in Scheme 3.



Scheme 3

2.10. 2-[1-Carboxy-2-(3,4-dimethoxyphenyl)-2-(phenylthio) ethyl]benzoic acid (12)

A mixture of compound **2** (3.1 g, 0.01 mole) and thiophenol (1.1 mL, 0.01 mole) in 40 mL dry benzene was refluxed for 10 hrs. After concentration of the solvent, a yellow oil was obtained which was solidified after trituration with diethyl ether. The separated solid was filtered off, dried and recrystallized from benzene/ethanol to give **12** as colorless crystals. M.p.: 170-173°C, yield 81%. IR (ν): br. 3480-2500 cm^{-1} (OH_{acid}), 1717, 1700 cm^{-1} (C=O). ^{1}H-NMR (DMSO-d_6) δ 7.6-6.5 (m, 12 $H_{arom.}$), 5.67-5.63 (d, IH, J = 12 Hz), 5.0-4.97 (d, IH, J = 9 Hz), 3.69 (s, 6H, 2OMe). MS: 328 ([M-PhSH], 5.5), 310 (13), 151 (19.9), 110 ([$PhSH^{+\cdot}$], 61.6), 91 (19.9), 65 (26). Anal. calcd. for $C_{24}H_{22}O_6S$ (438.49): C, 65.74; H, 5.06; S, 7.31. Found: C, 65.46; H, 5.0; S, 7.62.

2.11. (E)-2-(2-(4-methylbenzenesulfonamidocarbamoyl) phenyl)-3-(3,4-dimethoxyphenyl)-acrylic acid (13)

A mixture of compound **2** (3.1 g, 0.01 mole) and p-toluenesulfonohydrazide (1.86 g, 0.01 mole) in 30 mL dioxane was refluxed for 6 h. After concentration of the solvent, a yellow solid was deposited then filtered off, dried and recrystallized from benzene/ethanol to give **13** as yellow crystals. M.p.: 168-170°C, yield 68%. IR (ν): 3350-2530 cm^{-1} (OH), 3315 cm^{-1} (NH), 1699 cm^{-1} (C=O_{acid}), 1676 cm^{-1} (C=$O_{acid\ hydrazide}$). MS: 330 (11.3), 186 (28.0), 163 (47.5), 135 (94.7), 91 (100), 65 (65.5). Anal. calcd. for $C_{25}H_{24}N_2O_7S$ (496.53): C, 60.47; H, 4.87; N, 5.64; S, 6.46. Found: C, 60.73; H, 4.77; N, 5.45; S, 6.24.

2.12. Ethyl-α-cyano-3,4-dimethoxy cinnamate (14)

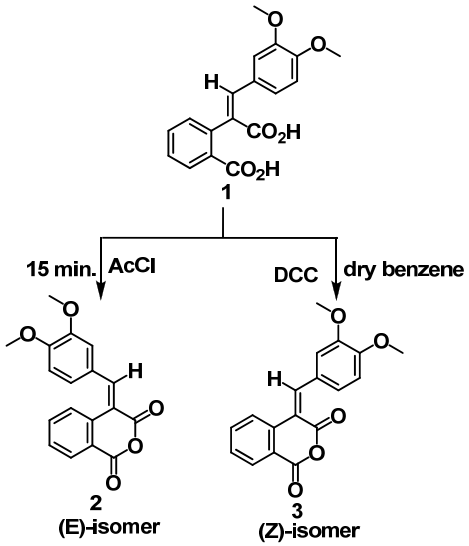
A mixture of compound **2** (3.1 g, 0.01 mole) and ethyl cyanoacetate (1.13 mL, 0.01 mole) in 30 mL dioxane in the presence of drops of triethylamine was refluxed for 3 h. After concentration of the solvent, a pale yellow solid was deposited then filtered off, dried and recrystallized from ethanol to give **14** as pale yellow crystals. M.p.: 138-140°C, yield 77%. IR (ν): 2221 cm^{-1} (C≡N), 1713 cm^{-1} (C=O_{ester}). MS: 261 (100). Anal. calcd. for $C_{14}H_{15}NO_4$ (261.27): C, 64.36; H, 5.79; N, 5.36. Found: C, 64.6; H, 5.71; N, 5.34.

3. Results and Discussion

3.1. Chemistry

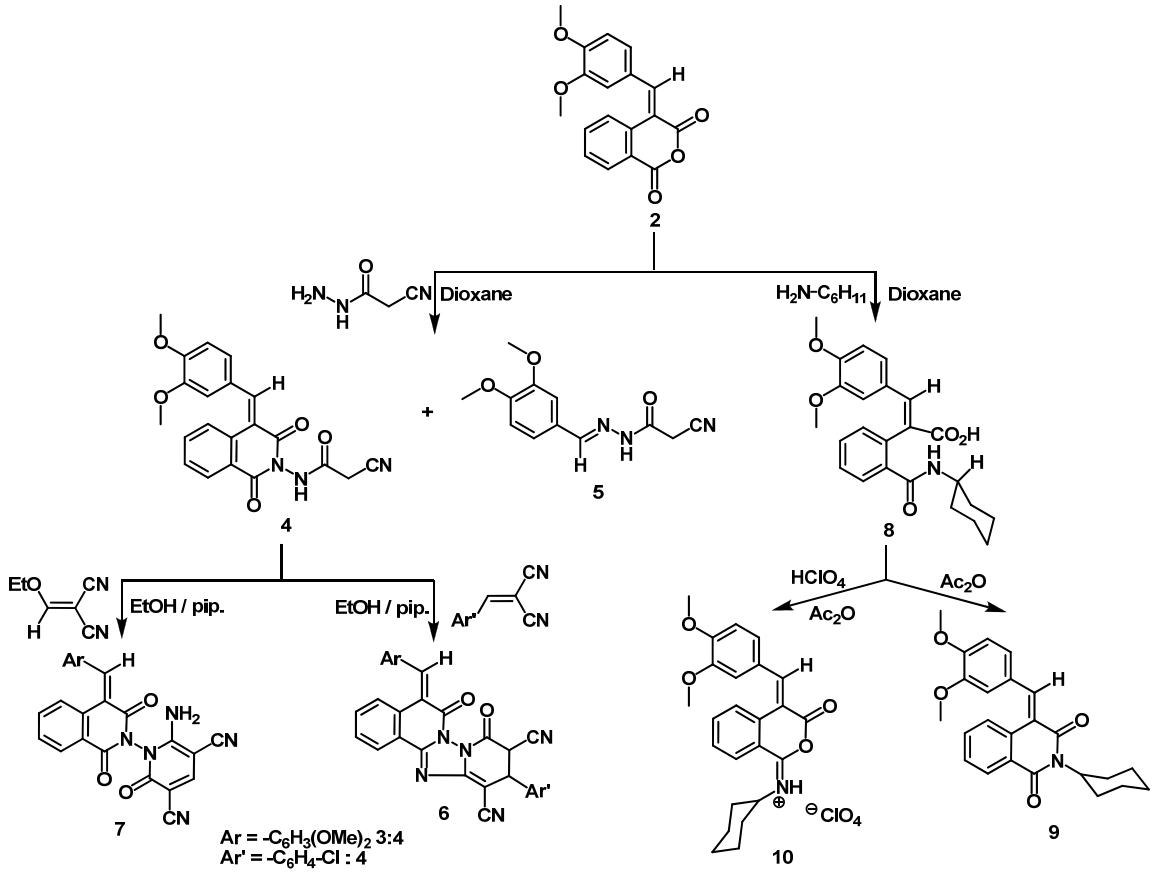
Attempted cyclization of **1** using acetyl chloride for 15 min. produced (E)-4-(3,4-dimethoxybenzylidene)-4H-isochromene-1,3-dione **2** as the sole product (yield 90%). The formation of the E-isomer **2** may be attributed to the cis-trans isomerization which understandable in view of the drastic acidic reaction conditions, being HCl liberated during anhydride formation. Complications due to isomerization were overcome by the use of DCC as dehydrating agent which gave **3** as the sole product (Scheme 4).

Cyclization to compounds **2** and **3** was inferred from the IR spectra which displayed the carbonyl coupling bands expected for the six-membered anhydride with α,β-conjugation. Analysis of the observed signals in the ^{1}H-NMR spectra of **2** and **3** revealed a singlet for ethylenic proton with down field chemical shift at δ = 6.9 ppm which may suffer from the shield effect of the carbonyl group, and that of the Z-isomer **3** at lower value (δ = 6.4 ppm). [29,30] Furthermore, the mass spectra of **2** and **3** show the correct molecular ion peak at m/z = 310 which is the base peak.



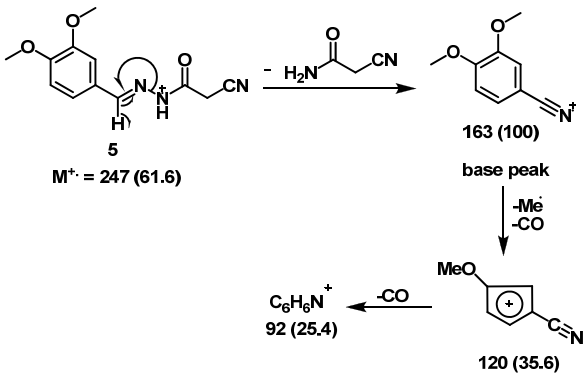
Scheme 4

A number of novel isoquinoline-1,3-diones have been synthesized using isochromen-1,3-dione **2** upon treatment with cyanoethanoic hydrazide or cyclohexylamine. Thus, refluxing **2** with cyanoethanoic hydrazide in dioxane gave isoquinoline derivative **4** together with a minor amount of the elimination product **5** (Scheme 5). The IR spectrum of **4** displayed υ_{NH} at 3210 cm^{-1}, $\upsilon_{C\equiv N}$ at 2257 cm^{-1}, $\upsilon_{C=O}$ at 1748 and 1682 cm^{-1} due to the carbonyl absorption bands of the vibrational coupling and saturated carbonyl group in the side chain. ^{1}H-NMR spectrum of **4** (DMSO-d_6) revealed signals at δ (ppm): 11.07 (s, 1H, NH, exchangeable with D_2O), 8.09-7.08 (m, $8H_{arom.}$ + olefinic H), 4.05 (s, 2H, $COCH_2CN$) and 3.85 (s, 6H, 2OMe). Furthermore, full analysis of the mass spectrum of compound **4** agrees well with the assigned structure. Thus, the EI fragmentation shows the correct molecular ion peak at m/z = 391 (71.3%) together with the base peak at m/z = 216.



Scheme 5

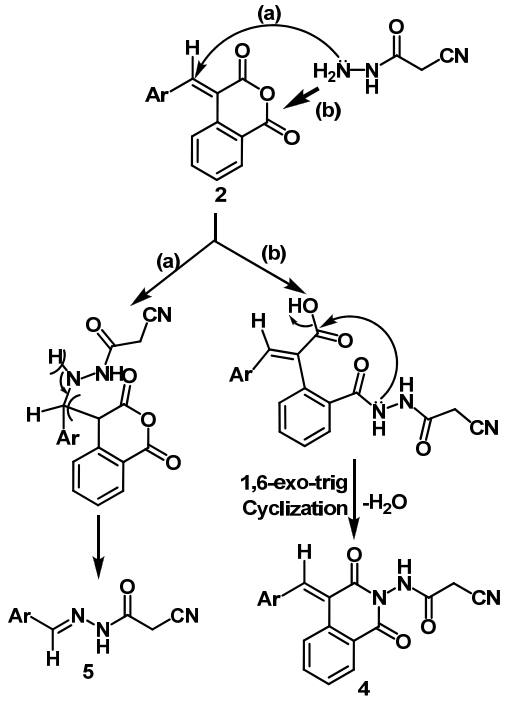
The structure of the minor product (13%) **5** was confirmed from the analytical and spectroscopic data. The highest recorded peak in the mass spectrum at m/z = 247 (61.6%) represent the molecular ion which upon loss of cyanoacetamide molecule afforded the base peak at m/z = 163 (Scheme 6).



Scheme 6

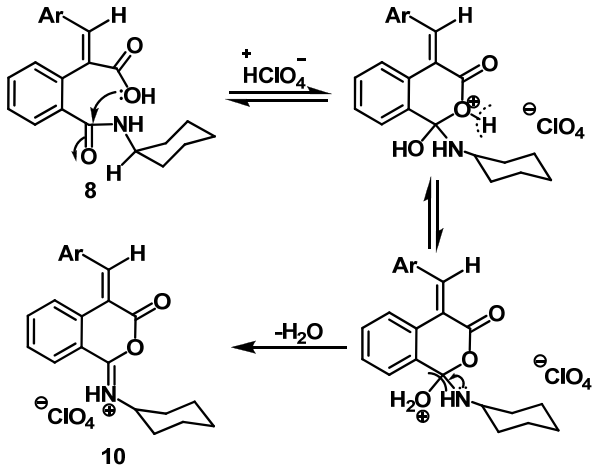
Adequate evidence for the structure **5** was performed by comparison (M.p., TLC and IR spectrum) with authentic sample prepared from the condensation of 3,4-dimethoxybenzaldehyde with cyanoethanoic hydrazide in refluxing ethanol. The formation of compounds **4** and **5** could be explained according to Scheme 7.

Compelling evidence for the isoquinolin-1,3-dione **4** is forthcoming by study of its reactivity towards some activated nitriles such as arylidenemalononitrile and ethoxymethylenemalononitrile. Thus, reaction of **4** with p-chlorobenzylidenemalononitrile in refluxing ethanol in the presence of piperidine afforded pyrido[1,2-b]-1,2,4-triazolo[5,1-a]isoquinolinone derivative **6**, whereas under the same conditions, treatment of **4** with ethoxymethylenemalononitrile yielded (E)-1-[4-(3,4-dimethoxybenzylidene)1,3-dioxo-3,4-dihydroisoquinolin-2-(1H)-yl]-6-amino-2-oxo-1,2-dihydro pyridin-3,5-dicarbonitrile **7** (Scheme 5).



Scheme 7

The reaction of isochromene **2** with cyclohexylamine in refluxing dioxane afforded (E)-2-[2-(N-cyclohexyl-carbamoyl)phenyl]-3-(3,4-dimethoxyphenyl) acrylic acid **8** which readily cyclized using freshly distilled acetic anhydride to give (E)-4-(3,4-dimethoxy benzylidene)-2-cyclohexyliso quinoline-1,3-(2H,4H)-dione **9**. Furthermore, the amido acid **8** in the presence of a mixture of acetic anhydride and perchloric acid at 0°C under the condition described by Boyed *et al.* [31] yielded the homophthalisoimidium perchlorate **10**. The IR spectrum of **10** exhibited typical carbonyl and iminium C=N$^+$ absorption frequencies at 1809 cm^{-1} and 1643 cm^{-1}, respectively. The highest recorded peak in the mass spectrum of **10** represents the radical cation [M$^{+\cdot}$ – $HClO_4$] (Scheme 5). A tentative explanation for the formation of **10** is represented in Scheme 5.



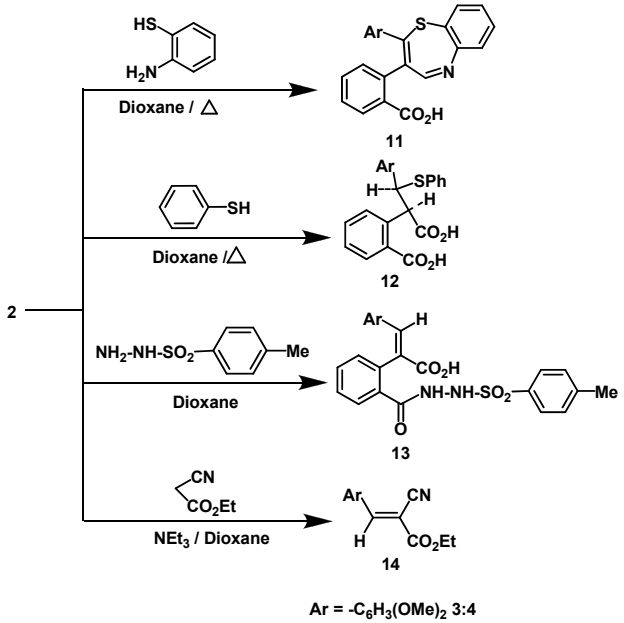
Scheme 8

Isochromen-1,3-dione **2** behave differently when reacted with 2-aminothiophenol. Reaction using a 1:1 molar ratio in refluxing dioxane afforded the adduct with molecular formula $C_{24}H_{19}NO_4S$ [M$^{+\cdot}$ = 417]. Many structures seemed possible for this product. The assigned benzo[1,4]-thiazepine structure **11** was validated on the basis of the following arguments: i- The compound is soluble in aqueous sodium bicarbonate solution, ii- The IR spectrum revealed the presence of carbonyl stretching absorption band at 1684 cm^{-1} together with the broad band for carboxylic-OH at 3480-2850 cm^{-1}, iii- The spot test for SH group is negative (Scheme 9).

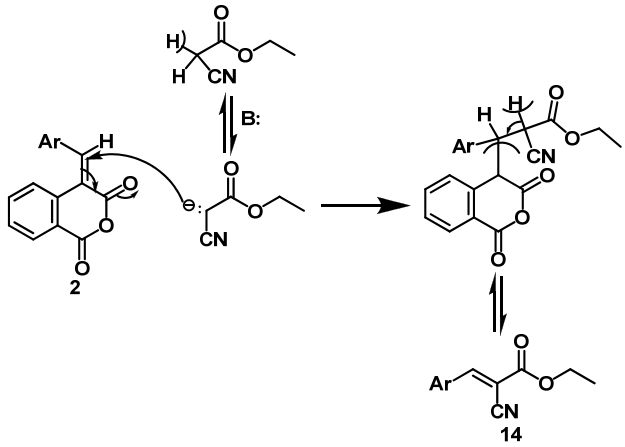
When equimolar amounts of compound **2** and thiophenol were refluxed in boiling dioxane evaporation of the solvent left a crude solid product which completely dissolved in an aqueous sodium bicarbonate solution and precipitated when treated with ice cold hydrochloric acid. This product was identified as 2-[1-carboxy-2-(3,4-dimethoxyphenyl)-2-(phenyl thio)ethyl]benzoic acid **12** as by spectroscopic data. Thus, the IR spectrum of **12** showed the typical stretching absorption bands at 3480-2500 (br) and 1717, 1700 cm^{-1} of the carboxylic acid group (ν_{OH}, ν_{CO}). ^{1}H-NMR spectrum (DMSO-d_6) of **12** revealed the signals at δ 7.6-6.5 (m, 12 $H_{arom.}$), 5.7-5.6 (d, IH, J = 12 Hz), 5.0-4.97 (d, IH, J = 9 Hz) and 3.69 (s, 6H, 2OMe). Treatment of the isochromene dione **2** with p-toluenesulfonohydrazide in refluxing dioxane yielded the hydrazide derivative **13** (Scheme 9).

The reactivity of compound **2** towards the carbon nucleophiles has been investigated too. Thus, isochromene-1,3-dione **2** when reacted with ethyl cyanoacetate in the presence of triethylamine in boiling dioxane afforded the unexpected ethyl-α-cyano-3,4-dimethoxy cinnamate [32] **14**.

The structure of **14** was confirmed besides the spectroscopic data by identity with an authentic sample obtained from the condensation of 3,4-dimethoxybenzaldehyde with ethyl cyanoacetate in refluxing ethanol in the presence of drops piperidine. The formation of **14** could be explained as outlined in Scheme 10.



Scheme 9



Scheme 10

3.2. Biological investigation-antimicrobial activity

The antimicrobial screening of some of the synthesized compounds was done using the agar diffusion assay. This screening was performed against the Gram-negative bacteria, *Escherichia coli* ATCC 10536 and Gram-positive bacteria, *Staphylococcus aureus* ATCC 06538 in addition to the pathogenic fungi *Candida albicans* ATCC 1023 and *Aspergilus flavus*. A strong activity was observed with compounds **4**, **9**, **11**, **12** and **13** which proved to possess marked activity against *E. coli*, *S. aurous* and *C. albicans*. The inhibitory concentration was determined for each of the active compounds along with *Tetracycline* Antibacterial agent and *Amphotericin B* Antifungal agent. No activity was detected for all the synthesized compounds towards *Aspergilus flavus*. Results are shown in Table 1.

Table 1. Antimicrobial screening results of the tested compounds at 1000 μg/mL[a].

Sample	Inhibition zone diameter (mm/mg sample)			
	***Escherichia coli* (G⁻)**	***Staphylococcus aureus* (G⁺)**	***Aspergilus flavus* (Fungus)**	***Candida albicans* (Fungus)**
Control : DMSO	0.0	0.0	0.0	0.0
Tetracycline Antibacterial agent (Standard)	32	30	--	--
Amphotericin B Antifungal agent (Standard)	--	--	18	20
4	15	15	0.0	0.0
9	17	18	0.0	16
11	15	14	0.0	0.0
12	16	17	0.0	0.0
13	18	16	0.0	15

[a] 0.0, no activity, (inhibition zone < 7 mm), weak activity (7-10 mm), moderate activity (11-15 mm), strong activity (> 15 mm). Solvent : DMSO.

References

[1]. Alien, G. R.; Littell, R.; McEvoy, F. J.; Sloboda, A. E. *J. Med. Chem.* **1972**, *15*, 934-937.
[2]. Barry, R. P. *Chem. Rev.* **1964**, *64*, 229-260.
[3]. Billingam, M. E. G. *J. Pharmacol.* **1981**, *72*, 523-526.
[4]. Kovacs, T.; Sonnenbichler, J. *Liebigs Ann. Rcl.* **1997**, *1*, 211-212.
[5]. Mali, R. S.; Babu, K.N . *J. Org. Chem.* **1998**, *63*, 2488.
[6]. Noguchi, S.; Kishimoto, S.; Minamida, I.; Obayashi, M. *Chem. Pharm. Bull.* **1974**, *22*, 529-536.
[7]. Stadler, M.; Anke, H.; Sterner, O. *J. Antibio.* **1995**, *48*, 261-266.
[8]. Chinworrungs, M.; Kittakoop, P.; Isaka, M.; Chanphen, R.; Anicharoen, M.; Thebtaranonth, Y. *J. Chem. Soc., Perkin Trans 1* **2002**, *22*, 2473-2476.
[9]. Rama, N. H.; Iqbal, R.; Zamani, K. *J. Chem. Soc. Pak.* **1998**, *20*, 62-65.
[10]. Stephen W. P.; Koslowski, M. C. *Tetrahedron Lett.* **2001**, *42*, 3567-3570.
[11]. Hajipour, A. ; Mallakpour, S. ; Imanzadeh, G. *Indian J. Chem.* **2001**, *40B*, 250-251.
[12]. Karina, F.; Devienna, M.; Sela Raddi, G.; Eliana, A.; Varand, Z. *Z. Naturforsch* **2002**, *57*, 85-88.
[13]. Gitto, R.; Barreca, M.; Francica, E.; Caruse, R.; Deluea, L.; Russo, E.; Desarro, G.; Chimirri, A. *Arkivoc* **2004**, *5*, 170-180.
[14]. Azizian, J.; Mohammadi, A. A.; Karami, A. R.; Mohammadizadeh, M. R. A. *J. Org. Chem.* **2005**, *70*, 350-352.
[15]. Kandinska, M. I.; Kozekov, I. D.; Palamareva, M. D. *Molecules* **2006**, *11*, 403-414.
[16]. Kane, T. R.; Ly, C. Q.; Kelly, D.E.; Denner, J. M. *J. Comb. Chem.* **2004**, *6*, 564-572.
[17]. Kim, H. J.; Yoon, U. C.; Young, S. Y.; Park, N. S.; Cedeistrom, E.M.; Marriano; P. S. *J. Org. Chem.* **1998**, *63*, 860-863.
[18]. Kozekov, I. D.; Koleva, R. I.; Palamareva, M. D. *J. Heterocyclic Chem*. **2002**, *39*, 229-236.
[19]. Stoyanova, M. P.; Kozekov, I. D.; Palamareva, M.D. *J. Heterocyclic Chem.* **2003**, *40*, 795-803.
[20]. Yadav, J. S.; Reddy, B. V. S.; Saritha Raj, K.; Prasad, A. R. *Tetrahedron* **2003**, *59*, 1805-1809.
[21]. Zalan, Z.; Martinek, T. A.; Lazar, L.; Fulop, F. *Tetrahedron* **2003**, *59*, 9117-9125.
[22]. Coelho, F.; Veronese, D.; Lopes, E. C. S.; Rossi, R. C. *Tetrahedron Lett.* **2003**, *44*, 5731-5735.
[23]. Shaabani, A.; Soleimani, E.; Khavasi, H. R. *Tetrahedron Lett.* **2007**, *48*, 4743-4747.
[24]. Knolker, H. J.; Agarwal, S. *Tetrahedron Lett.* **2005**, *46*, 1173-1175.
[25]. Scott, J. D.; Williams, R. M. *Chem. Rev.* **2002**, *102*, 1669-1730.
[26]. Mahmoud, M. R.; El-Shahawi, M. M.; Farahat, S. E. *J. Chem. Res.* **2008**, *2*, 86-88.
[27]. Mahmoud, M. R.; El-Shahawi, M. M.; Farahat, S. E. *J. Chem. Res.* **2008**, *1*, 59-60.
[28]. Mahmoud, M. R.; El-Shahawi, M. M.; El-Bordany, E. A. A.; Abu El-Azm, F.S . M. *Synth. Commun.* **2010**, *40*, 666-676.
[29]. Morgenstern, A. P.; Schuitj, C.; Nauta, W. T. *J. Chem. Soc. Chem. Commun.* **1969**, *7*, 321-322.
[30]. Morgenstern, A. P.; Schuitj, C.; Nauta, W. T. *J. Chem. Soc.* **1971**, 3706-3712.
[31]. Boyed, G. V.; Monteil, R. L. *J. Chem. Soc. Perkin Trans. 1* **1978**, *11*, 1338-1350.
[32]. Sausen, G. N.; Engelhardt, V. A.; Middleton, W. J. *J. Am. Chem. Soc.* **1958**, *80*, 2815-2822.