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***Research article***

# SPECTROSCOPY STUDIES, X-RAY CRYSTALLOGRAPHY, AND ANTITUMOR EVALUATION OF THE BEHAVIOR OF REACTIONS OF BISDIMEDONE DERIVATIVES WITH MALONONITRILE OR WITH BENZYLIDENEMALONONITILE IN ETHANOLIC PIPERIDINE

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**ABSTRACT**: A simple, environmentally acceptable, a one-pot method, which is efficient, inexpensive, and rapid, afforded excellent yields of the 4*H*-chromeme derivatives **5** and **6** from a three-component reaction of dimedone, arylaldehdes **2a-b**, and malononitrile and a two-component reaction of bisdimedones **3a-b** and malononitrile, respectively. Refluxing ethanolic piperidine was used as the catalyst for the 10-min reactions. A one-pot reaction of benzylidenemalononitrile, instead of malononitrile, with bisdimedones **3a-b**, using the aforementioned reaction, also provided the 4*H*-chromene derivative **5** in excellent yield. The structures of the newly synthesized compounds were elucidated by elemental analyses, X-ray crystallography, and a variety of spectroscopic methods, including proton and carbon nuclear magnetic resonance spectroscopy ($^{1}$H-NMR and $^{13}$C-NMR, respectively), correlation spectroscopy (COSY), heteronuclear single quantum coherence spectroscopy (HSQC), heteronuclear multiple-bond correlation spectroscopy (HMBC), and mass spectrometry (MS). The inhibitory effects of the 4*H*-chromeme derivatives **5** and **6** on the *in vitro* growth of human tumor cell and normal cell lines were greater than that of the reference drug doxorubicin.

**Keywords:** 2-Amino-4*H*-chromenes / Bisdimedone / Cytotoxicity / Multicomponent reactions (MCRs)/ Benzylidenemalononitrile

## INTRODUCTION

One-pot methods involving multicomponent reactions (MCRs) , using different reagents and catalysts have recently become popular for the synthesis of heterocyclic compounds. These heterocyclic compounds are extremely widely distributed in nature and have a wide range of biological activity (Ablajan *et al*.,2012 ; Müller *et al.* 2011) Among of these compounds, 4*H*-chromene and its derivatives have attracted a great deal of interest because of their extensive range of biological activity, including antibacterial, anticoagulant, spasmolytic, diuretic, anticancer, antianaphylactic, and antimicrobial properties (Abdelrazek *et al* .2007; Li *et al 2008* ;Kidwai *et al* 2005; Kumar *et al* 2009 ; Bonsignore *et al 1993* ; Langer *et al* 2008 ;Cai *et al* 2008 ; Cai *et al* 2008). In addition, these compounds are frequently used in cosmetics and pigments, and as potential biodegradable agrochemicals (Ding & Zhao 2010). Therefore, several publications have pointed out various methodologies for a one-pot reactions involving the three-component condensation of a 1,3-dicarbonyl compound, aldehydes, and malononitrile using different catalysts (Lande *et al* 2011; Gheath & Al-Orffi 2008; Xiangshsn *et al* 2004; Perumal *et al* 2013;Feng *et al* 2011;Beifuss *et al* 2011; Ziarani *et al* 2011;Abdelrazek *et al* 2004 ; Davood & Atefeh 2013; Zu-xing *et al* 2011; Abdollahi -Alibeik & Nezampour 2013). Most of these methodologies suffer from one or more disadvantage, such as, use of catalysts that are expensive or harmful to the environment, use of large amounts of non-reusable catalyst, prolonged reaction times, higher temperatures, and unsatisfactory yield. Therefore, the search for a better method for the synthesis of 4*H*-benzo [b]pyrans is still of prime importance to overcome the aforementioned disadvantages.

In continuation of our current interest in the synthesis of functionally substituted heteroaromatic compounds, using inexpensive materials, for use as potential pharmaceuticals (Al-Omran, *et al* 1998, 2001, 2002, 2011, 2013; Al-Omran & El-Khair 2004, 2005, 2008, 2009; Mohareb & Al-Omran 2012), we report herein the synthesis of 2-amino-4*H*-chromene derivatives using one-pot MCRs in ethanol containing a catalytic amount of piperidine (ethanolic piperidine). Piperidine seems to be an excellent candidate for the catalyst as it provided excellent yields of product in a short amount of time. We also report the results of comprehensive spectroscopic analyses of these compounds and evaluations of their ability to inhibit the *in vitro* growth of human tumor cell and normal cell lines.

## Experimental
## Chemistry

All mp. values are reported uncorrected and were determined on a Gallenkamp apparatus. The Fourier transform - infrared (FT- IR) spectra were recorded on a FT- IR ( Jasco FT/IR-6300) using a KBr disc. The $^{1}$H- and $^{13}$C-NMR spectra were recorded on a Bruker DPX 400 MHz spectrometer, with DMSO-$d_6$ or $CDCl_3$ as solvent and tetramethylsilane (TMS) as internal standard. Chemical shifts are reported as δ unit (ppm). Mass spectra were measured on GC/MS DFS, THERMO instrument. Microanalyses were perform on a CHN -Vario Micro Cube analyzer (Germany), a single-crystal X-ray crystallography instrument (Rigaku Rapid II, Japan), and a Bruker $X_8$ Prospector (Bruker, Germany) in the Chemistry Department of Kuwait University.

**Amino-7,7-dimethyl-5-oxo-4-phenyl-4H-5,6,7,8-tetrahydro-4H-chromene-3-carbonitrile (5):**

**Method A**: A solution of **3a** (3.68 g, 10.0 mmol) and malononitrile **(**0.66 g, 10 mmol) in ethanol (20 mL) containing a few drops of piperidine was refluxed for 10 min. The reaction mixture was allowed to cool to room temperature for 24 h. The solid product so formed was collected by filtration and crystallized from ethanol to afford 2.38 g (81% yield).

**Method B:** A solution of **3a** or **3b** (10.0 mmol) and benzylidenemalononitrile (1.54 g, 10 mmol) in ethanol (20 mL) containing a few drops of piperidine was heated under reflux for 10 min. The reaction mixture was allowed to cool to room temperature for 24 h. The solid product so formed was collected by filtration and crystallized from ethanol to afford 2.41 g (82% yield).

**Method C:** A solution of benzaldehyde **2a** (1.0 g, 10.0 mmol), malononitrile (0.66 g, 10 mmol) and dimedone **1**(1.4 g, 10 mmol) in ethanol (20 mL) was heated under reflux for 10 min. The reaction was allowed to cool to room temperature overnight. The solid product so formed was collected by filtration and crystallized from ethanol to afford 2.76 g (94% yield), as a white crystal, m.p. 228–230 $^{0}$C, Lit [4] m.p. 224–225$^{0}$C; FT-IR: $\nu_{max}$ cm$^{-1}$ 3396–3324 ($NH_2$), 2199 (CN), 1680 cm$^{-1}$ (C=O); $^{1}$ H -NMR (400 MHz, DMSO- $d_6$): $\delta_H$ 0.97 (s, 3H, $CH_3$), 1.05 (s, 3H, $CH_3$), 2.13 (d , 1H, *J* = 16 Hz, H-8), 2.28 (d , 1H, *J* = 16 Hz, H- 8'), 2.49–2.58 (m, 2H, H- 6), 4.23 (s, 1H, H-4), 7.05 (s, 1H, $NH_2$, $D_2O$ exchangeable), 7.12 (d, 2H, *J* = 8 Hz, H-2' & H-6'), 7.17 (t, 1H, *J* = 7.6 Hz, H-4'), 7.26 ppm (t, 2H, *J* = 8 Hz, H-3' & H-5'); $^{13}$C-NMR (400 MHz, DMSO): $\delta_C$ 195.5 (C-5), 162.4(C-2), 158.6 (C- 8a), 144.6 (C-1'), 128.4 (C-3' & C -5'), 127.1 (C -2' & C-6'), 126.5 (C-4), 119.5 (CN), 112.9 (C-4a), 58.7 (C -3), 50.1 (C-8), 40.2 (C-6), 35.6 (C-4), 31.7 (C-7), 28.5, 26.9 ($2CH_3$); MS m/z (%) 294.2 [$M^+$, 88%]. *Anal*. Calcd. for $C_{18}H_{18}N_2O_2$(294.35): C, 73.44; H, 6.16; N, 9.51%. Found: C, 73.66; H, 6.16, N, 9.48%.

***2-Amino-4(furan-2-yl)-7,7-dimethyl-5-oxo-5,6,7,8-tetrahydro-4H-chromene-3-carbonitrile* (6):**

**Method A**: A solution of **3b** (3.58 g, 10.0 mmol) and malononitrile **(**0.66 g, 10 mmol) in ethanol (20 mL) containing a few drops of piperidine was heated under reflux for 10 min. The reaction mixture was allowed to cool to room temperature for 24 h. The solid product so formed was collected by filtration and crystallized from ethanol to afford 2.38 g (81% yield).

**Method B**: A solution of furan-2-carbaldehyde **2b** (1.0 g, 10.0 mmol), malononitrile (0.66 g, 10 mmol) and dimedone **1** (1.4 g, 10 mmol) in ethanol (20 mL) was refluxed for 10 min. The reaction was allowed to cool to room temperature overnight. The solid product so formed was collected by filtration and crystallized from ethanol to afford 2.89 g (81%), as a brown crystal, m.p. 218–220$^{0}$C, Lit [4] m.p. 216–217$^{0}$C; FT-IR: $\nu_{max}$ cm$^{-1}$ 3370–3329 ($NH_2$), 2197 (CN), 1660 cm$^{-1}$ (C=O); $^{1}$H-NMR (400 MHz, DMSO- $d_6$ ): $\delta_H$ 0.99 (s, 3H, $CH_3$), 1.05 (s, 3H, $CH_3$), 2.13 (d, 1H, *J* = 16Hz, H -8), 2.26 (d, 1H, *J* = 16Hz, H-8'), 2.40 (d, 2H, *J* = 16 Hz, H-6), 2.51 (d, 2H, *J* = 16 Hz, H -6'), 4.17 (s, 1H, H-4 pyran), 6.06 (d, 1H, *J* = 3.20 Hz, H-3'), 6.33 (d, 1H, *J* = 4.0 Hz, H-4'), 7.11 (s, 1H, $NH_2$, $D_2O$ exchangeable ), 7.48 ppm (d, 1H, *J* = 1.2 Hz, H- 5').

$^{13}$C-NMR (DMSO-$d_6$): $\delta_C$ 195.5 (C-5), 163.3 (C-2), 159.3 (C -8a), 155.7 (C-2'), 141.8 ( C-5'), 119.6 (CN), 110.5 ( C-4'), 110.4 ( C-4a), 105.1 (C -3'), 56.1 (C-3), 49.9 (C-8), 40.1 (C -6), 31.8 (C-7), 29.0 (C-4), 28.4, 26.6 ($2CH_3$); MS m/z (%) 284 [$M^+$, 37%]. *Anal*. Calcd. for $C_{16}H_{16}N_2O_3$ (284.31): C, 67.59; H, 5.67; N, 9.85%. Found: C, 67.05; H, 5.81; N, 9.48%.

## Antitumor Activity Tests

**Reagents:** Fetal bovine serum (FBS) and L-glutamine were from Gibco Invitrogen Company (Scotland, UK). RPMI-1640 medium was from Cambrex (East Rutherford, NJ, USA). DMSO, doxorubicin, penicillin, streptomycin, and sulforhodamine B (SRB) were from Sigma Chemical Company (St. Louis, MO, USA).

**Tumor cell cultures:** Three human tumor cell lines MCF-7 (breast adenocarcinoma), NCI-H460 (non -small cell lung cancer), and SF-268 (human glioblastoma cells) were used. MCF-7 was obtained from the European Collection of Cell Cultures (ECACC, Salisbury, UK) and NCI -H460 and SF-268 were kindly provided by the National Cancer Institute (NCI, Cairo, Egypt). The cell lines were grown as monolayers and routinely maintained in RPMI-1640 medium supplemented with 5% heat -inactivated FBS, 2 µM glutamine, and antibiotics (penicillin at 100 U/mL, streptomycin at 100 µg/mL), at 37$^o$C in a humidified atmosphere containing 5% $CO_2$. Exponentially growing cells were obtained by plating 1.5 x $10^5$ cells/mL for MCF-7 and SF-268 and 0.75 x $10^4$ cells/mL for NCI- H460, followed by 24 h of incubation. The effect of the vehicle solvent (DMSO) on the growth of these cell lines was evaluated in all the experiments by exposing untreated control cells to the maximum concentration (0.5%) of DMSO used in each assay.

**Tumor cell growth assay:** The effects of **5 and 6** on the *in vitro* growth of human tumor cell lines were evaluated according to the procedure adopted by the National Cancer Institute (NCI, USA) in the '*In Vitro* Anticancer Drug Discovery Screen' that uses the protein-binding dye sulforhodamine B to assess cell growth [31]. Briefly, cells growing exponentially in 96-well plates were then exposed for 48 h to five serial concentrations of each compound, starting from a maximum concentration of 150 µM. Following this exposure period, adherent cells were fixed, washed, and stained. The bound stain was solubilized, and the absorbance was measured at 492 nm in a plate reader (Power wave XS, Bio-Tek Instruments Inc., Wincoski,VT, USA). A dose–response curve was obtained for each test compound and cell line, and the minimum concentration for inhibition of 50% of net cell growth ($IC_{50}$) was calculated as described elsewhere ( Monks *et al* 1991). We selected the breast adenocarcinoma (MCF-7), non-small cell lung cancer (NCI-H460), and CNS cancer (SF-268) cell lines to evaluate the effectiveness of our synthesized products compared to that of doxorubicin, which has been shown to be the best positive control against these three cell lines. doxorubicin was used as a positive control and tested in the same manner.

**Normal cell line cultures:** A human diploid normal human fibroblast (WI-38) and normal prostate epithelial cells (PrEC) were purchased from the American Type Culture collection (ATCC; Manassas, VA,USA). Normal colon mucosal (NCM 460) cells were obtained from INCELL Corporation LLC (San Antonio, TX,USA). All cell lines were tested regularly for *Mycoplasma* contamination by the DNA hybridization method using a Gen-Probe Kit. To further characterize the possible differential effects of the synthesized compounds on tumor and normal cells, we compared cell viability (scored as membrane integrity by the Trypan blue exclusion assay) after treatment of the cells with the compound.
Normal PrEC cells showed minimal loss of viability up to at least 25 µM of the tested compound (i.e., ~75 x IC50) even after a 24-h continuous treatment. Other normal cell lines showed similar marginal decreases in cell viability after the 48- h.

## RESULTS AND DISCUSSION

Syntheses
Treatment of dimedone **1** with arylaldehydes **2a -b** in refluxing ethanolic piperidine gave the corresponding bisdimedones **3a-b.** Treatment of the resultant **3a** with malononitrile for 10 min in refluxing ethanolic piperidine afforded a white crystal in excellent yield (94%), which was thought to be hexahydroisoquinoline-4-carbonitrile **4a** (*cf*. Scheme 1). The actual structure of the product, however, was assigned as *4H*-chromene-3 -carbonitrile derivative **5** on the basis of molecular mass spectrometry (MS), which revealed a molecular ion peak [$M^+$] with m/z = 294, corresponding to a molecular weight consistent with a formula of $C_{18}H_{18}N_2O_2$.

The structure of **5** was also established on the basis of its spectral data. The infrared (IR) spectrum of the isolated product showed amino, cyano, and carbonyl absorption bands at $\nu_{max}$ = 3396–3324, 2199, and 1680 $cm^{-1}$, respectively. The chemical shifts of protons for **5** were assigned using correlation spectroscopy (COSY) measurements, which provided the proton-proton coupling values (*cf*. Figure 1). The proton nuclear magnetic resonance ($^1$H-NMR) spectra show two singlets at $\delta_H$ 0.97 and 1.05 ppm for pairs of methyl groups attached to the same carbon. In fact, the conformation of two methyl groups will not be in the same environment. The geminal protons H-8 and H -8' are diastereotopic protons, resonating at $\delta_H$ 2.13 and 2. 28 ppm, respectively, and are coupled to each other with the largest coupling constant ($J$ = 16 $H_z$). The methylene protons adjacent to the carbonyl group appear as a multiple at δ 2.49–2.58 ppm. The $^1$H-NMR spectrum was also characterized by two singlet signals for one proton each at $\delta_H$ 4.23 and 7.05 ppm, which were assigned to the pyran proton and amino group, respectively. The latter signal underwent facile hydrogen deuterium exchange upon addition of deuterium oxide.

The chemical shifts of carbons for *4H*-chromene-3-carbonitrile derivative **5** were assigned using heteronuclear single quantum coherence (HSQC) and heteronuclear multiple bond coherence (HMBC) measurements (*cf*. Figure 2). The carbon nuclear magnetic resonance ($^{13}$ C- NMR) spectrum for **5** showed three downfield signals at δc 195.9, 162.5, and 158.5 ppm, which were assigned to the carbonyl carbon, C-2, and C-8a, respectively. The complete assignment of $^1$H and $^{13}$C chemical shifts for **5** are presented in Figure 3**.** The structure of **5** was unambiguously confirmed by X-ray crystallography as 2- amino-7,7-dimethyl-5-oxo-4-phenyl-*4H*-5,6,7,8-tetrahydro-*4H*-chromene-3-carbonitrile **5** (*cf*. Figure 4 and Table 1). The Cambridge Crystallographic Data Center (CCDC) file 934257 contains the supplementary crystallographic data for **5** reported here. These data can be obtained free of charge from the CCDC at www.ccdc.cam.ac.uk.

Encouraged by this successful result, the one-pot, three-component reaction of benzaldehyde **2a**, malononitrile, and dimedone **1 in** refluxing ethanolic piperidine was performed and afford the 4*H*-chromene derivative **5** (*cf*. Scheme 1). Analytical and spectral data of the product are in agreement with the proposed structure of **5** (see Experimental Section) and are identical to the data for the product obtained previously from the reaction of **3a** with malononitrile

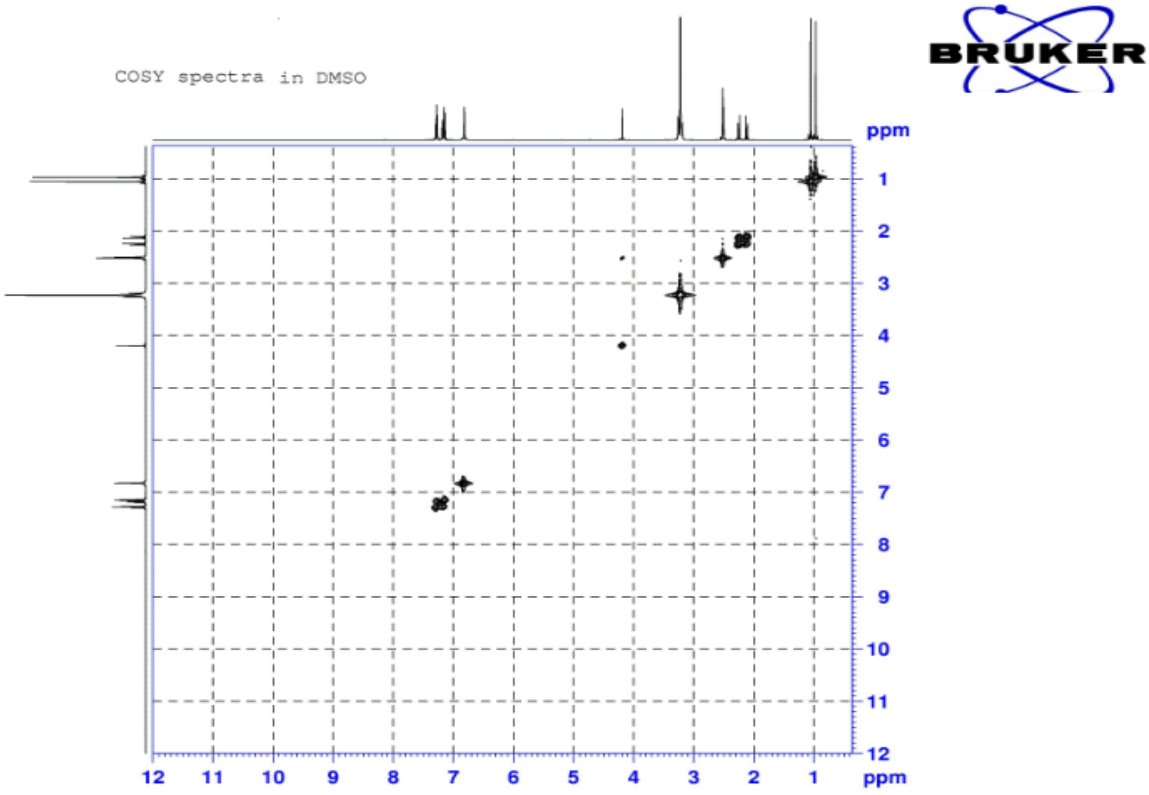


**Figure 1. The COSY spectrum for 2-Amino-4-phenyl -*4H*-chromene (derivative 5) in DMSO-$d_6$**

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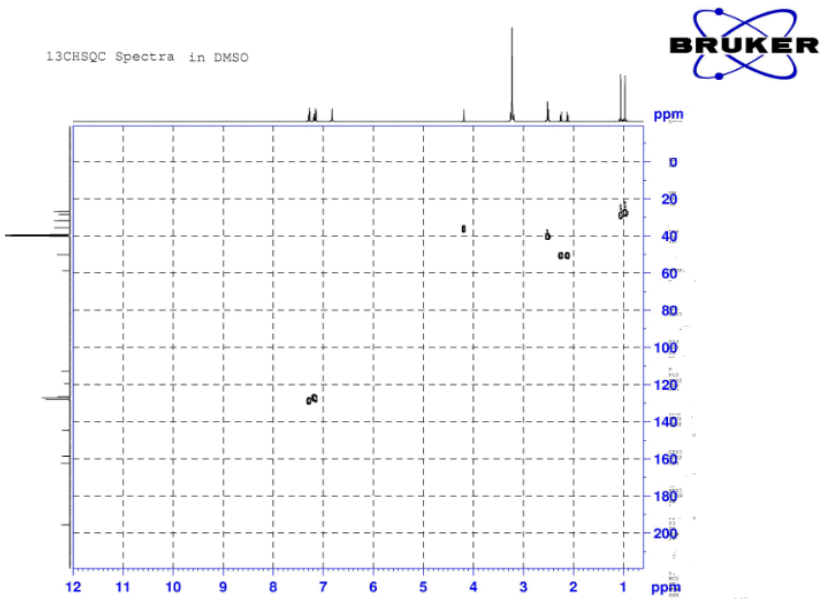


**Figure 2. The CHSQC spectrum for 2-Amino-4-phenyl-*4H*-chromene (derivative 5) in DMSO-$d_6$**

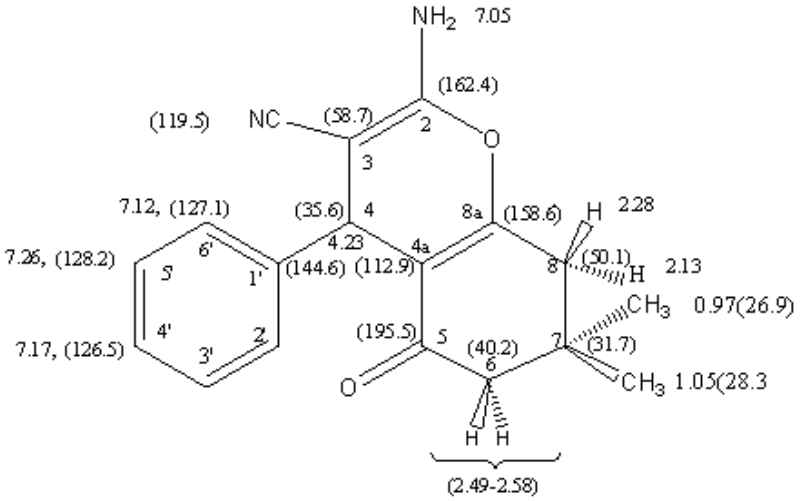


**Figure 3. Complete assignment of $^{1}H$ and $^{13}C$ chemical shift for 5 in DMSO-$d_6$ based on the COSY and HSQC experiments.**

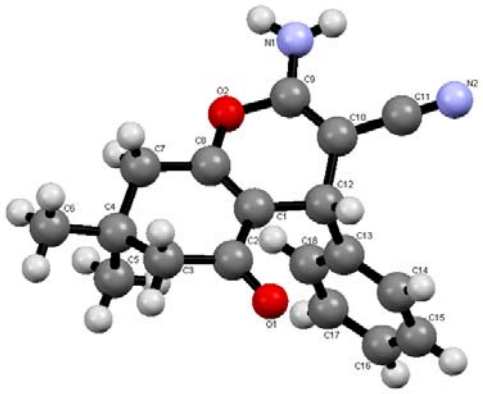

**Figure 4. Perspective view and atom labeling of the X-ray structure of compound 5.**

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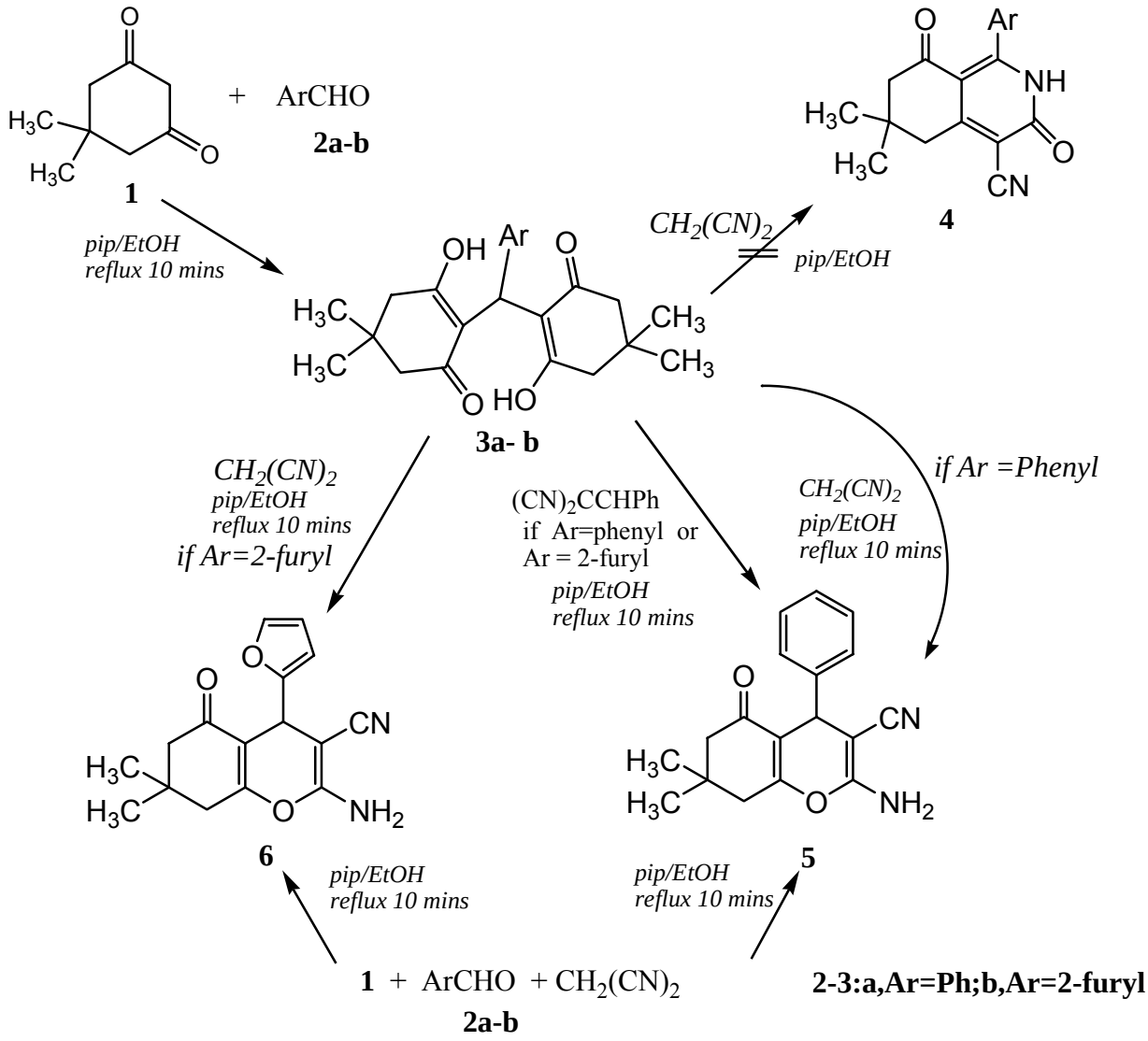


**Scheme 1. Three-component and one-pot synthesis of 2-amino-4*H*-chromene derivatives 5 and 6**

**Table 1: Selected bond lengths and angles for compound 5**

| Atom | distance | Atom | Angle |
|---|---|---|---|
| O2-C8 | 1.3734(15) | C8-O2-C9 | 118.46(10) |
| O1-C2 | 1.2208(16) | C4-C6-H6C | 109.5 |
| C12-C10 | 1.5201(18) | O1-C2-C3 | 121.67(12) |
| C4-C7 | 1.5255(19) | C14-C13-C12 | 120.95(12) |
| C4-C5 | 1.531(2) | C8-C7-H7A | 109.1 |
| C2-C1 | 1.4676(18) | C1-C12-C10 | 107.93(10) |
| C13-C14 | 1.3699(18) | C10-C12-H12 | 107.9 |
| C5-H5A | 0.96 | C7-C4-C3 | 108.31(12) |
| C10-C11 | 1.4137(18) | C2-C3-C4 | 114.09(11) |
| N1-H1A | 0.86 | C4-C3-H3A | 108.7 |
| C12-C13 | 1.5266(18) | O1-C2-C1 | 120.82(12) |
| N2-C11 | 1.1437(17) | C14-C15-H15 | 119.6 |
| C4-C3 | 1.529(2) | C9-N1-H1A | 120 |
| C1-C8 | 1.3389(17) | N2-C11-C10 | 178.20(14) |
| C9-N1 | 1.3341(17) | C13-C18-C17 | 121.36(16) |

In a similar manner, treatment of **3b** with malononitrile in refluxing ethanolic piperidine for 10 min afforded 2-amino -*4H*-chromene-3-carbonitrile derivative **6** in 81% yield (*cf.* Scheme 1)**.** The mass spectrum of **6** revealed a molecular ion peak [$M^+$] with m/z 284, which is compatible with the molecular formula $C_{16}H_{16}N_2O_3$**.** The isolated product was also identified on the basis of spectral data. The IR spectrum of **6** exhibits bands at 3370–3329, 2197, and 1660 $cm^{-1}$, indicating respectively, the presence of amino, cyano , and carbonyl functionalities. The chemical shifts of protons for **6** were assigned using COSY measurements, which provided the proton–proton coupling (*cf.* Figure 5). In addition to a furan proton, the $^1$H-NMR analysis revealed a singlet signal integrated for one proton at $\delta_H$ 4.17 ppm, corresponding to pyran C–H proton, and four doublets at $\delta_H$ 2.13, 2.26, 2.40, and 2.51 ppm. The doublets correspond to two methylene protons, H- 8 and H- 6, respectively, which indicates they are not equivalent protons. The chemical shifts of carbons for **6** were assigned using HSQC and HMBC measurements and are shown in Figure 6. The $^{13}$C-NMR spectrum for **6** shows three downfield signals at δc 195.5, 163.3, and 159.3 ppm, which are assignable to carbonyl carbon, C-2, and C-8a, respectively. The complete assignment of $^1$H and $^{13}$C chemical shifts for **6** are presented in Figure 7.

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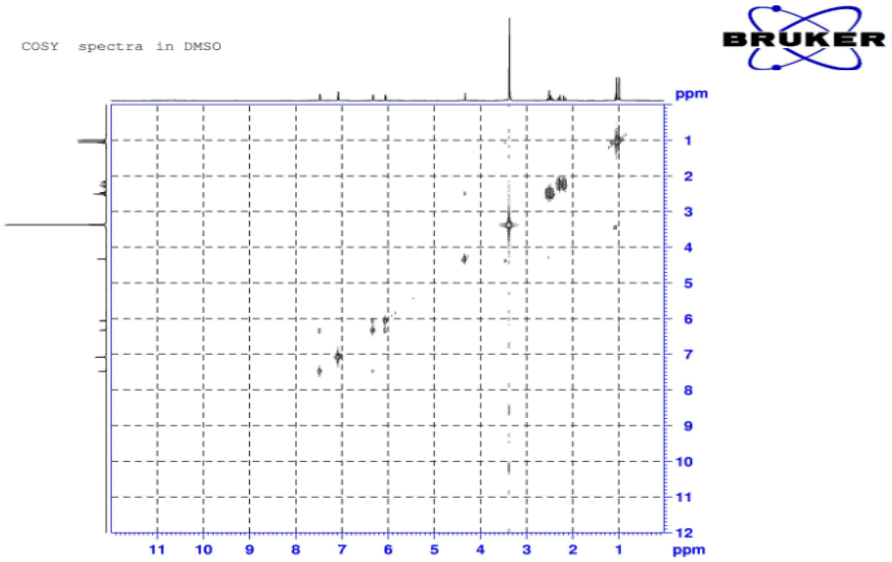


**Figure 5. The COSY spectrum for 2-Amino- 4-(furan-2yl)- *4H*-chromenes ( derivative 6) in DMSO-$d_6$**

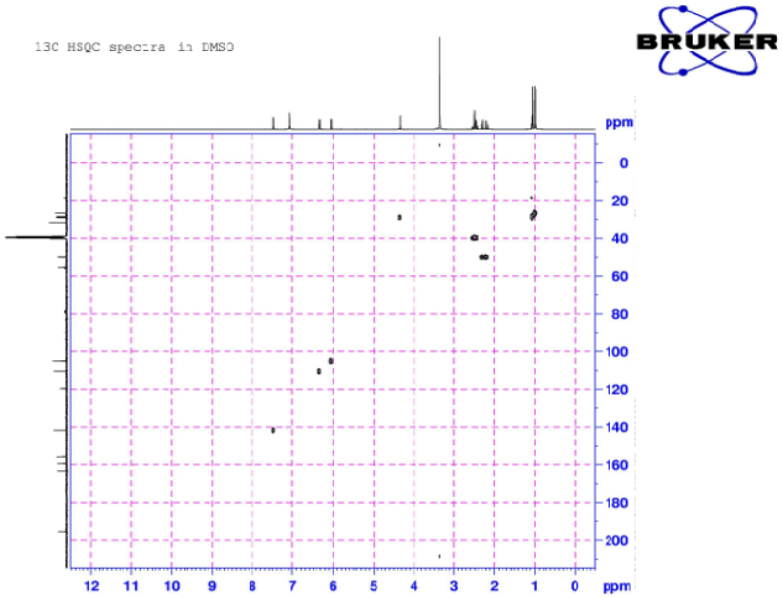


**Figure 6. The HSQC spectrum for 2-Amino- 4-(furan-2yl)- *4H*-chromenes ( derivative 6) in DMSO-$d_6$**

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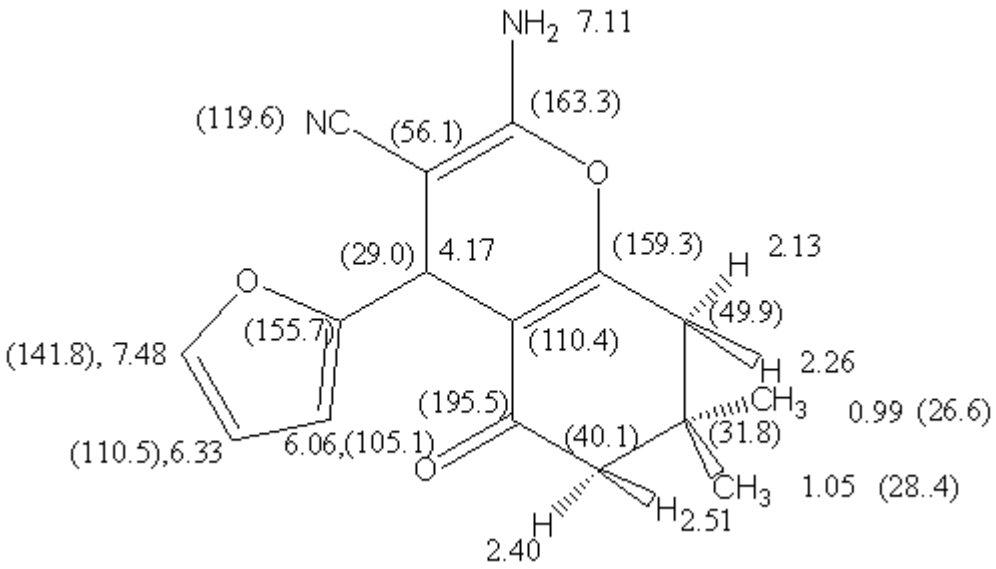


**Figure 7. Complete assignment of $^{1}H$ and $^{13}C$ chemical shift for 6 in DMSO-$d_6$ based on the COSY and HSQC experiments.**

The structure of **6** was unambiguously confirmed by X -ray crystallography as 2-amino-4-(furan-2-yl)-7,7-dimethyl-5-oxo-5,6,7,8-tetrahydro-*4H*-chromene-3-carbonitrile **6** (*cf*. Figure **8** and Table 2). The CCDC file 934258 contains the supplementary crystallographic data for **6** reported herein. These data can be obtained free of charge from the CCDC at www.ccdc.cam.ac.uk.

On the other hand, compound **6** was also obtained *in situ* by a one -step process in which three components, 2-furancarbaldehyde **2b**, malononitrile, and dimedone **1,** were heated in ethanolic piperidine. The product afforded by this process is identical in all respects [melting point (m.p) and spectra] to that obtained previously from the reaction of **3b** with malononitrile (*cf*. Scheme 1). The reaction of the bisdimedone derivatives with malononitrile was believed to be typical *via* Michael addition of active methylene of malononitrile to the methine carbon of bisdimedone, followed by the formation of intermediates **8** and **9** to generate dimedone and 4*H*-chromene derivatives (*cf*. Scheme 2).

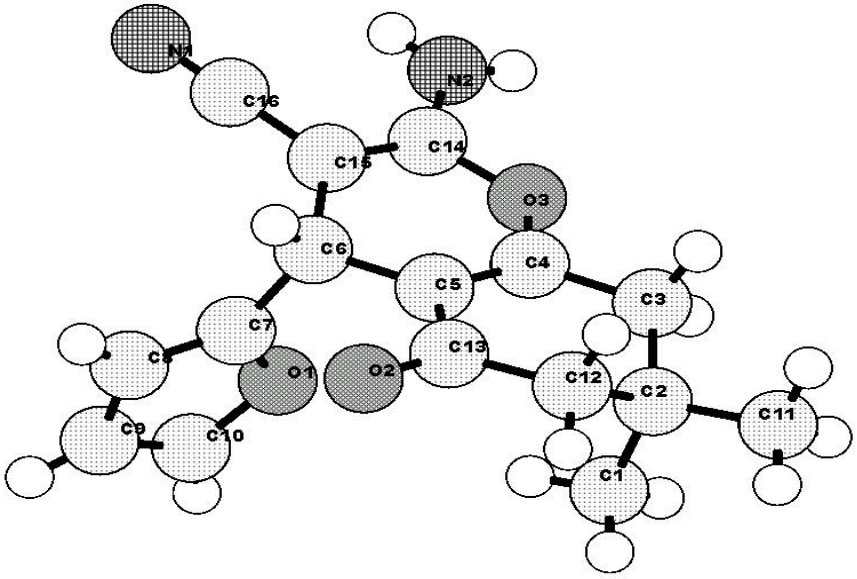


**Figure 8. Perspective view and atom labeling of the X-ray structure of compound 6.**

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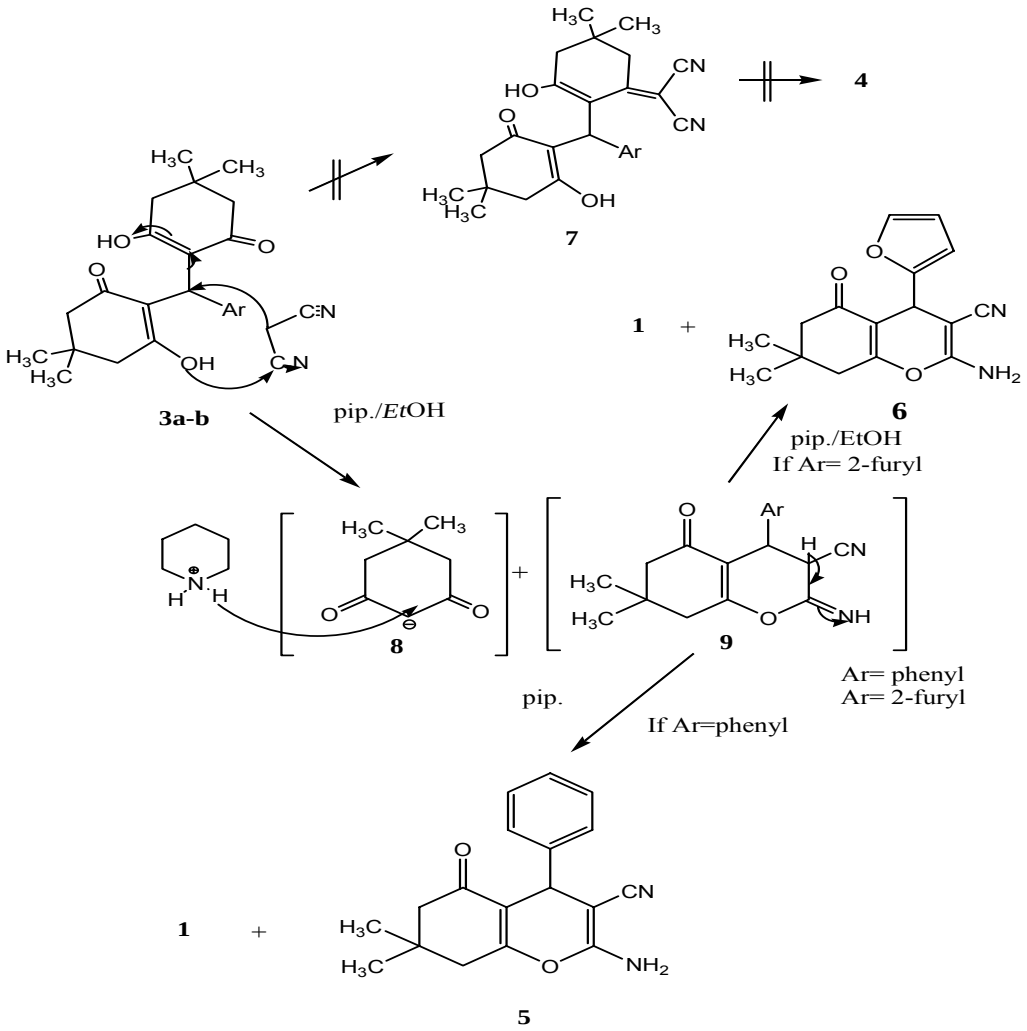
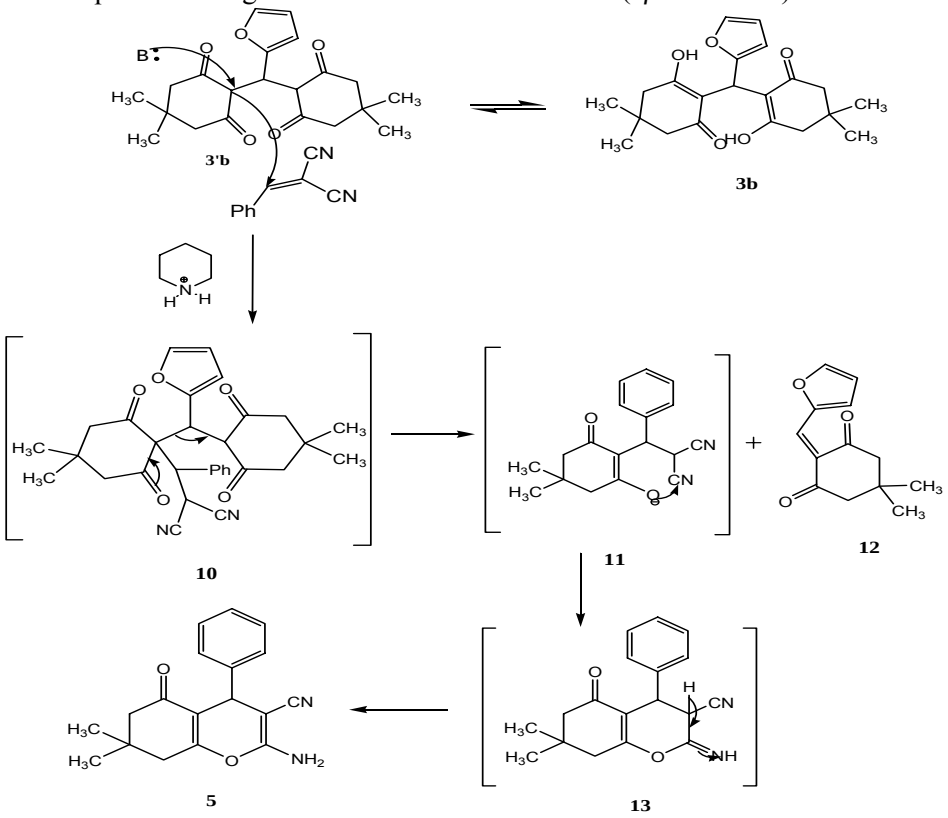



**Scheme 2. Proposed mechanisms for the formation 4-chromenes from the reaction of bisdimedone derivatives 3a-b with malononitrile**

**Table 2: Selected bond lengths and angles for compound 6**

| Atom | Distance | Atom | Angles |
|---|---|---|---|
| O1-C7 | 1.353(2) | C7-O1-C10 | 106.27(18) |
| O2-C13 | 1.2248(19) | C11-C2-C12 | 110.24(14) |
| O3-C4 | 1.3721(18) | C14-O3-C4 | 118.63(11) |
| C15-C16 | 1.410(2) | C11-C2-C12 | 110.24(14) |
| N2-C14 | 1.334(2) | C3-C2-C1 | 109.61(14) |
| C1-C2 | 1.530(3) | C5-C4-C3 | 125.97(14) |
| C4-C5 | 1.334(2) | C10-C9-H9 | 127 |
| C2-C3 | 1.524(2) | C8-C7-C6 | 133.76(18) |
| C3-C4 | 1.4946(19) | C5-C4-O3 | 123.07(13) |
| C4-C5 | 1.334(2) | C7-C6-C5 | 112.88(14) |
| C5-C6 | 1.5090(19) | C8-C7-O1 | 109.23(18) |
| C6-C7 | 1.492(2) | C13-C12-H12B | 108.8 |
| C7-C8 | 1.326(2) | O2-C13-C12 | 121.59(14) |
| C12-C13 | 1.501(2) | N1-C16-C15 | 178.79(19) |
| C12-C13 | 1.501(2) | C3-C2-C12 | 107.99(14) |
| C12-H12B | 0.97 | C3-C2-C12 | 107.99(14) |
| C16-N1 | 1.143(2) | | |

Moreover, treatment of both **3a**or **3b** with benzylidenemalononitrile in refluxing ethanolic piperidine afforded products that are identical in all respects (m.p. and spectra) to those obtained previously from the reactions of malononitrile either with **3a** or with dimidone **1** and benzaldehyde **2a**. To our knowledge, this is the first report for the formation of 2-amino- *4H*-chromene-3-carbonitrile **5** from the reaction of the bisdimedone **3b** with benzylidene - malononitrile in refluxing ethanolic piperidine. It is worth mentioning that it is assumed that the reaction under the reported conditions takes place *via* Michael addition of the α-carbon-2 of one dimedone to the α-β-unsaturated nitrile to give **10,** with subsequent rearrangement to form 5,5-dimethylcyclohexane-1,3-dione derivative **12** and intermediate **11** . The latter intermediate **11** undergoes intramolecular cyclization of the cyano group by the enolic hydroxyl group and subsequent rearrangement to form 4*H*-chromene **5** (*cf*. Scheme 3).




**Scheme 3: Proposed mechanism for the formation of 4-chromene derivative 5 from the reaction of bisdimedone derivative 3b with benzylidenemalononitrile.**

## Antitumor Evaluations and Structure–Activity Relationships of the Synthesized Products

The effects of the synthesized products on the *in vitro* growth of three human tumor cell lines [breast adenocarcinoma (MCF-7), non-small cell lung cancer (NCI-H460), CNS cancer (SF-268)] and three normal cell lines [human fibroblast (WI-38), normal prostate epithelial cells (PrEc), and normal colon mucosal (NCM)] were evaluated after a continuous exposure of 48 h. The results are summarized in Tables 1 and 2.

**Table 3. Effect of the synthesized compounds on the growth of three human tumor cell lines**

| | $IC_{50}$ (mol $L^{-1}$) | | |
|---|---|---|---|
| **Compound** | **MCF-7** | **NCI-H460** | **SF-268** |
| **5** | 2.0 ± 1.0 | 4.4 ± 1.2 | 1.8 ± 0.8 |
| **6** | 0.08 ± 0.001 | 0.2 ± 0.06 | 0.5 ± 0.08 |
| **Doxorubicin** | 0.04 ± 0.008 | 0.09 ± 0.008 | 0.09 ± 0.007 |

Results are given as the concentration that inhibits 50 % of cell growth (IC50) after a continuous exposure of 48 h and show means ± SEM of three-independent experiments, each performed in duplicate.

**Table 4. Effect of the synthesized compounds on the growth of three normal cell lines.**

| | $IC_{50}$ (mol $L^{-1}$) | | |
|---|---|---|---|
| **Compound** | **WI-38** | **PrEC** | **NCM 460** |
| **5** | 0.36 ± 0.02 | 0.22 ± 0.02 | 0.42 ± 0.01 |
| **6** | 0.17 ± 0.06 | 0.15 ± 0.01 | 0.09 ± 0.04 |

Results are given as the concentration that inhibits 50 % of cell growth (IC50) after a continuous exposure of 48 h and show means ± SEM of three-independent experiments, each performed in duplicate.

All the compounds were able to inhibit growth of the human tumor cell lines in a dose-dependent manner. Compounds **5** and **6** show the best inhibitor effects of the tested compounds toward the three tumor cell lines, which are very close to the effect of the reference compound doxorubicin. Comparing **5** and **6**, one can say that the latter showed higher inhibitory effect towards the three cancer cell lines based on the presence of the furan moiety present in **6**. In other words, the presence of the high oxygen content in **6** is responsible for its high potent effect towards the three cancer cell lines. Similarly, all the compounds were also able to inhibit growth of normal cell lines in a dose-dependent manner. Compounds **5** and **6** again exhibit the best inhibitor effects of the tested compounds toward the three normal cell lines which are higher than that of the reference compound doxorubicin.

## CONCLUSION

Synthesis of 2-amino-4*H*-chromenes in excellent yields and in short time periods was accomplished *via* one-pot MCRs of dimedone, arylaldehydes **2a-b**, and malononitrile or *via* a one-pot of benzylidenemalononitrile with bisdimedones **3a-b** in ethanolic piperidine with comprehensive spectroscopic analysis for the 2-amino-4*H*-chromenes derivatives . The cytotoxicity of the synthesized compounds against three human tumor cell lines and normal cell lines was tested and shown to be greater than the reference drug doxorubicin.

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