Full Paper

Design, Synthesis and Evaluation of Cytotoxicity of Novel Chromeno[4,3-*b*]quinoline Derivatives

Ramin Miri¹, Radineh Motamedi², Mohammad Reza Rezaei², Omidreza Firuzi¹, Azita Javidnia³, and Abbas Shafiee³

¹ Medicinal & Natural Products Chemistry Research Center, Shiraz University of Medical Sciences, Shiraz, Iran

² Department of Chemistry, Payam Noor University, Delijan, Iran

³ Department of Medicinal Chemistry, Faculty of Pharmacy, and Pharmaceutical Sciences Research Center, Tehran University of Medical Sciences, Tehran, Iran

In the present work 15 new 1,4-dihydropyridines (DHPs) bearing a coumarin ring were synthesized and assessed on 4 different human cancer cell lines (HeLa, K562, LS180, and MCF-7). Although, all the derivatives were inactive on LS180 cell line, the results on other cells showed that these compounds had weak to moderate antitumoral activities and their IC₅₀ ranged from 25 to >100 μ M. Among the synthesized compounds, 7-(2-nitrophenyl)-8,9,10,12-tetrahydro-7*H*-chromeno[4,3-*b*]quinoline-6,8-dione (**6a**) demonstrated the highest activity (IC₅₀ range in different cell lines: 25.4–58.6 μ M). Furthermore, the calcium channel antagonist activity of the derivatives, an undesired effect when these compounds are used as antitumoral agents, was much lower than nifedipine, a reference antagonist. In conclusion, this group of compounds seems to have promising biological properties and further investigation on this group could potentially lead to the discovery of cytotoxic agents with low calcium channel blocking activity.

Keywords: Calcium channel antagonist activity / Cytotoxicity / 1,4-Dihydropyridines / 4-Hydroxycoumarins

Received: July 6, 2010; Revised: August 16, 2010; Accepted: August 20, 2010

DOI 10.1002/ardp.201000196

Introduction

1,4-Dihydropyridines (DHPs), particularly 4-aryl-substituted-1,4-dihydropridines, exhibit a wide range of biological properties such as calcium channel blocking activity [1]. Recently, DHPs were recognized as reversing agents of multidrug resistance in cancer [2, 3]. Furthermore, there are some reports about their possible cytotoxic activity. Mainly, some derivatives including dexniguldipine and some dibenzoyl ones have shown significant cytotoxicity [4, 5]. There are also some reports on the effects of DHPs on the potentiation of antitumoral and antimetastatic activity of some common cytotoxic drugs [6]. This finding revitalized the interest of

Correspondence: Abbas Shafiee, Department of Medicinal Chemistry, Faculty of Pharmacy and Pharmaceutical Sciences Research Center, Tehran University of Medical Sciences, Tehran 14176, Iran E-mail: ashafiee@asms.ac.ir Fax: +98-21-66461178 synthetic community in the 1,4-dihydropyridine core [7]. It is well established that slight structural modifications on the DHP ring may result in remarkable changes in pharmacological effects [8–10]. For this reason, considerable effort has been focused on the synthesis of new 1,4-DHPs with wider applicability and higher efficiency. Hantzsch esters and acridines are two important DHPs' generation and NADH models (Fig. 1).

Hantzsch esters, first reported by Hantzsch in 1882 [11] were typically synthesized by the one-pot condensation of an aldehyde with alkyl acetoacetate and ammonia either in acetic acid or in alcohol [12]. Improved methods for the Hantzsch esters synthesis have been achieved by the use of an autoclave [13] and by microwave irradiation [14, 15]. A recent work on the thermal solvent free synthesis of Hantzsch esters has been reported [16]. The next group of DHPs, acridine-1,8-diones have been synthesized by the reactions of aldehyde with two equivalents of derivatives 1,3-cyclohexadione and appropriate amines *via* various methods

^{© 2011} WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim



Figure 1. Structure of Hantzsch esters and acridines.

[17]. Asymmetrical acridines have been reported less than symmetrical acridinones, which contain two identical cyclohexadione rings fused to the DHP [18].

Moreover, 4-hydroxycoumarin constitutes the structural nucleus of many natural products, drugs and pesticides [19–21]. It is the key intermediate for various widely used oral anticoagulants and rodenticides [22]. Moreover, some natural and synthetic derivatives of coumarins have demonstrated promising cytotoxic activity [23, 24]. These findings together with our interest in 1,4-dihydropyridines [25], prompted us to synthesize and study the cytotoxicity of some new DHPs heteroanalogues in which one of the 1,3-cyclohexadiones is replaced by a coumarin ring leading to the chromeno[4,3-*b*]quinoline or 7-aryl-8,9,10,12-tetrahydro-7*H*-chromeno[4,3-*b*]quinoline-6,11dione (**6a–m**). Additionally, the calcium channel antagonistic activity of the DHP derivatives was examined due to the known potential Ca²⁺ channel blocking activity of these molecules.

Chemistry

Scheme 1 outlines the synthetic pathway followed for the synthesis of the desired new ring system. 4-Aminocoumarin was obtained in 90% yield by melting 4-hydroxycoumarin in the presence of excess ammonium acetate for 30 min. This method compared to the ones using boiling acetic acid or ethoxyethanol has more yields and needs less time [26–29]. It seems 4-amino-coumarin was converted to 4-hydroxycoumarin in the presence of both solvents and heating, making to decrease yield.

Condensation of 1,3-cyclohexadione **3** and arylaldehydes **4**, in boiling dry benzene for 5 h yielded the 2-benzylidene-cyclohexane-1,3-dione derivatives **5a–m** (80–90%). These compounds were characterized by ¹H-NMR and EI-MS experiments [18]. The reaction of 4-aminocoumarin **2** with 2-benzylidene-cyclohexane-1,3-dione derivatives (**5a–m**) proceeded in solvent free system at 200–220°C, affording 7-aryl-8,9,10,12-tetrahydro-7*H*-chromeno[4,3-*b*]quinoline-6,8-dione (**6a–m**) in 30–50% yield after completion monitoring by TLC (Scheme 1).

In our protocol, no organic solvents or additives were used during the reaction process. The crude products were solid and the work up procedure was just washing by acetone. Desired products of high purity were obtained by column



Scheme 1. Preparation of chromeno[4,3-b]quinoline 6a-m.

chromatography and were characterized by ¹H-NMR, FT-IR, and EI-MS experiments. The ¹H-¹H shift COSY, HSQC, and HMBC of one of the product **6c**, were performed to establish the interfragment relationship and to assign the proton and carbon signals of compound **6** as shown in Scheme 1. This structure also correlated with ¹H-NMR experiments that have a singlet associated with the C₇-H between δ 4.8–5.1 ppm and multiple signals between δ 6.7–8.3 ppm and δ 1.8–2.9 ppm corresponding to aromatic and aliphatic protons. A broad signal between δ 9.6–9.7 ppm in the ¹H-NMR spectra and a strong absorption band at 3200–3300 cm⁻¹ in FT-IR analysis confirmed the secondary amine functional group.

The IR spectrum of product **6** showed a strong peak at 1701– 1720 cm⁻¹which is typical for a coumarin carbonyl group. A mechanism for the formation of **6a–m** is outlined in Scheme 2. The Micheal addition of 4-aminocoumarin **2** with α,β unsaturated ketones **5a–m** give the intermediates **A**. Isomerization of **A** to **B** followed by intermolecular cycloadditions and subsequent dehydrations afford compounds **6a–m**.

Results and discussion

Pharmacology

The *in vitro* calcium channel antagonist activities of novel derivatives were evaluated and their IC_{30} and IC_{50} values were calculated. IC_{30} and IC_{50} values were defined as the molar concentration of the test compounds required producing 30 or 50% inhibition of guinea pig ileal longitudinal smooth muscle (GPLISM) contractions. Results are summarized in the Table 1.

Arch. Pharm. Chem. Life Sci. 2011, 2, 111-118



Scheme 2. Possible mechanism pathway for the formation of 6.

These compounds demonstrated weak calcium channel antagonist activities ($IC_{50} = 10^{-4}-10^{-7}$ M) compared to the reference drug nifedipine ($IC_{50} = 9.14 \times 10^{-8}$ M). The most active compound **6f**, which is at least 3 folds stronger than the others, is nearly 7 times weaker than nifedipine.

As the Ca²⁺-channel antagonist activity was evaluated as an undesired effect for these compounds; their much lower activity in comparison with nifedipine would be a positive point in the future designs of similar derivatives.

Table 1. Physical properties of compounds 6a-m and their calcium channel antagonist activity assessed in the ileum of guinea-pigs.



Compound	R	М. р.	Yield (%)	Formula	$\text{IC}_{30} \text{ (Mean} \pm \text{SE}\text{)}^{\text{a}}$	IC_{50} (Mean \pm SE) ^a	N
6a	2-nitro	>300	35	C ₂₂ H ₁₆ N ₂ O ₅	$(7.07 \pm 5.03) imes 10^{-5}$	$(8.00 \pm 6.00) imes 10^{-4}$	3
6b	4-nitro	260-262	45	C22H16N2O5	$(2.96 \pm 1.33) imes 10^{-6}$	$(7.55 \pm 2.17) imes 10^{-6}$	3
6c	3-bromo	267-269	60	C22H16BrNO3	$(2.79 \pm 1.73) imes 10^{-6}$	$(6.55 \pm 4.20) imes 10^{-6}$	3
6d	4-bromo	294-296	65	C ₂₂ H ₁₆ BrNO ₃	$(7.69 \pm 4.82) imes 10^{-7}$	$(2.29 \pm 1.03) imes 10^{-6}$	3
6e	2-chloro	>310	50	C ₂₂ H ₁₆ ClNO ₃	$(6.06 \pm 2.12) imes 10^{-6}$	$(9.50 \pm 3.34) \times 10^{-6}$	4
6f	3-chloro	208-210	50	C ₂₂ H ₁₆ ClNO ₃	$(8.69 \pm 7.11) imes 10^{-8}$	$(5.99 \pm 4.00) \times 10^{-7}$	2
6g	4-chloro	214-216	55	C ₂₂ H ₁₆ ClNO ₃	$(1.39 \pm 0.91) imes 10^{-6}$	$(3.23 \pm 1.57) imes 10^{-6}$	3
6h	2-methoxy	>310	75	C ₂₃ H ₁₉ NO ₄	$(5.30 \pm 3.58) imes 10^{-6}$	$(2.00 \pm 2.00) imes 10^{-4}$	5
6i	3-methoxy	280-281	55	C ₂₃ H ₁₉ NO ₄	Inactive	Inactive	2
6j	4-methoxy	276-278	75	C ₂₃ H ₁₉ NO ₄	$(6.36 \pm 6.13) imes 10^{-8}$	$(1.83 \pm 1.79) imes 10^{-6}$	2
6k	2-methyl	188-190	45	C ₂₃ H ₁₉ NO ₃	$(9.16 \pm 5.21) \times 10^{-6}$	$(4.99 \pm 3.78) \times 10^{-5}$	4
61	3-methyl	310	74	C ₂₃ H ₁₉ NO ₃	$(4.10 \pm 2.96) \times 10^{-6}$	$(1.02 \pm 0.48) \times 10^{-5}$	3
6m	4-methyl	304-305	72	$C_{23}H_{19}NO_3$	$(2.97 \pm 0.70) imes 10^{-6}$	$(8.93 \pm 1.48) imes 10^{-6}$	4
Nifedipine	_	-	-	$C_{19}H_{22}N_2O_6$	$(1.49 \pm 0.83) \times 10^{-8}$	$(9.14 \pm 4.83) \times 10^{-8}$	4

^a Molar concentration.

© 2011 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

www.archpharm.com

Compound	IC ₅₀ (μM)					
	HeLa	K562	LS180	MCF-7		
6a	29.8 ± 7.7	25.4 ± 4.4	>100	58.6 ± 14.1		
6b	88.2 ± 35.4	43.8 ± 8.6	>100	>100		
6c	83.6 ± 18.9	38.8 ± 4.3	>100	31.4 ± 9.8		
6d	47.3 ± 11.1	47.5 ± 9.6	>100	46.2 ± 10.5		
6e	>100	>100	>100	>100		
6f	>100	50.7 ± 10.0	>100	71.7 ± 17.0		
6g	37.7 ± 11.0	37.5 ± 5.1	>100	41.8 ± 10.1		
6h	>100	>100	>100	>100		
6i	>100	>100	>100	>100		
6j	98.5 ± 74.9	>100	>100	62.0 ± 20.4		
6k	>100	>100	>100	>100		
61	>100	57.4 ± 10.9	>100	52.2 ± 12.6		
6m	43.6 ± 15.0	56.2 ± 23.8	>100	66.7 ± 16.6		
Doxorubicin	0.256 ± 0.057	0.137 ± 0.045	0.157 ± 0.117	0.180 ± 0.031		

Table 2. Cytotoxic activity of synthetic compounds assessed by the MTT reduction assay.

Values represent the mean \pm S.D. of 3 to 4 different experiments.

Cytotoxicity activity

The cytotoxic activity of synthesized compounds was evaluated in 4 human cancer cell lines and the data are demonstrated in Table 2. The lowest activity was observed over LS180 cells, since the IC₅₀ values of all compounds were higher than 100 μ M in this cell line. Compounds seemed to have similar effects on the other 3 cell lines (HeLa, K562, and MCF-7). Among these compounds, 7-(2-nitrophenyl)-8,9,10,12-tetrahydro-7*H*-chromeno[4,3-*b*]quinoline-6,8-dione (**6a**) was the most potent derivative with an IC₅₀ range of 25–58 μ M. Although, 7-(4-chlorophenyl)-8,9,10,12-tetrahydro-7*H*-chromeno[4,3-*b*] quinoline-6,8-dione (**6g**) showed to some extent similar activity (IC₅₀: 37–41 μ M). On the other hand, four compounds, **6e**, **6h**, **6i**, and **6k**, were the weakest ones showing no activity at concentrations lower than 100 μ M.

A survey on the basis of changing moieties showed that the methoxy group confers the lowest cytotoxic activity, since compounds **6h** and **6i** were among the weakest derivatives. Interestingly, the analysis of the correlation between cytotoxic activity in different cell lines demonstrated clear relationship between cytotoxic efficiency of these compounds in various cell lines (Table 3); HeLa and K562 data had the best correlation, while IC₅₀ values of HeLa and MCF-7

Table 3. Correlation coefficients (R^2) between IC₅₀ values of synthesized compounds obtained by the MTT assay in various cell lines and Ca channel antagonist activity (CCA).

	HeLa	K562	MCF-7	CCA
HeLa	1			
K562	0.720	1		
MCF-7	0.471	0.640	1	
CCA	0.246	-0.289	0.061	1

© 2011 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

cells did not show a strong correlation. These correlations may be a sign of possible similar cytotoxic mechanisms on different cancer cell lines that we used in this study. On the other hand, we did not find any significant correlation between the cytotoxic activity and the calcium channel antagonist action of the synthetic compounds.

Conclusion

In this study, we synthesized 15 new compounds having the features of 1,4-dihydropyridines and 4-hydroxycoumarins and tested for their cytotoxic activity on human cancer cell lines. Some of these derivatives showed moderate cytotoxic capacity and at the same time very low calcium channel antagonist activity, an undesirable effect when these compounds are used as antitumoral agents. This group of compounds seems to have promising properties and further investigation on this group could potentially lead to the discovery of potent cytotoxic agents with low calcium channel blocking activity.

Experimental

General

All chemicals and all solvents used in this study were purchased from Merck Company (Darmstadt, Germany) and Sigma-Aldrich Chemical Company (Steinhein, Germany). RPMI 1640, fetal bovine serum (FBS), trypsin and phosphate buffered saline (PBS) were purchased from Biosera (Ringmer, UK). Doxorubicin was obtained from EBEWE Pharma (Unterach, Austria). Melting points were determined on a Kofler hot stage apparatus (Reichert, Vienna, Austria) and are uncorrected. ¹H-NMR spectra were measured using a Bruker 500 spectrometer (Bruker, Rheinstetten, Germany) and chemical shifts are expressed as δ (ppm) with tetramethylsilane as internal standard. The IR spectra were acquired on a Nicollet FT-IR magna 550 spectrographs (KBr disks) (Nicollet, Madison, WI, USA). MS spectra were obtained with a Finnegan MAT TSQ-70 spectrometer (Finnegan Mat, Bremen, Germany). The purity of a compound was confirmed by TLC using different mobile phases. The results of elemental analyses (C, H, N) were within $\pm 0.4\%$ of theoretical values for C, H, and N.

4-Aminocoumarin (2)

A mixture of 4-hydroxycoumarin 1 (1 mmol) and ammonium acetate (2 mmol) were stirred after melting 4-hydroxycoumarin in 211°C for 30 min to complete the reaction (monitored by TLC), then cooled to room temperature. The crude was washed with ethyl acetate and dried *in vacuo* to give the pure compound **2**, a colorless solid (90%). Mp 232–233°C, reported 232–234°C [26].

Typical procedure for the preparation of 2-benzylidenecyclohexane-1,3-dione derivatives (**5a–m**)

A mixture of 1,3-cyclohexadione **3** (1 mmol) and arylaldehyde **4** (1.0 mmol) were refluxed in dry benzene (50 mL) for 5 h. The mixture was cooled to room temperature and the solvent was evaporated to dryness under reduced pressure, and the crude product was washed with cold ethyl acetate and dried *in vacuo* to give pure product **5** (70–90% yield). The products are known compounds and were identified by comparison of spectral and physical data [18].

General procedure for the preparation of 7-aryl-8,9,10,12-

tetrahydro-7H-chromeno[4,3-b]quinoline-6,8-dione (6a–m) 4-Aminocoumarin 2 (1mmol) and 2-benzylidene-cyclohexane-1,3dione derivatives **5a–m** (1 mmol) were thoroughly mixed in a beaker using spatula. Then the beaker is placed in an autoclave (200–220°C) for 45 min to complete the reaction (monitored by TLC). The solid crude was washed with acetone (50 mL) and purified by column chromatography using ethyl acetate/ petroleum ether (20:80) to give **6**.

7-(2-Nitrophenyl)-8,9,10,12-tetrahydro-7H-chromeno [4,3-b]quinoline-6,8-dione (6a)

Yiled 35%: m.p. >300°C. IR (KBr) cm⁻¹: ν 3313 (NH), 3088 (C–H aromatic), 2945 (C–H aliphatic), 1670 (C=O), 1526, 1352 (NO₂). ¹H-NMR (DMSO- d_6): δ 2.05–2.14 (m, 2H, H₁₀), 2.43–2.40 (m, 2H, H₁₁), 2.72–2.79 (m, 1H, H₉), 2.83–2.89 (m, 1H, H₉), 5.75 (s, 1H, H₇), 7.39 (d, 1H, *J* = 8.0 Hz, H₄), 7.49 (t, 1H, *J* = 8.0 Hz, H₂), 7.54 (t, 1H, *J* = 8.0 Hz, H₁₆), 7.64–7.73 (m, 3H, H₁₇, H₁₈, H₃), 8.00 (d, 1H, *J* = 8.0 Hz, H₁₅), 8.35 (d, 1H, *J* = 8.0 Hz, H₁), 9.90 (s, 1H, NH). EI-MS: *m*/*z* (%), 388 (M⁺, 51), 358 (34), 342 (17), 266 (100), 228 (74), 146 (20), 126 (40), 70 (48). Anal. calcd. for C₂₂H₁₆N₂O₅: C, 67.04; H, 4.15; N, 7.21. Found: C, 67.28; H, 4.02; N, 7.45.

7-(4-Nitrophenyl)-8,9,10,12-tetrahydro-7H-chromeno [4,3-b]quinoline-6,8-dione **(6b)**

Yield 45%: m.p. 260–262°C. IR (KBr) cm⁻¹: ν 3336 (NH), 3100 (C–H aromatic), 2934 (C–H aliphatic), 1685 (C=O), 1520, 1362 (NO₂). ¹H-NMR (DMSO- d_6): δ 1.83–2.02 (m, 2H, H₁₀), 2.20–2.43 (m, 2H, H₁₁), 2.72–2.79 (m, 1H, H₉), 2.83–2.87 (m, 1H, H₉), 5.10 (s, 1H, H₇), 7.39 (d, 1H, J = 8.0 Hz, H₄), 7.45 (t, 1H, J = 8.0 Hz, H₂), 7.52 (d, 2H, J = 9.0 Hz, H₁₄, H₁₈), 7.65 (t, 1H, J = 8.0 Hz, H₃), 8.08 (d, 1H, J = 9.0 Hz, H₁₅, H₁₇), 8.34 (d, 2H, J = 8.0 Hz, H₁), 9.90 (s, 1H,

NH). ¹³C-NMR (DMSO- d_6): δ 20.69 (C₁₀), 26.59 (C₉), 35.02 (C₇), 36.56 (C₁₁), 95.4–100.6 (C_{12a}, C_{6a}), 110.93 (C_{1a}, C_{7a}), 116.93 (C₄), 123.22–123.30 (C₁₅, C₁₇, C₁), 124.06 (C₂), 129.14 (C₁₄, C₁₈), 132.19 (C₃), 145.92 (C₁₃), 152.19 (C_{4a}, C_{1a}), 153.27 (C₁₆), 160.29 (C₆), 194.87 (C₈). EI-MS: m/z (%), 389 (M⁺ + 1, 30), 388 (M⁺, 10), 370 (17), 342 (22), 267 (100), 149 (15), 83 (30). Anal. calcd. for C₂₂H₁₆N₂O₅: C, 67.04; H, 4.15; N, 7.21. Found: C, 67.26; H, 4.34; N, 7.03.

7-(3-Bromophenyl)-8,9,10,12-tetrahydro-7Hchromeno[4,3-b]quinoline-6,8-dione (6c)

Yield 60%: m.p. 267–269°C. IR (KBr) cm⁻¹: ν 3436 (NH), 3078 (C–H aromatic), 2924 (C–H aliphatic), 1671 (C=O). ¹H-NMR (DMSO- d_6): δ 1.88–1.99 (m, 2H, H₁₀), 2.24–2.40 (m, 2H, H₁₁), 2.51–2.81 (m, 1H, H₉), 2.83–2.89 (m, 1H, H₉), 4.97 (s, 1H, H₇), 7.18–7.41 (m, 5H, H₄, H₁₄, H₁₆, H₁₇, H₁₈), 7.46 (t, 1H, J = 8.0 Hz, H₂), 7.64 (t, 1H, J = 8.0 Hz, H₃), 8.33 (d, 1H, J = 8.0 Hz, H₁), 9.95 (s, 1H, NH). EI-MS: m/z (%), 423 (M⁺ + 2, 24), 421 (M⁺, 25), 342 (34), 267 (100), 144 (20), 76 (8), 56 (15). Anal. calcd. for C₂₂H₁₆BrNO₃: C, 62.57; H, 3.82; N, 3.32. Found: C, 62.38; H, 3.96; N, 3.54.

7-(4-Bromophenyl)-8,9,10,12-tetrahydro-7Hchromeno[4,3-b]quinoline-6,8-dione (6d)

Yield 65%: m.p. 294–296°C. IR (KBr) cm⁻¹: ν 3334 (NH), 3025 (C–H aromatic), 2934 (C–H aliphatic), 1675 (C=O). ¹H-NMR (DMSO- d_6) δ : 1.84–1.89 (m, 1H, H₁₀), 1.97–2.02 (m, 1H, H₁₀), 2.24–2.31 (m, 2H, H₁₁), 2.65–2.72 (m, 1H, H₉), 2.80–2.85 (m, 1H, H₉), 4.95 (s, 1H, H₇), 7.18 (d, 2H, *J* = 8.5 Hz, H₁₄, H₁₈), 7.36 (d, 2H, *J* = 8.5 Hz, H₁₅, H₁₇), 7.41 (dd, 1H, *J* = 8.0, 1.0 Hz, H₄), 7.45 (td, 1H, *J* = 8.0, 1.0 Hz, H₂), 7.64 (td, 1H, *J* = 8.0, 1.5 Hz, H₃), 8.33 (dd, 1H, *J* = 8.0, 1.5 Hz, H₁), 9.79 (s, 1H, NH). EI-MS: *m*/*z* (%), 423 (M⁺ + 2, 15), 422 (M⁺, 15), 266 (100), 211 (25), 155 (38), 56 (27). Anal. calcd. for C₂₂H₁₆BrNO₃: C, 62.57; H, 3.82; N, 3.32. Found: C, 62.76; H, 3.95; N, 3.14.

7-(2-Chlorophenyl)-8,9,10,12-tetrahydro-7Hchromeno[4,3-b]quinoline-6,8-dione (6e)

Yield 50%: m.p. >310°C. IR (KBr) cm⁻¹: ν 3370 (NH), 3067 (C-H aromatic), 2934 (C-H aliphatic), 1685 (C=O). ¹H-NMR (DMSO- d_6) δ : 1.84–1.86 (m, 1H, H₁₀), 1.95–1.99 (m, 1H, H₁₀), 2.24–2.30 (m, 2H, H₁₁), 2.63–2.69 (m, 1H, H₉), 2.78–2.84 (m, 1H, H₉), 5.21 (s, 1H, H₇), 7.07 (t, 1H, J = 7.6 Hz, H₁₆), 7.14 (t, 1H, J = 7.6 Hz, H₁₇), 7.20 (d, 1H, J = 7.6, H₁₈), 7.32 (m, 2H, H₄, H₁₅), 7.40 (t, 1H, 8.0 Hz, H₂), 7.60 (t, 1H, J = 8.0 Hz, H₃), 8.29 (d, 1H, J = 8.0 Hz, H₁), 9.85 (s, 1H, NH). EI-MS: m/z (%), 379 (M⁺ + 2, 8), 377 (M⁺, 25), 342 (23), 266 (100), 56 (25). Anal. calcd. for C₂₂H₁₆CINO₃: C, 69.94; H, 4.27; N, 3.71. Found: C, 69.75; H, 4.09; N, 3.53.

7-(3-Chlorophenyl)-8,9,10,12-tetrahydro-7Hchromeno[4,3-b]quinoline-6,8-dione **(6f)**

Yield 50%: m.p. 208–210°C. IR (KBr) cm⁻¹: ν 3324 (NH), 3073 (C–H aromatic), 2939 (C–H aliphatic), 1675 (C=O). ¹H-NMR (DMSO- d_6): δ 1.83–1.99 (m, 2H, H₁₀), 2.25–2.32 (m, 2H, H₁₁), 2.62–2.72 (m, 1H, H₉), 2.82–2.89 (m, 1H, H₉), 4.97 (s, 1H, H₇), 7.11–7.27 (m, 4H, H₁₄, H₁₆, H₁₇, H₁₈), 7.39 (d, 1H, *J* = 8.0 Hz, H₄), 7.45 (t, 1H, *J* = 8.0 Hz, H₄), 7.45 (t, 1H, *J* = 8.0 Hz, H₄), 9.82 (s, 1H, NH). EI-MS: *m/z* (%), 379 (M⁺ + 2, 2), 377 (M⁺, 7), 342 (30), 266 (100), 111 (10), 56 (5). Anal. calcd. for C₂₂H₁₆CINO₃: C, 69.94; H, 4.27; N, 3.71. Found: C, 70.15; H, 4.49; N, 3.52.

7-(4-Chlorophenyl)-8,9,10,12-tetrahydro-7Hchromeno[4,3-b]quinoline-6,8-dione (6g)

Yield 55%: m.p. 214–216°C. IR (KBr) cm⁻¹: ν 3390 (NH), 3206 (C–H aromatic), 2939 (C–H aliphatic), 1675 (C=O). ¹H-NMR (DMSO- d_6): δ 1.86–1.87 (m, 1H, H₁₀), 1.97–2.01 (m, 1H, H₁₀), 2.26–2.31 (m, 2H, H₁₁), 2.65–2.71 (m, 1H, H₉), 2.80–2.85 (m, 1H, H₉), 4.97 (s, 1H, H₇), 7.12 (d, 2H, J = 8.5 Hz, H₁₄, H₁₈), 7.30 (d, 2H, J = 8.5 Hz, H₁₅, H₁₇), 7.36 (d, 1H, J = 8.0 Hz, H₄), 7.43 (t, 1H, J = 8.0 Hz, H₂), 7.63 (t, 1H, J = 8.0 Hz, H₃), 8.30 (d, 1H, J = 8.0 Hz, H₁), 9.77 (s, 1H, NH). EI-MS: m/z (%), 379 (M⁺ + 2, 17), 377 (M⁺, 41), 344 (15), 321 (30), 266 (100), 216 (75), 112 (65), 68 (38). Anal. calcd. for C₂₂H₁₆ClNO₃: C, 69.94; H, 4.27; N, 3.71. Found: C, 69.73; H, 4.48; N, 3.95.

7-(2-Methoxyphenyl)-8,9,10,12-tetrahydro-7Hchromeno[4.3-b]quinoline-6.8-dione (6h)

Yield 75%: m.p. >310°C. IR (KBr) cm⁻¹: ν 3359 (NH), 3053 (C-H aromatic), 2863 (C-H aliphatic), 1670 (C=O). ¹H-NMR (DMSO- d_6): δ 1.76–1.79 (m, 1H, H₁₀), 1.93–1.95 (m, 1H, H₁₀), 2.10–2.23 (m, 2H, H₁₁), 2.54–2.65 (m, 1H, H₉), 2.72–2.75 (m, 1H, H₉), 3.63 (s, 3H, CH₃), 5.06 (s, 1H, H₇), 6.7 (t, 1H, J = 8.0 Hz, H₁₇), 6.85 (d, 1H, J = 8.0 Hz, H₁₅), 7.06 (td, 1H, J = 8.0, 1.5 Hz, H₁₆), 7.23 (dd, 1H, J = 8.0 Hz, H₂), 7.59 (t, 1H, J = 8.0 Hz, H₄), 7.41 (t, 1H, J = 8.0 Hz, H₁), 9.68 (s, 1H, NH). EI-MS: m/z (%), 373 (M⁺, 36), 342 (10), 266 (100), 167 (25), 87 (30), 56 (12). Anal. calcd. for C₂₃H₁₉NO₄: C, 73.98; H, 5.13; N, 3.75. Found: C, 74.15; H, 5.01; N, 3.57.

7-(3-Methoxyphenyl)-8,9,10,12-tetrahydro-7Hchromeno[4,3-b]quinoline-6,8-dione (6i)

Yield 55%: m.p. 280–281°C. IR (KBr) cm⁻¹: ν 3324 (NH), 3100 (C–H aromatic), 2945 (C–H aliphatic), 1670 (C=O). ¹H-NMR (DMSO- d_6): δ 1.46–1.57 (m, 1H, H₁₀), 1.59–1.65 (m, 1H, H₁₀), 2. 05–2.24 (m, 2H, H₁₁), 2.48–2.59 (m, 1H, H₉), 2.67–2.70 (m, 1H, H₉), 3.68 (s, 3H, CH₃), 4.97 (s, 1H, H₇), 6.68 (d, 1H, J = 7.5 Hz, H₁₈), 6.76–6.79 (m, 2H, H₁₄, H₁₆), 7.11 (t, 1H, J = 7.5 Hz, H₁₇), 7.29 (d, 1H, J = 8.0 Hz, H₄), 7.43 (t, 1H, J = 8.0 Hz, H₂), 7.58 (t, 1H, J = 8.0 Hz, H₃), 8.30 (d, 1H, J = 8.0 Hz, H₁), 9.77 (s, 1H, NH). EI-MS: m/z (%), 373 (M⁺, 45), 342 (22), 266 (100), 93 (85), 76 (50). Anal. calcd. for C_{23} H₁₉NO₄: C, 73.98; H, 5.13, N, 3.75. Found: C, 73.72; H, 5.35; N, 3.56.

7-(4-Methoxyphenyl)-8,9,10,12-tetrahydro-7Hchromeno[4,3-b]quinoline-6,8-dione (6j)

Yield 75%: m.p. 276–278°C. IR (KBr) cm⁻¹: ν 3441 (NH), 3073 (C–H aromatic), 2934 (C–H aliphatic), 1670 (C=O). ¹H-NMR (DMSO- d_6): δ 1.92–2.04 (m, 2H, H₁₀), 2.35–2.41 (m, 2H, H₁₁), 2.45–2.55 (m, 1H, H₉), 2.56–2.63 (m, 1H, H₉), 3.64 (s, 3H, CH₃), 5.25 (s, 1H, H₇), 6.68 (d, 2H, J = 8.5 Hz, H₁₅, H₁₇), 7.23–7.27 (m, 2H, H₄, H₂),7.30 (d, 2H, J = 8.5 Hz, H₁₄, H₁₈), 7.50 (t, 1H, J = 7.5 Hz, H₃), 7.86 (d, 1H, J = 7.5 Hz, H₁), 8.28 (s, 1H, NH). EI-MS: m/z (%), 373 (M⁺, 25), 266 (100), 211 (10), 77 (10). Anal. calcd. for C₂₃H₁₉NO₄: C, 73.98; H, 5.13, N, 3.75. Found: C, 73.76; H, 5.02; N, 3.96.

7-(2-Methylphenyl)-8,9,10,12-tetrahydro-7Hchromeno[4,3-b]quinoline-6,8-dione (6k)

Yield 45%: m.p. 188–190°C. IR (KBr) cm⁻¹: ν 3405 (NH), 3057 (C–H aromatic), 2929 (C–H aliphatic), 1700 (C=O). ¹H-NMR (DMSO- d_6): δ 1.81–1.97 (m, 2H, H₁₀), 2.18–2.27 (m, 2H, H₁₁), 2.50 (s, 3H, CH₃), 2.65–2.90 (m, 2H, H₉), 5.03 (s, 1H, H₇), 6.94–6.99 (m, 2H, H₁₅, H₁₇),

7.07 (d, 1H, J = 7.5 Hz, H₁₈), 7.29 (t, 1H, J = 7.5 Hz, H₁₆), 7.34 (d, 1H, J = 8.0 Hz, H₄), 7.43 (t, 1H, J = 8.0 Hz, H₂), 7.60 (t, 1H, J = 8.0 Hz, H₃), 8.31 (d, 1H, J = 8.0 Hz, H₁), 9.67 (s, 1H, NH). EI-MS: m/z (%), 357 (M⁺, 21), 342 (15), 266 (100), 224 (32), 196 (10). Anal. calcd. for C₂₃H₁₉NO₃: C, 77.29; H, 5.36; N, 3.92. Found: C, 77.48; H, 5.08; N, 4.15.

7-(3-Methylphenyl)-8,9,10,12-tetrahydro-7Hchromeno[4,3-b]quinoline-6,8-dione (6I)

Yield 74%: m.p. 310°C. IR (KBr) cm⁻¹: ν 3339 (NH), 3052 (C–H aromatic), 2909 (C–H aliphatic), 1680 (C=O). ¹H-NMR (DMSO- d_6): δ 1.84–1.88 (m, 1H, H₁₀), 1.98–2.01 (m, 1H, H₁₀), 2.20 (s, 3H, CH₃), 2.24–2.31 (m, 2H, H₁₁), 2.65–2.72 (m, 1H, H₉), 2.80–2.85 (m, 1H, H₉), 4.95 (s, 1H, H₇), 6.89 (d, 1H, J = 8.0 Hz, H₁₆), 6.98 (d, 1H, J = 8.0 Hz, H₁₈), 7.03 (s, 1H, H₁₄), 7.07 (d, 1H, J = 8.0 Hz, H₁₇), 7.36 (d, 1H, J = 8.0 Hz, H₄), 7.41 (t, 1H, J = 8.0 Hz, H₂), 7.63 (t, 1H, J = 8.0 Hz, H₃), 8.31 (d, 1H, J = 8.0 Hz, H₁). EI-MS: m/z (%), 357 (M⁺, 5), 266 (100), 224 (23), 111 (45), 196 (40). Anal. calcd. for C₂₃H₁₉NO₃: C, 77.29; H, 5.36; N, 3.92. Found: C, 77.06; H, 5.53; N, 3.75.

7-(4-Methylphenyl)-8,9,10,12-tetrahydro-7H-

chromeno[4,3-b]quinoline-6,8-dione (6m)

Yield 72%: m.p. 304–305°C. IR (KBr) cm⁻¹: ν 3272 (NH), 3062 (C–H aromatic), 2950 (C–H aliphatic), 1710 (C=O). ¹H-NMR (DMSO- d_6): δ 1.82–1.88 (m, 1H, H₁₀), 1.98–2.03 (m, 1H, H₁₀), 2.17 (s, 3H, CH₃), 2.15–2.34 (m, 2H, H₁₁), 2.72 (m, 1H, H₉), 2.80–2.85 (m, 1H, H₉), 4.94 (s, 1H, H₇), 7.00 (d, 2H, J = 8.0 Hz, H₁₅, H₁₇), 7.11 (d, 2H, J = 8.0 Hz, H₁₄, H₁₈), 7.37 (d, 1H, J = 8.0 Hz, H₄), 7.43 (t, 1H, J = 8.0 Hz, H₁), 9.73 (s, 1H, NH). EI-MS: m/z (%), 357 (M⁺, 8), 343 (50), 266 (100), 224 (25), 83(23), 65 (35). Anal. calcd. for C₂₃H₁₉NO₃: C, 77.29; H, 5.36; N, 3.92. Found: C, 77.43; H, 5.18; N, 3.76.

Pharmacology

Calcium channel antagonist activity

Male albino guinea pigs (300-450 g) were purchased from Animal House Department of the Shiraz University of Medical Sciences. They had free access to standard rodent chow and tap water. The animals were housed in a room maintained at 23 \pm 2°C, 55 \pm 10% of humidity, and on a 12 h dark/light cycle. Feeding was stopped one day before starting the in-vitro tests. Guinea pigs were sacrificed and their intestines were removed above the ileocecal junction. Smooth muscle segments of about 1 cm length were mounted under a resting tension of 500 mg and were maintained at 37°C in a 20 mL jacketed organ bath containing oxygenated (95% O_2 and 5% CO_2) physiological saline solution of the following compositions: 137 mM NaCl, 1.8 mM CaCl₂, 2.7 mM KCl, 1.1 mM MgSO₄, 0.4 mM NaHPO₄, 12 mM NaHCO₃, and 5 mM glucose. The muscle was equilibrated for 1 h with a solution changing every 15 min. The contractions were recorded with a forced displacement transducer (Hugo Sachs, March-Hugstetten and Germany) on a

physiograph (Hugo Sachs). All compounds were dissolved in DMSO and the same volume of solvent was used as the negative control. Nifedipine was used as the positive control. The contraction was elicited with 80 mM KCl. The contractile response was taken as the 100% value for the tonic (slow) component of the response. Test compounds were added in cumulative doses after the dose response for KCl. Test compound-induced relaxation of contracted muscle was expressed as the percent of inhibition of the control. The IC₅₀ values were determined from the concentration–response curves [30, 31].

Cytotoxicity

Cell lines and cell culture

HeLa (human cervical adenocarsinoma), K562 (human chronic myelogenous leukemia), LS180 (human colon adenocarcinoma), and MCF-7 (human breast adenocarcinoma) cells were obtained from the National Cell Bank of Iran, Pasteur Institute, Tehran, Iran. All cell lines were maintained in RPMI 1640 supplemented with 10% FBS, and 100 units/mL penicillin-G and 100 μ g/mL streptomycin. Cells were grown in monolayer cultures at 37°C in humidified air containing 5% CO₂.

Cytotoxicity assay

Cell viability following exposure to synthetic compounds was estimated by using the MTT reduction assay [31-33]. MCF-7 and K562 cells were plated in 96-well micro-plates at a density of 5 \times 10⁴ cells/mL (100 μ L per well). LS180 and HeLa cells were plated at densities of 1×10^5 and 2.5×10^4 cells/mL, respectively. Control wells contained no drugs and blank wells contained only growth medium for background correction. After overnight incubation at 37°C, half of the growth medium was removed and 50 µL of medium supplemented with different concentrations of synthetic compounds dissolved in DMSO were added in quadruplicate. Plates with K562 cells were centrifuged before this procedure. Maximum concentration of DMSO in the wells was 0.5%. Cells were further incubated for 72 h, except for HeLa cells, which were incubated for 96 h. At the end of the incubation time, the medium was removed and MTT was added to each well at a final concentration of 0.5 mg/mL and plates were incubated for another 4 h at 37°C. Then formazan crystals were dissolved in 200 µL DMSO. The optical density was measured at 570 nm with background correction at 655 nm using a BioRad micro-plate reader (Model 680). The percentage of viability compared to control wells was calculated for each concentration of the compound and IC₅₀ values were calculated with the software Curve Expert version 1.34 for Windows. Each experiment was repeated at least 3 times and data were presented as mean \pm S.D.

This work was supported by research council of Tehran University of Medical Sciences, and INSF (Iran National Sciences Foundation). Financial support of the Research Council of Shiraz University of Medical Sciences is also acknowledged.

The authors have declared no conflict of interest.

References

- a) D. J. Triggle, *Mini Rev. Med. Chem.* 2003, 3, 215–223. b) A. M. Katz, N. M. Leach, *J. Clin. Pharmacol.* 1987, 27, 825–834. c) A. Davood, N. Mansouri, A. R. Dehpour, H. Shafaroudi, *et al.*, *Arch. Pharm. Chem. Life Sci.* 2006, 339, 299–304. d) A. Shafiee, N. Rastkary, M. Jorjani, B. Shafaghi, *Arch. Pharm.* 2002, 2, 69–76. e) A. Shafiee, N. Rastkary, M. Jorjani, B. Shafaghi, *Arch. Pharm.* 2002, 2, 69–76. e) A. Shafiee, N. Rastkary, M. Jorjani, A. Rolabchifar, A. R. Dehpour, M. Pirali Hamedani, A. Shafiee, *Arzneim. Forsch. Drug Res.* 2002, 52, 21–26.
- [2] a) F. Fusi, S. Saponara, M. Valoti, S. Dragoni, et al., Curr. Drug Targets 2006, 7, 949–959. b) L. Bazargan, S. Fouladdel, A. Shafiee, M. Amini, et al., Cell Biol. Toxicol. 2008, 24, 165– 174.
- [3] R. Miri, A. R. Mehdipour, Bioorg. Med. Chem. 2008, 16, 8329– 8334.
- [4] K. A. Hahn, A. M. Legendre, H. M. Schuller, J. Cancer Res. Clin. Oncol. 1997, 123, 34–38.
- [5] S. R. Morshed, K. Hashimoto, Y. Murotani, M. Kawase, et al., Anticancer Res. 2005, 25, 2033–2038.
- [6] L. Fedeli, M. Colozza, E. Boschetti, I. Sabalich, et al., Cancer 1989, 6, 1805–1811.
- [7] J. Jiang, J. Yu, X. X. Sun, Q. Q. Rao, L. Z. Gong, Angew. Chem. Int. Ed. 2008, 47, 2458–2462.
- [8] C. O. Kappe, W. M. F. Fabian, M. A. Semones, *Tetrahedron* 1997, 53, 2803–2816.
- [9] D. J. Triggle, Cell Mol. Neurobiol. 2003, 23, 293-303.
- [10] M. Schramm, G. Thomas, R. Tower, G. Franckowiak, Nature 1983, 303, 535–537.
- [11] A. Hantsch, Justus Liebigs Ann. Chem. 1982, 215, 1.
- [12] B. Love, K. M. Snader, J. Org. Chem. 1965, 30, 1914-1916.
- [13] Y. Watanabe, K. Shiota, T. Hoshiko, S. Ozako, *Synthesis* **1983**, 761.
- [14] R. Alajarin, J. J. Vaquero, J. L. G. Navio, J. Alvarez-Builla, Synlett. 1992, 297–298.
- [15] L. Öhberg, J. Westman, Synlett. 2001, 1296-1298.
- [16] M. A. Zolfigol, M. Safaiee, Synlett. 2004, 827-828.
- [17] S. Tu, C. Miao, Y. Gao, F. Fang, et al., Synlett. 2004, 255–258.
- [18] W. G. Wang, C. B. Miao, Green Chem. 2006, 8, 1080– 1085.
- [19] S. S. Kang, H. J. Kim, C. Jin, Y. S. Lee, Bioorg. Med. Chem. Lett. 2009, 19, 188–191.
- [20] A. O. Obaseki, W. R. Porter, J. Heterocycl. Chem. 1982, 19, 385– 390.
- [21] A. K. D. Gupta, R. M. Chaterjee, K. R. Das, Indian J. Chem. 1981, 20B, 511–515.

- [22] M. A. Hermodson, W. M. Barker, K. P. Link, J. Med. Chem. 1971, 14, 167–169.
- [23] C. R. Su, S. F. Yeh, C. M. Liu, A. G. Damu, et al., Bioorg. Med. Chem. 2009, 17, 6137–6143.
- [24] a) S. Stanchev, G. Momekov, F. Jensen, I. Manolov, *Eur. J. Med. Chem.* 2008, 43, 694–706. b) E. Azizi, M. H. Abdolmohammadi
 Sh. Fouladdel, A. Shafiee, *et al.*, *Daru* 2009, 17, 181–186.
- [25] L. Navidpour, H. Shafaroodi, R. Miri, A. R. Dehpour, A. Shafiee, *Il Farmaco* 2004, 59, 261–269.
- [26] I. C. Iranov, S. K. Karagiosov, I. Manolov, Arch. Pharm. 1991, 324, 61–62.
- [27] Y. Jacquot, L. Bermont, H. Giorgi, B. Refouvelet, et al., Eur. J. Med. Chem. 2001, 36, 127–136.

- [28] Y. Jacquot, A. Cleern, I. Loios, Y. Ma, et al., Biol. Pharm. Bull. 2002, 25, 335–341.
- [29] K. Tabakovic, I. Tabakovic, N. Ajdini, O. Leci, Synthesis 1987, 1987, 308–310.
- [30] R. Miri, H. Niknahad, G. Vesal, A. Shafiee, Farmaco 2002, 57, 123–128.
- [31] A. R. Mehdipour, K. Javidnia, B. Hemmateenejad, Z. Amirghofran, R. Miri, *Chem. Biol. Drug Des.* 2007, 70, 337– 346.
- [32] A. Shafiee, R. Motamedi, O. Firuzi, S. Meili, et al., Med. Chem. Res. 2010, published online. DOI 10.1007/s00044-010-9340-3
- [33] S. A. Jabbar, P. R. Twentyman, J. V. Watson, Br. J. Cancer 1989, 60, 523–528.