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Synthesis, Cytotoxicity and Calcium Antagonist Activity of Novel Imidazolyl Derivatives of 1,8-Acridinediones

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A series of novel imidazolyl derivatives of fully and partially hydrogenated 1,8-acridinediones were synthesized and assessed for their cytotoxic activity on four different human cancer cell lines (HeLa, MCF-7, LS-180, and Raji cells). Although being inactive on LS-180 and Raji cell lines, the compounds showed weak to moderate anti-tumor activities on other cell lines and their IC_{50} ranged from 31.7 to more than 100 μM. Among the synthesized compounds **12b**, **13b**, **12c** and **13c**, bearing an electron-attracting substituent on the imidazole ring, and **12f** and **13f**, with a benzyl substituent, showed higher activities. Furthermore, the calcium channel antagonist activity of the derivatives, an undesired effect when these compounds are used as anti-tumor agents, was much lower than that of Nifedipine, a reference antagonist. Imidazolyl derivatives of 1,8-acridinedione represent an interesting template, showing promising biological properties. Further investigation on this chemical scaffold could potentially lead to the discovery of cytotoxic agents with low calcium channel blocking activity.

Keywords: Imidazole, 1,8-Acridinedione, Cytotoxicity, Calcium channel antagonist activity

INTRODUCTION

Among the wide range of biological properties of 1,4-dihydropyridines, the calcium channel blocking activity has been closely studied and clearly pointed out, particularly in 4-aryl derivatives of 1,4-DHPs [1]. They have also been recognized as multidrug resistance reversing agents in cancer therapy [2]. Several reports are published regarding cytotoxic activities of the different derivatives of DHPs including dexniguldipine and some di-benzoyls [3,4]. Moreover, the effects of DHPs on the potentiation of anti-tumor and anti-metastatic activity of some common cytotoxic drugs have been explored [5].

As it is well established that slight structural modifications of the DHP ring may result in remarkable changes in pharmacological effects [6-8], the DHP core has revitalized the interest of the synthetic community [9]. Among different derivatives of 1,4-DHPs, 1,8-acridinedione is a known scaffold with wide spectrum of biological effects such as anti-malarial activity (compound **1** in Fig. 1) [10,11] and DNA active, anti-tumor, and cytotoxic activity (compound **2** in Fig. 1) [12].

Furhtermore, it has been shown that aminoalkyl side chain of acridindione, as a chromophoric group, plays an important role in the biological activity of agents like mitoxantrone and ametantrone (compounds **3** and **4** in Fig. 1, respectively) [13].

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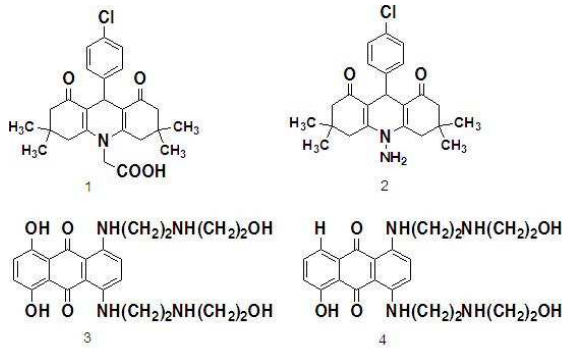


Fig. 1. Some examples of 1,8-acridinedione structure.

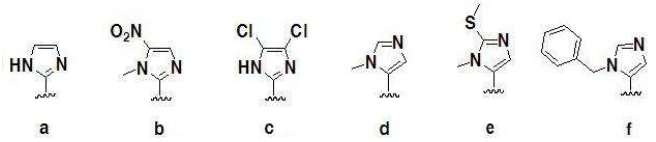


Fig. 2. Structures of the imidazolyl side-chains, (**a**) 1*H*-imidazole-2-yl, (**b**) 1-methyl-5-nitro-1*H*-imidazole-2-yl, (**c**) 4,5-dichloro-1*H*-imidazole-2-yl, (**d**) 1-methyl-1*H*-imidazole-5-yl, (**e**) 1-methyl-2-methyl-thio-1*H*-imidazole-5-yl, (**f**) 1-benzyl-1*H*-imidazole-5-yl substituents.

In addition, the side chains containing 2 or 3 methylenes between amino groups are linked to the highest biological activity observed in antheracenediones [14].

On the basis of the above investigations, synthesis of the "ring closed" version (imidazole) of the aminoalkyl side chain of 1,8-acridinediones was pursued and systematic substitution of the imidazolyl moiety, bearing different electron releasing or attracting groups, was carried out (Fig. 2). Additionally, the calcium channel antagonistic activity of the derivatives was examined due to the known potential Ca^{2+} channel blocking activity of the synthetic ligands.

EXPERIMENTAL

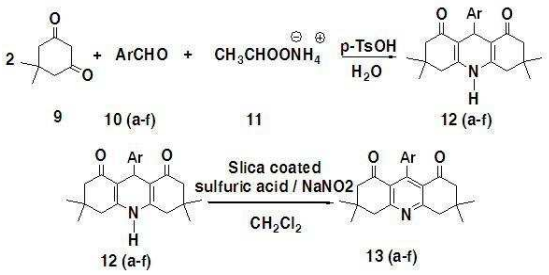
Chemicals and Reagents

Chemical reagents and solvents were purchased from Merck AG or Aldrich Chemical. Column chromatography purifications were performed on Merck silica gel (70-230 mesh). All melting points were determined with a Kofler hot stage apparatus and were uncorrected. IR spectra were recorded on a Nicolet FT-IR Magna 550 spectrophotometer. ^{1}H NMR spectra were measured using a Bruker FT-500 MHz, and chemical shifts are expressed as δ values (ppm) against tetramethylsilane as the internal standard. The mass spectra were run on a Finnigan TSQ-70 spectrometer at 70 ev. The purity of the compounds was confirmed by TLC using different mobile phases. The results of elemental analyses (C, H, N) were within ± 0.4% of the theoretical values for C, H and N. 5,5-Dimethyl-1,3-cyclohexanedione (dimedone), *ρ*-TsOH, 1H-imidazole-2-carbaldehyde (**a**) were obtained from commercial sources. 1-Methyl-2-(methylthio)-1H-imidazole-5-carbadehyde (**e**), 1-methyl-1H-imidazole-5-carbaldehyde (**d**), 1-benzyl-1H-imidazole-5-carbaldehyde (**f**) [15], 4,5-dichloro-1H-imidazole-2-carbaldehyde (c) [16], 1-methyl-5-nitro-1H-imidazole-2-carbaldehyde (b) [17] and their corresponding alcohols were synthesized following the methods previously reported in the literature [18].

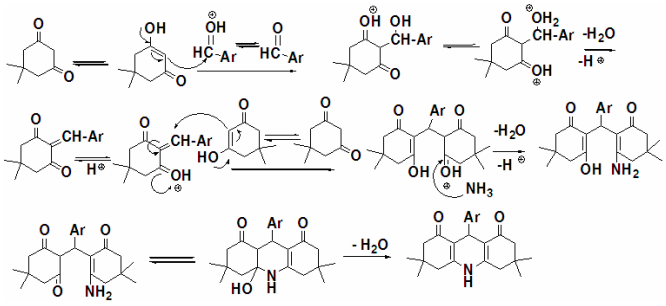
RPMI 1640, fetal bovine serum (FBS), trypsin and phosphate buffered saline (PBS) were purchased from Biosera. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was obtained from Sigma and penicillin/streptomycin was purchased from Invitrogen. Doxorubicin and dimethyl sulphoxide were obtained from EBEWE Pharma and Merck, respectively.

Chemistry

Dimedone and the corresponding aldehyde in aqueous ethanol, furnishing the tetraketone, refluxed subsequently with ammonium acetate in acetic acid [15], or with ammonia in methanol over vapor bath [16] were condensed. Nevertheless, in the case of imidazole aldehydes the method did not afford acceptable yield. Consequently, we investigated a one-pot method which had the advantage of not using organic solvent, refluxing dimedone and the corresponding aldehyde and ammonium acetate in water [17,19,20]. But again, no significant yield was produced. Finally, addition of catalytic amount of *ρ*-toluene sulfonic acid (*ρ*-TsOH) to the refluxing mixture in water [21], afforded an acceptable yield in shorter reaction times. Simple work-up condition, one-pot synthesis, higher yield, shorter reaction times, and most important of all, the "green" synthesis, encouraged us to use and report this



Scheme 1. Reagents and conditions: (a) *ρ*-TsOH (catalytic amount), water, reflux; (b) Silica-coated sulfuric acid, $NaNO_2$, CH_2Cl_2, reflux



Scheme 2. Mechanism proposed for the formation of 3,3,6, 6-tetramethyl-3,4,6,7,9,10-hexahydro-2H,5H-1, 8-acridinediones

facile and simple method of synthesis of imidazole-containing condensed acridinedione systems (Scheme 1).

Unexpectedly, oxidation of the central dihydropyridine ring of the 1,8-acridinedione was troublesome. Several oxidation methods were adopted [22-26]. Difficult work-up leading to low recovery yield and de-alkylation of the dihydropyridine ring (especially with DDQ) proved to be the problems with these methods.

Ultimately, stirring silica coated sulfuric acid with $NaNO_2$ refluxed in CH_2Cl_2 [27] resulted in the oxidized derivative in good yields (35-57%) after a simple filtration and recrystallization (Scheme 1). The desired products of high purity were characterized by ^{1}H NMR, FT-IR and EI-MS experiments. A possible mechanism for the formation of the target compounds is shown in Scheme 2.

General procedure for the synthesis of 9-Substituted-3, 3,6,6-tetramethyl-3,4,6,7,9,10-hexahydro-2H,5H-acridine-1,8-diones (12a-f). To a mixture of dimedone (5,5-dimethyl-1,3-cyclohexanedione) (280 mg, 2 mmol), the corresponding aldehyde (1 mmol), and ammonium acetate (excess) in water (5 ml), and catalytic amount of *ρ*-TsOH was added and the reaction mixture was refluxed for 8-12 h (monitored by TLC) [19]. After the completion of the reaction, the mixture was cooled and poured into crushed ice, the yellow solid precipitated which was then filtered, dried, and recrystalized from ethanol or a mixture of ethanol and chloroform (80:20) or purified through column chromatography using methanol-chloroform (20:80) as the mobile phase to afford the final compounds.

9-(1H-Imidazol-2-yl)-3,3,6,6-tetramethyl-3,4,6,7,9,10-hexahydro-2H,5H-acridine-1,8-dione (12a). This compound was obtained from 1H-imidazole-2-carbaldehyde (**10a**) which was obtained from commercial sources (96.0 mg, 1 mmol), and dimedone following the above general procedure by refluxing for 7 h (72% yield), m.p.: 295-297 ºC, IR (potassium bromide): 3439 (NH), 1648 (C=O) cm^{-1}, ^{1}H NMR (DMSO-d_6) δ: 0.91 and 1.03 (2s, 12H, gem-dimethyl), 2.13-2.33 (2d, 4H, C_4 and C_5-CH_2, J = 16.2 Hz), 2.48-2.62 (2d, 4H, C_2 and C_7-CH_2, J = 16.9 Hz), 4.79 (s, 1H, C_9-H), 7.43 (s, 1H, NH), 7.68-7.72 (dd, 2H, imidazole), 14.29 (s, 1H, imidazole NH), EI-MS *m/z* (%) 339 (M^+, 21), 218 (49), 174 (36), 133 (77), 55 (100).

9-(1-Methyl-5-nitro-1H-imidazol-2-yl)-3,3,6,6-tetra-methyl-3,4,6,7,9,10-hexahydro-2H,5H-acridine-1,8-dione (12b). This compound was obtained from 1-methyl-5-nitro-1H-imidazole-2-carbaldehyde (**10b**) (155.1 mg, 1 mmol) and dimedone following the above general procedure by refluxing for 12 h. (58% yield), m.p.: 300-303 ºC, IR (potassium bromide): 3435 (NH), 1647 (C=O), 1532 and 1379 (NO_2) cm^{-1}, ^{1}H NMR (DMSO-d_6) δ: 0.93 and 1.05 (2s, 12H, gem-dimethyl), 2.15-2.24 (2d, 4H, C_4 and C_5-CH_2, J = 16.2 Hz), 2.39-2.51 (2d, 4H, C_2 and C_7-CH_2, J = 16.8 Hz), 4.20 (s, 3H, N-CH_3), 4.64 (s, 1H, C_9-H), 7.91 (s, 1H, HC_4 imidazole), EI-MS *m/z* (%) 398 (M^+, 79), 381 (19), 315 (29), 272 (29), 160 (29), 82 (43), 68 (100).

9-(4,5-Dichloro-1H-imidazol-2-yl)-3,3,6,6-tetramethyl-3,4,6,7,9,10-hexahydro-2H,5H-acridine-1,8-dione (12c). This compound was obtained from 4,5-dichloro-1H-imidazole-2-carbaldehyde (**10c**) (164.98 mg, 1 mmol) and

dimedone following the above general procedure by refluxing for 4 h. (81% yield), m.p.: 278-280 ºC, IR (potassium bromide): 3433 (NH), 1677 (C=O) cm^{-1}, ^1H NMR (DMSO-d_6) δ: 0.99 and 1.07 (2s, 12H, gem-dimethyl), 2.02-2.20 (2d, 4H, C_4 and C_5-CH_2, J = 16.1 Hz), 2.30-2.44 (2d, 4H, C_2 and C_7-CH_2, J = 17.1 Hz), 4.77 (s, 1H, C_9-H), 9.36 (s, 1H, NH), EI-MS *m/z* (%) 409 (M^++2, 60) 407 (M^+, 100), 342 (73), 307 (49), 305 (18), 272 (15), 835 (15).

9-(1-Methyl-1H-imidazol-5-yl)-3,3,6,6-tetramethyl-3,4,6,7,9,10-hexahydro-2H,5H-acridine-1,8-dione (12d). This compound was obtained from 1-methyl-1H-imidazole-4-carbaldehyde (**10d**) (110.11 mg, 1 mmol) and dimedone following the above general procedure by refluxing for 6.5 h (79% yield), m.p.: 279-281 ºC, IR (potassium bromide): 3421 (NH), 1657 (C=O), 1530 (C=C) cm^{-1}, ^1H NMR (DMSO-d_6) δ: 0.88 and 1.00 (2s, 12H, gem-dimethyl), 2.02-2.10 (2d, 4H, C_4 and C_5-CH_2, J = 16.0 Hz), 2.33-2.48 (2d, 4H, C_2 and C_7-CH_2, J = 16.9 Hz), 3.04 (s, 3H, N-CH_3), 4.58 (s, 1H, C_9-H), 6.33 (s, 1H, imidazole C_3-H), 7.21 (s, 1H, imidazole C_5-H), 9.34 (s, 1H, NH), EI-MS *m/z* (%) 353 (M^+, 100), 268 (49), 226 (14), 143 (14), 98 (14), 82 (79).

9-(1-Methyl-2-methylthio-1H-imidazol-5-yl)-3,3,6,6-tetramethyl-3,4,6,7,9,10-hexahydro-2H,5H-acridine-1,8-dione (12e). This compound was obtained from 1-methyl-2-methylthio-1H-imidazole-4-carbaldehyde (**10e**) (156 mg, 1 mmol) and dimedone following the above general procedure by refluxing for 8 h (50% yield), m.p. > 300 ºC, IR (potassium bromide): 3445 (NH), 1645 (C=O) cm^{-1}, ^1H NMR (DMSO-d_6) δ: 0.93 and 1.05 (2s, 12H, gem-dimethyl), 2.15-2.24 (2d, 4H, C_4 and C_5-CH_2, J = 15.8 Hz), 2.39-2.1 (2d, 4H, C_2 and C_7-CH_2, J = 16.5 Hz), 3.8 (s, 3H, S-CH_3), 4.64 (s, 1H, C_9-H), 6.49 (s, 1H, C_3-H imidazole), 9.5 (s, 1H, NH), EI-MS *m/z* (%) 399 (M^+, 100), 385 (35), 314 (8), 294 (13), 271 (27), 182 (13), 82 (13).

9-(1-Benzyl-1H-imidazol-5-yl)-3,3,6,6-tetramethyl-3,4,6,7,9,10-hexahydro-2H,5H-acridine-1,8-dione (12f). This compound was obtained from 1-benzyl-1H-imidazole-5-carbaldehyde (**10f**) (186.21 mg, 1 mmol) and dimedone following the above general procedure by refluxing for 3.5 h. (82% yield), m.p. > 300 ºC, IR (potassium bromide): 3440 (NH), 1648 (C=O) cm^{-1}, ^1H NMR (DMSO-d_6) δ: 0.92 and 1.03 (2s, 12H, gem-dimethyl), 2.00-2.20 (2d, 4H, C_4 and C_5-CH_2, J = 16.0), 2.27-2.44 (2d, 4H, C_2 and C_7-CH_2, J = 16.7 Hz), 4.83 (s, 1H, C_9-H), 5.42 (s, 2H, benzyl CH_2), 6.45 (s, 1H, imidazole C_4-H), 6.98 (s, 1H, imidazole C_2-H). 7.31-7.43 (m, 5H, Ar-H), 8.28 (s, 1H, NH), EI-MS *m/z* (%) 429 (M^+, 9), 272 (100), 188 (11), 83 (5).

General procedure for synthesis of 9-substituted-3,3,6,6-tetramethyl-3,4,6,7-tetrahydro-2H,5H-acridine-1,8-dione (13a-f). To a solution of 9-substituted-3,3,6,6-tetramethyl-3,4,6,7,9,10-hexahydro-2H,5H-acridine-1,8-dione (1 mmol) in dichloromethane (5 ml) was added silica coated sulfuric acid (1.52 g, 4 mmoles), wet SiO_2 (50% w/w) (0.4 g) and $NaNO_2$ (0.257 g, 4 mmol) were added [16]. The resulting mixture was gradually heated and refluxed for variable time (3-7 h) monitored by TLC. The reaction mixture was filtered and the residue was washed with CH_2Cl_2. Anhydrous Na_2SO_4 (1 g) was added to the filtrate. The resulting mixture was filtered again. Dichloromethane was evaporated and the brownish to red solids were crystallized from methanol to afford the compound.

9-(1H-Imidazol-2-yl)-3,3,6,6-tetramethyl-3,4,6,7-tetrahydro-2H,5H-acridine-1,8-dione (13a). Compound **12a** (339.43 mg) was dissolved in dichloromethane. Following the above general procedure and refluxing for 7 h the desired compound was obtained in 53% yield, m.p. > 300 ºC, IR (potassium bromide): 1698 (C=O) cm^{-1}, ^1H NMR (DMSO-d_6) δ: 1.16 (s, 12H, gem-dimethyl), 2.08 and 2.49 (2s, 8H, C_2, C_4, C_5 and C_7-CH_2), 7.99-8.88 (2d, 2H, imidazole-H), EI-MS *m/z* (%) 337 (M^+, 8), 322 (3), 269 (53), 176 (66), 98 (36), 55 (100).

9-(1-Methyl-5-nitro-1H-imidazol-2-yl)-3,3,6,6-tetramethyl-3,4,6,7-tetrahydro-2H,5H-acridine-1,8-dione (13b). The acridinedione **12b** (397.53 mg, 1 mmol) was dissolved in dichloromethane. Following the above general procedure by refluxing for 11 h, the desired compound was obtained in 66% yield, m.p. > 300 ºC, IR (potassium bromide): 1677 (C=O), 1509 and 1378 (NO_2) cm^{-1}, ^1H NMR (DMSO-d_6) δ: 0.93 & 1.05 (2s, 12H, gem-dimethyl), 2.15 and 2.24 (2s, 8H, C_2, C_4, C_5 and C_7-CH_2), 4.19 (s, 3H, N-CH_3), 7.90 (s, 1H, imidazole-H), EI-MS *m/z* (%) 396 (M^+, 100), 381 (25), 312 (47), 271 (32), 215 (18), 161 (18), 81 (18), 53 (32).

9-(4,5-Dichloro-1H-imidazol-2-yl)-3,3,6,6-tetramethyl-3,4,6,7-tetrahydro-2H,5H-acridine-1,8-dione (13c). Compound **12c** (406.31 mg, 1 mmol) was dissolved in dichloromethane. Following the above general procedure by refluxing for 5 h, the desired compound was obtained in 50% yield, m.p.: 278-280 ºC, IR (potassium bromide): 3421 (NH),

1698 (C=O) cm^{-1}, ^1H NMR (DMSO-d_6) δ: 1.12 (s, 12H, gem-dimethyl), 2.57 and 3.09 (2s, 8H, C_2, C_4, C_5 and C_7-CH_2), 7.3 (s, 1H, imidazole N-H), EI-MS *m/z* (%) 407 (M^++2, 61), 405 (M^+, 100), 349 (65), 306 (53), 238 (11), 189 (11), 154 (11), 83 (11).

9-(1-Methyl-1H-imidazol-5-yl)-3,3,6,6-tetramethyl-3,4, 6,7-tetrahydro-2H,5H-acridine-1,8-dione (13d). Compound **12d** (353.46 mg, 1 mmol) was dissolved in dichloromethane. Following the above general procedure by refluxing for 5.5 h, compound **13d** was obtained in 53% yield, m.p.: 279-281 ºC, IR (potassium bromide): 1723 (C=O) cm^{-1}, ^1H NMR (DMSO-d_6) δ: 0.97 and 1.05 (2s, 12H, gem-dimethyl), 2.48-2.63 (2d, 4H, C_4 and C_5-CH_2, J = 15.9 Hz), 2.50 (s, 3H, CH_3), 3.02-3.09 (2d, 4H, C_2 and C_7-CH_2, J = 16.7 Hz), 7.37 (s, 1H, imidazole C-H), 8.41 (s, 1H, imidazole C-H), EI-MS *m/z* (%) 351 (M^+, 100), 336 (8), 285 (11), 269 (67), 253 (15), 81 (77).

9-(1-Methyl-2-methylthio-1H-imidazol-5-yl)-3,3,6,6-tetramethyl-3,4,6,7-tetrahydro-2H,5H-acridine-1,8-dione (13e). Compound **12e** (406.31 mg, 1 mmol) was dissolved in dichloromethane. Following the above general procedure by refluxing for 5 h compound **13e** was obtained in 75% yield, m.p.: 298-301 ºC, IR (potassium bromide): 1700 (C=O) cm^{-1}, ^1H NMR (DMSO-d_6) δ: 0.98 and 1.05 (2s, 12H, gem-dimethyl), 2.48-2.63 (2d, 4H, C_4 and C_5-CH_2, J = 16.2 Hz), 2.50 (s, 3H, S-CH_3), 3.02-3.09 (2d, 4H, C_2 and C_7-CH_2, J = 16.5 Hz), 3.87 (s, 3H, N-CH_3), 6.41 (s, 1H, imidazole-H), EI-MS *m/z* (%) 397 (M^+, 100), 350 (27), 235 (19), 270 (32), 172 (19), 81 (19).

9-(1-Benzyl-1H-imidazol-5-yl)-3,3,6,6-tetramethyl-3,4,6, 7-tetrahydro-2H,5H-acridine-1,8-dione (13f). Compound **12f** (339.43 mg, 1 mmol) was dissolved in dichloromethane. Following the above general procedure by refluxing for 12 h, compound **13f** was obtained in 73% yield, m.p. > 300 ºC, IR (potassium bromide): 1703 (C=O) cm^{-1}, ^1H NMR (DMSO-d_6) δ: 1.05 and 1.16 (2s, 12H, gem-dimethyl), 2.08 and 2.49 (2s, 8H, C_2, C_4, C_5 and C_7-CH_2), 5.45 (s, 2H, benzyl-CH_2), 7.06-7.14 (m, 5H, phenyl), 7.1 (s, 1H, C_4-H imidazole), 7.6 (s, 1H, C_2-H imidazole), EI-MS *m/z* (%) 427 (M^+, 5), 329 (6), 270 (100), 185 (15), 83 (11).

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 ± 2 ºC, 55 ± 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 ºC in a 20 ml Jackted organ bath containing oxygenated (95% O_2 and 5% CO_2) physiological saline solution of the following compositions: NaCl 137 mM; $CaCl_2$ 1.8 mM; $MgSO_4$ 1.1 mM; $NaHPO_4$ 0.4 mM, $NaHCO_3$ 12 mM, and glucose 5 mM. The muscle was equilibrated for 1 h with a solution changing every 15 min. The concentrations 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 percentage of inhibition of the control. The IC_{50} & IC_{30} values were determined from the concentration-response curves [28-31].

Cytotoxicity

Cell lines and cell culture. HeLa (human cervical adenocarcinoma), LS-180 (human colon cancer), MCF-7 (human breast adenocarcinoma) and Raji (human B lymphoma) 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 µg ml^{-1} streptomycin. Cells were grown in monolayer cultures, except for Raji cells, which were grown in suspension, at 37 °C in humidified air containing 5% CO_2.

Cytotoxicity assay. Cell viability following exposure to synthetic compounds was estimated by using the MTT reduction assay [32,33]. MCF-7 and Raji cells were plated in 96-well micro-plates at a density of 5×10^4 cells/ml (100 µl

per well). LS-180 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 the medium supplemented with different concentrations of synthetic compounds dissolved in DMSO were added in triplicate. Plates with Raji 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^{-1} and plates were incubated for another 4 h at 37 °C. Then formazan crystals were solubilized in 200 µl DMSO. The optical density was measured at 570 nm with background correction at 655 nm using a Bio-Rad micro-plate reader (Model 680). The percentage of viability compared to control wells was calculated for each concentration of the compound and IC_{16} and IC_{50} values were calculated with the software CurveExpert version 1.34 for Windows. Each experiment was repeated 4-5 times [34]. Data are presented as mean ± S.D.

RESULTS AND DISCUSION

Calcium Channel Antagonist Activity

The *in vitro* calcium channel antagonist activities (IC_{50} & IC_{30}) of all the synthesized ligands were determined as the molar concentration of the test compounds required to produce 50% or 30% inhibition of guinea pig ileal longitudinal smooth muscle. Results are summarized in Table 1.

All compounds were inactive on calcium channels or exhibited weak calcium channel antagonist activity (10^{-4} M range) relative to the reference drug Nifedipine ($IC_{50} = 2.95 \times 10^{-8}$ M). As shown in Table 1, the highest activity belongs to compound **13b** which is at least 2 fold stronger than others, while this compound is nearly 10^4 times weaker than Nifedipine as the reference drug. As the Ca^{2+} channel

Table 1. Calcium Channel Antagonist Activity of **12(a-f)** and **13(a-e)** Assessed in the Ileum of Guinea-Pigs

Compound	Ar	Calcium channel antagonist activity			
		IC_{30} (Mean ± SE[a]) (M)	n	IC_{50} (Mean ± SE[a]) (M)	n
12a	1H-Imidazole-2-yl	-[b]	4	-	4
12b	1-Methyl-5-nitro-1H-imidazole-2-yl	-	4	-	4
12c	4,5-Dichloro-1H-imidazole-2-yl	$(2.68 \pm 1.88) \times 10^{-5}$	2	-	4
12d	1-Methyl-1H-imidazole-5-yl	$(2.03 \pm 0.48) \times 10^{-5}$	3	-	4
12f	1-Benzyl-1H-imidazole-5-yl substituents	-	4	-	4
13a	1H-Imidazole-2-yl	$(4.52 \pm 1.64) \times 10^{-5}$	4	-	4
13b	1-Methyl-5-nitro-1H-imidazole-2-yl	$(1.34 \pm 1.18) \times 10^{-5}$	4	$(1.38 \pm 1.16) \times 10^{-4}$	4
13c	4,5-Dichloro-1H-imidazole-2-yl	$(4.09 \pm 1.41) \times 10^{-5}$	3	$(2.37 \pm 1.00) \times 10^{-4}$	3
13d	1-Methyl-1H-imidazole-5-yl	$(4.50 \pm 1.89) \times 10^{-5}$	2	-	4
13e	1-Methyl-2-methylthio-1H-imidazole-5-yl	$(2.42 \pm 1.90) \times 10^{-4}$	3	-	4
Nifedipine		$(5.06 \pm 1.25) \times 10^{-9}$	4	$(2.95 \pm 1.23) \times 10^{-8}$	4

[a]The molar concentration of antagonist test compound causing a 30 and 50% in the tonic contractile response in guinea-pig ileum smooth muscle by KCl (80 mM) was determined graphically from dose-response curve. [b]The blanks represent lack of calcium channel antagonist activity of the compounds and subsequently no IC_{50} or in some cases even no IC_{30} could be determined.

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.

Cytotoxic Activity

The cytotoxic activity of the synthesized compounds was evaluated in 4 human cancer cell lines and the data are demonstrated in Table 2. The lowest activity was observed over LS-180 and Raji cells, since the IC_{50} values of all compounds were higher than 100 μM on these cell line.

Different electron-withdrawing substituents, such as NO_2 and Cl, and electron-donating groups, such as CH_3 and S-CH_3 were introduced to the imidazole ring in position 9 of the 3,3,6,6-tetramethyl-tetra and hexahydro-2H,5H-acridine-1,8-diones core to provide the opportunity to investigate the electronic effect of the substituents on the biological activity.

The introduction of NO_2 to position 5 of the imidazole ring (compounds **12b**, and **13b**) resulted in the best activity against HeLa and MCF-7 cell lines (40.5, 33.2 μM in HeLa cells and 31.7, 46.2 μM in MCF-7 cells, respectively). Compounds **12c** and **13c** (bearing two Cl atoms at positions 4 and 5 of the imidazole ring) also showed good cytotoxicity on MCF-7 cells (54.0 and 47.6 μM), respectively. Replacement of the electron-attracting groups with N-CH_3 (in **12d** and **13d**) or N-CH_3 and S-CH_3 (in **12e** and **13e)** significantly decreased the cytotoxic potency in all cell lines. (With the exception of **12e** on HeLa cells, IC_{50} = 34.2 μM). N-Benzyl imidazolyl substitution (**12f** and **13f**) revealed an increase in the cytotoxic activity against HeLa and MCF-7 cells (57.6 and 49.3 μM in HeLa cells and 54.8 and 52.0 μM on MCF-7 cells, respectively). It can be deduced from the data that the introduction of electron-attracting groups such as NO_2 and Cl on the imidazole ring at position 9 of the 1,8-acridinediones can significantly improve the cytotoxic potency against HeLa and MCF-7 cancer cell lines.

In parallel, the addition of flexible and planar substituents such as benzyl group on the imidazole ring may spatially orient the molecule to a conformation more favorable for cytotoxic activity. Electron-donating substitution on the imidazole ring is not suggested as it clearly diminished the cytotoxic potency according to our experiments.

Table 2. Cytotoxic Activity of the Synthetic Compounds Assessed by the MTT Reduction Assay

Compound	HeLa cells		LS-180 cells		MCF-7 cells		Raji cells	
	IC_{16} (μM)	IC_{50} (μM)	IC_{16} (μM)	IC_{50} (μM)	IC_{16} (μM)	IC_{50} (μM)	IC_{16} (μM)	IC_{50} (μM)
12a	> 10	> 10	> 10	> 10	> 10	> 10	> 10	> 10
13a	17.6 ± 2.9	85.9 ± 23.2	> 100	> 100	33.6 ± 42.6	> 100	35.3 ± 14.5	> 100
12b	21.5 ± 16.8	40.5 ± 12.3	> 25	> 25	5.0 ± 2.7	31.7 ± 10.9	> 25	> 25
13b	8.0 ± 1.0	33.2 ± 17.7	> 25	> 25	7.3 ± 5.6	46.2 ± 20.0	> 25	> 25
12c	23.1 ± 7.1	> 100	> 100	> 100	10.5 ± 3.3	54.0 ± 24.6	16.2 ± 5.5	> 100
13c	25.9 ± 2.1	> 100	> 100	> 100	8.0 ± 8.6	47.6 ± 7.6	85.0 ± 37.6	> 100
12d	37.5 ± 14.1	> 100	> 100	> 100	10.6 ± 11.5	> 100	> 100	> 100
13d	53.2 ± 6.5	> 100	> 100	> 100	55.7 ± 7.8	> 100	> 100	> 100
12e	8.6 ± 2.6	34.2 ± 12.1	> 25	> 25	16.9 ± 23.0	> 25	18.0 ± 4.8	> 25
13e	> 100	> 100	> 100	> 100	82.3 ± 18.7	> 100	> 100	> 100
12f	19.1 ± 3.2	57.6 ± 0.6	> 100	> 100	10.0 ± 2.2	54.8 ± 19.5	26.1 ± 10.3	> 100
13f	65.6 ± 6.6	49.3 ± 11.7	> 100	> 100	15.7 ± 5.0	52.0 ± 11.5	> 100	> 100
Doxorubicin	0.043 ± 0.015	0.187 ± 0.028	0.026± 0.005	0.230 ±0.031	0.044 ± 0.042	0.205 ± 0.103	0.041 ± 0.008	0.144 ± 0.007

*Values represent the mean ± S.D. of at least 3 different experiments. The maximum concentration of the compound tested for cytotoxicity was 10 μM for compounds **12a**, while compounds **13b**, **12e** and **12b** were tested at 25 μM. The rest of the compounds were tested at the maximum final concentration of 100 μM.

CONCLUSIONS

Synthesis of a series of novel imidazolyl derivatives of fully and partially hydrogenated 1,8-acridinediones bearing different substituents on the imidazole ring has been reported. The ligands were assessed for their cytotoxic activity on human cancer cell lines. Some of the derivatives were identified as cytotoxic agents with moderate-to-good cytotoxic potencies with no or a very low calcium channel antagonist activity, an undesired side effect when used as anti-tumor agents. It was found that decreasing the electron density of the imidazole ring by the introduction of an electron-withdrawing substituent (such as NO_2 or Cl) improved the cytotoxicity of the ligand. It was also shown that the introduction of a flexible planar substituent such as benzyl on the imidazole ring significantly increased the pharmacological activity of the ligand, probably due to the induction of a desired conformation for pharmacological effect. In summary, the chemical template of 1,8-acridinedione attached to an imidazole ring seems to have promising pharmacological effects and further investigations on this chemical structure can potentially lead to the discovery of potent cytotoxic agents with low calcium channel blocking activities.

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