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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Investigation of myorelaxant activity of 9-aryl-3,4,6,7-tetrahydroacridine-1,8-(2H,5H,9H,10H)-diones in isolated rabbit gastric fundus

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Abstract In this study, twelve compounds having 9-aryl-3,4,6,7-tetrahydroacridine-1,8-(2H,5H,9H,10H)-dione structure were synthesized by reaction of 5-methyl-1,3-cyclohexanedione, the appropriate aromatic aldehydes, and ammonium acetate in methanol. The structures of the compounds were elucidated by infrared, ^{1}H- and ^{13}C-nuclear magnetic resonance spectroscopy (-NMR), mass spectroscopy, and elemental analysis. The maximum relaxant effects (E_{max}) and pD2 values of the compounds **3a–l** and pinacidil were tested on isolated strips of rabbit gastric fundus smooth muscle.

Keywords Myorelaxant activity · Acridinedione · Pinacidil

Introduction

Ion channels are very important for cell function and responsible for physiological effects. Potassium ion channels regulate some functions in both excitable and non-excitable cells. With respect to the effects of potassium ion channels on autonomic nerve functions, different research groups have recently shown that big conductance Ca^{2+}-activated potassium ion channels operate in the release of acetylcholine from vagal nerve terminals (Tagaya *et al*., 1998). The properties of KATP channels in guinea pig gastric myocytes were similar to those of KATP channels in other smooth muscles (Sim *et al*., 2002).

Potassium channel opening is a physiological mechanism by which excitable cells exploit to maintain or restore their resting state (Grissmer, 1997; Jaggar *et al*., 1998; Klöckner and Trieschmann, 1989; Lawson, 2000; Firth *et al*., 2000; Loussouarn *et al*., 2001; Robertson and Steinberg, 1990). Potassium ion channels are now classified into ten types according to their electrophysiological and pharmacological properties (Sanguinetti and Spector, 1997). Several types of potassium ion channels including voltage-activated potassium ion channels (Kv channel), calcium-activated potassium ion channels (KCa channel) and adenosine 5′-triphosphate (ATP)-sensitive potassium ion channels (KATP channel) are found in central and peripheral nervous system (Roeper and Pongs, 1996; Aronson, 1992). Potassium ion channels play an important role in a number of different aspects of the electrical responses of the nervous system (Nicoll *et al*., 1990; Cook, 1988; Mathie *et al*., 1998). For example, potassium ion channel opener activity is involved in setting the membrane resting potential, determining the frequency of action-potentials and the shape of the action-potential wave forms. Most of these channels permit the potassium ion efflux from the neurons, thereby tending to oppose depolarization or to use repolarization or hyperpolarization and resulting in a decrease of neurotransmitter release (Nakamura *et al*., 2004).

It was shown that these KATP channels play critical roles in modulating physiological processes such as insulin secretion, leptin release, synaptic transmission, and excitability of cardiac, vascular, and nonvascular smooth muscle (Davis-Taber *et al*., 2003).

KATP channel openers are a structurally diverse group of drugs with a broad spectrum of potential therapeutic

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applications. These drugs interact with KATP channels in numerous tissues and increase their activity, thereby hyperpolarizing the plasma membrane and reducing electrical excitability (Ashcroft and Gribble, 2000). Potassium channel openers might be useful as therapeutic agents in the treatment of various diseases such as hypertension, asthma, peripheral vascular disease, right heart failure, congestive heart failure, angina, ischemic heart disease, cerebrovascular disease, glaucoma, renal cholic, disorders associated with kidney stones, overactive bladder, irritable bowel syndrome, male pattern baldness, premature labor, and peptic ulcers (Ohnmacht *et al*., 1995; Carroll *et al*., 2004a, b). Calcium channel blockers having 1,4-dihydropyridine (DHP) structure which nifedipine is the prototype and their condensed analogs such as bicyclo (quinoline) and tricyclo (acridine) have vasodilator and antihypertensive effects (Özturk *et al*., 2008; Simsek *et al*., 2008, 2004; Saraç *et al*., 2002; Berkan *et al*., 2002; Carroll *et al*., 2004a, b; Ashworth *et al*., 2002). Some DHP derivatives have also potassium channel opener activity (Klöckner and Trieschmann, 1989; Davis-Taber, *et al*., 2003; Grissmer and Cahalan, 1989; Mannhold, 2004; Gopalakrishnan *et al*., 2003). Frank and coworkers synthesized 9-(3-nitrophenyl)-3,4,6,7-tetrahydroacridine-1,8-(2*H*,5*H*,9*H*,10*H*)-dione and showed its potassium channel opener activity (Frank *et al*. 1993). It has been shown that imidazolylacridinedione derivatives possess potassium channel modulatory activity (Hadizadeh and Mehri, 2006). In addition, we synthesized some compounds having similar structure in our previous work (Gündüz *et al*., 2009). Although those compounds have disubstituted phenyl ring in 3,4,6,7-tetrahydroacridine-1,8-dione structure, monosubstituted analogs of them were synthesized in this study. In this study, we aimed to synthesize twelve derivatives and investigate their effects on isolated strips of rabbit gastric fundus smooth muscle. Compounds **3a** and **3c** were synthesized earlier by Bossert and Vater (1971). Since there was no data about potassium channel modulatory activity of these two compounds, we also synthesized them in order to investigate their activities in this study. Therefore, we planned to synthesize new tricyclic analog of 1,4-DHP to explain the contribution of the position of electron withdrawing substituents on phenyl ring of acridine and to the methyl groups substituted to acridine ring to the mentioned activity.

Materials and methods

Chemistry

All chemicals used in this study were purchased from Sigma-Aldrich. Melting points were determined by using a Thomas Hoover capillary melting point apparatus (Philadelphia, PA, USA); the values are uncorrected. Infrared (IR) spectra were recorded by using a Perkin Elmer Fourier-transform FT–IR Systems Spectrum BX. ^{1}H-nuclear magnetic resonance (^{1}H-NMR) and ^{13}C-nuclear magnetic resonance (^{13}C-NMR) spectra were recorded by using a Varian Mercury 400, 400 MHz high-performance digital FT–NMR spectrometer (methanol-d4; tetramethylsilane as internal standard). Chemical shift values are given as ppm. Mass spectra (MS) were recorded by using a Waters 2996 photodiode array detector. Thin layer chromatography was run on aluminum sheets Merck silica gel 60 F254, and short wavelength ultraviolet (UV) light (254 nm) was used to detect the UV absorption spots. Elemental analysis was carried out by using a Leco CHNS-932 elemental analyzer. The elemental analysis results were within ±0.4% of the theoretical values.

*General procedure for 9-aryl-3,4,6,7-tetrahydroacridine-1,8-(2H,5H,9H,10H)-diones (**3a–3l**)*

The mixture of 5-methyl-1,3-cyclohexanedione (0.252 g, 0.002 mol) and appropriate aromatic aldehyde (0.001 mol) were refluxed in methanol (20 ml) in 65°C in the presence of ammonium acetate (0.385 g, 0.005 mol) for 4 h. Then the forming crystals were filtered off and crystallized from alcohol.

*3,6-Dimethyl-9-(2-nitrophenyl)-3,4,6,7-tetrahydroacridine-1,8-(2H,5H,9H,10H)-dione (**3a**)* M.p. > 300°C. Yield: 0.278 g (76%). IR (cm^{-1}): 3302, 1616. ^{1}H-NMR δ 0.93, 0.97 (3H; d; J: 6.0 Hz, 3-CH_3, 6-CH3), 1.85–2.50 (10H; m; H-2,3,4,5,6,7), 5.55 (1H; s; 9-H), 7.24–7.76 (4H; m; Ar–H), 9.40 (1H; s; NH). ^{13}C-NMR δ 20.4, 20.7, 27.9, 28.3, 33.1, 33.9, 38.2, 49.6, 50.0, 105.8, 106.2, 124.6, 126.3, 127.8, 131.5, 140.5, 146.8, 153.3, 153.6, 196.5, 196.7. MS (*m/z*) 366, 365, 320, 244. Analysis for $C_{21}H_{22}N_2O_4$: calculated C: 68.84, H: 6.05, N: 7.65; found C: 68.52, H: 5.99, N: 7.48.

*3,6-Dimethyl-9-(3-nitrophenyl)-3,4,6,7-tetrahydroacridine-1,8-(2H,5H,9H,10H)-dione (**3b**)* M.p. 274°C Yield: 0.292 g (80%). IR (cm^{-1}): 3319, 1613. ^{1}H-NMR δ 0.93, 0.97 (3H; d; J: 6.2 Hz, 3-CH3, 6-CH3), 1.99–2.56 (10H; m; H-2,3,4,5,6,7), 5.00 (1H; s; 9-H), 7.46-8.03 (4H; m; Ar–H), 9.52 (1H; s; NH). ^{13}C-NMR δ 19.7, 20.1, 27.4, 27.8, 32.3, 32.8, 39.2, 52.2, 52.9, 104.8, 105.4, 126.3, 127.2, 128.4, 132.6, 142.6, 147.2, 151.0, 151.8, 195.1, 195.9. MS (*m/z*) 366, 365, 320, 244. Analysis for $C_{21}H_{22}N_2O_4$: calculated C: 68.84, H: 6.05, N: 7.65; found C: 69.10, H: 6.01, N: 7.56.

*3,6-Dimethyl-9-(4-nitrophenyl)-3,4,6,7-tetrahydroacridine-1,8-(2H,5H,9H,10H)-dione (**3c**)* M.p. 186°C. Yield: 0.314 g

(86%). IR (cm^{-1}): 3298, 1611. ^{1}H-NMR δ 0.93, 0.95 (3H; d; J: 6.1 Hz, 3-CH3, 6-CH3), 1.94–2.55 (10H; m; H-2,3,4,5,6,7), 4.95 (1H; s; 9-H), 7.38 (2H; d; J: 8.60 Hz, Ar–H2, Ar–H6), 8.17 (2H; d; J: 8.60 Hz, Ar–H3, Ar–H5), 9.52 (1H; s; N–H). ^{13}C-NMR δ 21.2, 21.8, 28.7, 29.0, 32.5, 32.7, 39.9, 50.6, 50.8, 106.2, 106.7, 125.8, 127.4, 129.4, 132.8, 142.4, 147.1, 150.2, 150.8, 194.3, 194.6. MS (*m/z*) 366, 365, 320, 244. Analysis for $C_{21}H_{22}N_2O_4$: calculated C: 68.84, H:6.05, N: 7.65; found C: 68.55, H: 5.97, N: 7.52.

3,6-Dimethyl-9-(2-fluorophenyl)-3,4,6,7-tetrahydroacridine-1,8-(2H,5H,9H,10H)-dione ***(3d)*** M.p. > 300°C. Yield: 0.261 g (77%). IR (cm^{-1}): 3275, 1608. ^{1}H-NMR δ 0.93, 0.97 (3H; d; J: 6.1 Hz, 3-CH3, 6-CH3), 1.91–2.56 (10H; m; H-2,3,4,5,6,7), 4.97 (1H; s; 9H), 6.88–7.62 (4H; m; Ar–H), 9.42 (1H; s; N–H) ^{13}C-NMR δ 20.3, 20.8, 28.2, 28.9, 33.2, 33.8, 40.2, 49.5, 50.2, 99.8, 100.2, 115.6, 124.3, 126.7, 131.5, 143.8, 153.1, 153.6, 160.8, 195.9, 196.1. MS (*m/z*) 339, 338, 320, 244. Analysis for $C_{21}H_{22}FNO_2$: calculated C: 74.31, H: 6.53, N: 4.13; found C: 74.04, H: 6.46, N: 3.87.

3,6-Dimethyl-9-(3-fluorophenyl)-3,4,6,7-tetrahydroacridine-1,8-(2H,5H,9H,10H)-dione ***(3e)*** M.p. > 300°C. Yield: 0.288 g (85%). IR (cm^{-1}): 3284, 1611. ^{1}H-NMR δ 0.93, 0.97 (3H; d; J: 6.0 Hz, 3-CH3, 6-CH3), 1.97–2.56 (10H; m; H-2,3,4,5,6,7), 4.91 (1H; s; 9-H), 6.89–7.71 (4H; m; Ar–H), 9.49 (1H; s; NH). ^{13}C-NMR δ 21.2, 21.7, 27.9, 28.1, 32.9, 33.5, 41.3, 50.1, 50.6, 100.8, 101.2, 118.9, 125.4,126.8, 133.4, 141.7, 150.2, 150.8, 159.3, 196.4, 196.6. MS (m/z) 339, 338, 320, 244. Analysis for-$C_{21}H_{22}FNO_2$: calculated C: 74.31, H: 6.53, N: 4.13; found C: 73.93, H: 6.27, N: 4.05.

3,6-Dimethyl-9-(4-fluorophenyl)-3,4,6,7-tetrahydroacridine-1,8-(2H,5H,9H,10H)-dione ***(3f)*** M.p. 279°C. Yield: 0.298 g (88%). IR (cm^{-1}): 3306, 1618. ^{1}H-NMR δ 0.94, 0.99 (3H; d; J: 6.1 Hz, 3-CH3, 6-CH3), 1.92–2.55 (10H; m; H-2,3,4,5, 6,7), 5.01 (1H; s; 9-H), 7.02 (2H; d; J: 8.72 Hz, Ar–H2, Ar–H6), 7.35 (2H; d; J: 8.72 Hz, Ar–H3, Ar–H5), 9.60 (1H; s; N–H). ^{13}C-NMR δ 19.5, 20.0, 26.3, 26.8, 31.8, 32.1, 42.5, 51.2, 51.8, 102.3, 102.9, 121.6, 126.8, 128.2, 132.6, 139.8, 150.4, 150.6, 156.7, 196.0, 196.4. MS (*m/z*) 339, 338, 320, 244. Analysis for $C_{21}H_{22}FNO_2$: calcu lated C:74.31, H: 6.53, N: 4.13; found C: 74.23, H: 6.46, N: 4.08.

3,6-Dimethyl-9-(2-trifluoromethylphenyl)-3,4,6,7-tetrahydroacridine-1,8-(2H,5H,9H,10H)-dione ***(3g)*** M.p. > 300°C. Yield: 0.280 g (72%). IR (cm^{-1}): 3303, 1610. ^{1}H-NMR δ 0.92, 0.97 (3H; d; J: 6.4 Hz, 3-CH3, 6-CH3), 1.81–2.50 (10H; m; H-2,3,4,5,6,7), 5.35 (1H; s; 9-H), 7.04–7.58 (4H; m; Ar–H), 9.35 (1H; s; NH). ^{13}C-NMR δ 19.4, 19.8, 28.9, 29.2, 33.9, 34.2, 40.1, 51.2, 51.9, 105.8, 106.3, 121.3, 124.2, 125.7, 128.2, 132.7, 134.5, 146.8, 153.2, 153.7, 196.4, 196.8 MS (*m/z*) 389, 388, 320, 244. Analysis for $C_{22}H_{22}F_3NO_2$: calculated C: 67.86, H: 5.69, N: 3.60; found C: 67.55, H: 5.54, N: 3.48.

3,6-Dimethyl-9-(3-trifluoromethylphenyl)-3,4,6,7-tetrahydroacridine-1,8-(2H,5H,9H,10H)-dione ***(3h)*** M.p. 287°C. Yield: 0.311 g (80%). IR (cm^{-1}): 3298, 1612. ^{1}H-NMR δ 0.93, 0.96 (3H; d; J: 6.5 Hz, 3-CH3, 6-CH3), 1.95–2.56 (10H; m; H-2,3,4,5,6,7), 4.90 (1H; s; 9-H), 7.36–7.96 (4H; m; Ar–H), 9.47 (1H; s; NH). ^{13}C-NMR δ 20.3, 20.7, 27.8, 28.0, 35.2, 35.7, 42.3, 52.2, 52.7, 109.3, 109.5, 122.4, 125.8, 127.2, 129.0, 132.5, 134.7, 139.8, 150.1, 150.7, 194.2, 194.9. MS (*m/z*) 389, 388, 320, 244. Analysis for $C_{22}H_{22}F_3NO_2$: calculated C: 67.86, H: 5.69, N: 3.60; found C: 67.45, H: 5.43, N: 3.52.

3,6-Dimethyl-9-(4-trifluoromethylphenyl)-3,4,6,7-tetrahydroacridine-1,8(2H,5H,9H,10H)-dione ***(3i)*** M.p. 128°C. Yield: 0.346 g (89%). IR (cm^{-1}): 3295, 1608. ^{1}H-NMR δ 0.93, 0.96 (3H; d; J: 6.5 Hz, 3-CH3, 6-CH3), 1.96–2.53 (10H; m; H-2,3,4,5,6,7), 4.91 (1H; s; 9-H), 7.35 (2H; d; J: 8.32 Hz, Ar–H2, Ar–H6), 7.71 (2H; d; J: 8.32 Hz, Ar–H3, Ar–H5), 9.46 (1H; s; N–H). ^{13}C-NMR δ 21.1, 21.8, 28.3, 28.9, 31.9, 32.3, 43.6, 50.4, 50.8, 104.3, 104.7, 122.6, 125.8, 128.4, 129.2, 135.7, 136.8, 140.4, 150.2,150.9, 193.5, 193.9. MS (*m/z*) 389, 388, 320, 244. Analysis for $C_{22}H_{22}F_3NO_2$: calculated C: 67.86, H: 5.69, N: 3.60; found C: 67.55, H: 5.50, N: 3.49.

9-(2-Cyanophenyl)-3,6-dimethyl-3,4,6,7-tetrahydroacridine-1,8-(2H,5H,9H,10H)-dione ***(3j)*** M.p. > 300°C. Yield: 0.262 g (76%). IR (cm^{-1}): 3288, 1615. ^{1}H-NMR δ 0.94, 0,97 (3H; d; J: 6.4 Hz, 3 CH3, 6-CH3), 1.87–2.32 (10H; m; H-2,3,4,5,6,7), 5.04 (1H; s; 9-H), 7.18–7.96 (4H; m; Ar–H), 9.59 (1H; s; NH). ^{13}C-NMR δ 20.2, 20.7, 28.1, 28.6, 33.9, 34.4, 37.9, 49.6, 50.1, 98.7, 99.2, 107.8, 112.9, 126.6, 128.2, 132.5, 134.8, 143.1, 153.1, 153.6, 196.0, 196.4. MS (*m/z*) 346, 345, 244. Analysis for $C_{22}H_{22}N_2O_2$: calculated C: 76.28, H: 6.40, N: 8.09; found C: 76.51, H: 6.00, N: 8.03.

9-(3-Cyanophenyl)-3,6-dimethyl-3,4,6,7-tetrahydroacridine-1,8-(2H,5H,9H,10H)-dione ***(3k)*** M.p. 277°C. Yield: 0.276 g (80%). IR (cm^{-1}): 3290, 1614. ^{1}H-NMR δ 0.92, 0.97 (3H; d; J: 6.2 Hz, 3-CH3, 6-CH3), 1.94–2.55 (10H; m; H-2,3,4,5,6,7), 4.84 (1H; s; 9-H), 7.35–7.52 (4H; m; Ar–H), 9.50 (1H; s; NH). ^{13}C-NMR δ 21.3, 21.8, 27.4, 27.9, 32.8, 33.5, 38.1, 50.6, 51.2, 99.7, 100.3, 108.3, 113.6,125.8, 128.9, 133.5, 135.7, 143.8, 152.2, 152.8, 195.2, 195.9. MS (m/z)

346, 345, 244. Analysis for $C_{22}H_{22}N_2O_2$: calculated C: 76.28, H: 6.40, N: 8.09; found C: 75.90, H: 6.27, N: 7.78.

*9-(4-Cyanophenyl)-3,6-dimethyl-3,4,6,7-tetrahydroacridine-1,8-(2H,5H,9H,10H)-dione (**3l**)* M.p. 261°C. Yield: 0.290 g (84%). IR (cm^{-1}): 3284, 1618. ^{1}H-NMR δ 0.93, 0.97 (3H; d; J: 6.1 Hz, 3-CH3, 6-CH3), 1.91–2.50 (10H; m; H-2,3,4,5,6,7), 5.03 (1H; s; 9-H), 7.43 (2H; d; J: 8.80 Hz, Ar–H2, Ar–H6), 7.64 (2H; d; J: 8.80 Hz, Ar–H3, Ar–H5), 9.54 (1H; s; N–H). ^{13}C-NMR δ 210.4, 20.9, 26.6, 26.9, 33.2, 34.1, 38.4, 51.3, 51.6, 100.6, 100.9, 109.0, 112.7, 126.1, 129.4, 138.1, 139.3, 142.5, 151.1, 151.4, 195.3, 195.7. MS (*m/z*) 346, 345, 244. Analysis for $C_{22}H_{22}N_2O_2$: calculated C: 76.28, H: 6.40, N: 8.09; found C: 76.08, H: 6.11, N: 7.96.

Pharmacology

New Zealand white rabbits (weighing 2.5–3 kg.) were used in this study. The study was approved by the Ethics Committee at Gazi University, Faculty of Medicine. Rabbits were killed with i.v. injection of sodium pentobarbital (30–40 mg/kg, i.v.) then stomachs were removed by abdominal incision. The fundal part of the stomach was dissected parallel to the longitudinal muscle wall. One muscle strip which is approximately 15–20 mm long and 2 mm wide was obtained and allowed to equilibrate for a period of 60 min in 20 ml organ baths containing normal Krebs'–Henseleit solution (KHS). The composition of the Krebs' solution was as follows (in mmol/l): NaCl (118); KCl (4.7); $CaCl_2$ (1.26); $NaHCO_3$ (25); $MgCl_2$ (0.54); $NaHPO_4$ (0.9); glucose (10.04). The solution was gassed with 95% O_2 and 5% CO_2 and temperature was maintained at 37°C by a thermoregulated water circuit during the study. The pH of the saturated solution was 7.4. Each strip was connected to a force transducer (FDT 10-A, May IOBS 99, COMMAT Iletisim Co., Ankara, Turkey) for the measurement of isometric force. It was displaced continuously and recorded on online computer via four-channel transducer data acquisition system (MP30B-CE, BIOPAC Systems Inc., Santa Barbara, CA using software (BSL PRO v 3.6.7, BIOPAC Systems Inc.). After mounting, each strip was allowed to equilibrate with a basal tension of 1 g for 60 min. KHS was replaced with fresh solution every 15 min. *N*-(γ)-nitro-L-arginine methyl ester (L-NAME) hydrochloride (the nitric oxide synthase inhibitor, 10^{-4} M), indomethacin (COX inhibitor, 10^{-5} M), and guanethidine (adrenergic blocker, 10^{-6} M) were added into the organ bath 20 min before the precontraction in order to eliminate the effects of nitric oxide, prostaglandins, and adrenergic agonists. These contribute to the gastric fundus smooth muscle relaxation induced by compounds and pinacidil. Rabbit fundus smooth muscle strips were precontracted with submaximal concentration of noradrenaline (10^{-5} M). Concentration–relaxation for compounds and pinacidil were obtained by adding into the bath in cumulative manner. Dimethylsulfoxide (DMSO) was also tested in noradrenaline precontracted rings. Cumulative concentration–response curve was constructed in a stepwise manner after the response to previous concentration had reached a plateau. The same experimental procedure was performed in presence of glibenclamide (KATP channel blocker, 10^{-6} M), tetraethylammonium (TEA) (Ca^{2+}-activated K^+ channel blocker, 10^{-4} M), 4-aminopyridine (4AP) (KV blocker, 10^{-4} M), apamine (a selective blocker of small conductance Ca^{2+}-activated potassium ion channels, 10^{-6} M), iberiotoxin (large-conductance Ca^{2+}-activated potassium ion channels-blocker, 10^{-8} M).

Drugs

L-NAME, indomethacin, guanethidine, noradrenaline, glibenclamide, TEA, 4AP, apamine, iberiotoxin, pinacidil, and DMSO were supplied by Sigma. Stock solutions of L-NAME, guanethidine, TEA, 4-AP, iberiotoxin, and noradrenaline were prepared in distilled water. Compounds, pinacidil, glibenclamide, apamine, and indomethacin were dissolved in DMSO.

Data analysis

The relaxant effects of the compounds and pinacidil on the tissues precontracted with noradrenaline were expressed as percentage of the obtained precontraction by using noradrenaline. To evaluate the effects of the compounds, the maximum response (E_{max}) and p*D*2 values [the negative logarithm of the concentration for the half-maximal response (EC50)] were calculated according to *Scatchard* equation for drug–receptor interaction. While E_{max} is the parameter for efficacy, pD2 is the parameter for potency. Agonist p*D*2 values (apparent agonist affinity constants) were calculated from each agonist concentration–response curve by linear regression of the linear part of the curve. This value is taken as a measure of the sensitivity of the tissues to each agonist. All data are expressed as mean ± standard error.

Statistical analysis

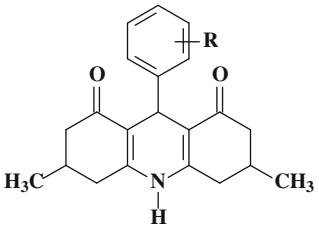
Statistical comparison between groups was performed using general linear models by *Scheffe's F* test and *P* values of less than 0.05 were considered to be statistically significant.

Results and discussion

In this study, acridinedione derivatives were obtained by the reaction of 5-methyl-1,3-cyclohexanedione (**1**) with the appropriate aromatic aldehydes (**2**) (Scheme 1). The structures of the compounds were elucidated by IR, ^{1}H-NMR, ^{13}C-NMR, mass spectra, and elemental analysis. In the IR spectra, N–H and C=O stretching bands were seen at the expected values. In the ^{1}H-NMR spectra, protons in three and six positions of the acridinedione ring were seen about 0.92–0.97 ppm as doublet. The peaks belonging to the protons of aromatic ring and alkyl groups were seen at the expected chemical shift values. The N–H signal of the compounds was observed at about 9.35–9.59 ppm. The ^{13}C-NMR spectra of the compounds displayed the appropriate number of resonances that exactly fitted the number of carbon atoms. The mass spectra of the compounds were recorded using the electrospray ionization technique. Molecular ion peaks were seen for all compounds. In further fragmentation, the peaks formed by cleavage of the aryl ring from the parent molecule. These findings are in accordance with the literature (Mathie *et al*., 1998). In addition, the structure of the compounds was confirmed by elemental analysis.

The maximum relaxant effects (E_{max}) and pD2 values of the compounds **3a–l** and pinacidil on isolated strips of rabbit gastric fundus smooth muscle are given in Table 1. The values indicate that compounds **3b**, **3c**, **3f**, **3i**, **3k**, **3l**, and pinacidil produced concentration-dependent relaxation in rabbit gastric fundus smooth muscle strips. Compounds **3a**, **3d**, **3g**, and **3h** did not show any relaxation effect. DMSO had no significant relaxant effect. Some compounds and pinacidil exerted concentration-dependent relaxation responses precontracted with submaximal concentration of noradrenaline in the gastric fundus smooth muscle strips with efficacy order: pinacidil $\geq$ **3b > 3k** $\geq$ **3c > 3l > 3f** $\geq$ **3i.** Compound **3b** has similar relaxation activity with pinacidil. Since compounds **3e** and **3j** did not dissolve in distilled water or DMSO, their relaxant responses could not be tested. To investigate whether relaxation induced by test compounds was due to interaction with the cyclooxygenase,

Table 1 Maximum relaxant responses(E_{max}) and pD2 values of the compounds and pinacidil on isolated strips of rabbit gastric fundus smooth muscle

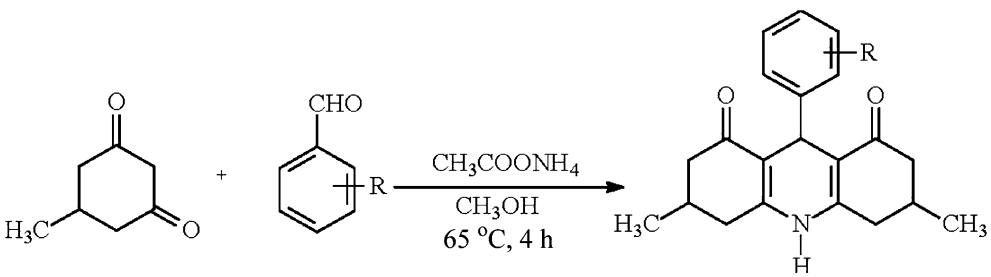


Compounds	R	E_{max}	pD_2
3a	2-Nitro	No effect	No effect
3b*	3-Nitro	62.08 ± 5.81	4.57 ± 0.35
3c*	4-Nitro	53.93 ± 7.49	4.66 ± 0.42
3d	2-Fluoro	No effect	No effect
3e[a]	3-Fluoro	–	–
3f	4-Fluoro	37.23 ± 9.23	4.60 ± 0.24
3g	2-Trifluoromethyl	No effect	No effect
3h	3-Trifluoromethyl	No effect	No effect
3i*	4-Trifluoromethyl	33.76 ± 8.54	4.68 ± 0.51
3j[a]	2-Cyano	–	–
3k*	3-Cyano	56.02 ± 7.5	4.70 ± 0.29
3l*	4-Cyano	45.99 ± 4.29	4.57 ± 0.33
Pinacidil	–	73.55 ± 6.11	4.90 ± 0.15

Relaxant responses are expressed as a percentage of the precontraction induced by noradrenaline. The negative logarithm of the concentration for the half-maximal response (pD2) value represent mean value ± SEM

* $P < 0.05$, compared with control responses ($n = 6$)

[a] Since it did not dissolve in distilled water or DMSO, their relaxant responses could not be tested



1 **2** **3a-l**

R: NO2, F, CF3, CN

Scheme 1 Synthesis of compounds **3a–l**

adrenergic or nitric oxide pathways, tissues were pretreated with indomethacin (COX inhibitor), guanethidine (adrenergic blocker) or L-NAME hydrochloride (the nitric oxide synthase inhibitor), respectively. Pretreatment of the strips with indomethacin, guanethidine and L-NAME did not significantly alter the relaxant responses of the compounds. These findings indicate that cyclooxygenase, adrenergic and nitric oxide (NO) pathways do not play a role in relaxations evoked by these substances. When the E_{max} values were investigated, it was seen that the compounds having substituent in 2 position of the phenyl ring do not possess any response. The compounds having nitrophenyl (**3a–c)** and cyanophenyl groups) (**3j–l)** showed similar activity pattern. Among these two groups, 3-substituted derivatives show higher activity than their 2-substituted analogs. The pD2 and E_{max} values of the compounds **3b**, **3c**, **3f**, **3i**, **3k**, and **3l** are closer to pinacidil. The results obtained in the presence of potassium channel blockers (TEA, glibenclamid, 4AP, apamine, and iberitoxin) are in accordance with the E_{max} value of the compounds compared with pinacidil (Table 2). The relaxant response of **3b** was significantly inhibited by apamine. Compound **3c** induced relaxation response was altered by either TEA or iberiotoxin. The enhancement of **3c** induced relaxation responses driven by these mentioned antagonists points out the possibility that **3c** exerts its effect via mechanisms other than potassium channels. This requires further investigation. Either 4AP or TEA inhibited **3f** induced relaxation responses. Compound **3i** induced relaxation response was inhibited by 4AP. Compound **3k** induced relaxation response was inhibited by 4AP or iberiotoxin. Relaxation responses induced by **3k** were not inhibited by glibenclamide despite the fact that **3k** is a dimethyl analog of KATP channel activators. The introduction of dimethyl groups to the acridine ring might have changed the action characteristics of this agent on potassium channels. All potassium ion channel blockers used in this study did not alter **3l** induced relaxation response. The results of the study showed that the introduction of the electron withdrawing substituent to 3 position of the phenyl ring increased mentioned activity. The opening of the potassium (K^+) channels, causing hyperpolarisation of the cell membrane, is a physiological means of decreasing cell excitability. Thereby, drugs having this property will demonstrate a broad clinical potential. New molecules evoke physiological responses such as smooth muscle relaxation by the opening of potassium ion channels led to a new direction in the pharmacology of ion channels. The term "potassium channel openers" was initially associated with a group of chemically diverse agents (for example, cromakalim, pinacidil, and nicorandil) that evoke potassium ion efflux through KATP channels. KATP channels are inhibited by intracellular ATP. These channels play a role in linking cell metabolic state to membrane potential. KATP channels are associated with diverse cellular functions such as shortening of action-potential duration in cardiac myocytes, insulin release in pancreatic β-cells, regulation of excitability in skeletal muscle, and regulation of vascular smooth muscle contractility (Aronson, 1992; Ashcroft and Gribble, 2000; Noma, 1983; Ashcroft and Rorsman, 1989). But KATP blocker glibenclamide did not alter compounds-induced relaxation responses in this study. The physiological role of potassium ion channels is not clear in gastrointestinal smooth muscle. KATP channels may play a role in regulation in membrane potential and/or in change of contractility

Table 2 In the presence of potassium channel blockers, maximum relaxant responses (E_{max}) values of the compounds on isolated strips of rabbit gastric fundus smooth muscle

Compounds	Control	In the presence of potassium channel blockers, E_{max} values of compounds				
	E_{max}	TEA (10^{-4} M)	Glibenclamide (10^{-6} M)	4-Aminopyridine (10^{-4} M)	Apamine (10^{-6} M)	Iberiotoxin (10^{-8} M)
3a	No effect	No effect	No effect	No effect	No effect	No effect
3b	62.08 ± 5.81	54.63 ± 8.43	51.82 ± 7.64	54.08 ± 6.88	35.30 ± 5.55*	54.44 ± 5.23
3c	53.93 ± 7.49	74.43 ± 4.63*	51.97 ± 9.72	51.37 ± 4.53	54.45 ± 5.65	82.53 ± 2.40*
3d	No effect	No effect	No effect	No effect	No effect	No effect
3f	37.23 ± 9.23	18.83 ± 6.82*	46.68 ± 5.31	21.84 ± 7.72	39.3 ± 5.99	39.08 ± 5.11
3g	No effect	No effect	No effect	No effect	No effect	No effect
3h	No effect	No effect	No effect	No effect	No effect	No effect
3i	33.76 ± 8.54	35.11 ± 7.39	43.75 ± 4.22	14.04 ± 6.21*	26.74 ± 6.34	43.3 ± 5.44
3k	56.02 ± 7.5	44.61 ± 7.00	66.05 ± 5.72	32.26 ± 4.08*	60.6 ± 8.01	30.88 ± 4.57*
3l	45.99 ± 4.29	45.19 ± 6.27	45.24 ± 8.24	51.08 ± 7.88	45.74 ± 5.01	52.02 ± 6.09

Relaxant responses are expressed as a percentage of the precontraction induced by noradrenaline. The maximum relaxant responses (E_{max}) value represents mean value ± SEM

* $P < 0.05$, compared with control responses ($n = 6$)

mediated by neurotransmitters in guinea pig stomach (Sim *et al*., 2002). In the present study, Ca^{2+}-activated potassium ion channel blocker TEA, KV blocker 4AP, small conductance Ca^{2+}-activated potassium ion channel blocker apamine, large-conductance Ca^{2+}-activated potassium ion channel blocker iberiotoxin significantly inhibited compounds-induced relaxation responses. Every blocker showed different effect on different compounds. It has been shown that several types of potassium ion channels, including voltage sensitive potassium ion channels, big conductance Ca^{2+}-activated potassium ion channels and small conductance Ca^{2+}-activated potassium ion channels are present on nerve cells (MacKinnon and Yellen, 1990; Reinhart et al., 1989; Blatz and Magleby, 1986). Most of these channels permit the potassium ion efflux from within the neurons, thereby tending to oppose depolarization or to cause repolarization or hyperpolarization, and resulting in a decrease of neurotransmitter release (Nakamura *et al*., 2004). In conclusion, there is need of more direct evidence to enlighten the potassium channel opening mechanisms of these compounds.

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