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Original article

Microwave-assisted synthesis and myorelaxant activity of 9-indolyl-1,8-acridinedione derivatives

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ABSTRACT

In this study a microwave-assisted method was applied for the synthesis of novel 9-(substituted indolyl)-3,4,6,7-tetrahydroacridine-1,8-(2H,5H,9H,10H)-dione derivatives. The structures of the compounds were confirmed by spectral methods including X-ray studies and elemental analysis.

The E_{max} and pD_2 values of the compounds and pinacidil were determined on noradrenaline pre-contracted tissues of isolated strips of rabbit gastric fundus smooth muscle. The obtained results indicated that some compounds and pinacidil produced concentration-dependent relaxation on the strips. The efficacy of compound **9** was higher than pinacidil.

Docking studies were carried out to understand the interactions of the compounds with the active site of potassium channel. Methyl substituents on the acridine backbone and bromine atom on the indole ring led to more active compounds.

1. Introduction

Ion channels are pore-forming protein tunnels that span the lipid bilayer of the cell membrane and help to establish and control the small voltage gradient that exists across the plasma membrane of all living cells by allowing the flow of ions down their electrochemical gradient [1,2]. Potassium ions are selectively concentrated in the interior part of the cells and are especially important in controlling the resting membrane potential in most excitable cells and maintaining the transmembrane voltage [3]. Potassium channels selectively conduct potassium ions across the cell membrane along its electrochemical gradient [4]. This diverse and ubiquitous channel family plays important role in cellular signaling processes, neuronal excitability, neurotransmitter release, insulin secretion, smooth muscle contraction, heart rate and cell volume regulation [5–7]. Potassium channels exist as several types with multiple subtypes [8]. The control of these channels is regulated physiologically through several means and their classification is often according to their electrophysiological and pharmacological properties [9]. Some major classes of potassium channels are voltage-gated potassium ion channels (K_v channel) that open or close in response to alterations in the transmembrane voltage field, calcium activated potassium ion channels (K_{Ca} channel) that open in response to the presence of calcium ions or other signaling molecules and inward-rectifying potassium channels (K_{ir} channel) that pass current positive charge easily into the cell, including adenosine triphosphate (ATP)-sensitive potassium channels (K_{ATP}) [10–13].

Potassium channel opening is a physiological mechanism by which excitable cells exploit to maintain or restore their resting state. Thus drugs that open vascular potassium channels have the potential to restrain or prevent contractile responses to excitatory stimuli or clamp the vessel in a relaxed condition. Hence, potassium channel openers (KCOs) such as cromakalim and pinacidil (Fig. 1), relax precontracted vascular smooth muscles and lower systemic and regional vascular resistances [14,15]. Potassium channel openers are also under investigation as potential therapeutic agents for nonvascular indications such as bladder dysfunction [16,17].

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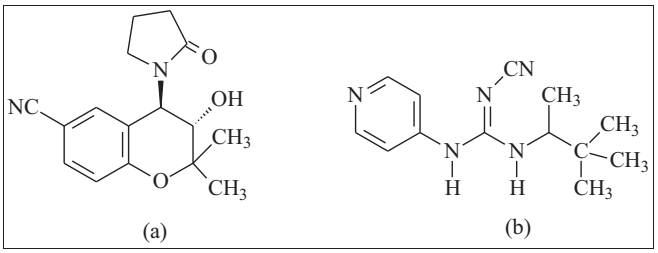


Fig. 1. Chemical structures of cromakalim (a) and pinacidil (b).

1,4-Dihydropyridines (DHPs) present a well-known class of calcium antagonists and are commercially employed for the treatment of cardiovascular diseases particularly hypertension and angina [18,19]. It has been reported that some 1,4-DHP derivatives that were originally developed as long-acting calcium channel blockers, such as niguldipine (Fig. 2), increased the open probability of Ca-activated potassium channels [20].

Tricyclic dihydropyridine-based analogues (Fig. 3), comprising a variety of heterocyclic rings fused to the dihydropyridine nucleus, were also found to be active as potassium channel openers [21–23].

Although the phenyl ring is generally preferred as the aromatic substituent, also tricyclic dihydropyridine containing KCOs bearing different heteroaromatic rings like imidazole have been synthesized to elucidate the structure–activity relationships [24].

The indole nucleus is a ubiquitous nitrogen heterocyclic structure found in numerous natural and synthetic compounds with a wide variety of biological activities and considerable pharmaceutical importance [25]. It is an essential part of the amino acid tryptophan and the neurotransmitter serotonin. Several plant based alkaloids bearing indole as their basic ring are also found to be therapeutically active agents [26]. In recent years lots of indole derivatives have been synthesized exhibiting versatile pharmacological properties such as antihypertensive [27], antitumor [28], anti-inflammatory [29], antimicrobial [30] and anticonvulsant activities [31].

Microwave (MW) irradiation as an energy source for the activation of chemical reactions has been recently introduced and gained great popularity compared to conventional reactions because of its ability to reduce reaction times, to improve yields and to simplify the work-up processes [32].

The heating characteristics of a solvent under microwave irradiation conditions are dependent on its dielectric properties. The ability of a solvent to convert electromagnetic energy into heat at a given frequency and temperature is determined by the so-called loss factor tan δ, which is a measure of the amount of microwave energy that is lost by dissipation as heat [33]. Conventional reactions to obtain 1,4-DHP derivatives were also performed by applying this technique; short-chain alcohols (methanol and ethanol) were proved to be much better solvents in terms of yield than other ones including tetrahydrofuran, acetonitrile and water [34–36].

The aim of this work is to report an efficient and rapid synthetic route based on microwave irradiation for twelve novel 1,4-DHP derivatives in which (substituted) cyclohexane rings are fused to the DHP ring, and to determine how the indole moiety attached to this backbone affects the myorelaxant activities of these compounds.

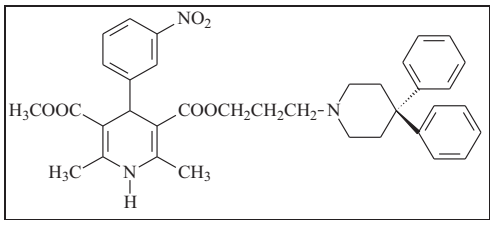


Fig. 2. Niguldipine.

2. Results and discussion

2.1. Chemistry

The synthetic route used to synthesize (2,2,7,7/3,3,6,6-tetramethyl substituted)-9-(substituted-1*H*-indol-2/3-yl)-3,4,6,7-tetrahydroacridine-1,8-(2H,5H,9H,10H)-diones (compound **1**–**12**) have been outlined in Fig. 4. In order to prepare the target compounds; appropriate 1,3-cyclic dicarbonyl compound, substituted indole carboxaldehyde and ammonium acetate were heated under microwave irradiation in methanol.

Methanol, which is one of the most preferred solvents for the synthesis of 1,4-DHPs, with high tan δ value and/or dielectric constant was classified as excellent microwave-absorbing solvent [32,37,38].

The appearance of the products was monitored by TLC and the reaction time was determined as 10 min, which is quite a short time compared to conventional heating [39].

In previous papers, we reported the conventional synthesis of some compounds, which have similar structures to compound **1**–**12**, so it is obvious that this method reduces the solvent use and reaction time [23,40].

Structures and chemical characteristics of the synthesized compounds are given in Table 1.

The structures of the synthesized compounds were elucidated by spectral methods (^{1}H NMR, ^{13}C NMR, X-ray analysis and mass spectra) and confirmed by elemental analysis.

In the ^{1}H NMR spectra, the methylene groups of the acridine ring were at 1.69–2.70 ppm. The protons of the methyl substituents on the same ring were seen at 0.79–0.99 ppm separately and as singlets. The methine protons on the acridine ring were observed as singlet at 4.85–5.10 ppm. The aromatic protons of the indole ring were at 6.23–7.90 ppm. The N–H protons of the acridine ring and indole ring were seen at 9.35–9.59 ppm and 10.71–10.84 ppm, respectively.

The mass spectra of the compounds were recorded via the electron ionization technique. The molecular ion peak (M^+) or the M−1 peak due to the aromatization of the tetrahydroacridine ring were seen in the spectra of all compounds. Cleavage of indole ring from the parent molecule is the next most observed fragmentation.

Elemental analysis results were within ±0.4% of the theoretical values for all compounds.

2.2. Pharmacology

The myorelaxant effects of the compound **1**–**12** were investigated on isolated strips of rabbit gastric fundus muscle.

The maximum relaxant effects (E_{max}) and pD_2 values [the negative logarithm of the concentration for the half-maximal response (EC_{50})] of compounds **1**–**12** and pinacidil on isolated strips of rabbit gastric fundus smooth muscle are given in Table 2.

The cumulative concentration–response curves, which were achieved after the response to the previous concentration had reached a plateau, were given for pinacidil and Compound **3**, **9** and **12** (Fig. 5). The compounds were selected according to their activities compared to the standard compound pinacidil. Compound **9** was the most active compound with higher efficacy than pinacidil while compound **12** had nearly the same efficacy and compound **3** had less myorelaxant effect than pinacidil.

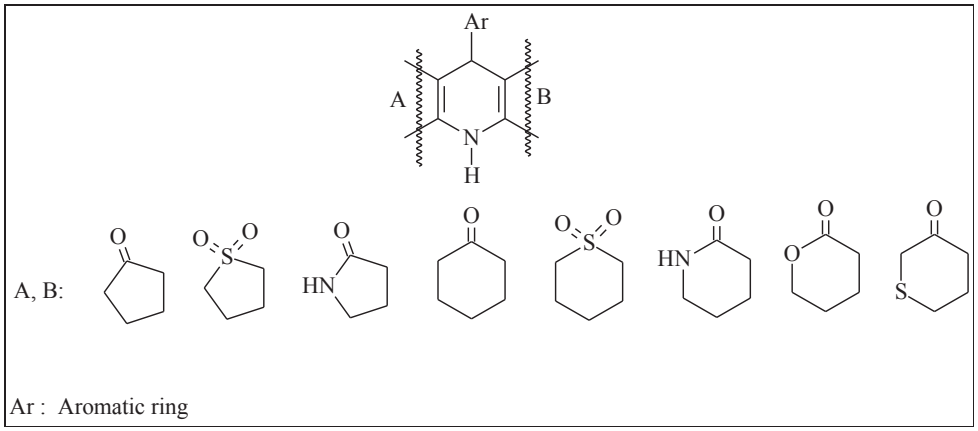


Fig. 3. Tricyclic DHP-based derivatives possessing potassium channel opening activity.

The pharmacological results indicated that compound **2**, **3**, **6**, **8**, **9**, **11** and **12** produced concentration-dependent relaxation responses precontracted with noradrenaline in the gastric fundus smooth muscle strips with the efficacy order: **9** ≥ pinacidil > **12** > **3** ≥ **6** ≥ **8** > **11** = **2**. It is interesting that the efficacy of compound **9** was higher than pinacidil.

On the other hand, compound **1**, **4**, **5**, **7** and **10** caused slightly relaxant responses which were not statistically significant. Tissues were pretreated with indomethacin (cyclooxygenase inhibitor), propranolol (β-adrenergic receptor blocker) or N-ω-nitro-L-arginine methyl ester (L-NAME) hydrochloride (the nitric oxide synthase inhibitor) to investigate whether the relaxation induced by the compounds were through cyclooxygenase, adrenergic system or nitric oxide pathways, respectively.

In this study, pretreatment of the strips with indomethacin, propranolol and L-NAME did not significantly alter the relaxant responses of the compounds so these findings explained that cyclooxygenase, adrenergic and nitric oxide (NO) pathways did not play a role on relaxations evoked by these substances.

Glibenclamide (10^{-6} M), a K_{ATP} blocker and tetraethylammonium chloride (TEA) (10^{-4} M), a KCa blocker did not reverse the myorelaxant effects of the compound **2**, **3**, **6**, **8**, **9**, **11** and **12**. These results suggested that myorelaxant effects of the mentioned compounds were not mediated by K_{ATP} and K_{Ca} channels. We suppose that calcium channels or other types of potassium channels may mediate these effects.

2.3. Molecular modeling

Molecular docking studies of the compounds **1**–**12** in the active site of potassium channel (PDB code: 1BL8) were performed in order to get further information about the type of interactions between the compounds and the active site amino acids to rationalize the obtained biological results. Binding conformation of the most active compound (compound **9**) and space occupied (yellow box around the ligand) in the 1BL8 binding pocket is shown in Fig. 6.

One carbonyl oxygen of the acridine ring is placed close to the –OH group of Thr75 for the formation of hydrogen bond. The indole ring is positioned in the hydrophobic binding pocket surrounded by Thr74, Thr75, Ile100 and Phe103.

When the obtained findings were analyzed with respect to the indole ring, it was observed that the substitution at the nitrogen atom of the indole ring by a methyl group led to less potent compounds while the introduction of bromine on the indole ring made a positive contribution to the activity. The unsubstituted nitrogen atom on the indole ring was important for the hydrogen bonding with the carbonyl group of Thr75 and the bromine atom was employed for the hydrophobic interactions with the protein. The methyl groups substituted to the acridine ring are oriented in the cavities of the protein for the additional hydrophobic interactions.

Pharmacophore features and both 2D and 3D interactions of the Compound **9** with the binding site of the protein have been showed in Figs. 7 and 8.

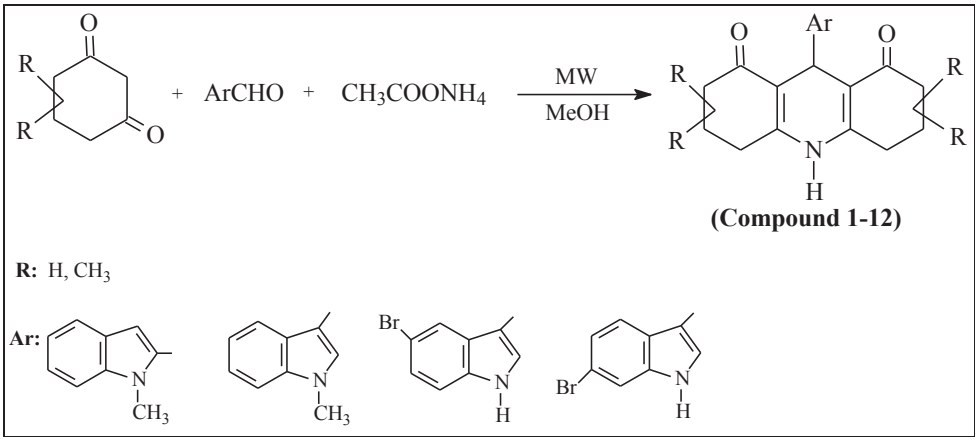
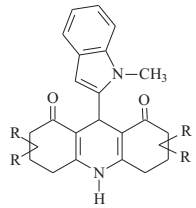
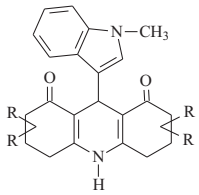
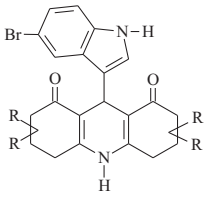
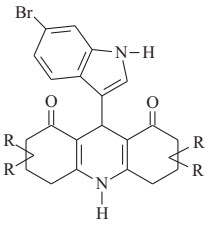


Fig. 4. Synthesis of the compound **1**–**12.**

Table 1
Structural data of the synthesized compounds.

Compound	R	Melting point (°C)	Empirical formula	Molecular weight
				
1	H	293–295	$C_{22}H_{22}N_2O_2$	346
2	2,2,7,7-tetraCH_3	203–205	$C_{26}H_{30}N_2O_2$	402
3	3,3,6,6-tetraCH_3	280–282	$C_{26}H_{30}N_2O_2$	402
				
4	H	246–248	$C_{22}H_{22}N_2O_2$	346
5	2,2,7,7-tetraCH_3	248–250	$C_{26}H_{30}N_2O_2$	402
6	3,3,6,6-tetraCH_3	273–275	$C_{26}H_{30}N_2O_2$	402
				
7	H	213–215	$C_{21}H_{19}N_2O_2$	411
8	2,2,7,7-tetraCH_3	238–240	$C_{25}H_{27}N_2O_2$	467
9	3,3,6,6-tetraCH_3	265–267	$C_{25}H_{27}N_2O_2$	467
				
10	H	218–220	$C_{21}H_{19}N_2O_2$	411
11	2,2,7,7-tetraCH_3	205–207	$C_{25}H_{27}N_2O_2$	467
12	3,3,6,6-tetraCH_3	281–283	$C_{25}H_{27}N_2O_2$	467

Docking studies were also carried out on the pharmacologically inactive compounds with the aim of explaining their inactivity. For the best obtained pose of compound **1** (Fig. 9), there is primarily a hydrophobic interaction between its indole ring and the protein. The methyl substituent at the nitrogen atom of the indole ring and the nonsubstituted acridine backbone could be the reason for the inactivity of this compound due to the lacking further interactions with the protein.

2.4. X-ray studies

The X-ray crystallographic data of compound **3** (Fig. 10) demonstrated that both cyclohex-2-enone rings adopt sofa conformations in the acridine system, while the indole ring system is essentially planar. There is an intramolecular C–H·····O hydrogen bond and the molecules assemble into C(6) chains in the crystal by way of N–H·····O hydrogen bonds.

For compound **7** (Fig. 11), it was observed that the two cyclohex-2-enone rings adopt half-chair conformations. In the crystal, molecules are linked by N–H·····O hydrogen bonds and there is also an intramolecular C–H·····O hydrogen bond. The solvent molecule (dimethyl sulfoxide) exhibits minor disorder of the S atom.

Detailed descriptions of the structures have been presented in Refs. [41,42].

3. Conclusions

We reported herein an easy, very rapid and convenient method for the preparation of tricyclic 1,4-DHPs. This method also offers a reduction of solvent use, simplification of the work-up procedures and low energy consumption in addition to higher yields.

The obtained pharmacological results showed that several 9-indolyl-1,8-acridinedione derivatives have myorelaxant activity on isolated rabbit gastric fundus smooth muscle precontracted by noradrenaline. Glibenclamide and TEA did not change the relaxation responses of the effective compounds. These results point out that there is no contribution of ATP sensitive and calcium activated

Table 2
Maximum relaxant responses (E_{max}) and pD_2 values on precontracted tissues by noradrenaline of the compounds and pinacidil on isolated strips of rabbit gastric fundus smooth muscle.

Compound	E_{max}	pD_2
1	No effect	No effect
2	11.64 ± 4.40[a]	4.60 ± 0.34[a]
3	33.95 ± 4.36[a]	4.10 ± 0.34[a]
4	No effect	No effect
5	No effect	No effect
6	28.58 ± 9.61[a]	4.28 ± 0.68[a]
7	No effect	No effect
8	17.51 ± 2.59[a]	4.46 ± 0.11[a]
9	71.50 ± 6.27[a]	3.85 ± 0.50[a]
10	No effect	No effect
11	11.98 ± 2.26[a]	4.54 ± 0.05[a]
12	52.71 ± 4.13[a]	4.25 ± 0.31[a]
Pinacidil	63.82 ± 3.39[a]	3.90 ± 0.23[a]

Relaxation is expressed as a percentage of the precontraction induced by noradrenaline (10^{-5} M). The negative logarithm of the concentration for the half-maximal response (pD_2) and E_{max} values represent mean value ± S.E.
[a] $p < 0.05$, compared with control responses ($n = 6$).

potassium channels to myorelaxant effects of compounds. As a result the potassium channel opening ability of these effective agents need further investigations.

4. Experimental section

4.1. General

All chemicals used in this study were purchased from Aldrich and Fluka (Steinheim, Germany). All reactions were carried out in Discover Microwave Apparatus (CEM). Thin layer chromatography (TLC) was run on Merck aluminum sheets (Darmstadt, Germany), Silica gel 60 F_{254}, mobile phase ethyl acetate–hexane: (1:1) and ultraviolet (UV) absorbing spots were detected by short-wavelength (254 nm) UV light (Camag UV Cabinet, Wiesloch, Germany). Melting points were determined on a Thomas Hoover Capillary Melting Point Apparatus (Philadelphia, PA, USA) and were uncorrected. ^{1}H NMR and ^{13}C NMR spectra were obtained in dimethyl sulfoxide (DMSO) solutions on a Varian Mercury 400, 400 MHz High Performance Digital FT-NMR Spectrometer (Palo Alto, CA, USA). Chemical shifts are reported in parts per million (ppm) relative to tetramethylsilane. The X-ray crystallographic analysis was carried out on an Agilent Xcalibur (Ruby, Gemini) diffractometer. Mass spectra were obtained on an Agilent 5973 Network Mass Selective Detector by electron ionization (Philadelphia, PA, USA). Elemental analyses were performed on a Leco CHNS-932 Elemental Analyzer (Philadelphia, PA, USA).

4.2. General procedure for the preparation of (2,2,7,7/3,3,6,6-tetramethyl substituted)-9-(substituted-1H-indol-2/3-yl)-3,4,6,7-tetrahydroacridine-1,8-(2H,5H,9H,10H)-diones (Compound 1–12)

One-pot three component mixture of 2 mmol appropriate 1,3-cyclic dicarbonyl compound (1,3-cyclohexanedione/4,4-dimethyl-1,3-cyclohexanedione or 5,5-dimethyl-1,3-cyclohexanedione), 1 mmol substituted indole carboxaldehyde (1-methyl-1H-indole-2-carbaldehyde/1-methyl-1H-indole-3-carbaldehyde/5-bromo-1H-indole-3-carbaldehyde or 6-bromo-1H-indole-3-carbaldehyde) and 5 mmol ammonium acetate was filled into 10 mL-microwave pressure vial and heated under microwave irradiation (power 50 W, maximum temperature 120 °C) for 10 min in 5 mL methanol. After the reaction was completed, the reaction mixture was poured into ice-water, the obtained precipitate was filtered and crystallized from ethanol–water.

4.2.1. 9-(1-Methyl-1H-indol-2-yl)-3,4,6,7-tetrahydroacridine-1,8-(2H,5H,9H,10H)-dione (Compound 1)

Yield: 83%. m.p. 293–295 °C. ^{1}H NMR (δ, DMSO-d_6): 1.72–2.70 (12H; m; acridine $H^{2,3,4,5,6,7}$), 3.39 (3H; s; N–CH_3), 4.91 (H; s; acridine H^9), 6.23 (H; s; indole H^3), 6.86–6.90 (H; m; indole H^6), 7.01–7.04 (H; m; indole H^5), 7.30–7.37 (2H; m; indole $H^{4,7}$), 9.59 (H; s;

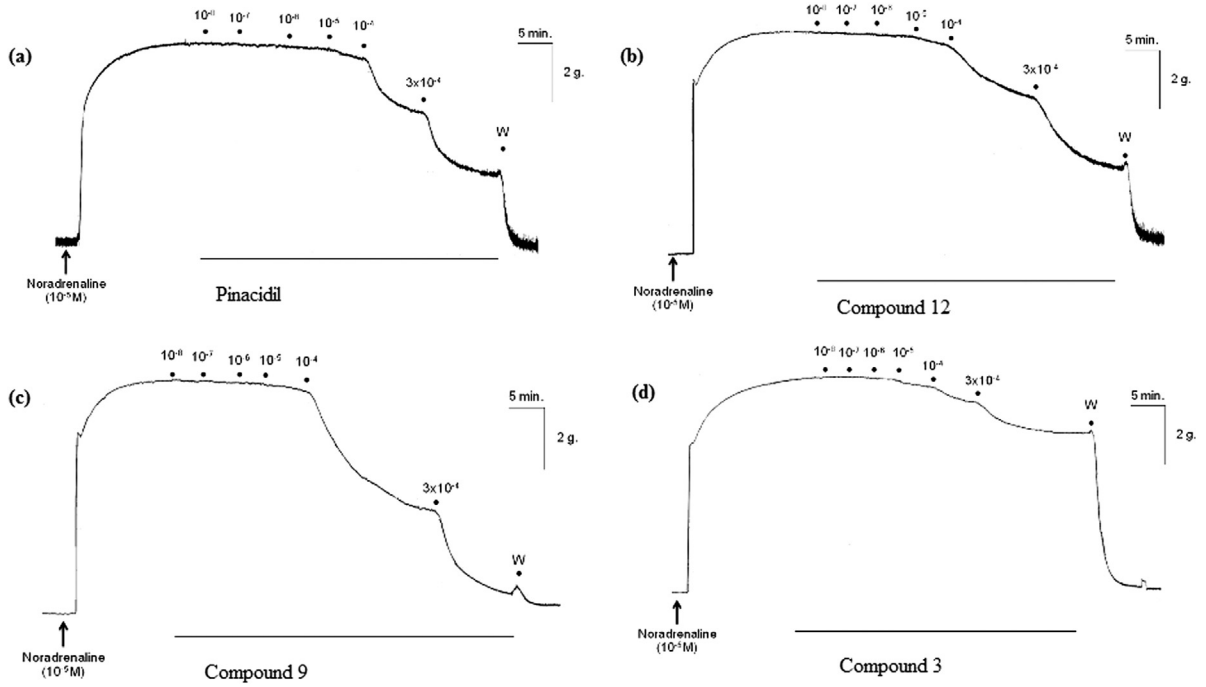


Fig. 5. Typical traces showing the relaxant effects of pinacidil (a), compound **12** (b), compound **9** (c) and compound **3** (d) (10^{-8}–3×10^{-4} M) on noradrenaline (10^{-5} M) precontracted isolated smooth muscle strips of rabbit gastric fundus.

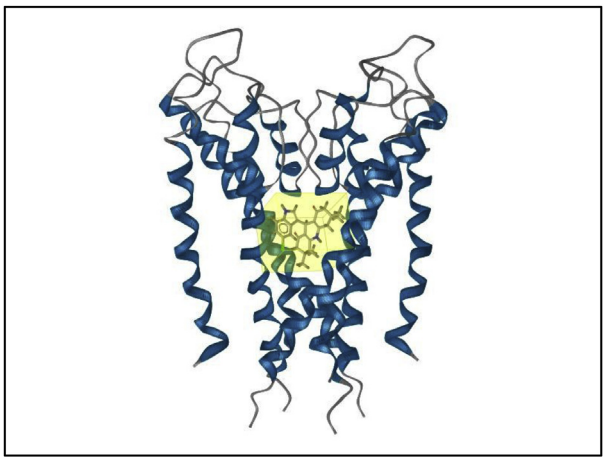


Fig. 6. Binding conformation of compound **9** and the space occupied in the 1BL8 binding pocket.

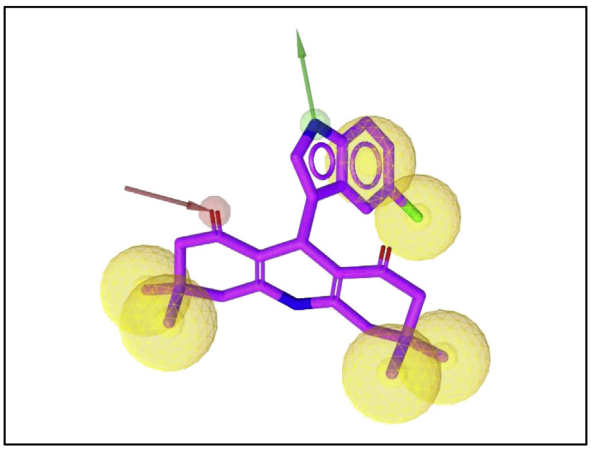


Fig. 7. Color-coded pharmacophore features of compound **9**: hydrophobic feature (yellow sphere), electron donor group (red vector) and hydrogen bonding domain feature (green vector). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

NH). MS (*m*/*z*): 346 $[M]^+$. Anal. Calcd. for $C_{22}H_{22}N_2O_2$ (C, H, N, O): C, 76.28; H, 6.40; N, 8.09. Found: C, 76.33; H, 6.36; N, 8.05.

4.2.2. 2,2,7,7-Tetramethyl-9-(1-methyl-1H-indol-2-yl)-3,4,6,7-tetrahydroacridine-1,8-(2H,5H,9H,10H)-dione (Compound 2)

Yield: 78%. m.p. 203–205 °C. ^{1}H NMR (*δ*, DMSO-d_6): 0.89 (6H; s; 2 × CH_3), 0.98 (6H; s; 2 × CH_3), 1.90–2.53 (8H; m; acridine $H^{3,4,5,6}$), 3.67 (3H; s; N–CH_3), 5.03 (H; s; acridine H^9), 6.25 (H; s; indole H^3), 6.79-6.84 (H; m; indole H^6), 7.00–7.05 (H; m; indole H^5), 7.35–7.42 (2H; m; indole $H^{4,7}$), 9.51 (H; s; NH). MS (*m*/*z*): 402 $[M]^+$. Anal. Calcd. for $C_{26}H_{30}N_2O_2$ (C, H, N, O): C, 77.58; H, 7.51; N, 6.96. Found: C, 77.55; H, 7.53; N, 7.02.

4.2.3. 3,3,6,6-Tetramethyl-9-(1-methyl-1H-indol-2-yl)-3,4,6,7-tetrahydroacridine-1,8-(2H,5H,9H,10H)-dione (Compound 3)

Yield: 73%. m.p. 280–282 °C. ^{1}H NMR (*δ*, DMSO-d_6): 0.89 (6H; s; 2 × CH_3), 0.96 (6H; s; 2 × CH_3), 1.91–2.56 (8H; m; acridine $H^{2,4,5,7}$), 3.65 (3H; s; N–CH_3), 4.85 (H; s; acridine H^9), 6.28 (H; s; indole H^3), 6.80–6.86 (H; m; indole H^6), 6.99–7.03 (H; m; indole H^5), 7.32–7.39 (2H; m; indole $H^{4,7}$), 9.43 (H; s; NH). MS (*m*/*z*): 401 $[M-1]^+$. Anal. Calcd. for $C_{26}H_{30}N_2O_2$ (C, H, N, O): C, 77.58; H, 7.51; N, 6.96. Found: C, 77.61; H, 7.50; N, 6.98.

4.2.4. 9-(1-Methyl-1H-indol-3-yl)-3,4,6,7-tetrahydroacridine-1,8-(2H,5H,9H,10H)-dione (Compound 4)

Yield: 80%. m.p. 246–248 °C. ^{1}H NMR (*δ*, DMSO-d_6): 1.69-2.49 (12H; m; acridine $H^{2,3,4,5,6,7}$), 3.61 (3H; s; N–CH_3), 5.10 (H; s; acridine H^9), 6.80 (H; s; indole H^2), 6.87-6.91 (H; m; indole H^5), 6.98–7.02 (H; m; indole H^6), 7.21 (H; d; J: 8 Hz; indole H^7), 7.64 (H; d; J: 7,6 Hz; indole H^4), 9,47 (H; s; NH). MS (*m*/*z*): 345 $[M-1]^+$. Anal. Calcd. for $C_{22}H_{22}N_2O_2$ (C, H, N, O): C, 76.28; H, 6.40; N, 8.09. Found: C, 76.24; H, 6.43; N, 8.11.

4.2.5. 2,2,7,7-Tetramethyl-9-(1-methyl-1H-indol-3-yl)-3,4,6,7-tetrahydroacridine-1,8-(2H,5H,9H,10H)-dione (Compound 5)

Yield: 73%. m.p. 248–250 °C. ^{1}H NMR (*δ*, DMSO-d_6): 0.88 (6H; s; 2 × CH_3), 0.99 (6H; s; 2 × CH_3), 1.69–2.53 (8H; m; acridine $H^{3,4,5,6}$), 3.64 (3H; s; N–CH_3), 5.09 (H; s; acridine H^9), 6.79 (H; s; indole H^2), 6.90–6.94 (H; m; indole H^5), 7.01–7.05 (H; m; indole H^6), 7.24 (H; d; J: 8 Hz; indole H^7), 7.68 (H; d; J: 8 Hz; indole H^4), 9.39 (H; s; NH). MS (*m*/*z*): 402 $[M]^+$. Anal. Calcd. for $C_{26}H_{30}N_2O_2$ (C, H, N, O): C, 77.58; H, 7.51; N, 6.96. Found: C, 77.62; H, 7.49; N, 6.93.

4.2.6. 3,3,6,6-Tetramethyl-9-(1-methyl-1H-indol-3-yl)-3,4,6,7-tetrahydroacridine-1,8-(2H,5H,9H,10H)-dione (Compound 6)

Yield: 79%. m.p. 273–275 °C. ^{1}H NMR (*δ*, DMSO-d_6): 0.79 (6H; s; 2 × CH_3), 0.97 (6H; s; 2 × CH_3), 1.92–2.51 (8H; m; acridine $H^{2,4,5,7}$), 3.66 (3H; s; N–CH_3), 5.08 (H; s; acridine H^9), 6.89–6.93 (H; m; indole H^5), 6.91 (H; s; indole H^2), 7.00–7.04 (H; m; indole H^6), 7.25 (H; d; J: 8 Hz; indole H^7), 7.55 (H; d; J: 8 Hz; indole H^4), 9.35 (H; s; NH). ^{13}C NMR (*δ*, DMSO-d_6): 24.6 (3,3,6,6-tetraCH_3), 27.1 (C^3, C^6), 29.4 (C^4, C^5), 32.4 (C^2, C^7), 32.5 (C^9), 50.8 (N–CH_3), 109.6 (C^{8a}, C^{9a}), 111.6, 118.3, 120.2, 120.3, 120.7, 126.6, 127.8, 136.9 (indole carbons), 148.9 (C^{4a}, C^{10a}), 194.9 (C^1, C^8). MS (*m*/*z*): 401 $[M-1]^+$. Anal. Calcd. for $C_{26}H_{30}N_2O_2$ (C, H, N, O): C, 77.58; H, 7.51; N, 6.96. Found: C, 77.61; H, 7.48; N, 6.98.

4.2.7. 9-(5-Bromo-1H-indol-3-yl)-3,4,6,7-tetrahydroacridine-1,8-(2H,5H,9H,10H)-dione (Compound 7)

Yield: 86%. m.p. 213–215 °C. ^{1}H NMR (*δ*, DMSO-d_6): 1.71-2.59 (12H; m; acridine $H^{2,3,4,5,6,7}$), 5.08 (H; s; acridine H^9), 6.87 (H; d; J: 2 Hz; indole H^4), 7.08 (H; dd; J: 8/2 Hz; indole H^6), 7.21 (H; d; J: 8 Hz, indole H^7), 7.90 (H; d; J: 1,2 Hz; indole H^2), 9.53 (H; s; NH), 10.84 (H; s; indole NH). MS (*m*/*z*): 410 $[M-1]^+$. Anal. Calcd. for $C_{21}H_{19}N_2O_2$ (C, H, N, O): C, 61.33; H, 4.66; N, 6.81. Found: C, 61.37; H, 4.68; N, 6.85.

4.2.8. 2,2,7,7-Tetramethyl-9-(5-bromo-1H-indol-3-yl)-3,4,6,7-tetrahydroacridine-1,8-(2H,5H,9H,10H)-dione (Compound 8)

Yield: 80%. m.p. 238–240 °C. ^{1}H NMR (*δ*, DMSO-d_6): 0.88 (6H; s; 2 × CH_3), 0.98 (6H; s; 2 × CH_3), 1.71–2.54 (8H; m; acridine $H^{3,4,5,6}$), 5.02 (H; s; acridine H^9), 6.83 (H; d; J: 2,4 Hz; indole H^4), 7.07 (H; dd; J: 8,4/2,4 Hz; indole H^6), 7.19 (H; d; J: 8,4 Hz, indole H^7), 7.85 (H; d; J: 1,6 Hz; indole H^2), 9.43 (H; s; NH), 10.77 (H; s; indole NH). MS (*m*/*z*): 467 $[M]^+$. Anal. Calcd. for $C_{25}H_{27}N_2O_2$ (C, H, N, O): C, 64.24; H, 5.82; N, 5.99. Found: C, 64.29; H, 5.85; N, 6.03.

4.2.9. 3,3,6,6-Tetramethyl-9-(5-bromo-1H-indol-3-yl)-3,4,6,7-tetrahydroacridine-1,8-(2H,5H,9H,10H)-dione (Compound 9)

Yield: 86%. m.p. 265–267 °C. ^{1}H NMR (*δ*, DMSO-d_6): 0.90 (6H; s; 2 × CH_3), 0.98 (6H; s; 2 × CH_3), 1.72–2.55 (8H; m; acridine $H^{2,4,5,7}$), 5.05 (H; s; acridine H^9), 6.82 (H; d; J: 2,4 Hz; indole H^4), 7.11 (H; dd; J: 8,4/2,4 Hz; indole H^6), 7.24 (H; d; J: 8,4 Hz, indole H^7), 7.86 (H; d; J: 1,2 Hz; indole H^2), 9.45 (H; s; NH), 10.76 (H; s; indole NH). MS (*m*/*z*): 466 $[M-1]^+$. Anal. Calcd. for $C_{25}H_{27}N_2O_2$ (C, H, N, O): C, 64.24; H, 5.82; N, 5.99. Found: C, 64.29; H, 5.84; N, 5.97.

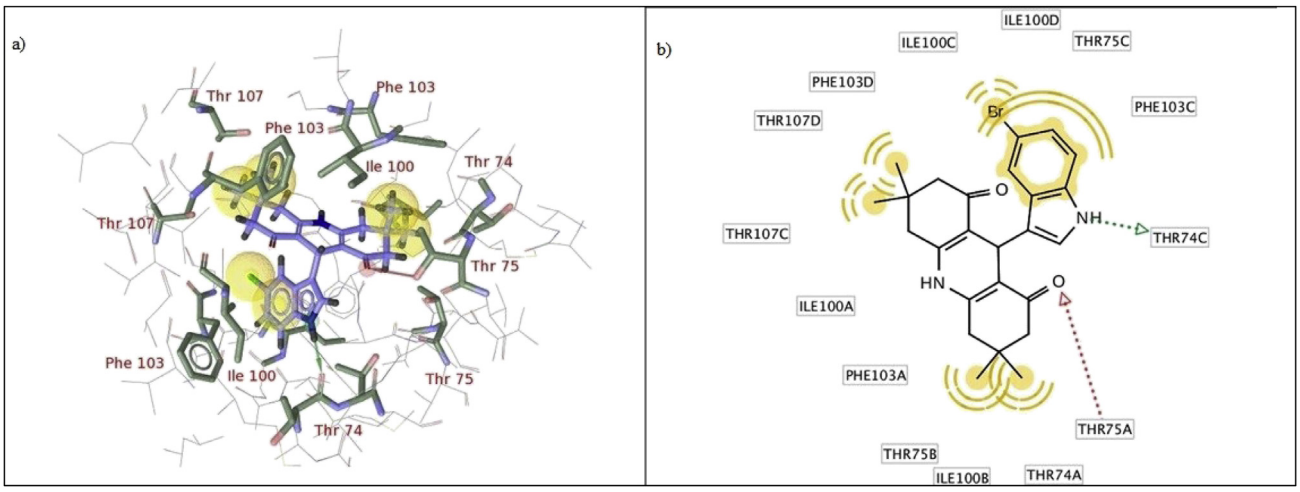


Fig. 8. The orientation, 3D (a) and 2D (b) interactions of the compound **9** (shown as blue) with the binding site of 1BL8. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

4.2.10. 9-(6-Bromo-1H-indol-3-yl)-3,4,6,7-tetrahydroacridine-1,8-(2H,5H,9H,10H)-dione (Compound 10)

Yield: 77%. m.p. 218–220 °C. ^{1}H NMR (δ, DMSO-d_6): 1.69-2.50 (12H; m; acridine $H^{2,3,4,5,6,7}$), 5.09 (H; s; acridine H^9), 6.82 (H; d; J: 2,4 Hz; indole H^7), 7.00 (H; dd; J: 8,4/2,4 Hz; indole H^5), 7.38 (H; d; J: 1,6 Hz, indole H^2), 7.61 (H; d; J: 8,4 Hz; indole H^4), 9.47 (H; s; NH), 10.73 (H; s; indole NH). ^{13}C NMR (δ, DMSO-d_6): 21.2 (C^3, C^6), 23.6 (C^4, C^5), 26.7 (C^2, C^7), 37.3 (C^9), 112.9 (C^{8a}, C^{9a}), 113.6, 113.9, 121.2, 121.8, 122.0, 124.2, 125.3, 137.5 (indole carbons), 151.2 (C^{4a}, C^{10a}), 195.2 (C^1, C^8). MS (*m*/*z*): 411 $[M]^+$. Anal. Calcd. for $C_{21}H_{19}N_2O_2$ (C, H, N, O): C, 61.33; H, 4.66; N, 6.81. Found: C, 61.37; H, 4.62; N, 6.85.

4.2.11. 2,2,7,7-Tetramethyl-9-(6-bromo-1H-indol-3-yl)-3,4,6,7-tetrahydroacridine-1,8-(2H,5H,9H,10H)-dione (Compound 11)

Yield: 84%. m.p. 205–207 °C. ^{1}H NMR (δ, DMSO-d_6): 0.86 (6H; s; 2 × CH_3), 0.97 (6H; s; 2 × CH_3), 1.69–2.53 (8H; m; acridine $H^{3,4,5,6}$), 5.06 (H; s; acridine H^9), 6.81 (H; d; J: 2 Hz; indole H^7), 7.02 (H; dd; J: 8,8/2 Hz; indole H^5), 7.39 (H; d; J: 1,2 Hz, indole H^2), 7.63 (H; d; J: 8,8 Hz; indole H^4), 9.39 (H; s; NH), 10.71 (H; s; indole NH). MS (*m*/*z*): 467 $[M]^+$. Anal. Calcd. for $C_{25}H_{27}N_2O_2$ (C, H, N, O): C, 64.24; H, 5.82; N, 5.99. Found: C, 64.20; H, 5.85; N, 6.02.

4.2.12. 3,3,6,6-Tetramethyl-9-(6-bromo-1H-indol-3-yl)-3,4,6,7-tetrahydroacridine-1,8-(2H,5H,9H,10H)-dione (Compound 12)

Yield: 76%. m.p. 281–283 °C. ^{1}H NMR (δ, DMSO-d_6): 0.87 (6H; *s*; 2 × CH_3), 0.99 (6H; s; 2 × CH_3), 1.70-2.55 (8H; m; acridine $H^{2,4,5,7}$), 5.04 (H; s; acridine H^9), 6.82 (H; d; J: 2,4 Hz; indole H^7), 7.05 (H; dd; J: 8,8/2,4 Hz; indole H^5), 7.42 (H; d; J: 1,2 Hz, indole H^2), 7.68 (H; d; J: 8,8 Hz; indole H^4), 9.42 (H; s; NH), 10.75 (H; s; indole NH). MS (*m*/*z*): 466 $[M-1]^+$. Anal. Calcd. for $C_{25}H_{27}N_2O_2$ (C, H, N, O): C, 64.24; H, 5.82; N, 5.99. Found: C, 64.27; H, 5.85; N, 5.95.

4.3. Pharmacology

Nω-nitro-L-arginine-methyl ester (L-NAME) hydrochloride, indomethacin, propranolol hydrochloride, glibenclamide, TEA, noradrenaline and pinacidil were supplied by Sigma. While L-NAME, TEA, propranolol hydrochloride, glibenclamide and noradrenaline were dissolved in distilled water; the compounds, pinacidil and indomethacin were dissolved in DMSO. DMSO did not alter the responses.

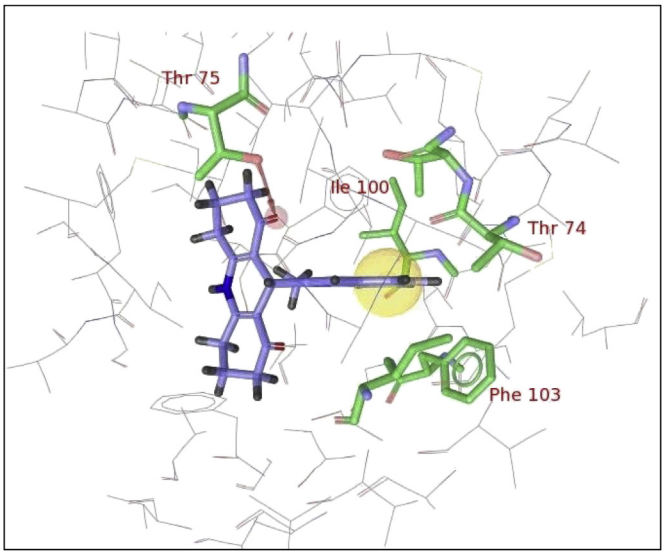


Fig. 9. The orientation of compound **1** (shown as blue) docked into the binding site of 1BL8. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

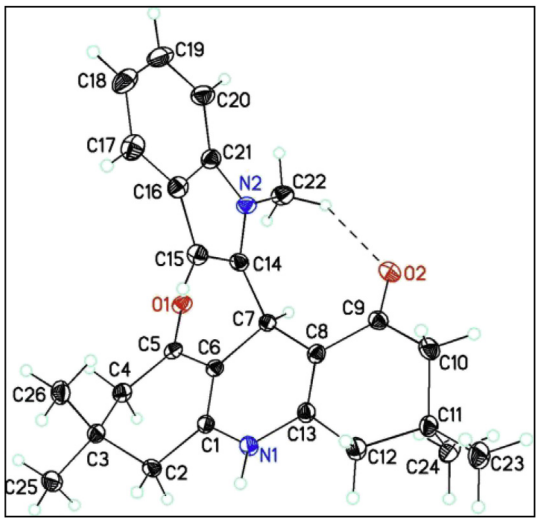


Fig. 10. The molecular structure of compound **3.**

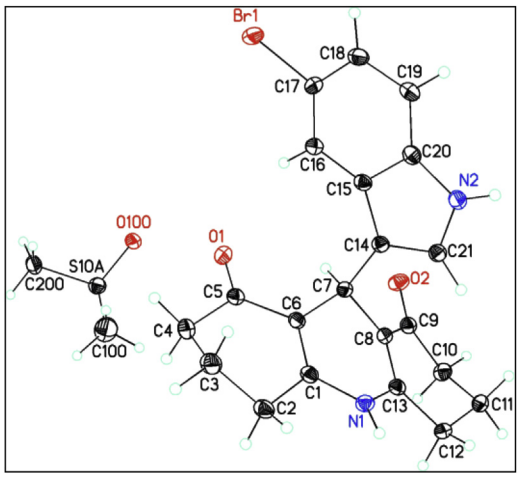


Fig. 11. The molecular structure of compound **7.**

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. Procedures involving animals and their care were conducted in conformity with international laws and policies. The rabbits were sacrificed with i.v. injection of sodium pentobarbital (30–40 mg/kg, i.v.), followed by removal of the stomach through abdominal incision. The fundal part of the stomach was then dissected parallel to the longitudinal muscle wall. One muscle strip 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 filled with 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; $Mg\ Cl_2$ 0.54; $NaHPO_4$ 0.9; glucose 10.04. The solution was gassed with 95% O_2 and 5% CO_2 during the study and temperature was maintained at 37 °C by a thermoregulated water circuit. 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, which was continuously displaced and recorded on an online computer via four-channel transducer data acquisition system (MP30B-CE, BIOPAC Systems Inc., Santa Barbara, CA) using a software (BSL PRO v 3.6.7, BIOPAC Systems Inc.) which also had the capacity to analyze the data. 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 during this time period. N-ω-nitro-L-arginine methyl ester (L-NAME) hydrochloride (the nitric oxide synthase inhibitor, 10^{-4} M), indomethacin (COX inhibitor, 10^{-5} M) and propranolol (β adrenergic receptor blocker, 10^{-6}) were added into the organ bath 20 min before the precontraction with noradrenaline in order to eliminate the effects of nitric oxide, cyclooxygenase and adrenergic pathways, respectively. Rabbit gastric fundus smooth muscle strips were precontracted with noradrenaline (10^{-5} M) to obtain submaximal contraction. Concentration–relaxation responses of Compounds 1–12, pinacidil and DMSO were obtained by adding these into the bath in a cumulative manner. A cumulative concentration–response curve was constructed in a stepwise manner after the response to the previous concentration had reached a plateau. This experimental protocol was repeated in the presence of glibenclamide and TEA.

4.3.1. Data analysis

The relaxant effects of the compounds and pinacidil (K^+ channel opener) were expressed as percentage of the precontraction with noradrenaline.

To evaluate the effects of the compounds, the maximum response (E_{max}) [each drug's E_{max} value has been established at 3×10^{-4} M concentration] and pD_2 values [the negative logarithm of the concentration for the half-maximal response (EC_{50})] were calculated, as predicted from the Scatchard equation for drug–receptor interaction. Agonist pD_2 values (apparent agonist affinity constants) were calculated from each agonist concentration–response curve by linear regression of the linear part of the curve and taken as a measure of the sensitivity of the tissues to each agonist. While E_{max} is the parameter for efficacy, pD_2 is the parameter for potency. All data are expressed as mean ± standard error.

4.3.2. Statistical analysis

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

4.4. Computational methodology

4.4.1. Ligand preparation

The formulas of the compounds were drawn in Chembiodraw Ultra 12.0 and saved as Simplified Molecule Input Entry System (SMILES) file. The file was transfered to LigandScout 3.1. [43] in order to prepare the appropriate file needed for the docking study. For this purpose, the structures were geometrically optimized and energy minimized to 3D structure using the MMFF94x force field in LigandScout 3.1.

4.4.2. Protein preparation

The reported X-ray crystal structure of potassium channel receptor (KcsA) of Streptomyces lividans (PDB code: 1BL8) was obtained from the Protein Data Bank of Brookhaven (PDB, www.rcsb.org/pdb) [44]. The protein was imported to GOLD (Genetically Optimized Ligand Docking) and prepared by removing water molecules and metal ions and adding hydrogen atoms using GOLDMINE before docking.

4.4.3. Docking procedure

The binding region was identified by the help of recent studies about the same protein [44,45]. Dockings were performed under 'standard default settings mode' and for each run; maximum number of 100,000 operations were performed with a population size of 100 individuals. Ten docking poses were obtained for each ligand and the scoring function GoldScore implemented in GOLD was used to rank the docking positions of the molecules. LigandScout was used for the further analysis of the conformation of molecules based on the best fitness scores.

Conflict of interest

All authors of the article declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2014.01.059.

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