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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 precontracted 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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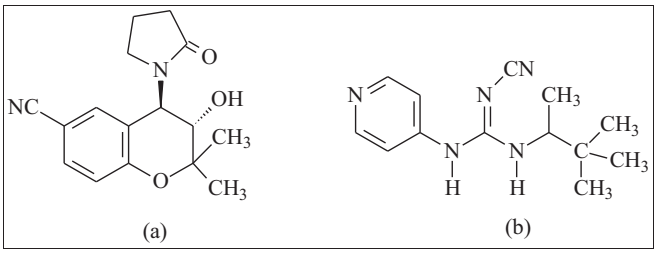


**Fig. 1.** Chemical structures of cromakalim (a) and pinacidil (b).

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.

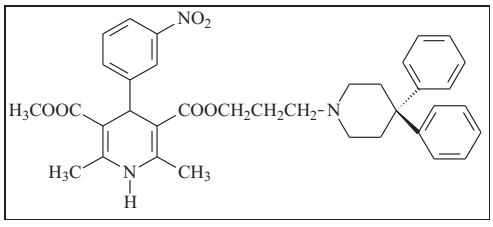


**Fig. 2.** Niguldipine.

## 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.