drugs and drug candidates

MDPI

Article

Relaxant Activity of 4H-Pyran and 1,6-Dihydropyridine Derivatives on Isolated Rat Trachea

Samuel Estrada-Soto [1,*,†], Soledad Alemán-Pantitlán [1], Emmanuel Gaona-Tovar [1], Fernando Hernández-Borja [2], Yolanda Alcaraz [3], Rafael Villalobos-Molina [4] and Miguel A. Vázquez [2,*,†]

[1] Facultad de Farmacia, Universidad Autónoma del Estado de Morelos, Cuernavaca 62209, Mexico
[2] Departamento de Química, División de Ciencias Naturales y Exactas, Universidad de Guanajuato, Guanajuato 36050, Mexico
[3] Departamento de Farmacia, Universidad de Guanajuato, Guanajuato 36050, Mexico
[4] Unidad de Biomedicina, Facultad de Estudios Superiores-Iztacala, Universidad Nacional Autónoma de México, Tlalnepantla 54090, Mexico
* Correspondence: enoch@uaem.mx (S.E.-S.); mvazquez@ugto.mx (M.A.V.)
† These authors contributed equally to this work.

Abstract: Derivatives of 4H-pyrans and 1,6-dihydropyridines have generated considerable attention due to their interesting biological and therapeutic values. Their pharmacological activities include vasorelaxant, anticarcinogenic, antimicrobial, and antioxidant activities. Thus, the aim of the current work is to determine the relaxant effect of synthesized 4H-pyran and 1,6-dihydropyridine derivatives with potential anti-asthmatic properties on the smooth muscle airway, with a possible Ca^{2+}-channel blockade as a mechanism of action due to their analogy with 1,4-dihidropyridines. 4*H*-pyrans and 1,6-dihydropyridines were achieved using multicomponent reactions by microwave and conventional heating. Also, test samples were evaluated ex vivo to determine their relaxant effect on isolated rat tracheal rings pre-contracted with carbachol. All compounds evaluated showed a significant relaxant effect on carbachol-induced contraction in tracheal rat rings. Compounds **4b**, **4e**, **7a**, and **8d** were the most potent from the entire series and were also more potent than theophylline, used as a positive control. In conclusion, in the current work some relaxant compounds of the airway smooth muscle with potential to be developed as anti-asthmatic drugs were obtained.

Keywords: 4H-pyrans; 1,6-dihydropyridines; trachea-relaxant activity; asthma; calcium channels

Citation: Estrada-Soto, S.; Alemán-Pantitlán, S.; Gaona-Tovar, E.; Hernández-Borja, F.; Alcaraz, Y.; Villalobos-Molina, R.; Vázquez, M.A. Relaxant Activity of 4H-Pyran and 1,6-Dihydropyridine Derivatives on Isolated Rat Trachea. *Drugs Drug Candidates* **2024**, *3*, 342–352. https://doi.org/10.3390/ddc3020020

Academic Editor: Jean Jacques Vanden Eynde

Received: 19 January 2024
Revised: 28 March 2024
Accepted: 2 April 2024
Published: 11 April 2024

1. Introduction

Derivatives of 4H-pyrans have generated considerable attention due to their interesting biological and therapeutic value. Their pharmacological activities include vasorelaxant [1], anticarcinogenic [2], antimicrobial [3], and antioxidant [4] activities, among others. Furthermore, several types of 4H-pyrans have been synthesized searching for new chemical entities, such as inhibitors of acetylcholinesterase/butyrylcholinesterase and modulators of Ca^{2+} channels and nicotinic receptors [5]; some of them have been identified as potent and specific IK(Ca) channel blockers [6,7]. In contrast, 1,6-dihydropyridine derivatives have been poorly studied from the pharmacological point of view, and those few studies have shown their vasorelaxing effect as L-type calcium channel blockers [1,8]. However, their analogues from the 1,4-dihydropyridines moiety are very important heterocyclic rings that possess prominent therapeutic effects, mainly a potent Voltage-Gated Calcium Channel (VGCC) blocker derivative, which acts as an anti-hypertensive, anti-anginal, anti-tumor, anti-inflammatory, anti-tubercular, anti-cancer, anti-hyperplasic, anti-mutagenic, anti-dyslipidemic, and anti-ulcer agent [9]. Thus, in the current study, 4H-pyran and 1,6-dihydropyridine derivatives were synthesized as potential calcium channel blockers. The Ca^{2+} channels are present in the vascular smooth muscle and are targeted for the development of anti-hypertensive drugs; currently, we describe the importance of these

channels in the airway smooth muscle [10] and their possible therapeutic implications in some pathologies mediated by TH2 cells, such as in asthma [11,12]. Asthma traits involve fluctuating airflow obstruction and non-specific hyper sensibility to a diversity of bronchoconstrictors, which are both traits mediated by airway smooth muscle (ASM). The ASM is also a target in the airway inflammation and airway wall remodeling noticed in asthma. The World Health Organization (WHO) reported that about 300 million people worldwide are affected by asthma; therefore, it is considered a public health problem. The most important therapy for asthma is the inhibition of constriction on the ASM [13] and inflammation. In this context, drugs used in the treatment of asthma are those that prevent symptoms (inhaled corticosteroids, leukotriene inhibitors, tromboxane A2 inhibitors, and mast cell stabilizers) as well as those that relieve symptoms (short acting β2 agonists, systemic corticosteroids, anti-cholinergic drugs, and methylxanthines) [14,15]. Although a lot of efficient drugs for the treatment of asthma do exist, it is necessary to develop new bioactive compounds that serve as alternatives for treating symptoms more effectively than the current therapeutic drugs.

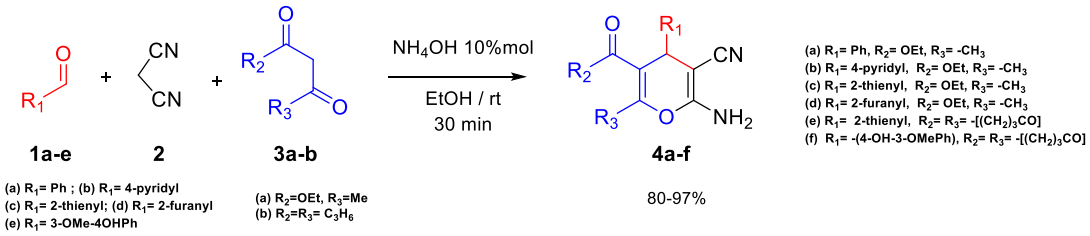
Thus, the aim of this work was to determine the relaxant activity of some 4H-pyran and 1,6-dihydropyridine derivatives on isolated tracheal rat rings, in order to find new or known ASM-relaxing molecules to be used as potential drugs in the treatment of asthma.

2. Results and Discussion

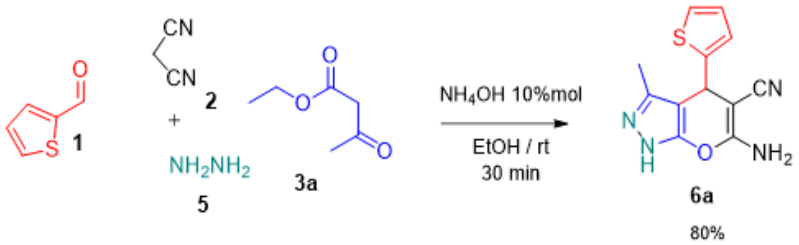
2.1. Synthesis of 4H-Pyran Derivatives **4a–f** *and* **6a**

The traditional route for the synthesis of 4H-pyrans is the cyclocondensation of 5-oxonitriles. These compounds are generated by Michael addition between α,β-unsaturated carbonyl derivatives and compounds with active methylene groups. By this method, we prepared the intermediate **4a–f** and target compounds (**8b–d** and **8g–h**) (Schemes 1–3). The compounds **4a–f** and **6a** were obtained in good yields using multicomponent reactions between aldehydes, malononitrile, and the appropriate 1,3-dicarbonyl systems, using ammonium hydroxide as the catalyst.

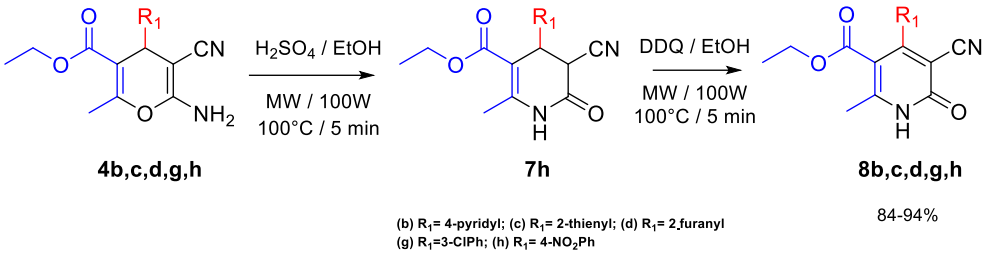
Previously, a procedure to obtain 4H-pyrans [8,16] using a multicomponent reaction between aldehydes, malononitrile, and 1,3-dicarbonyl systems was reported. This reaction proceeded with high yields of 80–97% (Scheme 1).



Scheme 1. Synthesis of 4H-pyran analogues **4a–f.**



Scheme 2. Synthesis of 4H-pyran analogue **6a**.



Scheme 3. Synthesis of 1,6-dihydropyridines **8b**–**d** and **8g**–**h**.

The synthesis of 4H-pyran derivatives occurred either directly with 1,3-dicarbonyl systems like ethyl acetoacetate and 1,3-cyclohexanodione, or with other substrates like pyrazolone (Scheme 2). It is noticeable that the malleability of these routes led to the construction of an extensive collection of 4H-pyrans, changing the substitution pattern in positions 2 and 3. The 4H-pyran core served as a scaffold to produce other nuclei of biological interest; then, based on this feature, we synthetized 2-pyridones.

2.2. Synthesis of 1,6-Dihydropyridine Derivatives **8b**–**d** *and* **8g**–**h**

Likewise, for the preparation of compounds **8b**–**d** and **8g**–**h** the 4H-pyran analogues (**4a**–**f**) were used. In this respect, reacting 4-derivatives in acid medium gave rise to product 8-derivatives through an opening–closure ring process, followed by oxidation. Both processes were promoted by microwave radiation (Scheme 3).

Moreover, in previous works the synthesis of 1,6-dihydropyridines using microwaves [1] and infrared irradiations was reported (Scheme 3) [16]. By using microwave energy, 90–92% yields were obtained.

The first step in the reaction was promoted by H_2SO_4 (10 mol%), yielding a mixture of diastereoisomers, in a ratio ≈80/20 trans/cis. In this mixture, both isomers were oxidized by DDQ. Microwave radiation promoted an efficient reaction in comparison with infrared radiation, with shorter times (10 min) and better yields. NMR and HRMS spectra of compounds studied are available as Supplementary Materials. NMR and HRMS spectra of compounds studied are available as Supplementary Materials.

2.3. Relaxant Effect

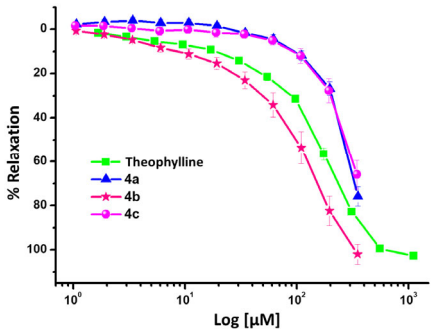
Smooth muscle is the main tissue that plays an important role in asthmatic pathways that involve bronchoconstriction processes, bronchial hyper-reactivity, and airway remodeling [17].

Bronchoconstriction by smooth muscle is the main consequence of damage to the epithelium and, consequently, mucosal wear. These factors together cause the nerve endings to be exposed, and due to being easily activated by the cholinergic and adrenergic systems, be susceptible to the stimulation of cytokines; neuropeptide types A and P, resulting in glandular secretion; and increased smooth muscle contraction. Current molecules for anti-asthmatic drug development include anti-inflammatory and/or bronchodilator drugs. Thus, by exploring direct airway relaxation by 4H-pyranes and 1,6-dihydropyridines on carbachol-pre-contracted rat tracheal rings, an emergent model similar to the guinea-pig tracheal model was obtained [18].

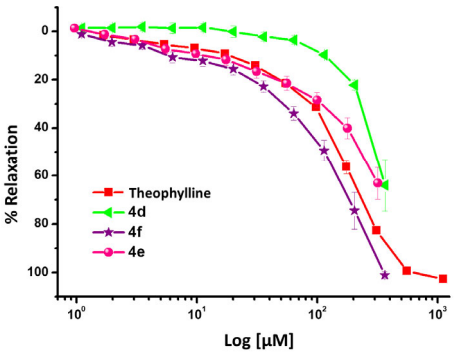
All compounds showed a concentration-dependent relaxant effect on the contraction induced by carbachol (1 μM, Table 1). Concentration–response curves revealed that compounds **4b** (EC50: 96.3 ± 7.5 μM) and **4f** (EC50: 25.9 ± 4.5) were the most potent and efficient (100% of Emax) of the entire series, even more than theophylline (phosfodiesterase inhibitor, EC50: 158 μM), which was used as positive control (Figure 1A,B; Table 1).

Table 1. Relaxing effect by theophylline and compounds **4a–f**, **6a**, **7a**, and **8b–d**, and **8g–h** on the contraction induced by carbachol (1 μM).

Compound	Emax (%)	EC50 (μM)
Theophylline	102.9 ± 1.6	158 ± 3.0
4a	77.1 ± 4.9	236.8 ± 4.9
4b	102.3 ± 4.9	96.3 ± 7.5
4c	75.9 ± 8.8	143.1 ± 8.8
4d	74.5 ± 9.4	259 ± 9.4
4e	64.6 ± 7.1	101.9 ± 7.1
4f	101.2 ± 1.2	25.9 ± 4.5
6a	48.63 ± 5.9	ND
7a	82.6 ± 2.1	98.2 ± 3.1
8b	47.1 ± 6.1	ND
8c	75.3 ± 4.2	152.7 ± 3.6
8d	87.2 ± 3.8	109.6 ± 7.5
8g	46.5 ± 4.6	ND
8h	78.4 ± 3.9	126.4 ± 6.5



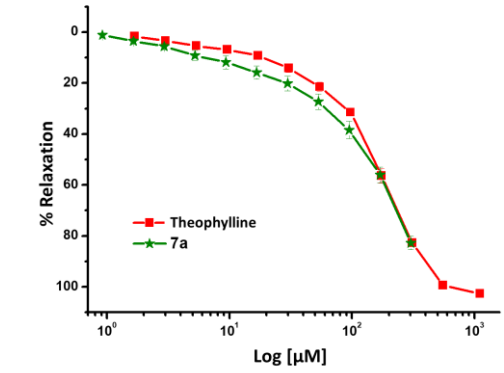
(**A**)



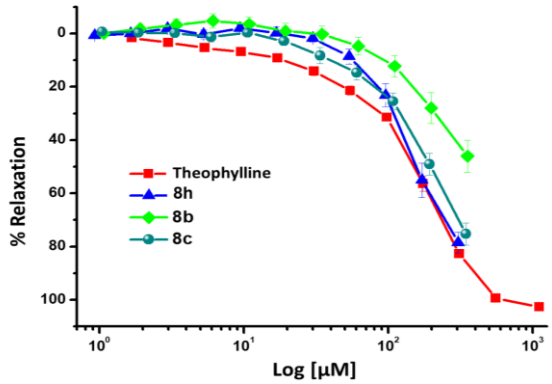
(**B**)

Figure 1. Concentration–response curves of compounds **4a–c** (**A**) and **4d–f** (**B**) on rat trachea rings pre-contracted with carbachol (1 μM).

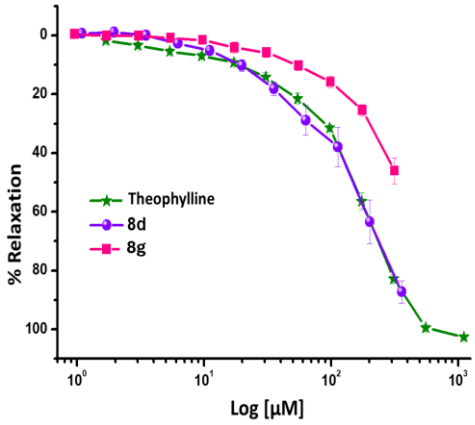
On the other hand, compounds **7a** (EC50: 98.2 ± 3.1 μM), **8c** (EC50: 152.7 ± 3.6 μM), **8d** (EC50: 109.6 ± 7.5 μM) and **8h** (EC50: 126.4 ± 6.5 μM) showed the same potency as the positive control, but were less efficient (Figure 2A–C, Table 1). Finally, the remaining compounds (**4a**, **4c**, **4d**, **4e**, **6a**, **8b**, and **8e**) were less potent and less efficient than theophylline (Figures 1 and 2, Table 1).



(**A**)



(**B**)



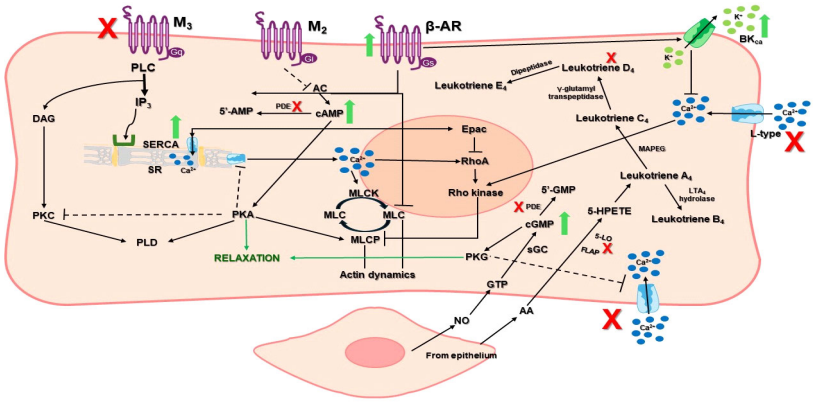
(**C**)

Figure 2. Concentration–response curves of compounds **7a** (**A**), **8b, c** and **h** (**B**), and **8d** and **g** (**C**) on rat trachea rings pre-contracted with carbachol (1 µM).

The smooth muscle cell relaxant effect shown by the test samples could be related with the interference with contraction or induce a direct relaxant effect on airway smooth muscle (Scheme 4).

Smooth muscle contraction can be initiated by depolarization (electro–mechanical coupling), or by stimulation with contractile agonists (pharmaco–mechanical coupling), which are closely related to an increase in intracellular Ca^{2+} concentrations; subsequently, the formation of a Ca^{2+}–calmodulin complex activates the myosin light chain kinase (MLCK), which phosphorylates myosin light chains at serine 19 residue. This phosphorylation

activates myosin ATPase, with the subsequent formation of cross-bridges between actin and myosin triggering the contractile process.



Scheme 4. General mechanisms of relaxation in the airway smooth muscle cells.

Therefore, the mechanisms of relaxation of 1,6-dihydropyridine derivatives could be related with different pathways, such as β2-adrenergic stimulation that increases intracellular cyclic AMP (by adenylate cyclase activation) and induces ASM relaxation by protein kinase A activation. Other mechanisms involved are K+-channel opening, which causes the entry of K^+ and blocks the Ca^{2+} channels, inducing hyperpolarization with consequent relaxation; cAMP increment by PDE4-phosphodiesterase inhibition; leukotriene and muscarinic receptor antagonism; Ca^{2+} channel blockade, including the voltage-operated calcium channel (VOC), receptor-operated Ca^{2+} channels (ROC), and reverse-mode Na^+/Ca^{2+} exchangers and/or NO/cGMP system activation; and the augmented production of cGMP on rat trachea, which interferes with the contraction mechanism of smooth muscle cells in the airways (Scheme 4) [8,15,17].

With the obtained results, we cannot establish an SAR pattern with the number of compounds studied, nor due to the substituents included because of their great structural variation, but we can attribute it to the similarity between the 2-pyridones and 4H-pyrans with the 1,4-dihydropyridines, such as nifedipine. 1,4 dihydropyridines (pharmacophore) are compounds with powerful Ca^{2+} channel blocking activity, producing significant relaxation on smooth muscle cells from different tissues, such as that present in the trachea. That is why our compounds show a similar mechanism, i.e., a blockade of L-type Ca^{2+} channels activated by voltage or by the agonism of G protein-coupled receptors. Recently, we reported the trachea-relaxing effect of a compound very similar to those here described, showing that, through functional and in silico studies, the effect was caused by Ca^{2+} channel blockades [19].

Thus, we present results that offer solid evidence of the pharmacological efficacy of 4H-pyran and 1,6-dihydropyridine derivatives as potential agents for the treatment of respiratory diseases.

Further experiments are necessary to establish the mechanism of action of most active compounds; however, considering their analogy with 1,4-dihydropyridines, they could probably be L-type Ca^{2+} channel blockers [8,9].

The development of drugs with various pharmacological properties that may be useful in therapy requires a series of preclinical studies that ensure the effectiveness and safety of the molecules to be developed. In pharmaceutical chemistry, the first step is to find a molecule that can behave as a leader or a hit (a molecule that shows relevant potency to be selected) whose EC50 values are in the order of nM to μM. After that, it is necessary to establish its mechanism of action, preferably multitarget (with more than one mechanism of action) in in vivo, ex vivo and in silico approaches, which suggests how the bioactive compound produces its effect, and after that evaluate the effect in a related murine model to establish if its effect is dose-dependent. If they are effective at the in vivo level, their

toxicity is studied in animal models and at the cellular level and at the same time their pharmacokinetics are established. With this information generated, we can begin to carry out pharmaceutical development studies to have an established pharmaceutical form with the appropriate administration route to proceed with clinical studies in their different phases [20,21].

3. Materials and Methods

The synthesis reagents and chemicals were obtained from Sigma-Aldrich and used as received without any further purification. On the other hand, pharmacological reagents such as carbamylcholine (carbachol), theophylline, and KCl were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). All compounds and positive control were freshly prepared on the same day of experimentation. Microwave irradiation was conducted in a Discover SP CEM Activent microwave apparatus (CEM corporation, 3100 Smith Farm Road, Matthews, NC, USA).

Melting points were determined on an Electrothermal digital 90100 melting point apparatus and were uncorrected. The progress of the reaction and the purity of compounds were monitored by TLC with Merck silica gel 60-F254-coated aluminum sheets, in n-hexane/ethyl acetate, and visualized by a 254 nm UV lamp. NMR spectra were recorded, for solutions in DMSO-d6 with Me4Si as internal standard, on Varian Mercury (200 MHz), Varian Gemini (300 MHz), Bruker UltraShield (400 MHz), and Bruker UltraShield (500 MHz) instruments (Bruker corporation, Billerica, MA, USA). Mass studies were performed using a spectrometer Bruker ESI-QTOFMS maXis impact (Bruker corporation, Billerica, MA, USA) and the samples were analyzed in combination with methyl stearate as internal standard.

Chemical shifts are given in ppm (δ); multiplicities are indicated by s (singlet), d (doublet), dd (double doublet), t (triplet), q (quartet), m (multiplet) or bs (broad singlet).

3.1. General Procedure for the Synthesis of Compounds **4a**–**f**

A mixture of aldehyde **1a**–**e** (3.0 mmol), malononitrile 2 (3.0 mmol), an appropriate 1,3-dicarbonyl compound (**3a**–**b**, 3.0 mmol) and an ammonium hydroxide solution 28% *w*/*w* (10% mmol) was placed in a 25 mL round-bottom flask with 10 mL of EtOH. The mixture was stirred at room temperature for 30 min until the consumption of the substrates. The reaction was monitored by TLC. The precipitated product formed was filtered and successively washed with warm hexane; the residue was purified by recrystallization in ethanol to provide pure **4a**–**f**.

Ethyl-6-amino-5-cyano-2-methyl-4-phenyl-4H-pyran-3-carboxylate (**4a**)

Yield: 95%; Yellow solid; mp 179–180 °C, 1H NMR (DMSO-d6, 400 MHz) δ 7.31 (d, J = 6.7 Hz, 2H, H-2′), 7.27 (d, J = 6.6 Hz, 1H, H-4′), 7.17 (t, J = 8, 6.7 Hz, 2H, H-3′), 6.93 (s, 2H, -NH_2), 4.30 (s, 1H, H-4), 3.97 (q, 2H, OCH_2CH_3), 2.32 (s, 3H, -CH_3), 1.03 (t, 3H, -OCH2CH3). 13C NMR (DMSO-d6, 100 MHz) δ 165.9 (C=O), 158.9 (C-6), 157.1 (C-2), 145.4 (C-1′), 128.9 (C-2′,6′), 127.7 (C-3′,5′), 127.3 (C-4′), 120.2 (-CN), 107.7 (C-3), 60.6 (-OCH2CH3), 57.7 (C-5), 39.3 (C-4), 18.6 (-CH_3), 14.2 (-OCH_2CH_3), HRMS (ESI+, [M+1]+) calculated for [C16H17N2O3]+: 285.1239, found 285.1234.

Ethyl-6-amino-5-cyano-2-methyl-4-(pyridin-4-yl)-4H-pyran-3-carboxylate (**4b**)

Yield: 86%; White solid; mp 157–158 °C, 1H NMR (DMSO-d6, 400 MHz) δ 8.51 (d, J =7 Hz, 2H, H-3′), 7.14 (d, J =7 Hz, 2H, H-2′), 7.08 (s, 2H, -NH_2), 4.32 (s, 1H, H-4), 3.97 (q, 2H, -OCH_2CH_3), 2.35 (s, 3H, -CH_3), 1.02 (t, 3H, -OCH_2CH_3). 13C NMR (DMSO-d6, 100 MHz) δ 165.6 (C=O), 159.1 (C-6), 158.5 (C-2), 153.8 (C-3′), 150.3 (C-1′), 122.8 (C-2′), 119.8 (-CN), 106.2 (C-3), 60.8 (-OCH_2CH_3), 56.3 (C-5), 38.7 (C-4), 18.7 (-CH_3), 14.1 (-OCH_2CH_3). HRMS (ESI+, [M+1]+) calculated for [C15H16N3O3]+: 286.1186, found 286.1192.

Ethyl-6-amino-5-cyano-2-methyl-4-(thiophen-2-yl)-4H-pyran-3-carboxylate (**4c**)

Yield: 97%; Yellow solid; mp 180–182 °C, 1H NMR (DMSO-d6, 200 MHz) δ 7.37 (dd, J = 0.8, 1.2 Hz, 1H, H-3′), 7.05 (s, 2H, -NH_2), 6.93 (dd, J = 3.3, 3.6 Hz, 1H, H-4′) 6.86 (d,

J = 3.8 Hz, 1H, H-5′), 4.65 (s, 1H, H-4), 4.09 (q, 2H, -OCH_2CH_3), 2.28 (s, 3H, -CH_3), 1.14 (t, 3H, -OCH_2CH_3). 13C NMR (DMSO-d6, 50 MHz) δ 165.2 (C=O), 159.0 (C-6), 156.7 (C-2), 149.4 (C-1′), 126.9 (C-3′), 124.8 (C-5′), 124.0 (C-4′), 119.6 (-CN), 107.7 (C-3), 60.4 (-OCH_2CH_3), 56.9 (C-5), 33.8 (C-4), 18.1 (-CH_3), 13.8 (-OCH_2CH_3). HRMS (ESI+, [M+1]+) calculated for [C14H15N2O3S]+: 291.0798, found 291.0795.

Ethyl-6-amino-5-cyano-4-(furan-2-yl)-2-methyl-4H-pyran-3-carboxylate (**4d**)

Yield: 84%; Red solid; mp 189–190 °C, 1H NMR (DMSO-d6, 200 MHz) δ 7.51 (d, J = 1.6 Hz, 1H, H-3′), 7.01 (s, 2H, -NH_2), 6.24 (m, 1H, H-5´), 6.07 (d, J = 3.4 Hz, 1H, H-4′), 4.44 (s, 1H, H-4), 4.05 (q, 2H, -OCH_2CH_3), 2.28 (s, 3H, -CH_3), 1.12 (t, 3H, -OCH_2CH_3). 13C NMR (DMSO-d6, 50 MHz) δ 165.2 (C=O), 159.5 (C-6), 157.7 (C-2), 155.9 (C-1′), 142.1 (C-3´), 119.6 (-CN), 110.5 (C-5′), 105.4 (C-4′), 105.0 (C-3), 60.3(-OCH_2CH_3), 56.1(C-5), 32.5 (C-4), 18.2 (-CH3), 13.9(-OCH_2CH_3). HRMS (ESI+, [M+1]+) calculated for [C14H15N2O4]+: 275.1026, found 275.1025.

2-amino-5-oxo-4-(thiophen-2-yl)-5,6,7,8-tetrahydro-4H-chromene-3-carbonitrile (**4e**)

Yield: 80%; Yellow solid; mp 219–220 °C, 1H NMR (DMSO-d6, 200 MHz) δ 8.30 (dd, J = 1.2, 4.8 Hz, 1H, H-3′),7.13 (s, 2H, -NH_2), 6.68 (m, 2H, H-4′, 5′), 4.53 (s, 1H, H-4), 2.58 (t, 2H, H-6), 2.32 (m, 2H. H-8), 1.92 (m, 2H, H-7). 13C NMR (DMSO-d6, 50 MHz) δ 195.7(C=O), 164.3 (C-8a), 159.0 (C-2), 149.2 (C-1′), 126.8 (C-5′), 124.4 (C-3′), 123.9 (C-4′), 119.6 (-CN), 114.1 (C-4a), 57.8 (C-3), 36.2 (C-4), 30.3 (C-6), 26.4 (C-8), 19.7 (C-7). HRMS (ESI+, [M+1]+) calculated for [C14H13N2O2S]+: 273.0698, found 273.0695.

2-amino-4-(4-hydroxy-3-methoxyphenyl)-5-oxo-5,6,7,8-tetrahydro-4H-chromene-3-carbonitrile (**4f**)

Yield: 81%; Red solid; mp 233–234 °C, 1H NMR (DMSO-d6, 200 MHz) δ 8.82 (s, 1H, -OH), 6.92 (s, 2H, -NH_2), 6.68 (m, 2H, H-2′,6′), 6.50 (dd, J = 1.8 Hz, 1H, H-3′), 4.10 (s, 1H, H-4), 3.72 (s, 3H, -OCH_3), 2.58 (m, 2H, H-6), 2.27 (m, 2H, H-8), 1.93 (m, 2H, H-7). 13C NMR (DMSO-d6, 50 MHz) δ 195.7(C=O), 164.1 (C-8a), 158.4 (C-2), 147.2 (C-4′), 145.2 (C-5′), 135.8 (C-1′), 119.9 (-CN), 119.2 (C-4a), 115.4 (C-2′), 114.1 (C-3´), 111.5 (C-6′), 58.6 (-OCH_3), 55.66 (C-3), 36.4 (C-4), 34.8 (C-6), 26.5 (C-8), 19.9 (C-7). HRMS (ESI+, [M+1]+) calculated for [C17H17N2O4]+: 313.1183, found 313.1175 (2.5 ppm error).

*3.2. Synthesis of 6-amino-3-methyl-4-(thiophen-2-yl)-1,4-dihydropyrano [2,3-c]pyrazole-5-carbonitrile (***6a***)*

A mixture of 2-thiophenecarboxaldehyde (336 mg, 3.0 mmol), malononitrile 2 (197 mg, 3.0 mmol), ethyl acetoacetate **3a** (389 mg, 3.0 mmol), hydrazine solution 50% 4 (192 mg, 3.0 mmol), and ammonium hydroxide solution 28% *w*/*w* (37 mg, 10% mmol) was placed in a 25 mL round-bottom flask, with 10 mL of EtOH. The mixture was stirred at room temperature for 30 min until the consumption of the substrates. The reaction was monitored by TLC. The precipitated product formed was filtered and successively washed with warm hexane; the residue was purified by recrystallization in ethanol to provide pure **6a**.

Yield: 80%; Yellow solid; mp 238–239 °C, 1H NMR (DMSO-d6, 200 MHz) δ 12.13 (bs, 1H, NH), 7.39 (d, J = 4.8 Hz, 1H, H-5′), 6.96 (m, 4H, -NH_2, H-3′, 4′), 4.49 (s, 1H, H-4), 1.97 (s, 3H, -CH_3). 13C NMR (DMSO-d6, 50 MHz) δ 160.6 (C-6), 154.2 (C-7a), 149.7 (C-3), 136.0 (C-2′), 126.4 (C-3′), 124.9 (C-5′), 124.3 (C-4′), 120.6 (-CN), 97.5 (C-3a), 57.5 (C-5), 31.3 (C-4), 9.7 (-CH_3). HRMS (ESI+, [M+1]+) calculated for [C12H11N4OS]+: 259.0648, found 259.0651 (1.1 ppm error).

3.3. General Procedure for the Synthesis of Compounds **8b**–**d** *and* **8g**–**h**

In a pressure tube for microwave reactions, 4H-pyran **4a**–**f** (1.50 mmol) was placed and 3 mL of EtOH was added. Concentrated sulfuric acid (10 mol%) was added to the reaction mixture. The reaction mixture was irradiated with microwave irradiation until a reaction temperature of 100 °C for 5 min, 39 psi, 100 W. The end of the reaction was confirmed by TLC using a 7:3 (Hex/AcOEt). The reaction was continued to carry out recrystallization using a ratio of H_2O/EtOH (95/5), to obtain the mixture of the two diastereoisomers.

The obtained solid was collected by vacuum filtration; the product was allowed to dry and then quantified.

A mixture of 1,4,5,6-tetrahydropyridine (1.50 mmol), ethanol (3 mL), and DDQ (1.50 mmol) was irradiated with microwaves up to 100 °C, 39 psi, 10 W for 5 min. The progress of the reaction was monitored by TLC (EtOAc/hexane 5:5). The crude product was further purified by silica gel column chromatography using (hexane/AcOEt, 1:1) as eluent to afford the pure product **8b**–**d** and **8g**–**h**.

Ethyl-5-cyano-2-methyl-6-oxo-4-(pyridin-4-yl)-1,6-dihydropyridine-3-carboxylate (**8b**)

Yield: 84%; Brown solid; mp 174–176 °C; 1H NMR (DMSO-d6, 200 MHz) δ 8.71 (d, J = 5.4 Hz, 2H, H3′), 7.08 (d, J = 5.6 Hz, 2H, H-2′), 3.82 (q, J = 7 Hz, 2H, -OCH_2CH_3), 2.64 (3H, s, -CH_3), 0.71 (3H, t, J = 7Hz, -OCH_2CH_3). 13C NMR (DMSO-d6, 50 MHz): δ 164.2 (C=O), 159.5 (C-2), 157.1 (C-6), 155.0 (C-4), 149.8 (C-2′), 144.1 (C-1′), 121.9 (C-3′), 114.9 (-CN), 110.7 (C-5), 100.7 (C-3), 61.1 (-OCH_2CH_3), 18.6 (-CH_3), 12.9 (-OCH_2CH_3). HRMS (ESI+, [M+1]+) calculated for [C15H14N3O3]+: 284.1035, found 284.1030.

Ethyl-5-cyano-2-methyl-4-(thiophen-2-yl)-6-oxo-1,6-dihydropyridine-3-carboxylate (**8c**)

Yield: 94%; Brown solid; mp 212–214 °C; 1H NMR (DMSO-d6, 200 MHz) δ 13.00 (bs, 1H, NH), 7.86 (d, J = 4.8 Hz, 1H, H-3′), 7.31 (s, 1H, H-4′), 7.20 (t, J = 4.2 Hz, 1H, H-5′), 3.92 (q, J = 7 Hz 2H, -OCH_2CH_3), 2.36 (s, 3H, -CH_3), 0.89 (t, J =7 Hz, 3H, -OCH_2CH_3). 13C NMR (DMSO-d6, 50 MHz): δ 165.0 (C=O), 159.7 (C-2); 152.7 (C-6), 151.7 (C-4), 134.9 (C-1′), 129.8 (C-5′), 129.5 (C-3′), 127.7 (C-4′), 115.4 (-CN), 112.5 (C-5), 100.6 (C-3), 61.3 (-OCH_2CH_3), 18.1 (-CH_3), 13.3 (-OCH_2CH_3). HRMS (ESI+, [M+1]+) calculated for [C14H13N2O3S]+: 289.0647, found 289.0643.

Ethyl-5-cyano-4-(furan-2-yl)-2-methyl-6-oxo-1,6-dihydropyridine-3-carboxylate (**8d**)

Yield: 90%; Yellow solid; mp 223–225 °C; 1H NMR (DMSO-d6, 200 MHz) δ 7.93 (d, J = 1.6 Hz, 1H, H-3′), 7.24 (d, J = 3.8 Hz, 1H, H-5′), 6.07 (m, 1H, H-4′), 4.10 (q, J = 7 Hz, 2H, -OCH2CH3), 2.35 (s, 3H, -CH_3), 1.05 (t, J = 7 Hz, 3H, -OCH_2CH_3). 13C NMR (DMSO-d6, 50 MHz): δ 165.3 (C=O), 160.1 (C-2), 152.8 (C-6), 146.3 (C-4), 146.1 (C-3′), 145.1 (C-1′), 115.8 (-CN), 115.6 (C-5′), 112.6 (C-4′), 109.6 (C-5), 96.1 (C-3), 61.4 (-OCH_2CH_3), 17.9 (-CH_3), 13.7 (-OCH_2CH_3). HRMS (ESI+, [M+1]+) calculated for [C14H13N2O4]+: 273.0870, found 273.0875.

Ethyl-4-(3-chlorophenyl)-5-cyano-2-methyl-6-oxo-1,6-dihydropyridine-3-carboxylate (**8g**)

Yield: 95%; Red solid; mp 230–232 °C; 1H NMR (DMSO-d6, 200 MHz) δ 7.59 (m, 1H), 7.26 (m, 1H), 7.48 (m, 2H), 3.83 (q, J = 7 Hz, 2H, -OCH_2CH_3), 2.41 (s, 3H, -CH_3), 0.77 (t, J = 7 Hz, 3H, -OCH_2CH_3). 13C NMR (DMSO-d6, 50 MHz): δ 164.5 (C=O), 159.6 (C-2), 157.9 (C-6), 153.9, 138.0, 130.5, 129.3, 127.0, 126.1, 115.2 (-CN), 111.6 (C-5), 100.9 (C-3), 60.9 (-OCH_2CH_3), 18.4 (-CH3), 13.1 (-OCH_2CH_3). HRMS (ESI+, [M+1]+) calculated for [C16H14ClN2O3]+: 317.0693, found 317.0687.

Ethyl-5-cyano-2-methyl-4-(4-nitrophenyl)-6-oxo-1,6-dihydropyridine-3-carboxylate (**8h**)

Yield: 91%; Brown solid; mp 208–210 °C; 1H NMR (DMSO-d6, 200 MHz) δ 13.13 (bs, 1H, -NH), 8.46 (d, J = 8.4 Hz, 2H, H-3′), 7.74 (d, 2H, J = 8.4Hz, H-2′), 3.92 (q, 2H, J = 7 Hz, -OCH_2CH_3), 2.54 (s, 3H, -CH_3), 0.80 (t, 3H, J = 7 Hz, -OCH_2CH_3). 13C NMR (DMSO-d6, 50 MHz): δ 164.2 (C=O), 159.4 (C-2), 157.8 (C-6), 154.9 (C-4), 147.9 (C-4′), 142.8 (C-1′), 128.9 (C-2′), 123.6 (C-3′), 115.0 (-CN), 110.9 (C-5), 101.0 (C-3), 61.05 (-OCH_2CH_3), 18.7 (-CH_3), 13.1 (-OCH_2CH_3). HRMS (ESI+, [M+1]+) calculated for [$C_{16}H_{14}N_3O_5$]+: 328.0928, found 328.0932.

3.4. Pharmacological Evaluation

3.4.1. Rats

Healthy male Wistar rats (250–300 g) were used and maintained under standard laboratory conditions with free access to food and water. All experiments were carried out using six animals per group. Animals used were euthanized by cervical dislocation.

3.4.2. Rat Tracheal Ring Assay

For this purpose, a previous protocol was used [18]. After death, trachea was dissected, cleaned of connective tissue and mucus, and immediately cut into 4–5 mm length rings. Then, tissue segments were mounted by stainless steel hooks, under an optimal tension of 2 g in 10 mL organ baths containing warmed (37 °C) and oxygenated (O_2/CO_2 19:1) Krebs solution. Changes in tension were recorded by isometric force transducers (Grass FT03) connected to an MP100 analyzer (Biopac® System Inc., Goleta, CA, USA). After stabilization, rings were stimulated with carbachol (1 μM) for 10 min and they were washed with fresh Krebs solution. This procedure was repeated at intervals of 30 min for 2 h before starting the experiment. Later, all tissues were contracted with carbachol (1 μM) and test samples (pure compounds, vehicle, and positive control) were added to the bath in quarter-log cumulative concentration (evaluation period); then, cumulative concentration–response curves were obtained for each ring. The relaxant effect of the compound and positive control (theophylline, 1.67–557 μM) was determined by comparing the muscular tone of the contraction before and after the addition of the materials. Muscular tone was calculated from the tracing, using Acknowledge 3.9 software version (Biopac® System, Inc., Goleta, CA, USA).

3.5. Data Analysis

Data were expressed as means ± standard error of the mean (S.E.M.). Concentration–response curves were plotted, and the obtained experimental data were adjusted by nonlinear curves fitting program Origin® 8.0 (Massachusetts, USA, 2007). Statistical analysis was conducted using one-way ANOVA ($p < 0.05$), followed by Bonferroni post hoc test with SigmaStat® 3.0 software (San José, CA, USA, 2003).

4. Conclusions

In conclusion, some 4H-pyran and 1,6-dihydropyridine derivatives were synthesized with significant, potent, and efficient trachea-relaxant effects, and could be used to develop potential calcium channel blockers as candidates for the treatment of asthma.

Supplementary Materials: The NMR and HRMS spectra of compounds studied can be downloaded at: https://www.mdpi.com/article/10.3390/ddc3020020/s1.

Author Contributions: Conceptualization, S.E.-S. and M.A.V.; methodology, S.A.-P., E.G.-T., F.H.-B. and Y.A.; software, S.A.-P. and E.G.-T.; validation, S.A.-P., E.G.-T. and F.H.-B.; formal analysis, S.A.-P., E.G.-T., F.H.-B. and Y.A.; investigation, R.V.-M., S.E.-S., M.A.V., S.A.-P. and F.H.-B.; resources, S.E.-S., R.V.-M. and M.A.V.; data curation, S.A.-P., F.H.-B. and Y.A.; writing—original draft preparation, S.E.-S. and M.A.V.; writing—review and editing, R.V.-M., S.E.-S. and M.A.V.; visualization, S.A.-P., F.H.-B. and Y.A.; supervision, S.E.-S. and M.A.V.; project administration, S.E.-S. and M.A.V.; funding acquisition, S.E.-S., R.V.-M. and M.A.V. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by SEP-CONACYT (Ciencia Básica A1-S-13540, A1-S-27694), CONACYT FORDECYT-PRONACES (Ciencia de Frontera 377882/2020), IN210222, PAPIIT, DGAPA, UNAM, and DAIP-UGTO (063/2024), and fellowship grants from CONACyT (267615 and 482137) for the Ph.D. Studies of S. Alemán-Pantitlán and F. Hernandez-Borja, respectively.

Institutional Review Board Statement: This study was conducted in accordance with the Declaration of Helsinki and in accordance with our Federal Regulations for Animal Experimentation and Care (SAGARPA, NOM-062-ZOO-1999, Mexico), and it was approved by the Institutional Animal Care and Use Committee (Protocol 1497, F.E.S. Iztacala).

Data Availability Statement: Data is contained within the article and Supplementary Materials.

Acknowledgments: We are thankful for the support of the National Laboratory of Molecular Spectroscopy of the Universidad de Guanajuato (CONACyT grant 260373) and of Julia Solano-Chopin for making diagram 4, which shows the various mechanisms of relaxing actions in the airway smooth muscle cells.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Hernández, F.; Sánchez, A.; Rendón-Vallejo, P.; Millán-Pacheco, C.; Alcaraz, Y.; Delgado, F.; Vázquez, M.A.; Estrada-Soto, S. Synthesis, ex vivo and in silico studies of 3-cyano-2-piridone derivates with vasorelaxant activity. *Eur. J. Med. Chem.* **2013**, *70*, 669–676. [CrossRef] [PubMed]
2. Farard, J.; Lanceart, G.; Logé, C.; Nourrison, M.R.; Cruzzalegui, F.; Pfeiffer, B.; Duflos, M. Design, synthesis and evaluation of new 6-substituted-5-benzyloxy-4-oxo-4H-pyran-2-carboxamidas as potential Src inhibitors. *J. Enzyme Inhib. Med. Chem.* **2008**, *5*, 629–640. [CrossRef] [PubMed]
3. Lenaerts, A.J.; Bitting, C.; Woolhiser, L.; Gruppo, V.; Marietta, K.S.; Johnson, C.M.; Orme, I.M. Evaluation of a 2-Pyridone, KRQ-10018, against Mycobacterium tuberculosis in vitro and in vivo. *Antimicrob. Agents Chemother.* **2008**, *52*, 1513–1515. [CrossRef] [PubMed]
4. Saundane, A.; Vijaykumar, K.; Vaijinath, A.V. Syntesis of novel 2-amino-4-(5′-substituted 2′-phenyl-1H-indol-3′-yl)-6-aryl-4H-pyran-3-cabonitrile derivates as antimicrobial and antioxidant agents. *Biorg. Med. Chem. Lett.* **2013**, *23*, 1978–1984. [CrossRef] [PubMed]
5. Marco, J.L.; de los Ríos, C.; García, A.G.; Villarroya, M.; Carreiras, M.C.; Martins, C.; Eleutério, A.; Morreale, A.; Orozco, M.; Luque, F.J. Synthesis, biological evaluation and molecular modelling of diversely functionalized heterocyclic derivatives as inhibitors of acetylcholinesterase/butyrylcholinesterase and modulators of Ca2+ channels and nicotinic receptors. *Bioorg. Med. Chem.* **2004**, *12*, 2199–2218. [CrossRef] [PubMed]
6. Urbahns, K.; Horváth, E.; Stasch, J.P.; Mauler, F. 4-Phenyl-4H-pyrans as IK(Ca) channel blockers. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 2637–2639. [CrossRef] [PubMed]
7. Kang, S.; Cooper, G.; Dunne, S.F.; Luan, C.H.; James Surmeier, D.; Silverman, R.B. Antagonism of L-type Ca2+ channels CaV1.3 and CaV1.2 by 1,4-dihydropyrimidines and 4H-pyrans as dihydropyridine mimics. *Bioorg. Med. Chem.* **2013**, *21*, 4365–4373. [CrossRef] [PubMed]
8. Rendón-Vallejo, P.; Estrada-Soto, S.; Vázquez, M.A.; Hernández-Borja, F.; Villalobos-Molina, R.; Ibarra-Barajas, R. Design, Synthesis and ex vivo study of the vasorelaxant activity induced by isosteric derivatives of dihydropyridines (NH→O). *Lett. Drug Des. Discov.* **2016**, *13*, 353–359. [CrossRef]
9. Abhinav Prasoon, M.; Ankit, B.; Awani Kumar, R. 1,4-Dihydropyridine: A dependable heterocyclic ring with the promising and the most anticipable therapeutic Effects. *Mini Rev. Med. Chem.* **2019**, *19*, 1219–1254.
10. Hernandez, J.M.; Janssen, L.J. L-type Ca+2 channels, Ca+2-induced Ca+2 release, and BKca channels in airway stretch-induced contraction. *Eur. J. Pharmacol.* **2012**, *696*, 161–165. [CrossRef]
11. Pelletier, L.; Savignac, M. Ca+2 signaling in T-cell subsets with a focus on the role of Cav1 channels: Possible implications in therapeutics. *Front. Immunol.* **2013**, *4*, 150. [CrossRef] [PubMed]
12. Gomes, B.; Cabral, M.; Gallard, A.; Savignac, M.; Paulet, P.; Druet, P.; Mariamé, M.; Moreau, M.; Leclerc, C.; Guéry, J.; et al. Calcium channel blocker prevents T helper type 2 cell-mediated airway inflamation. *Am. J. Respir. Crit. Care Med.* **2007**, *175*, 1117–1124. [CrossRef] [PubMed]
13. O'Byrne, P.M. Global guidelines for asthma management: Summary of the current status and future challenges. *Pol. Arch. Med. Wewn.* **2010**, *120*, 511–517. [CrossRef] [PubMed]
14. Nagai, H. Recent research and developmental strategy of anti-asthma drugs. *Pharmacol. Ther.* **2012**, *133*, 70–78. [CrossRef]
15. Siddiqui, S.; Redhu, N.S.; Ojo, O.O.; Liu, B.; Irechukwu, N.; Billington, C.; Janssen, L.; Moir, L.M. Emerging airway smooth muscle targets to treat asthma. *Pulm. Pharmacol. Ther.* **2013**, *26*, 132–144. [CrossRef] [PubMed]
16. Sánchez, A.; Hernández, F.; Cruz, P.; Alcaraz, Y.; Tamariz, J.; Delgado, F.; Vázquez, M.A. Infrared irradiation-assisted multicomponent synthesis of 2-amino-3-cyano-4H-pyran derivatives. *J. Mex. Chem. Soc.* **2012**, *56*, 121–127. [CrossRef]
17. Satori, N.A.; Pacini, E.S.A.; Godinho, R.O. Impact of the cAMP efflux and extracellular cAMP-adenosine pathway on airway smooth muscle relaxation induced by formoterol and phosphodiesterase inhibitors. *Chem. Biol. Interact.* **2023**, *382*, 110630. [CrossRef]
18. Uka, A.; Krasniqi, D.; Beretta, G.; Daci, A. Assessment of in vitro airway smooth muscle relaxant activity of *Rhus coriaria* L. fruit ethanolic extract and its possible mechanisms. *J. Med. Food* **2023**, *26*, 820–830. [CrossRef]
19. Alemán-Pantitlán, S.; Millán-Pacheco, C.; Vázquez, M.A.; Hernández-Borja, F.; Villalobos-Molina, R.; Bazán-Perkins, B.; Estrada-Soto, S. Mechanism of Relaxant action of ethyl 6-amino-5-cyano-2-methyl-4-(pyridin-4-yl)-4H-pyran-3-carboxylate mainly through calcium channel blockade in isolated rat trachea. *J. App. Pharm. Sci.* **2016**, *6*, 29–36.
20. Hughes, J.P.; Rees, S.; Kalindjian, S.B.; Philpott, K.L. Principles of early drug discovery. *Br. J. Pharmacol.* **2011**, *162*, 1239–1249. [CrossRef]
21. Brodniewicz, T.; Grynkiewicz, G. Preclinical drug development. *Acta Pol. Pharm.* **2010**, *67*, 578–585. [PubMed]